Functions, transmission and emission of the canopy microbiota

Tania Fort

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Par **Tania Fort**

Fonctions, transmission et émission du microbiote de la canopée

Sous la direction de Corinne Vacher

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Membres du jury :

Mme. Anne-Marie DELORT	Directrice de recherche	Institut de Chimie de Clermont-Ferrand	Rapporteuse
M. Stéphane Uroz	Directeur de recherche	INRA Nancy	Rapporteur
Mme. Patricia Luis	Maître de conférence	Université de Lyon 1	Rapporteuse
Mme. Annabel Porté	Directrice de recherche	INRA Bordeaux	Présidente
Mme. Corinne Vacher	Directrice de recherche	INRA Bordeaux	Directrice

Fonctions, transmission et émission du microbiote de la canopée.

Les arbres interagissent avec des communautés microbiennes diversifiées qui influencent leur fitness et le fonctionnement des écosystèmes terrestres. Contrairement aux micro-organismes associés aux racines et au sol, les micro-organismes qui colonisent la canopée forestière sont encore mal connus. L'objectif de cette thèse est de mieux comprendre les fonctions des micro-organismes associés aux parties aériennes des arbres (feuilles, tiges, graines) ainsi que leur dynamique de transmission verticale (de l'arbre à ses descendants) et horizontale (émission de l'arbre vers l'atmosphère), en combinant des analyses d'écologie moléculaire et d'écophysiologie végétale. Le **chapitre II** montre que les communautés fongiques de la phyllosphère du hêtre (*Fagus Sylvatica*) sont fortement structurées par l'arbre hôte, contrairement aux bactéries qui sont plus fortement influencées par les gradients environnementaux verticaux à l'intérieur de la canopée. Dans les deux cas, les variations de composition microbienne sont mieux expliquées par la chimie foliaire que par la morphologie ou physiologie. Ce chapitre révèle aussi la prédominance des champignons pathogènes dans la phyllosphère du hêtre, ainsi qu'une forte abondance de saprotrophes en bas de la canopée. La phyllosphère du hêtre contenait très peu de bactéries diazotrophes, contrairement à d'autres espèces d'arbres. Le **chapitre III** met en évidence la présence de champignons dans les tissus internes des glands du chêne sessile (*Quercus petraea*), y compris l'embryon, suggérant que le microbiote peut être transmis verticalement de l'arbre mère à ses descendants et influencer la régénération forestière. Les glands contiennent en particulier plusieurs espèces de champignons pathogènes, en association avec leurs mycoparasites. Ces communautés fongiques varient significativement en fonction de l'arbre mère et de la population de chêne. Enfin, le **chapitre IV** teste un prototype de mesure des flux d'émissions bactériennes au-dessus des couverts végétaux. Il montre que la moitié des espèces capturées dans l'atmosphère est présente sur la surface des feuilles et sugg ère que la composition des bioaérosols est fortement influencée par la plante cultivée dominante en Nouvelle-Aquitaine, la vigne (*Vitis vinifera*). Cette thèse apporte donc des éléments pour modéliser la dynamique et l'évolution du système arbre-microbiote-atmosphère, qu'il conviendra de renforcer et d'intégrer aux connaissances sur le sol afin de répondre aux défis posés par le changement climatique.

Functions, transmission and emission of the canopy microbiota.

Trees interact with diverse microbial communities that influence their fitness and the functioning of terrestrial ecosystems. Unlike microorganisms associated with roots and soil, microorganisms that colonize the forest canopy are still poorly understood. The objective of this thesis is to better understand the functions of microorganisms associated with the aerial parts of trees (leaves, stems, seeds) as well as their vertical (from the tree to its descendants) and horizontal (emission from the tree to the atmosphere) transmission dynamics, by combining molecular ecology and plant ecophysiology analyses. The **chapter II** shows that fungal communities in the beech phyllosphere (*Fagus Sylvatica*) are strongly structured by the host tree, unlike bacteria, which are more strongly influenced by the vertical environmental gradients within the canopy. In both cases, variations in microbial composition are better explained by foliar chemistry than by morphology or physiology. This chapter also reveals the predominance of pathogenic fungi in the beech phyllosphere, as well as a high abundance of saprotrophic fungi at the bottom of the canopy. The beech phyllosphere contained very few diazotrophic bacteria, unlike other tree species. The **chapter III** highlights the presence of fungi in the internal tissues of acorns of sessile oak (*Quercus petraea*), including the embryo, suggesting that the mycobiota can be transmitted vertically from the mother tree to its descendants and influence forest regeneration. Acorns contain several species of pathogenic fungi, in association with their mycoparasites. These fungal communities differ significantly among mother trees and oak populations. Finally, the **chapter IV** tests a prototype for measuring bacterial emission fluxes over plant canopies. It shows that half of the species captured in the atmosphere are present on leaf surfaces and suggests that the composition of bioaerosols is strongly influenced by the dominant cultivated plant in New Aquitaine, the vine (*Vitis vinifera*). This thesis therefore provides elements for modelling the dynamics and evolution of the tree-microbiota-atmosphere system, which will need to be strengthened and integrated into knowledge about soil in order to meet the challenges posed by climate change.

Unité de recherche

UMR 1202 Biodiversité, Gènes et Communautés (BIOGECO), INRA, 33610, Pessac, France

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Publications scientifiques

Publications acceptées

- Desprez-Loustau ML, Massot M, Feau N, **Fort T**, De Vincente A, Tores J, Fernandez-Ortuno D, Further Support of Conspecificity of Oak and Mango Powdery Mildews and First Report of Erysiphe quercicola and Erysiphe alphitoides on Mango in Mainland Europe. Pant Disease.

- Desprez-Loustau ML et al., 2018, "From leaf to continent: The multi-scale distribution of an invasive cryptic pathogen complex on oak". Fungal Ecology.

Publications soumises ou en préparation

- **Fort T.**, Pauvert C, Zanne AE, Ovaskainen O, Caignard T, Barret M, Compant S, Hampe A, Delzon S, and Vacher C (2019). Maternal Effects and Environmental Filtering Shape Seed Fungal Communities in Oak Trees. In : bioRxiv, p. 691121. doi:10.1101/691121. *En révision pour New Phytologist*.

- **Fort T.**, Pauvert C., Louisanna E., Burlett R., Chancerel E., Linares-Maurizi A., Balias P., Gibbon Y., Zanne AE., Hampe A., Schimann H., Wingate L., and Vacher C. Within-canopy variations in phyllosphere microbial communities of European beech (*Fagus sylvatica*): magnitude, drivers and functional consequences. *En préparation pour ISME J*.

- **Fort T**, Vacher C., Faivre d'Arcier J., Lalanne C., Chancerel E., Bonnefond JM, Garrigou D., Delmas F., Brunet Y. Quantitative and qualitative assessment of bacterial fluxes between among soil, plant phyllosphere and near-surface atmosphere. *En préparation pour Science of the total environment*.

- Pauvert C., Vallance J., **Fort T**., Calonnec A., Faivre d'Arcier J., Chancerel E., Massot M., Bohan DA., Chiquet J., Robin S., and Vacher C. Microbial association networks give relevant insights into plant pathobiomes. *En préparation*.

- Ma Y., **Fort T**., Marais A., Lefebvre M., Theil S., Vacher C. and Candresse T. Different patterns in leaf-associated viromes and mycobiomes of wild plant populations between cultivated and natural ecosystems. *En préparation*.

- Cartry, D., **Fort, T**., Delzon, S., Bonal D., Buee M., Chancerel, E., Coste S., Louisanna E., Roy M., Stahl C., Ziegler L., Schimann H., Vacher C. Relationships between the canopy microbiota and tree tolerance to drought. *En préparation*.

- Schimann H., Coste S., **Fort T**., Louisanna E., Sagne A., Vacher C., Zinger L. Vertical stratification and functioning of the phyllosphere microbial communities in tropical rainforest. *En préparation*.

Communications scientifiques

- Functional ecology of microbial communities of forest trees. Communication orale à la Journée des doctorants en 2017.

- Vertical transmission of the microbiota in sessil oak (*Quercus petraea*). Communication orale à la Journée des doctorants en 2018.

- Histoires passées et futures de mystérieuses microsociétés. Communication orale pour un événement de vulgarisation scientifique, Pint of Science en 2018.

- Vertical transmission of the pathobiome in sessile oak (*Quercus petraea*). Communication orale à la conférence Pathobiome en mars 2018, Ajaccio.

- Seed mycobiota is influenced by maternal and environmental effects in sessile oak (*Quercus petraea*). Communication d'un poster à la conférence Phytobiomes en décembre 2018, Montpellier. Prix INRA jeunes chercheurs.

- Structures et fonctions des communautés associées aux plantes. Communication orale pour un événement de vulgarisation scientifique à La Maison pour la Science en 2019.

- Mobility to Washington DC. Communication orale au Labex Day en 2019.

Formations suivies

- Intégrité scientifique dans les secteurs de la recherche organisée par l'Université de Bordeaux en 2017

- Formation avec Patrick Leger et Adline Delcamp (techniciens à l'UMR Biogeco) à l'extraction, l'amplification par PCR et le dosage d'ADN (Nanodrop et Qubit) en novembre 2016.

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- Formation à l'utilisation du package R metacoder lors d'un workshop "Analysis of microbiome community data in R" organisé par Phytobiomes Alliance à Montpellier

- Formation à l'utilisation de modèles Poisson log-normal à AgroParistech en février 2019.

Autres activités

- Participation à la Journée de lancement du réseau Phytobiome INRA à Paris le 16 septembre 2016

- Participation à plusieurs campagnes d'échantillonnage dans l'UMR EcoFog en Guyane française en mars et en octobre 2017

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Animations scientifiques

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- Participation aux Zanne Lab Meetings hebdomadaires: partage de connaissances en méthodologie; présentation de premiers résultats de biologie moléculaire.

- Participation au GWU Eco Evo Biogeo Journal Club hebdomadaire animé par Amy Zanne: présentations animées par différents scientifiques (doctorants, post-doctorants, chercheurs) sur l'organisation du projet de thèse (rédaction de demandes de subvention, rédaction du manuscrit de thèse) et les perspectives d'insertion professionnelle (rédaction d'un CV, postuler à un post-doctorat, rédaction de lettre de recommandation).

Sommaire

I Introduction

I. Les micro-organismes, des éléments essentiels au fonctionnement du système Terre

Les micro-organismes ont peuplé, dominé et façonné notre planète et ses habitants depuis plus de 3,5 milliards d'années (Blaser *et al.*, 2016). Lors de ces nombreuses années d'évolution, ils se sont diversifiés et sont maintenant retrouvés dans les trois domaines du vivant, qui sont les bactéries, les archées et les eucaryotes (Figure 1). Les micro-organismes remplissent de nombreuses fonctions essentielles au fonctionnement des écosystèmes et contribuent notamment à une productivité végétale durable et un environnement stable pour la vie humaine (Gilbert & Neufeld, 2014). Les micro-organismes sont l'un des principaux moteurs du cycle du carbone de la Terre. Dans l'océan, le phytoplancton (bactéries et algues unicellulaires photosynthétiques) est responsable d'environ la moitié du carbone fixé dans l'atmosphère chaque année. Leur participation à la fixation du carbone atmosphérique est bien moindre dans les écosystèmes terrestres puisqu'une grande partie du carbone est séquestrée par la végétation terrestre (Field *et al.*, 1998). Néanmoins, les micro-organismes jouent également un rôle clé dans la stabilisation et le recyclage du carbone fixé. Dans les sols, les micro-organismes transforment les polymères végétaux (cellulose et hemicellulose) et permettent l'accumulation du carbone, formant ainsi la base d'une grande partie des stocks de carbone terrestre (Schmidt *et al.*, 2011). La fixation de l'azote est un autre processus chimique remarquable réalisé par les micro-organismes. Les micro-organismes catalysent cette réaction coûteuse en énergie à température et pression ambiantes, formant fréquemment des symbioses avec des organismes supérieurs tels que les plantes (Vitousek *et al.*, 2002) et les insectes (Douglas, 2009). A l'échelle de la planète, les bactéries métabolisent 92% de l'azote atmosphérique dont 88% qu'elles transforment en protoxyde d'azote. Au-delà du cycle du carbone et de l'azote, les micro-organismes purifient l'eau des rivières, ruisseaux et lacs et contrôlent le flux de nutriments comme le phosphore qui peut réguler le développement et la stabilité des écosystèmes, ainsi que l'établissement de réseaux trophiques complexes. Au cours des dernières années, l'avènement des techniques moléculaires a conduit à la découverte d'une multitude d'espèces non cultivables, révélant ainsi la diversité et l'omniprésence des micro-organismes à travers le globe (Thompson *et al.*, 2017; Flemming & Wuertz, 2019).

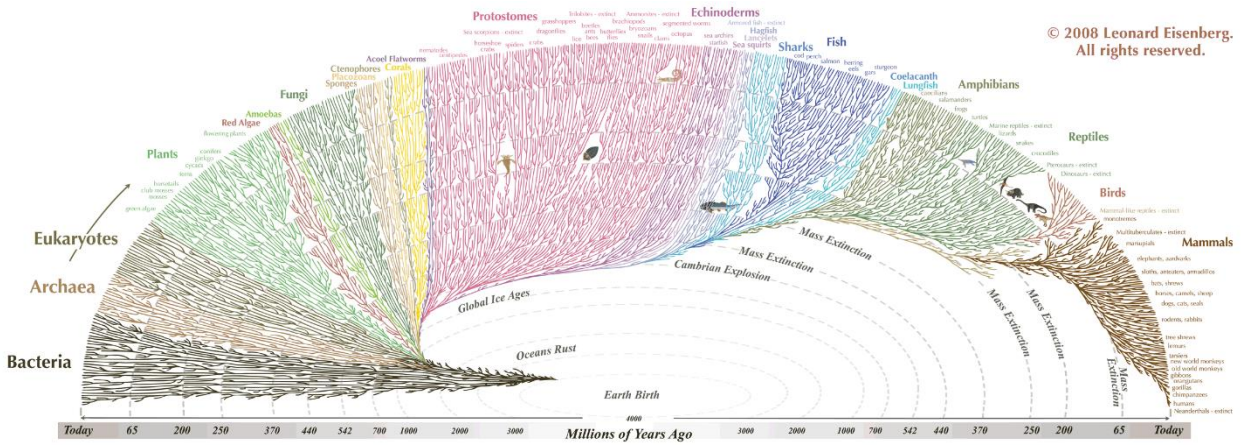


Figure 1. Arbre phylogénétique représentant les ordres majeurs du vivant (Interactive Tree of Life). L'arbre illustre l'évolution des organismes vivants de la formation de la Terre (intérieur du demi-cercle) à nos jours (extérieur de demi-cercle). Chaque couleur représente un règne ou sous-règne. Les bactéries (vert foncé) sont les premiers organismes à avoir colonisées la planète. L'explosion cambrienne produite par l'accumulation d'oxygène qu'elles ont produit a permis l'établissement d'une myriade de nouvelles espèces.

Les micro-organismes colonisent un large éventail d'habitats microbiens. Leurs capacités d'adaptation et leurs résistances à des conditions extrêmes leur permettent de proliférer dans des milieux hostiles tels que la fosse des Mariannes (Bartlett, 2009) et les lacs acides du parc du Yellowstone (Quatrini & Johnson, 2018). Ils sont également communément retrouvés dans les sols ou en association avec des hôtes incluant les animaux et les plantes (Zilber-Rosenberg & Rosenberg, 2008). La colonisation et la prolifération des micro-organismes dans ces habitats conduit à la formation de microbiomes. Le microbiome désigne l'ensemble formé par l'habitat microbien, les micro-organismes qui le colonisent (microbiote), leurs génomes et les conditions environnementales qui les entourent (Marchesi & Ravel, 2015). Le microbiote s'organise sous forme de communauté, définie comme un assemblage multi-spécifique, dans lequel les individus partagent un environnement commun et interagissent les uns avec les autres (ou ont le potentiel et la possibilité d'interagir) de diverses manières (Konopka, 2009). Ces communautés sont structurées par 4 processus : la dispersion, la diversification évolutive, la sélection et la dérive (Nemergut *et al.*, 2013; Vacher *et al.*, 2016b). La dispersion et la diversification évolutive façonnent la diversité fonctionnelle des micro-organismes qui arrivent dans la communauté. La sélection externe favorise celles qui sont les plus adaptées aux conditions locales. La sélection interne, par le biais d'interactions biotiques telles que la compétition ou le parasitisme, régule davantage la structure de la communauté microbienne. La dérive entraîne des changements stochastiques dans la structure

communautaire. La structure de la communauté microbienne résulte donc de l'influence de la plante, de l'atmosphère et de la communauté elle-même. Ces processus ne sont pas indépendants les uns des autres et peuvent s'influencer mutuellement. Comprendre les contributions relatives de ces processus dans les microbiomes associés à l'hôte est une première étape importante pour mieux comprendre le rôle des communautés microbiennes sur l'hôte (Costello *et al.*, 2012).

II. La forêt, un écosystème diversifié en habitats microbiens indispensables à son fonctionnement

Les forêts offrent une grande variété d'habitats pour les micro-organismes tels que le sol, la litière et les organes des arbres (feuilles, bois, écorces, racines) (Tableau 1, Figure 2 ; Baldrian, 2017). Ces écosystèmes occupent environ 38 millions de kilomètres carrés de superficie terrestre et représentent donc d'importants microbiomes à l'échelle de la planète (Perry, 2009; Crowther *et al.*, 2013). Les habitats microbiens forestiers diffèrent de par leur taille et leurs propriétés : le sol et les feuilles représentent des habitats considérables en termes de masse et de surface. La disponibilité en éléments nutritifs, les conditions environnementales affectent également l'abondance et la composition des microbiomes forestiers (Baldrian, 2017). Jusqu'à présent, les recherches en écologie microbienne forestière étaient fortement axées sur le microbiome du sol et des racines des arbres et, dans une moindre mesure, sur le microbiome du bois mort et la litière, tandis que les autres habitats étaient largement sous-explorés (Baldrian, 2017).

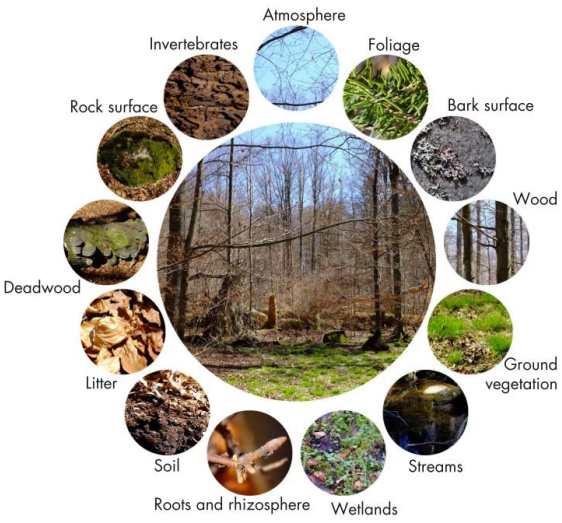


Figure 2. Diversité des habitats microbiens au sein d'un écosystème forestier. Les photos représentent différents habitats de la forêt naturelle mixte de la réserve naturelle Zoffin, en Europe centrale (Baldrian, 2017). Les graines des plantes ne sont pas représentées, mais elles constituent également un habitat microbien.

Table 1. Propriétés des habitats au sein des écosystèmes forestiers adapté de Baldrian et al. (2017). Les valeurs sont représentatives des forêts tempérées et boréales de l'hémisphère nord, qui sont les plus etudiées.

Habitat	Taille de l'habitat	Biomasse microbienne	Taxa dominants et groupes fonctionnels	Références
Litière	2–50 t C ha^{-1} (production de 1–5 t ha^{-1} y^{-1})	10^8–10^9 cellules bactériennes g^{-1}, 0.7–7 mg g^{-1} biomasse fongique	Dominance des champignons, des bactéries à phyla multiples, écologie diverse, grande abondance de saprotrophes, de bactéries et de champignons mycophages	Lindahl *et al.* (2007); Voříšková and Baldrian (2013); López-Mondéjar *et al.* (2015); Žifčáková *et al.* (2016)
Bois mort	1.5–300 t ha^{-1} (5–1000 m^3 ha^{-1}), production de 0.5–1 t ha^{-1} y^{-1}	Généralement très élevé, jusqu'à 0.15 g g^{-1} de biomasse fongique ; plusieurs t ha^{-1} de fructifications fongiques	Basidiomycota et Ascomycota saprotrophes ; lichens ; Myxomycota ; abondance élevée de Protéobactéries, Acidobactéries et Actinobactéries ; saproparasites	Rayner and Boddy (1988); Stokland, Siitonen and Jonsson (2012); Clissmann *et al.* (2015); Hoppe *et al.* (2015); Johnston, Boddy and Weightman (2016); Svensson *et al.* (2016)
Sol	50–300 t ha^{-1} matière organique	10^7–10^9 cellules bactériennes g^{-1}, 0.1–0.6 t ha^{-1} de biomasse ecotmycorrhizienne ; 0.2–0.7 mg g^{-1} de mycelium fongique ; production de 2 t ha^{-1} y^{-1} mycelium ectomycorrhizien, 0.2^{-1} t ha^{-1} y^{-1} de fructifications fongiques	Champignons mycorhiziens, champignons saprotrophes et bactéries à phyla multiples	Lindahl *et al.* (2007); Baldrian *et al.* (2012); Clemmensen *et al.* (2013); Žifčáková *et al.* (2016)
Racines et rhizosphère	Dizaines de t ha-1 de racines (production de racines 1,0-5,0 t ha-1 an-1)	Plus élevé que dans le sol, très élevé dans les racines mycorhiziennes, 10^8–10^{10} cellules bactériennes g^{-1}	Champignons ectomycorhiziens et arbusculaires, endophytes, présence de décomposeurs du bois, de bactéries spécifiques de la rhizosphère et de bactéries mycorhiziennes auxiliaires	Prescott and Grayston (2013); Hardoim *et al.* (2015); Kohler *et al.* (2015); Lukešová *et al.* (2015); Marupakula, Mahmood and Finlay (2016)
Feuille	1–11 ha ha^{-1}; 0.5–6.0 t ha^{-1}	Faible biomasse, faible diversité, niveau élevé de fluctuation temporelle	Proteobacteria, Firmicutes, Actinobacteria, Acidobacteria, plusieurs taxa fongiques, e.g. Dothideomycetes and Leotiomycetes	Vorholt (2012); Carrell and Frank (2015); Hardoim *et al.* (2015); Peršoh (2015)

II.1. Les micro-organismes associés à la formation des sols

1.A. Les micro-organismes décomposeurs des litières

En forêt, la matière organique morte représente un groupe hétérogène de résidus comprenant le bois mort, les tiges et petits rameaux, les feuilles et les aiguilles des arbres tombées au sol (Robertson & Paul, 2000; Didion *et al.*, 2014). La litière végétale, en particulier la litière d'arbres forestiers, est la principale source d'accumulation de matière organique à la surface du sol forestier (Tableau 1). Les tissus foliaires peuvent représenter plus de 70 % de la litière en surface dans les forêts (Robertson & Paul, 2000) et représentent 3 à 5 tonnes de matière produite par hectare dans les forêts tempérées chaque année (Bray & Gorham, 1964). Dans les forêts de feuillus, à l'automne, les feuilles sénescentes commencent à tomber et créent la première strate de la litière. La présence de cette couche de litière a de multiples fonctions importantes dans le sol forestier : Elle représente une étape cruciale pour le cycle des nutriments et la formation des sols (Krishna & Mohan, 2017). Elle protège les sols en diminuant leur érosion et limite les fluctuations du microclimat ainsi que le compactage du sol (Sayer *et al.*, 2006).

Cet habitat est colonisé par un grand nombre de saprotrophes dégradant la matière organique morte et jouant ainsi un rôle significatif sur le cycle du carbone (Krishna & Mohan, 2017). Les micro-organismes saprotrophes responsables de la décomposition de la litière sont majoritairement des basidiomycètes (Osono & Takeda, 2001; Eichlerová *et al.*, 2015). Les communautés bactériennes, dominées par des protéobactéries, bactéroides, et archées sont souvent moins efficaces dans le processus de décomposition (Kellner & Vandenbol, 2010; Schneider *et al.*, 2012; López-Mondéjar *et al.*, 2015). La composition chimique des litières change au fur et à mesure de leur décomposition. Ainsi les litières de différents âges offrent des nutriments différents aux organismes qui les consomment (Šnajdr *et al.*, 2011). Par conséquent, la décomposition de la litière est caractérisée par une succession de communautés microbiennes dont la diversité augmente au cours de la décomposition (Šnajdr *et al.*, 2011; Voriskova & Baldrian, 2013; Tlaskal *et al.*, 2016). En général, les bactéries et les champignons de la phyllosphère ne sont présents que dans les premières étapes de la décomposition des litières, puis sont rapidement remplacées par des espèces produisant des enzymes protéolytiques et cellulolytiques tels que les endocellulases et les endoxylanases, produites par exemple par *Frigobacterium* et *Sphingomonas* (Purahong *et al.*, 2016; Tlaskal *et al.*, 2016).

Lors des dernières phases de décomposition de la litière, les communautés microbiennes sont caractérisées par une fraction croissante de Basidiomycètes. Ceux-ci sont capables de dégrader la lignine et les acides humiques (Voriskova & Baldrian, 2013; Purahong *et al.*, 2016). Ainsi, les enzymes produites par les micro-organismes capables de dégrader ces composés tels que la peroxydase de manganèse, la laccase et la peroxydase de lignine sont les principales enzymes actives lors des dernières phases de décomposition des litières (Fioretto *et al.*, 2007; Šnajdr *et al.*, 2011; Purahong *et al.*, 2014). Ces phases avancées de décomposition sont également caractérisées par l'augmentation en abondance de divers taxa bactériens tels que *Bradyzhorium*, *Burkholderia* et *Streptomyces* (Purahong *et al.*, 2016; Tlaskal *et al.*, 2016).

1.B. Les micro-organismes décomposeurs du bois mort

Le bois mort représente l'autre composante clé des débris forestiers. La plupart du bois mort est représenté par du bois grossier (arbres tombés et grosses branches) et par des débris ligneux fins totalisant 2-8 t ha^{-1} (Domke *et al.*, 2016). Ces débris supportent un grand nombre d'organismes, y compris des plantes, des insectes, des champignons, des lichens, des oiseaux et des mammifères (Heilmann-Clausen & Christensen, 2005). Dans les écosystèmes où la litière se décompose rapidement, le bois mort joue un rôle important pour la conservation de la couche organique. Il fournit également une source d'humidité qui favorise le développement de la végétation pendant les périodes sécheresse (Harmon *et al.*, 1986). Le bois mort représente un habitat spécifique, dont la quantité varie grandement d'une forêt à l'autre (Tableau 1). Alors que le volume de bois mort dans les forêts naturelles peut atteindre jusqu'à 1200 m^3 ha^{-1} et peut dépasser la biomasse des arbres vivants, le stock est généralement de 2-65 m^3 ha^{-1} dans les forêts aménagées où le bois est récolté (Hahn & Christensen, 2004; Stokland *et al.*, 2012).

En raison de ses propriétés physiques et chimiques, telles que l'imperméabilité, la forte teneur en lignine et les faibles concentrations d'azote, le bois frais est résistant à la colonisation de la plupart des bactéries (De Boer *et al.*, 2005). Ainsi, les champignons, en particulier les basidiomycètes formant des cordons mycéliens, dominent le bois en décomposition, ce qui reflète la capacité de ces micro-organismes à coloniser et à décomposer efficacement la matière organique complexe (Eichlerová *et al.*, 2015). Ainsi, tout comme la litière, la décomposition du bois mort est caractérisée par une succession de communautés microbiennes. Après une première phase dominée par des champignons de pourriture molle et fibreuse blanche (ou champignons lignivores), le processus de décomposition est favorisé par des champignons de pourriture cubique brune (Fukasawa *et al.*, 2011; Rajala *et al.*, 2011) tels que *Mycena haematopus* et *Pluteus* spp. (Fukasawa *et al.*, 2009). Peu

d'études ont exploré les changements des communautés bactériennes lors de la décomposition du bois et leurs possibles interactions avec les communautés fongiques (Johnston *et al.*, 2016). Il a été néanmoins suggéré que l'activité enzymatique de certaines espèces fongiques et les changements des conditions de pH pourraient favoriser le développement des espèces bactériennes tolérantes à un pH faible (Kielak *et al.*, 2016).

1.C. Les sols représentent le plus important des habitats microbiens en forêt

Le sol forestier est considéré comme un habitat particulièrement conséquent en termes de biomasse, diversité et activité microbienne (Serna-Chavez *et al.*, 2013). La dégradation de la matière organique ainsi que de nombreux autres processus biogéochimiques dépendent de l'activité des micro-organismes dans les sols (Serna-Chavez *et al.*, 2013). Les sols des forêts tempérées et boréales sont caractérisés par la présence de grandes quantités de mycéliums de champignons ectomycorhiziens (ECM) qui peuvent représenter jusqu'à un tiers de la biomasse microbienne totale et produire jusqu'à 50% du carbone organique (Högberg & Högberg, 2002; Ekblad *et al.*, 2013). Les mycorhizes transfèrent une grande quantité de nutriments aux arbres et peuvent former des réseaux complexes qui les relient entre eux (Högberg *et al.*, 1999). Les mycéliums eux-mêmes abritent et servent de ressource à un grand nombre d'organismes tels que les collemboles, les nématodes, les microarthropodes, les enchytraeidae (Schröter *et al.*, 2003; Kanters *et al.*, 2015) et des endobactéries principalement des alphaprotéobactéries (Bertaux *et al.*, 2005). En plus des champignons ECM et des organismes qui leur sont associés, les sols abritent également des champignons et des bactéries saprotrophes. Parmis les phylums bactériens, les *Acidobactéries*, *Actinobactéries*, *Protéobactéries*, *Bactéroïdes* et *Firmicutes*, semblent être abondants dans la plupart des sols (Lauber *et al.*, 2009) tandis que les communautés fongiques sont majoritairement dominées par des Basidiomycètes (Sun *et al.*, 2016).

La composition de ces communautés microbiennes varie selon la quantité de matière organique dans le sol et selon son taux de renouvellement (O'Brien *et al.*, 2005; López-Mondéjar *et al.*, 2015). Ce taux de renouvellement de matière organique varie lui-même en fonction de la profondeur du sol, et par conséquent la composition microbienne des sols varie entre les différentes strates du sol : Les saprotrophes sont présents dans les parties supérieures du sol tandis que les couches profondes du sol sont caractérisées par une biomasse mycorhizienne, une abondance relative des bactéries et une diversité des archées plus importantes (Lindahl *et al.,* 2007; Vala *et al.*, 2008). La composition microbienne des sols forestiers varie également selon un gradient horizontal à l'échelle continentale et à l'échelle locale. Les propriétés édaphiques du sol, les précipitations et la température

constituent les facteurs les plus déterminants dans la composition de la communauté microbienne du sol à de grandes échelles (Lauber *et al.*, 2009; Talbot *et al.*, 2014). A l'échelle locale, les arbres créent des gradients physicochimiques (c.-à-d. l'humidité, la température, le pH et la disponibilité des éléments nutritifs) qui influencent la distribution spatiale des communautés microbiennes du sol (Lavoie *et al.*, 2012).

II.2. La plante et son microbiote forment une entité fonctionnelle : l'holobionte

Depuis leur colonisation terrestre, il y a plus de 450 millions d'années, les plantes sont devenues le foyer d'un grand nombre de micro-organismes qui sont indispensables à leur survie (Kiers & Heijden, 2006). Les plantes ont assimilé plusieurs fonctions clés assurées par les procaryotes. Les exemples les plus éloquents étant la mitochondrie et le chloroplaste, tous deux probablement issus de l'endosymbiose de bactéries autrefois libres et servants, à présent, de producteurs d'énergie dans le règne animal et végétal (Blaser *et al.*, 2016). Les plantes sont associées à une multitude d'autres micro-organismes qui peuvent influencer leur croissance, survie et capacité à se reproduire, ou fitness (Vandenkoornhuyse *et al.*, 2015; Lafferty, 2017). La plante et son microbiote peuvent par conséquent être considérés comme une entité fonctionnelle appelée "holobionte" (Lynn & Fester, 1991). Cette vision de l'hôte a donné naissance à la théorie évolutive de l'hologénome (Zilber-Rosenberg & Rosenberg, 2008), selon laquelle l'hôte et les génomes microbiens qui lui sont associés agissent comme un consortium qui fait face aux changements environnementaux en évoluant comme un tout. Lors d'un changement environnemental rapide, la diversité des micro-organismes associés à l'hôte pourrait l'aider à survivre et à se reproduire dans les nouvelles conditions, et à « gagner le temps nécessaire » pour que son génome évolue (Zilber-Rosenberg & Rosenberg, 2008). Néanmoins cette théorie est débattue car elle ne considère pas les nombreuses formes d'associations existantes entre les micro-organismes et la plante (Douglas & Werren, 2016). Certains micro-organismes peuvent, en effet, former des interactions durables avec leur hôte conduisant à une dépendance totale de l'un envers l'autre (Bright & Bulgheresi, 2010), tandis que d'autres peuvent habiter la plante de manière temporaire et ont peu d'effet sur l'adaptation ou l'évolution de leur hôte. Entre ces deux extrêmes, se trouve un gradient d'interactions de force variable encore difficile à distinguer (Hassani *et al.*, 2018). En outre, la plante interagit avec plusieurs communautés microbiennes entre lesquelles les interactions restent à élucider. En effet, les micro-organismes colonisent la surface de tous les organes des plantes incluant les fleurs (anthosphère) (Aleklett *et al.*, 2014), les fruits (carposphère) (Heywood, 1969), les graines (habitat

appelé spermosphère lorsque la graine germe; Nelson, 2004), le tronc (dermosphère) (Lambais *et al.*, 2006), les feuilles (phyllosphère; Ruinen, 1956) et les racines (rhizosphère) (Hiltner, 1904), ainsi que les tissus intérieurs de tous ces organes (endosphère). La composition des communautés microbiennes diffère d'un organe à l'autre (Amend *et al.*, 2019) ce qui conduit à une compartimentation du microbiote au sein de l'holobionte.

2.A. Les micro-organismes colonisent les organes reproducteurs des plantes

Les graines représentent l'une des étapes les plus cruciales du cycle biologique d'une plante. Elles servent non seulement à amorcer le cycle de vie et à reproduire les espèces, mais aussi à faciliter leur dispersion, leur adaptation et leur persistance dans de nouveaux environnements (Fenner & Thompson, 2005). Les graines et les semis en germination sont particulièrement vulnérables à la mortalité due à la sécheresse, aux granivores et aux champignons pathogènes (Bever *et al.*, 2015). Ainsi, la transition de graines à plantule représente un des plus importants goulots d'étranglement du cycle biologique des plantes (Leck *et al.*, 2008). Par conséquent, la nature et l'impact des interactions microbiennes qui ont lieu avant et pendant ces stades vulnérables de développement des plantes sont essentiels pour établir les trajectoires de la dynamique des communautés végétales dans les systèmes naturels et la réussite ou l'échec des cultures dans les systèmes agricoles (Nelson, 2017). Le microbiote des graines est constitué de communautés riches en espèces bactériennes et fongiques (Rodriguez *et al.*, 2009; Malfanova *et al.*, 2013; Hodgson *et al.*, 2014; Truyens *et al.*, 2015) mais aussi en virus (Sastry, 2013) et oomycètes (Thines, 2014) qui colonisent la surface comme les tissus internes des graines (Nelson, 2017). Néanmoins il contient, en moyenne, moins d'espèces bactériennes et fongiques que les communautés microbiennes associées à la rhizosphère et possède un niveau de diversité microbienne comparable à la phyllosphère (Links *et al.*, 2014; Barret *et al.*, 2015; Klaedtke *et al.*, 2016).

Les graines acquièrent leur microbiote via la transmission verticale, définie comme la transmission de micro-organismes des parents à leurs descendants et la transmission horizontale, définie comme la transmission de micro-organismes du milieu environnant à la plante (Gundel *et al.*, 2011) (Figure 3). Le type de transmission est, en partie, liée au chemin emprunté par les micro-organismes. Il existe deux chemins utilisés par les micro-organismes pour être transmis verticalement (Maude, 1996). Le premier chemin appelé voie interne permets aux micro-organismes de passer à travers le xylème ou le tissu non vasculaire de la plante mère et de coloniser les graines (Truyens *et al.*, 2015). Le second chemin, la voie florale, fait passer les micro-organismes dans la graine à partir de la

colonisation des stigmates de la plante mère (Ambika *et al.*, 2018). Selon le chemin emprunté, les micro-organismes sont situés à des endroits distincts dans la graine (Singh & Mathur, 2004). Ainsi, ces deux voies sont limitées aux micro-organismes qui se développent dans les parties internes de la graine (Maude, 1996). La transmission verticale est un mécanisme courant chez les champignons (Hardoim, 2012) et plus variable chez les bactéries (Frank *et al.*, 2017) qui peut apporter un avantage direct à la plante hôte, comme une meilleure tolérance à la sécheresse de *Festuca rubra* lorsqu'elle est associée à son endophytre fongique *Epichloe festucae* (Clay & Schardl, 2002a; Faeth, 2002). Un troisième chemin, la voie externe (Figure 3) permet la colonisation de la graine via la transmission horizontale. Les graines sont contaminées par contact avec l'inoculum microbien présent sur les fruits ou autres organes de la plante (Maude, 1996). Pour les plantes, l'importance relative de la transmission horizontale et verticale demeure incertaine (Vandenkoornhuyse *et al.*, 2015), mais les deux types de transmission sont susceptibles de contribuer à la composition finale du microbiote de la graine et du semis (Shade *et al.*, 2017). A la surface de la feuille, l'immigration de micro-organismes est déterminée par plusieurs vecteurs de transmission incluant les graines, les animaux, la pluie et l'atmosphère (Vacher *et al.*, 2016b).

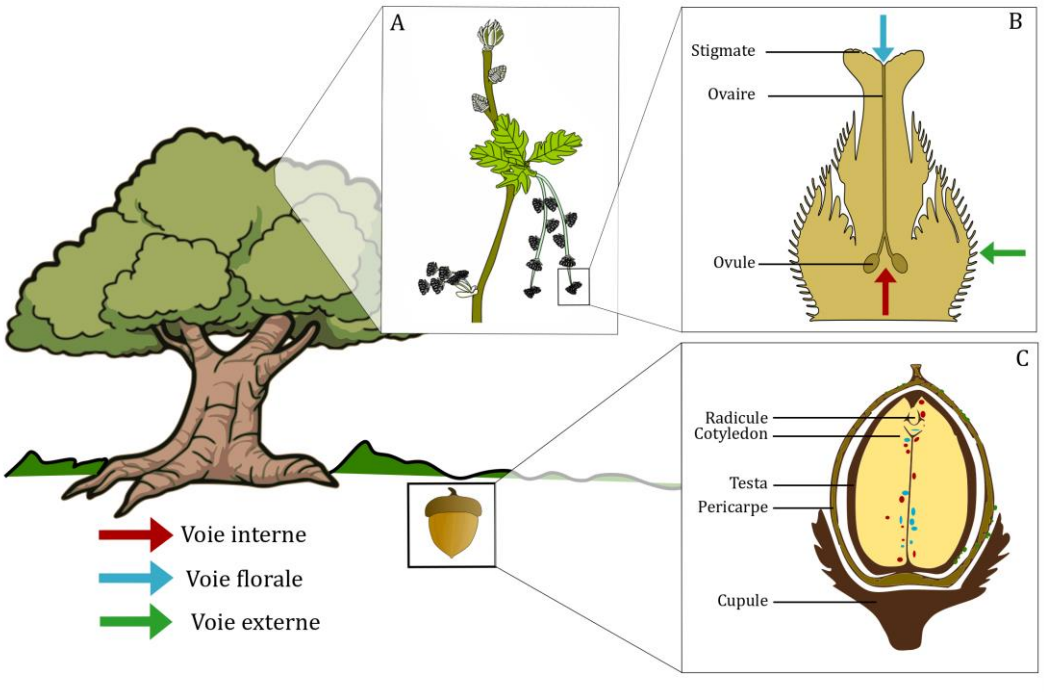


Figure 3. Les trois voies de transmission verticale et horizontale de micro-organismes à la graine. Les micro-organismes peuvent être transmis par voie florale (flèche bleue), voie interne (flèche rouge) et voie externe (flèche verte). A : Feuilles et fleures de chêne. B : Coupe transversale de fleure femelle de chêne. C : Coupe transversale de gland de chêne.

2.B. Les micro-organismes colonisent les organes végétatifs des plantes

a. Les racines et la rhizosphère représentent un habitat riche en nutriments

Les racines, ainsi que la zone de sol à la surface des racines sous influence des exsudats racinaires, appelée rhizosphère (Bent, 2006; Lugtenberg *et al.*, 2015), représentent un habitat vaste caractérisé par une activité microbienne intense (Lebeis, 2015). Les divers composés libérés par les racines des plantes sous forme d'exsudats, comme les sucres, les acides aminés, les flavonoïdes, les acides aliphatiques, les protéines et les acides gras, créent un environnement unique autour des racines (Badri *et al.*, 2009). Ces composés attirent des micro-organismes symbiotiques et pathogènes ce qui génère un réseau d'interactions biotiques autour des racines (Bais *et al.*, 2006). Certains de ces micro-organismes, en pénétrant ou en entourant le système racinaire, favorisent la croissance des plantes, comme les mycorhizes arbusculaires, les ectomycorhizes et autres champignons endophytes (Das & Varma, 2009), ainsi que certaines bactéries (Bashan & Holguin, 1998) et rhizobactéries (Kloepper & Schroth, 1978). Compte tenu de l'importance du transfert de carbone des producteurs primaires vers le sol, la rhizosphère représente probablement le compartiment forestier le plus important en termes d'activité bactérienne dans les sols. Les nutriments disponibles fournis par les plantes et les champignons mycorhiziens conduisent à la formation d'un réseau interactif composé de divers micro-organismes provenant du sol, ce qui fait de la rhizosphère un système fascinant et très complexe, dont la disponibilité en ressource et les conditions environnementales sont différents du sol environnant (Lladó *et al.*, 2017).

Par conséquent, les communautés microbiennes associées à la rhizosphère présentent des différences marquées par rapport aux communautés des sols environnants. On suppose que les plantes peuvent influencer activement la diversité et la composition du microbiote associé à leurs racines. Les microorganismes de la rhizosphère sont tout d'abord influencés par la secrétion de composés organiques par la plante hôte, appelé rhizodéposition et par le génotype de la plante hôte (Bulgarelli *et al.*, 2013; Cregger *et al.*, 2018; Gallart *et al.*, 2018). Ainsi, en comparaison avec le sol environnant, les communautés de la rhizosphère sont enrichies en champignons mycorhiziens qui présentent souvent un certain degré de spécificité à leur plante hôte, alors que les saprotrophes sont beaucoup moins abondants (Baldrian, 2017). Les racines des graminées sont principalement colonisées par des champignons arbusculaires, alors que de nombreux arbres forestiers sont engagés dans des relations mutualistes avec les champignons ectomycorhiziens (Terhonen *et al.*, 2019).

b. La phyllosphère est un habitat oligotrophe exposé à de multiples contraintes

La surface des feuilles constitue un habitat considérable pour les micro-organismes. Cet habitat foliaire, communément appelé la phyllosphère, est colonisé par des centaines d'espèces de champignons et de bactéries ainsi que par des oomycètes et protozoaires (Lindow & Brandl, 2003; Vorholt, 2012; Bulgarelli *et al.*, 2013; Hardoim *et al.*, 2015; Vacher *et al.*, 2016b; Compant *et al.*, 2019; Schlechter *et al.*, 2019). Les bactéries sont les habitants les plus nombreux et les plus diversifiés, avec une densité estimée à environ 10^6 à 10^7 bactéries par cm^2 de surface foliaire. Ainsi, en supposant que la surface foliaire à l'échelle du globe peut atteindre une superficie deux fois plus grande que la surface terrestre (environ 1 x 10^9 km^2), la population bactérienne mondiale présente dans les feuilles pourrait comprendre jusqu'à 10^{26} cellules (Vorholt, 2012). Les micro-organismes qui colonisent les feuilles peuvent être trouvés à la fois comme épiphytes, à la surface des feuilles, et comme endophytes, à l'intérieur des tissus végétaux (Vorholt, 2012). La distinction entre endophytes et épiphytes n'est pas toujours marquée car certains micro-organismes peuvent changer d'habitat foliaire au cours de leur cycle de vie, passant d'épiphyte à endophyte, comme c'est le cas d'un grand nombre de champignons pathogènes qui traversent l'épiderme de la feuille via leur appressorium (*Erysiphe*, *Podosphaera*, *Phyllactinia*, *Blumeria*, *Ascochyta*, *Colletotrichum*, *Phomopsis*) (Osono & Mori, 2003, 2004; Osono, 2006). La définition du terme phyllosphère varie d'une étude à l'autre. Le terme a été introduit pour la première fois par le phytopathologiste Last en 1955 et le microbiologiste Ruinen en 1956 (Vacher *et al.*, 2016b). Ces auteurs ont défini la phyllosphère, par analogie avec la rhizosphère, comme la surface externe des feuilles (Ruinen, 1956). Depuis, certains chercheurs ont élargi la définition et considèrent que la phyllosphère représente l'ensemble des parties aériennes de la plante (Leveau & Lindow, 2001; Lindow & Brandl, 2003; Whipps *et al.*, 2008; Vorholt, 2012; Turner *et al.*, 2013). D'autres proposent d'étendre sa définition aux parties internes de la feuille (Newton *et al.*, 2010; Müller & Ruppel, 2014; Vacher *et al.*, 2016b).

Les micro-organismes de la phyllosphère peuvent provenir de diverses sources environnementales, y compris le sol et la litière du milieu de germination (Barret *et al.*, 2015; Copeland *et al.*, 2015) et l'atmosphère (Bulgarelli *et al.*, 2013). La survie des micro-organismes dans la phyllosphère exige une adaptation aux conditions oligotrophes ainsi qu'imposées par les stress abiotiques et biotiques des feuilles (Lindow & Brandl, 2003). Ainsi, les communautés microbiennes de la phyllosphère sont susceptibles de posséder des caractéristiques fonctionnelles qui leur confèrent un avantage à la colonisation des feuilles. L'établissement des micro-organismes dépend donc en partie de leurs capacités métaboliques, c'est-à-dire la capacité à absorber, métaboliser et sécréter une gamme de nutriments et d'autres composés (Vorholt, 2012).

Caractéristiques anatomiques et chimiques foliaires

Lorsque les micro-organismes se déposent sur l'épiderme foliaire, ils entrent d'abord en contact avec la cuticule, une couche hydrophobe de cire recouvrant l'épiderme (Vacher *et al.*, 2016b). La composition chimique de cette cire peut affecter la croissance des bactéries de la phyllosphère (Bodenhausen *et al.*, 2013; Reisberg *et al.*, 2013). Bien que les cires cuticulaires agissent comme barrière physique, entre la surface et l'intérieur de la feuille, certains métabolites peuvent se déplacer à la surface des feuilles, favorisant ainsi la croissance microbienne (Tukey, 1970; Mercier & Lindow, 2000). Ces composés peuvent arriver à la surface de la feuille par excrétion des cellules foliaires ou par pression osmotique lorsque la feuille est humide (Tukey, 1970). Ce processus peut être amplifié par la production de biosurfactants (agents tensioactifs produits biologiquement) synthétisés par certaines bactéries comme *Pseudomonas syringae* (Knoll & Schreiber, 2000; Burch *et al.*, 2014).

Les principaux facteurs de sélection des communautés foliaires incluent la disponibilité en nutriment et en eau à la surface et à l'intérieur des feuilles (Wilson *et al.*, 1995; Lindow & Brandl, 2003). La principale source de nutriments sur la feuille est constituée de photoassimilats qui se diffusent à travers la cuticule hydrophobe tapissant la couche cellulaire épidermique des feuilles (Van Der Wal & Leveau, 2011). Les micro-organismes des feuilles sont généralement des oligotrophes qui peuvent tolérer les faibles teneurs en nutriments de la feuille ou des micro-organismes qui peuvent interagir avec la plante hôte pour obtenir plus de nutriments (Beattie & Lindow, 1995). Le méthanol peut être utilisé, comme source de carbone, par des micro-organismes dits méthylotrophiques comme la bactérie, *Methylobacterium extorquens*, et la levure, *Candida boidinii* (Sy *et al.*, 2005; Kawaguchi *et al.*, 2011).

La distribution hétérogène d'autres nutriments et la production de composés chimiques foliaires antimicrobiens (alkaloïdes, phénols et terpénoïdes) structurent également la diversité des micro-organismes de la phyllosphère (Junker & Tholl, 2013; Radulovic *et al.*, 2013). L'épiderme est jonché de nervures, stomates, hydathodes et trichomes répartis de manière hétérogène à la surface de la feuille. Du fait de leur forte teneur en eau et en nutriments, ces structures représentent les sites de colonisation privilégiés des bactéries (Figure 4) (Lindow & Brandl, 2003; Monier & Lindow, 2003; Baldotto & Olivares, 2008; Peredo & Simmons, 2018). Les pores formés par les deux cellules de garde (stomates et hydathodes) permettent aux micro-organismes d'entrer à l'intérieur des tissus foliaires (Whipps *et al.*, 2008) et ainsi d'avoir accès aux glucides produits par la photosynthèse (Rennie & Turgeon, 2009). Les trichomes sont des excroissances qui sécrètent une variété de

métabolites secondaires capables de limiter la croissance d'herbivores et pathogènes (Karamanoli *et al.*, 2005) et favoriser la croissance de certains micro-organismes (Monier & Lindow, 2003).

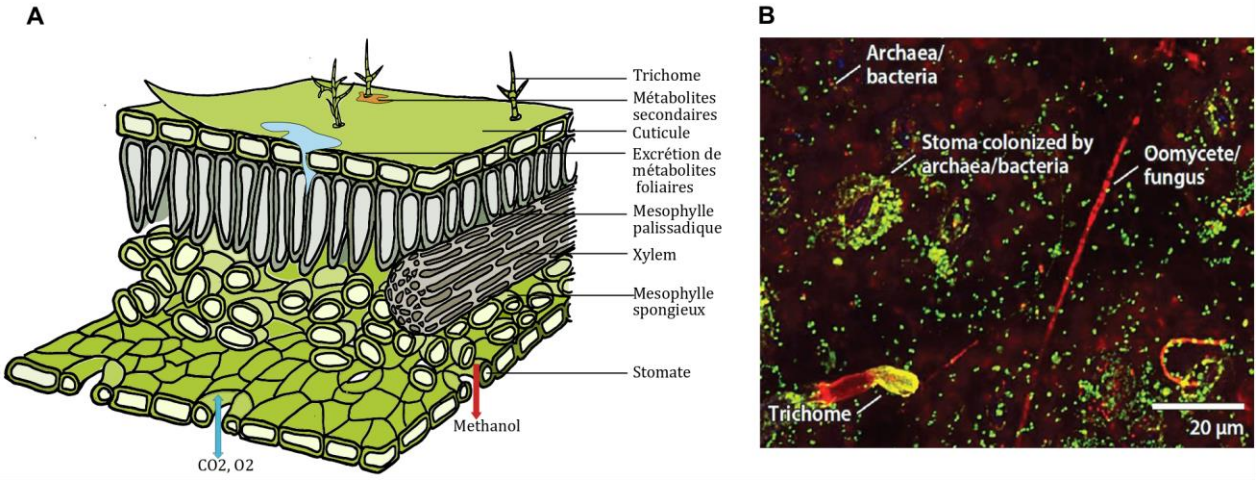


Figure 4. Schéma des attributs structurels et fonctionnels de la phyllosphère (A) et image de micro-organismes colonisant la surface des feuilles (B). Le schéma représente une coupe transversale de feuille illustrant les caractéristiques chimiques et anatomiques foliaires qui influencent la distribution des micro-organismes incluant les flux de plusieurs métabolites utilisés comme nutriments et de la répartition des stomates et hydathodes qui représentent les principaux points d'entrée des micro-organismes dans les tissus foliaires internes. La photo a été prise sur la surface inférieure d'une feuille de chêne (*Quercus robur*) colonisées par l'oïdium (*Erysiphe sp.*) par marquage avec les fluorophores SYTO-9 et l'iodure de propidium (B). Les bactéries et archées vivantes apparaissent en vert, les bactéries et les archées mortes apparaissent en jaune, les champignons et les oomycètes apparaissent en rouge. Le fond rouge foncé correspond à la chlorophylle de la feuille (Adapté de Vacher et al. 2016).

Conditions microclimatiques foliaires

La phyllosphère est soumise à des facteurs climatiques importants tels que la pluie et le vent, auxquels s'ajoutent des fluctuations diurnes considérables de température, d'humidité et d'intensité lumineuse (Morris *et al.*, 2001; Bailey *et al.*, 2006; Vacher *et al.*, 2016b). De telles variations microclimatiques peuvent avoir une grande influence sur la croissance et la survie des micro-organismes. La disponibilité en eau est un des facteurs limitant le plus la croissance microbienne à la surface des feuilles (Beattie & Marcell, 2002). L'exposition aux rayons UV pose un défi particulier aux épiphytes des feuilles, et les populations bactériennes et fongiques de la phyllosphère sont généralement plus pigmentées que celles du sol (Stout, 1960; Sundin *et al.*, 2002). Cette pigmentation peut augmenter la survie dans l'environnement de la phyllosphère (Sundin *et al.*, 2002). De plus, certaines bactéries peuvent produire des surfactants pour augmenter la teneur en

eau à la surface de la feuille (Knoll & Schreiber, 2000). Les micro-organismes de la phyllosphère peuvent également exprimer des enzymes pour manipuler les espèces réactives de l'oxygène générées par le rayonnement solaire, ainsi que pour produire des protéines de protection de l'ADN (Delmotte *et al.*, 2009). L'expression rapide des mécanismes de réparation de l'ADN après une exposition aux UV peut être un déterminant crucial de la survie de la phyllosphère (Sundin, 2002).

Les interactions biotiques à l'intérieur de la phyllosphère

Une fois les micro-organismes établis dans la phyllosphère, des interactions complexes se développent entre les bactéries, les champignons, les macroorganismes (insectes et autres eucaryotes) et la plante hôte. Les interactions entre micro-organismes couvrent toute une gamme d'interactions incluant la compétition, le parasitisme, la prédation, le mutualisme, le commensalisme et l'amensalisme (Faust & Raes, 2012). Les interactions biotiques peuvent faciliter ou entraver l'établissement d'une espèce dans une communauté. Le mutualisme et le commensalisme sont des interactions positives qui sont bénéfiques pour au moins une espèce et qui ne nuisent pas à l'autre. Dans le cas du mutualisme, les deux espèces bénéficient l'une de l'autre, tandis que dans le cas du commensalisme, l'un des partenaires profite et l'autre n'est pas affecté (Schlechter *et al.*, 2019). Les micro-organismes sont dits commensalistes lorsque, par exemple, un des partenaires produit des substrats et/ou des facteurs de croissance qui affectent positivement son partenaire (Faust & Raes, 2012; Großkopf & Soyer, 2014). L'effet de ces interactions sur la structure des communautés microbiennes de la phyllosphère est encore peu connu. Cependant, un exemple de coopération a été observé en utilisant des communautés synthétiques de la rhizosphère du maïs, où l'élimination d'une espèce clé a entraîné l'effondrement d'autres populations bactériennes (Niu *et al.*, 2017).

La compétition, le parasitisme, la prédation sont des interactions négatives qui nuisent à au moins une espèce. Le parasitisme dans la phyllosphère est principalement dû aux interactions virus-bactéries. Les virus infectant les bactéries (c.-à-d. les bactériophages ou phages) peuvent modifier la dynamique des communautés en influençant la diversité bactérienne (Koskella & Brockhurst, 2014). La compétition peut être le résultat d'une interaction directe entre les micro-organismes ou d'une interaction indirecte via le prélèvement d'une ressource ou d'un espace commun aux deux protagonistes. La compétition indirecte entraîne, quant à elle, soit une exclusion spatiale complète,

soit une coexistence (Freilich *et al.*, 2011). Le fructose et le saccharose, par exemple, limitent les ressources pendant la colonisation de la phyllosphère (Leveau & Lindow, 2001).

Le type d'interaction entre micro-organismes dépend en grande partie de leurs capacités métaboliques, ainsi le degré de ressources partagées entre les espèces peut façonner la composition des communautés microbiennes (Freilich *et al.*, 2011). En outre, d'autres facteurs peuvent influencer les interactions entre les espèces. Les espèces microbiennes qui colonisent d'abord la phyllosphère peuvent déterminer la gamme d'espèces microbiennes qui peuvent ensuite la coloniser. Ces effets, appelés effets prioritaires, ont été observés par Maignien (2014) sur des plantes d'*A.thaliana* appartenant au même génotype et vivant dans la même chambre climatique. L'assemblage des communautés a suivi des trajectoires divergentes, probablement en raison de différents événements lors de la colonisation des feuilles.

III. Influence des micro-organismes de la canopée des arbres sur le fonctionnement des forêts

III.1. Effets positifs et négatifs des micro-organismes sur la fitness des arbres

Les micro-organismes peuvent influencer le fonctionnement des écosystèmes via leurs effets sur la santé et la productivité des plantes hôtes en influençant la germination, la croissance et la survie des plantes (Friesen *et al.*, 2011). Les microbiomes foliaires ont un répertoire métabolique beaucoup plus vaste que leur hôte, synthétisant des produits chimiques biologiquement actifs que les plantes ne peuvent secréter. Ils peuvent également modifier les voies physiologiques des plantes en produisant ou en manipulant les phytohormones produites par la plante hôte (Tsavkelova *et al.*, 2006; Friesen *et al.*, 2011) ayant ainsi des effets bénéfiques ou néfastes sur la croissance et la survie de la plante hôte tout au long de son cycle de vie.

1.A. Stimulation et inhibition de la germination des graines et de la croissance des plantes

a. Attaques de pathogènes et d'herbivores sur les graines

La germination et la croissance des semis représentent les étapes du cycle de vie des plantes où toutes les interactions avec les micro-organismes ont potentiellement le plus d'impact néfaste ou bénéfique sur la fitness de la plante (Nelson, 2017; Shade *et al.*, 2017). Le microbiote des graines est

souvent reconnu comme néfaste pour la germination des graines (Munkvold, 2009). La transmission d'agents phytopathogènes par les graines est un moyen de dispersion important chez les pathogènes qui joue un rôle dans l'émergence des maladies (Baker & Smith, 1966; Barret *et al.*, 2016). En forêt, la majorité des graines dispersées proches de l'arbre mère meurent fréquemment d'infections par des espèces fongiques comme *Pythium torulosum* et *Pythium aphanidermatum* (Augspurger & Kelly, 1984; Lemanceau *et al.*, 2017).

Néanmoins, certains micro-organismes transmis de la plante à ses descendants sont également connus pour avoir un effet bénéfique sur la survie des graines. Certaines bactéries bénéfiques inoculées sur des graines de maïs peuvent induire une augmentation significative de la tolérance de la plante à la sécheresse (Naveed *et al.*, 2014). Cette transmission verticale est également fréquente chez les espèces fongiques endophytes qui protègent les plantes contre les herbivores (Rodriguez *et al.*, 2009; Hodgson *et al.*, 2014). Les champignons endophytes peuvent aussi réduire l'herbivorie des graines et des feuilles. Par exemple, chez les graminées, *Néotyphodium* produit des alcaloïdes, des lolitres, des lolines et des peramines dans les graines et les feuilles (Clay & Schardl, 2002). Les alcaloïdes produits par les endophytes sont souvent concentrés dans les graines et peuvent dissuader les prédateurs de consommer la plante hôte (Knoch *et al.*, 1993).

b. Régulation hormonale de la croissance des plantes

La capacité des micro-organismes à synthétiser des phytohormones, régulant ainsi la croissance et le développement des plantes, peut avoir des effets délétères sur la survie de plante hôte. De nombreux agents pathogènes produisent des régulateurs de croissance en quantités dépassant les niveaux nécessaires pour les plantes. Par exemple, la bactérie *Burkholderia* (*Pseudomonas*) *solanacearum* augmente cent fois la teneur en auxine (acide indole-3-acétique) des plantes infectées (Agrios, 1997). L'hypersynthèse des phytohormones par des micro-organismes pathogènes perturbe l'équilibre hormonal des plantes, provoquant l'apparition d'une variété de maladies (Tsavkelova *et al.*, 2006). De plus, la fermeture stomatique est un mécanisme majeur qui confère aux plantes une tolérance à la sécheresse (Sussmilch et McAdam, 2017). Ce mécanisme est principalement contrôlé par la production d'acide abscissique (ABA). Les épiphytes peuvent synthétiser de l'auxine et cytokine qui maintient l'ouverture des stomates (Tanaka *et al.*, 2006) alors que les endophytes foliaires peuvent augmenter les concentrations endogènes d'ABA pour favoriser la fermeture stomatique (Forchetti *et al.*, 2007).

Ainsi, la stimulation ou la production de phytohormones par le microbiote foliaire peut également avoir un effet bénéfique sur la croissance et la survie des plantes. De nombreuses bactéries endophytes favorisent la croissance des plantes via la biosynthèse d'auxine. Une étude a, par exemple, reporté que la production d'auxine était un trait commun de stimulation de la croissance chez des bactéries isolées à partir de bourgeons de pommier (Miliūtė & Buzaitė, 2011). La synthèse de gibbérellines par la bactérie endophyte *Sphingomonas* sp LK11 isolée sur feuille de *Tephrosia apollinea* a également augmenté significativement la teneur en chlorophylle, la hauteur et la biomasse des semis de tomate (Khan *et al.*, 2014).

1.B. Contribution à la nutrition azotée

La fixation biologique d'azote atmosphérique représente plus de 97 % des apports d'azote dans les écosystèmes terrestres non gérés (Galloway *et al.*, 2004). Ce processus est une étape clé dans le cycle de l'azote et influence également la production primaire terrestre et donc joue un rôle dans le cycle du carbone (Elser *et al.*, 2007; LeBauer, D; Treseder, 2008). La disponibilité d'azote (N) est un facteur majeur dans la croissance et le développement des arbres. L'assimilation, le stockage et la remobilisation de l'azote sont des processus importants qui déterminent le développement vasculaire et la productivité des arbres (Cánovas *et al.*, 2018). En effet l'azote est nécessaire à la production de protéines chez les plantes, en particulier pour la Rubisco (ribulose-1,5-bisphosphate carboxylase/oxygénase), une enzyme qui fixe le dioxyde de carbone (CO_2) lors du processus de photosynthèse ayant lieu dans la membrane des thylakoïdes. La capacité de fixer l'azote est totalement absente des plantes terrestres, mais elle est présente dans au moins six phylums bactériens (comme les *Protéobactéries*, qui comprennent à la fois les rhizobactéries et le genre *Frankia*), plusieurs lignées archéennes (Martinez-romero, 2006) et chez certains champignons mycorhiziens arbusculaires (Requena *et al.*, 2001).

Dans les feuilles, les bactéries et les archées fixent l'azote atmosphérique à l'aide d'une enzyme appelée nitrogénase (*nifH*) (Lafferty doty, 2017). Dans le cas du palmier *Welfia georgii*, on estime que 10 à 20 % de l'azote des feuilles provient de cyanobactéries (Bentley & Carpenter, 1984). La présence et l'activité de bactéries fixant l'azote ont été rapportées dans la phyllosphère de plusieurs arbres forestiers tels que diverses plantes tropicales (Fürnkranz *et al.*, 2008), le chêne vert (Rico *et al.*, 2014), le pin flexible (Moyes *et al.*, 2016a) et le peuplier de l'Ouest (Doty *et al.*, 2016). Une étude récente a également démontré que les endophytes des aiguilles d'une espèce de *Picea* et *Pinus* contribuaient à la croissance des arbres hôtes en fixant l'azote (Carrell & Frank, 2014). De plus, chez le chêne vert la richesse des bactéries fixatrices d'azote atmosphérique augmente lors de périodes

de sécheresse, ce qui suggère que leur recrutement de ces micro-organismes pourrait prolonger la capacité de la plante à s'adapter à l'environnement (Rico *et al.*, 2014).

1.C. Régulation des stress biotiques

Les agents pathogènes peuvent pénétrer dans les feuilles des plantes par divers points d'entrée, y compris les stomates, les plaies ou les hydatodes (Melotto *et al.*, 2008). L'inhibition de l'attaque de pathogène de la phyllosphère implique des interactions entre la plante hôte, le pathogène potentiel, ses antagonistes et d'autres membres de la communauté microbienne sur ou dans la feuille (Elad, 1996). Le pathogène et les organismes - incluant les micro-organismes et l'hôte - avec qui il interagit représente le pathobiome (Vayssier-Taussat *et al.*, 2014; Brader *et al.*, 2017). Cette inhibition peut être réalisée par l'induction d'une réponse immunitaire de la plante hôte par des micro-organismes non pathogènes résultant d'une compétition directe entre micro-organismes, ou par la production de molécules antimicrobiennes.

a. Effets directs : Interactions biotiques entre micro-organismes

Qu'ils soient produits en réponse à la compétition ou de manière constante, les molécules antimicrobiennes produites par les micro-organismes de la phyllosphère peuvent inhiber la colonisation des pathogènes (Müller *et al.*, 2016). Les molécules antimicrobiennes produites par les bactéries ont souvent un large spectre d'action, agissant contre une gamme de micro-organismes, y compris les pathogènes bactériens et fongiques (Berg & Raaijmakers, 2018). L'exemple le plus courant de compétition directe est la sécrétion de composés toxiques par l'un des deux partenaires (Schlechter *et al.*, 2019) comme cela a été démontré chez *P. agglomerans* E325 qui supprime la croissance du phytopathogène *Erwinia amylovora* sur les fleurs de pommier par son activité antibiotique (Pusey *et al.*, 2011). Certaines bactéries (p. ex. *Bacillus*, *Arthrobacter* et *Klebsiella*) peuvent également protéger les plantes en produisant des lactonases dégradant des molécules impliquées dans le *quorum sensing* (mécanisme de synchronisation de l'expression ou de la répression de gènes particuliers) et interférant ainsi avec la communication pathogène-pathogène et empêchant l'expression des gènes de virulence (Reading & Sperandio, 2006). Les champignons sont également connus pour produire de l'acide nonanoïque dans les tissus internes de la plante. Les plantules inoculées avec le champignon *Trichoderma harzianum* inhibent ainsi les pathogènes du cacao (*Theobroma cacao*) (Aneja *et al.*, 2006). Les membres de microbiote associés aux plantes peuvent également entrer en compétition pour la ressource et les nutriments de manière indirecte. Ce type d'interaction biotique appelé compétition par exclusion est un mécanisme couramment

utilisé pour lutter contre l'infection d'agents pathogènes de la phyllosphère (Sullivant & Gara, 1992; Graham *et al.*, 2012). La forte compétitivité du champignon *Aureobasidium pullulans* est par exemple utilisée en agriculture comme méthode de lutte biologique (Chi *et al.*, 2009).

De manière générale, une plus grande diversité des communautés résidentes de la phyllosphère est souvent associée à une certaine stabilité et ainsi à une meilleure résistance contre les perturbations (Balint-Kurti *et al.*, 2010; Kemen *et al.*, 2015). L'un des processus importants de ce principe est l'effet de sélection, dans lequel l'augmentation de la diversité entraîne une probabilité plus élevée qu'un antagoniste de bio-agresseur soit présent dans la communauté (Fargione *et al.*, 2007). Ainsi la composition même du microbiote des graines et des feuilles peut jouer un rôle important dans la résistance aux agents pathogènes des plantes (Innerebner *et al.*, 2011; Agler *et al.*, 2016). Néanmoins toujours dans un contexte de sélection, un microbiome diversifié impliquant de nombreuses interactions de compétition entre micro-organismes défavorise les espèces moins bien adaptées à l'environnement, qu'elles soient bénéfiques ou pathogènes pour l'hôte (Foster *et al.*, 2017).

b. Effets indirects : Activation du système immunitaire de la plante hôte

Le système immunitaire inné des plantes peut reconnaître les agents pathogènes microbiens et réduire ou arrêter leur croissance (Hacquard *et al.*, 2015). Les structures conservées des micro-organismes appelées motifs moléculaires associés aux micro-organismes (MAMP), comme la flagelline bactérienne ou la chitine fongique (Boller & Felix, 2009; Zipfel, 2009), sont reconnus par des récepteurs de reconnaissance de motifs moléculaires (PRR) de la plante hôte qui activent le système immunitaire. Plusieurs études ont montré que les micro-organismes peuvent utiliser ces voies de signalisation pour activer le système immunitaire de la plante et diminuer la croissance d'autres micro-organismes. Chez *Arabidopsis*, l'infection de *Sphingomonas* sur les feuilles et limiter par l'activation du système immunitaire de la plante par *Sphingomonas* (Innerebner *et al.*, 2011; Vogel *et al.*, 2016).

III.2. Contribution aux cycles biogéochimiques

2.A. Cycle des nutriments

En agissant sur la croissance et la survie des plantes et sur les processus de décomposition de la litière, les micro-organismes de la phyllosphère peuvent influencer la production primaire des forêts et ainsi affecter le cycle des nutriments (Bailey *et al.*, 2006; Friesen *et al.*, 2011; Laforest-Lapointe *et al.*, 2017). En effet, la décomposition de la litière est étroitement associée au cycle du carbone et de l'azote et à l'accumulation de matière organique dans les sols forestiers. Elle représente donc un processus clé en ce qui concerne le cycle du carbone et des nutriments à l'échelle des écosystèmes et à l'échelle mondiale (Schneider *et al.*, 2012). Un grand nombre de champignons habitant les feuilles vivantes est également retrouvé dans les litières, indiquant que certaines espèces fongiques de la phyllosphère sont des saprotrophes dormants (Osono, 2006; Peršoh, 2013a; Unterseher *et al.*, 2013; Voriskova & Baldrian, 2013). Ces espèces sont impliquées dans la décomposition de la cellulose, de l'hémicellulose et de la lignine dans les feuilles sénescentes et la litière. Ils sont ensuite remplacés par de nouveaux colonisateurs ayant une plus grande capacité de décomposition de la lignine; ces colonisateurs appartiennent pour la plupart aux *Basidiomycota* (Voriskova & Baldrian, 2013). Les micro-organismes de la phyllosphère peuvent aussi influencer la composition chimique et la vitesse de décomposition des litières comme c'est le cas des champignons endophytes de la fétuque (*Lolium arundinaceum*) qui, en conférant une résistance aux herbivores à la plante hôte modifie la composition chimique des feuilles et donc entraîne une décomposition plus lente de la litière et modifie également le microbiote du sol (Lemons *et al.*, 2005).

2.B. Cycle de l'eau

Les aérosols biologiques primaires (PBA), ou bioaérosols, jouent un rôle vital dans le système terrestre, en particulier dans les interactions entre l'atmosphère, la biosphère et le climat. Ils représentent un sous-ensemble de particules atmosphériques, dont la taille varie entre 0.05 et 100 µm, qui sont directement libérées de la biosphère dans l'atmosphère et comprennent des micro-organismes vivants ou morts (algues, archées, bactéries), des unités de dispersion (spores fongiques et pollen de plantes) et divers fragments ou excrétions (débris végétaux) (Ariya & Amyot, 2004; Womack *et al.*, 2010; Després *et al.*, 2012; Fröhlich-Nowoisky *et al.*, 2016).

Les cellules microbiennes ont des propriétés anatomiques et métaboliques qui leur confèrent la capacité d'interagir avec des processus physiques et chimiques de l'atmosphère. Les micro-organismes peuvent agir comme noyaux de condensation des nuages ou comme noyaux de glaciation responsables de la formation de gouttelettes dans les nuages, de cristaux de glace et de

précipitations. Ils peuvent donc influencer le cycle de l'eau et le climat. En plus du rôle direct que les micro-organismes jouent en tant que bioaérosols, ils peuvent également contribuer ou modifier la formation de noyaux de condensation en produisant des biosurfactants qui facilitent la formation de gouttelettes dans les nuages (Morris *et al.*, 2014a; Fröhlich-Nowoisky *et al.*, 2016).

Un large éventail de particules dans l'atmosphère peut agir en tant que noyaux de condensation en favorisant la condensation de la vapeur d'eau sur la surface des micro-organismes. Une importante fraction des communautés microbiennes de la phyllosphère peuvent se comporter comme des noyaux de condensation tels que *Erwinia carotovora*, *Micrococcus agilis*, *Mycoplana bullata* ou *Brevundimonas* (Franc & Demott, 1998; Bauer *et al.*, 2003). De la même manière, de nombreuses espèces bactériennes telles que les genres *Pseudomonas*, *Pantoea* et *Xanthomonas* et fongiques telles que les espèces *Boletus zelleri* (Haga *et al.*, 2013), *Hemileia vastatrix* (Morris *et al.*, 2013), *Isaria farinosa* (Huffman *et al.*, 2013) peuvent agir comme noyaux de glaciation grâce à leurs protéines transmembranaires. Dans l'air pur au-dessus des forêts ou dans des milieux isolés, les bioaérosols pourraient représenter une fraction importante du CCN et du IN et sont susceptibles d'être un facteur régulateur essentiel dans la formation des nuages et des précipitations (Pöschl *et al.*, 2010; Huffman *et al.*, 2013).

IV. Métagénomique ciblée : une méthode devenue incontournable pour étudier les communautés microbiennes

L'importance des communautés microbiennes pour la santé humaine et environnementale a incité les chercheurs à mettre au point plusieurs méthodes afin de caractériser efficacement ces communautés. La plus courante et la plus rentable est le séquençage de gènes cible (Gevers *et al.*, 2012). Différentes techniques de séquençage permettent d'identifier les micro-organismes. Le séquençage Sanger permet l'identification d'une seule espèce dans chaque échantillon. Il est donc souvent associé à la mise en culture et aux prélèvements de chaque souche microbienne. Néanmoins la majorité des micro-organismes ne se développent pas sur les milieux nutritifs et n'est donc pas détectée via cette technique de séquençage (Müller & Ruppel, 2014).

En revanche, la technique de séquençage haut débit, appelée metabarcoding, simplifie considérablement l'analyse de ces communautés parce qu'elle élimine le besoin de cultiver les micro-organismes et accroît le nombre d'espèces détectées simultanément (Taberlet & Coissac,

2012). Cette approche permet l'identification des micro-organismes sur la base d'une séquence d'ADN appelée barcode (Hebert *et al.*, 2003). Cette technique nécessite différentes étapes de préparation des échantillons avant séquençage et de traitements des séquences obtenues. Les étapes de préparation des échantillons regroupent la collecte des échantillons, l'extraction de l'ADN total qu'ils contiennent appelé ADN environnemental (Taberlet & Coissac, 2012), l'amplification par PCR (Polymerase Chain Reaction) de gènes taxonomiques ou fonctionnels microbiens. Après séquençage, l'application de différents filtres permettent de vérifier la qualité des séquences et d'identifier les espèces microbiennes. La succession de ces différentes étapes, appelé pipeline-bioinformatique, permet d'obtenir des comptages de séquences dans chaque échantillon.

IV.1. Préparation des échantillons

Afin de limiter la contamination des échantillons, la collecte sur le terrain se fait, le plus souvent, à l'aide de gants et d'instruments stériles autoclavés et nettoyés à l'éthanol pour tuer les micro-organismes et une solution d'hypochlorite de sodium ou de DNAway pour dégrader l'ADN microbien entre chaque collecte d'échantillon (Champlot *et al.*, 2010; Greenstone *et al.*, 2012). Le stockage à froid des échantillons (-80, -20 ou 4 °C) après collecte et avant extraction de l'ADN permet d'une part d'arrêter le développement des communautés microbiennes et d'autre part d'assurer une meilleure qualité des ADNs (Rissanen *et al.*, 2010; Rubin *et al.*, 2013). Après stockage, les étapes de mise en plaque sont réalisées sous une hotte à flux laminaire (flux d'air ascendant) préalablement stérilisée par l'action d'UV.

L'extraction de l'ADN total (végétal et microbien) permet, par le biais d'actions mécaniques (broyage et température) et chimiques, de libérer l'ADN contenu dans les cellules et d'éliminer une partie des débris cellulaires inhibant les étapes d'amplification et de séquençage. L'extraction de l'ADN peut être réalisée à l'aide de kits commerciaux ou de protocoles classiques à base de phénol-chloroforme. Les procédures d'extraction peuvent affecter la composition de la communauté microbienne (Fliegerova *et al.*, 2014; Pollock *et al.*, 2018) et le choix de la technique d'extraction se fait selon la quantité et le type d'échantillon collecté et l'organisme cible.

IV.2. Amplification des gènes microbiens

L'étape d'amplification par PCR permet de dupliquer le nombre de copies des séquences microbiennes. Le principe de la méthode est de réaliser une succession de réactions de réplication d'une matrice d'ADN double brin. Chaque cycle met en œuvre 3 réactions : (1) la dénaturation de

l'ADN double brin, (2) L'hybridation d'amorces (séquences d'environ 15 à 25 nucléotides) et (3) l'élongation de la séquence complémentaire. Les amorces ou «primers» en anglais définissent, en la bornant, la séquence à amplifier. L'hybridation des amorces en amont de la zone à séquencer permet à l'ADN polymérase, un complexe enzymatique, de répliquer la séquence complémentaire à la région d'intérêt (Mullis *et al.*, 1986). Chaque réaction est réalisée à une température déterminée. Un thermocycleur, déterminant la température de la solution, permet de déterminer le temps de réaction et le nombre de cycle à répéter. Le choix de la séquence à amplifier (marqueur) dépend des objectifs de l'étude : Le marqueur idéal pour les études à l'échelle des communautés microbiennes doit : (1) avoir des sites d'amorçage communs à tous les micro-organismes, (2) être d'une longueur appropriée pour une amplification et un séquençage efficace, (3) présenter une variation interspécifique élevée mais une faible variation intraspécifique (Schoch *et al.*, 2012; Lindahl *et al.*, 2013; Tedersoo *et al.*, 2019).

Les genres bactériens sont généralement identifiés sur la base du gène 16S. Ce gène code la sous-unité 16S de l'ARN ribosomal (ARNr) et est essentiellement utilisé en raison de sa structure très conservée dans le règne des bactéries. Il est constitué d'une succession de régions conservées qui représentent des sites idéals pour la liaison des amorces et de régions hypervariables qui permettent d'identifier le genre et le sous-genre des bactéries présentes dans l'échantillon (Yarza *et al.*, 2014). Néanmoins, il n'existe actuellement aucun consensus sur la ou les régions hypervariables les plus appropriées, et plusieurs études ont été menées pour déterminer les avantages et les inconvénients de chacune d'elles (Pollock *et al.*, 2018). Le choix de la ou des régions hypervariables et le choix du couple d'amorces utilisé ont un effet sur la résolution phylogénétique des communautés bactériennes étudiées (Ghyselinck *et al.*, 2013; Cruaud *et al.*, 2014; Yang *et al.*, 2016). Parmi les eucaryotes, les champignons sont systématiquement identifiés à partir de la région de l'Internal Transcribed Spacer (ITS). Cette région est composée de deux gènes hypervariables, ITS1 et ITS2 (Begerow *et al.*, 2010; Schoch *et al.*, 2012) situées entre les gènes très conservées de la petite sous-unité (SSU : 16S/18S) et de la grande sous-unité (LSU : 23S/25S/28S). Ainsi, la région des ITS a récemment été proposée comme marqueur officiel pour l'identification des champignons (Schoch *et al.*, 2012). Néanmoins bien qu'utile pour l'identification des espèces, la région est trop variable pour étudier la phylogénie des rangs supérieurs, c'est-à-dire au niveau des familles et des ordres. Comme pour le 16S bactérien, le choix de la région hypervariable étudiée dépend des objectifs de l'étude et du type d'échantillon étudié. Le couple d'amorces utilisé pour amplifier l'ITS2 permet une meilleure résolution taxonomique tandis que celui qui est utilisé pour l'amplification de l'ITS1 est plus spécifique des champignons limitant donc l'amplification de l'ITS de la plante (Tedersoo *et al.*,

2019). La diversité fonctionnelle des communautés peut également être étudiée en ciblant les gènes codant pour des protéines nécessaires à la métabolisation de molécules importantes dans les interactions plante-micro-organismes. Dans les communautés microbiennes foliaires, plusieurs gènes fonctionnels souvent ciblés tel que le gène *nif*H qui code pour la nitrogénase et permet aux micro-organismes de fixer l'azote atmosphérique et la famille de gènes GH qui code pour la glycolyse hydrolase et permet de cataboliser les sucres de la plante (Barbi *et al.*, 2014; Lafferty doty, 2017; Nguyen *et al.*, 2019).

IV.3. Séquençage des gènes microbiens

L'ADN amplifié est ensuite séquencé par des méthodes de séquençage haut débit (HTS). Ce terme regroupe plusieurs méthodes de séquençage qui permettent d'obtenir des millions de séquences courtes appelé lectures ou *reads*. Les technologies de séquençage se distinguent principalement par leurs modes de détection produisant un signal qui dépend des bases polymérisées lors de l'opération de séquençage. Ainsi, la société Roche s'est appuyée sur la méthode du pyroséquençage (Roche 454), la société Solexa sur le séquençage par synthèse de nucléotides fluorescents (Illumina), et la société Ion Torrent sur la lecture du pH suite à la libération d'un ion H+ après la polymérisation d'un nucléotide (Ion Torrent) (Goodwin *et al.*, 2016; Heather & Chain, 2016). La technologie Illumina est devenue la plateforme de séquençage la plus couramment utilisée pour l'étude des communautés microbiennes (Nilsson *et al.*, 2019). En effet, le système MiSeq, en général, produit les lectures les plus longues et les plus précises pour l'étude des communautés microbiennes et a un débit beaucoup plus élevé que les autres plates-formes ce qui permet une plus grande profondeur de séquençage (nombre de séquences dans un échantillon) à moindre coût (D'Amore *et al.*, 2016). De plus cette technique permet le séquençage en *paired-end* de l'ADN microbien, ce qui consiste à séquencer le fragment d'ADN dans les deux sens (*forward* et *reverse*). Le chevauchement des deux séquences permet de détecter les duplicats générés lors de l'amplification et les indels (insertion ou une délétion dans une séquence) (Nakazato *et al.*, 2013).

Le séquençage Illumina MiSeq des ADNs microbiens se réalise en 3 étapes : (1) Préparation de l'ADN (ou préparation des librairies), (2) PCR en pont (*bridge-PCR*) et (3) séquençage par synthèse. La préparation des librairies consiste à ajouter aux extrémités des amorces un index ou *tag* propre à chaque échantillon et un adaptateur qui permet l'adhésion des fragments d'ADN au support solide (*flowcell*) utilisé lors du séquençage. La préparation des librairies consiste à ajouter les adaptateurs et index aux ADNs microbiens. Les adaptateurs et les tags peuvent être ajoutés par ligation ou PCR

aux fragments d'ADN (Lindahl *et al.*, 2013). Ces amorces peuvent également comporter une succession de paires de base (0 à 7) appelée *heterogeneity spacer* qui diminue le nombre de faux-positifs obtenus lors du séquençage (Fadrosh *et al.*, 2014). L'ensemble de ces étapes peut être également réalisé dès la première amplification des gènes d'intérêt (après extraction de l'ADN) en utilisant des amorces spécifiques contenant les *tags*, les adaptateurs et les *heterogeneity spacer*. La seconde étape du séquençage consiste à fixer les fragments d'ADNs sur la surface de la *flow cell*. Pour se faire les adaptateurs des fragments amplifiés s'hybrident à leurs séquences complémentaires, elles-mêmes fixées sur la surface de la *flow cell*. Les fragments d'ADN sont donc fixés à leurs deux extrémités. Chaque « lien » est ensuite amplifier par Bridge-PCR afin d'obtenir des clusters d'amplicons. Durant le séquençage, des nucléotides fluorescents s'associent à chaque nucléotide du fragment d'intérêt. Chaque type de nucléotide (A, T, G et C) possède un fluorochrome spécifique qui, une fois excité, permet d'identifier le type d'émission lumineuse et à quelle intensité la lumière est renvoyée. L'opération se répète pour chaque nucléotide de la séquence d'intérêt permettant d'obtenir son code génétique.

IV.4. Traitements bioinformatiques des séquences

L'analyse des séquences obtenues nécessite l'utilisation d'outils bioinformatiques afin d'identifier et limiter les biais introduits lors des étapes d'extraction, amplification et séquençage des ADNs microbien et d'identifier à quelle espèce ou genre appartiennent les séquences obtenues (Bálint *et al.*, 2014; Pollock *et al.*, 2018). Plusieurs filtres de qualité peuvent être appliquées afin d'éliminer les séquences de mauvaise qualité : Les séquences peuvent être alignées entre elles ou sur une base de référence contenant des séquences connues ce qui permet de reconnaître les séquences artéfactuelles (Zhang *et al.*, 2014; Edgar & Flyvbjerg, 2015). En fonction du score de qualité attribué à chaque base séquencée, les séquences peuvent être supprimée ou tronquée (Edgar & Flyvbjerg, 2015). Dans le cas d'un séquençage *paired-end*, les séquences *forward* et *reverse* qui ne s'assemblent pas ensemble (Nguyen *et al.*, 2014) et les séquences chimériques, résultant de l'association de plusieurs séquences sont supprimées. Enfin, après l'étape d'inférence de variants, des seuils peuvent être calculés sur la base du nombre de séquences détectées dans les témoins négatifs et de témoins positifs (mock).

Les séquences ou les variants ne dépassant pas ces seuils sont supprimés (Galan *et al.*, 2016). Enfin les séquences plus ou moins similaires peuvent être regroupées sous forme d'Unité Taxonomique Opérationnelle (OTU) ou *Amplicon Sequence Variant* (ASV). Ces unités, appelées espèces

moléculaires (Lindahl *et al.*, 2013), permettent d'étudier la structure des communautés microbiennes en s'affranchissant de la qualité des assignations taxonomiques. Ainsi deux OTUs ou ASVs peuvent être considérés comme deux substituts bioinformatiques différents d'un genre ou d'une espèce microbienne bien qu'ils ne soient pas connus des bases de données actuelles. Dans le cas des OTUs, le seuil de similarité entre séquences est souvent de 97% (Balint *et al.*, 2016) tandis que dans le cas des ASVs il est de 100% (Callahan *et al.*, 2017). Les OTUs ou ASVs se voient ensuite attribuer une taxonomie en fonction de leur similarité avec des séquences stockées dans des bases de données comme Greengenes (McDonald *et al.*, 2012), UNITE (Abarenkov *et al.*, 2018) et SILVA (Quast *et al.*, 2013). Des bases de données contenant les guildes ou les voies métaboliques employées par les micro-organismes permettent sur la base des assignations taxonomiques d'identifier les fonctions des espèces fongiques ou bactériennes détectées dans le jeu de données (Louca *et al.*, 2016; Nguyen *et al.*, 2016). Il existe de nombreux pipelines disponibles pour traiter et analyser les données de séquençage incluant MOTHUR (Schloss *et al.*, 2009), QIIME (Caporaso *et al.*, 2010), VSEARCH (Rognes *et al.*, 2016), FROGS (Escudié *et al.*, 2018) et DADA2 (Callahan *et al.*, 2016). Dans l'ensemble, ces pipelines réalisent majoritairement les mêmes étapes de traitement de séquence. Néanmoins, ils utilisent des outils différents ce qui conduit à des fortes variations dans les estimations de diversité et composition microbienne obtenues (Nilakanta *et al.*, 2014; Plummer & Twin, 2015; Pauvert *et al.*, 2019).

V. Objectifs de la thèse

L'ensemble de la littérature présentée ici montre que la canopée forestière (feuilles, branches, graines) héberge des communautés microbiennes diversifiées qui pourraient influencer la fitness des arbres et, à plus grande échelle, le fonctionnement des écosystèmes terrestres. De nombreuses études ont permis d'identifier et quantifier certains facteurs qui contrôlent les variations de composition taxonomique de ces communautés (Peršoh, 2013a; Kembel & Mueller, 2014; Agler *et al.*, 2016; Laforest-Lapointe *et al.*, 2016a). Néanmoins, les liens entre le microbiote de la canopée et le fonctionnement des forêts sont encore mal connus. L'objectif de cette thèse est (1) d'identifier les facteurs qui influencent la structure du microbiote de la canopée ainsi que (2) quantifier l'effet du microbiote de la canopée sur le compartiment sol (chapitre II), le compartiment plante (chapitre III) et le compartiment atmosphérique (IV) en étudiant les dynamiques de transmission verticale (de l'arbre à ses descendants) et horizontale (émission de l'arbre vers l'atmosphère) du microbiote de la canopée (Figure 6).

Le chapitre II de la thèse s'intéresse aux causes et conséquences fonctionnelles de la stratification verticale des communautés microbiennes au sein de la canopée (Leff *et al.*, 2015; Harrison *et al.*, 2016; Laforest-Lapointe *et al.*, 2016b; Stone & Jackson, 2019). Son objectif est d'identifier les traits foliaires morphologiques, chimiques et physiologiques qui contrôlent les variations de la composition taxonomique et fonctionnelle des communautés bactériennes et fongiques au sein de la canopée du hêtre européen (*Fagus sylvatica*) et d'évaluer à quel point ces variations perdurent après abscission des feuilles et affectent le processus de décomposition de la litière.

Le chapitre III s'intéresse à la transmission verticale du microbiote de l'arbre via la graine (Gundel *et al.*, 2017). Son objectif est d'identifier l'identité et la fonction des espèces fongiques associées aux différents tissus des glands du chêne sessile (*Quercus petraea*), de décrire l'évolution de la composition de la communauté fongique lorsque le gland passe de la canopée au sol, et d'évaluer l'importance relative de l'environnement et des effets maternels sur l'assemblage de cette communauté. L'implication des résultats sur le processus de régénération forestière est discutée.

Le chapitre IV porte sur l'émission des micro-organismes depuis la canopée vers l'atmosphère (Morris *et al.*, 2014b). Son objectif est de mesurer les variations spatiales et temporelles du flux d'émission bactérienne et d'évaluer l'importance relative de la canopée et du sol à cette émission. Notre capacité à analyser quantitativement et qualitativement les flux bactériens a été testée au-dessus de différents écosystèmes de la région Aquitaine (vigne, prairie) et pourra être étendue plus tard aux écosystèmes forestiers. Les méthodes de mesure mises au point dans ce chapitre pourront contribuer à une meilleure compréhension du cycle de l'eau.

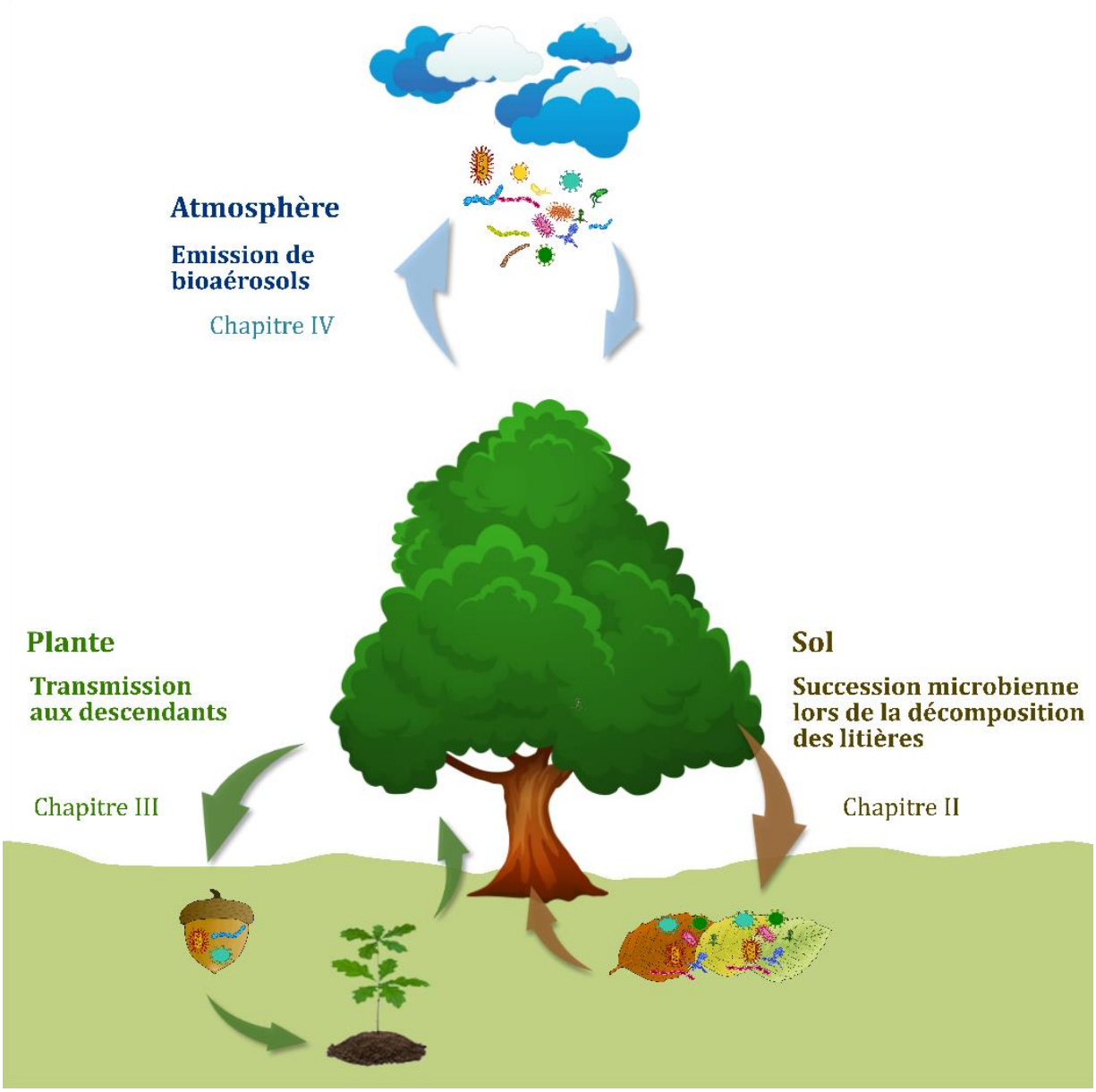


Figure 6. Schéma illustrant les objectifs de chaque chapitre de thèse. L'objectif de cette thèse est d'identifier et quantifier les effets des facteurs biotiques et abiotiques sur la structure du microbiote de la canopée et son effet sur les microbiotes associés au sol (chapitre II), à la plante (chapitre III) et l'atmosphère (chapitre IV).

VI. Références

Abarenkov K, Nilsson RH, Larsson K-H, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E, Pennanen T, *et al.* 2018. The UNITE database for molecular identification of fungi – recent updates and future perspectives. *New Phytologist* **219**: 5.

Afkhami ME, Rudgers JA, Afkhami ME, Rudgers JA. **2015**. Symbiosis Lost : Imperfect Vertical Transmission of Fungal Endophytes in Grasses. **172**: 405–416.

Agler MT, Ruhe J, Kroll S, Morhenn C, Kim ST, Weigel D, Kemen EM. **2016**. Microbial Hub Taxa Link Host and Abiotic Factors to Plant Microbiome Variation. *PLoS Biology* **14**: 1–31.

Agrios GN. **1997**. Control of Plant Diseases. In: Plant Pathology. San Diego, 200–216.

Ahn JH, Kim BC, Kim SJ, Lee GH, Song J, Kwon SW, Weon HY. **2015**. Sphingomonas parvus sp. nov. isolated from a ginseng-cultivated soil. *Journal of Microbiology* **53**: 673–677.

Aleklett K, Hart M, Shade A. **2014**. The microbial ecology of flowers: An emerging frontier in phyllosphere research1. *Botany* **92**: 253–266.

Amato P, Joly M, Besaury L, Oudart A, Taib N, Deguillaume L, Delort A, Debroas D, Mone I. **2017**. Active microorganisms thrive among extremely diverse communities in cloud water. *PLoS ONE* **3**: 1–22.

Amend AS, Cobian GM, Laruson AJ, Remple K, Tucker SJ, Poff KE, Antaky C, Boraks A, Jones CA, Kuehu D, *et al.* 2019. Phytobiomes are compositionally nested from the ground up. *PeerJ* **2019**: 1–18.

Aneja M, Gianfagna TJ, Hebbar PK. **2006**. Trichoderma harzianum produces nonanoic acid, an inhibitor of spore germination and mycelial growth of two cacao pathogens. *Physiological and Molecular Plant Pathology* **67**: 304–307.

Ariya PA, Amyot M. **2004**. New Directions: The role of bioaerosols in atmospheric chemistry and physics. *Atmospheric Environment* **38**: 1231–1232.

Arnold AE, Mejia LC, Kyllo D, Rojas EI, Maynard Z, Robbins N, Herre EA. **2003**. Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences* **100**: 15649–15654.

Arrigoni E, Antonielli L, Pindo M, Pertot I, Perazzolli M. **2018**. Tissue age and plant genotype affect the microbiota of apple and pear bark. *Microbiological Research* **211**: 57–68.

Atamna-Ismaeel N, Finkel O, Glaser F, von Mering C, Vorholt JA, Koblížek M, Belkin S, Béjà O. **2012a**. Bacterial anoxygenic photosynthesis on plant leaf surfaces. *Environmental Microbiology Reports* **4**: 209–216.

Atamna-Ismaeel N, Finkel OM, Glaser F, Sharon I, Schneider R, Post AF, Spudich JL, von Mering C, Vorholt JA, Iluz D, *et al.* 2012b. Microbial rhodopsins on leaf surfaces of terrestrial plants. *Environmental Microbiology* **14**: 140–146.

Augspurger CK, Kelly CK. **1984**. Pathogen mortality of tropical tree seedlings: experimental studies of the effects of dispersal distance, seedling denisty, and light conditions. *Oecologia.* **61**: 211–217.

Aydogan EL, Moser G, Müller C, Kämpfer P, Glaeser SP. **2018**. Long-term warming shifts the composition of bacterial communities in the phyllosphere of Galium album in a permanent grassland field-experiment. *Frontiers in Microbiology* **9**: 1–17.

Badri D V., Weir TL, van der Lelie D, Vivanco JM. **2009**. Rhizosphere chemical dialogues: plant-microbe interactions. *Current Opinion in Biotechnology* **20**: 642–650.

Bailey MJ, Lilley AK, Timms-Wilson TM, Spencer-Phillips PTN. **2006**. Microbial ecology of aerial plant surfaces. *Microbial Ecology of Aerial Plant Surfaces*: 1–321.

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. **2006**. the Role of Root Exudates in Rhizosphere Interactions With Plants and Other Organisms. *Annual Review of Plant Biology* **57**: 233–266.

Baker KF, Smith SH. **1966**. Dynamics of seed transmission of plan pathogens. *Annual Review of Phytopathology* **4**: 311 – 332.

Baldotto LEB, Olivares FL. **2008**. Phylloepiphytic interaction between bacteria and different plant species in a tropical agricultural system. *Canadian Journal of Microbiology* **54**: 918–931.

Baldrian P. **2017**. Forest microbiome: Diversity, complexity and dynamics. *FEMS Microbiology Reviews* **41**: 109–130.

Balint-Kurti P, Simmons SJ, Blum JE, Ballaré CL, Stapleton AE. **2010**. Maize leaf epiphytic bacteria diversity patterns are genetically correlated with resistance to fungal pathogen infection. *Molecular Plant-Microbe Interactions* **23**: 473–484.

Balint M, Bahram M, Eren AM, Faust K, Fuhrman JA, Lindahl B, O'Hara RB, Ï¿½pik M, Sogin ML, Unterseher M, *et al.* 2016. Millions of reads, thousands of taxa: Microbial community structure and associations analyzed via marker genesa. *FEMS Microbiology Reviews* **40**: 686–700.

Bálint M, Schmidt PA, Sharma R, Thines M, Schmitt I. **2014**. An Illumina metabarcoding pipeline for fungi. *Ecology and Evolution* **4**: 2642–2653.

Barbeta A, Jones SP, Clavé L, Wingate L, Gimeno TE, Fréjaville B, Wohl S, Ogee J. **2019**. Unexplained hydrogen isotope offsets complicate the identification and quantification of tree water sources in a riparian forest. *Hydrology and Earth System Sciences* **23**: 2129–2146.

Barbi F, Bragalini C, Vallon L, Prudent E, Dubost A, Fraissinet-Tachet L, Marmeisse R, Luis P.

2014. PCR primers to study the diversity of expressed fungal genes encoding lignocellulolytic enzymes in soils using high-throughput sequencing. *PLoS ONE* **9**: 1–22.

Barret M, Briand M, Bonneau S, Préveaux A, Valière S, Bouchez O, Hunault G, Simoneau P, Jacquesa MA. **2015**. Emergence shapes the structure of the seed microbiota. *Applied and Environmental Microbiology* **81**: 1257–1266.

Barret M, Guimbaud JF, Darrasse A, Jacques MA. **2016**. Plant microbiota affects seed transmission of phytopathogenic microorganisms. *Molecular plant pathology* **17**: 791–795.

Bartlett DH. **2009**. Microbial life in the trenches. *Marine Technology Society Journal* **43**: 128–131.

Bashan Y, Holguin G. **1998**. Proposal for the division of plant growth-promoting rhizobacteria into two classifications: Biocontrol-PGPB (plant growth-promoting bacteria) and PGPB. *Soil Biology and Biochemistry* **30**: 1225–1228.

Bassas-Galia M, Nogales B, Arias S, Rohde M, Timmis KN, Molinari G. **2012**. Plant original Massilia isolates producing polyhydroxybutyrate, including one exhibiting high yields from glycerol. *Journal of Applied Microbiology* **112**: 443–454.

Bauer H, Giebl H, Hitzenberger R, Kasper-giebl A, Reischl G, Zibuschka F, Puxbaum H. **2003**. Airborne bacteria as cloud condensation nuclei. **108**: 1–5.

Beattie GA, Lindow SE. **1995**. The Secret Life of Foliar Bacterial Pathogens on Leaves. *Annual Review of Phytopathology* **33**: 145–172.

Beattie GA, Marcell LM. **2002**. Effect of alterations in cuticular wax biosynthesis on the physicochemical properties and topography of maize leaf surfaces. *Plant, Cell and Environment* **25**: 1–16.

Beckers B, De Beeck MO, Weyens N, Boerjan W, Vangronsveld J. **2017**. Structural variability and niche differentiation in the rhizosphere and endosphere bacterial microbiome of field-grown poplar trees. *Microbiome* **5**: 1–17.

Begerow D, Nilsson H, Unterseher M, Maier W. **2010**. Current state and perspectives of fungal DNA barcoding and rapid identification procedures. *Applied Microbiology and Biotechnology* **87**: 99–108.

Bent E. **2006**. *Induced Systemic ResistanceMediated by PlantGrowth-Promoting Rhizobacteria(PGPR) and Fungi (PGPF)*.

Bentley BL, Carpenter EJ. **1984**. Direct transfer of newly-fixed nitrogen from free-living epiphyllous microorganisms to their host plant. *Oecologia* **63**: 52–56.

Berg G, Raaijmakers JM. **2018**. Saving seed microbiomes. *ISME Journal* **12**: 1167–1170.

Bertaux J, Schmid M, Hutzler P, Hartmann A, Garbaye J, Frey-Klett P. **2005**. Occurrence and

distribution of endobacteria in the plant-associated mycelium of the ectomycorrhizal fungus Laccaria bicolor S238N. *Environmental Microbiology* **7**: 1786–1795.

Bever JD, Mangan SA, Alexander HM. **2015**. Maintenance of Plant Species Diversity by Pathogens. *Annual Review of Ecology, Evolution, and Systematics* **46**: 305–325.

Bjorn D. Lindahl Johanna Boberg, Susan E. Trumbore, KI, Peter Hogberg Roger D. Finlay JS. **2007**. Spatial separation of litter decomposition and mycorrhizal nitrogen \nuptake in a boreal forest. *New Phytologist*: 611–620.

Blaser MJ, Cardon ZG, Cho MK, Dangl JL, Donohue TJ, Green JL, Knight R, Maxon ME, Northen TR, Pollard KS, *et al.* 2016. Toward a predictive understanding of earth's microbiomes to address 21st century challenges. *mBio* **7**: 1–16.

Bodenhausen N, Horton MW, Bergelson J. **2013**. Bacterial Communities Associated with the Leaves and the Roots of Arabidopsis thaliana. *PLoS ONE* **8**.

De Boer W, Folman LB, Summerbell RC, Boddy L. **2005**. Living in a fungal world: Impact of fungi on soil bacterial niche development. *FEMS Microbiology Reviews* **29**: 795–811.

Boller T, Felix G. **2009**. A Renaissance of Elicitors: Perception of Microbe-Associated Molecular Patterns and Danger Signals by Pattern-Recognition Receptors. *Annual Review of Plant Biology* **60**: 379–406.

Brader G, Compant S, Vescio K, Mitter B, Trognitz F, Ma L-J, Sessitsch A. **2017**. Ecology and Genomic Insights into Plant-Pathogenic and Plant-Nonpathogenic Endophytes. *Annual Review of Phytopathology* **55**: 61–83.

Bradley DJ, Gilbert GS, Martiny JBH. **2008**. Pathogens promote plant diversity through a compensatory response. *Ecology Letters* **11**: 461–469.

Bray JR, Gorham E. **1964**. Litter Production in Forests of the World†. *Advances in Ecological Research* **2**: 101–157.

Bright M, Bulgheresi S. **2010**. A complex journey: Transmission of microbial symbionts. *Nature Reviews Microbiology* **8**: 218–230.

Bringel F, Couée I. **2015**. Pivotal roles of phyllosphere microorganisms at the interface between plant functioning and atmospheric trace gas dynamics. *Frontiers in Microbiology* **6**: 1–14.

Brouwer B, Ziolkowska A, Bagard M, Keech O, Gardeström P. **2012**. The impact of light intensity on shade-induced leaf senescence. *Plant, Cell and Environment* **35**: 1084–1098.

Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P. **2013**. Structure and Functions of the Bacterial Microbiota of Plants. *Annual Review of Plant Biology* **64**: 807–838.

Burch AY, Zeisler V, Yokota K, Schreiber L, Lindow SE. **2014**. The hygroscopic biosurfactant

syringafactin produced by Pseudomonas syringae enhances fitness on leaf surfaces during fluctuating humidity. *Environmental Microbiology* **16**: 2086–2098.

Burrows SM, Butler T. **2009**. Bacteria in the global atmosphere – PartPart 2 : Modeling of emissions and transport between different ecosystems. : 9281–9297.

Burrows SM, Elbert W, Lawrence MG. **2009**. Bacteria in the global atmosphere – Part 1: Review and synthesis of literature data for different ecosystems. *Atmospheric Chemistry and Physics*: 9263–9280.

Busse HJ, Denner EB, Buczolits S, Salkinoja-Salonen M, Bennasar A, Kämpfer P. **2003**. Sphingomonas aurantiaca sp . nov ., Sphingomonas aerolata sp . nov . and Sphingomonas faeni sp . nov ., air- and dustborne and Antarctic , orange- pigmented , psychrotolerant bacteria , and emended description of the genus Sphingomonas. *International journal of systematic and evolutionary microbiology* **53**: 1253–1260.

Callahan BJ, McMurdie PJ, Holmes SP. **2017**. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME Journal* **11**: 2639–2643.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. **2016**. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**: 581–583.

Campisano A, Ometto L, Compant S, Pancher M, Antonielli L, Yousaf S, Varotto C, Anfora G, Pertot I, Sessitsch A, *et al.* 2014. Interkingdom transfer of the acne-causing agent, propionibacterium acnes, from human to grapevine. *Molecular Biology and Evolution* **31**: 1059–1065.

Cánovas FM, Cañas RA, de la Torre FN, Pascual MB, Castro-Rodríguez V, Avila C. **2018**. Nitrogen metabolism and biomass production in forest trees. *Frontiers in Plant Science* **9**: 1–9.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman F d, Costello elizabeth K, Fierer N, Peña antonio G, Goodrich JK, Gordon JI, *et al.* 2010. QIIME allows analysis of high- throughput community sequencing data. *Nature Publishing Group* **7**: 335–336.

Cappelletti. **2018**. Update on the biopesticide regulation and development of protein-based products for the biocontrol of crop diseases.

Carrell AA, Frank AC. **2014**. Pinus flexilis and Picea engelmannii share a simple and consistent needle endophyte microbiota with a potential role in nitrogen fixation. *Frontiers in Microbiology* **5**: 1–11.

Cernava T, Chen X, Krug L, Li H, Yang M, Berg G. **2019**. The tea leaf microbiome shows specific responses to chemical pesticides and biocontrol applications. *Science of the Total Environment* **667**: 33–40.

Chakravorty S, Helb D, Burday M, Connell N, Alland D. **2007**. A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. *Journal of Microbiological Methods* **69**: 330–339.

Champlot S, Berthelot C, Pruvost M, Andrew Bennett E, Grange T, Geigl EM. **2010**. An efficient multistrategy DNA decontamination procedure of PCR reagents for hypersensitive PCR applications. *PLoS ONE* **5**.

Chi Z, Wang F, Chi Z, Yue L, Liu G, Zhang T. **2009**. Bioproducts from Aureobasidium pullulans, a biotechnologically important yeast. *Applied Microbiology and Biotechnology* **82**: 793–804.

Clay K, Schardl C. **2002a**. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *American Naturalist* **160**.

Clay K, Schardl C. **2002b**. Evolutionary Origins and Ecological Consequences of Endophyte Symbiosis with Grasses. *The American Naturalist* **160**: S99–S127.

Coble AP, Vanderwall B, Mau A, Cavaleri MA. **2016**. How vertical patterns in leaf traits shift seasonally and the implications for modeling canopy photosynthesis in a temperate deciduous forest. *Tree Physiology* **36**: 1077–1091.

Coleman-Derr D, Desgarennes D, Fonseca-Garcia C, Gross S, Clingenpeel S, Woyke T, North G, Visel A, Partida-Martinez LP, Tringe SG. **2016**. Plant compartment and biogeography affect microbiome composition in cultivated and native Agave species. *New Phytologist* **209**: 798–811.

Compant S, Samad A, Faist H, Sessitsch A. **2019**. A review on the plant microbiome : Ecology , functions , and emerging trends in microbial application. *Journal of Advanced Research journal* **9**: 29–37.

Copeland JK, Yuan L, Layeghifard M, Wang PW, Guttman DS. **2015**. Seasonal community succession of the phyllosphere microbiome. *Molecular Plant-Microbe Interactions* **28**: 274–285.

Cordier T, Robin C, Capdevielle X, Fabreguettes O, Desprez-Loustau ML, Vacher C. **2012**. The composition of phyllosphere fungal assemblages of European beech (fagus sylvatica) varies significantly along an elevation gradient. *New Phytologist* **196**: 510–519.

Costello EK, Stagaman K, Dethlefsen L, Bohannan BJM, Relman DA. **2012**. The application of ecological theory toward an understanding of the human microbiome. *Science* **336**: 1255–1262.

Crawford I, Robinson NH, Flynn MJ, Foot VE, Gallagher MW, Huffman JA, Stanley WR, Kaye PH. **2014**. Characterisation of bioaerosol emissions from a Colorado pine forest: Results from the beachon-rombas experiment. *Atmospheric Chemistry and Physics* **14**: 8559–8578.

Cregger MA, Veach AM, Yang ZK, Crouch MJ, Vilgalys R, Tuskan GA, Schadt CW. **2018**. The Populus holobiont: Dissecting the effects of plant niches and genotype on the microbiome.

Microbiome **6**: 1–14.

Crowther TW, Stanton DWG, Thomas SM, A'Bear AD, Hiscox J, Jones TH, Voříšková J, Baldrian P, Boddy L. 2013. Top-down control of soil fungal community composition by a globally distributed keystone consumer. *Ecology* **94**: 2518–2528.

Cruaud P, Vigneron A, Lucchetti-Miganeh C, Ciron PE, Godfroy A, Cambon-Bonavita MA. 2014. Influence of DNA extraction method, 16S rRNA targeted hypervariable regions, and sample origin on microbial diversity detected by 454 pyrosequencing in marine chemosynthetic ecosystems. *Applied and Environmental Microbiology* **80**: 4626–4639.

D'Amore R, Ijaz UZ, Schirmer M, Kenny JG, Gregory R, Darby AC, Shakya M, Podar M, Quince C, Hall N. 2016. A comprehensive benchmarking study of protocols and sequencing platforms for 16S rRNA community profiling. *BMC Genomics* **17**.

Darlington AB, Dat JF, Dixon MA. 2001. The biofiltration of indoor air: Air flux and temperature influences the removal of toluene, ethylbenzene, and xylene. *Environmental Science and Technology* **35**: 240–246.

Das A, Varma A. 2009. Symbiosis: The Art of Living. In: Symbiotic Fungi Principles and Practice. 1–28.

Delmotte N, Knief C, Chaffron S, Innerebner G, Roschitzki B, Schlapbach R, Von Mering C, Vorholt JA. 2009. Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 16428–16433.

Després V, Huffman JA, Burrows SM, Hoose C, Safatov A, Buryak G, Fröhlich-nowoisky J, Andreae M, Pöschl U, Jaenicke R, *et al.* 2012. Primary biological aerosol particles in the atmosphere : a review. *Tellus* **0889**.

Didion M, Frey B, Rogiers N, Thürig E. 2014. Validating tree litter decomposition in the Yasso07 carbon model. *Ecological Modelling* **291**: 58–68.

Domke GM, Perry CH, Walters BF, Woodall CW, Russell MB, Smith JE. 2016. Estimating litter carbon stocks on forest land in the United States. *Science of the Total Environment* **557–558**: 469–478.

Doty SL, Sher AW, Fleck ND, Khorasani M, Bumgarner RE, Khan Z, Ko AWK, Kim SH, DeLuca TH. 2016. Variable nitrogen fixation in wild Populus. *PLoS ONE* **11**: 1–22.

Douglas AE. 2009. The microbial dimension in insect nutritional ecology. *Functional Ecology* **23**: 38–47.

Douglas AE, Werren JH. 2016. Holes in the hologenome: Why host-microbe symbioses are not

holobionts. *mBio* **7**: 1–7.

Duran P, Thiergart T, Garrido-Oter R, Agler M, Kemen E, Schulze-Lefert P, Hacquard S. **2018**. Microbial interkingdom interactions in roots promote Arabidopsis survival. *bioRxiv*: 354167.

Edgar RC, Flyvbjerg H. **2015**. Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics* **31**: 3476–3482.

Eichlerová I, Homolka L, Žifčáková L, Lisá L, Dobiášová P, Baldrian P. **2015**. Enzymatic systems involved in decomposition reflects the ecology and taxonomy of saprotrophic fungi. *Fungal Ecology* **13**: 10–22.

Ekblad A, Wallander H, Godbold DL, Cruz C, Johnson D, Baldrian P, Björk RG, Epron D, Kieliszewska-Rokicka B, Kjøller R, *et al.* 2013. The production and turnover of extramatrical mycelium of ectomycorrhizal fungi in forest soils: Role in carbon cycling. *Plant and Soil* **366**: 1–27.

Elad Y. **1996**. Mechanisms involved in the biological control of Botrytis cinerea incited diseases. *European Journal of Plant Pathology* **102**: 719–732.

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE. **2007**. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters* **10**: 1135–1142.

Escudié F, Auer L, Bernard M, Mariadassou M, Cauquil L, Vidal K, Maman S, Hernandez-Raquet G, Combes S, Pascal G. **2018**. FROGS: Find, Rapidly, OTUs with Galaxy Solution. *Bioinformatics* **34**: 1287–1294.

Ewald PW. **1989**. Transmission modes and evolution of the parasitism-mutualism continuum. *Annals of the New York Academy of Sciences* **503**: 295–306.

Fadrosh DW, Ma B, Gajer P, Sengamalay N, Ott S, Brotman RM, Ravel J. **2014**. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the IlluminaMiSeqplatform. *Microbiome* **2**: 6.

Faeth SH. **2002**. Are endophytic fungi defensive plant mutualists? *Oikos* **98**: 25–36.

Fang Z, Gong C, Ouyang Z, Liu P, Sun L, Wang X. **2014**. Characteristic and Concentration Distribution of Culturable Airborne Bacteria in Residential Environments in Beijing , China. : 943–953.

Fargione J, Tilman D, Dybzinski R, Lambers JHR, Clark C, Harpole WS, Knops JMH, Reich PB, Loreau M. **2007**. From selection to complementarity: Shifts in the causes of biodiversity-productivity relationships in a long-term biodiversity experiment. *Proceedings of the Royal Society B: Biological Sciences* **274**: 871–876.

Farré-Armengol G, Filella I, Llusia J, Peñuelas J. **2016**. Bidirectional Interaction between

Phyllospheric Microbiotas and Plant Volatile Emissions. *Trends in Plant Science* **21**: 854–860.

Faust K, Raes J. **2012**. Microbial interactions: From networks to models. *Nature Reviews Microbiology* **10**: 538–550.

Fehsenfeld F, Calvert J, Fall R, Goldan P, Guenther AB, Hewitt CN, Lamb B, Trainer M, Westberg H. **1992**. Emissions of volatile organic compounds from vegetation and the implications ofr atmospheric chemistry. *Global biogeochemical cylces* **6**: 389–430.

Feldman TS, Brien HEO, Arnold AE. **2008**. Moths that Vector a Plant Pathogen also Transport Endophytic Fungi and Mycoparasitic Antagonists. *Microbial Ecology* **56**: 742–750.

Fenner M, Thompson K. **2005**. *The ecology of seeds*.

Field CB, Behrenfeld MJ, Randerson JT, Falkowski P. **1998**. Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science* **281**: 237–240.

Fioretto A, Papa S, Pellegrino A, Fuggi A. **2007**. Decomposition dynamics of Myrtus communis and Quercus ilex leaf litter: Mass loss, microbial activity and quality change. *Applied Soil Ecology* **36**: 32–40.

Flemming HC, Wuertz S. **2019**. Bacteria and archaea on Earth and their abundance in biofilms. *Nature Reviews Microbiology* **17**: 247–260.

Fliegerova K, Tapio I, Bonin A, Mrazek J, Callegari ML, Bani P, Bayat A, Vilkki J, Kopečný J, Shingfield KJ, *et al.* 2014. Effect of DNA extraction and sample preservation method on rumen bacterial population. *Anaerobe* **29**: 80–84.

Forchetti G, Masciarelli O, Alemano S, Alvarez D, Abdala G. **2007**. Endophytic bacteria in sunflower (Helianthus annuus L.): Isolation, characterization, and production of jasmonates and abscisic acid in culture medium. *Applied Microbiology and Biotechnology* **76**: 1145–1152.

Foster KR, Schluter J, Coyte KZ, Rakoff-Nahoum S. **2017**. The evolution of the host microbiome as an ecosystem on a leash. *Nature* **548**: 43–51.

Franc GD, Demott PJ. **1998**. Cloud activation characteristics of airborne Erwinia carotovora cells. *Journal of Applied Meteorology* **37**: 1293–1300.

Frank A, Saldierna Guzmán J, Shay J. **2017**. Transmission of Bacterial Endophytes. *Microorganisms* **5**: 70.

Franzetti A, Gandolfi I, Bestetti G, Padoa Schioppa E, Canedoli C, Brambilla D, Cappelletti D, Sebastiani B, Federici E, Papacchini M, *et al.* 2020. Plant-microorganisms interaction promotes removal of air pollutants in Milan (Italy) urban area. *Journal of Hazardous Materials* **384**.

Freiberg E. **1998**. Microclimatic parameters influencing nitrogen fixation in the phyllosphere in a Costa Rican premontane rain forest. *Oecologia* **117**: 9–18.

Freilich S, Zarecki R, Eilam O, Segal ES, Henry CS, Kupiec M, Gophna U, Sharan R, Ruppin E. **2011**. Competitive and cooperative metabolic interactions in bacterial communities. *Nature Communications* **2**.

Friesen ML, Porter SS, Stark SC, von Wettberg EJ, Sachs JL, Martinez-Romero E. **2011**. Microbially Mediated Plant Functional Traits. *Annual Review of Ecology, Evolution, and Systematics* **42**: 23–46.

Fröhlich-Nowoisky J, Kampf CJ, Weber B, Huffman JA, Pï¿½hlker C, Andreae MO, Lang-Yona N, Burrows SM, Gunthe SS, Elbert W, *et al.* 2016. Bioaerosols in the Earth system: Climate, health, and ecosystem interactions. *Atmospheric Research* **182**: 346–376.

Fukasawa Y, Osono T, Takeda H. **2009**. Dynamics of physicochemical properties and occurrence of fungal fruit bodies during decomposition of coarse woody debris of Fagus crenata. *Journal of Forest Research* **14**: 20–29.

Fukasawa Y, Osono T, Takeda H. **2011**. Wood decomposing abilities of diverse lignicolous fungi on nondecayed and decayed beech wood. *Mycologia* **103**: 474–482.

Fürnkranz M, Wanek W, Richter A, Abell G, Rasche F, Sessitsch A. **2008**. Nitrogen fixation by phyllosphere bacteria associated with higher plants and their colonizing epiphytes of a tropical lowland rainforest of Costa Rica. *ISME Journal* **2**: 561–570.

Galan M, Razzauti M, Bard E, Bernard M, Brouat C, Charbonnel N, Dehne-Garcia A, Loiseau A, Tatard C, Tamisier L, *et al.* 2016. 16S rRNA Amplicon Sequencing for Epidemiological Surveys of Bacteria in Wildlife. *mSystems* **1**: e00032-16.

Gallart M, Adair KL, Love J, Meason DF, Clinton PW, Xue J, Turnbull MH. **2018**. Host Genotype and Nitrogen Form Shape the Root Microbiome of Pinus radiata. *Microbial Ecology* **75**: 419–433.

Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, *et al.* 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry 70:*: 153–226.

Gesheva V, Vasileva-Tonkova E. **2012**. Production of enzymes and antimicrobial compounds by halophilic Antarctic Nocardioides sp. grown on different carbon sources. *World Journal of Microbiology and Biotechnology* **28**: 2069–2076.

Gevers D, Knight R, Petrosino JF, Huang K, McGuire AL, Birren BW, Nelson KE, White O, Methé BA, Huttenhower C. **2012**. The Human Microbiome Project: A Community Resource for the Healthy Human Microbiome. *PLoS Biology* **10**: 6–10.

Ghyselinck J, Pfeiffer S, Heylen K, Sessitsch A, De Vos P. **2013**. The effect of primer choice and short read sequences on the outcome of 16S rRNA gene based diversity studies. *PloS one* **8**: 1–14.

Gilbert GS. **2002**. Evolutionary ecology of plant diseases in natural ecosystems. *Annual Review of Phytopathology* **40**: 13–43.

Gilbert JA, Neufeld JD. **2014**. Life in a World without Microbes. *PLoS Biology* **12**: 1–3.

Gimenez-Ibanez S, Boter M, Ortigosa A, Garcï¿½a-Casado G, Chini A, Lewsey MG, Ecker JR, Ntoukakis V, Solano R. **2017**. JAZ2 controls stomata dynamics during bacterial invasion. *New Phytologist* **213**: 1378–1392.

Goodwin S, McPherson JD, McCombie WR. **2016**. Coming of age: Ten years of next-generation sequencing technologies. *Nature Reviews Genetics* **17**: 333–351.

Graham JH, Colburn GC, Chung KR, Cubero J. **2012**. Protection of citrus roots against infection by Phytophthora spp. by hypovirulent P. nicotianae is not related to induction of systemic acquired resistance. *Plant and Soil* **358**: 39–49.

Greenstone MH, Weber DC, Coudron TA, Payton ME, Hu JS. **2012**. Removing external DNA contamination from arthropod predators destined for molecular gut-content analysis. *Molecular Ecology Resources* **12**: 464–469.

Großkopf T, Soyer OS. **2014**. Synthetic microbial communities. *Current Opinion in Microbiology* **18**: 72–77.

Gundel PE, Rudgers JA, Ghersa CM. **2011**. Incorporating the process of vertical transmission into understanding of host-symbiont dynamics. *Oikos* **120**: 1121–1128.

Gundel PE, Rudgers JA, Whitney KD. **2017**. Vertically transmitted symbionts as mechanisms of transgenerational effects. *American Journal of Botany* **104**: 787–792.

Hacquard S, Garrido-Oter R, González A, Spaepen S, Ackermann G, Lebeis S, McHardy AC, Dangl JL, Knight R, Ley R, *et al.* 2015. Microbiota and host nutrition across plant and animal kingdoms. *Cell Host and Microbe* **17**: 603–616.

Haga DI, Iannone R, Wheeler MJ, Mason R, Polishchuk EA, Fetch T, Van Der Kamp BJ, McKendry IG, Bertram AK. **2013**. Ice nucleation properties of rust and bunt fungal spores and their transport to high altitudes, where they can cause heterogeneous freezing. *Journal of Geophysical Research Atmospheres* **118**: 7260–7272.

Hahn K, Christensen M. **2004**. Dead Wood in European Forest Reserves – A Reference for Forest Management. In: Monitoring and Indicators of Forest Biodiversity in Europe – From Ideas to Operationality. 181.

Hania W Ben, Godbane R, Postec A, Hamdi M, Ollivier B, Fardeau M. **2019**. Defluviitoga tunisiensis gen. nov., sp. nov., a thermophilic bacterium isolated from a mesothermic and anaerobic whey digester. *International Journal of Systematic and Evolutionary Microbiology* **62**: 1377–1382.

Hardoim PR, Hardoim CCP, van Overbeek LS, van Elsas JD. **2012**. Dynamics of seed-borne rice endophytes on early plant growth stages. *PLoS ONE* **7**.

Hardoim PR, van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M, Sessitsch A. **2015**. The Hidden World within Plants: Ecological and Evolutionary Considerations for Defining Functioning of Microbial Endophytes. *Microbiology and Molecular Biology Reviews* **79**: 293–320.

Haritash AK, Kaushik CP. **2009**. Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A review. **169**: 1–15.

Harmon ME, Franklin JF, Swanson FJ, Sollins P, Gregory S V., Lattin JD, Anderson NH, Cline SP, Aumen NG, Sedell JR, *et al.* 1986. Ecology of Coarse Woody Debris in Temperate Ecosystems. *Advances in Ecological Research* **34**: 59–234.

Harrison JG, Forister ML, Parchman TL, Koch GW. **2016**. Vertical stratification of the foliar fungal community in the world's tallest trees. *American Journal of Botany* **103**: 2087–2095.

Hassani MA, Durán P, Hacquard S. **2018**. Microbial interactions within the plant holobiont. : 1–17.

Heather JM, Chain B. **2016**. The sequence of sequencers: The history of sequencing DNA. *Genomics* **107**: 1–8.

Hebert PDN, Cywinska A, Ball SL, DeWaard JR. **2003**. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences* **270**: 313–321.

Heilmann-Clausen J, Christensen M. **2005**. Wood-inhabiting macrofungi in Danish beech-forests - Conflicting diversity patterns and their implications in a conservation perspective. *Biological Conservation* **122**: 633–642.

Heywood VH. **1969**. Scanning electron microscopy in the study of plant materials. *Micron (1969)* **1**: 1–14.

Hodgson S, de Cates C, Hodgson J, Morley NJ, Sutton BC, Gange AC. **2014**. Vertical transmission of fungal endophytes is widespread in forbs. *Ecology and Evolution* **4**: 1199–1208.

Högberg MN, Högberg P. **2002**. Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. *New Phytologist* **154**: 791–795.

Högberg P, Plamboeck AH, Taylor AFS, Fransson PMA. **1999**. Natural 13 C abundance reveals trophic status of fungi and host-origin of carbon in mycorrhizal fungi in mixed forests. *Proceedings of the National Academy of Sciences of the United States of America* **96**: 8534–8539.

Huffman JA, Prenni AJ, Demott PJ, Pöhlker C, Mason RH, Robinson NH, Fröhlich-Nowoisky J, Tobo Y, Després VR, Garcia E, *et al.* 2013. High concentrations of biological aerosol particles and

ice nuclei during and after rain. *Atmospheric Chemistry and Physics* **13**: 6151–6164.

Iguchi H, Sato I, Sakakibara M, Yurimoto H, Sakai Y. **2012**. Distribution of methanotrophs in the phyllosphere. *Bioscience, Biotechnology and Biochemistry* **76**: 1580–1583.

Innerebner G, Knief C, Vorholt JA. **2011**. Protection of Arabidopsis thaliana against leaf-pathogenic Pseudomonas syringae by Sphingomonas strains in a controlled model system. *Applied and Environmental Microbiology* **77**: 3202–3210.

Ivanova EG, Doronina N V., Shepelyakovskaya AO, Laman AG, Brovko FA, Trotsenko YA. **2000**. Facultative and obligate aerobic methylobacteria synthesize cytokinins. *Microbiology* **69**: 646–651.

Ivanova EG, Doronina N V., Trotsenko YA. **2001**. Aerobic methylobacteria are capable of synthesizing auxins. *Microbiology* **70**: 392–397.

Ivanova EG, Fedorov DN, Doronina N V., Trotsenko YA. **2006**. Production of vitamin B12 in aerobic methylotrophic bacteria. *Microbiology* **75**: 494–496.

Johnston SR, Boddy L, Weightman AJ. **2016**. Bacteria in decomposing wood and their interactions with wood-decay fungi. *FEMS microbiology ecology* **92**: 1–12.

Jumpponen A, Jones KL. **2009**. Massively parallel 454 sequencing indicates hyperdiverse fungal communities in temperate Quercus macrocarpa phyllosphere. *New Phytologist* **184**: 438–448.

Junker RR, Tholl D. **2013**. Volatile Organic Compound Mediated Interactions at the Plant-Microbe Interface. *Journal of Chemical Ecology* **39**: 810–825.

Kämpfer P, Rainey FA, Andersson MA, Nurmiaho Lassila E-L, Ulrych U, Busse H., Weiss N, Mikkola R, Salkinoja-Salonen M. **2019**. Frigoribacterium faeni gen . nov ., sp . nov ., a novel psychrophilic genus of the family Microbacteriaceae. : 355–363.

Kanters C, Anderson IC, Johnson D. **2015**. Chewing up the wood-wide web: Selective grazing on ectomycorrhizal fungi by collembola. *Forests* **6**: 2560–2570.

Karamanoli K, Menkissoglu-Spiroudi U, Bosabalidis AM, Vokou D, Constantinidou HIA. **2005**. Bacterial colonization of the phyllosphere of nineteen plant species and antimicrobial activity of their leaf secondary metabolites against leaf associated bacteria. *Chemoecology* **15**: 59–67.

Kawaguchi K, Yurimoto H, Oku M, Sakai Y. **2011**. Yeast methylotrophy and autophagy in a methanol-oscillating environment on growing Arabidopsis thaliana leaves. *PLoS ONE* **6**: 1–9.

Kellner H, Vandenbol M. **2010**. Fungi unearthed: Transcripts encoding lignocellulolytic and chitinolytic enzymes in forest soil. *PLoS ONE* **5**.

Kembel SW, Mueller RC. **2014**. Plant traits and taxonomy drive host associations in tropical phyllosphere fungal communities [1]. *Botany* **92**: 303–311.

Kembel SW, O'Connor TK, Arnold HK, Hubbell SP, Wright SJ, Green JL. **2014**. Relationships

between phyllosphere bacterial communities and plant functional traits in a neotropical forest. *Proceedings of the National Academy of Sciences* **111**: 13715–13720.

Kemen E, Kemen AC, Agler MT, Kemen E. **2015**. Host – microbe and microbe – microbe interactions in the evolution of obligate plant parasitism. *New Phytologist*: 1–18.

De Kempeneer L, Sercu B, Vanbrabant W, Van Langenhove H, Verstraete W. **2004**. Bioaugmentation of the phyllosphere for the removal of toluene from indoor air. *Applied Microbiology and Biotechnology* **64**: 284–288.

Kesselmeier J, Staudt M. **1999**. An Overview on Emission, Physiology and Ecology.pdf. *Journal of Atmospheric Chemistry* **33**: 23–88.

Khan AL, Waqas M, Kang SM, Al-Harrasi A, Hussain J, Al-Rawahi A, Al-Khiziri S, Ullah I, Ali L, Jung HY, *et al.* 2014. Bacterial endophyte Sphingomonas sp. LK11 produces gibberellins and IAA and promotes tomato plant growth. *Journal of Microbiology* **52**: 689–695.

Kielak AM, Scheublin TR, Mendes LW, van Veen JA, Kuramae EE. **2016**. Bacterial community succession in pine-wood decomposition. *Frontiers in Microbiology* **7**: 1–12.

Kiers ET, Heijden MGA Van Der. **2006**. Mutualistic stability in the arbuscular mycorrhizal symbiosis: Exploring hypothesis of evolutionary cooperation. *Ecology* **87**: 1627–1636.

Klaedtke S, Jacques MA, Raggi L, Préveaux A, Bonneau S, Negri V, Chable V, Barret M. **2016**. Terroir is a key driver of seed-associated microbial assemblages. *Environmental microbiology* **18**: 1792–1804.

Klassen JL, Foght JM. **2011**. Characterization of Hymenobacter isolates from Victoria Upper Glacier, Antarctica reveals five new species and substantial non-vertical evolution within this genus. *Extremophiles* **15**: 45–57.

Kloepper J, Schroth M. 1978. Plant growth-promoting rhizobacteria on radishes. IV international conference on plant pathogenic bacteria Article. *Reads*: 879.

Knief C, Chaffron S, Stark M, Innerebner G. **2012**. Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. *The ISME Journal*: 1378–1390.

Knoch TR, Faeth SH, Arnott DL. **1993**. Endophytic fungi alter foraging and dispersal by desert seed-harvesting ants. *Oecologia* **95**: 470–473.

Knoll D, Schreiber L. **2000**. Plant-microbe interactions: Wetting of ivy (Hedera helix L.) leaf surfaces in relation to colonization by epiphytic microorganisms. *Microbial Ecology* **40**: 33–42.

Konopka A. **2009**. What is microbial community ecology. *ISME Journal* **3**: 1223–1230.

Koskella B, Brockhurst MA. **2014**. Bacteria-phage coevolution as a driver of ecological and evolutionary processes in microbial communities. *FEMS Microbiology Reviews* **38**: 916–931.

Kraft NJB, Ackerly DD. **2010**. Functional trait and phylogenetic tests of community assembly across spatial scales in an Amazonian forest. *Ecological Monographs* **80**: 401–422.

Krishna MP, Mohan M. **2017**. Litter decomposition in forest ecosystems: a review. *Energy, Ecology and Environment* **2**: 236–249.

L H. **1904**. Über neuere Erfahrungen und Probleme aufdem Gebiete der Bodenbakteriologie unter besonderer Berücksichtigung der Gründüngung und Brache. : 59–78.

Lafferty doty S. **2017**. *Functional Importance of the Plant Microbiome*.

Laforest-Lapointe I. **2017**. Exploring the temperate tree leaf microbiome: From natural forests to controlled experiments and urban environments.

Laforest-Lapointe I, Messier C, Kembel SW. **2016a**. Host species identity, site and time drive temperate tree phyllosphere bacterial community structure. *Microbiome* **4**: 1–10.

Laforest-Lapointe I, Messier C, Kembel SW. **2016b**. Tree phyllosphere bacterial communities: exploring the magnitude of intra- and inter-individual variation among host species. *PeerJ* **4**: 1–18.

Laforest-Lapointe I, Paquette A, Messier C, Kembel SW. **2017**. Leaf bacterial diversity mediates plant diversity and ecosystem function relationships. *Nature* **546**: 145–147.

Lambais MR, Crowley DE, Cury JC, Büll RC, Rodrigues RR. **2006**. Bacterial diversity in tree canopies of the Atlantic Forest. *Science* **312**: 1917.

Lange M, Habekost M, Eisenhauer N, Roscher C, Bessler H, Engels C, Oelmann Y, Scheu S, Wilcke W, Schulze ED, *et al.* 2014. Biotic and abiotic properties mediating plant diversity effects on soil microbial communities in an experimental grassland. *PLoS ONE* **9**.

Larsson J. **2018**. eulerr: Area-Proportional Euler Diagrams with Ellipses.

Last FT. **1955**. Seasonal incidence of Sporobolomyces on cereal leaves. *Transactions of the British Mycological Society*: 221–239.

Lauber CL, Hamady M, Knight R, Fierer N. **2009**. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology* **75**: 5111–5120.

Lavoie M, Mack MC, Hiers JK, Pokswinski S. **2012**. The effect of restoration treatments on the spatial variability of soil processes under longleaf pine trees. *Forests* **3**: 591–604.

LeBauer, D; Treseder K. **2008**. Nitrogen Limitation of Net Primary Productivity. *Ecology* **89**: 371–379.

Lebeis SL. **2015**. Greater than the sum of their parts: Characterizing plant microbiomes at the community-level. *Current Opinion in Plant Biology* **24**: 82–86.

Leck MA, Parker VT, Simpson RL. **2008**. Seedling ecology and evolution. *Seedling Ecology and*

Evolution: 1–514.

Lecours PB, Veillette M, Marsolais D, Duchaine C. **2012**. Characterization of bioaerosols from dairy barns: Reconstructing the puzzle of occupational respiratory diseases by using molecular approaches. *Applied and Environmental Microbiology* **78**: 3242–3248.

Lee YM, Kim EH, Lee HK, Hong SG. **2014**. Biodiversity and physiological characteristics of Antarctic and Arctic lichens-associated bacteria. *World Journal of Microbiology and Biotechnology* **30**: 2711–2721.

Lee SD, Lee DW, Ko Y. **2011**. Marmoricola korecus sp. nov. *International Journal of Systematic and Evolutionary Microbiology* **61**: 1628–1631.

Lee HS, Madhaiyan M, Kim CW, Choi SJ, Chung KY, Sa TM. **2006**. Physiological enhancement of early growth of rice seedlings (Oryza sativa L.) by production of phytohormone of N2-fixing methylotrophic isolates. *Biology and Fertility of Soils* **42**: 402–408.

Leff JW, Del Tredici P, Friedman WE, Fierer N. **2015**. Spatial structuring of bacterial communities within individual Ginkgo biloba trees. *Environmental Microbiology* **17**: 2352–2361.

Lemanceau P, Barret M, Mazurier S, Mondy S, Pivato B, Fort T, Vacher C. **2017**. *Plant Communication With Associated Microbiota in the Spermosphere, Rhizosphere and Phyllosphere*. Elsevier Ltd.

Lemons A, Clay K, Rudgers JA. **2005**. Connecting plant-microbial interactions above and belowground: A fungal endophyte affects decomposition. *Oecologia* **145**: 595–604.

Leveau JHJ, Lindow SE. **2001**. Appetite of an epiphyte: Quantitative monitoring of bacterial sugar consumption in the phyllosphere. *Proceedings of the National Academy of Sciences of the United States of America* **98**: 3446–3453.

Li Y, Wu X, Chen T, Wang W, Liu G, Zhang W, Li S, Wang M, Zhao C, Zhou H, *et al.* 2018. Plant phenotypic traits eventually shape its microbiota: A common garden test. *Frontiers in Microbiology* **9**: 1–13.

Lighthart B, Shaffer BT. **1994**. Bacterial flux from chaparral into the atmosphere in mid-summer at a high desert location. *Atmospheric Environment* **28**: 1267–1274.

Lindahl BD, Nilsson RH, Tedersoo L. **2013**. Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide - Lindahl - 2013 - New Phytologist - Wiley Online Library. *New …*.

Lindemann J, Constantinidou HA, Barchet WR, Upper CD. **1982**. Plants as sources of airborne bacteria, including ice nucleation-active bacteria. *Applied and Environmental Microbiology* **44**: 1059–1063.

Lindow SE, Brandl MT. **2003**. Microbiology of the Phyllosphere. *Applied and Environmental Microbiology* **69**: 1875–1883.

Links MG, Demeke T, Gräfenhan T, Hill JE, Hemmingsen SM, Dumonceaux TJ. **2014**. Simultaneous profiling of seed-associated bacteria and fungi reveals antagonistic interactions between microorganisms within a shared epiphytic microbiome on Triticum and Brassica seeds. *New Phytologist* **202**: 542–553.

Liu Y, Zuo S, Zou Y, Wang J, Song W. **2012**. Investigation on diversity and population succession dynamics of indigenous bacteria of the maize spermosphere. *World Journal of Microbiology and Biotechnology* **28**: 391–396.

Lladó S, López-Mondéjar R, Baldrian P. **2017**. Forest Soil Bacteria: Diversity, Involvement in Ecosystem Processes, and Response to Global Change. *Microbiology and Molecular Biology Reviews* **81**: 1–27.

López-Mondéjar R, Voříšková J, Větrovský T, Baldrian P. **2015**. The bacterial community inhabiting temperate deciduous forests is vertically stratified and undergoes seasonal dynamics. *Soil Biology and Biochemistry* **87**: 43–50.

Lopez-Velasco G, Carder PA, Welbaum GE, Ponder MA. **2013**. Diversity of the spinach (Spinacia oleracea) spermosphere and phyllosphere bacterial communities. *FEMS Microbiology Letters* **346**: 146–154.

Louca S, Jacques SMS, Pires APF, Leal JS, Srivastava DS, Parfrey LW, Farjalla VF, Doebeli M. **2016**. High taxonomic variability despite stable functional structure across microbial communities. *Nature Ecology & Evolution* **1**: 0015.

Lugtenberg B, Kamilova F, Lugtenberg B, Kamilova F. **2015**. Plant-Growth-Promoting Rhizobacteria Plant-Growth-Promoting Rhizobacteria.

Lynn M, Fester R. **1991**. *Symbiogenesis and Symbionticism*.

Lyons JA, Bemis DA, Bryant MJ, Kania SA, Abd-Eldaim M, Newman SJ. **2009**. Isolation of Propionibacterium acnes from a case of placentitis and abortion in a cow. *Journal of Veterinary Diagnostic Investigation* **21**: 274–277.

Maignien L, DeForce EA, Chafee ME, Murat Eren A, Simmons SL. **2014**. Ecological succession and stochastic variation in the assembly of Arabidopsis thaliana phyllosphere communities. *mBio* **5**.

Malfanova N, Lugtenberg BJJ, Berg G. **2013**. Bacterial Endophytes: Who and Where, and What are they doing there? *Molecular Microbial Ecology of the Rhizosphere* **1**: 391–403.

Manirajan BA, Maisinger C, Ratering S, Rusch V, Schwiertz A, Cardinale M, Schnell S. **2018**. Diversity, specificity, co-occurrence and hub taxa of the bacterial–fungal pollen microbiome. *FEMS*

Microbiology Ecology: 1–11.

Marchesi JR, Ravel J. **2015**. The vocabulary of microbiome research: a proposal. *Microbiome* **3**: 1–3.

Martinez-romero E. **2006**. Dinitrogen-Fixing Prokaryotes. *Prokaryotes*: 793–817.

Maude RB. **1996**. *Seedborne Diseases and Their Control: Principles and Practice*.

Maus I, Gabriela K, Wibberg D, Winkler A, Stolze Y, König H, Pühler A, Schlüter A. **2015**. Complete genome sequence of the strain Defluviitoga tunisiensis L3 , isolated from a thermophilic , production-scale biogas plant. *Journal of Biotechnology* **203**: 17–18.

McDonald D, Price MN, Goodrich J, Nawrocki EP, Desantis TZ, Probst A, Andersen GL, Knight R, Hugenholtz P. **2012**. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME Journal* **6**: 610–618.

Melotto M, Underwood W, He SY. **2008**. Role of Stomata in Plant Innate Immunity and Foliar Bacterial Diseases. *Annual Review of Phytopathology* **46**: 101–122.

Mercier J, Lindow SE. **2000**. Role of leaf surface sugars in colonization of plants by bacterial epiphytes. *Applied and Environmental Microbiology* **66**: 369–374.

Miliūtė I, Buzaitė O. **2011**. IAA production and other plant growth promoting traits of endophytic bacteria from apple tree. *Biologija* **57**: 98–102.

Mitter B, Pfaffenbichler N, Flavell R, Compant S, Antonielli L, Petric A, Berninger T, Naveed M, Sheibani-Tezerji R, Maltzahn G von, *et al.* 2017. A new approach to modify plant microbiomes and traits by introducing beneficial bacteria at flowering into progeny seeds. *Frontiers in Microbiology* **8**.

Miura T, Sánchez R, Castañeda LE, Godoy K, Barbosa O. **2019**. Shared and unique features of bacterial communities in native forest and vineyard phyllosphere. *Ecology and Evolution* **9**: 3295–3305.

Monier JM, Lindow SE. **2003**. Differential survival of solitary and aggregated bacterial cells promotes aggregate formation on leaf surfaces. *Proceedings of the National Academy of Sciences of the United States of America* **100**: 15977–15982.

Morris CE, Conen F, Alex Huffman J, Phillips V, Pöschl U, Sands DC. **2014a**. Bioprecipitation: A feedback cycle linking Earth history, ecosystem dynamics and land use through biological ice nucleators in the atmosphere. *Global Change Biology* **20**: 341–351.

Morris CE, Leyronas C, Nicot PC. **2014b**. Movement of Bioaerosols in the Atmosphere and the Consequences for Climate and Microbial Evolution. *Aerosol Science: Technology and Applications*: 393–415.

Morris CE, National I, Recherche D. **2001**. Phyllosphere. : 1–8.

Morris CE, Sands DC, Glaux C, Samsatly J, Asaad S, Moukahel AR, Gonçalves FLT, Bigg EK. **2013**. Urediospores of rust fungi are ice nucleation active at >-10 °c and harbor ice nucleation active bacteria. *Atmospheric Chemistry and Physics* **13**: 4223–4233.

Moyes AB, Kueppers LM, Pett-Ridge J, Carper DL, Vandehey N, O'Neil J, Frank AC. **2016a**. Evidence for foliar endophytic nitrogen fixation in a widely distributed subalpine conifer. *New Phytologist* **210**: 657–668.

Moyes AB, Kueppers LM, Pett-Ridge J, Carper DL, Vandehey N, O'Neil J, Frank AC. **2016b**. Evidence for foliar endophytic nitrogen fixation in a widely distributed subalpine conifer. *New Phytologist* **210**: 657–668.

Müller T, Ruppel S. **2014**. Progress in cultivation-independent phyllosphere microbiology. *FEMS Microbiology Ecology* **87**: 2–17.

Müller DB, Vogel C, Bai Y, Vorholt JA. **2016**. The Plant Microbiota: Systems-Level Insights and Perspectives. *Annual Review of Genetics* **50**: 211–234.

Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. **1986**. Specific enzymatic amplification of DNA in vitro: The polymerase chain reaction. : 11.

Munkvold GP. **2009**. Seed Pathology Progress in Academia and Industry. *Annual Review of Phytopathology* **47**: 285–311.

Nakazato T, Ohta T, Bono H. **2013**. Experimental Design-Based Functional Mining and Characterization of High-Throughput Sequencing Data in the Sequence Read Archive. *PLoS ONE* **8**.

Naveed M, Mitter B, Reichenauer TG, Wieczorek K, Sessitsch A. **2014**. Increased drought stress resilience of maize through endophytic colonization by Burkholderia phytofirmans PsJN and Enterobacter sp. FD17. *Environmental and Experimental Botany* **97**: 30–39.

Nelson EB. **2004**. Microbial Dynamics and Interactions in the Spermosphere. *Annual Review of Phytopathology* **42**: 271–309.

Nelson EB. **2017**. The seed microbiome: Origins, interactions, and impacts. *Plant and Soil* **422**: 7–34.

Nelson EB, Simoneau P, Barret M, Mitter B, Compant S. **2018**. The soil, the seed, the microbes and the plant. *Plant Soil* **422**: 1–5.

Nemergut DR, Schmidt SK, Fukami T, O'Neill SP, Bilinski TM, Stanish LF, Knelman JE, Darcy JL, Lynch RC, Wickey P, *et al.* 2013. Patterns and Processes of Microbial Community Assembly. *Microbiology and Molecular Biology Reviews* **77**: 342–356.

Neubauer M, Sourek J, Ryc M, Bohacek J, Mara M, Mnukova J. **1991**. Corynebacterium accolens sp . nov ., a Gram-Positive Rod Exhibiting Satellitism , from Clinical Material. **51**: 46–51.

Newton AC, Gravouil C, Fountaine JM. **2010**. Managing the ecology of foliar pathogens: Ecological tolerance in crops. *Annals of Applied Biology* **157**: 343–359.

Nguyen SN, Flores A, Talamantes D, Dar F, Valdez A, Schwans J, Berlemont R. **2019**. GeneHunt for rapid domain-specific annotation of glycoside hydrolases. *Scientific Reports* **9**: 1–10.

Nguyen NH, Smith D, Peay K, Kennedy P. **2014**. Parsing ecological signal from noise in next generation amplicon sequencing. *New p*.

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG. **2016**. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology* **20**: 241–248.

Nilakanta H, Drews KL, Firrell S, Foulkes MA, Jablonski KA. **2014**. A review of software for analyzing molecular Sequences. *BMC Research Notes* **7**.

Nilsson RH, Anslan S, Bahram M, Wurzbacher C, Baldrian P, Tedersoo L. **2019**. Mycobiome diversity: high-throughput sequencing and identification of fungi. *Nature Reviews Microbiology* **17**: 95–109.

Nisbet M, Briggs S, Ellis-Pegler R, Thomas M, Holland D. **2007**. Propionibacterium acnes: An under-appreciated cause of post-neurosurgical infection. *Journal of Antimicrobial Chemotherapy* **60**: 1097–1103.

Niu B, Paulson JN, Zheng X, Kolter R. **2017**. Simplified and representative bacterial community of maize roots. *Proceedings of the National Academy of Sciences of the United States of America* **114**: E2450–E2459.

Norros V, Penttilä R, Suominen M, Ovaskainen O. **2012**. Dispersal may limit the occurrence of specialist wood decay fungi already at small spatial scales. *Oikos* **121**: 961–974.

O'Brien HE, Parrent JL, Jackson JA, Moncalvo JM, Vilgalys R. **2005**. Fungal community analysis by large-scale sequencing of environmental samples. *Applied and Environmental Microbiology* **71**: 5544–5550.

Ofek M, Hadar Y, Minz D. **2011**. Colonization of cucumber seeds by bacteria during germination. *Environmental Microbiology* **13**: 2794–2807.

Oksanen AJ, Kindt R, Legendre P, Hara BO, Simpson GL, Stevens MHH, Wagner H. **2008**. The vegan Package; Community Ecology Package (Version 1.15-1).

Osono T. **2006**. Role of phyllosphere fungi of forest trees in the development of decomposer fungal communities and decomposition processes of leaf litter. *Canadian Journal of Microbiology* **52**: 701–716.

Osono T, Mori A. **2003**. Colonization of Japanese beech leaves by phyllosphere fungi. *Mycoscience*

44: 437–441.

Osono T, Mori A. **2004**. Distribution of phyllosphere fungi within the canopy of giant dogwood. *Mycoscience* **45**: 161–168.

Osono T, Takeda H. **2001**. Organic chemical and nutrient dynamics in decomposing beech leaf litter in relation to fungal ingrowth and succession during 3-year decomposition processes in a cool temperate deciduous forest in Japan. *Ecological Research* **16**: 649–670.

Ottesen AR, White JR, Skaltsas DN, Newell MJ, Walsh CS. **2009**. Impact of organic and conventional management on the phyllosphere microbial ecology of an apple crop. *Journal of Food Protection* **72**: 2321–2325.

Ouayjan A, Hampe A. **2018**. Extensive sib-mating in a refugial population of beech (Fagus sylvatica) growing along a lowland river. *Forest Ecology and Management* **407**: 66–74.

Pascazio S, Crecchio C, Ricciuti P, Palese AM, Xiloyannis C, Sofo A. **2015**. Phyllosphere and carposphere bacterial communities in olive plants subjected to different cultural practices. *International Journal of Plant Biology* **6**: 15–19.

Pauvert C, Buée M, Laval V, Edel-Hermann V, Fauchery L, Gautier A, Lesur I, Vallance J, Vacher C. **2019**. Bioinformatics matters: The accuracy of plant and soil fungal community data is highly dependent on the metabarcoding pipeline. *Fungal Ecology* **41**: 23–33.

Penuelas J, Llusia J. **2001**. The complexity of factors driving volatile organic compound emissions by plants. *Biologia plantarum* **44**: 481–487.

Peredo EL, Simmons SL. **2018**. Leaf-FISH: Microscale imaging of bacterial taxa on phyllosphere. *Frontiers in Microbiology* **8**: 1–14.

Pereira SIA, Castro PML. **2014**. Diversity and characterization of culturable bacterial endophytes from Zea mays and their potential as plant growth-promoting agents in metal-degraded soils. : 14110–14123.

Perry DA. **2009**. Forest Ecosystems. *Forest Ecosystems*.

Peršoh D. **2013a**. Factors shaping community structure of endophytic fungi-evidence from the Pinus-Viscum-system. *Fungal Diversity* **60**: 55–69.

Peršoh D. **2013b**. Factors shaping community structure of endophytic fungi-evidence from the Pinus-Viscum-system. *Fungal Diversity* **60**: 55–69.

Pettersson BMF, Behra PRK, Manduva S, Das S, Dasgupta S, Bhattacharya A, Kempf A, Grund E, Rm K, Hj K. **2014**. Draft Genome Sequence of Saccharopolyspora rectivirgula. *Genome Announc* **2**: 5–6.

Plummer E, Twin J. **2015**. A Comparison of Three Bioinformatics Pipelines for the Analysis of

Preterm Gut Microbiota using 16S rRNA Gene Sequencing Data. *Journal of Proteomics & Bioinformatics* **8**: 283–291.

Pochon S, Simoneau P, Pigné S, Balidas S, Bataillé-Simoneau N, Campion C, Jaspard E, Calmes B, Hamon B, Berruyer R, *et al.* 2013. Dehydrin-like Proteins in the Necrotrophic Fungus Alternaria brassicicola Have a Role in Plant Pathogenesis and Stress Response. *PLoS ONE* **8**.

Pollock J, Glendinning L, Wisedchanwet T, Watson M. **2018**. The Madness of Microbiome : Attempting To Find Consensus. *Applied and Environmental Microbiology* **84**: 1–12.

Pöschl U, Martin ST, Sinha B, Chen Q, Gunthe SS, Huffman JA, Borrmann S, Farmer DK, Garland RM, Helas G, *et al.* 2010. Rainforest Aerosols as Biogenic Nuclei of Clouds and Precipitation in the Amazon. *Science* **329**: 1513–1517.

Purahong W, Kapturska D, Pecyna MJ, Schulz E, Schloter M, Buscot F, Hofrichter M, Kruğer D. **2014**. Influence of different forest system management practices on leaf litter decomposition rates, nutrient dynamics and the activity of ligninolytic enzymes: A case study from Central European forests. *PLoS ONE* **9**: 1–11.

Purahong W, Wubet T, Lentendu G, Schloter M, Pecyna MJ, Kapturska D, Hofrichter M, Krüger D, Buscot F. **2016**. Life in leaf litter: novel insights into community dynamics of bacteria and fungi during litter decomposition. *Molecular ecology* **25**: 4059–4074.

Pusey PL, Stockwell VO, Reardon CL, Smits THM, Duffy B. **2011**. Antibiosis activity of Pantoea agglomerans biocontrol strain E325 against Erwinia amylovora on apple flower stigmas. *Phytopathology* **101**: 1234–1241.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. **2013**. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research* **41**: 590–596.

Quatrini R, Johnson DB. **2018**. Microbiomes in extremely acidic environments: functionalities and interactions that allow survival and growth of prokaryotes at low pH. *Current Opinion in Microbiology* **43**: 139–147.

Radulovic NS, Blagojevic PD, Stojanovic-Radic ZZ, Stojanovic NM. **2013**. Antimicrobial Plant Metabolites: Structural Diversity and Mechanism of Action. *Current Medicinal Chemistry* **20**: 932–952.

Rajala T, Peltoniemi M, Hantula J, Mäkipää R, Pennanen T. **2011**. RNA reveals a succession of active fungi during the decay of Norway spruce logs. *Fungal Ecology* **4**: 437–448.

Rasmussen TT. **2000**. Resident aerobic microbiota of the adult human nasal cavity.

Rastogi G, Coaker GL, Leveau JHJ. **2013**. New insights into the structure and function of

phyllosphere microbiota through high-throughput molecular approaches. *FEMS Microbiology Letters* **348**: 1–10.

Reading NC, Sperandio V. **2006**. Quorum sensing: The many languages of bacteria. *FEMS Microbiology Letters* **254**: 1–11.

Redford AJ, Bowers RM, Knight R, Linhart Y, Fierer N. **2010**. The ecology of the phyllosphere: Geographic and phylogenetic variability in the distribution of bacteria on tree leaves. *Environmental Microbiology* **12**: 2885–2893.

Reed SC, Cleveland CC, Townsend AR. **2011**. Functional Ecology of Free-Living Nitrogen Fixation: A Contemporary Perspective. *Annual Review of Ecology, Evolution, and Systematics* **42**: 489–512.

Reisberg EE, Hildebrandt U, Riederer M, Hentschel U. **2013**. Distinct phyllosphere bacterial communities on Arabidopsis wax mutant leaves. *PLoS ONE* **8**.

Rennie EA, Turgeon R. **2009**. A comprehensive picture of phloem loading strategies. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 14162–14167.

Requena N, Perez-solis E, Azcon-aguilar C, Jeffries P, Barea J. **2001**. Management of Indigenous Plant-Microbe Symbioses Aids Restoration of Desertified Ecosystems. *Applied and environmental microbiology* **67**: 495–498.

Rezki S, Campion C, Simoneau P, Jacques MA, Shade A, Barret M. **2018**. Assembly of seed-associated microbial communities within and across successive plant generations. *Plant and Soil* **422**: 67–79.

Rico L, Ogaya R, Terradas J, Peñuelas J. **2014**. Community structures of N2-fixing bacteria associated with the phyllosphere of a Holm oak forest and their response to drought. *Plant Biology* **16**: 586–593.

Riegel P, Heller R, Prevost G, Jehl F, Monteil H. **2019**. Corynebacterium durum sp . nov ., from Human Clinical SDecimens. : 1107–1111.

Rissanen AJ, Kurhela E, Aho T, Oittinen T, Tiirola M. **2010**. Storage of environmental samples for guaranteeing nucleic acid yields for molecular microbiological studies. *Applied Microbiology and Biotechnology* **88**: 977–984.

Robertson GP, Paul EA. **2000**. Decomposition and Soil Organic Matter Dynamics. *Methods in Ecosystem Science*: 104–116.

Rodriguez RJ, White JF, Arnold a E, Redman RS. **2009**. Fungal endophytes: diversity and functional roles. *New phytologist* **182**: 314–330.

Rognes T, Flouri T, Nichols B, Quince C, Mahé F. **2016**. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* **4**: e2584.

Roh SW, Quan Z, Nam Y, Chang H, Kim K, Kim M, Im W, Jin L, Kim S, Lee S, *et al.* 2019. Pedobacter agri sp . nov ., from soil. : 1640–1643.

Rosado BHP, Almeida LC, Alves LF, Lambais MR, Oliveira RS. **2018**. The importance of phyllosphere on plant functional ecology: A phyllo trait manifesto. *New Phytologist*.

Rubin BER, Gibbons SM, Kennedy S, Hampton-Marcell J, Owens S, Gilbert JA. **2013**. Investigating the Impact of Storage Conditions on Microbial Community Composition in Soil Samples. *PLoS ONE* **8**: 1–9.

Ruinen J. **1956**. Occurrence of Beijerinckia species in the 'phyllosphere'. *Nature* **177**: 220–221.

Ryffel F, Helfrich EJN, Kiefer P, Peyriga L, Portais JC, Piel J, Vorholt JA. **2016**. Metabolic footprint of epiphytic bacteria on Arabidopsis thaliana leaves. *ISME Journal* **10**: 632–643.

Ryu J, Madhaiyan M, Poonguzhali S, Yim W, Indiragandhi P, Kim K, Anandham R, Yun J, Kim KH, Sa T. **2006**. Plant growth substances produced by methylobacterium spp. and their effect on tomato (lycopersicon esculentum L.) and red pepper (capsicum annuum L.) growth. *Journal of Microbiology and Biotechnology* **16**: 1622–1628.

Sastry KS. **2013**. *Seed-borne Plant Virus Diseases*.

Sayer EJ, Tanner EVJ, Lacey AL. **2006**. Effects of litter manipulation on early-stage decomposition and meso-arthropod abundance in a tropical moist forest. *Forest Ecology and Management* **229**: 285–293.

Schlechter RO, Miebach M, Remus-emsermann MNP. **2019**. Driving factors of epiphytic bacterial communities : A review. *Journal of Advanced Research* **19**: 57–65.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, *et al.* 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* **75**: 7537–7541.

Schmidt MWI, Torn MS, Abiven S, Dittmar T, Guggenberger G, Janssens IA, Kleber M, Kögel-Knabner I, Lehmann J, Manning DAC, *et al.* 2011. Persistence of soil organic matter as an ecosystem property. *Nature* **478**: 49–56.

Schneider T, Keiblinger KM, Schmid E, Sterflinger-Gleixner K, Ellersdorfer G, Roschitzki B, Richter A, Eberl L, Zechmeister-Boltenstern S, Riedel K. **2012**. Who is who in litter decomposition Metaproteomics reveals major microbial players and their biogeochemical functions. *ISME Journal* **6**: 1749–1762.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Bolchacova E, Voigt K, Crous PW, *et al.* 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a

universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences* **109**: 6241–6246.

Schröter D, Wolters V, De Ruiter PC. **2003**. C and N mineralisation in the decomposer food webs of a European forest transect. *Oikos* **102**: 294–308.

Serna-Chavez HM, Fierer N, Van Bodegom PM. **2013**. Global drivers and patterns of microbial abundance in soil. *Global Ecology and Biogeography* **22**: 1162–1172.

Sessitsch A, Reiter B, Berg G. **2004**. Endophytic bacterial communities of field-grown potato plants and their plant-growth-promoting and antagonistic abilities. **249**: 239–249.

Shade A, Jacques MA, Barret M. **2017**. Ecological patterns of seed microbiome diversity, transmission, and assembly. *Current Opinion in Microbiology* **37**: 15–22.

Shivlata L, Satyanarayana T. **2015**. Thermophilic and alkaliphilic Actinobacteria : biology and potential applications. **6**: 1–29.

Sibley CD, Peirano G, Church DL. **2012**. Molecular methods for pathogen and microbial community detection and characterization: Current and potential application in diagnostic microbiology. *Infection, Genetics and Evolution* **12**: 505–521.

Singh D, Mathur SB. **2004**. *Histopathology of Seed-Borne Infections*. CRC Press.

Sittenfeld A, Uribe-lorío L, Mora M, Nielsen V, Arrieta G, Janzen DH. **2002**. Does a polyphagous caterpillar have the same gut microbiota when feeding on different species of food plants ? **50**: 547–560.

Šnajdr J, Cajthaml T, Valášková V, Merhautová V, Petránková M, Spetz P, Leppänen K, Baldrian P. **2011**. Transformation of Quercus petraea litter: Successive changes in litter chemistry are reflected in differential enzyme activity and changes in the microbial community composition. *FEMS Microbiology Ecology* **75**: 291–303.

Staley JT, Irgens RL, Brenner DJ. **2019**. Enhydrobacter aerasaccus gen. nov. , sp. nov. a Gas-Vacuolated, Facultatively Anaerobic, Heterotrophic Rod. *International journal of systematic bacteriology* **37**: 289–291.

Stokland JN, Jonsson BG, Information C. **2012**. Biodiversity in Dead Wood. *Cambridge University Press*.

Stone BWG, Jackson CR. **2019**. Canopy position is a stronger determinant of bacterial community composition and diversity than environmental disturbance in the phyllosphere. : 1–11.

Stout JD. **1960**. Bacteria of soil and pasture leaves at Claudelands Showgrounds. *New Zealand Journal of Agricultural Research* **3**: 413–430.

Štursová M, Žifčáková L, Leigh MB, Burgess R, Baldrian P. **2012**. Cellulose utilization in forest

litter and soil: Identification of bacterial and fungal decomposers. *FEMS Microbiology Ecology* **80**: 735–746.

Su S, Chen M, Teng C, Jiang S, Zhang C, Lin M, Zhang W. **2014**. Hymenobacter kanuolensis sp. nov., a novel radiation-resistant bacterium. *International Journal of Systematic and Evolutionary Microbiology* **64**: 2108–2112.

Sullivant DJO, Gara FO. **1992**. Traits of Fluorescent Pseudomonas spp . Involved in Suppression of Plant Root Pathogens. *Microbiological Reviews* **56**: 662–676.

Sun H, Terhonen E, Kovalchuk A, Tuovila H, Chen H, Oghenekaro AO, Heinonsalo J, Kohler A, Kasanen R, Vasander H, *et al.* 2016. Dominant tree species and soil type affect the fungal community structure in a boreal peatland forest. *Applied and Environmental Microbiology* **82**: 2632–2643.

Sy A, Timmers ACJ, Knief C, Vorholt JA. **2005**. Methylotrophic metabolism is advantageous for Methylobacterium extorquens during colonization of Medicago truncatula under competitive conditions. *Applied and Environmental Microbiology* **71**: 7245–7252.

Taberlet P, Coissac E. **2012**. Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology* **21**: 2045–2050.

Talbot JM, Bruns TD, Taylor JW, Smith DP, Branco S, Glassman SI, Erlandson S, Vilgalys R, Liao HL, Smith ME, *et al.* 2014. Endemism and functional convergence across the North American soil mycobiome. *Proceedings of the National Academy of Sciences of the United States of America* **111**: 6341–6346.

Tanaka Y, Sano T, Tamaoki M, Nakajima N, Kondo N, Hasezawa S. **2006**. Cytokinin and auxin inhibit abscisic acid-induced stomatal closure by enhancing ethylene production in Arabidopsis. *Journal of Experimental Botany* **57**: 2259–2266.

Tancos MA, Chalupowicz L, Barash I, Manulis-sasson S, Smart D. **2013**. Tomato Fruit and Seed Colonization by Clavibacter michiganensis subsp . michiganensis through External and Internal Routes. **79**: 6948–6957.

Tedersoo L, Drenkhan R, Anslan S, Morales-Rodriguez C, Cleary M. **2019**. High-throughput identification and diagnostics of pathogens and pests: Overview and practical recommendations. *Molecular Ecology Resources* **19**: 47–76.

Terhonen E, Blumenstein K, Kovalchuk A. **2019**. Forest Tree Microbiomes and Associated Fungal Endophytes : Functional Roles and Impact on Forest Health. : 1–32.

Thines M. **2014**. Phylogeny and evolution of plant pathogenic oomycetes-a global overview. *European Journal of Plant Pathology* **138**: 431–447.

Thompson LR, Sanders JG, McDonald D, Amir A, Ladau J, Locey KJ, Prill RJ, Tripathi A, Gibbons SM, Ackermann G, *et al.* 2017. A communal catalogue reveals Earth's multiscale microbial diversity. *Nature* **551**: 457–463.

Tlaskal V, Vorıskov J, Baldrian P. **2016**. Bacterial succession on decomposing leaf litter exhibits a specific occurrence pattern of cellulolytic taxa and potential decomposers of fungal mycelia. *FEMS Microbiology Ecology* **92**: 1–10.

Trotsenko YA, Ivanova EG, Doronina N V. 2001. Aerobic methylotrophic bacteria as phytosymbionts. *Mikrobiologiya* **70**: 725–736.

Truyens S, Weyens N, Cuypers A, Vangronsveld J. **2015**. Bacterial seed endophytes: Genera, vertical transmission and interaction with plants. *Environmental Microbiology Reports* **7**: 40–50.

Tsavkelova EA, Klimova SY, Cherdyntseva TA, Netrusov AI. **2006**. Microbial producers of plant growth stimulators and their practical use: A review. *Applied Biochemistry and Microbiology* **42**: 117–126.

Tukey HB. **1970**. The Leaching of Substances from Plants. *Annual Review of Plant Physiology* **21**: 305–324.

Turner TR, James EK, Poole PS, Gilbert J, Meyer F, Jansson J, Gordon J, Pace N, Tiedje J, Ley R, *et al.* 2013. The plant microbiome. *Genome Biology* **14**: 209.

Unterseher M, Peršoh D, Schnittler M. **2013**. Leaf-inhabiting endophytic fungi of European Beech (Fagus sylvatica L.) co-occur in leaf litter but are rare on decaying wood of the same host. *Fungal Diversity* **60**: 43–54.

Urbanová M, Šnajdr J, Baldrian P. **2015**. Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. *Soil Biology and Biochemistry* **84**: 53–64.

Vacher C, Cordier T, Vallance J. **2016a**. Phyllosphere Fungal Communities Differentiate More Thoroughly than Bacterial Communities Along an Elevation Gradient. *Microbial Ecology*: 1–3.

Vacher C, Hampe A, Porté AJ, Sauer U, Compant S, Morris CE. **2016b**. The Phyllosphere: Microbial Jungle at the Plant–Climate Interface. *Annual Review of Ecology, Evolution, and Systematics* **47**: 1–24.

Vala V, Baldrian P, Herinkova J. **2008**. Soil Biology & Biochemistry Spatial variability of enzyme activities and microbial biomass in the upper layers of Quercus petraea forest soil. **40**: 2068–2075.

Valverde A, Peix A, Rivas R, Velázquez E, Salazar S, Santa-Regina I, Rodríguez-Barrueco C, Igual JM. **2008**. Paenibacillus castaneae sp. nov., isolaled from the phyllosphere of Castanea sativa Miller. *International Journal of Systematic and Evolutionary Microbiology* **58**: 2560–2564.

Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A. **2015**. The importance of the microbiome of the plant holobiont. *New Phytologist* **206**: 1196–1206.

Vayssier-Taussat M, Albina E, Citti C, Cosson JF, Jacques MA, Lebrun MH, Le Loir Y, Ogliastro M, Petit MA, Roumagnac P, *et al.* 2014. Shifting the paradigm from pathogens to pathobiome new concepts in the light of meta-omics. *Frontiers in Cellular and Infection Microbiology* **5**: 1–7.

Verma VC, Gange AC. **2014**. *Advances in endophytic research*.

Vitousek PM, Cassman K, Cleveland C, Crews T, Field CB, Grimm NB, Howarth RW, Marino R, Martinelli L, Rastetter EB, *et al.* 2002. Towards an ecological understanding of biological nitrogen fixation. *Biogeochemistry* **57–58**: 1–45.

Vogel C, Bodenhausen N, Gruissem W, Vorholt JA. **2016**. The Arabidopsis leaf transcriptome reveals distinct but also overlapping responses to colonization by phyllosphere commensals and pathogen infection with impact on plant health. *New phytologist* **212**: 192–207.

Vorholt JA. **2012**. Microbial life in the phyllosphere. *Nature Reviews Microbiology* **10**: 828–840.

Voriskova J, Baldrian P. **2013**. Fungal community on decomposing leaf litter undergoes rapid successional changes. *ISME Journal* **7**: 477–486.

Waight K, Pinyakong O, Luepromchai E. **2007**. Degradation of phenanthrene on plant leaves by phyllosphere bacteria. *Journal of General and Applied Microbiology* **53**: 265–272.

Van Der Wal A, Leveau JHJ. **2011**. Modelling sugar diffusion across plant leaf cuticles: The effect of free water on substrate availability to phyllosphere bacteria. *Environmental Microbiology* **13**: 792–797.

Wellner S, Lodders N, Kämpfer P. **2011**. Diversity and biogeography of selected phyllosphere bacteria with special emphasis on Methylobacterium spp. *Systematic and Applied Microbiology* **34**: 621–630.

Whipps JM, Hand P, Pink D, Bending GD. **2008**. Phyllosphere microbiology with special reference to diversity and plant genotype. *Journal of Applied Microbiology* **105**: 1744–1755.

Whitham TG, Bailey JK, Schweitzer| JA, Shuster SM, Bangert RK, LeRoy CJ, Lonsdorf E V., Allan GJ, DiFazio SP, Potts BM, *et al.* 2006. A framework for community and ecosystem genetics : from genes to ecosystems. *Nature Reviews Genetics* **7**: 510–523.

Wilson M, Savka MA, Hwang I, Farrand SK, Lindow SE. **1995**. Altered epiphytic colonization of mannityl opine-producing transgenic tobacco plants by a mannityl opine-catabolizing strain of Pseudomonas syringae. *Applied and Environmental Microbiology* **61**: 2151–2158.

Witzell J, Martín JA. **2018**. Endophytes of Forest Health. In: Endophytes of Forest Trees. 261–282.

Womack AM, Bohannan BJM, Green JL. **2010**. Biodiversity and biogeography of the atmosphere.

Philosophical Transactions of the Royal Society B: Biological Sciences **365**: 3645–3653.

Wong SCY, Poon RWS, Chen JHK, Tse H, Lo JYC, Ng T, Au JCK, Tse CWS. Corynebacterium kroppenstedtii Is an Emerging Cause of Mastitis Especially in Patients With Psychiatric Illness on Antipsychotic Medication. : 1–4.

Yang B, Wang Y, Qian PY. **2016**. Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. *BMC Bioinformatics* **17**: 1–8.

Yao H, Sun X, He C, Maitra P, Li XC, Guo LD. **2019**. Phyllosphere epiphytic and endophytic fungal community and network structures differ in a tropical mangrove ecosystem. *Microbiome* **7**: 1–15.

Yarza P, Yilmaz P, Pruesse E, Glöckner FO, Ludwig W, Schleifer KH, Whitman WB, Euzéby J, Amann R, Rosselló-Móra R. **2014**. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nature Reviews Microbiology* **12**: 635–645.

Yasumura Y, Hikosaka K, Matsui K, Hirose T. **2002**. Leaf-level nitrogen-use efficiency of canopy and understorey species in a beech forest. *Functional Ecology* **16**: 826–834.

Zeng Q, Liu Y, Zhang H, An S. **2019**. Fast bacterial succession associated with the decomposition of Quercus wutaishanica litter on the Loess Plateau. *Biogeochemistry* **144**: 119–131.

Zhang J, Kobert K, Flouri T, Stamatakis A. **2014**. PEAR: A fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* **30**: 614–620.

Zilber-Rosenberg I, Rosenberg E. **2008**. Role of microorganisms in the evolution of animals and plants: The hologenome theory of evolution. *FEMS Microbiology Reviews* **32**: 723–735.

Zipfel C. **2009**. Early molecular events in PAMP-triggered immunity. *Current Opinion in Plant Biology* **12**: 414–420.

II Within-canopy variation in phyllosphere microbial communities of European beech (Fagus sylvatica): magnitude, drivers and functional consequences

Tania Fort[1], Charlie Pauvert[1], Eliane Louisanna[2], Régis Burlett[1], Emilie Chancerel[3], Amandyne Linares- Maurizi[1], Patricia Balias[4], Yves Gibon[4], Amy Zanne[5], Arndt Hampe[1], Heidy Schimann[2], Lisa Wingate[6], Corinne Vacher*[1]

1. BIOGECO, INRA, Univ. Bordeaux, 33615 Pessac, France
2. ECOFOG, INRA, University of French Guyana, France
3. CGFB, Bordeaux Genome Transcriptome facility, Cestas, France
4. BFP, INRA, Villenave d'Ornon, France
5. George Washington University, Biological Sciences Department, Washington DC, USA
6. ISPA, INRA, Villenave d'Ornon, France

Abstract

Forest canopies represent hotspots of biodiversity and play a crucial role in the functioning of the Earth system. Owing to the strong light gradient from the top to the bottom of the canopy, tree leaves harbor steep changes in their morphology, physiology and chemistry and therefore favor various animal, vegetal and microbial species within the canopy, that may in turn affect tree performance and forest functioning. The objective of the study was to quantify the magnitude, drivers and functional consequences of within-canopy variation in phyllosphere bacterial and fungal communities in European beech (*Fagus sylvatica*), by combining leaf traits measurements and high-throughput sequencing of microbial barcode regions and functional genes. Our results showed that fungal pathogens and their mycoparasites dominated beech phyllosphere. Fungal saprotrophs were also abundant, particularly at the bottom of the canopy and the end of the growing season. Diazotrophic bacteria were rare, contrasting with the results found for other tree species. Between-tree variation in fungal community composition was larger than within-canopy variation that was larger than within-leaf variation. In contrast, within-canopy variation in bacterial community composition was larger than both between-tree variation and within-leaf variation. The vertical stratification was significant for both epiphytic and endophytic fungal and bacterial communities, and was stronger at the beginning of the growing season for bacteria. For both fungi and bacteria, the leaf traits that best explained variation in community composition were chlorophyll a concentration, specific leaf area, stomatal conductance, and fructose and glucose concentrations. Variation in community composition remained significant throughout leaf decomposition. Overall, our results suggest that phyllosphere fungal and bacterial communities are not shaped by the same processes, that fungal communities interact more intimately with their host tree, and that the phyllosphere microbiota responds to or interfere with major physiological processes such as photosynthesis and transpiration.

Introduction

Forest canopies are hotspots of biodiversity that provide habitat and resources for a wide range of organisms, including animals (mammals, birds, amphibians and insects), plants (bryophytes and lichens) and microorganisms (Nakamura *et al.*, 2017). Prokaryotic (bacteria and archaea) and eukaryotic microorganisms (fungi, oomycetes and nematodes) colonize both the surface and internal tissues of the leaves. They form complex, taxonomically and functionally diverse communities (Vorholt, 2012; Bulgarelli *et al.*, 2013; Hardoim *et al.*, 2015; Compant *et al.*, 2019; Schlechter *et al.*, 2019) that are shaped simultaneously by leaf traits (Turner *et al.*, 2013; Kembel & Mueller, 2014; Kembel *et al.*, 2014) and climatic and microclimatic factors (Cordier *et al.*, 2012b; Coince *et al.*, 2014; Bálint *et al.*, 2015). There is now a large body of evidence showing that the influence of these foliar communities, termed phyllosphere microbial communities (PMCs), surpasses by far their microscale (Vacher *et al.*, 2016). PMCs can increase tree tolerance to biotic and abiotic stresses (Arnold *et al.*, 2003; Rosado *et al.*, 2018), and thus influence tree health and growth and forest productivity (Laforet-Lapointe et al. 2017; Stone *et al.*, 2018; Compant *et al.*, 2019; Terhonen *et al.*, 2019; Witzell & Martín, 2018). Foliar diazotrophic bacteria, that use atmospheric nitrogen (N2) as a source of nitrogen, may for instance contribute to N-acquisition by trees (Cusack *et al.,* 2009; Reed *et al.*, 2011; Moyes et al. 2016; Doty et al. 2017a; Doty et al. 2017b). In addition, PMCs play a role in carbon cycling through early leaf decomposition (Osono, 2006; Unterseher *et al.*, 2013; Voriskova & Baldrian, 2013) and in global water cycle (Morris *et al.*, 2014).

Forests harbor a steep vertical gradient of microclimatic conditions within their canopy. This microclimate gradient, due mainly to leaf interception of sunlight and precipitation (Anhuf & Rollenbeck, 2001; Storck *et al.*, 2002), triggers spatial variations in leaf chemistry, physiology and morphology (Niinemets, 2016). For instance, sun and shade leaves differ in nitrogen and pigment content, in Non-Structural Carbohydrates (NSC) concentration, in photosynthetic capacity and in transpiration rate (Meir *et al.*, 2002; Niinemets, 2007; Druebert *et al.*, 2009). These spatial variations in both microclimate and leaf traits account for the within-canopy variations in PMCs diversity and composition observed in several tree species (Cordier *et al.*, 2012a; Ottesen *et al.*, 2013; Leff *et al.*, 2015; Laforest-Lapointe *et al.*, 2016a; Stone & Jackson, 2019). Some studies showed for instance that leaves growing at branch tips or at the top of canopies harbor a lower bacterial richness (Leff *et al.*, 2015; Stone & Jackson, 2019) and a lower fungal richness (Izuno *et al.*, 2016). Interestingly in *Gingko biloba*, bacterial phyla had distinct distributions within the tree crown. *Proteobacteria* and *Bacteroidetes* increased in abundance at the branch tips, while *Actinobacteria*

were more common deeper in the crown (Leff *et al.*, 2015). The cascading effects of these within-canopy variations in PMCs on tree performance and forest ecosystem functioning have hardly been assessed so far (Laforest-Lapointe *et al.*, 2016b).

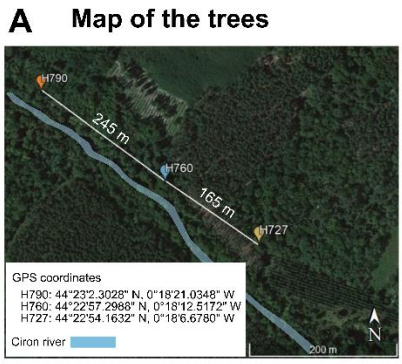
In this study, we analyzed the magnitude, drivers and functional consequences of within-canopy variation in phyllosphere microbial communities in European beech (*Fagus sylvatica*). We tested the hypothesis that (**H1**) bacterial and fungal communities are spatially structured within the canopy, in terms of both taxonomic composition and putative functions, with, in particular, (**H2**) significant variations in the nitrogen-fixing and carbohydrate-decomposing potentials. We also investigated whether variation in community composition (**H3**) is accounted for by variations in leaf morphology, physiology and chemistry and (**H4**) increase along the vegetative season, due to within-canopy leaf differentiation in response to light gradients (Coble *et al.*, 2016). We also tested if within-canopy variation in community composition is (**H5**) larger than within-leaf variation and (**H6**) larger in epiphytes than endophytes, because the former directly experience light and temperature gradients (Peršoh, 2013b; Yao *et al.*, 2019). Finally, to assess the functional consequences of within-canopy variation in phyllosphere microbial communities, we investigated whether this variation (**H7**) remain significant after leaf abscission and during early decomposition.

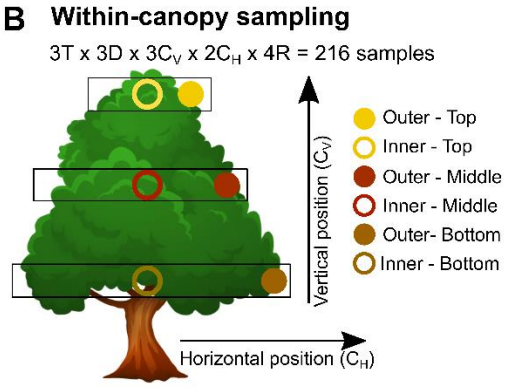
Materials and methods

Study site and sampling design

Samples were collected in the canopy and the surrounding environment of three European beech (*Fagus sylvatica*) trees growing in the Ciron Valley forest (44 ° 23 '2 "North, 0 ° 18 '21 "West), which is approximately 50 km South-East of Bordeaux (France). The trees were located 14 to 55 meters from the Ciron River and 200 to 400 meters from each other (Figure 1A). Samples were collected on three sampling dates corresponding to the beginning, the middle and the end of the beech vegetative season (30th to 31st of May, 4th to 5th of July and 19th to 20th of September, respectively). During each sampling campaign, six branches facing southwestern direction were collected on each tree by tree climbers, without touching the leaves with hands. Branches were collected at three heights, corresponding to the top, middle and bottom crown. At each height, one branch was sampled in the inner part of the crown and another in the outer part of the crown (Figure 1B and 1C). Branches were immediately placed in a water bottle and brought back to the field lab, which was set up close to the three trees to process the leaves for PMC analyses et to perform leaf traits measurements.

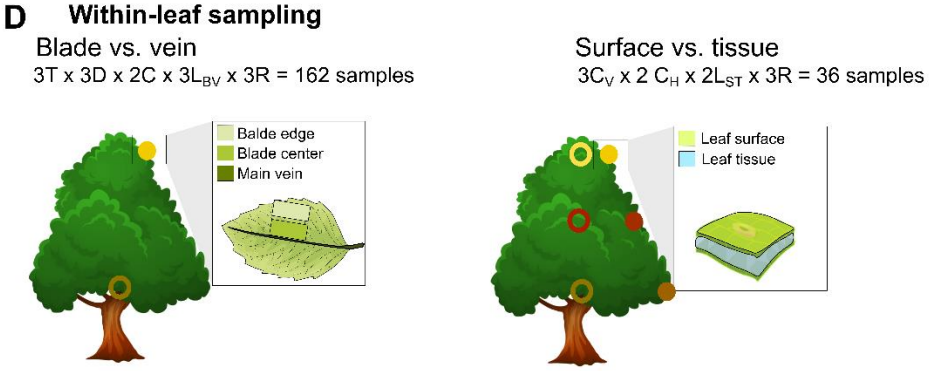
Figure 1. Sampling design. (A) Map of the three beech trees sampled. (B) Sampling design for the analysis of within-canopy variations (T: tree, D: date, C_V: within-canopy vertical position, C_H: within-canopy horizontal position, R: leaf replicate). (C) Position of the sampled branches in each tree. (D) Sampling design for the analysis of within-leaf variations (C: within-canopy position, L_{BV}: within-leaf position – blade *vs* vein, L_{ST}: within-leaf position – surface *vs* internal tissues). (E) Sampling design for the monitoring of leaf decomposers (IT: incubation time).

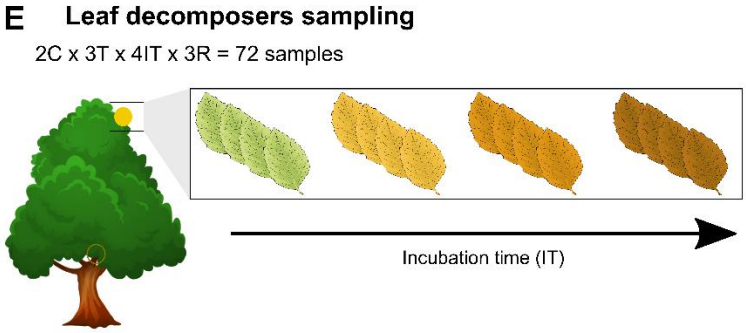




C Location of the sampled branches

	Tree H727		Tree H760		Tree H790	
Branch position	Height (m)	Distance to the trunk (m)	Height (m)	Distance to the trunk (m)	Height (m)	Distance to the trunk (m)
Outer - Top	17.4	4.8	17.3	3.9	16	5.5
Outer - Middle	13.2	7.1	12.15	3.7	8.71	3.6
Outer - Bottom	1.95	9	3.82	4.1	4.82	5.7
Inner - Top	15.9	3.5	17.7	2.3	15.5	4.2
Inner - Middle	12.3	4.9	11.9	1.8	8.81	1.6
Inner - Bottom	2.4	3.1	3.62	2.2	6.34	2.6





Leaf processing for the analysis of within-canopy variations in microbial communities

On each branch, four leaves were randomly chosen (Figure 1B), collected using sterilized nitrile gloves and placed on autoclaved filter papers. Each leaf was then wrapped with pliers, placed in a 20mL high-density polyethylene (HDPE) vial (Zinsser Analytic®) and frozen with liquid nitrogen. Vials were then stored in a Nitrogen dry shipper (Voyageur, Air liquide ®). They were cold ground using a GenoGrinder (30s at 1750 RPM), after adding two autoclaved stainless steel balls to the tubes. Aliquots of 20mg of powder were then transferred in individual tubes (Micronic, MP32022, In Vitro A/S, Fredensborg, Denmark) along with two sterile stainless steel balls (TissueLyser II, Retsch, Qiagen®) and stored at -80° until DNA extraction.

Leaf processing for the analysis of within-leaf variations in microbial communities

Moreover, three leaves were collected on the two most distant branches (cut from the lower inner part and higher outer part of the crown, respectively) with pliers and a pair of scissors cleaned with DNaway and 70% alcohol between each use. On each leaf, two 1cm x 3cm pieces of leaf were cut; one in the blade edge and the other in the blade center, avoiding the main rib. The entire rib was also removed and cut into 3 to 4 pieces (Figure 1D). The samples were then stored in Eppendorf tubes. Samples were transported in a dry-shipper and stored at -80°C until further analysis.

Leaf processing for the comparison of epiphytic and endophytic microbial communities

Finally, three leaves per branch were collected to compare variations in epiphytic and endophytic communities (Figure 1E). Each leaf was collected with sterile nitrile gloves and placed in a sterile 50ml Falcon tube in a cooler. Leaves were stored in the lab at -20°C until the epiphytic fractions of microbial communities could be detached by sonication and vortexing (sonication 3min, vortex 5sec, shake 10sec), after adding 20mL of PBS-Tween 20 in each tube under a laminar flow hood. The resulting solution was then centrifuged during 15min at 4,000g. The supernatant was removed using a PipetBoy (Integra Biosciences, Fernwald, Germany), by leaving the pellet and approximately 2ml of solution. For each date, solutions of the first replicate of each tree were re-suspended, pooled and centrifuged a second time. The second and third replicates were processed similarly, resulting in three replicates of each foliar habitat for each date and position in the canopy. The supernatant was removed, by leaving out 800µL of solution. The remaining solution was transferred into a 1.1 mL Micronic tube (NovaZine, Lyon, France, ref: MP32033L) itself placed in a 2ml Eppendorf tube. The latter was centrifuged during 5min at 10 000g and the supernatant was removed. Two stainless steel balls were added to each sample and stored at -80C° until DNA extraction. To recover the

endophyte fraction of beech leaves, leaves were removed from each falcon tube after sonication and surface-sterilized by immersion for 3 min in a 70% ethanol solution, immersion for 2 min in a 3% calcium hypochlorite solution and rinsing with DNAway and sterilized water. After drying on sterilized filter papers, leaves were cold-ground in Zinsser vials (as previously described).

Leaf processing for the analysis of decomposer communities

On the last sampling campaign, additional leaves were collected on each tree to characterize the succession of microbial communities throughout leaf decomposition (Figure 1F). Twelve leaves were collected on two branches, cut from the lower inner part and higher outer part of the canopy, respectively. Each leaf was placed in a sterile Petri Dish with 2mL of sterile water and incubated in a climatic chamber at 17.33°C with 85.9% of relative humidity, corresponding to the mean climatic conditions recorded on the sampling site in September. Four leaves were then cold-ground in Zinsser vials (as previously described) 1 day, 15 days, 30 days and 60 days after sampling for each branch and tree sampled. Leaf powders from the same branch on the same tree were pooled resulting in three replicates per branch and tree.

Environmental sampling for the characterization of microorganism habitat range

Seven types of environmental samples were collected to characterize the microbial communities in the environment of each tree: (1) twigs cut from the branches collected in the canopy, (2) common ivy (*Hedera helix L.*) and (3) bryophytes growing on the tree trunk, (4) dead wood bark, (5) litter and (6) butcher's-broom leaves and twigs *(Ruscus aculeatus*) beneath each tree, and (7) river bedding. All samples were collected in triplicates with sterile latex gloves and stored in 50mL Falcons tubes, transported in coolers and stored at -20°C until the epiphytic microorganisms could be detached by sonication and vortexing (as previously described, except that the first solution was filtered with a sterile cell strainer (100µm of porosity, nylon; Dutscher) to remove bark and litter fragments).

Leaf traits measurements

Twenty-three leaf traits representing leaf morphology, chemistry and physiology were measured on each sampled branch (Table 1). All traits were measured on several leaves per branch and then averaged at the branch level.

Table 1. Leaf chemical, physiological and morphological traits measured on each sampled branch. NM indicates the number of independent measurements per branch. Traits highly correlated ($R^2 > 0.7$) to each other were removed from the dataset (Fig. S1). Foliar traits selected for further analysis are marked with a cross.

Trait	Abbreviation	NM	Traits selected
Leaf chemistry			
Chlorophyll a and b concentration (mg/gFW)	Chl a. Chl b	4	X
Glucose concentration (µmol/gFW)	Gc	4	X
Fructose concentration (µmol/gFW)	Fc	4	X
Sucrose concentration (µmol/gFW)	Sc	4	X
Amino acids concentration (µmol/gFW)	AA	4	X
Nitrogen Balance Index	NBI	6	X
Chlorophyll content	Chl index	6	
Flavonol content	Flv index	6	X
Leaf physiology			
Leaf water content (%)	LWC	6	X
Stomatal conductance (mol m−2 s−1)	Gs	6	X
Leaf turgor loss point (MPa)	πtlp	4	X
Osmotic potential (MPa)	πosm		
Leaf water potential (MPa)	LWP	3	X
Leaf morphology			
Leaf surface (cm2)	Surface	6	
Fresh weight (mg)	FW	6	
Dry weight (mg)	DW	6	
Specific leaf area (cm2.mg-1)	SLA	6	X
Total leaf thickness (µm)	Tot. Thickness	3	X
Upper epidermis cuticle thickness (µm)	Up. Ep. Thickness	3	
Palisade mesophyll thickness (µm)	P. Thickness	3	
Spongy mesophyll thickness (µm)	S. Thickness	3	
Lower epidermis cuticle thickness (µm)	Lo. Ep. Thickness	3	
Ratio palisade:spongy mesophyll (µm)	P/S Ratio	3	X

Chemical traits measurement

The four leaves collected on each branch for the analysis of within-canopy variations in microbial communities (Figure 1B) were also used to quantify leaf chlorophyll a and b, amino acids and non-structural carbohydrates (fructose, glucose and sucrose) at the HiTMe platform of the MetaboHUB Bordeaux facility (France). Chemical compounds were extracted from aliquots of 20 to 50 mg leaf powder, by shaking them with 250µL of 80% ethanol then heating at 80°C for 20 minutes and centrifuging at 13,400 rpm for 5 minutes. This step was repeated a second time with 150µL of 80% ethanol and a third time with 250µL of 50% ethanol. At each step, the ethanolic supernatant was collected, representing at the end a total volume of 650 µL of solution. Leaf chlorophyll a and b concentrations were determined as in Arnon (1949) by measuring the absorbance at 645 and 665nm of 35µL of ethanolic extract mixed with 120µL of 98% ethanol. Concentrations of glucose, fructose and sucrose were quantified from the ethanolic extract using a method adapted from Stitt et al. (1989). Aliquots of 5µL of ethanolic extract were added to a 160µL mix containing 0.1 M Hepes-KOH buffer (pH 7), 3 mM $MgCl_2$, 100 mM ATP, 45 mM NADP, 1% polyvinylpyrrolidone and 5 glucose-6-phosphate dehydrogenase units. The glucose, fructose and sucrose contents were quantified from the change in absorbance triggered by the sequential addition of one unit of hexokinase, one unit of phosphoglucose isomerase and 30 invertase units , respectively. The quantification of total free amino acids was based on the reaction of amino acids with fluorescamine (Bantan-Polak *et al.*, 2001). The final 213µL reaction mixture contained 8µL of ethanolic extract mixed with 15µL of sodium borate at 0.1 mM, pH 8.0, 90 µL of 0.1% of fluorescamine and 100 µL of water. The solution was excited at 405 nm and read at 485 nm. Absorbance and fluorescence measurements were performed using SAFAS MP96 and SAFAS Xenius microplate readers, respectively. Flavonol content and Nitrogen Balance Index were measured in the field on 6 leaves per branch, using the Dualex forceps (Dualex®. ForceOne CNRS-LURE. France).

Physiological traits measurement

These six leaves were also used to measure leaf stomatal conductance and water content. Leaf stomatal conductance was measured in the field using a Porometer (SC-1, Decagon Devices Inc., Pullman. WA. USA). Fresh weight was measured on the day of sampling using a balance and dry weight was measured after 48 h of desiccation in an oven at 60°C (Sayer, 2006). Leaf water content was calculated as *LWC (%) = 100 x (Fresh weight – Dry Weight) / Fresh weight*.

Two additional leaves were taken from each branch to measure leaf osmotic potential. They were immediately immersed in liquid nitrogen using pliers and then placed on filter paper. For each leaf,

two 6mm diameter foliar discs were cut with a punch on each side of the midrib, placed in an Eppendorf in a dry-shipper and then stored at -80°C. The osmotic potential (π_{osm}) was measured by placing a frozen foliar disc in a calibrated osmometer chamber (C52, Wescor, USA) connected to a datalogger (Psypro, Wescor, USA). The turgor loss point (π_{tlp}) was then estimated as $\pi_{tlp} = 0.832\ \pi_{osm} - 0.631$ (Bartlett et al. 2012). The turgor loss point represents the leaf water potential at wilting and is a more reliable predictor of plant drought tolerance than the osmotic potential (Bartlett et al. 2008; Bartlett et al. 2012).

Leaf water potential was measured in the field at predawn and at the time of sampling using a Scholander-type pressure chamber (DGMeca, France), on two to four leaves per branch.

Morphological trait measurements

Three leaves per branch were also collected to measure the thickness of the upper epidermis, palisade mesophyll, spongy mesophyll, lower epidermis and the total leaf thickness. These leaves were placed in plastic bags, transported in a cooler and stored at 5°C. A transversal, 1cm large strip was cut with scissors in the middle of each leaf. Each strip was then dehydrated by a series of 5 graduated ethanol baths containing 30, 50, 70, 85 and 100% of ethanol for 30, 30, 60, 50 and 30min, respectively (Chen *et al.*, 2016). Ultra-fine cross sections (between 30 and 40 µm) were then finely cut using a microtome (GSL1, Schweingruber inst, Switzerland), avoiding the midrib, by holding the samples between two plastic lamellae. The foliar strips were then stained by adding a solution composed of 300 µL of methylene blue, 100µL of safranin and 1.6mL of distilled water diluted in 9mL of methylene blue. Microscopic pictures (magnification x 200) were taken using a microscopic imaging software (LasX, Leica Microsystems, Germany) and a microscope (DM2500, Leica Microsystems, Germany) equipped with a camera (MC190HD, Leica Microsystems, Germany). For each cut, the thickness of 4 types of leaf tissue and the total thickness were measured using the ImageJ software (http://imagej.nih.gov/ij/). Specific leaf area was estimated by dividing the leaf surface by the dry matter.

Metabarcoding of bacterial and fungal communities

DNA extraction and amplification were performed under a hood in a confined laboratory. Total genomic DNA of leaf and environmental samples was extracted using the Qiagen DNeasy plant kit (Qiagen) accordingly to the manufacturer's protocol, except that samples were incubated with AP1

solution during one hour at 65°C and that DNA extracts were eluted twice, the first time with 50µL of Buffer APE and the second time with the first elution solution.

The V5-V6 region of the bacterial 16S rDNA gene was amplified using 16S primers 799F-1115R (Redford et al., 2010; Chelius et al., 2001) to exclude chloroplast DNA. To avoid a two-stage PCR protocol and reduce sequencing biases, each primer contained the Illumina adaptor sequence, a tag and a heterogeneity spacer, as described in Laforest-Lapointe (2017) (799F: 5'-CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTxxxxxxxxxxxxHS-AACMGGATTAGATACCCKG-3'; 1115R: 5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTxxxxxxxxxxxxHS-AGGGTTGCGCTCGTTG-3'). For 16S primers, HS represented a 0–7-base-pair heterogeneity spacer and for all primers "x" a 12 nucleotides tag. The PCR mixture (20µL of final volume) consisted of 2µL of each of the forward and reverse primers (2µM), 2µL of dNTPs (2mM), 4µL of 5X HotStart Phusion HF Mix, 0.6µL of DMSO, 0.2 µL of Phusion Hot Start II polymerase (ThermoScientific), 1µL of template and water up to 20 µl. PCR cycling reactions were conducted on a Veriti 96-well Thermal Cycler (Applied Biosystems) using the following conditions: initial denaturation at 98 °C for 30 s followed by 35 cycles at 98°C for 15 s, 60°C for 30s, 72°C for 30s with final extension of 72 °C for 10 min.

The fungal ITS1 region of the nuclear ribosomal internal transcribed spacer, considered the universal barcode marker for fungi (Schoch *et al.*, 2012), was amplified using the ITS1F (5'-CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTxxxxxxxxxxxx-3') and ITS2 (5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTxxxxxxxxxxxx-3') primers. The PCR mixture (20 µL of final volume) consisted of 1µL of each of the forward and reverse primers (2µM), 4µL of 5X HotFirePol Mix (Solis BioDyne), 3µL of template and water up to 20 µl. PCR cycling reactions were conducted on a Veriti 96-well Thermal Cycler (Applied Biosystems) using the following conditions: initial denaturation at 95 °C for 15 min followed by 35 cycles at 95°C for 15 s, 55.8°C for 30 s, 72°C for 45 s with final extension of 72 °C for 10 min.

Each PCR plate had 2 positive controls and 5 to 6 negative controls. Bacterial positive controls were represented by the DNA of two marine bacterial strains (*Sulfitobacter pontiacus* and *Vibrio splendidus*); the first positive control included 1 µL of 10 ng. µL-1 DNA of *Vibrio splendidus* only and the second included 1µL of an equimolar mixture of both strains. Fungal positive controls were represented by the DNA of one fungal strain growing on hyper saline environments (*Debaryomyces*

hansenii) and second strain growing on hyper sweet or salty environments (*Wallemia sebi*); the first positive control included 3 µL of 10 ng. µL-1 DNA of *Debaryomyces hanseni* only and the second included 3µL of an equimolar mixture of both strains. Negative controls were represented by one to two controls containing PCR mix without any DNA template and four to five controls containing DNA extraction buffer solution in each PCR plate. Four additional controls were performed each time samples had been opened under non-steel conditions. Potential contamination during leaf weighing and grouding was analysed by opening three empty tubes and rinsing them with 1mL of sterile water. The putative contaminants present in the PBS-Tween solution used to separate the epiphyte and endophyte fraction of the leaves and in the sterile water added to the petri dishes during the decomposition of the leaves were analyzed in 1mL of each solution. Each type of negative control was represented by 1ml of solution of 3 replicates pooled together.

Amplifications were confirmed by electrophoresis on a 2% agarose gel. PCR products were purified, quantified (Quant-it dsDNA assay kit; Invitrogen) and equimolarly pooled (Hamilton Microlab STAR robot). Average size fragment was checked using Tapestation instrument (Agilent Technologies). Libraries were sequenced on four runs of the Miseq Instrument (Illumina) with the reagent kit v2 (500-cycles). Sequence demultiplexing (with exact index search) was performed at the PGTB sequencing facility (Genome Transcriptome Facility of Bordeaux, Pierroton, France) using DoubleTagDemultiplexer.

Amplification and sequencing of microbial functional genes

The bacterial nitrogenase reductase *nifH* gene was amplified on DNA extracts from the whole-leaf samples collected for the analysis of within-canopy variations in microbial communities (Figure 1B), using IGK3 (5'-GCIWTHTAYGGIAARGGIATHGGIAA-3') and DVV (5'-ATIGCRAAICCICCRCAIACIACRTC-3') (Ando *et al.*, 2005). In silico tests showed that this primer pair provides a very good coverage of diazotrophic bacteria (Gaby et al. 2012). It has already been used for amplifying endophytic diazotrophic bacteria (Ando et al., 2005) and outperforms the *nifH* b1-forB primer pair used recently for characterizing nitrogen-fixing endophytic bacteria in poplar (Doty et al. 2016). Both the forward and reverse primers were tagged according to the Fasteris MetaFast protocol (https://www.fasteris.com/dna/?q=content/metafast-protocol-amplicon-metagenomic-analysis). The PCR mixture (20 µL of final volume) consisted of 1µL each of the forward and reverse primers (30 µM), 1µL of BSA (10 mg.mL-1), 4 µL of Blend Mmix 5X (HOT FIREPOL Blend Master Mix with MgCl2, Solis Biodyne), 2 µL of DNA template and water up to 20 µl.

PCR cycling reactions were conducted on a S1000 Thermal Cycler (BioRad) using the following conditions: initial denaturation at 95 °C for 15 min followed by 40 cycles at 95°C for 30 s, 55°C for 30 s, 72°C for 1 min with final extension of 72 °C for 10 min.

The fungal glucoside hydrolase GH11 gene was amplified on the same DNA samples (Figure 1B) and on the DNA extracts of the decomposition experiment samples (Figure 1E), using FungGH11F (5'-GGVAAGGGITGGAAYCCNGG-3') and FungGH11R (5'-TGKCGRACIGACCARTAYTG-3') (Barbi et al., 2014). The fungal GH11 encodes endoxylanases. Xylan constitutes the major component of hemicelluloses, which are the first compounds decomposed by fungal saprotrophs (Snadjr et al. 2011); its proportion is generally high in broad-leaved trees (Barbi et al. 2014). Both the forward and reverse primer were tagged according to the Fasteris MetaFast protocol. The PCR mixture (25 µL of final volume) consisted of 6µL each of the forward and reverse primers (10 µM), 2µL of BSA (10 mg.mL-1), 5 µL of Blend Mmix 5X (HOT FIREPOL Blend Master Mix), 5 µL of DNA template at 300 ng.µL-1 and water up to 25 µl. PCR cycling reactions were conducted on a S1000 Thermal Cycler (BioRad) using the following conditions: initial denaturation at 95 °C for 15 min followed by 45 cycles at 95°C for 30 s, 50°C for 30 s, 72°C for 30 s. Each PCR plate had 3 positive controls and 4 to 7 negative controls represented by PCR mix without any DNA template. The bacterial *nifH* positive controls included the 2 µL of *Bradyrhizobium* sp. at 33 ng.µl^{-1} and the fungal GH11 positive controls included the 5 µl of *Trichoderma harzianum* at 38 ng.µl-1.

Amplifications were checked by electrophoresis on a 1% agarose gel. The *nifH* amplicons were not sequenced due to the low amplification rate. PCR products of the GH11 gene were purified (Sephadex G-50 Superfine, Sigma), quantified (Quant-it Picogreen dsDNA assay kit; Invitrogen) with a plate spectrophotometer reader (Victor 3, Perkin Elmer) and manually equimolarly pooled (Hamilton Microlab STAR robot). Sequencing adapters were added by ligation by Fasteris SA (Switzerland). Libraries were sequenced on one run of the Miseq Instrument (Illumina) with the reagent kit v3 (2 x 300 bp).

Bioinformatic analysis

Primers of bacterial 16S and fungal ITS1 reads were removed using cutadapt (Martin, 2011) and sequences were then analyzed using DADA2 v1.12.1 (Callahan *et al.*, 2016). Only sequences with less than two expected errors and longer than 50 bp were retained. Quality reads were then assembled into Amplicon sequence variants (ASVs) and chimeric sequences were removed using

the consensus method of the *removeBimeras* function. Taxonomic assignments were performed using the RDP classifier (Wang *et al.*, 2007) implemented in DADA2 and trained with the UNITE fungal database v7.2 (UNITE Community 2017) and SILVA bacterial database v132 (Quast *et al.*, 2013), with an 80% confidence threshold. The ASV tables were then imported in R using the phyloseq package v1.26.0 (McMurdie & Holmes, 2013) and filtered. Only ASVs assigned to a bacterial and fungal phylum were kept. ASVs assigned to chloroplast were also removed from the bacterial amplicons dataset. Positive and negative controls were used to remove contaminants (as described in Galan *et al.*, 2016). The cross-contamination threshold (T_{CC}) was defined as the maximal number of sequences of each ASV found in negative or positive control samples. The false-assignment threshold (T_{FA}) was defined as the highest sequence count of a positive control strain in a non-control sample, divided by the total number of sequences of the strain in the whole run and multiplied by the total number of sequences of each ASV. ASVs were removed from all samples where they harbored fewer sequences than either threshold (T_{FA} or T_{CC}). Functional assignation of all bacterial taxa was obtained using the "functional annotation of prokaryotic taxa" (FAPROTAX) program on the ASV table (Louca *et al.*, 2016). The putative trophic mode of each fungal ASV was determined using the FUNGuild database (Nguyen *et al.*, 2016).

Bioinformatic analysis of GH11 amplicons is ongoing. Sequence demultiplexping (with exact index search) and clustering were performed by Fasteris SA (Switzerland) using Perl script (v. 2.10) with no mismatch allowed in the barcode sequences and the number of degenerated bases plus 2 mismatches allowed in the primer sequences. The obtained reads were filtered and trimmed with TRIMMOMATIC (v.0.32) for Q (quality) 15 along a sliding window of 4 bp. Read pairs for which both forward and reverse reads did not pass the quality criterion were removed. Paired-end reads were assembled using ea-utils tool (v. 1.1.2). Only paired-end reads with a minimum overlap of 6 bp and up to 8% of mismatches were kept. Sequences will be clustered into Operational Functional Units (OFU) using a 95% cutoff value and singletons will be removed before diversity analyses (Barbi et al. 2014; 2016).

Statistical analysis

H1: Bacterial and fungal communities are spatially structured within the canopy, in terms of both taxonomic composition and putative function

To begin with, bacterial and fungal species and functions were ranked according to their relative abundance in the whole-leaf dataset (Figure 1B) and the ecology of the most abundant species was described. Beech leaf specialists were identified by performing a differential abundance analysis between beech leaf samples and the other environmental samples, using the ALDEx2 package (Fernandes et al., 2013).

Then, to test H1, the effects of within-canopy vertical (C_V) and horizontal (C_H) positions (Figure 1B) on PMC composition were assessed using permutational multivariate analyses of variance (PERMANOVAs), performed with 999 permutations with the *adonis* function of the R vegan package (Oksanen *et al.*, 2008). The models had C_V, C_H, sampling date (D), tree (T) and all their interactions as fixed effects. Variance percentages explained by each factor were represented on Venn diagrams with the function *euler* from the eulerr package (Larsson, 2018). To account for the compositional nature of metabarcoding data (Gloor *et al.*, 2017), dissimilarities among samples were estimated using the Aitchinson distance (Euclidean distance on centered-log ratio (clr) sequence counts; Aitchison, 1986) and visualized with Principal Component Analyses (PCA). Clr-transformation was performed after transforming zero counts with the "CZM" function of the zComposition R package (Palarea-albaladejo & Martín-fernández, 2015) and, if necessary, after removing ASVs present in less than 3% of the samples.

Then, the spatial variations in the relative abundance of putative bacterial nitrogen fixers (according to FAPROTAX) and fungal saprotrophs (according to FUNGUILD) were analyzed by modelling clr-transformed sequence counts with linear regressions performed with the *lmer* function of the *lme4* package (v.1.1.21) (Bates et al., 2015), followed by Tukey's post-hoc pairwise comparisons performed with the *multcomp* package (v.1.4.10) (Hothorn, Bretz, & Westfall, 2008). The models had within-canopy position (C), D, T and their interactions as fixed effects.

H2: Nitrogen-fixing and carbohydrate-decomposing potentials of microbial communities vary within the canopy

To test H2, within-canopy variation in OFU alpha-diversity will be analyzed using a generalized linear model having C_V, C_H, D, T and their interactions as fixed effects, and the sampling depth (defined as the total number of raw sequences per sample) as an offset (Bálint et al., 2015; McMurdie and Holmes 2014). Within-canopy variation in OFU composition (e.g. Barbi et al. 2016) will be analyzed using PERMANOVAs, as described above. These analyses will only be performed for GH11 OFUs as *nifH* amplicons were not sequenced.

H3: Variation in community composition is accounted for by variations in leaf morphology, physiology and chemistry

To test H3, relationships between leaf traits and PMC composition were deciphered using Redundancy Analyses (RDA; Legendre & Legendre, 1998) performed on clr-transformed sequence counts. A forward selection of leaf traits was performed on the 15 selected leaf traits (Table 1) using the function *ordiR2step* from the vegan package (Blanchet et al., 2008). Variance partitioning was performed with the *varpart* function of the vegan package to determine variation percentages explained by leaf chemistry, physiology and morphology, respectively. The coefficient of determination of each set of traits was adjusted to account for the number of predictor variables (Peres-Neto et al., 2006).

H4: Within-canopy variation in community composition increase along the vegetative season

To test H4, the effect of sampling date on within-canopy community heterogeneity was assessed using the *betadisper* and *permutest* functions of the vegan package (Oksanen et al. 2018), followed by Tukey's post-hoc pairwise comparisons. For each sampling date, community heterogeneity was defined as the sample dispersion within each tree, calculated as the distances between samples and the centroid corresponding to the tree on PCAs.

H5: Within-canopy variation in community composition is larger than within-leaf variation

To test H5, the effects of within-canopy position (C), within-leaf position (L_{BV}: blade edge, blade central part, main vein) and their interaction were tested using PERMANOVA, as previously described. Permutations were constrained by date (D) and tree (T).

H6: Within-canopy variation is larger for epiphytes than endophytes

To test H6, the effects of within-canopy position (C), within-leaf position (L_{ST}: surface *vs* internal tissue) and their interaction were tested using PERMANOVA, as previously described. Then, the effect of within-canopy position on community composition was assessed separately for epiphytes and endophytes.

H7: Within-canopy variation remain significant after leaf abscission and during early decomposition

To test H7, the effects of within-canopy position (C), incubation time (IT) and their interaction on community composition were tested using PERMANOVA, with permutations were constrained by tree (T). The effect of within-canopy position (C) was also tested for each incubation time separately as previously mentioned. Then, the effect of incubation time on community heterogeneity was

assessed using *betadisper* and *permutest* functions, as previously described. All statistical analyses were performed with the R software (v.3.6.1).

Results

Bioinformatic analysis generated 9,657,993 16S bacterial pairs of reads and 13,485,654 ITS1 fungal pairs of reads for all datasets, among which 12,970 bacterial and 11,222 fungal ASVs were detected after quality filtering and error correction. Overall 45.6% of bacterial and 36.6% of fungal ASVs were assigned at the species level. Beech leaves were dominated by Proteobacteria (49.3%), Actinobacteria (20.4%), Ascomycota (65.3%) and Basidiomycota (21.2%). Out of the 7,989 bacterial ASVs detected in beech leaves, 2,060 (25.7%) were successfully assigned to at least one functional group using FAPROTAX and out of the 5,944 fungal sequences, 2746 (45.5%) were successfully assigned to at least one guild using FUNGuild. Beech leaves were dominated by bacterial chemoheterotrophs (22.9 %) and putative fungal pathogens (48.4%) (Table 2). The ten most abundant bacterial genus of the beech phyllosphere were previously found in various habitats such as sea and drinking water, rhizosphere, soil, dust and leaves, and only three ASVs assigned to these bacterial genera were specialist of beech leaves. In contrast, the ten most abundant fungal taxa were isolated from the phyllosphere, litter and soil of forest ecosystems and eight of them were significantly more abundant in beech leaves than in the surrounding environment (Table 3).

Table 2. Putative functions of bacteria and fungi colonizing beech leaves. Bacterial metabolic pathways were assigned using FAPROTAX (Louca et al., 2016) and fungal guilds were assigned using FUNGuild (Nguyen et al., 2016). Proportions represent the percentage of bacterial and fungal sequences assigned to each pathway and guild, respectively.

Bacterial metabolic pathway	Proportion
aerobic chemoheterotrophy	22.922%
chemoheterotrophy	23.281%
phototrophy	0.031%
aromatic compound degradation	1.208%
aromatic hydrocarbon degradation	0.002%
cellulolysis	0.109%
xylanolysis	0.001%
chitinolysis	0.001%
fermentation	0.344%
dark hydrogen oxidation	0.020%
hydrocarbon degradation	0.030%
iron respiration	0.001%
manganese oxidation	0.202%
methanol oxidation	0.020%
methanotrophy	0.028%
methylotrophy	0.048%
cyanobacteria	0.031%
nitrogen fixation	0.018%
denitrification	0.020%
nitrate denitrification	0.020%
nitrate reduction	0.193%
nitrate respiration	0.025%
nitrite denitrification	0.020%
nitrite respiration	0.020%
nitrogen respiration	0.025%
nitrous oxide denitrification	0.020%
respiration of sulfur compounds	0.002%
sulfate respiration	0.001%
sulfur respiration	0.000%

Fungal guild	Proportion
Dung Saprotroph	0.012%
Litter Saprotroph	0.131%
Plant Saprotroph	1.017%
Soil Saprotroph	0.029%
Wood Saprotroph	1.421%
Leaf Saprotroph	0.000%
Saprotroph	13.477%
Plant Pathogen	48.413%
Plant Parasite	0.000%
Lichen Parasite	0.093%
Fungal Parasite	1.635%
Ectomycorrhizal	0.005%
Endophyte	0.367%
Epiphyte	0.127%
Lichenized	0.875%

Table 3. Most abundant microbial ASV colonizing beech leaves. Only Amplicon sequence variants (ASV) assigned to fungi and bacteria phyla with the UNITE and SILVA databases were kept. Average relative abundances of all ASVs were computed after merging ASVs assigned to the same bacterial genus and fungal species. Only ASVs assigned at the bacterial genus and fungal species level are shown in the table. Beech leaf specialists (S) were identified by comparing beef leaf samples to other environmental samples with differential abundance analysis (Aldex2; Fernandes et al., 2013).

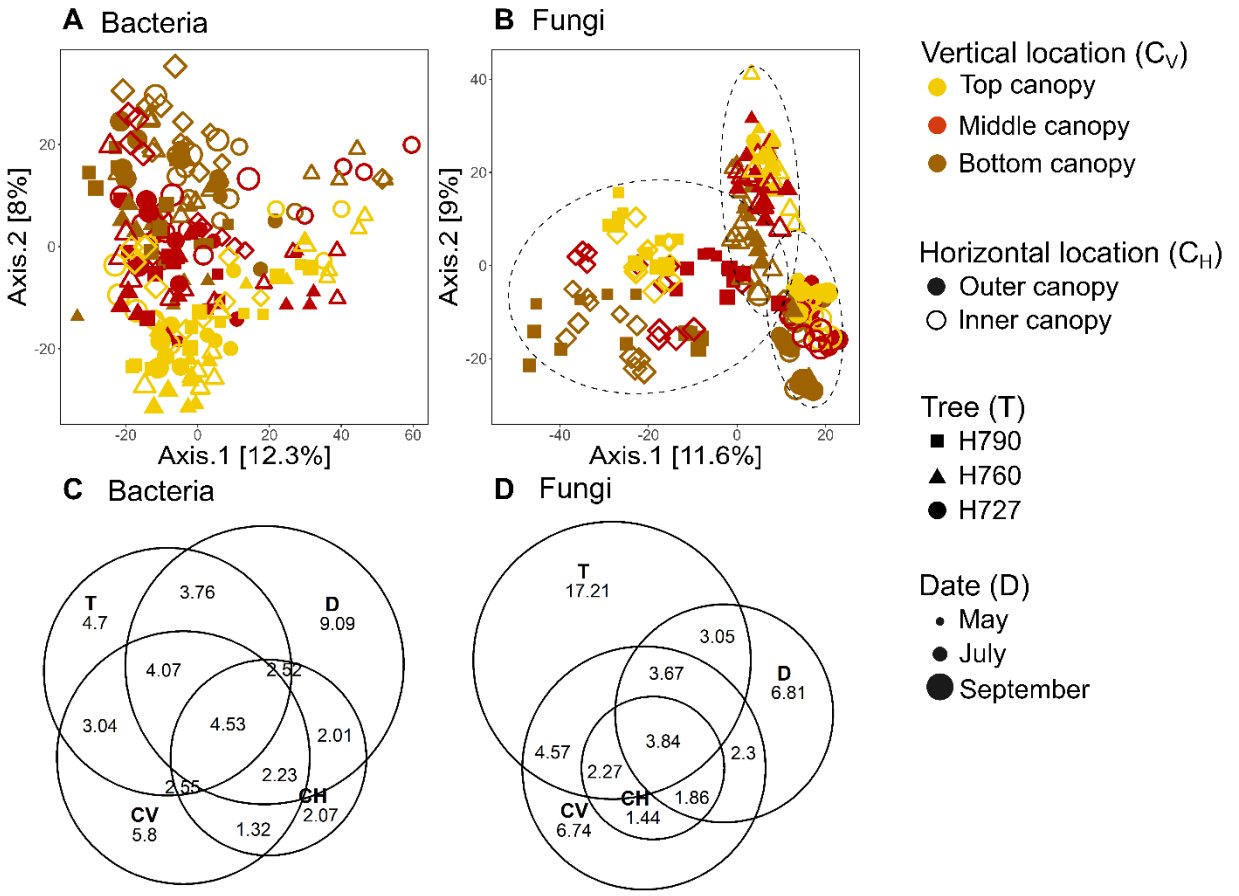
Taxa name	Relative abundance	Lifestyle	References
Hymenobacter sp. (S)	20.3	Common inhabitants of the phyllosphere. Some members are radiation tolerant and psychrophilic or psychrotolerant.	Ottesen (2009); Leveau & Tech (2011); Leff *et al.*, 2015; Klassen & Foght (2011); Lee (2014); Su *et* (2014); Aydogan (2018)
Sphingomonas sp. (S)	10.4	Leaf and seed symbiont protecting against *Pseudomonas syringaei.*	Innerebner (2011); Vogel (2016)
Massilia sp.	4.8	Commonly retrieved in phyllospheric communities of different plants, including lettuce and apple and common spermosphere colonists.	(Ofek *et al.*, 2011; Bassas-Galia *et al.*, 2012; Liu *et al.*, 2012; Lopez-Velasco *et al.*, 2013; Rastogi *et al.*, 2013)
Methylobacterium sp. (S)	4.8	Metabolize methanol emitted by plants and promote seed germination, root development and the yield of agricultural plants by producing substances like auxins, cytokinins and vitamin B12.	Ivanova (2000, 2001, 2006); Trotsenko (2001); Lee (2006); Ryu (2006)
Amnibacterium sp.	3.5	Detected in stem, leaf and bark samples.	Beckers (2017); Arrigoni (2018); Franzetti (2020)
Kineococcus sp.	2.0	Microbial rhodopsin isolated on *Tamarix nilotica* leaves.	Atamna-Ismaeel (2012a)
Acidiphilium sp.	1.8	Thrives in cloud water.	Amato (2017)
Frondihabitans sp.	1.6	Detected in fruit, leaves and litter. Also isolated in extreme ecosystems.	Lee (2014); Pascazio (2015); Laforest-Lapointe (2016b); Arrigoni (2018); Zeng (2019)
Jatrophihabitans sp.	1.2	Endophyte inhabiting plant leaves.	Verma & Gange (2014); Laforest-Lapointe (2017); Cernava (2019)
Nocardioides sp.	1.1	Prominent in alkaline environments and associated to plant tissues (phyllosphere and rhizosphere). Some members can produce antimicrobial compounds.	Gesheva & Vasileva-Tonkova (2012); Reisberg (2013); Shivlata & Satyanarayana (2015); Beckers (2017); Aydogan (2018); Cernava (2019)
Taphrina inositophila (S)	19.3	Frequently isolated from plant surfaces, potentially saprobic lifestyle	Moore (1990); Inácio (2004)
Aureobasidium pullulans (S)	4.4	Ubiquitous, widespread oligotrophe found in water, phyllosphere, bathrooms, food and feeds	Zalar (2008)
Genolevuria amylolytica (S)	1.4	Isolated from the phyllosphere of temperate forest trees	Buzzini (2017)
Vishniacozyma victoriae	1.1	Yeast isolated from the galls in the phyllosphere temperate forest trees	Glushakova (2017)
Taphrina carpini (S)	1.1	Common pathogen encountered on Fagaceae leaves.	Bacigálová (1991); Inácio et al. (2004); Cordier et al. (2012)
Erythrobasidium hasegawianum (S)	1	Isolated from the phyllosphere of tropical forest trees	Nakase (2000)
Mycosphaerella tassiana (S)	1	Common pathogen found in the phyllosphere including that of oak.	Schubert et al. (2007); Jakuschkin et al. (2016)
Filobasidium wieringae (S)	0.9	Yeast found in the phyllosphere. The genus Filobasidium have hyphal structures with haustoria only in the dikaryotic phase suggesting mycoparasites capacities	Glushakova & Kachalkin (2017); Bills, Muller & Foster (2004)
Buckleyzyma aurantiaca	0.8	Yeast found in the soil and litter of temperate forest, with antibiosis capacity against foliar pathogens	Mašínová (2019)
Mycosphaerella punctiformis (S)	0.7	Isolated from living and fallen leaves in temperate forest	Verkley (2004); Pauvert et al (this thesis) Voriskova (2013); Scholtysik (2012)

In accordance with H1, the taxonomic composition of bacterial and fungal communities varied significantly within the canopy, both vertically and horizontally. Community composition also differed significantly among dates and trees (Table 4 and Figure 2). Variance partitioning indicated that the vertical stratification was stronger than the horizontal variation, for both bacteria (Fig. 2C) and fungi (Fig. 2D). Moreover, it demonstrated that the community drivers were not the same for bacteria and fungi. The tree was the strongest driver of fungal community composition (Fig. 2B and Fig. 2D), while it was not the case for bacteria (Fig. 2A and Fig. 2C).

Table 4. Effect of within-canopy position on the composition of phyllosphere microbial communities of beech, tested using permutational multivariate analyses of variance (PERMANOVA). Compositional dissimilarities among leaves were estimated using the Aitchinson distance.

	Bacteria				**Fungi**			
	d.f.	**F-value**	**R2**	**P-value**	**d.f.**	**F-value**	**R2**	**P-value**
Vertical position (C_V)	2	9.146	0.058	0.001	2	12.472	0.067	0.001
Horizontal position (C_H)	1	6.525	0.021	0.001	1	5.067	0.014	0.001
Date (D)	2	14.322	0.091	0.001	2	12.594	0.068	0.001
Tree (T)	2	7.414	0.047	0.001	2	31.846	0.172	0.001
C_V x C_H	2	2.092	0.013	0.002	2	2.661	0.014	0.001
C_V x D	4	2.487	0.032	0.001	4	2.128	0.023	0.001
C_H x D	2	3.172	0.020	0.001	2	2.179	0.012	0.001
C_V x T	4	2.401	0.030	0.001	4	4.231	0.046	0.001
C_H x T	2	2.305	0.015	0.001	2	2.402	0.013	0.001
D x T	4	2.969	0.038	0.001	4	2.822	0.031	0.001
C_V x C_H x D	4	1.760	0.022	0.001	4	1.724	0.019	0.001
C_V x C_H x T	4	2.009	0.026	0.001	4	2.096	0.023	0.001
C_V x D x T	8	1.607	0.041	0.001	8	1.697	0.037	0.001
C_H x D x T	4	1.991	0.025	0.001	4	1.717	0.019	0.001
C_H x C_H x D x T	8	1.787	0.045	0.001	8	1.778	0.038	0.001

Figure 2. Variation in PMC composition of beech phyllosphere, depending on leaf position within the canopy, tree and date. Dissimilarities among samples were estimated using Aitchinson distance and represented with a PCA plot (A and B). Colors indicate within-canopy vertical positions and shapes indicate the tree sampled and within-canopy horizontal positions and size indicate the date of sampling. Confidence ellipses (95%) are drawn around tree (T) for fungal communities. All factors had significant effects on microbial composition (Table 1). Variance partitions were represented on Venn diagrams (C and D).

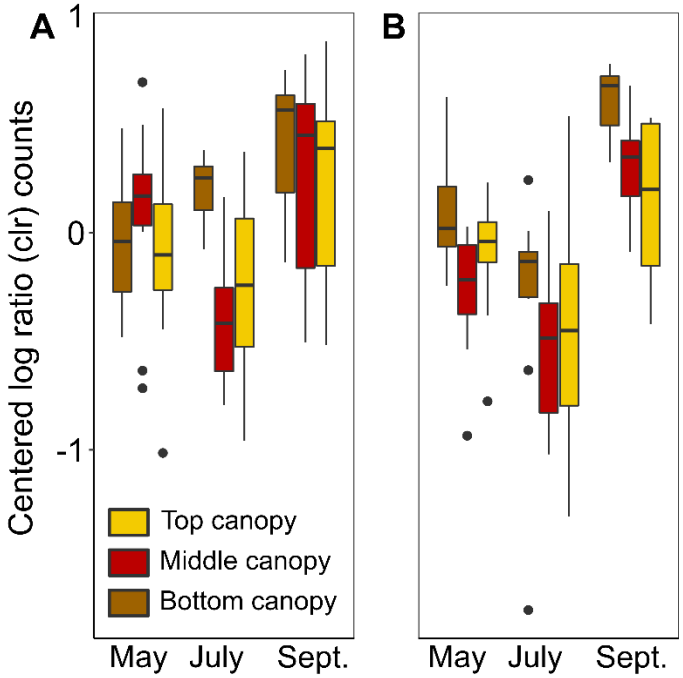


The relative abundance of fungal saprotrophs varied significantly within the canopy (Table 5). Their relative abundance was higher at the end of the growing season, especially in the inner, bottom canopy (Figure 2). According to FAPROTAX assignments, nitrogen fixers represented only 0.018% of the 16S sequences and were present in only 63 samples (out of 216). The amplification of the *nifH* gene supported these findings, as only 4 samples were successfully amplified.

Table 5. Effect of within-canopy position on the relative abundance of fungal saprotrophs in the beech phyllosphere, tested using linear models (LM) of clr-transformed sequence counts.

	d.f.	F-value	P-value
Vertical position (C_V)	2	19.8319	< 0.001***
Horizontal position (C_H)	1	6.8385	< 0.01**
Date (D)	2	92.8117	< 0.001***
Tree (T)	2	51.4887	< 0.001***
C_V x C_H	2	0.8236	0.44
C_V x D	4	2.7445	< 0.05*
C_H x D	2	6.5219	< 0.01**
C_V x T	4	5.2324	< 0.001***
C_H x T	2	3.4600	< 0.05*
D x T	4	5.3234	< 0.001***
C_V x C_H x D	4	5.0301	< 0.001***
C_V x C_H x T	4	1.6219	0.17
C_V x D x T	8	1.1825	0.31
C_H x D x T	4	7.6225	< 0.001***
C_V x C_H x D x T	8	1.9034	0.06

Figure 3. Relative abundance of fungal saprotrophs in the beech phyllosphere, depending on leaf position within the canopy and date. Fungal saprotrophs were identified using FUNGuild (Nguyen *et al.*, 2016). Their relative abundance (estimated using CLR-transformed sequence counts) varied significantly among positions in the canopy (A: Outer-top canopy location, B: Inner-bottom canopy location), dates and trees (Table 5).



RDA models indicated that all leaf traits were significantly related to variations in PMC taxonomic composition (H3) (Figure 4; Table S1). Leaf traits explained 18.8% and 25.5% of bacterial and fungal community composition, respectively (Table 6). Leaf chlorophyll a concentration best explained variations in fungal community composition, while the specific leaf area (SLA) was the factor that best explained the variation in bacterial communities (Figure 4). However, leaf chemical traits, taken together, better explained the variations in both fungal and bacterial composition than physiological and morphological traits (Table 6).

Figure 4. Effect of leaf traits on variation in PMC composition of beech phyllosphere, assessed using Redundancy Analysis (RDAs). Values represent the percentage of variance (unadjusted) explained by each leaf trait. Dark large circles indicate a stronger relation between leaf traits and PMC composition. Colors represent the category of trait (Green: Leaf morphology. Blue: Leaf physiology. Orange: Leaf chemistry). All traits significantly influenced bacterial and fungal community composition (Table S1).

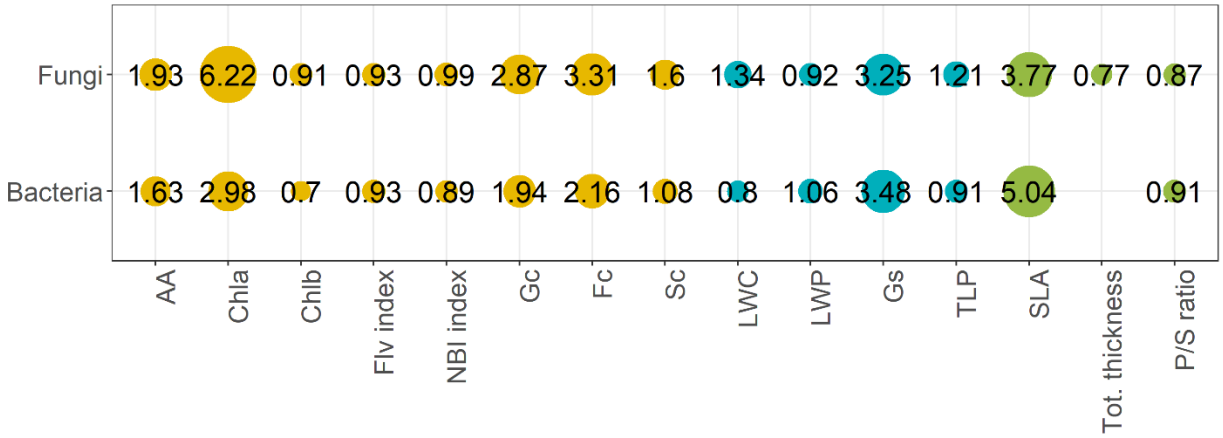


Table 6. Effect of leaf traits on variation in PMC composition of beech phyllosphere, assessed using Redundancy Analysis (RDA) and variance partitioning. Values represent the percentage of variance (adjusted) explained by each leaf trait category.

	Bacteria		**Fungi**	
	d.f.	**R^2 adjusted**	**d.f.**	**R^2 adjusted**
Leaf morphology	2	1.90	3	1.87
Leaf physiology	4	2.26	4	2.96
Leaf chemistry	8	7.89	8	12.04
All leaf traits	15	18.80	15	25.25

In contrast to H4, bacterial community homogenized during the growing season (Figure 5), while within-canopy fungal heterogeneity remained stable. Moreover, in contrast to H5, within-leaf position (blade edge, blade central part or main nerve) did not have any significant effect on community composition (Table 7), indicating within-canopy variations were larger than within-leaf variations. Finally, in contrast to H7, within-canopy variation in community composition was not larger for epiphytes than endophytes. Both communities were vertically stratified (Table 8).

Figure 5. PMC heterogeneity within the canopy of beech throughout the growing season. Community heterogeneity within the canopy was calculated for each tree using *betadisper* function from the vegan package (Oksanen et al. 2007).

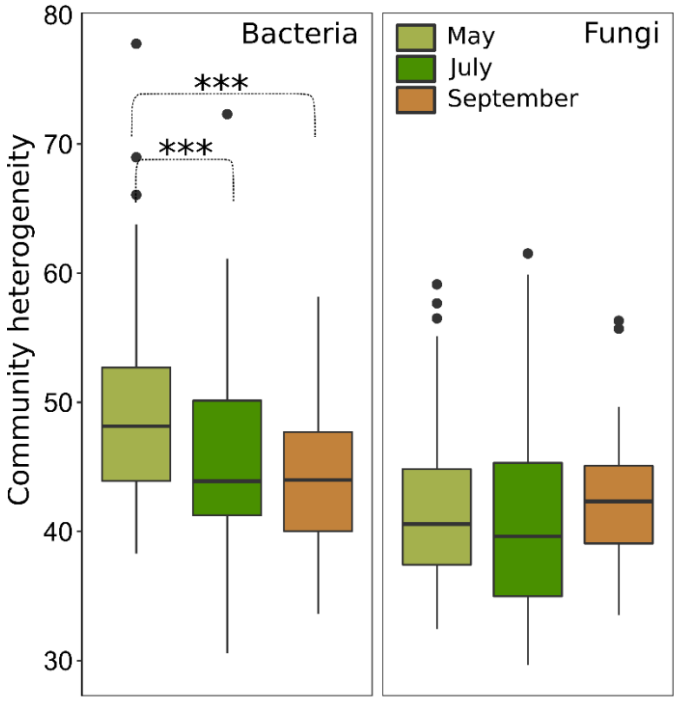


Table 7. Effects of within-canopy and within-leaf positions on the composition of phyllosphere microbial communities of beech, tested using permutational multivariate analyses of variance (PERMANOVA). Compositional dissimilarities among samples were estimated using the Aitchinson distance. Within-leaf position corresponds to blade edge, blade central part or main nerve.

	Bacteria				**Fungi**			
	d.f.	**F-value**	**R^2**	**P-value**	**d.f.**	**F-value**	**R^2**	**P-value**
Within-leaf position (L_{BV})	2	1.378	0.017	0.136	2	1.359	0.017	0.135
Within-canopy position (C)	1	4.498	0.028	0.001**	1	5.864	0.036	0.002**
L_{BV} x C	2	1.183	0.015	0.226	2	0.910	0.011	0.374

Table 8. Permutational multivariate analyses of variance (PERMANOVA) of within-leaf position (L_{ST}: Surface vs. tissue) of bacterial and fungal composition dissimilarities. Dissimilarities among samples were estimated using the Aitchinson distance. The effects of within-canopy cation (C), within-leaf position (L_{ST}) and their interaction were tested on all data while the effects of within-canopy position (C) was tested separately on epiphyte and endophyte communities and permutations were constrained by date of sampling (D) and tree identity (T).

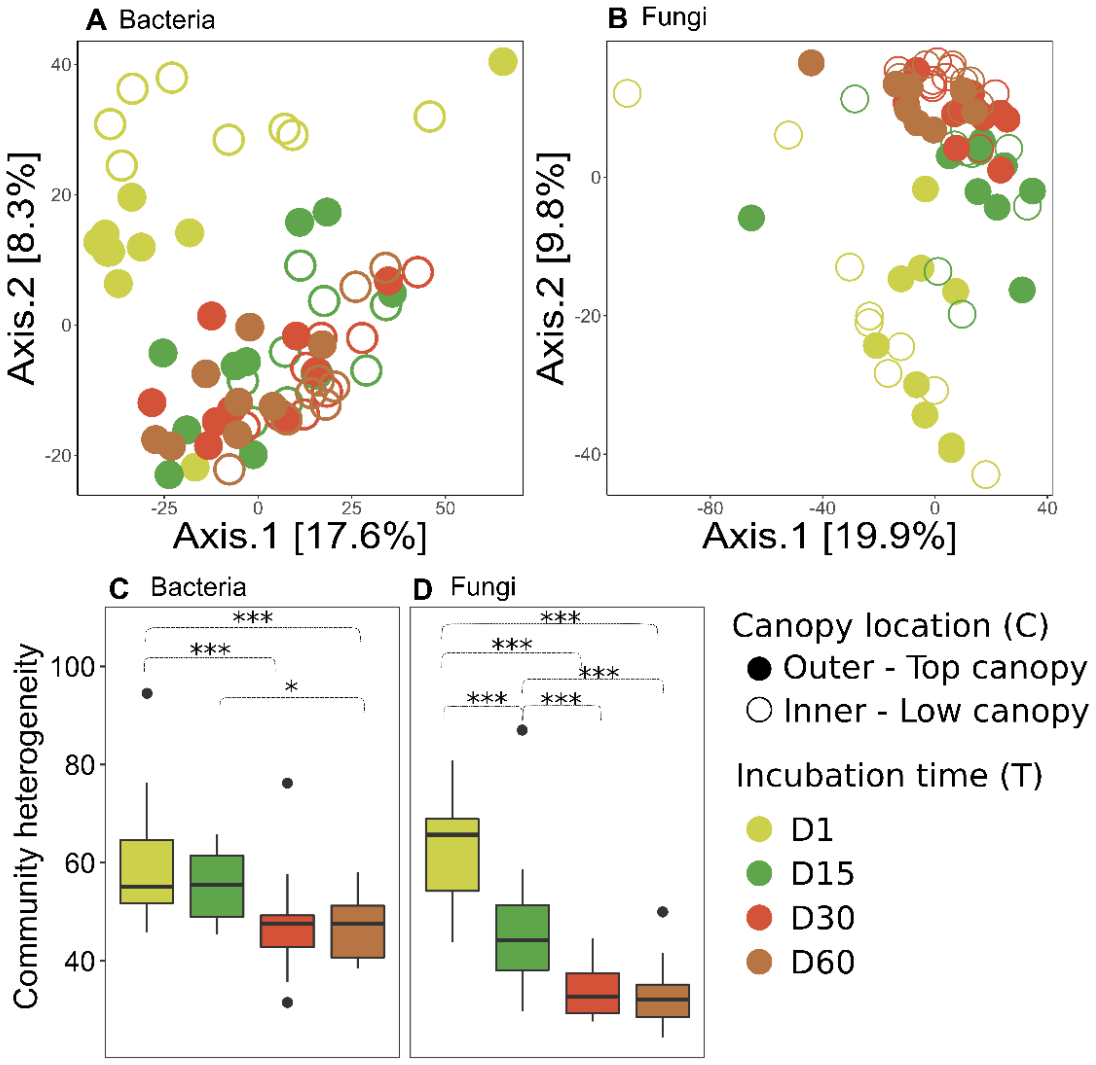
	Bacterial community				Fungal community			
	d.f.	**F-value**	**R^2**	**P-value**	**d.f.**	**F-value**	**R^2**	**P-value**
Within-leaf								
Within-leaf position (L_{ST})	1	5.39	0.11	0.001***	1	7.07	0.14	0.001***
Within-canopy position (C)	5	1.88	0.19	0.023*	5	2.33	0.23	0.003**
L_{ST} x C	5	2.16	0.22	0.016*	5	1.63	0.16	0.024*
Epiphyte								
Within-canopy position (C)	5	1.93	0.47	0.001***	5	1.79	0.43	0.001***
Endophyte								
Within-canopy position (C)	5	2.03	0.46	0.037*	5	1.80	0.43	0.021*

In accordance with H7, within-canopy position significantly influenced PMC composition during decomposition, in interaction with incubation time (Table 9). The incubation time (IT) had a greater effect on community composition than the within-canopy position (C) (Table 9). PCA plots revealed a steep change in microbial composition after 15 days of leaf incubation (Figure 6A and Figure 6B) and the PMC composition significant decreased of over leaf decomposition (Figure 6C and Figure 6D; Permutest bacteria: F-value = 7.84, P-value = 0.001***; Permutest fungi*: F-value* = 33.6, *P-value* = 0.001***). However, compositional variation analyses for each incubation time indicated that the effect of the within-canopy position persisted during the two months of leaf decomposition for bacterial and fungal communities (Table 9).

Table 9. Effect of within-canopy initial position on microbial communities of beech leaves during decomposition, tested using permutational analyses of variance (PERMANOVAs). Dissimilarities among leaves were estimated using the Aitchinson distance. The incubation time (IT), within-canopy position (C) and their interaction were tested on all data while the effects of within-canopy position (C) was tested separately on each incubation and permutations were constrained by tree identity (T).

	Bacteria				**Fungi**			
	d.f.	**F-value**	**R^2**	**P-value**	**d.f.**	**F-value**	**R^2**	**P-value**
Leaf decomposition								
Incubation time (IT)	3	2.892	0.110	0.001***	3	3.518	0.131	0.001***
Within-canopy position (C)	1	4.260	0.054	0.001***	1	3.310	0.041	0.001***
IT x C	3	1.320	0.050	0.033*	3	1.499	0.056	0.01**
One day of incubation								
Within-canopy position (C)	1	2.440	0.132	0.007**	1	2.747	0.147	0.003**
15 days of incubation								
Within-canopy position (C)	1	1.849	0.104	0.014*	1	2.778	0.148	0.001**
30 days of incubation								
Within-canopy position (C)	1	1.741	0.098	0.002**	1	4.427	0.217	0.008**
60 days of incubation								
Within-canopy position (C)	1	1.454	0.094	0.015*	1	4.232	0.232	0.015*

Figure 6. Variations in community composition (A and B) and heterogeneity (C and D) during the decomposition of beech leaves, depending on leaf within-canopy initial position. Dissimilarities among leaves were estimated using Aitchinson distance and represented with a PCA plot. Colors indicate incubation time. Shapes indicate the leaf initial position. PMC composition differed significantly among incubation time and within-canopy position (Table 9). PMC heterogeneity significantly decreased throughout incubation time.



Discussion

In this study, we thoroughly analyzed phyllosphere microbial communities in a beech (*Fagus Sylvatica*) forest located in a climate refugium at the species range margin in South West of France (Ouayjan & Hampe, 2018). This refugium, which is crossed by the Ciron river, has a special microclimate marked by abundant morning mists (Barbeta *et al.*, 2019). Bacterial chemoheterotrophs dominated the phyllosphere (23.2%). They correspond to bacteria that consume organic molecules, such as leaf leachates (Van Der Wal & Leveau, 2011), as a source of carbon and energy (*Parker, in* Encyclopedia of Genetics*, 2001*). Among these chemoheterotrophs, several functional groups have already been described in the phyllosphere of various plant species (Delmotte et al., 2009; Knief et al., 2012; Fürnkranz et al. 2008), including bacteria involved in the degradation of aromatic compounds (Farré-Armengol *et al.*, 2016), fermentation (Miura *et al.*, 2019), nitrate reduction (Valverde *et al.*, 2008), nitrogen fixation (Fürnkranz *et al.*, 2008; Rico *et al.*, 2014; Doty *et al.*, 2016; Moyes *et al.*, 2016a), hydrocarbon degradation (Darlington *et al.*, 2001; De Kempeneer *et al.*, 2004; Waight *et al.*, 2007) such as methanotrophs (Iguchi *et al.*, 2012) and methylotrophs (Delmotte *et al.*, 2009; Wellner *et al.*, 2011; Knief *et al.*, 2012) (Table 2). Phototrophs were also found (Atamna-Ismaeel *et al.*, 2012b,a), but represented a very small fraction of our dataset (0.031%). Functional assignments with FAPROTAX (Louca *et al.*, 2016) and *nifH* gene low amplification success both indicated that the beech phyllosphere was poor in nitrogen-fixing bacteria, contrasting with the results found for other tree species (Fürnkranz *et al.*, 2008; Reed *et al.*, 2011; Doty *et al.*, 2016). Phyllosphere fungal communities were dominated by putative plant pathogens (48.4%) and mycoparasites (1.6%). They were also rich in saprotrophs (13.47%), as found in other tree species (Jumpponen & Jones, 2009; Peršoh, 2013a).

Our analyses revealed a vertical and horizontal stratification of microbial communities within the canopy of beech trees. These results are consistent with several studies exploring the vertical variation in PMC composition within the canopy of other forest trees (Harrison *et al.*, 2005; Leff *et al.*, 2015; Izuno *et al.*, 2016; Laforest-Lapointe *et al.*, 2016a; Stone & Jackson, 2019) and indicate that the leaf position within the canopy of forest trees should be taken into account in sampling designs. Moreover, our study also showed that, unlike bacterial communities, the composition of fungal communities varied more between trees than within their canopy. The variance in fungal community composition explained by the host identity (about 20%) confirmed the results of a previous study on beech phyllosphere conducted in the same geographical region (Cordier *et al.*,

2012b). The redundancy analysis indicated that a significant part of the variation in fungal community composition was explained by the host phenotype. Leaf traits accounted for 25% of the variation in fungal community composition. The variance explained was lower for bacteria (18%), and bacterial and fungal communities were not influenced by the same leaf traits. Bacterial composition was mostly influenced by SLA while fungal composition was driven by chlorophyll a concentration. Interestingly, stomatal conductance also appeared to be an important factor in the structure of microbial communities. Stomata opening can regulate the plant-pathogen interactions. The plant can close the stomatal aperture in response to pathogen detection. In return, some bacterial strains such as *Pseudomonas syringae* (Melotto *et al.*, 2008) have the ability to reopen stomata to enter into the leaf tissues. Our results suggest that these interactions seem to be decisive in the structure of bacterial and fungal communities in forest trees. Overall, the leaf chemistry better explained the variation of PMC composition than leaf anatomy or physiology. These results are in line with studies conducted on other plant species (Kembel & Mueller, 2014; Kembel *et al.*, 2014).

Our results showed that within-canopy variation in phyllosphere communities was much larger that within-leaf variation. Community composition did not differ among leaf parts (main vein, blade center, blade edge) in our study. Nevertheless, the analyses of PMC composition revealed great differences in community composition between epiphytes and endophytes, for both fungi and bacteria. These results suggest that the factors shaping PMC at the leaf scale do not operate across leaf parts but between leaf surface and leaf internal tissue. They confirm that depending on whether they colonize the surface or the interior of leaf tissue, microorganisms do not undergo the same environmental pressures (Vorholt 2012). However, the endophytic and epiphytic communities were both vertically stratified, indicating that they both respond to within-canopy gradients in microclimate and leaf traits. These results indicate that when analyzing microbial communities associated to the phyllosphere of forest trees, sampling only leaf pieces may be sufficient to assess microbial composition of entire leaves, but that it is important to separate epiphytes and endophytes.

Overall, our study revealed that beech phyllosphere includes microorganisms able to influence tree performance and forest functioning. Bacteria involved in methanol and aromatic compounds degradation may affect tree-tree communication and atmospheric chemistry, while fungal decomposers involved in litter degradation influence nutrient cycle. As methanol and aromatic

compounds can be emitted by the plants in the form of volatile organic compounds (VOC) and play a key role in plant defense and communication and to a greater extent in chemical, physical and climatic properties of the atmosphere (Fehsenfeld *et al.*, 1992; Kesselmeier & Staudt, 1999; Penuelas & Llusia, 2001), bacteria which modulate these emissions can have various effects on plant-plant, plant-insect and plant-atmosphere exchanges (Farré-Armengol *et al.*, 2016; Bringel & Couée, 2015). The analysis of fungal saprotrophs within the canopy confirms that some phyllosphere fungal species are dormant saprotrophs (Osono 2006, Unterseher et al. 2013, Vorıskova & Baldrian 2013) and emphasizes that their relative abundance is higher at the bottom of the canopy which may be driven by a greater number of senescent leaves at these within-canopy positions (Hikosaka et al. 2013). Our analyses indicated that the effect of within-canopy position persists during the two months of leaf decomposition suggesting that the initial composition of PMCs may influence microbial succession during litter decomposition.

Conclusion

Our study confirmed that phyllosphere bacterial and fungal communities are not shaped by the same processes (Vacher *et al.*, 2016a). Fungal communities were more strongly influenced by tree identity and leaf traits, while bacterial communities were more influenced by the date of sampling. At the canopy scale, we found a significant vertical and horizontal stratification of both fungal and bacterial communities associated with beech leaves. This spatial heterogeneity decreased over the vegetative season for bacterial communities. At the leaf scale, bacterial and fungal community composition was found to be homogeneous over the leaf but differed significantly between leaf surface and leaf internal tissues. Bacterial nitrogen fixers were absent or very scarcely abundant within the leaf of beech trees. Fungal saprotrophs were more abundant in the inner bottom canopy at the end of the vegetative season and the differences in community composition persisted over the leaf decomposition process. In order to have a more accurate picture, future research should use a combination of multiple strategies such as protein or metabolite production analysis that would provide better insight into the functions of microbial communities within forest trees (Delmotte *et al.*, 2009; Rastogi *et al.*, 2013). A combination of leaf microclimate measures and their effects on microbial community functions may be further used to parameterize models and predict the consequences of climate-driven changes in PMCs on nutrient cycling under climate change scenarios.

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Authors contributions

TF organized the sampling campaigns, processed all samples, performed the extractions and amplifications of barcode regions, measured most leaf traits, performed the bioinformatic and statistical analyses, and wrote the first draft of the manuscript. TF, CP, RB, LW and CV participated to the sampling campaigns. CP provided scripts for bioinformatic and statistical analyses. EL performed the amplification of functional genes. EC performed the sequencing and demultiplexing of metabarcoding data. ALM performed the microscopic measurements of leaf thickness. RB developped protocols for leaf physiological traits measurements. PB and YG developped protocols for leaf chemistry measurements. AEZ, AH and HS provided scientific advice throughout the project as part of the TF's PhD committee. LW and CV had the original idea for the project. CV supervised all

stages of the work and made a major contribution to the statistical analyses and writing of the manuscript. All authors contributed to the final version.

References

Aitchison J. **1986**. The statistical analysis of compositional data. *Journal of the Royal Statistical Society. Series A (General)* **150**: 396.

Amato P, Joly M, Besaury L, Oudart A, Taib N, Deguillaume L, Delort A, Debroas D, Mone I. **2017**. Active microorganisms thrive among extremely diverse communities in cloud water. *PLoS ONE* **3**: 1–22.

Arrigoni E, Antonielli L, Pindo M, Pertot I, Perazzolli M. **2018**. Tissue age and plant genotype affect the microbiota of apple and pear bark. *Microbiological Research* **211**: 57–68.

Atamna-Ismaeel N, Finkel OM, Glaser F, Sharon I, Schneider R, Post AF, Spudich JL, von Mering C, Vorholt JA, Iluz D, *et al.* 2012b. Microbial rhodopsins on leaf surfaces of terrestrial plants. *Environmental Microbiology* **14**: 140–146.

Aydogan EL, Moser G, Müller C, Kämpfer P, Glaeser SP. **2018**. Long-term warming shifts the composition of bacterial communities in the phyllosphere of Galium album in a permanent grassland field-experiment. *Frontiers in Microbiology* **9**: 1–17.

Ando S, Goto M, Hayashi H, Yoneyama T, Meunchang S, Thongra-ar P, Fujiwara T. **2005**. Detection of *nifH* sequences in sugarcane (*Saccharum officinarum* L.) and pineapple (*Ananas comosus* [L.] Merr.). *Soil Science and Plant Nutrition* **51**: 303–308.

Anhuf D, Rollenbeck R. **2001**. Canopy structure of the Rio surumoni rain forest (Venezuela) and its influence on microclimate. *Ecotropica* **7**: 21–32.

Arnold AE, Mejia LC, Kyllo D, Rojas EI, Maynard Z, Robbins N, Herre EA. **2003**. Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences* **100**: 15649–15654.

Arnon DI. **1949**. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant Physiology* **24**: 1–15.

Atamna-Ismaeel N, Finkel O, Glaser F, von Mering C, Vorholt JA, Koblížek M, Belkin S, Béjà O. **2012a**. Bacterial anoxygenic photosynthesis on plant leaf surfaces. *Environmental Microbiology Reports* **4**: 209–216.

Bacigálová K. **1991**. New localities of *Taphrina carpini* (Rostr.) Johans, on *Carpinus betulus* in Slovakia. *Czech Mycology* **46**: 296–302.

Bálint M, Bartha L, O'Hara RB, Olson MS, Otte J, Pfenninger M, Robertson AL, Tiffin P, Schmitt I. **2015**. Reposition, high-latitude warming and host genetic identity shape the foliar fungal microbiome of poplars. *Molecular Ecology* **24**: 235–248.

Bálint M, Bartha L, O'Hara RB, Olson MS, Otte J, Pfenninger M, Robertson AL, Tiffin P, Schmitt I. **2015**. Relocation, high-latitude warming and host genetic identity shape the foliar fungal microbiome of poplars. *Molecular Ecology* **24**: 235–248.

Barbeta A, Jones SP, Clavé L, Wingate L, Gimeno TE, Fréjaville B, Wohl S, Ogee J. **2019**. Unexplained hydrogen isotope offsets complicate the identification and quantification of tree water sources in a riparian forest. *Hydrology and Earth System Sciences* **23**: 2129–2146.

Bassas-Galia M, Nogales B, Arias S, Rohde M, Timmis KN, Molinari G. **2012**. Plant original Massilia isolates producing polyhydroxybutyrate, including one exhibiting high yields from glycerol. *Journal of Applied Microbiology* **112**: 443–454.

Beckers B, De Beeck MO, Weyens N, Boerjan W, Vangronsveld J. **2017**. Structural variability and niche differentiation in the rhizosphere and endosphere bacterial microbiome of field-grown poplar trees. *Microbiome* **5**: 1–17.

Bringel F, Couée I. **2015**. Pivotal roles of phyllosphere microorganisms at the interface between plant functioning and atmospheric trace gas dynamics. *Frontiers in Microbiology* **6**: 1–14.

Bantan-Polak T, Kassai M, Grant KB. **2001**. A comparison of fluorescamine and naphthalene-2, 3-dicarboxaldehyde fluorogenic reagents for microplate-based detection of amino acids. *Analytical Biochemistry* **297**: 128–136.

Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P. **2013**. Structure and functions of the bacterial microbiota of plants. *Annual Review of Plant Biology* **64**: 807–838.

Buzzini P, Lachance MA, Yurkov A. **2017**. Yeasts in natural ecosystems: Diversity. *Cham, Switzerland: Springer*.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. **2016**. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**: 581–583.

Cernava T, Chen X, Krug L, Li H, Yang M, Berg G. **2019**. The tea leaf microbiome shows specific responses to chemical pesticides and biocontrol applications. *Science of the Total Environment*

667: 33–40.

Chelle M. **2005**. Phylloclimate or the climate perceived by individual plant organs: What is it? How to model it? What for? *New Phytologist* **166**: 781–790.

Chen TK, Yang HT, Fang SC, Lien YC, Yang TT, Ko SS. **2016**. Hybrid-cut: An improved sectioning method for recalcitrant plant tissue samples. *Journal of Visualized Experiments* **2016**: 1–11.

Coble AP, Vanderwall B, Mau A, Cavaleri MA. **2016**. How vertical patterns in leaf traits shift seasonally and the implications for modeling canopy photosynthesis in a temperate deciduous forest. *Tree Physiology* **36**: 1077–1091.

Coince A, Cordier T, Lengellé J, Defossez E, Vacher C, Robin C, Buée M, Marçais B. **2014**. Leaf and root-associated fungal assemblages do not follow similar elevational diversity patterns. *PLoS ONE* **9**.

Compant S, Samad A, Faist H, Sessitsch A. **2019**. A review on the plant microbiome : Ecology, functions, and emerging trends in microbial application. *Journal of Advanced Research* **9:** 29-37

Coleman-Derr D, Desgarennes D, Fonseca-Garcia C, Gross S, Clingenpeel S, Woyke T, North G, Visel A, Partida-Martinez LP, Tringe SG. **2016**. Plant compartment and biogeography affect microbiome composition in cultivated and native Agave species. *New Phytologist* **209**: 798–811.

Cordier T, Robin C, Capdevielle X, Desprez-Loustau ML, Vacher C. **2012a**. Spatial variability of phyllosphere fungal assemblages: Genetic distance predominates over geographic distance in a European beech stand (*Fagus sylvatica*). *Fungal Ecology* **5**: 509–520.

Cordier T, Robin C, Capdevielle X, Fabreguettes O, Desprez-Loustau ML, Vacher C. **2012b**. The composition of phyllosphere fungal assemblages of European beech (fagus sylvatica) varies significantly along an elevation gradient. *New Phytologist* **196**: 510–519.

Cusack DF, Silver W, McDowell WH. **2009**. Biological nitrogen fixation in two tropical forests: Ecosystem-level patterns and effects of nitrogen fertilization. *Ecosystems* **12**: 1299–1315.

Darlington AB, Dat JF, Dixon MA. **2001**. The biofiltration of indoor air: Air flux and temperature influences the removal of toluene, ethylbenzene, and xylene. *Environmental Science and Technology* **35**: 240–246.

Delmotte N, Knief C, Chaffron S, Innerebner G, Roschitzki B, Schlapbach R, Von Mering C, Vorholt JA. **2009**. Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. *Proceedings of the National Academy of Sciences of the United States of*

America **106**: 16428–16433.

De Kempeneer L, Sercu B, Vanbrabant W, Van Langenhove H, Verstraete W. **2004**. Bioaugmentation of the phyllosphere for the removal of toluene from indoor air. *Applied Microbiology and Biotechnology* **64**: 284–288.

Druebert C, Lang C, Valtanen K, Polle A. **2009**. Beech carbon productivity as driver of ectomycorrhizal abundance and diversity. *Plant, Cell and Environment* **32**: 992–1003.

Doty SL, Sher AW, Fleck ND, Khorasani M, Bumgarner RE, Khan Z, Ko AWK, Kim SH, DeLuca TH. **2016**. Variable nitrogen fixation in wild Populus. *PLoS ONE* **11**: 1–22.

Farré-Armengol G, Filella I, Llusia J, Peñuelas J. **2016**. Bidirectional interaction between phyllospheric microbiotas and plant volatile emissions. *Trends in Plant Science* **21**: 854–860.

Fehsenfeld F, Calvert J, Fall R, Goldan P, Guenther AB, Hewitt CN, Lamb B, Trainer M, Westberg H. **1992**. Emissions of volatile organic compounds from vegetation and the implications ofr atmospheric chemistry. *Global biogeochemical cylces* **6**: 389–430.

Franzetti A, Gandolfi I, Bestetti G, Padoa Schioppa E, Canedoli C, Brambilla D, Cappelletti D, Sebastiani B, Federici E, Papacchini M, *et al.* 2020. Plant-microorganisms interaction promotes removal of air pollutants in Milan (Italy) urban area. *Journal of Hazardous Materials* **384**.

Friesen ML, Porter SS, Stark SC, von Wettberg EJ, Sachs JL, Martinez-Romero E. **2011**. Microbially Mediated Plant Functional Traits. *Annual Review of Ecology, Evolution, and Systematics* **42**: 23–46.

Fürnkranz M, Wanek W, Richter A, Abell G, Rasche F, Sessitsch A. **2008**. Nitrogen fixation by phyllosphere bacteria associated with higher plants and their colonizing epiphytes of a tropical lowland rainforest of Costa Rica. *ISME Journal* **2**: 561–570.

Fürnkranz M, Müller H, Berg G. **2009**. Characterization of plant growth promoting bacteria from crops in Bolivia. *Journal of Plant Diseases and Protection* **116**: 149–155.

Galan M, Razzauti M, Bard E, Bernard M, Brouat C, Charbonnel N, Dehne-Garcia A, Loiseau A, Tatard C, Tamisier L, *et al.* 2016. 16S rRNA amplicon sequencing for epidemiological surveys of bacteria in wildlife. *mSystems* **1**: 1-16.

Gesheva V, Vasileva-Tonkova E. **2012**. Production of enzymes and antimicrobial compounds by halophilic Antarctic Nocardioides sp. grown on different carbon sources. *World Journal of*

Microbiology and Biotechnology **28**: 2069–2076.

Gillespie AR, Kahle AB, Walker RE. **1987**. Color enhancement of highly correlated images. II. Channel ratio and 'chromaticity' transformation techniques. *Remote Sensing of Environment* **22**: 343–365.

Gloor GB, Macklaim JM, Pawlowsky-Glahn V, Egozcue JJ. **2017**. Microbiome datasets are compositional: And this is not optional. *Frontiers in Microbiology* **8**: 1–6.

Glushakova AM, Kachalkin A V. 2017. Endophytic yeasts in leaf galls. *Microbiology (Russian Federation)* **86**: 250–256.

Hacquard S. **2016**. Disentangling the factors shaping microbiota composition across the plant holobiont. *New Phytologist* **209**: 454–457.

Hardoim PR, van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M, Sessitsch A. **2015**. The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and Molecular Biology Reviews* **79**: 293–320.

Harrison RM, Jones AM, Biggins PDE, Pomeroy N, Cox CS, Kidd SP, Hobman JL, Brown NL, Beswick A. **2005**. Climate factors influencing bacterial count in background air samples. : 167–178.

Hendry TA, Hunter MS, Baltrus DA. **2014**. The facultative symbiont Rickettsia protects an invasive whitefly against entomopathogenic *Pseudomonas syringae* strains. *Applied and Environmental Microbiology* **80**: 7161–7168.

Hunter PJ, Hand P, Pink D, Whipps JM, Bending GD, Al HET, 2010. Both leaf properties and microbe-microbe interactions influence within-species variation in bacterial population diversity and structure in the lettuce (*Lactuca Species*). *Applied and environmental microbiology* **76**: 8117–8125.

Iguchi H, Sato I, Sakakibara M, Yurimoto H, Sakai Y. **2012**. Distribution of methanotrophs in the phyllosphere. *Bioscience, Biotechnology and Biochemistry* **76**: 1580–1583.

Inácio J, Rodrigues MG, Sobral P, Fonseca Á. **2004**. Characterisation and classification of phylloplane yeasts from Portugal related to the genus *Taphrina* and description of five novel Lalaria species. *FEMS Yeast Research* **4**: 541–555.

Innerebner G, Knief C, Vorholt JA. **2011**. Protection of *Arabidopsis thaliana* against leaf-pathogenic *Pseudomonas syringae* by *Sphingomonas* strains in a controlled model system. *Applied*

and Environmental Microbiology **77**: 3202–3210.

Ivanova EG, Doronina N V., Shepelyakovskaya AO, Laman AG, Brovko FA, Trotsenko YA. **2000**. Facultative and obligate aerobic methylobacteria synthesize cytokinins. *Microbiology* **69**: 646–651.

Ivanova EG, Doronina N V., Trotsenko YA. **2001**. Aerobic methylobacteria are capable of synthesizing auxins. *Microbiology* **70**: 392–397.

Ivanova EG, Fedorov DN, Doronina N V., Trotsenko YA. **2006**. Production of vitamin B12 in aerobic methylotrophic bacteria. *Microbiology* **75**: 494–496.

Izuno A, Kanzaki M, Artchawakom T, Wachrinrat C, Isagi Y, Arnold A, Rodriguez R, Jr JW, Arnold A, Redman R, *et al.* 2016. Vertical structure of phyllosphere fungal communities in a tropical forest in Thailand uncovered by high-throughput sequencing. *PLoS ONE* **11**: 1-23.

Jakuschkin B, Fievet V, Schwaller L, Fort T, Robin C, Vacher C. **2016**. Deciphering the pathobiome: intra- and interkingdom interactions involving the pathogen *Erysiphe alphitoides*. *Microbial Ecology* **72**: 870–880.

Jumpponen A, Jones KL. **2010**. Seasonally dynamic fungal communities in the *Quercus macrocarpa* phyllosphere differ between urban and nonurban environments. *New Phytologist* **186**: 496–513.

Kämpfer P, Rainey FA, Andersson MA, Nurmiaho Lassila E-L, Ulrych U, Busse H., Weiss N, Mikkola R, Salkinoja-Salonen M. **2019**. *Frigoribacterium faeni* gen . nov ., sp . nov ., a novel psychrophilic genus of the family Microbacteriaceae. **50**: 355–363.

Kembel SW, Mueller RC. **2014**. Plant traits and taxonomy drive host associations in tropical phyllosphere fungal communities. *Botany* **92**: 303–311.

Kembel SW, O'Connor TK, Arnold HK, Hubbell SP, Wright SJ, Green JL. **2014**. Relationships between phyllosphere bacterial communities and plant functional traits in a neotropical forest. *Proceedings of the National Academy of Sciences* **111**: 13715–13720.

Kim KK, Lee KC, Lee JS. **2012**. Nakamurella panacisegetis sp. nov. and proposal for reclassification of Humicoccus flavidus Yoon et al., 2007 and Saxeibacter lacteus Lee et al., 2008 as Nakamurella flavida comb. nov. and Nakamurella lactea comb. nov. *Systematic and Applied Microbiology* **35**: 291–296.

Kesselmeier J, Staudt M. **1999**. Biogenic volatile organic compounds (VOC): an overview on emission, physiology and ecology. *Journal of Atmospheric Chemistry* **33**: 23–88.

Klassen JL, Foght JM. **2011**. Characterization of Hymenobacter isolates from Victoria Upper Glacier, Antarctica reveals five new species and substantial non-vertical evolution within this genus. *Extremophiles* **15**: 45–57.

Knief C, Chaffron S, Stark M, Innerebner G. **2012**. Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. *The ISME Journal* **6:** 1378–1390.

Laforest-Lapointe I, Messier C, Kembel SW. **2016a**. Tree phyllosphere bacterial communities: exploring the magnitude of intra- and inter-individual variation among host species. *PeerJ* **4**, e2367.

Laforest-Lapointe I, Messier C, Kembel SW. **2016b**. Host species identity, site and time drive temperate tree phyllosphere bacterial community structure. *Microbiome* **4**: 1–10.

Laforest-Lapointe I, Paquette A, Messier C, Kembel SW. **2017**. Leaf bacterial diversity mediates plant diversity and ecosystem function relationships. *Nature* **546**: 145–147.

Larsson J. **2018**. eulerr: Area-Proportional Euler Diagrams with Ellipses.

Lee YM, Kim EH, Lee HK, Hong SG. **2014**. Biodiversity and physiological characteristics of Antarctic and Arctic lichens-associated bacteria. *World Journal of Microbiology and Biotechnology* **30**: 2711–2721.

Lee SD, Lee DW, Ko Y. **2011**. Marmoricola korecus sp. nov. *International Journal of Systematic and Evolutionary Microbiology* **61**: 1628–1631.

Lee HS, Madhaiyan M, Kim CW, Choi SJ, Chung KY, Sa TM. **2006**. Physiological enhancement of early growth of rice seedlings (Oryza sativa L.) by production of phytohormone of N2-fixing methylotrophic isolates. *Biology and Fertility of Soils* **42**: 402–408.

Leff JW, Del Tredici P, Friedman WE, Fierer N. **2015**. Spatial structuring of bacterial communities within individual Ginkgo biloba trees. *Environmental Microbiology* **17**: 2352–2361.

Li Y, Wu X, Chen T, Wang W, Liu G, Zhang W, Li S, Wang M, Zhao C, Zhou H, *et al.* 2018. Plant phenotypic traits eventually shape its microbiota: A common garden test. *Frontiers in Microbiology* **9**: 1–13.

Liu Y, Zuo S, Zou Y, Wang J, Song W. **2012**. Investigation on diversity and population succession dynamics of indigenous bacteria of the maize spermosphere. *World Journal of Microbiology and Biotechnology* **28**: 391–396.

Lopez-Velasco G, Carder PA, Welbaum GE, Ponder MA. **2013**. Diversity of the spinach (Spinacia

oleracea) spermosphere and phyllosphere bacterial communities. *FEMS Microbiology Letters* **346**: 146–154.

Louca S, Jacques SMS, Pires APF, Leal JS, Srivastava DS, Parfrey LW, Farjalla VF, Doebeli M. **2016**. High taxonomic variability despite stable functional structure across microbial communities. *Nature Ecology & Evolution* **1**: 0015.

Martin M. **2011**. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet journal* **17**: 2803–2809.

Mašínová T, Bahnmann BD, Větrovský T, Tomšovský M, Merunková K, Baldrian P. **2017**. Drivers of yeast community composition in the litter and soil of a temperate forest. *FEMS microbiology ecology* **93**: 1–10.

McMurdie PJ, Holmes S. **2013**. Phyloseq: An R Package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* **8**: e61217.

Meir P, Kruijt B, Broadmeadow M, Barbosa E, Kull O, Carswell F, Nobre A, Jarvis PG. **2002**. Acclimation of photosynthetic capacity to irradiance in tree canopies in relation to leaf nitrogen concentration and leaf mass per unit area. *Plant, Cell and Environment* **25**: 343–357.

Melotto M, Underwood W, He SY. **2008**. Role of stomata in plant innate immunity and foliar bacterial diseases. *Annual Review of Phytopathology* **46**: 101–122.

Miura T, Sánchez R, Castañeda LE, Godoy K, Barbosa O. **2019**. Shared and unique features of bacterial communities in native forest and vineyard phyllosphere. *Ecology and Evolution* **9**: 3295–3305.

Montesinos E, Bonaterra A, Badosa E, Francés J, Alemany J, Llorente I, Moragrega C. **2002**. Plant-microbe interactions and the new biotechnological methods of plant disease control. *International Microbiology* **5**: 169–175.

Moore RT. **1990**. The genus Lalaria gen. nov.: Taphrinales anamorphosum. *Mycotaxon*.

Morris CE, Conen F, Alex Huffman J, Phillips V, Pöschl U, Sands DC. **2014**. Bioprecipitation: A feedback cycle linking Earth history, ecosystem dynamics and land use through biological ice nucleators in the atmosphere. *Global Change Biology* **20**: 341–351.

Moyes AB, Kueppers LM, Pett-Ridge J, Carper DL, Vandehey N, O'Neil J, Frank AC. **2016**. Evidence for foliar endophytic nitrogen fixation in a widely distributed subalpine conifer. *New Phytologist* **210**: 657–668.

Mueller, G.M., Bills, G.F., Foster MSB. **2004**. *Biodiversity of Fungi : Inventory and monitoring methods* Elsevier.

Nakamura A, Kitching RL, Cao M, Creedy TJ, Fayle TM, Freiberg M, Hewitt CN, Itioka T, Koh LP, Ma K, *et al.* 2017. Forests and Their Canopies: Achievements and Horizons in Canopy Science. *Trends in Ecology and Evolution* **32**: 438–451.

Nakase T. **2000**. Expanding world of ballistosporous yeasts: Distribution in the phyllosphere, systematics and phylogeny. *Journal of General and Applied Microbiology* **46**: 189–216.

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG. **2016**. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology* **20**: 241–248.

Niinemets Ü. **2007**. Photosynthesis and resource distribution through plant canopies. *Plant, Cell and Environment* **30**: 1052–1071.

Niinemets Ü. **2016**. Leaf age dependent changes in within-canopy variation in leaf functional traits: a meta-analysis. *Journal of Plant Research* **129**: 313–338.

Ofek M, Hadar Y, Minz D. **2011**. Colonization of cucumber seeds by bacteria during germination. *Environmental Microbiology* **13**: 2794–2807.

Oksanen AJ, Kindt R, Legendre P, Hara BO, Simpson GL, Stevens MHH, Wagner H. **2008**. The vegan Package; Community Ecology Package (Version 1.15-1).

Osono T. **2006**. Role of phyllosphere fungi of forest trees in the development of decomposer fungal communities and decomposition processes of leaf litter. *Canadian Journal of Microbiology* **52**: 701–716.

Ottesen AR, González Peña A, White JR, Pettengill JB, Li C, Allard S, Rideout S, Allard M, Hill T, Evans P, *et al.* 2013. Baseline survey of the anatomical microbial ecology of an important food plant: *Solanum lycopersicum* (tomato). *BMC Microbiology* **13**: 114

Ouayjan A, Hampe A. **2018**. Extensive sib-mating in a refugial population of beech (Fagus sylvatica) growing along a lowland river. *Forest Ecology and Management* **407**: 66–74.

Palarea-albaladejo J, Martín-fernández JA. **2015**. zCompositions — R package for multivariate imputation of left-censored data under a compositional approach. *Chemometrics and Intelligent Laboratory Systems* **143**: 85–96.

Pascazio S, Crecchio C, Ricciuti P, Palese AM, Xiloyannis C, Sofo A. **2015**. Phyllosphere and

carposphere bacterial communities in olive plants subjected to different cultural practices. *International Journal of Plant Biology* **6**: 15–19.

Penuelas J, Llusia J. **2001**. The complexity of factors driving volatile organic compound emissions by plants. *Biologia plantarum* **44**: 481–487.

Pereira SIA, Castro PML. **2014**. Diversity and characterization of culturable bacterial endophytes from Zea mays and their potential as plant growth-promoting agents in metal-degraded soils. **21**: 14110–14123.

Pérez CA, Carmona MR, Armesto JJ. **2010**. Non-symbiotic nitrogen fixation during leaf litter decomposition in an old-growth temperate rain forest of Chiloé Island, southern Chile: Effects of single *versus* mixed species litter. *Austral Ecology* **35**: 148–156.

Peršoh D. **2013**. Factors shaping community structure of endophytic fungi-evidence from the Pinus-Viscum-system. *Fungal Diversity* **60**: 55–69.

Purahong W, Wubet T, Lentendu G, Schloter M, Pecyna MJ, Kapturska D, Hofrichter M, Krüger D, Buscot F. **2016**. Life in leaf litter: novel insights into community dynamics of bacteria and fungi during litter decomposition. *Molecular ecology* **25**: 4059–4074.

Rajsnerová P, Klem K, Holub P, Vec K, Kozác M, Rivas-ubach A, Sardans J, Marek M V, Peñuelas J, Urban O. **2015**. Morphological, biochemical and physiological traits of upper and lower canopy leaves of European beech tend to converge with increasing altitude. **35**: 47–60.

Rastogi G, Coaker GL, Leveau JHJ. **2013**. New insights into the structure and function of phyllosphere microbiota through high-throughput molecular approaches. *FEMS Microbiology Letters* **348**: 1–10.

Rauha J-PP, Remes S, Heinonen M, Hopia A, Kähkönen M, Kujala T, Pihlaja K, Vuorela H, Vuorela P, Kahkonen M. **2000**. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *International Journal of Food Microbiology* **56**: 3–12.

Redford AJ, Bowers RM, Knight R, Linhart Y, Fierer N. **2010**. The ecology of the phyllosphere: Geographic and phylogenetic variability in the distribution of bacteria on tree leaves. *Environmental Microbiology* **12**: 2885–2893.

Reed SC, Cleveland CC, Townsend AR. **2011**. Functional ecology of free-living nitrogen fixation: A contemporary perspective. *Annual Review of Ecology, Evolution, and Systematics* **42**: 489–512.

Reisberg EE, Hildebrandt U, Riederer M, Hentschel U. **2012**. Phyllosphere bacterial communities of trichome-bearing and trichomeless *Arabidopsis thaliana* leaves. **101**: 551–560.

Reisberg EE, Hildebrandt U, Riederer M, Hentschel U. **2013**. Distinct phyllosphere bacterial communities on Arabidopsis wax mutant leaves. *PLoS ONE* **8**.

Rico L, Ogaya R, Terradas J, Peñuelas J. **2014**. Community structures of N2-fixing bacteria associated with the phyllosphere of a Holm oak forest and their response to drought. *Plant Biology* **16**: 586–593.

Robson RL, Postgate JR. **1980**. Oxygen and Hydrogen. *Annual Review of Microbiology* **34**: 183–207.

Rosado BHP, Almeida LC, Alves LF, Lambais MR, Oliveira RS. **2018**. The importance of phyllosphere on plant functional ecology: A phyllo trait manifesto. *New Phytologist*. **4:** 1145-1149.

Sanchez-Azofeifa A, Oki Y, Fernandes GW, Ball RA, Gamon J. **2012**. Relationships between endophyte diversity and leaf optical properties. *Trees - Structure and Function* **26**: 291–299.

Sayer EJ, Tanner EVJ, Lacey AL. **2006**. Effects of litter manipulation on early-stage decomposition and meso-arthropod abundance in a tropical moist forest. *Forest Ecology and Management* **229**: 285–293.

Schlechter RO, Miebach M, Remus-emsermann MNP. **2019**. Driving factors of epiphytic bacterial communities : A review. *Journal of Advanced Research* **19**: 57–65.

Scholtysik A, Unterseher M, Otto P, Wirth C. **2012**. Spatio-temporal dynamics of endophyte diversity in the canopy of European ash (*Fraxinus excelsior*) **12**: 291-304.

Schubert K, Groenewald JZ, Braun U, Dijksterhuis J, Starink M, Hill CF, Zalar P, De Hoog GS, Crous PW. **2007**. Biodiversity in the *Cladosporium herbarum* complex (Davidiellaceae, Capnodiales), with standardisation of methods for *Cladosporium* taxonomy and diagnostics. *Studies in Mycology* **58**: 105–156.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Bolchacova E, Voigt K, Crous PW, *et al.* 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences* **109**: 6241–6246.

Shahzad R, Khan AL, Bilal S, Asaf S, Lee I. **2018**. What is there in seeds? Vertically transmitted endophytic resources for sustainable improvement in plant growth. *Frontiers in Plant Science* **9,** 24.

Shivlata L, Satyanarayana T. **2015**. Thermophilic and alkaliphilic Actinobacteria : biology and

potential applications. **6**: 1–29.

Sonnentag O, Hufkens K, Teshera-Sterne C, Young AM, Friedl M, Braswell BH, Milliman T, O'Keefe J, Richardson AD. **2012**. Digital repeat photography for phenological research in forest ecosystems. *Agricultural and Forest Meteorology* **152**: 159–177.

Su S, Chen M, Teng C, Jiang S, Zhang C, Lin M, Zhang W. **2014**. Hymenobacter kanuolensis sp. nov., a novel radiation-resistant bacterium. *International Journal of Systematic and Evolutionary Microbiology* **64**: 2108–2112.

Trotsenko YA, Ivanova EG, Doronina N V. 2001. Aerobic methylotrophic bacteria as phytosymbionts. *Mikrobiologiya* **70**: 725–736.

Verma VC, Gange AC. **2014**. *Advances in endophytic research*.

Vogel C, Bodenhausen N, Gruissem W, Vorholt JA. **2016**. The Arabidopsis leaf transcriptome reveals distinct but also overlapping responses to colonization by phyllosphere commensals and pathogen infection with impact on plant health. *New phytologist* **212**: 192–207.

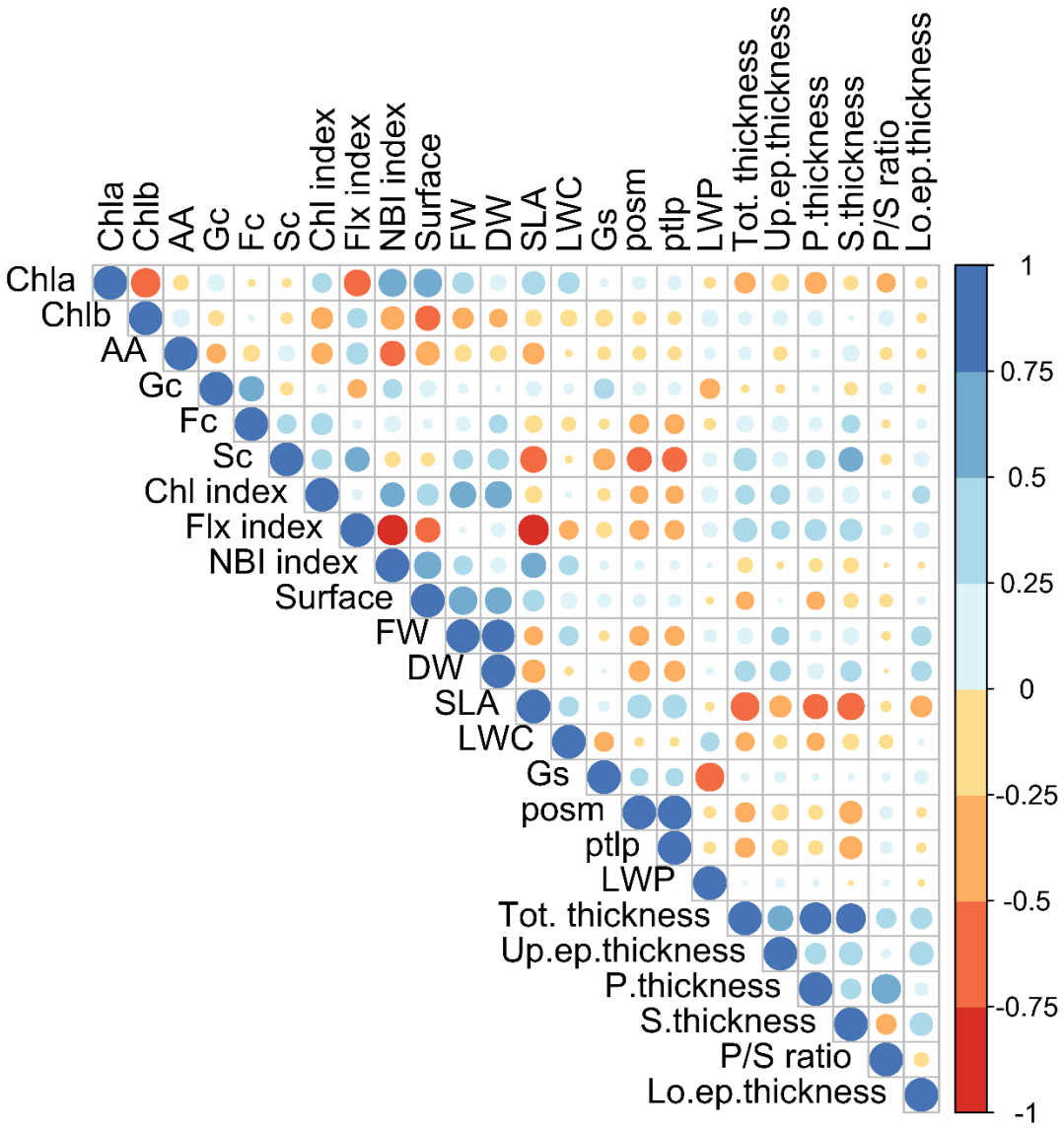
Zeng Q, Liu Y, Zhang H, An S. **2019**. Fast bacterial succession associated with the decomposition of Quercus wutaishanica litter on the Loess Plateau. *Biogeochemistry* **144**: 119–131.

Supplementary

Table S1. Redundancy analysis (RDA) results evaluating the correlation between leaf traits and microbial community structure. Partial RDAs perfomed on normalized microbial taxa counts present in at least 3% of the samples. Taxa counts were normalized as the relative abundance of the total sequence count in each sample adjusted to their centered log-ratio (CLR). Significance was evaluated with permutational ANOVA. Model variance values represent the proportion of variance (unadjusted R2) in microbial community composition associated with leaf traits. All leaf trait values were averaged by tree, position with the canopy and date and chose in a manner that they were poorly correlated with each other ($R^2 < 0.7$; Figure S1). A forward selection of leaf traits was performed on a set of leaf traits (described in table S1) using the function *ordiR2step* from the vegan package (Blanchet et al., 2008).

Bacteria					**Fungi**			
	d.f.	**Unadjusted R^2**	**F-value**	**P-value**	**d.f.**	**Unadjusted R^2**	**F-value**	**P-value**
SLA	1	135.98	12.36	0.001	1	92.83	10.03	0.001
P/S ratio	1	24.57	2.23	0.002	1	21.33	2.3	0.001
Tot. Thickness					1	19.03	2.06	0.004
Gs	1	93.87	8.53	0.001	1	80.15	8.66	0.001
Cha	1	80.39	7.31	0.001	1	153.31	16.56	0.001
Chlb	1	18.9	1.72	0.012	1	22.34	2.41	0.002
Fc	1	58.37	5.31	0.001	1	81.56	8.81	0.001
Gc	1	52.43	4.77	0.001	1	70.72	7.64	0.001
Sc	1	29.15	2.65	0.001	1	39.49	4.27	0.001
AA	1	43.83	3.98	0.001	1	47.67	5.15	0.001
Flv index	1	25	2.27	0.002	1	22.91	2.47	0.001
NBI index	1	23.93	2.17	0.002	1	24.28	2.62	0.001
LWP	1	28.57	2.6	0.001	1	22.77	2.46	0.001
πtlp	1	24.47	2.22	0.003	1	29.89	3.23	0.001
LWC	1	21.61	1.96	0.005	1	33.12	3.58	0.001
Residual	185	2035.46			184	1703.45		

Figure S1. Correlogram of all leaf traits measured within the canopy of the beech trees. All leaf trait values were averaged by tree, position within the canopy and date. Only leaf traits poorly correlated with each other ($R^2 < 0.7$) were selected to perform the variable selection using *ordiR2step* function.



III Maternal effects and environmental filtering shape seed fungal communities in oak trees

Tania Fort[1], Charlie Pauvert[1], Amy E. Zanne[2], Otso Ovaskainen[3,4], Thomas Caignard[1], Matthieu Barret[5], Stéphane Compant[6], Arndt Hampe[1], Sylvain Delzon[7] and Corinne Vacher[1]*

1. BIOGECO, INRA, Univ. Bordeaux, 33615 Pessac, France

2. George Washington University, Biological Sciences Department, 800 22nd St. Washington DC 20052, USA

3. Organismal and Evolutionary Biology Research Programme, P.O. Box 65, 00014 University of Helsinki, Finland.

4. Center for Biodiversity Dynamics, Department of Biology, Norwegian University of Science and Technology, 7491 Trondheim, Norway

5. IRHS, Agrocampus-Ouest, INRA, Université d'Angers, SFR4207 QuaSaV, 49071 Beaucouzé, France.

6. AIT Austrian Institute of Technology GmbH, Center for Health & Bioresources,

Bioresources Unit, Konrad-Lorenz Straße 24, 3430 Tulln, Austria

7. Univ. Bordeaux, INRA, BIOGECO, Pessac France.

Abstract

Trees, as foundation species, play a pivotal role in the species interaction networks that constitute forest ecosystems. From the seed stage, they interact with microbial communities that affect their growth, health and fitness. Despite their eco-evolutionary importance, the processes shaping seed microbial communities in natural forests have received little attention. To unravel these processes, we analyzed the microbial communities of seeds collected in populations of sessile oak (*Quercus petraea*) growing at two distinct elevations. We focused on the fungal communities as this group includes seed pathogens. Ecological processes shaping the communities were quantified using joint species distribution models. Fungi were present in all seed tissues, including the embryo. Fungal communities differed significantly among oak populations along the elevation, and among mother trees within the same population. These maternal effects remained significant after seed fall, despite colonization by fungal species on the ground. Associations between tree pathogens and their antagonists were detected in the seeds. Our results demonstrate that both maternal effects and environmental filtering shape seed microbial communities of sessile oak. They provide a starting point for future research aimed at identifying the seed extended phenotypic traits that influence seed dispersal and germination, and seedling survival and growth across environments.

Keywords

Microbial ecology; disease ecology; community genetics; ecological networks; joint species distribution models; endophyte; host-parasite interaction; vertical transmission

Introduction

Seeds are colonized by a wide range of microorganisms that positively and negatively influence plant growth, health and fitness. They associate with many endophytic and epiphytic microorganisms that foster the growth of seedlings and protect them against natural enemies (Baker & Smith, 1966; Nelson, 2004; Links, *et al.* 2014). For example, the seed-borne endophytic fungus *Epichloë festucae* limits pest attacks and pathogen development by producing alkaloids (Clay & Schardl, 2002; Faeth, 2002). Seeds are also colonized by pathogens that reduce germination rate (Nelson, 2017) and seedling survival (Kremer, 1987; Gilbert, 2002). For example, the fungal pathogen *Ciboria batschiana*, the causal agent of acorn black rot, can damage up to 80% of acorns when conditions are wet (Prochazkova *et al.*, 2005). By affecting the early stages of the plant life cycle, seed pathogens have significant impacts on natural ecosystems, including forests in which they shape tree species diversity and spatial structure (Janzen, 1970; Gilbert, 2002). Understanding how pathogens and other microorganisms are acquired and transmitted to the seeds of foundation tree species (i.e. tree species that structure and stabilize forest communities and ecosystem processes; Whitham *et al.,* 2006), and how these microorganisms associate to regulate seed germination and seedling development, is thus crucial to our ability to predict and manage the regeneration of forest ecosystems.

Up to now, processes of seed microbiota assembly have received more attention in crop plants than in natural ecosystems. In crops, microorganisms are transmitted by the mother plant to its seeds at the floral and during early seed development stages, through vascular tissues or contact between vegetative and reproductive organs (Maude, 1996). They can also be transmitted from the pollen of the father plant, insect vectors, or bioaerosols (Escobar Rodríguez *et al.,* 2018; Frank *et al.*, 2017). Once seeds fall on the ground, epiphytic microbial communities coalesce with microbial communities of litter and upper soil (Rillig *et al.*, 2015). Germinating seeds then release molecules that attract soil microbes, surrounding themselves with a microbiologically active soil area called the spermosphere (Nelson, 2004; Schiltz *et al.*, 2015). The emergence of the plant radicle creates cracks in the seed tegument, enabling microbes to colonize internal tissues (Nelson *et al.*, 2018). These events lead to intense biotic interactions among microorganisms (Nelson, 2004) and drastic changes in seed microbiota composition and function (Ofek *et al.*, 2011; Yang *et al.*, 2017; Torres-Cortés *et al.*, 2018). Recent studies suggest that seed colonization by soil microorganisms represents the most influential microbial acquisition for seedling growth and health (Nelson *et al.*, 2018).

The seed microbiota is therefore a dynamic entity that is shaped, like all ecological communities, by four fundamental processes: selection by the abiotic environment (environmental filtering) and

biotic interactions (biotic filtering), dispersal, ecological drift and evolutionary diversification (Vellend, 2010; Nemergut *et al.*, 2013; Ovaskainen *et al.*, 2017; Zhou & Ning, 2017). Unraveling these assembly processes is particularly important because they govern seed and seedling extended phenotypes (defined as the diversity and composition of associated communities; Whitham *et al.,* 2006), as well as the performance and eventually fitness of the plant host (Compant *et al.*, 2010; Truyens *et al.*, 2015; Brader *et al.*, 2017). Some processes are deterministic and depend on taxon-specific functional traits (Minard *et al.,* 2019), such as the response to selection. Other processes are partly or purely stochastic (Zhou & Ning, 2017; Rezki *et al.*, 2018) and generate divergences among communities occupying identical environments (Chase & Myers, 2011). The dispersal process is particular in the case of seed microbial communities because seeds are mobile. Microorganisms are recruited from a sequence of species pools: first from the microbiota of the mother tree's aboveground organs, and later from surrounding microbial sources such as bioaerosols, litter and soil (Barret *et al.,* 2016). The microorganisms that are directly transferred from the vascular system of mother plant to the seedlings through seeds are termed vertically-transmitted (Truyens *et al.,* 2015), while the others are called horizontally-acquired. Vertically-transmitted microorganisms can trigger maternal effects (defined as the causal influence of the maternal genotype or phenotype on the offspring phenotype; Wolf & Wade, 2009) in seed and seedling extended phenotypes (Vivas *et al.*, 2015).

To gain insight into processes of seed microbiome assembly in natural ecosystems, we analyzed the microbial turnover among acorns of sessile oak (*Quercus petraea*) populations growing at two different elevations. We collected acorns in the canopy and on the ground beneath individual trees, and used Hierarchical Models of Species Communities (HMSC; Ovaskainen *et al.,* 2017) to quantify the ecological processes shaping seed microbial communities at each spatial level (oak population, mother tree and seed) and to generate hypotheses of interactions among microorganisms. We focused on fungal communities as they include pathogens that are detrimental to seed survival (such as *Ciboria batschiana*; Prochazkova *et al.*, 2005). After having described and visualized the fungal communities associated to acorn tissues, we tested the following hypotheses: (**H1**) acorn fungal communities are shaped by environmental filtering and maternal effects, (**H2**) the maternal effects tend to disappear after acorn fall because of horizontal acquisition of fungi from the ground, (**H3**) the response of fungal species to variations in the acorn environment depend on their lifestyle, and (**H4**) biotic interactions among fungal species play a role in the protection of acorns against pathogens.

Materials and methods

Sampling design

Acorns were collected on October 21st and 22nd 2015 in four populations of sessile oak located in the Pyrenees Mountains (France). Two populations (Ade and Bager) were at an elevation of ~400 m a.s.l. and the other two (Gedre-Bas and Gabas) at ~1200 m a.s.l. The sampling date was chosen as close as possible to the fruiting peak at both elevations (see Caignard *et al.,* 2017 for a more detailled description of the sampling sites and fruiting phenology). In each site, acorns were collected from three trees randomly selected among the dominant adults. For each tree, we collected four acorns from the canopy using a slingshot and four acorns from the ground beneath the crown (within a distance of 2 m from the trunk). We also collected the biotic microenvironment of canopy acorns, defined as all tree tissues present in a cylinder of 4 cm diameter and 6 cm length around the acorn (including the acorn cupule, the cupules of other acorns, the twig to which the acorn was attached, the leaf petioles and the base of leaves) and the biotic microenvironment of ground acorns, defined as all substrates beneath the acorn within a cylinder of 4 cm diameter and 1 cm depth (including dead oak leaves, dead leaves of other plant species, acorn caps, twigs, pieces of bark, granules of soil, mosses, lichens or herbs). Each sample was collected aseptically, using new plastic gloves and scissors cleaned with 96% ethanol to minimize contamination. Samples were stored in individual plastic vials in a cooler with ice until they could be stored at -80°C.

Ten additional acorns were harvested from five mother trees (two in Gedre-Bas, two in Gabas and one in Ade). For each tree, one acorn was harvested in the canopy and one acorn was harvested on the ground. These acorns were surface-sterilized and dissected to characterize the endophytic fungal communities associated with acorn internal tissues. Surface-sterilization was completed using a three-step process: immersion for 3 min in a 70% ethanol solution, immersion for 2 min in a 3% calcium hypochlorite solution and rinsing with DNAway and sterilized water. After drying the acorns on sterilized filter papers, the fruit walls and the embryos were collected using a sterilized nutcracker and pliers and stored at -80°C.

DNA extraction, amplification and sequencing of fungal communities

Samples were ground into a homogeneous powder using liquid nitrogen. Mortars and pestles were cleaned using DNAway and autoclaved for 20 min at 121°C between each sample. Approximately 45 mg of powder from each sample was transferred to a microplate under a laminar flow hood. Microplates were stored at -80°C until DNA extraction. Total DNA was extracted using DNeasy Plant

Mini Kits (Qiagen, USA) according to the manufacturer's protocol except that DNA extracts were eluted twice with 50µL of elution buffer (10mM Tris-Cl, 0.5mM EDTA; pH 9.0).

The ITS1 region of the nuclear ribosomal internal transcribed spacer, considered the universal barcode marker for fungi (Schoch *et al.*, 2012), was amplified using the ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′; Gardes & Bruns, 1993) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′; White, Bruns, Lee, & Taylor, 1990) primers. The reaction mixture (12.5 µL of final volume) consisted of 1.25 µL of template DNA, 2.5µL of 1µM of each of the forward and reverse primers and 6.25µL of 2X KAPA HiFi HotStart ReadyMix (Kapa Biosystems) containing 0.6 mM of each dNTP, 5 mM $MgCl_2$, and 2 units of Kapa Taq DNA polymerase. PCR cycling reactions were conducted on a Veriti 96-well Thermal Cycler (Applied Biosystems) using the following conditions: initial denaturation at 95 °C for 3 min followed by 20 cycles at 98°C for 20 s, 55°C for 45 s, 72°C for 15 s with final extension of 72 °C for 1 min. ITS1 amplification was confirmed by electrophoresis on a 2% agarose gel. Templates that were not successfully amplified using this protocol were amplified again after DNA dilution to 20ng/µL or 10ng/µL. Two marine fungal species (*Yamadazyma barbieri* and *Candida oceani*) were used as positive controls as they were unlikely to be found in our samples. One positive control included DNA of a strain of *C. oceani*, and the other included an equimolar mixture of the DNA of both species. A first negative control was represented by 1 mL of water washes of 4 empty plastic vials opened during the sampling campaign and washed with sterile water. The PCR mix was used as a second negative control.

PCR products were diluted five times in PCR-grade water and used as a DNA template for a second PCR performed using the tailed primers ITS1F_PlaGe (5'-CTTTCCCTACACGACGCTCTTCCGATCTCTTGGTCATTTAGAGGAAGTAA-3') and ITS2_PlaGe (5'-GGAGTTCAGACGTGTGCTCTTCCGATCTGCTGCGTTCTTCATCGATGC-3') designed by the Get-PlaGe sequencing facility (Toulouse, France). The second PCR was performed twice (once in 12.5 µL and once in 25µL of final volume) using the same reaction mixture as the first PCR. The PCR conditions were as follows: initial denaturation at 95 °C for 3 min followed by 10 cycles at 98°C for 20 s, 60°C for 15 s, 72°C for 15 s and a final extension step of 72°C for 1 min. PCR products were purified (CleanPCR, MokaScience), multiplex identifiers and sequencing adapters were added, and library sequencing on an Illumina MiSeq platform (v3 chemistry, 2x250 bp) and sequence demultiplexing (with exact index search) were performed at the Get-PlaGe sequencing facility (Toulouse, France).

Bioinformatic analyses

Paired-end sequences were joined using PEAR v0.9.10 (Zhang *et al.*, 2014). Only pairs with a minimum overlap of 50 bp and without any uncalled bases were kept. Assembled sequences were filtered using DADA2 v1.4.0 (Callahan *et al.*, 2016). Only sequences with less than one expected error and longer than 100 bp were retained in the dataset. Amplicon sequence variants (ASVs) were inferred using DADA2 and chimeric sequences were removed using the consensus method of the *removeBimeras* function. Taxonomic assignments were performed using the RDP classifier (Wang *et al.*, 2007) implemented in DADA2 and trained with the UNITE database 7.2 (UNITE Community 2017), with an 80% confidence threshold. The ASV table was then imported in R using the phyloseq package v1.26.0 (McMurdie & Holmes, 2013) and filtered. Due to the high number of the sequences originating from the host plant, only ASVs assigned to a fungal phylum were kept. Positive and negative controls were used to remove contaminants (Galan *et al.,* 2016). The cross-contamination threshold (T_{CC}) was defined as the maximal number of sequences of each ASV found in negative or positive control samples. The false-assignment threshold (T_{FA}) was defined as the highest sequence count of a positive control strain in a non-control sample, divided by the total number of sequences of the strain in the whole run and multiplied by the total number of sequences of each ASV. ASVs were removed from all samples where they harbored fewer sequences than either threshold (T_{FA} or T_{CC}).

SNP genotyping and maternity exclusion analyses

Acorns collected on the ground were genotyped to confirm that they belonged to the mother tree above them. Genotyping was performed using 39 polymorphic single nucleotide polymorphism (SNP) markers (Gerzabek *et al.,* 2017). DNA was diluted to a final concentration of 15 to 20 ng/µL and sequenced using the iPLEX Gold Genotyping kit (Agena, San Diego, CA, USA) at the Genome Transcriptome Facility of Bordeaux (PGTB, Bordeaux, France) according to the manufacturer's instructions (see Chancerel *et al.*, 2013 for more details). Two samples of aboveground tissues of each tree were genotyped and compared to estimate the typing error rate of false calls during genotyping. Loci with poor performance during the clustering procedure (call rates <60%) were excluded, resulting in a final set of 28 loci. Acorn genotypes were compared to the genotype of their putative mother tree. Considering the low error rate of these SNPs (Gerzabek *et al.,* 2017), we took a deliberately conservative approach and assumed that if tree and acorn shared no alleles for at least

one locus, the mother-offspring relationship was not confirmed and the acorn sample was removed from the dataset.

Confocal microscopy

Sixteen additional acorns were collected in autumn 2017 from the ground of the oak forest of Bellebat (44°43'36.4"N 0°13'22.5"W, Southwest of France) to visualize fungal colonization outside and inside acorns. Acorns were cut in half with secators and fixed overnight at 4°C in a paraformaldehyde solution (4% w/v in PBS, pH 7.2). Samples were then rinsed three times with PBS, immersed in 15 mL PBS containing 50 µg ml^{-1} of wheat germ agglutinin (WGA)-AlexaFluor488 conjugate (Life Technologies, USA), incubated 2 hours at 37°C, and rinsed again three times with PBS. The samples were observed under a confocal microscope (Olympus Fluoview FV1000 with multiline laser FV5-LAMAR-2 and HeNe(G) laser FV10-LAHEG230-2). Pictures were taken with X, Y, Z coordinates at 405, 488, 594 nm and with 10X, 20X, 40X or 60X objectives. Images were merged (RGB) using Image J software (Schneider *et al.* 2012). Pictures were created using Z Project Stacks (Campisano *et al.* 2014), then cropped, and the light/contrast balance was improved (Glassner *et al.* 2015). Images presented in this publication correspond to the average colonization level observed.

Statistical analyses

Comparison of acorn fungal communities among mother trees and environments

To test hypotheses H1 and H2, we analyzed the effects of mother tree, local environment (oak population), and microenvironment (acorn position in the canopy *versus* on the ground) on acorn fungal community richness and composition. Fungal richness was defined as the total number of ASVs per acorn sample and was modelled using generalized linear mixed models (GLMMs) with a negative binomial distribution and a log-link function. The first model had oak population, acorn position and their interaction as fixed effects and the mother tree as a random effect. We then tested the effect of the mother tree on fungal richness for acorns in the canopy and acorns on the ground separately. The two models had the mother tree as fixed effect and the population as random effect. The natural logarithm of the total number of sequences per sample (sequencing depth) was introduced as an offset in all models.

We then analyzed the effects of the three same factors on fungal community composition by using permutational multivariate analyses of variance (PERMANOVAs) with 9999 permutations.

Compositional dissimilarities among acorn samples were estimated using quantitative and binary versions of the Jaccard index (Jaccard, 1901) and visualized with principal coordinate analyses (PCoA). We first tested the effects of oak population, acorn position and their interaction on compositional dissimilarities among samples, by constraining or not constraining the permutations by elevation. We then assessed the effect of mother tree on community composition for acorns in the canopy and on the ground separately. The two models had the mother tree as a fixed effect and permutations were constrained by population. The natural logarithm of the total number of sequences per sample (sequencing depth) was introduced as the first effect in all models.

In addition, we investigated whether changes in fungal community composition after acorn fall were due to either the substitution of canopy-associated fungal species by ground-associated fungal species or gain of ground-associated fungal species without loss of canopy-associated fungal species, by partitioning Jaccard binary dissimilarities among acorns of the same mother tree using the betapart package v1.5.1 (Baselga *et al.,* 2018). The proportion of fungal species of acorns on the ground also found in acorns in the canopy was calculated for each mother tree.

Quantification of maternal effects, environmental filtering and biotic interactions

Hierarchical Models of Species Communities (HMSC; Ovaskainen *et al.,* 2017) were then used to quantify processes of community assembly. The models assumed that variation in fungal community composition among acorns (i.e. the **Y** matrix in the HMSC framework) was accounted for by four ecological predictors (*Mother mycobiota*, *Microenv mycobiota*, *Acorn position* and *Site elevation*) introduced as fixed effects in the **X** matrix. The **Y** matrix represented ASV sequence counts in all acorn samples, out of which we included only the ASVs that were present in five or more acorns. *Mother mycobiota* and *Microenv mycobiota* represented fungal communities in the canopy of mothers trees and in the microenvironment of acorns, respectively. They were ASV-specific predictors and thus the **X** matrix was different for every ASV of the **Y** matrix. *Mother mycobiota* was calculated for all acorn samples as the average relative abundance of the focal ASV in the twigs and leaves of mother trees, and was included at the tree level to model vertical transmission of fungi from the mother tree to its acorns. *Microenv mycobiota* was calculated as the residuals of the regression of the relative abundance of the focal ASV in the microenvironment of each acorn over its relative abundance in the canopy of the mother tree, and was included at the sample level to model horizontal acquisition of fungi from the materials surrounding each acorn. *Site elevation* and *Acorn position* represented filtering of fungal communities by climate and microclimate, respectively. *Site elevation* was included at the site level to model selection exerted by site-level abiotic factors, such as average air temperature, on acorn fungal communities. *Acorn*

position (canopy vs. ground) was included at the sample level to model selection exerted by microclimate, such as higher humidity on the ground, on acorn fungal communities. *Mother mycobiota* and *Microenv mycobiota* were included in interaction with *Acorn position* to test the hypothesis that their contribution to fungal communities differ between acorns in the canopy and acorns on the ground (H2). We also introduced the log-transformed sequencing depth of each sample (*Sequencing depth*) as a fixed effect in the **X** matrix, to take into account methodological biases influencing ASV sequence counts. Random effects at each hierarchical level (oak population, mother tree and acorn sample) were also introduced to model variations in ASV sequence counts that can neither be attributed to the four ecological predictors nor sequencing depth. In addition, we tested the hypothesis that fungal lifestyle modulates fungal ASV responses to environmental variations (H3), by including the trophic mode (saprotroph, plant pathogen or other) and the degree of specialization toward acorns of each ASV in the **T** matrix. The putative trophic mode of each ASV was determined using the FUNGuild database (Nguyen *et al.*, 2016). Their degree of specialization toward acorns was defined as the log-ratio of ASV relative abundance in acorns *versus* other sample types (i.e. branches, leaves, litter and upper soil), calculated using DESeq2 (Love *et al.*, 2014). To account for the zero-inflated nature of the data, we applied a hurdle modelling approach. We first fitted a probit model on ASV presence-absence data and then fitted a linear model on sequence count data conditional on presence, in which counts were log-transformed and scaled to zero mean and unit variance for each ASV and absences masked as non-available data. We fitted both models with default prior distributions (Ovaskainen *et al.,* 2017). For each of the four MCMC chains, we sampled the posterior for 1,500,000 iterations, out of which we excluded the first 500,000 as burn-in and thinned the remaining iterations by 1000, thus producing a total of 4,000 posterior samples. We examined MCMC convergence through the distributions of potential scale reduction factors (PSRF) of the model parameters. To examine model fit, we applied a two-fold cross validation across the samples and evaluated predictive performance by AUC for the presence-absence model and R^2 for the linear model. Finally, residual correlations among fungal ASV sequence counts at the acorn level were interpreted as hypothetical biotic interactions among fungal strains (see Ovaskainen *et al.,* 2017). We examined associations among ASVs assigned at the species level to test hypothesis H4.

Results

All acorn tissues, including the fruit wall, seed coat, and embryo were colonized by fungi, with a dense colonization under the endocarp (Fig. 1A-E). Ascomycota represented 91.1% and 89.4% of

the sequences of canopy and ground acorns, respectively. *Dothideomycetes*, *Leotiomycetes* and *Sordariomycetes* were the three main classes of ascomycetes present (Fig. S1). Among the ten most abundant species associated to whole acorns, five are known as plant pathogens (*Gnomoniopsis paraclavulata, Taphrina carpini*, *Epicoccum nigrum*, *Mycosphaerella tassiana* and *Polyscytalum algarvense*) and two are known as antagonists of other microorganisms (*Cladosporium delicatulum, Cylindrium elongatum*) (Table 1; Tables including all ASVs and subset of ASVs used in HMSC models available at https://doi.org/10.15454/SM6OCR). The ubiquitous fungi *Curvibasidium cygneicolum* and *Epicoccum nigrum* were dominant in the internal tissues of acorns, including the embryo (Table S1).

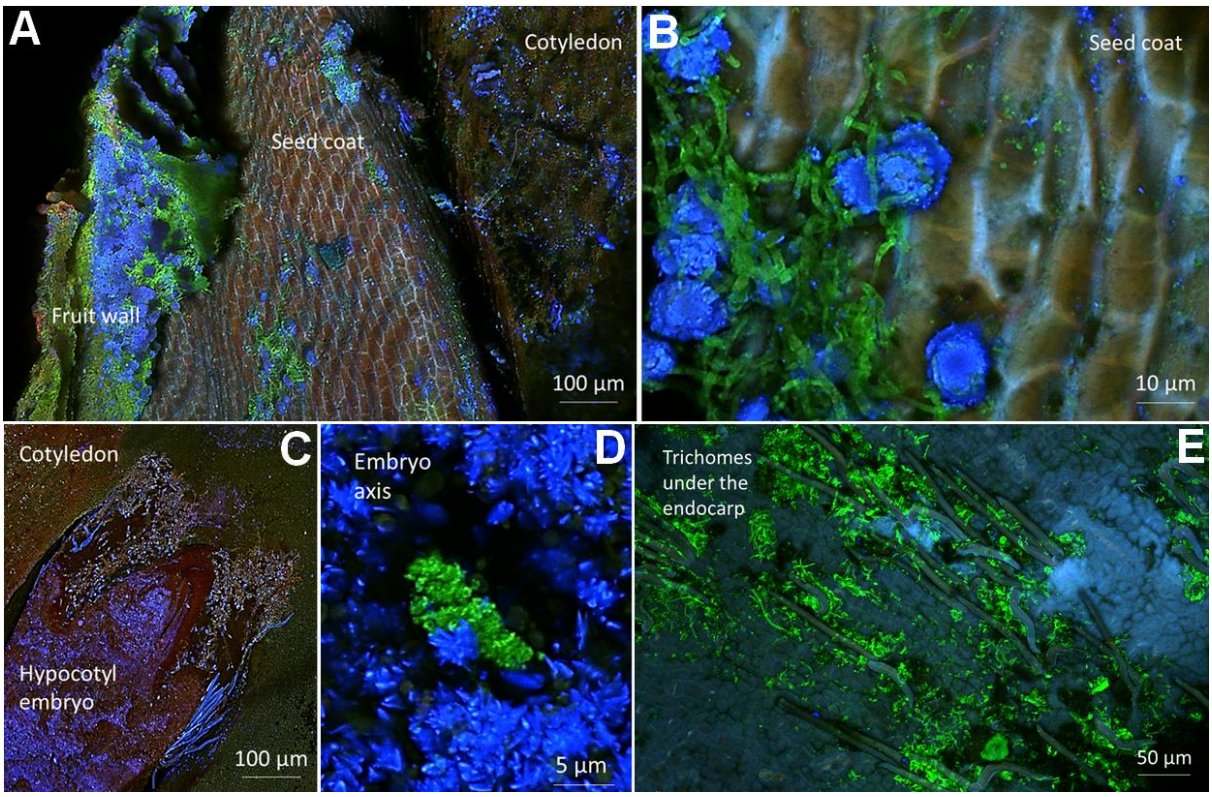


Figure 1. Fungi in cross sections of seeds of sessile oak collected on the ground. Fungi (green fluorescent) were revealed by confocal microscopy and WGA-ALEXA fluor488 staining. (A) Fruit wall and seed coat. (B) Zoom-in view of panel A. (C) Embryo and cotyledon. (D) Zoom-in view of panel C. (E) Internal surface of the endocarp.

Table 1. Most abundant fungal species associated with seeds of sessile oak and their microenvironment. Seeds were either collected in the tree canopy or on the ground. Materials from the seed microenvironment (twigs and leaves, or litter and upper soil) were also collected. The fungal community of all 4 sample types was analyzed using a metabarcoding approach. Only Amplicon sequence variants (ASV) assigned to Ascomycota (A) or Basidiomycota (B) with the UNITE database were kept. Average relative abundances of all ASVs were computed for each sample type, after merging ASVs assigned to the same fungal species. Only ASVs identified at the species level are shown in the table.

Fungal species	**Average relative abundance per sample type (%)**					**Lifestyle**	**Reference(s)**
	All seeds	Seeds in the canopy	Canopy samples (twigs, leaves)	Seeds on the ground	Ground samples (litter, upper soil)		
Gnomoniopsis paraclavulata (A)	13.4	5.3	1.5	21.9	0	Pathogen isolated in leaves, buds, cupules and shoots of *Castenea sativa*. Commonly isolated from overwintered leaves of *Quercus* sp.	Tosi *et al.* (2014) ; Sogonov *et al.* (2008)
Stromatoseptoria castaneicola (A)	2.7	5.2	0.9	0	0.2	Causes leaf spots on *Castenea sativa*.	Quaedvlieg *et al.* (2013)
Taphrina carpini (A)	2.4	3.4	0.7	1.4	0.3	Common pathogen encountered on *Fagaceae* leaves.	Bacigálová (1991); Inácio *et al.* (2004); Cordier *et al.* (2012)
Epicoccum nigrum (A)	2.3	0.4	0.1	4.2	0.2	Ubiquitous fungus found in soil, leaves and seeds described as primary saprotroph and plant pathogen.	Ahumada-Rudolph *et al.* (2014)
Mycosphaerella tassiana (A)	1.7	1.5	0.1	1.9	0.3	Common pathogen found in the phyllosphere including that of oak.	Schubert *et al.* (2007); Jakuschkin *et al.* (2016)
Curvibasidium cygneicollum (B)	1.5	1.2	0.2	1.9	0.1	Endophyte of fruits, leaves, trunk and soil behaving as a phytopathogen or a mycoparasite. The species is insensitive to the mycocins produce by *Filobasidium* and *Cystofilobasidium*.	Sampaio *et al.* (2004); Mašínová *et al.* (2017) ; Sampaio *et al.* (2004)
Cylindrium elongatum (A)	1.5	1.4	0.1	1.5	4.3	Bacterial and fungal antagonist found on oak leaves.	Reyes-Estebanez (2011) ; Duarte *et al.* (2015)
Polyscytalum algarvense (A)	1.5	1.9	0.1	1	5.3	Necrotroph fungi found on *Eucalyptus* leaves.	Cheewangkoon et al. (2009); Crous (2018)
Fusarium pseudensiforme (A)	1.3	1.4	0	1.2	0	Found on bark of trees.	Nalim *et al.* (2011)
Cladosporium delicatulum (A)	1.3	1.3	0.2	1.3	0.3	Found in cereal seeds, mycoparasite of *Taphrina spp* and *Magnaporthe oryzae*	Amanelah Baharvandi & Zafari (2015) ; Chaibub *et al.* (2016)

Acorn fungal communities are shaped by environmental filtering and maternal effects

Acorn fungal community richness (Table 2) and composition (Tables 3 and S2) differed significantly among oak populations at two different elevations, and among mother trees within the same population, confirming the hypothesis that both environmental filtering and maternal effects shape acorn fungal communities (H1). PCoAs suggested that fungal communities were more similar between populations at the same elevation (Fig. 2). However, the population effect remained significant when PERMANOVAs were constrained by elevation (Pseudo-F = 1.24, P < 0.01), implying that selection exerted by abiotic conditions between the two elevations was not the only process triggering variation in fungal community composition among oak populations.

Table 2. Generalized linear mixed-effects models (GLMM) of fungal community richness of seeds of sessile oak. Richness was defined as the number of amplicon sequence variants (ASV) per sample. The total number of sequences per sample (sequencing depth, SD) was introduced as an offset in all models. The effects of tree population (T), seed position (canopy *versus* ground, P) and their interaction were tested on the whole seed dataset while the effect of mother tree was tested separately on canopy seeds and ground seeds.

	d.f.	chi-square	*P*-value
All seeds			
Tree population (T)	3	12.5	**<.01**
Seed position (P)	1	12.6	**<.001**
T × P	3	5.74	0.12
Seeds in the canopy			
Mother Tree	10	30.6	**<.01**
Seeds on the ground			
Mother Tree	11	28.3	**<.01**

Table 3. Permutational multivariate analyses of variance (PERMANOVA) of compositional dissimilarities among fungal communities of seeds of sessile oak. Dissimilarities among seeds were estimated using the binary Jaccard distance. The total number of sequences per sample (sequencing depth, SD) was log-transformed and introduced as the first explanatory variable in all models. The effects of tree population (T), seed position (canopy *versus* ground, P) and their interaction were tested on the whole seed dataset while the effect of mother tree was tested separately on canopy seeds and ground seeds.

	d.f.	F-value	*P*-value	R^2
All seeds				
Log(SD)	1	1.3	**<.01**	0.01585
Tree population (T)	3	2.4	**<.001**	0.08711
Seed position (P)	1	1.8	**<.001**	0.02226
S x P	3	1.2	**<.001**	0.04494
Seeds in the canopy				
Log(SD)	1	1.3	0.191	0.03052
Mother tree	10	1.4	**0.010**	0.32742
Seeds on the ground				
Log(SD)	1	1.1	0.319	0.02834
Mother tree	11	1.2	**0.025**	0.34149

PCoAs and HMSC models gave different estimates of the relative contribution of environmental filtering and maternals effects. PCoAs suggested that mother tree identity had a lower influence on fungal community composition than elevation (Fig. 2), whereas HMSC models indicated the opposite trend (Table 4). The model of ASV presence-absence explained (in units of AUC, averaged over the ASVs) 80% of the variation for the model fitted to all data and 69% of the variation based on the two-fold cross-validation approach. According to this model, elevation was a minor direct driver of fungal community composition (only 2% of the explained variance). In contrast, the average relative abundance of a fungal ASV in the tissues of a mother tree (*Mother mycobiota*) was the second most important predictor of the occurrence of this ASV in an acorn from this tree, in interaction with the acorn position (39% of the explained variance). *Mother mycobiota* was the unique predictor of occurrence for several ASVs belonging to the orders *Helotiales*, *Venturiales* and *Xylariales* (Fig. S2). A similar ranking of the predictors was obtained for the model of ASV sequence

counts (Table 4), except that sequencing depth and random effects had a much larger influence on ASV sequence counts than on ASV presence-absence (17% *versus* 1% and 21% *versus* 6%, respectively). This model only explained 34% of the variation for the model fitted to all data and 18% of the variation based on the two-fold cross-validation approach.

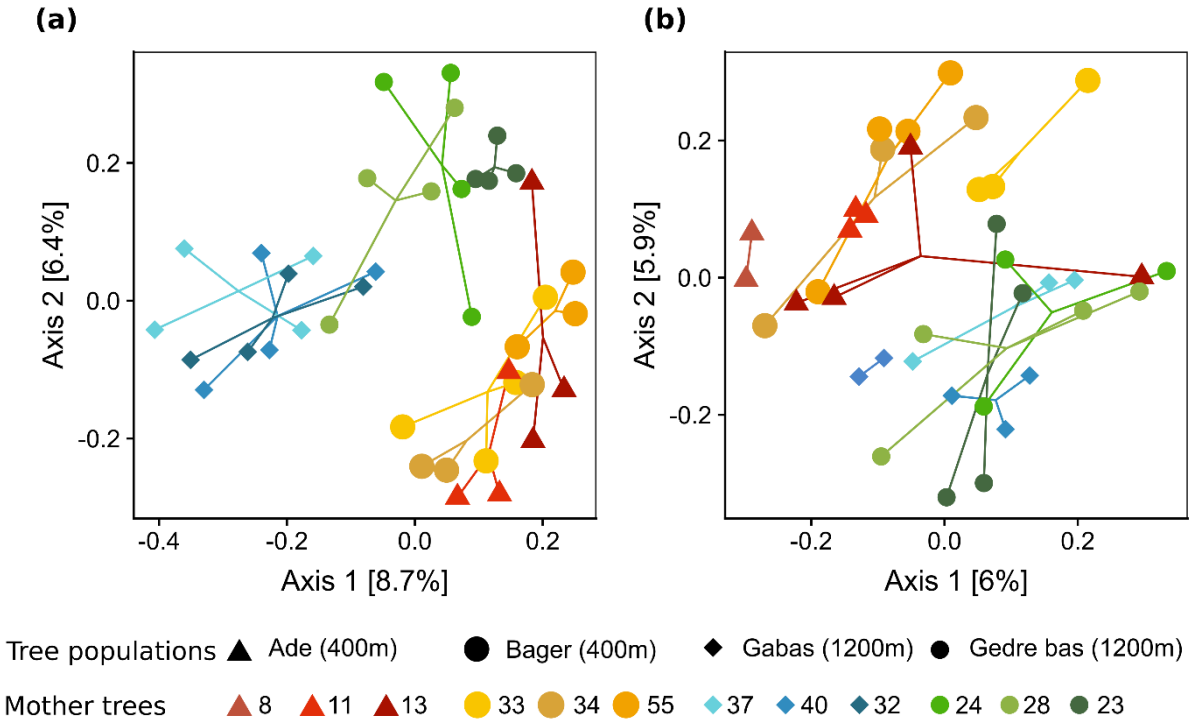


Figure 2. Compositional dissimilarities among fungal communities of seeds collected (A) in the canopy and (B) on the ground in four populations of sessile oak. Dissimilarities among seeds were estimated using binary Jaccard distance and represented with a PCoA plot. Colors and numbers represent mother trees and symbols represent tree populations. Fungal community composition differed significantly among tree populations and among mother trees (Table 3).

Table 4. Partitioning of the variance in fungal community composition of seeds of sessile oak. Four fixed effects were included in the HMSC models to explain variations in the presence-absence (PA) or the sequence count (SC) of a focal fungal ASV among seeds: *Sequencing depth* (total number of sequences per sample), *Seed position* (canopy or ground), *Microenv. mycobiota* (relative abundance of the focal ASV in the seed biotic microenvironment), *Mother mycobiota* (average relative abundance of the focal ASV in the mother tree aboveground tissues), and *Elevation*. Random effects were included at each spatial scale (seed, tree and population). Results of variance partitioning are given as percentages (%) of total explained variance.

Predictor			Explained variance (%)	
Type	**Level**	**Name**	**PA model**	**SC model**
Fixed	Seed	Sequencing depth	1	17
Fixed	Seed	Seed position (P)	4	8
Fixed	Seed	Microenv. mycobiota * P	47	23
Fixed	Tree	Mother mycobiota * P	39	25
Fixed	Population	Elevation	2	6
Random	Seed	Seed	3	9
Random	Tree	Tree	1	6
Random	Population	Population	2	6

Maternal effects persist after acorn fall despite horizontal acquisition of fungi from the ground

Fungal community richness (Table 2) and composition (Table 3 and S2) differed significantly between acorns in the canopy and acorns on the ground. For instance, *Gnomoniopsis paraclavulata* was four times more abundant in acorns on the ground than in acorns in the canopy, while *Stromatospheria castaneicola* was only present in canopy acorns (Table 1). Fungal richness increased and composition shifted toward that of ground materials after acorn fall (Fig. 3), confirming the horizontal acquisition of fungi from the ground. Partitioning of Jaccard beta-diversity indicated that these temporal changes in community composition were mainly driven by turnover (replacement of fungal species rather than net gains or losses in species number; Table S4). HMSC models confirmed the large influence of horizontal transmission on acorn fungal communities. The relative abundance of a fungal ASV in the microenvironment of an acorn (*Microenv mycobiota*), in

interaction with the acorn position, was generally the best predictor of ASV occurrence (47% of the explained variance), especially for the *Capnodiales*, *Dothideales* and *Taphrinales* orders (Fig. S2).

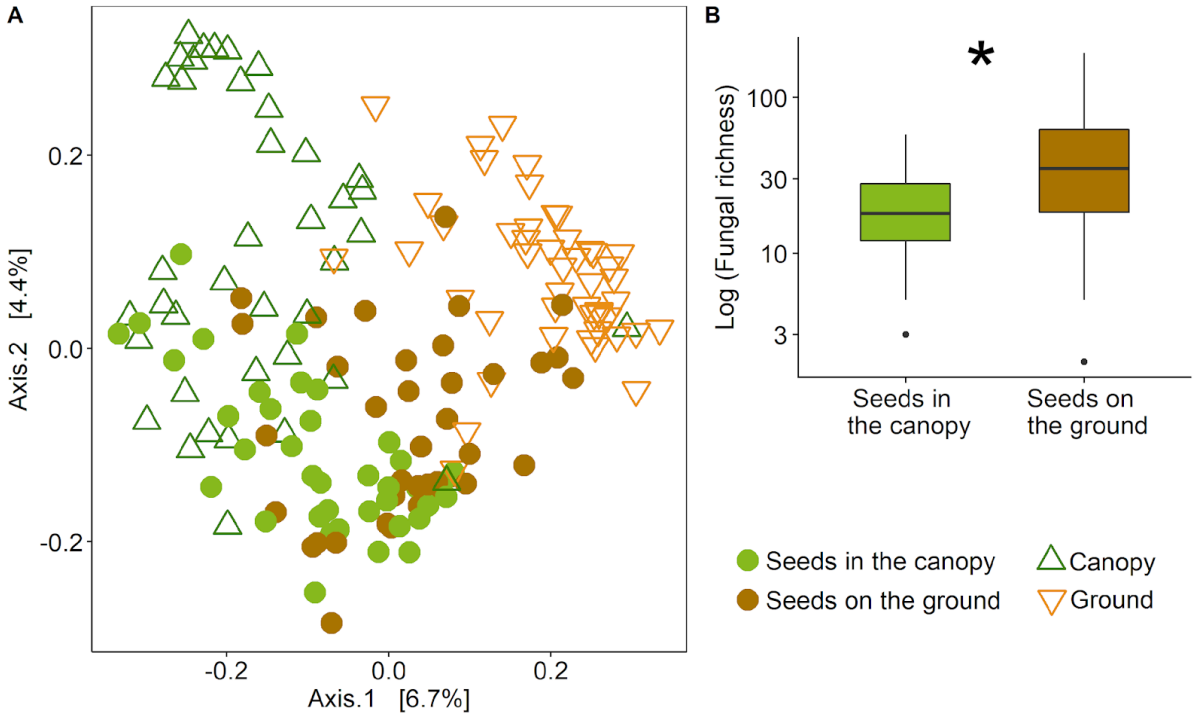


Figure 3. Fungal community composition and richness of seeds of sessile oak collected in the canopy and on the ground. (A) PCoA plot of compositional dissimilarities among fungal communities associated to seeds, canopy (leaves and twigs) and ground materials (litter and upper soil). Dissimilarities among samples were estimated using binary Jaccard distance. Fungal community composition differed significantly among the four sample types (PERMANOVA; F=4.3, *P*<0.001). (B) Richness (log-transformed) of seed fungal communities, defined as the number of ASVs per sample. Richness was significantly higher in seeds on the ground (Table 2).

Despite the turnover in fungal community composition after acorn fall and in contrast with our hypothesis (H2), the maternal effects were significant for acorns on the ground (Table 3), indicating that some maternal species were retained after acorn fall. Overall, acorns on the ground shared 10 to 40% of their fungal community with acorns in the canopy of the same mother tree, and 21 to 50% with mother tree tissues (Table S5). On average, 38% of fungal ASVs of acorns on the ground were present in both acorns in the canopy and mother tree tissues. Fungal species most often retained after acorn fall were *Taphrina* sp., *Cladosporium delicatulum*, a mycoparasite of *Taphrina* sp. (Baharvandi & Zafari, 2015), and the ubiquitous *Epicoccum nigrum*.

The response of fungal species to variations in the acorn environment depend on their lifestyle

HMSC models showed that fungal lifestyle influenced the response of acorn fungal communities to environmental variations, in accordance with our hypothesis (H3). High elevation selected for saprotroph species and seed specialists, increasing their proportion in acorn fungal communities (Table S3). Acorn fall favored pathogen species and saprotrophs. Their proportion increased after acorn fall while their abundance (conditional on presence) was not altered (Table S3). These findings suggest that vertically-transmitted pathogens did not increase in abundance after acorn fall, and that acorns were colonized by pathogen and saprotroph species of the ground.

Biotic interactions among fungal species might play a role in the protection of acorns against pathogens

Fungal colonization was very dense on acorn external surfaces (Fig. 1A) but also on internal surface of the fruit wall (Fig. 1E), indicating that fungal colonizers might enter into contact and compete for space and eventually resources. In contrast to this expectation, residual co-occurrence patterns of HMSC models at the acorn level revealed only positive associations between fungal ASVs. Among fungal ASVs associated with each other, 14 could be assigned at the species level (Fig. 5). Six of them are described as plant pathogens and two of them, *Mycosphaerella tassiana* and *Taphrina carpini*, have already been found in association with oak (Table S6). Four of the species are described as mycoparasites, including *Cladosporium delicatulum*. The positive association between *Taphrina carpini* and *Cladosporium delicatulum* (Fig. 5), which are both found in the embryo and the fruit wall (Table S3), might therefore represent a vertically-transmitted host-parasite interaction. This interaction might play a role in oak disease regulation, in accordance with our hypothesis (H4).

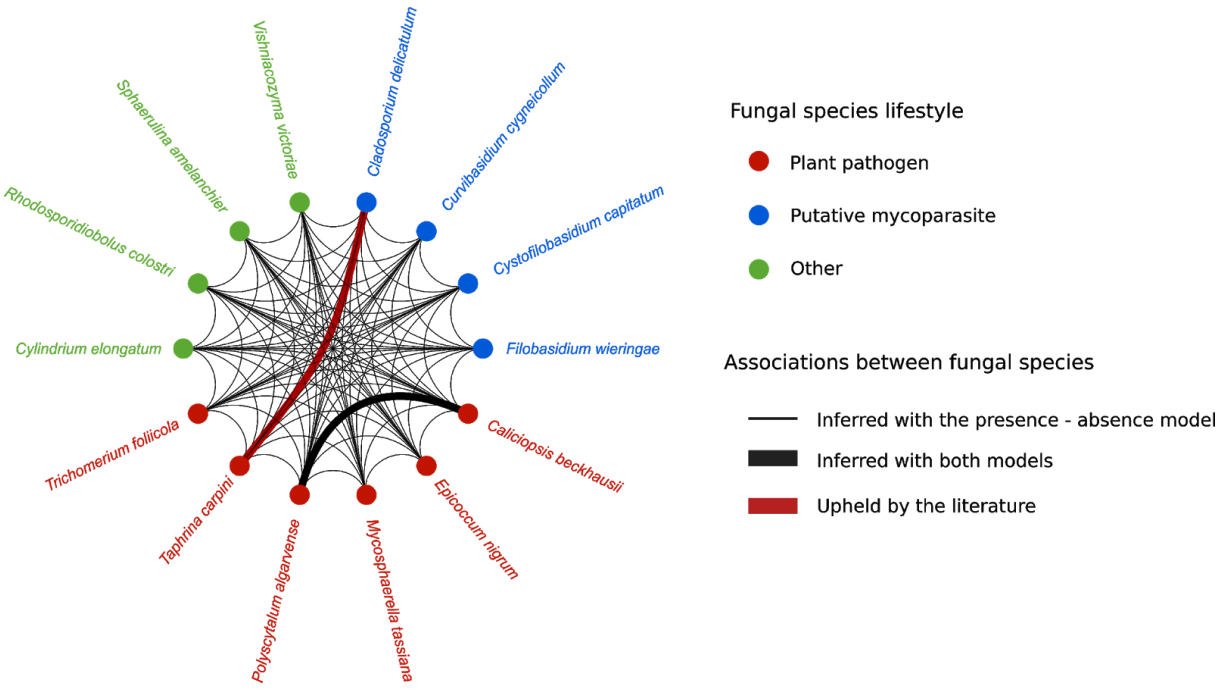


Figure 4. Network of fungal associations estimated by HMSC models. Associations were all positive. Network nodes correspond to fungal ASVs assigned to the species level and their color correspond to putative functions according to FUNGuild and the literature (Table S6). Network links indicate associations with at least 95% posterior probability estimated by the presence-absence model (thin black plain line) or by both the presence-absence model and the sequence count model (thick black plain line). The positive association between *Taphrina carpini* and *Cladosporium delicatulum* is indicated in red because it is described in the literature as a host-parasite interaction (Table S6).

Discussion

Seed microbial communities of trees have received little attention so far despite their potential influence on forest dynamics and evolution. In this study, we compared fungal communities of seeds among four populations of sessile oak (*Quercus petraea*), a dominant tree species of deciduous temperate European forests (McShea *et al.,* 2006). The populations were located at two different elevations in the Pyrenees Mountains (France). They differed by up to 800 m in elevation. Our analyses revealed that the richness and composition of seed fungal communities differed significantly among oak populations, suggesting that abiotic filtering was a major driver of community assembly. However, Hierarchical Models of Species Communities (HMSC; Ovaskainen et al. 2017) revealed that the direct influence of elevation on seed fungal communities was small. Elevation accounted for only 2% of the explained variance in fungal species presence-absence, and 6% of the variance in species abundance. The apparently large effects of elevation suggested by multivariate analyses might therefore be indirect effects. HMSC models estimated that fungal

community composition in the microenvironment of each seed (i.e. twigs and leaves surrounding seeds in the canopy, or ground materials surrounding dropped seeds), explained 47% of the variance in species presence-absence, and was by far the strongest driver of seed fungal communities. These findings suggest that environmental filtering act directly on the fungal communities of leaves, litter and soil (as shown by Cordier et al., 2012; Coince et al., 2014; Vacher et al., 2016), to then indirectly shape seed communities.

Seed fungal communities also differed among mother trees within the same population and these differences remained significant after seed fall. Such maternal effects have already been found for the abundance of some fungal genera in seeds (Johnston-Monje & Raizada, 2011; Pinciroli *et al.*, 2013). Here we showed that maternal effects extend to the whole fungal community of seeds through to seed fall. HMSC indicated that the fungal community composition of mother tree tissues (twigs and leaves) had a major, direct influence on that of seeds. It accounted for 39% of the explained variance in fungal species presence-absence, and 25% of the variance in species abundance. Recent findings by Vivas *et al.,* (2017) on *Eucalyptus* trees indicate that these maternal effects can persist in seedlings and influence their growth and resistance to pathogens. Together, these results suggest that maternal effects in seed and seedling extended phenotypes could be a major driver of forest regeneration success.

In addition, our confocal microscopy analyses revealed, for the first time, the presence of fungal aggregates within embryos of acorns of sessile oak, as well as a dense fungal colonization on internal surfaces of fruit walls. These endophytic fungal populations contained foliar pathogens of *Fagaceae* tree species, such as *Mycosphaerella tassiana* and *Taphrina carpini* (Schubert *et al.,* 2007; Bacigálová, 1991), ubiquitous fungal species, such as *Epicoccum nigrum* (Andrews & Harris, 2000), and endophytic yeasts previously described in other fruits, such as *Curvibasidium cygneicollum* (Sampaio *et al.,* 2004; Mašínová *et al.,* 2017). Network inference analyses revealed a positive association between the foliar pathogen *Taphrina carpini* and the mycoparasite *Cladosporium delicatulum,* suggesting that mother trees do not only transmit pathogens but also pathogen antagonists. Our results hence confirm that fungal pathogens use seeds for their own dispersion, and that the fungal pathogen's parasites can follow them using the same dispersion mode (Ewald, 1989; Feldman *et al.*, 2008). Unraveling the genetic architecture of these tripartite interactions, involving tree seedlings, seed-borne pathogens and their hyperparasites, could improve our understanding of forest ecosystem dynamics and evolution.

Finally, our analyses confirmed that seed fall corresponds to a major transition in seed fungal communities. Our results showed that fungal community richness significantly increased and that

composition shifted toward that of ground materials after seed fall, confirming that seeds on the ground are rapidly colonized by the species present in the surrounding microenvironment (Crist, 2009, Qin *et al.,* 2016; Truyens *et al.,* 2015; Klaedtke *et al.,* 2016). For instance, the species *Gnomoniopsis paraclavulata*, that was previously found in association with oak litter (U'Ren *et al.,* 2016), drastically increased in abundance after acorn fall. Our analyses also suggested that seed fall triggers a replacement of canopy-inherited species by ground-derived species, rather than an addition of species associated to ground materials. This replacement was however only partial. Seeds on the ground shared up to 50% of their fungal colonizers with the twigs and leaves of their mother tree. The mechanisms of community filtration during vertical transmission (Vannier *et al.*, 2018) will have to be investigated in future studies.

Conclusion

Our study revealed that acorns of sessile oak harbor diversified fungal communities in their internal tissues, including the embryo, and on their surfaces. These communities were shaped by maternal effects, environmental filtering and biotic interactions. Maternal effects persisted after seed fall, despite seed colonization by soil and litter fungi. Environmental filtering did not shape directly seed fungal communities, but rather influenced communities in the microenvironments surrounding seeds. Biotic interactions included several host-parasite interactions between tree pathogens and their antagonists, one of which was likely to be vertically-transmitted. Future research will have to investigate the maternal and environmental drivers of the rate of vertical transmission of microorganisms (e.g. Cavazos *et al.,* 2018; Sneck *et al.,* 2017; Leff *et al.,* 2017), and assess the role of these microorganisms on seed survival and germination, seedling growth and health (e.g. Vivas *et al.,* 2017; Leroy *et al.,* 2019), and ultimately tree fitness. The influence of vertically-transmitted microorganisms on seed and seedling secondary metabolites (Chen *et al.,* 2018; Shazad *et al.,* 2018), and their cascading effects on tree biotic interactions (e.g. Peris *et al.,* 2018) will also have to be investigated. Previous research on oak trees showed for instance a significant relationship between a fungus-like pathogen associated with acorns and the abundance of several oak-dependant bird species, including seed dispersers (Monahan & Koenig, 2006). A combination of germination experiments in controlled conditions (e.g. Leroy *et al.,* 2019), seed microbiome analyses in common gardens (Vivas *et al.,* 2015), and seed microorganisms manipulation across environments (Gundel *et al.,* 2017) will be required to integrate seed microbial ecology into predictive models of forest dynamics and evolution.

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Author contributions

TF participated in the sampling campaigns, processed all samples, extracted and amplified DNA, performed the statistical analyses, interpreted the results and wrote the first draft of the manuscript. CP helped process the samples, performed the bioinformatic analyses and provided statistical analysis scripts. AEZ assisted with supervision and writing. OO conducted the HMSC analysis and helped interpreting the results. TC organized the sampling campaigns and provided environmental data. MB provided advice on molecular biology protocols, bioinformatics and statistical analyses. SC took the microscopy pictures and interpreted the results. AH provided advice and analysis tools for SNP genotyping. SD, AEZ and CV had the original idea for the study. CV participated in the sampling campaigns, coordinated and supervised all stages of the work, and made a major contribution to the writing of the manuscript. All authors revised the manuscript and approved the final version.

Data availability

All raw sequences obtained from the sequencing of acorns and their biotic microenvironment are available from the National Center for Biotechnology Information Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra) under the accession number PRJNA551388. The code and ASV tables are available as an archive at https://doi.org/10.15454/SM6OCR.

References

Ahumada-Rudolph R, Cajas-Madriaga D, Rudolph A, Reinoso R, Torres C, Silva M, Becerra J. 2014. Variation of sterols and fatty acids as an adaptive response to changes in temperature, salinity and pH of a marine fungus *Epicoccum nigrum* isolated from the Patagonian Fjords. *Revista de biología marina y oceanografía* **49**: 293–305.

Amanelah Baharvandi H, Zafari D. 2015. Identification of *Cladosporium delicatulum* as a mycoparasite of *Taphrina pruni*. *Archives of Phytopathology and Plant Protection* **48**: 688–697.

Andrews JH, Harris RF. 2000. The ecology and biogeography of microorganisms on plant surfaces. *Annual Review of Phytopathology* **38**: 145–180.

Bacigálová K. 1991. New localities of *Taphrina carpini* (Rostr.) Johans, on *Carpinus betulus* in Slovakia. *Czech Mycology* **46**: 296–302.

Baker KF, Smith SH. 1966. Dynamics of seed transmission of plant pathogens. *Annual Review of Phytopathology* **4**: 311 – 332.

Barret M, Guimbaud JF, Darrasse A, Jacques MA. 2016. Plant microbiota affects seed transmission of phytopathogenic microorganisms. *Molecular plant pathology* **17**: 791–795.

Baselga A, Orme CDL. 2012. Betapart: An R package for the study of beta diversity. *Methods in Ecology and Evolution* **3**: 808–812.

Brader G, Compant S, Vescio K, Mitter B, Trognitz F, Ma L-J, Sessitsch A. 2017. Ecology and genomic insights into plant-pathogenic and plant-nonpathogenic endophytes. *Annual Review of Phytopathology* **55**: 61–83.

Caignard T, Kremer A, Firmat C, Nicolas M, Venner S, Delzon S. 2017. Increasing spring temperatures favor oak seed production in temperate areas. *Scientific Reports* **7**: 1–8.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. **2016**. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**: 581–583.

Campisano A, Ometto L, Compant S, Pancher M, Antonielli L, Yousaf S, Varotto C, Anfora G, Pertot I, Sessitsch A, et al. 2014. Interkingdom transfer of the acne-causing agent, propionibacterium acnes, from human to grapevine. *Molecular Biology and Evolution* **31**: 1059–1065.

Cavazos BR, Bohner TF, Donald ML, Sneck ME, Shadow A, Omacini M, Rudgers JA, Miller TEX. 2018. Testing the roles of vertical transmission and drought stress in the prevalence of heritable fungal endophytes in annual grass populations. *New Phytologist*: 1075–1084.

Chaibub AA, de Carvalho JCB, de Sousa Silva C, Collevatti RG, Gonçalves FJ, de Carvalho Barros Côrtes MV, de Filippi MCC, de Faria FP, Lopes DCB, de Araújo LG. **2016**. Defence responses in rice plants in prior and simultaneous applications of *Cladosporium* sp. during leaf blast suppression. *Environmental Science and Pollution Research* **23**: 21554–21564.

Chancerel E, Lamy JB, Lesur I, Noirot C, Klopp C, Ehrenmann F, Boury C, Provost G Le, Label P, Lalanne C, et al. 2013. High-density linkage mapping in a pine tree reveals a genomic region associated with inbreeding depression and provides clues to the extent and distribution of meiotic recombination. *BMC Biology* **11**: 50.

Chase JM, Myers JA. **2011**. Disentangling the importance of ecological niches from stochastic processes across scales. *Philosophical Transactions of the Royal Society* **366**: 2351–2363.

Cheewangkoon R, Groenewald JZ, Summerell BA, Hyde KD, To-Anun C, Crous PW. **2009**. Myrtaceae, a cache of fungal biodiversity. *Persoonia: Molecular Phylogeny and Evolution of Fungi* **23**: 55–85.

Chen H, Wu H, Yan B, Zhao H, Liu F, Zhang H, Sheng Q, Miao F, Liang Z. **2018**. Core microbiome of medicinal plant *Salvia miltiorrhiza* seed : A rich reservoir of beneficial microbes for secondary metabolism? *Molecular Sciences* **19**: 672–687.

Chomnunti P, Bhat DJ, Jones EBG. 2012. Trichomeriaceae, a new sooty mould family of Chaetothyriales. *Fungal Diversity* **56**:63-76

Clay K, Schardl C. **2002**. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *The American Naturalist* **160**: S99–S127.

Compant S, Van Der Heijden MGA, Sessitsch A. **2010**. Climate change effects on beneficial plant-microorganism interactions. *FEMS Microbiology Ecology* **73**: 197–214.

Coince A, Cordier T, Lengellé J, Defossez E, Vacher C, Robin C, Buée M, Marçais B. **2014**. Leaf and root-associated fungal assemblages do not follow similar elevational diversity patterns. *PLoS ONE* **9**.

Cordier T, Robin C, Capdevielle X, Fabreguettes O, Desprez-Loustau ML, Vacher C. **2012**. The composition of phyllosphere fungal assemblages of European beech (*Fagus sylvatica*) varies significantly along an elevation gradient. *New Phytologist* **196**: 510–519.

Crist T, Friese F. **2009**. The Impact of Fungi on Soil Seeds : Implications for plants and granivores in a semiarid shrub-steppe. *Ecological Society of America* **74**: 2231–2239.

Crous PW, Schumacher RK, Wingfield MJ, Akulov A, Denman S, Roux J, Braun U, Burgess TI, Carnegie AJ, Váczy KZ, et al. 2018. New and Interesting Fungi. *Fungal Systematics and Evolution* **1**: 169–215.

Desjardins AE. **2003**. Gibberella from A(venaceae) to Z(eae). *Annual Review of Phytopathology* **41**: 177–198.

Douglas Boyette C, Lynn Walker H. **1986**. Evaluation of *Fusarium lateritium* as a Biological Herbicide for Controlling Velvetleaf (Abutilon theophrasti) and Prickly Sida (Sida spinosa). *Weed Science Society of America* **34**: 106–109.

Duarte S, Bärlocher F, Trabulo J, Cássio F. **2015**. Stream-dwelling fungal decomposer communities along a gradient of eutrophication unraveled by 454 pyrosequencing. *Fungal Diversity* **70**: 127–148.

Escobar Rodriguez C, Mitter B, Barret M, Sessitsch A, Compant S. **2018**. Commentary: seed bacterial inhabitants and their routes of colonization. *Plant and Soil* **422**: 129–134.

Ewald PW. **1989**. Transmission modes and evolution of the parasitism-mutualism continuum. *Annals of the New York Academy of Sciences* **503**: 295–306.

Faeth SH. **2002**. Fungal Endophytes: Common Host Plant Symbionts but Uncommon Mutualists. *Integrative and Comparative Biology* **42**: 360–368.

Feldman TS, Brien HEO, Arnold AE. **2008**. Moths that vector a plant pathogen also transport endophytic fungi and mycoparasitic antagonists. *Microbial Ecology* **56**: 742–750.

Frank A, Saldierna Guzmán J, Shay J. **2017**. Transmission of bacterial endophytes. *Microorganisms* **5**: 70.

Galan M, Razzauti M, Bard E, Bernard M, Brouat C, Charbonnel N, Dehne-Garcia A, Loiseau A, Tatard C, Tamisier L, et al. 2016. 16S rRNA amplicon sequencing for epidemiological surveys of bacteria in wildlife. *mSystems* **1**: e00032-16.

Gardes M, Bruns TD. **1993**. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113–118.

Garrido-benavent I, Pérez-ortega S. **2015**. Unravelling the diversity of European Caliciopsis (Coryneliaceae, Ascomycota): *Caliciopsis valentina* sp. nov. and *C. beckhausii* comb. nov., with a worldwide key to Caliciopsis. *Mycological Progress* **14**: 1–11.

Gerzabek G, Oddou-Muratorio S, Hampe A. **2017**. Temporal change and determinants of maternal reproductive success in an expanding oak forest stand. *Journal of Ecology* **105**: 39–48.

Gilbert GS. **2002**. Evolutionary ecology of plant diseases in natural ecosystems. *Annual Review of Phytopathology* **40**: 13–43.

Glassner H, Zchori-Fein E, Compant S, Sessitsch A, Katzir N, Portnoy V, Yaron S. **2015**. Characterization of endophytic bacteria from cucurbit fruits with potential benefits to agriculture in melons (Cucumis melo L.). *FEMS Microbiology Ecology* **91**: 1–13.

Glushakova AM, Kachalkin A V. 2017. Endophytic yeasts in *Malus domestica* and *Pyrus communis* fruits under anthropogenic impact. *Microbiology* **86**: 128–135.

Golubev WI, Tomashevskaya MA. **2009**. Characterization of Mycocin Secreted by *Rhodotorula colostri* (Castelli) Lodder. *Biology bulletin* **36**: 373–378.

Gundel PE, Rudgers JA, Whitney KD. 2017. Vertically transmitted symbionts as mechanisms of transgenerational effects. *American Journal of Botany* 104: 787–792.

Jaccard P. **1901**. Étude comparative de la distribution florale dans une portion des Alpes et du Jura. *Bulletin de la Société Vaudoise des Sciences Naturelles* **37**: 547–579.

Inácio J, Rodrigues MG, Sobral P, Fonseca Á. **2004**. Characterisation and classification of phylloplane yeasts from Portugal related to the genus *Taphrina* and description of five novel *Lalaria* species. *FEMS Yeast Research* **4**: 541–555.

Isaeva O V., Glushakova AM, Yurkov AM, Chernov IY. **2009**. The yeast *Candida railenensis* in the fruits of English oak (Quercus robur L.). *Microbiology* **78**: 355–359.

Jakuschkin B, Fievet V, Schwaller L, Fort T, Robin C, Vacher C. **2016**. Deciphering the pathobiome: Intra- and interkingdom interactions involving the pathogen *Erysiphe alphitoides*. *Microbial Ecology* **72**: 870–880.

Janzen DH. **1970**. Herbivores and the Number of Tree Species in Tropical Forests. *The American Naturalist* **104**: 501–528.

Johnston-Monje D, Raizada MN. **2011**. Conservation and diversity of seed associated endophytes in Zea across boundaries of evolution, ethnography and ecology. *PLoS ONE* **6**:e20396.

Klaedtke S, Jacques MA, Raggi L, Préveaux A, Bonneau S, Negri V, Chable V, Barret M. **2016**. Terroir is a key driver of seed-associated microbial assemblages. *Environmental microbiology* **18**: 1792–1804.

Leff JW, Lynch RC, Kane NC, Fierer N. 2017. Plant domestication and the assembly of bacterial and fungal communities associated with strains of the common sunflower, *Helianthus annuus*. *New Phytologist* **214**: 412–423.

Leroy C, Maes AQ, Louisanna E, Séjalon-Delmas N. **2019**. How significant are endophytic fungi in bromeliad seeds and seedlings ? Effects on germination, survival and performance of two epiphytic plant species. *Fungal Ecology* **39**: 296–306.

Love MI, Huber W, Anders S, Love MI, Huber W, Anders S. **2014**. Moderated estimation of fold change and dispersion for RNA-Seq data with DESeq2. *Genome biology* **15**: 521–550.

Mašínová T, Bahnmann BD, Větrovský T, Tomšovský M, Merunková K, Baldrian P. **2017**. Drivers of yeast community composition in the litter and soil of a temperate forest. *FEMS microbiology ecology* **93**: 1–10.

Maude RB. **1996**. *Seedborne Diseases and Their Control: Principles and Practice*.

McMurdie PJ, Holmes S. **2013**. Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE* **8**: e61217.

Mcshea WJ, Healy WM, Devers P, Fearer T, Frank H, Stauffer D, Waldon J. **2019**. Forestry matters : Decline of oaks will impact wildlife in hardwood forests. *Journal of Wildlife management* **71**: 1717–1728.

Minard G, Tikhonov G, Ovaskainen O, Saastamoinen M. Variation in Melitaea cinxia gut microbiota is phylogenetically highly structured but 2only mildly driven by host plant microbiota, sex or parasitism. *bioRxiv,* in press.

Monahan WB, Koenig WD. **2005**. Estimating the potential effects of sudden oak death on oak-dependent birds. *Biological Conservation* **127**: 146–157.

Mueller, G.M., Bills, G.F., Foster MSB. **2004**. *Biodiversity of Fungi : Inventory and monitoring methods*.

Nalim FA, Samuels GJ, Wijesundera RL, Geiser DM. **2011**. New species from the *Fusarium solani* species complex derived from perithecia and soil in the old world tropics. *Mycologia* **103**: 1302–1330.

Nelson EB. **2004**. Microbial dynamics and interactions in the spermosphere. *Annual Review of Phytopathology* **42**: 271–309.

Nelson EB. **2017**. The seed microbiome: origins, interactions, and impacts. *Plant and Soil* **422**: 7–34.

Nelson EB, Simoneau P, Barret M, Mitter B, Compant S. **2018**. The soil, the seed, the microbes and the plant. *Plant Soil* **422**: 1–5.

Nemergut DR, Schmidt SK, Fukami T, O'Neill SP, Bilinski TM, Stanish LF, Knelman JE, Darcy JL, Lynch RC, Wickey P, *et al.* 2013. Patterns and processes of microbial community assembly. *Microbiology and Molecular Biology Reviews* **77**: 342–356.

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG. **2016**. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology* **20**: 241–248.

Oberwinkler F, Bandoni R, Blanz P. **1983**. Cystofilobasidium : a New Genus in the *Filobasidiaceae* *. *Systematic and applied microbiology* **4**: 114–122.

Ofek M, Hadar Y, Minz D. **2011**. Colonization of cucumber seeds by bacteria during germination. *Environmental Microbiology* **13**: 2794–2807.

Ovaskainen O, Tikhonov G, Norberg A, Guillaume Blanchet F, Duan L, Dunson D, Roslin T, Abrego N. **2017**. How to make more out of community data? A conceptual framework and its implementation as models and software. *Ecology Letters* **20**: 561–576.

Palou L, Guardado A, Monstesinos-Herrero C. **2010**. First report of *Penicillium* spp. and *Pilidiella granati* causing postharvest fruit rot of pomegranate in Spain. *New disease reports* **22**: 21.

Peris JE, Rodríguez A, Peña L, Fedriani JM. **2017**. Fungal infestation boosts fruit aroma and fruit removal by mammals and birds. *Scientific Reports*: 1–9.

Pertot I, Giovannini O, Benanchi M, Caf T, Rossi V, Mugnai L. **2017**. Combining biocontrol agents with different mechanisms of action in a strategy to control *Botrytis cinerea* on grapevine. **97**: 85–93.

Pinciroli M, Gribaldo A, Vidal A, Bezus R, Sisterna M. **2013**. Mycobiota evolution during storage of paddy, brown and milled rice in different genotypes. *Summa phytopathologica* **39**: 157–161.

Prochazkova Z, Sikorova A, Peskova V. **2005**. Preliminary observations on the occurrence of *Ciboria batschiana* (Zopf) Buchwald in the Czech Republic. *Working Papers of the Finnish Forest Research Institute* **11**: 13–18.

Qin Y, Pan X, Yuan Z. **2016**. Seed endophytic microbiota in a coastal plant and phytobeneficial properties of the fungus *Cladosporium cladosporioides*. *Fungal Ecology* **24**: 53–60.

Quaedvlieg W, Verkley GJM, Shin HD, Barreto RW, Alfenas AC, Swart WJ, Groenewald JZ, Crous PW. **2013**. Sizing up *Septoria*. *Studies in Mycology* **75**: 307–390.

Reyes-Estebanez M. **2011**. Antimicrobial and nematicidal screening of anamorphic fungi isolated from plant debris of tropical areas in Mexico. *African Journal of Microbiology Research* **5**: 1083–1089.

Rezki S, Campion C, Simoneau P, Jacques MA, Shade A, Barret M. **2018**. Assembly of seed-associated microbial communities within and across successive plant generations. *Plant and Soil* **422**: 67–79.

Rillig MC, Antonovics J, Caruso T, Lehmann A, Powell JR, Veresoglou SD, Verbruggen E. **2015**. Interchange of entire communities: Microbial community coalescence. *Trends in Ecology and Evolution* **30**: 470–476.

Sampaio JP, Golubev WI, Fell JW, Gadanho M, Golubev NW. **2004**. *Curvibasidium cygneicollum* gen. nov., sp. nov. and *Curvibasidium pallidicorallinum* sp. nov., novel taxa in the microbotryomycetidae (Urediniomycetes), and their relationship with *Rhodotorula fujisanensis* and *Rhodotorula nothofagi*. *International Journal of Systematic and Evolutionary Microbiology* **54**: 1401–1407.

Santiago IF, Rosa CA, Rosa LH. **2016**. Endophytic symbiont yeasts associated with the Antarctic angiosperms Deschampsia antarctica and Colobanthus quitensis. *Polar Biology* **40**:177-183

Schiltz S, Gaillard I, Pawlicki-Jullian N, Thiombiano B, Mesnard F, Gontier E. 2015. A review: what is the spermosphere and how can it be studied? *Journal of Applied Microbiology* **119**: 1467–1481.

Schneider C, Rasband W, Eliceiri K. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **9**: 671–675.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Bolchacova E, Voigt K, Crous PW, et al. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences* **109**: 6241–6246.

Schubert K, Groenewald JZ, Braun U, Dijksterhuis J, Starink M, Hill CF, Zalar P, De Hoog GS, Crous PW. 2007. Biodiversity in the *Cladosporium herbarum* complex (*Davidiellaceae*, *Capnodiales*), with standardisation of methods for *Cladosporium* taxonomy and diagnostics. *Studies in Mycology* **58**: 105–156.

Shahzad R, Khan AL, Bilal S, Asaf S, Lee I. 2018. What Is There in Seeds? Vertically Transmitted Endophytic Resources for Sustainable Improvement in Plant Growth. *Frontiers in Plant Science* **9**.

Sneck ME, Rudgers JA, Young CA, Miller TEX. 2017. Variation in the prevalence and Transmission of heritable symbionts across host populations in heterogeneous environments. Microbial Ecology 74: 640–653.

Sogonov M V., Castlebury LA, Rossman AY, Mejía LC, White JF. 2008. Leaf-inhabiting genera of the *Gnomoniaceae*, *Diaporthales*. *Studies in Mycology* **62**: 1–77.

Thambugala KM, Hyde KD, Tanaka K, Tian Q, Wanasinghe DN, Ariyawansa HA, Jayasiri SC, Boonmee S, Camporesi E, Hashimoto A, *et al.* 2015. Towards a natural classification and backbone tree for Lophiostomataceae, Floricolaceae, and Amorosiaceae fam. nov. *Fungal Diversity* **74**: 199–266.

Torres-Cortés G, Bonneau S, Bouchez O, Genthon C, Briand M, Jacques M-A, Barret M. 2018. Functional microbial features driving community assembly during seed germination and emergence. *Frontiers in Plant Science* **9**: 902.

Truyens S, Weyens N, Cuypers A, Vangronsveld J. 2015. Bacterial seed endophytes: Genera, vertical transmission and interaction with plants. *Environmental Microbiology Reports* **7**: 40–50.

U'Ren JM, Arnold AE. **2016**. Diversity, taxonomic composition, and functional aspects of fungal communities in living, senesced, and fallen leaves at five sites across North America. *PeerJ* **4**: e2768.

Vacher C, Hampe A, Porté AJ, Sauer U, Compant S, Morris CE. **2016**. The Phyllosphere: Microbial jungle at the plant–climate interface. *Annual Review of Ecology, Evolution, and Systematics* **47**: 1–24.

Vannier N, Mony C, Bittebiere A, Michon-coudouel S, Biget M, Vandenkoornhuyse P. **2018**. A microorganisms' journey between plant generations. *Microbiome* **6**: 1–11.

Vellend M. **2010**. Conceptual synthesis in community ecology. *The quarterly review of biology* **85**: 183–206.

Vivas M, Kemler M, Slippers B. 2015. Maternal effects on tree phenotypes : Considering the microbiome. *Trends in Plant Science* 20: 541–544.

Vivas M, Kemler M, Mphahlele MM, Wingfield MJ, Slippers B. **2017**. Maternal effects on phenotype, resistance and the structuring of fungal communities in *Eucalyptus grandis*. *Environmental and Experimental Botany* **140**: 120–127.

Wang Q, Garrity GM, Tiedje JM, Cole JR. **2007**. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* **73**: 5261–5267.

Whitham TG, Bailey JK, Schweitzer| JA, Shuster SM, Bangert RK, LeRoy CJ, Lonsdorf E V., Allan GJ, DiFazio SP, Potts BM, et al. 2006. A framework for community and ecosystem genetics : from genes to ecosystems. *Nature Reviews Genetics* **7**: 510–523.

Wolf JB, Wade MJ. **2009**. What are maternal effects (and what are they not)? **364**: 1107–1115.

Zhang J, Kobert K, Flouri T, Stamatakis A. **2014**. PEAR: A fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* **30**: 614–620.

Zhou J, Ning D. **2017**. Stochastic community assembly : Does it matter in microbial ecology? *Microbiology and Molecular Biology Reviews* **81**: 2-17.

Supplementary Materials

Table S1. Most abundant fungal species found as endophytes in seeds of sessile oak. Seeds were collected in the tree canopy or on the ground and were surface sterilized. The fruit wall and embryo were separated and their fungal community was analyzed using a metabarcoding approach. Only Amplicon sequence variants (ASV) assigned to Ascomycota (A) or Basidiomycota (B) with the UNITE database were kept. Average relative abundances of all ASVs were computed for each sample type, after merging ASVs assigned to the same fungal species. Only ASVs identified at the species level are shown in the table.

Fungal species	**Average relative abundance (%)**			**Lifestyle**	**Reference(s)**
	All internal tissues	Embryos	Fruit walls		
Curvibasidium cygneicollum (B)	8.7	6.8	10.4	Endophyte of fruits, leaves, trunk and soil behaving as a phytopathogen or a mycoparasite. The species is insensitive to the mycocins produce by *Filobasidium* and *Cystofilobasidium*.	Sampaio *et al.* (2004); Mašínová *et al.* (2017)
Epicoccum nigrum (A)	6.1	7.2	5	Ubiquitous fungus found in soil, leaves and seeds described as primary saprotroph and plant pathogen.	Ahumada-Rudolph *et al.* (2014)
Mycosphaerella tassiana (A)	4.7	1	8.2	Common pathogen found in the phyllosphere including that of oak.	Schubert *et al.* (2007); Jakuschkin *et al.* (2016)
Gibberella baccata (A)	4.5	4.5	4.5	Worldwide pathogen found in the phyllosphere and rarely found in seeds or soils.	Douglas Boyette & Lynn Walker (1986); Desjardins, (2003)
Cladosporium delicatulum (A)	3.2	3.5	3	Found in cereal seeds, mycoparasite of *Taphrina spp* and *Magnaporthe oryzae*.	Amanelah Baharvandi & Zafari (2015) ; Chaibub *et al.* (2016)
Caliciopsis beckhausii (A)	1.9	1.7	2	Most species of the genus are saprotrophs or parasites growing on bark of *Quercus* sp.	Garrido-Benavent & Perez-Ortega (2015)
Rhodosporidiobolus colostri (B)	1.5	0.7	2.2	Ubiquitious yeast behaving as a fungal antagonist.	Golubev and Tomashevskaya (2009)
Angustimassarina acerina (A)	1.4	2.4	0.5	Found on twigs of *Acer platanoides* and in bioaerosols.	Thambugala (2015); Banchi *et al.* (2018)
Penicillium paczoskii (A)	1.1	1.1	1.1	Pathogen causing post-harvest fruit rots recovered from harvested seeds.	Palou *et al.* (2010)
Taphrina carpini (A)	1	1	1.1	Common pathogen encountered on *Fagaceae* leaves.	Bacigálová (1991); Inácio *et al.* (2004); Cordier *et al.* (2012)

Table S2. Permutational multivariate analyses of variance (PERMANOVA) of compositional dissimilarities among fungal communities of seeds of sessile oak. Dissimilarities among seeds were estimated using the Jaccard quantitative distance. The total number of sequences per sample (sequencing depth, SD) was log-transformed and introduced as the first explanatory variable in all models. The effects of tree population (T), seed position (canopy *versus* ground, P) and their interaction were tested on the whole acorn dataset while the effect of mother tree was tested separately on canopy seeds and ground seeds.

	d.f.	F-value	*P*-value	R^2
All seeds				
Log(SD)	1	1.3	0.018	0.01630
Tree population (T)	3	2.1	**<.001**	0.07967
Seed position (P)	1	1.8	**<.001**	0.02240
T x P	3	1.2	**<.01**	0.04619
Seeds in the canopy				
Log(SD)	1	1.0	0.626	0.02588
Mother tree	10	1.2	**0.011**	0.30870
Seeds on the ground				
Log(SD)	1	1.1	0.238	0.02935
Mother tree	11	1.2	0.150	0.34904

Table S3. Response of fungal ASVs to site elevation and seed position depending on their lifestyle, in the HMSC presence-absence (PA) and sequence count (SC) models. In the PA model, values are the posterior probabilities that the community weighted mean specialization, or the proportion of pathogens or saprotrophs is higher in the reference modality of the ecological factor. In the SC model, values are the posterior probabilities that the community weighted mean specialization, or the abundance of pathogens or saprotrophs (conditional on presence) is higher in the reference modality of the ecological factor. Probabilities higher than 0.9 are in bold.

		PA model			SC model		
Ecological factor	Reference modality	Specialization	Pathogen	Saprotroph	Specialization	Pathogen	Saprotroph
Elevation	High	**0.96875**	0.57875	**0.97825**	0.71475	0.84475	0.75225
Seed position	Ground	0.78575	**0.92525**	**0.9225**	0.38525	0.42925	0.33575

Table S4. Nestedness and turnover components of compositional dissimilarities between fungal communities of seeds in the canopy and seeds on the ground. Turnover values represent the replacement of fungal species between the canopy and the ground, whereas nestedness values represent species loss or gain between the canopy and the ground. Compositional dissimilarities among fungal communities were estimated for each mother tree using the binary Jaccard dissimilarity index and partitioned with the *beta.temp* function of the betapart package (Baselga et al. 2018). A mother tree at the Bager site was excluded from the analysis because it had only two seeds on the ground.

		Dissimilarity between ground seeds and canopy seeds		
Tree population	**Mother tree**	**Turnover**	**Nestedness**	**Total**
Ade	33	0.75	0.06	0.81
	34	0.78	0.09	0.86
	55	0.85	0.08	0.92
Bager	11	0.66	0.09	0.75
	13	0.54	0.35	0.90
Gabas	32	0.62	0.17	0.79
	37	0.84	0.03	0.87
	40	0.76	0.08	0.84
Gedre bas	23	0.77	0.13	0.90
	24	0.91	0.01	0.92
	28	0.84	0.05	0.89

Table S5. Number and percentage of fungal ASVs of seeds on the ground also found in seeds in the canopy and mother tree aboveground tissues. A mother tree at the Bager site was excluded from the analysis because it had only two acorns on the ground.

Site	Mother tree	Total number of ASVs in seeds on the ground	Number (%) of ASVs in seeds on the ground shared with	
			Seeds in the canopy	Mother tree aboveground tissues
Ade	33	35	14 (40%)	17 (48%)
	34	142	26 (18%)	30 (21%)
	55	109	11 (10%)	44 (40%)
Bager	11	121	39 (32%)	59 (48%)
	13	247	27 (10%)	58 (23%)
Gabas	32	101	26 (25%)	51 (50%)
	37	67	18 (26%)	30 (44%)
	40	169	37 (21%)	56 (33%)
Gedrebas	23	105	12 (11%)	30 (28%)
	24	42	6 (14%)	11 (26%)
	28	102	16 (15%)	25 (24%)

Table S6. Fungal ASVs positively associated with another other ASV at the seed level according to HMSC models. Putative functions were inferred using FUNGuild and literature search. Only ASVs assigned at the species level are shown. The network of positive associations is represented on Figure 4.

Fungal species	Guild inference		Lifestyle	References
	FUNGuild	Literature		
Caliciopsis beckhausii	Pathotroph	Plant pathogen	Most species of the genus are saprotrophs or parasites growing on bark of *Quercus* sp.	Garrido-Benavent & Perez-Ortega (2015)
Cladosporium delicatulum	Unknown	Seed endophyte Putative mycoparasite	Found in cereal seeds, mycoparasite of *Taphrina spp* and *Magnaporthe oryzae*.	Amanelah Baharvandi & Zafari (2015) ; Chaibub *et al.* (2016).
Curvibasidium cygneicollum	Unknown	Generalist ubiquitous Putative mycoparasite	Endophyte of fruits, leaves, trunk and soil behaving as a phytopathogen or a mycoparasite. The species is insensitive to the mycocins produce by *Filobasidium* and *Cystofilobasidium*.	Sampaio *et al.* (2004); Mašínová *et al.* (2017)
Cylindrium elongatum	Unknown	Fungal antagonist Ubiquitous	Bacterial and fungal antagonist found on oak leaves.	Reyes-Estebanez (2011) ; Duarte *et al.* (2015)
Cystofilobasidium capitatum	Unknown	Seed endophyte Putative mycoparasite	Ubiquitious yeast which colonize the cotyledon after germination of english oak. The genus *Cystofilobasidium* have hyphal structures with haustoria only in the dikaryotic phase suggesting mycoparasites capacities.	Oberwinkler *et al.* (1983); Isaeva *et al.* (2009); Bills, Muller & Foster (2004)
Epicoccum nigrum	Pathotroph	Plant pathogen	Ubiquitous fungus found in soil, leaves and seeds described as primary saprotroph and plant pathogen.	Ahumada-Rudolph *et al.* (2014)
Filobasidium wieringae	Saprotroph	Generalist ubiquitous Putative mycoparasite	Yeast found in the phyllosphere. The genus *Filobasidium* have hyphal structures with haustoria only in the dikaryotic phase suggesting mycoparasites capacities.	Glushakova & Kachalkin (2017); Bills, Muller & Foster (2004)
Mycosphaerella tassiana	Pathotroph	Plant pathogen	Common pathogen found in the phyllosphere including that of oak.	Schubert *et al.* (2007); Jakuschkin *et al.* (2016)
Polyscytalum algarvense	Pathotroph	Plant pathogen	Necrotroph fungi found on *Eucalyptus* leaves.	Cheewangkoon *et al.* (2009); Crous (2018)
Rhodosporidiobolus colostri	Unknown	Fungal antagonist Ubiquitous	Ubiquitious yeast behaving as a fungal antagonist.	(Golubev & Tomashevskaya, 2009)
Sphaerulina amelanchier	Symbiotroph	Leaf and litter endophyte	Found in leaf litter of *Amelanchier*, *Betula*, *Castanea* and *Quercus*.	Quaedvlieg *et al.* (2013)
Taphrina carpini	Pathotroph	Plant pathogen	Common pathogen encountered on *Fagaceae* leaves.	Bacigálová (1991); Inácio *et al.* (2004); Cordier *et al.* (2012)
Trichomerium foliicola	Symbiotroph	Leaf epiphyte	Foliar epiphytes. *Trichomerium* is a genus apparently gaining their nutrients from insect exudates.	Chomnunti *et al.* (2012)
Vishniacozyma victoriae	Unknown	Generalist ubiquitous Fungal antagonist	Microbial antagonist of *Penicillium expansum* and *Botrytis cinerea*. Ubiquitous yeast able to tolerate stressful environments.	Pertot *et al.* (2017); Santiago *et al.* (2016)

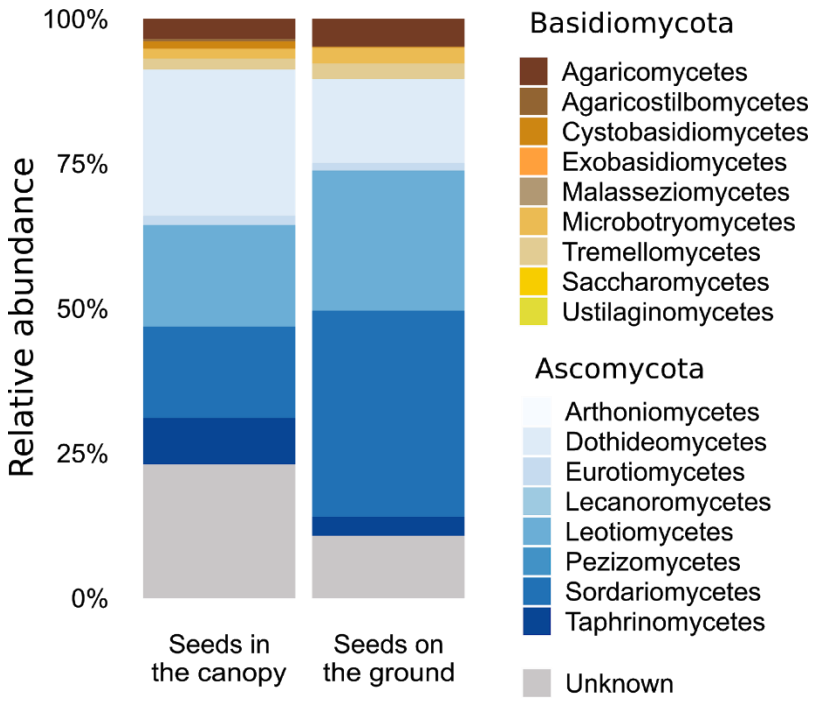


Figure S1. Taxonomic composition of fungal communities of seeds of sessile oak. The barplot indicates the average sequence percentage assigned to each fungal class.

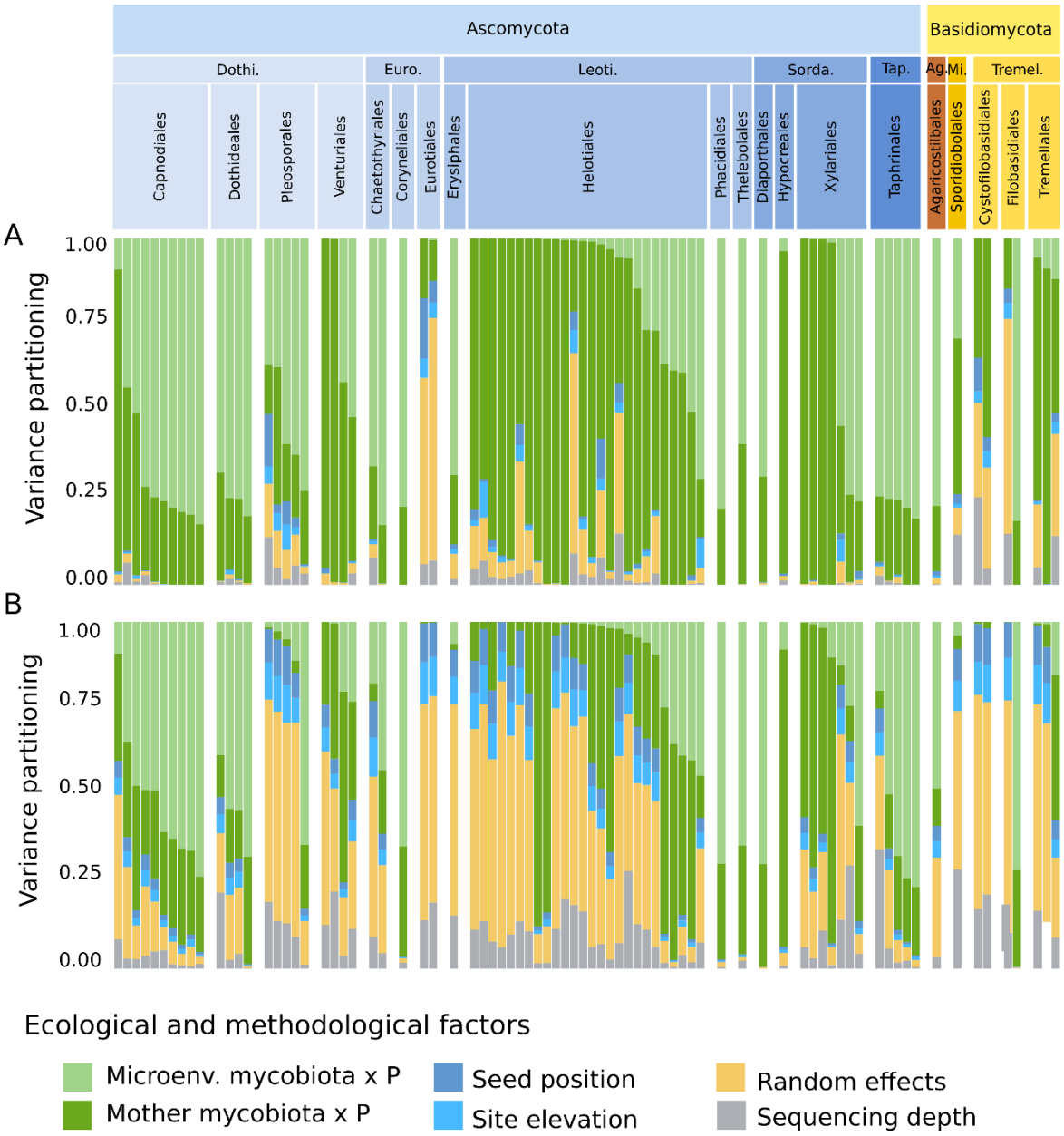


Figure S2. Partitioning of the variance in the composition of seed fungal communities of sessile oak. Four fixed effects were included in the HMSC models to explain variations in (A) the presence-absence or (B) the sequence count of a focal fungal ASV among seed samples: *Sequencing depth* (total number of sequences per sample), *Seed position* (P, canopy or ground), *Microenv. mycobiota* (relative abundance of the focal ASV in the seed microenvironment), *Mother mycobiota* (relative abundance of the focal ASV in the mother tree aboveground tissues), and *Site elevation*. *Mother mycobiota* and *Microenv. mycobiota* were introduced in interaction with P. Random effects were included at each spatial scale (seed, tree and population). Results of variance partitioning are given as percentages (%) of total explained variance. ASV are ranked by fungal phylum, class and order (Dothi: *Dothideomycetes*, Euro: *Eurotiomyecetes*, Leo: *Leotiomyecetes*, Sorda: *Sordariomycetes*, Tap: *Taphrinomycetes*, Ag: *Agaricostilbmoyecetes*, Mi: *Microbotryomycetes*, Tremel: *Tremellomycetes*). Only ASVs assigned at the order level are shown.

IV Quantitative and qualitative assessment of bacterial fluxes among soil, plant phyllosphere and near-surface atmosphere

Tania Fort[1], Corinne Vacher[1], Julie Faivre d'Arcier[1], Céline Lalanne[1], Emilie Chancerel[1], Jean-Marc Bonnefond[2], Didier Garrigou[2], Frédéric Delmas[3], Yves Brunet[2]

1. BIOGECO, INRA, Univ. Bordeaux, 33615 Pessac, France
2. ISPA, INRA, 33140 Villenave d'Ornon, France
3. BFP, INRA, 33140 Villenave d'Ornon, France

Abstract

Epiphytic microbes colonizing plant leaf surfaces represent important sources of airborne microorganisms. As their emission into the atmosphere can trigger the formation of clouds and precipitation, they play a crucial role in climate regulation especially in the water cycle. However, estimates of bioaerosol emission rates vary widely, and the temporal and spatial variations in the emission fluxes are poorly understood. These uncertainties complicate the identification of local sources of bioaerosol emissions and their composition. In this context, we assessed simultaneously bacterial fluxes and their composition over two land covers during two days each, using a measurement system based on the flux-gradient method. The composition of airborne communities was compared to leaf and soil bacterial communities in order to assess their similarity over the day at the regional and local scale. Our results demonstrate that airborne communities represent a heterogeneous pool of bacterial species of which half of the species are present on the leaf surface and the soil. Our results also suggest that the composition of bioaerosols is strongly influenced by a widely cultivated plant in New Aquitaine, the vine (*Vitis vinifera*).

Introduction

The surface of plant leaves, or phyllosphere, contains a wide variety of microorganisms including bacteria, filamentous fungi, yeasts and, to a lesser extent, archaea (Lindow & Brandl, 2003; Vorholt, 2012; Turner *et al.*, 2013; Hardoim *et al.*, 2015; Vacher *et al.*, 2016; Compant *et al.*, 2019). Among these microorganisms, many can be aerosolized. This aerosol matter of biological origin, referred to as bioaerosols (Morris *et al.*, 2014b), represents an important fraction of the particles suspended in the atmosphere, reaching up to 25% of the particles in the size range 0.2–50.0 μm (Fröhlich-Nowoisky *et al.*, 2016). Bacteria are among the most abundant organisms in bioaerosols, with a concentration ranging from 10^4 to 10^8 cells per m^3 of air (Lange *et al.*, 1997; Bauer *et al.*, 2003; Burrows & Butler, 2009; Després *et al.*, 2012). They play an important role in Earth functioning because bacteria emitted by the land surface to the atmosphere may influence atmospheric chemistry, trigger the formation of cloud condensation nuclei (CNN) and ice nuclei (IN), and thereby mediate cloud formation and precipitation (Bauer *et al.*, 2003; Mortazavi *et al.*, 2008; Morris *et al.*, 2014a). In turn, airborne bacteria deposited on the land surface influence the assembly trajectory of phyllosphere microbial communities (Lindow & Brandl, 2003; Laforest-Lapointe *et al.*, 2017) and therefore microbially-mediated plant functional traits (Friesen *et al.*, 2011).

In addition to the phyllosphere, the upper soil and the litter can contribute significantly to bacterial emissions (Lindemann *et al.*, 1982; Lighthart & Shaffer, 1994; Lymperopoulou *et al.*, 2016). The relative contribution of soil and leaves is still debated: it depends on abiotic factors such as temperature (Harrison *et al.*, 2005; Barberán *et al.*, 2015), relative humidity, solar radiation (Jones & Harrison, 2004), wind speed (Upper & Hirano, 1991; Lighthart & Shaffer, 1995; Lymperopoulou *et al.*, 2016) and rainfall events (Joung *et al.*, 2017), and on biotic factors including bacteria size (Després *et al.*, 2012; Wolf *et al.*, 2017; Wei *et al.*, 2019), generation and residence time (Womack *et al.*, 2010). These biotic and abiotic factors trigger spatial and temporal variations in bioaerosol composition and concentration (Harrison *et al.*, 2005; Bowers *et al.*, 2010; Striluk *et al.*, 2017). For instance, bioaerosols vary with vegetation type (Lymperopoulou *et al.*, 2016; Mhuireach *et al.*, 2016), the intensity of human activities (Fang *et al.*, 2014) and local climate (Jones & Harrison, 2004; Rossi et al., 2005; Klarić and Pepeljnjak, 2006).

Reliable, temporal *in situ* measurements of bacterial atmospheric flux (number of bacteria emitted or deposited m^{-2} s^{-1}) are required to estimate net bacterial emission by the surface and better understand the relative contribution of plant leaves and soil. Surprisingly, there have been very few

attempts so far at performing direct measurements of bacteria flux between the surface and the atmosphere. This was achieved by combining vertical gradients of bacteria concentrations (CFU m^{-3}) in the atmosphere with gradients of wind velocity, air temperature or other micrometeorological variables: Lindemann *et al.* (1982) provided estimates of the vertical bacteria flux over several surface types (winter wheat, bean, alfalfa, bare soil). However, their results were limited to a unique flux value for each of these surfaces, taken as the average of 4 to 8 sequential samples of 10 to 30 min duration each, collected on a single day. Lighthart & Shaffer (1994) later showed a set of 1-hr flux estimates measured during 4 days on a tower located at a desert site with sparse vegetation. Their results, plotted on a single graph, revealed a large run-to-run variability, with positive and negative flux values during the day, and no clear pattern. Chen *et al.* (2001) investigated bacteria fluxes measured at 30 and 50 m on a tower located at an urban site. Most of the ten values showed were negative, indicating an apparent net deposition of bacteria. More recently, Carotenuto et al. (2017) performed flux measurements over a Mediterranean grassland and showed time series of fluxes on a few summer days. In order to increase our knowledge of bacterial emission processes, such scarcity of bacterial flux measurements encourages us to carry out new observation campaigns.

In this study we performed quantitative and qualitative assessments of bacterial emissions in the near-surface atmosphere by combining flux measurements made using a micrometeorological method with barcoding and metabarcoding approaches. The objectives were to analyze the daily variations of bacterial community composition in upper soil, plant leaves and near-surface atmosphere, and estimate the intensity and origin of bacterial emissions throughout the day. We focused on two seasons (summer and autumn) and two ecosystems (a vineyard and a grasslang site). Several hypotheses were specifically tested: (**H1**) airborne bacterial communities are more diverse than phyllosphere or soil communities (because they are a mixture of bacteria from various environmental sources); (**H2**) the composition of airborne bacterial communities vary with season and land cover but (**H3**) it is more heterogeneous than that of leaves and soil; (**H4**) the similarity between airborne bacterial communities and that of environmental sources (leaves, soil) increases with bacterial emissions over the day.

Materials & methods

Bacterial flux measurements

As mentioned previously, bacterial flux measurements have rarely been reported in the literature. In all previous studies, the bacterial flux F_b was deduced from a vertical difference in bacterial concentration and additional micrometeorological measurements. Lindemann *et al.* (1982) used simple flux-profile relationships in the atmospheric surface layer to estimate F_b as a function of the differences in concentration and mean wind velocity measured between two levels. However, they did not account for the influence of stability forces, which may affect significantly the exchange coefficient, and their equation is only valid if both wind speed and bacterial concentrations are measured at the same levels, which may not always be the case. Carotenuto et al. (2017) used an improved version of this method, which accounts for stability forces. It has to be mentioned that this flux-gradient method is only valid in conditions were the field is large enough for the classical flux-profile relationships to apply. Lighthart & Shaffer (1994) and Chen *et al.* (2001) preferred to bypass the latter requirement by using the so-called "Bowen ratio" method, based on a similarity hypothesis between heat and particle transport. In this method, the bacterial flux is equal to the product of the concentration difference with the ratio between the sensible heat flux and the temperature difference measured between the two sampling levels. This method is simpler when sensible heat flux measurements can be performed (which has become easily feasible with sonic anemometry), but the quality of the flux estimate directly depends on the accuracy with which the temperature difference between the two levels is measured. It cannot be used in neutral conditions (e.g. early morning, late afternoon, conditions with high wind speed and low radiation), where this temperature difference vanishes.

Nowadays, the most widely used method for flux measurements is the "eddy-covariance" method, which combines high-frequency measurements (at least 10 Hz, typically) of concentration and wind velocity components. However, this method cannot be used for bacteria by lack of sensors able to measure concentration at such short time intervals. A possible alternative would be to use the so-called "eddy-accumulation" method, which associates high-frequency wind velocity measurements and low-frequency concentration measurements. A prototype of such a system was tested during the experiment reported here, but the results will be the object of a separate, methodological article. In the present study, we used a more conventional approach based on flux-profile relationships, which can be considered as an improved version of the method used by Lindemann *et al.*, (1982), close to that of Carotenuto *et al.* (2017).

Here, the vertical bacterial flux F_b (N_{CFU} m^{-2} s^{-1}), defined as positive when it leaves the surface, is calculated as:

$$F_b / \Delta C_b = - k u_* [\ln(z_1/z_2) - \psi_b(z_1/L) + \psi_b(z_2/L)]^{-1}$$

where ΔC_b is the concentration difference (N_{CFU} m^{-3}) between the lower (1) and upper (2) concentration measurement levels, k is the von Kármán constant taken as 0.4, u_* is the friction velocity (m s^{-1}) , z_1 and z_2 are the heights (above the zero-plane displacement) of the measurement levels (m), ψ_b is a non-dimensional stability function for scalars (see for instance Garratt, 1992). L is the Obukhov length (m) defined as:

$$L = - u_*^3 / [k (g/T) (H/\rho c_p)]$$

where g is the acceleration due to gravity (m s^{-2}), T is the mean air temperature (K), H is the sensible heat flux (W m^{-2}), ρ is the air density (kg m^{-3}), and c_p the specific heat capacity of air (J kg^{-1} K^{-1}). For the ψ_b functions we use the classical form: for $z/L < 0$ $\psi_b = 2 \ln[(1+x^2)/2]$ where $x = (1 - 16 z/L)^{¼}$, and for $0 < z/L < 1$: $\psi_b = -5 z/L$.

This method requires a sonic anemometer to measure u_* and H, as well as air samplers for collecting bacteria.

Study sites and instrumentation

All measurements were carried out in summer and autumn 2017 in a grassland (fallow) field and a vineyard located near Bordeaux in the South-West of France. The vineyard site (200 m x 140 m) was located at the INRA experimental station of La Grande Ferrade (44°47'31'' N, 0°34'35'' W, Villenave d'Ornon, France). The grassland site (280 m x 100 m) was an unmanaged field mainly colonized by *Trifolium sp.* and *Lolium perenne* located at the INRA experimental station of L'Hermitage (44°44'30'' N, 0°47'60'' W, Cestas-Pierroton, France). Mean canopy height was measured as 1.60 m in the vineyard, and 0.20 m in the grass field. Two days of sampling were performed for each ecosystem: July 4 and October 12 for the grassland, and July 11 and October 6 for the vineyard. These sampling dates were chosen so as to operate under fair weather conditions, with wind directions allowing a long enough fetch for the micrometeorological measurements.

On each day of sampling, a measurement mast was set up in the center of the study site. The mast was equipped with a sonic anemometer (Campbell CSAT3B) for measuring wind velocity as well as momentum and sensible heat flux, and two impactors (SKC BioStage single-stage cascade impactor) for collecting bacteria on Petri dishes (containing LB media with cycloheximide at 50 mg/L) and providing a vertical gradient of air concentration in cultivable bacteria. For the lower concentration, the sonic anemometer and the upper concentration, the measurement heights were 1.65 m, 2.31 m

and 3.58 m, respectively, over the vineyard; 0.52 m, 1.19 m and 2.45 m for grassland in summer; and 0.77 m, 1.43 m and 2.70 m for grassland in autumn. On each sampling day, two filter holders (Swinner 47 Millipore) were set up at the same levels as the bioimpactors, allowing additional air samples to be collected on sterile cellulose filters (Advantec, mixed cellulose ester membrane, pore size 0.45 μm). Air temperature was also measured next to the bioimpactors using shielded thermocouples, and at each site a nearby meteorological station provided ancillary meteorological variables of interest.

Sampling design

Petri dishes were inserted in the bioimpactors every hour, and exposed to a flow rate of 28,4 l min^{-1}) for an hour. They were then collected and replaced by new ones. The dishes were sealed in a plastic film and stored in a cooler for the rest of the day. They were then incubated for four days at about 25°C. After incubation, a photograph of each dish was taken through a microscope, and the number of colonies formed were later determined. Control samples were taken before and after each observation period, using the same protocol as for the "true" ones, but with no exposure to the incoming flow.

The filters were collected and replaced every two hours. Only on the first day (July 4), they were changed every hour but the flow rate through the filters (6.8 l min^{-1}) was found insufficient to ensure good enough sampling. They were collected with gloves, wrapped in a plastic film and stored in a cooler for later processing.

In between each changeover (on average 14 minutes after each filter collection), plant leaves, litter and upper soil were sampled in four plots distant of 2 m from one another. At each sampling time, plots were chosen in the upstream region of the mast to maximize the probability that the bacteria emitted by the plots be captured by the air samplers. The distance between each plot and the mast was approximately 10 m in the vineyard, and 30 m in the grassland. The position of the plots changed at each sampling time on July 11, October 6 and October 11 because wind direction changed over the day, while the same plots were sampled every hour on July 4. All grassland plots measured approximately 1 m x 1 m. In each of them, 10 leaves of *Trifolium sp.*, 10 leaves of *Lolium perenne* and 25 mL of a mixture of litter and upper soil were collected. Each vineyard plot corresponded to four stocks (two vine stocks on two vine rows). On each stock, three vine leaves (one from the top of the canopy, one from the middle, and one from the bottom) and 25 mL of a

mixture of litter and upper soil were collected. All samples were collected with gloves in sterile Falcon tubes, stored in a cooler during transportation in the field and then stored at -20°C until further processing. Filters were also stored at -20°C. On July 4, two of the Petri dishes impacted for flux measurements (between 13:14 and 14:14) were later used for DNA extraction, after incubation and CFU counting. A total of 113 bacterial colonies were then collected from the two plates and placed in autoclaved Eppendorf tubes with sterilized tools. The strains were stored at -80°C until extraction.

Barcoding and metabarcoding of airborne bacteria

Barcoding of isolated strains

Each bacterial strain isolated on July 4 was resuspended in 100 µL of sterile water and 20 µL of the solution was boiled during 20 min at 99°C. The V1-V6 region of the bacterial 16S was amplified using A1-1115R primers (A1: GAGTTTGATCATGGCTCAG; Manceau & Horvais, 1997; 1115R: AGGGTTGCGCTCGTTG; Redford *et al.*, 2010). The PCR reaction mixtures (25 µL of final volume) contained 5 µL of Green Taq buffer 5X, 0.25 µL of GoTaq Flexi DNA Polymerase 5U (Promega), 1.5 µL of MgCl2 at 25 mM, 2µL of dNTP at 2.5 mM, 0.5 µL of each primer at 20 µM, 2.5 µL of DNA template and water up to 25 ml. PCR cycling reactions were conducted on a Veriti 96-well Thermal Cycler (Applied Biosystems) using the following conditions: initial denaturation at 94°C for 5 min followed by 35 cycles at 94°C for 30 s, 59°C for 45 s, 72°C for 2 min with final extension of 72°C for 10 min. 16S amplification was confirmed by electrophoresis on a 2% agarose gel. The PCR products were purified and subjected to Sanger sequencing at the Genewiz facility (Beckman Coulter Genomics, UK). Out of the 218 forward and reverse sequenced, 138 were trimmed and assembled to produce 69 contigs using Geneious Prime 2019.2.1 (Kearse *et al.*, 2012). Taxonomic assignments of the consensus sequences were performed by megablast against NCBI nucleotide database. Only sequences assigned at the species rank were kept.

Metabarcoding of environmental samples

Air filters were cut in four quarters under the microbiology hood with sterilized clamps and scissors. Two quarters of each filter were then cut in small pieces in two autoclaved Eppendorf tubes. Epiphytes were collected from both sides of the leaves by rubbing them with a piece of autoclaved paper filter measuring 1 cm by 1 cm previously soaked in sterile water. Leaf rubbing was performed under the hood with gloves and sterile tools, by placing the leaves on autoclaved paper

sheets. All leaves of the same sampling plot were rubbed with the same piece of paper, which was then cut into smaller pieces into an Eppendorf tube. Soil samples were immersed in 40 mL of cold (4°C) PBS-Tween, agitated by hand and sonicated during 480 s at 42,000 Hz. The solution was then poured into a sterile nylon cell strainer (Dutscher, pore size 100 µm) to remove soil aggregates. The filtered solution was then centrifuged for 20 min at 4000 g and the supernatant was removed using a 25 mL pipette with a PipetBoy (Integra Biosciences, Fernwald, Germany). The remaining solution (corresponding to approximately 2 mL) was transferred into an autoclaved Eppendorf tube, which was then centrifuged during 5 min at 10 000 g. The supernatant was removed, and all samples were stored at -80°C until DNA extraction.

Total DNA was extracted using the PowerSoil 96 well DNA isolation kit (MoBio Laboratories, Carlsbad, CA). Transfer of filter samples into the bead beating plate containing the lyse buffer was performed under the microbiology hood with sterile tools. The well plate was heated during ten minutes at 65°C and shaked during 2 min. Soil and leaf samples were first re-suspended in 500 µL of lyse buffer to facilitate transfer. DNA extracts were eluted a first time with 60 µL of sterile water and a second time with the first elution solution. The C1 solution was preheated at 60°C before transfer and well plates were shake at 800 rpm for 10 min using a Grinder. The other steps were performed accordingly to the manufacturer's protocol. The V5-V6 region of the bacterial 16S rDNA gene was amplified using 16S primers 799F-1115R (Chelius & Triplett, 2001; Redford *et al.*, 2010) to exclude chloroplast DNA. To avoid a two-stage PCR protocol and reduce sequencing biases, each primer contained the Illumina adaptor sequence, a tag and a heterogeneity spacer, as described in Laforest-Lapointe *et al.*, (2017) (799F: 5'-CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTxxxxxxxxxxxxHS-AACMGGATTAGATACCCKG-3'; 1115R: 5'-AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTxxxxxxxxxxxxHS-AGGGTTGCGCTCGTTG-3', where HS represents a 0–7-base-pair heterogeneity spacer and "x" a 12 nucleotides tag). The PCR mixture (20 µL of final volume) consisted of 2 µL each of the forward and reverse primers (1 µM), 10 µL of 2X QIAGEN Multiplex PCR Master Mix, 1 µL of 10 mg ml^{-1} BSA, template volumes ranging from 1 to 5 µL according to the sample type (1 µL of soil, 2 µL of leaf and 5 µL of filter templates) and water up to 25 µl. PCR cycling reactions were conducted on a Veriti 96-well Thermal Cycler (Applied Biosystems) using the following conditions: initial denaturation at 95°C for 15 min followed by 30 cycles at 94°C for 30 s, 57°C for 90 s, 72°C for 90 s with final extension of 72°C for 10 min. 16S amplification was confirmed by electrophoresis on a 2% agarose gel. PCR were performed twice on each filter template to ensure sequence recovery after Illumina

sequencing. Two marine bacterial strains (*Sulfitobacter pontiacus* and *Vibrio splendidus*) were used as positive controls as they were unlikely to be found in our samples. One positive control included 1 µL of 10 ng μL^{-1} DNA of *Vibrio splendidus* only and the other included an equimolar mixture of both strains. Three negative controls were represented by PCR mix without any DNA template. PCR products were purified, quantified (Invitrogen Quant-it dsDNA assay kit) and equimolarly pooled (Hamilton Microlab STAR robot). The average size fragment was checked using Tapestation instrument (Agilent Technologies). Libraries were sequenced on the Miseq Instrument (Illumina) with the reagent kit v2 (500-cycles). Sequence demultiplexing (with exact index search) was performed at the PGTB sequencing facility (Genome Transcriptome Facility of Bordeaux, Pierroton, France) using DoubleTagDemultiplexer.

MiSeq sequencing degenerated primers were removed from bacterial 16S sequences using *cutadapt* (Martin, 2011). Only sequences with less than two expected errors and longer than 50 bp were retained in the dataset. Quality-filtered reads were assembled into Amplicon sequence variants (ASVs) using DADA2 v1.12.1 (Callahan *et al.*, 2016) and chimeric sequences were removed using the consensus method of the removeBimeras function. Taxonomic assignments were performed using the RDP classifier (Wang *et al.*, 2007) implemented in DADA2 and trained with the SILVA database v132 (Quast *et al.*, 2013), with an 80% confidence threshold. We used the Sanger sequences of isolated strains combined with the SILVA database v132 (Quast *et al.*, 2013) as a reference database. The ASV table was then imported in R using the phyloseq package v1.26.0 (McMurdie & Holmes, 2013) and filtered. Only ASVs assigned to a bacterial phylum were kept. When inspecting the reads found in negative controls, we found two ASVs assigned to *Janthinobacterium* and two other assigned to *Rhodoccocus* classes, present in two to three negative controls. These four ASVs were removed from the dataset as they represent PCR contaminants often detected in multiple amplification kits (Salter *et al.*, 2014). One sample, considered as highly contaminated by the two marine strains from the positive control next to it, was removed from the data set. Positive and negative controls were then used to remove contaminants from all environmental samples (as described in Galan *et al.* (2016). The cross-contamination threshold (TCC) was defined as the maximal number of sequences of each ASV found in negative or positive control samples. The false-assignment threshold (TFA) was defined as the highest sequence count of a positive control strain in a non-control sample, divided by the total number of sequences of the strain in the whole run and multiplied by the total number of sequences of each ASV. ASVs were removed from all samples where they harbored fewer sequences than either threshold (TFA or TCC). The four replicates of each air sample were pooled in the final ASV table. Functional assignation of all bacterial taxa

(Sanger and MiSeq sequencing) was obtained using the "functional annotation of prokaryotic taxa" (FAPROTAX) program on the ASV table (Louca *et al.*, 2016).

Statistical analyses

To investigate whether the airborne bacterial community retrieved by bacterial cultivation and Sanger sequencing was similar to the community retrieved by air filtering and MiSeq sequencing, the Sanger and the corresponding Illumina Miseq sequence datasets were visually compared using the package METACODER v.0.3.3 (Foster *et al.*, 2017), by representing two heat trees showing sequence counts across bacterial lineages.

Then, to investigate whether sequencing depth was sufficient to estimate and compare bacterial richness among samples, rarefaction curves were computed for all samples using the *ggrare* function from the ranacapa package (v.0.1.0). In order to test H1, generalized linear models (LM and GLM) were used to assess the effect of microbial habitat (soil *vs.* leaf *vs.* air, MH) on the taxonomic richness and diversity of bacterial communities, by including only the samples for which the rarefaction curves began to reach a plateau. The models included the microbial habitat as a fixed treatment effect, and the sampling depth (defined as the total number of raw sequences per sample) as an offset (Bálint *et al.*, 2014; McMurdie & Holmes, 2014). Community richness was defined as the number of ASVs per sample. Although count data often follows a Poisson or negative binomial distribution, in our study, species richness was better modelled using a linear regression model. Community diversity was measured with the inverse Simpson index (Simpson, 1949) and modelled with a Gaussian distribution and the logarithmic link function. Linear regression model and generalized linear model were performed using the *lm* and *glm* functions from the stats package (v.3.6.1). The offset was transformed according to the link function. The significance of the fixed treatment effect was finally assessed with the Wald χ^2 test (Bolker *et al.*, 2009). Post-hoc pairwise comparisons with Tukey's contrast method in the *multcomp* package (v.1.4.10) (Hothorn *et al.*, 2008) was performed to compare microbial diversity index across microbial habitats.

To test H2, the effects of land cover (LC) and season (S) on bacterial community composition for each microbial habitat were tested using permutational multivariate analyses of variance (PERMANOVAs) with 9999 permutations, performed with the *adonis* function of the R vegan package (Oksanen *et al.*, 2008) (Oksanen et al. 2017). Compositional dissimilarities among samples

were estimated using binary Jaccard index (Jaccard, 1901) and visualized with principal coordinate analyses (PCoA).

To test H3, the effect of microbial habitat (soil, leaf, air) on bacterial community heterogeneity was tested using the *betadisper* and *permutest* functions in the vegan package. Community heterogeneity (i.e. dispersion) was calculated as the distances between samples and the multivariate centroids in PCoAs ordination.

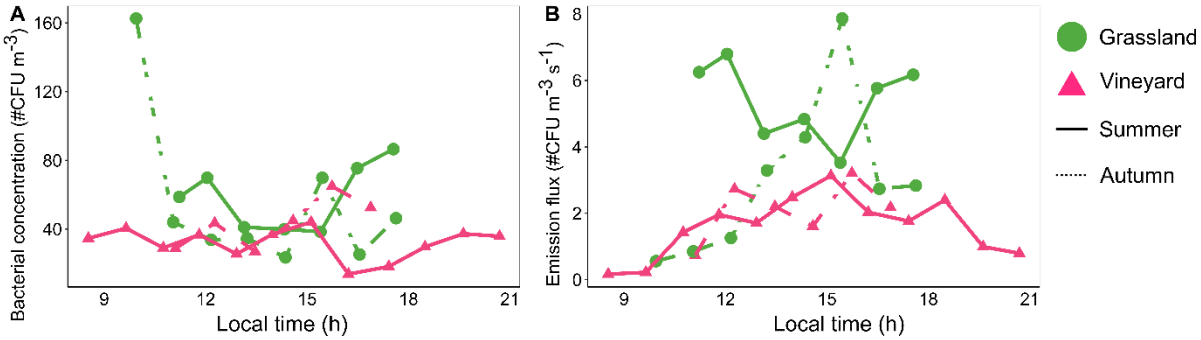
H4 was tested using the two most complete time series, which was collected in July in the vineyard and in October in grassland. Community similarity between airborne bacterial communities and the biosphere (leaf and soil) communities was defined as 1-*J* where *J* is the Jaccard binary index. The effect of bacterial fluxes on community similarity was assessed using GLM model with a binomial distribution. The bacterial fluxes (BF), environmental source (ES), the sampling campaign (C) and the interaction between all factors were implemented as fixed effects and post-hoc tests were performed as previously described.

Results

Bacterial concentrations and fluxes

At both sites, most concentrations range between 20 and 80 CFU m^{-3} (Figure 1A). No marked hourly trends were observed throughout the four days, except in the afternoon of the summer day in grassland. A rather high value of bacterial concentration (163 CFU m^{-3}) followed by a sudden drop was observed on the first measurement hour at the grassland site in autumn. All (positive) flux values showed that the exchange of bacteria between the crops and the atmosphere over the day results in a net emission flux (Figure 1B). Over the vineyard the emission flux was low in the early morning and late afternoon, and reached a maximum one or two hours after noon (solar time). Bacterial fluxes variations over the day in grassland showed opposite trends: on the summer day, bacterial fluxes were high in the morning, decreased during the afternoon and increased again at the end of the day. The autumn day was characterized by a very low flux that progressively increases to reach a maximum between 15:00 and 16:00 local time before it decreases.

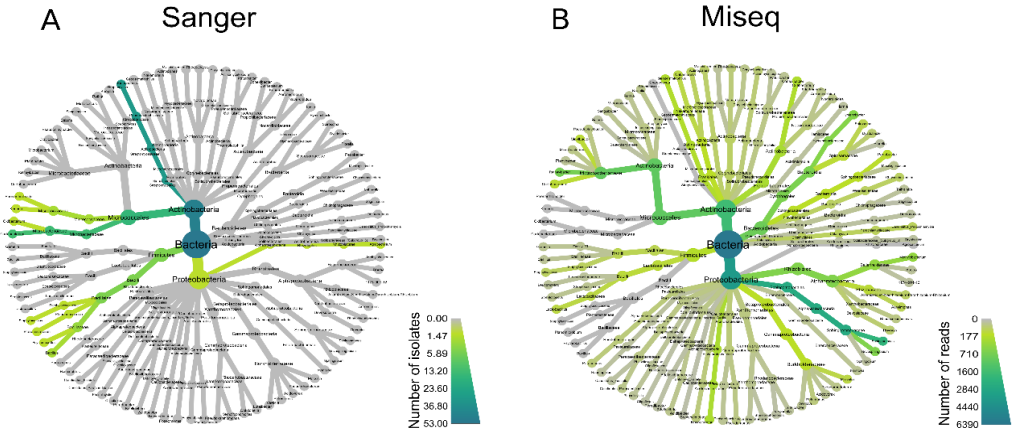
Figure 1. Time series of bacterial concentrations (A) and fluxes (B). Air samples were collected with two impactors placed on a micrometeorological mast allowing flow measurements using the flux-gradient technique. Hourly variations in bacterial concentrations and fluxes during four days were estimated above a grassland (green circles) and a vineyard (pink triangles) in summer (solid lines) and autumn (dotted lines). The hourly concentrations are the average values obtained from the two measurement levels.



Comparison of culture-dependent and culture-independent methods for bioaerosol analysis

Bacterial community composition differed greatly between culture-dependent (bacterial culture and Sanger sequencing) and culture-independent (air filtering and MiSeq sequencing) approaches (Figure 2). The number of bacterial species detected using the culture-independent approach was higher, with 182 bacterial species detected against only 19 bacterial species observed using the culture-dependent approach. Bacterial taxa overlapped at the genus but not at the species level.

Figure 2. Airborne bacterial composition detected with (A) Sanger and (B) MiSeq sequencing. Node size and color indicate the number of sequences across bacterial lineages retrieved from the bioaerosol samples at noon in the grassland ecosystem on July 4. Heat trees were plotted using Metacoder package v.0.3.3 (Foster *et al.,* 2017).



Taxonomic and functional composition of airborne bacterial communities

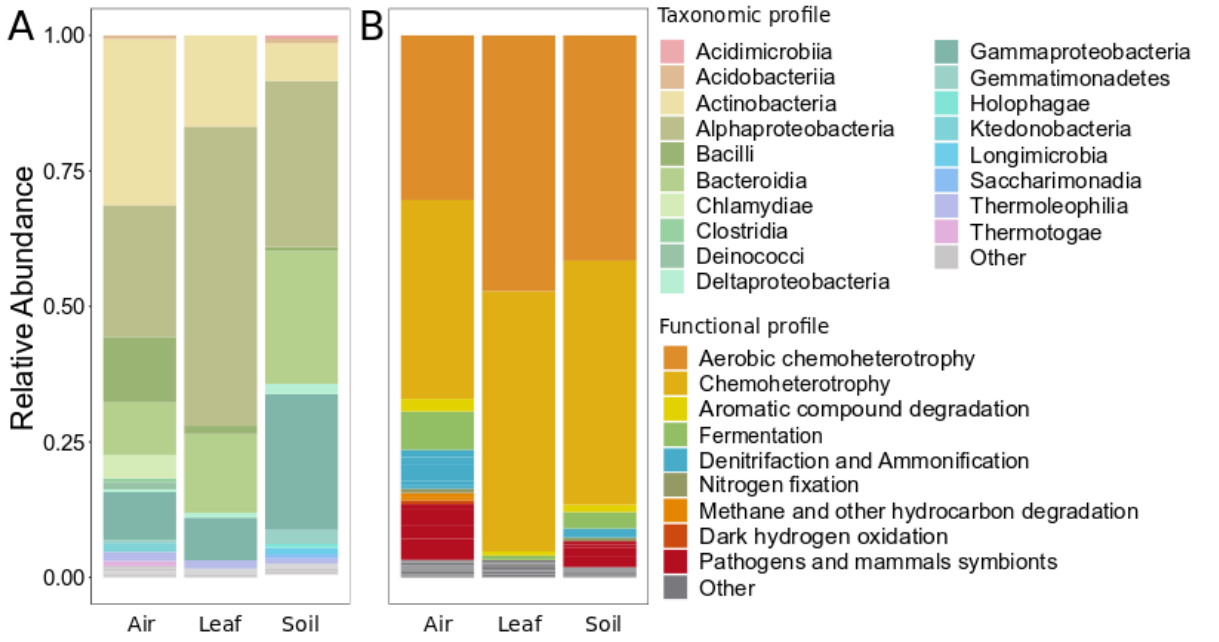
The sequence dataset contained 9,356,727 bacterial pairs of reads among which 12,121 bacterial sequences were unique. Of these bacterial ASVs, 97.43% were classified to the phylum level, 45.3% at the species rank and 23.21% were successfully assigned to at least one functional group using FAPROTAX. The ten most abundant bacterial genus in air samples represented a heterogeneous pool of taxa with human, plant, soil and outdoor associated taxa (Table 1). The most abundant bacterial genus, *Cutibacterium sp.*, was recently described as a grapevine obligate endophyte that was likely transferred from human to vine during the domestication of the latter around 7 500 years ago (Campisano *et al.*, 2014). Three additional human pathogens were identified (*Protochlamydia sp., Corynebacterium sp., Chryseobacterium sp.*). We also found bacterial species commonly associated to plants and soil, as well as extremophiles adapted to low or high temperatures (Table 1).

Table 1. The ten most abundant bacterial genus found in the air samples by applying metabarcoding approaches to air filters placed in grassland and vineyard ecosystems. Bacterial communities of leaves and upper soil (including litter) were analyzed simultaneously. Only bacteria identified at the genus level are shown in the table.

	Average relative abundance (%)					Lifestyle	References
Bacterial genus	**All filters**	**Filters**		**Leaf**	**Soil**		
		Grassland	**Vineyard**				
Cutibacterium sp.	9.9	8.4	11.6	$4.18.10^{-3}$	0	Human pathogen exhibiting opportunistic pathogenicity. Occasionally found in other animals, in the atmosphere and recently found as endophyte of grapevine plants (Vitis vinifera L.).	Nisbet (2007) ; Lyons (2009); Campisano (2014); Qiu (2018)
Sphingomonas sp.	9.3	9.8	8.6	0	0	Leaf and seed symbiont protecting against *Pseudomonas syringaei.*	Innerebner (2011); Vogel (2016)
Lactobacillus sp.	5.5	4.1	7.3	0	0	Convert sugars to lactic acid in human intestin and degrade hemicellulose during litter decomposition. Could also have direct biocontrol effects against *Erwinia amylovora* when associated to plants	Bernardeau (2008); Roselló (2013); Krishna & Mohan, (2017)
Protochlamydia sp.	5	8.9	0.3	0	0	Obligate intracellular bacteria, commonly described as human and animal pathogens and amoeba symbionts.	Sixt (2013)
Methylobacterium sp.	3.7	4.2	3.1	11.6	0.9	Metabolize methanol emitted by plants and promote seed germination, root development and the yield of agricultural plants by producing substances like auxins, cytokinins and vitamin B12.	Ivanova (2000, 2001, 2006); Trotsenko (2001); Ryu (2006); Lee (2007)
Corynebacterium sp.	3.5	2.5	4.7	0	0	Often identified as commensals bacteria associated to animals microbiota but can also be opportunistic pathogens	Collins (2004)
Staphylococcus sp.	3.2	2	4.7	0	0	Ubiquitous genera, isolated from inner seed tissues, leaves of several plant species	Odunbaku (2008); Truyens (2015); Jakuschkin (2016)
Hymenobacter sp.	3.1	3.7	2.4	6	3.6	Common inhabitants of the phyllosphere. Some members are radiation tolerant and psychrophilic or psychrotolerant.	Ottesen (2009); Leveau & Tech, (2011); Lee (2014); Su (2014); Leff *et al.*, (2015); Aydogan (2018)
Frigoribacterium sp.	2.4	0.2	5	0.6	0.1	Identified in the phyllosphere of various plant species especially grapevine	Perazzolli (2014); Copeland (2015); Compant (2019)
Chryseobacterium sp.	2.3	3	1.6	0	0	Opportunistic human pathogen detected in the atmosphere, plant roots, soil, seed and the phyllosphere of various plant species	Ottesen (2009, 2013); Barret (2015); Niu (2017); Cernava (2019); Yang (2019)

Airborne bacterial communities were dominated by *Actinobacteria* (30.6%), *Alphaproteobacteria* (24.3%) and *Bacilli* (12.0%) (Figures 3A). In comparison to air bacterial communities, leaves contained a higher proportion of *Alphaproteobacteria* (24.3% in air *vs.* 55.1% on leaves) and soils contained a higher proportion of *Gammaproteobacteria* (8.8% in air *vs.* 25.0% in soil). Whatever the compartment (air, leaves or soil), bacterial communities were dominated by aerobic and non-aerobic chemoheterotrophs (Figure 3B). Chemoheterotrophs represent organisms that obtain their source of carbon and energy by consuming organic molecules (Gu *et al.*, 2011), suggesting that bacteria present in the air, leaf surface and soil depend on the availability of organic compounds. The relative abundance of bacteria able to perform denitrification and ammonification, fermentation, and methane and other hydrocarbon degradation was higher in the air samples than in the plant and soil samples (Figure 3B).

Figure 3. Taxonomic and functional composition of bacterial communities and bacterial diversity of air, leaf and soil samples. The barplot indicates the average sequence percentage assigned to each (A) bacterial class and (B) functions observed in air, leaf and soil samples. Distance from group centroids (dispersion) was calculated using binary Jaccard distance (C) for each microbial habitat. Bacterial classes and functions present with a proportion of less than 0.005% have been grouped under the category "other". Bacterial functions were assigned using FAPROTAX (Louca *et al.*, 2016).



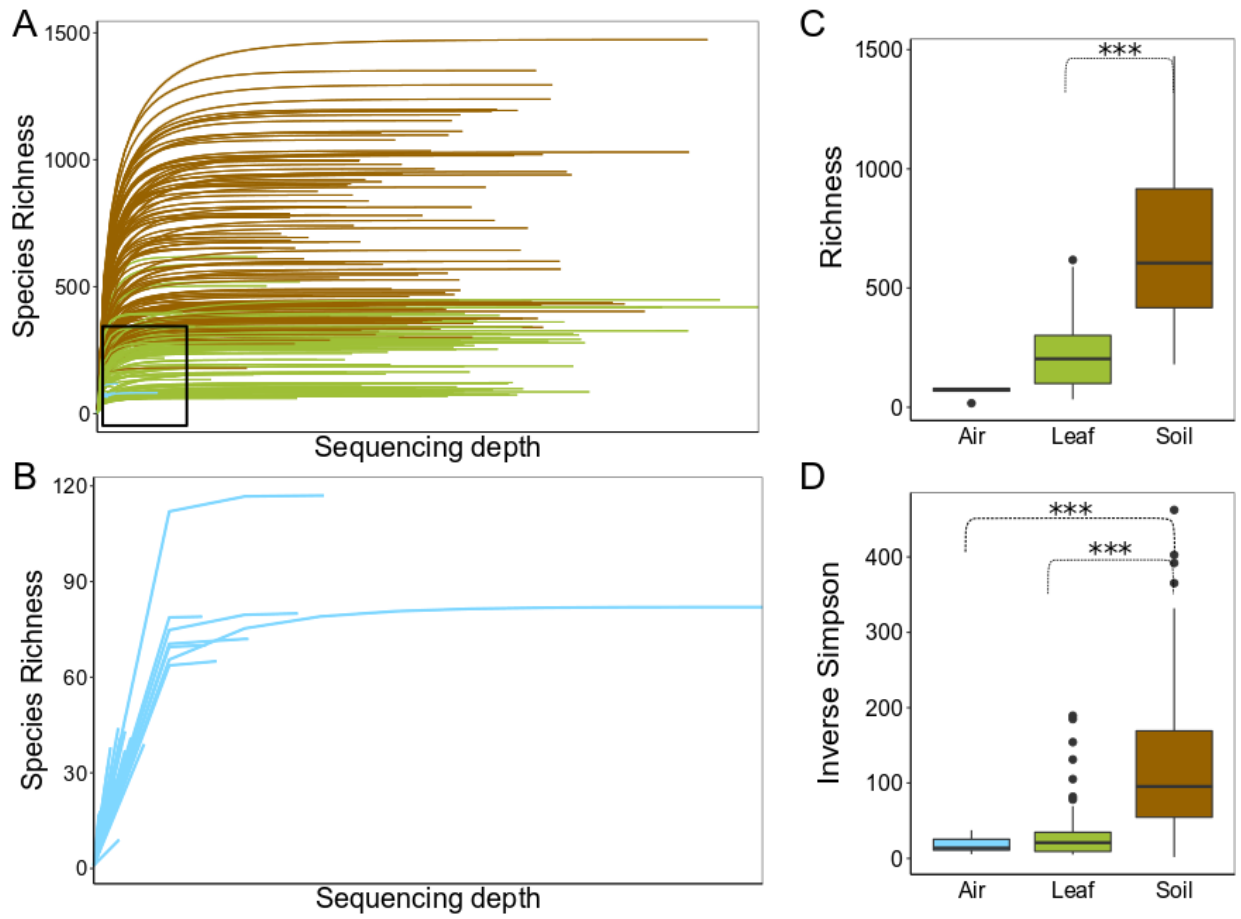
Comparison of bacterial community alpha-diversity among compartments (air, leaves and soil)

All rarefaction curves reached a plateau (Figure 4A) except for air samples, suggesting that the sequencing depth was large enough to estimate the bacterial diversity of leaf and soil samples, but not all air samples. Bacterial alpha-diversity taxonomic indices were therefore only calculated for the air samples that were sufficiently sequenced according to the rarefaction curve (n=7), and then compared to those of leaf and soil samples. In contrast to H1, bacterial richness and diversity in the air were significantly lower compared to those of the soil (Figure 4C; Table 2). There was no significant difference between air and leaf alpha-diversity index (richness and inverse Simpson).

Table 2. Bacterial alpha-diversity across microbial habitat. Bacterial richness was defined as the number of ASVs and bacterial diversity was measured as with the inverse Simpson index (Simpson, 1949). The effect of microbial habitat on bacterial richness was tested only for samples reaching an assum and modelled using a linear regression and inverse Simpson index using a GLM with a Gaussian distribution. The logarithm of the sequencing depth (SD) was introduced as an offset in all models.

	d.f.	**F-value**	**P-value**
Richness			
Microbial habitat (MH)	2	113.24	<0.001***
	d.f.	**Chisq**	**P-value**
Inverse of Simpson index			
Microbial habitat (MH)	2	2	<0.001***

Figure 4. Bacterial richness and diversity across microbial habitats. The dataset was composed of 78 air samples (blue), 100 leaf samples (green) and 112 soil samples (brown). The first column represents rarefaction curves (A and B), and the second column represents bacterial richness (C) and inverse Simpson index (D) estimated only on samples that reach a plateau. Rarefaction curves were performed using *ggrare* function from the ranacapa package (v.0.1.0). Lines represent the estimated bacterial ASV richness depending on the total number of Miseq sequencing reads in the sample (sequencing depth). Bacterial richness was defined as the number of ASVs. Bacterial richness and inverse Simpson significantly differed across microbial habitats (Table 2). Colors indicate the microbial habitats (blue: air samples, green: leaf samples, brown: soil samples).



Comparison of bacterial community beta-diversity between compartments (air, leaves and soil)

In accordance with H2, land cover, season and their interaction had significant effects on airborne bacterial community composition. However, this composition was poorly explained by any of the factors (less than 6% of variation explained by the two factors taken together; see Table 3). Land cover, season and their interaction also had significant effects on leaf and soil bacterial communities, with season explaining the largest part of the variance. The PCoAs are in line with

these results and indicated that airborne bacterial communities were close to that of vine leaves in terms of taxonomic composition, regardless of the ecosystem (Figure 5A). The airborne communities were more similar to that of vine leaves in summer (Figure 5B) than in autumn (Figure 5C). Overall, more than half of the bacterial ASVs detected in the air samples were also present on the phyllosphere (53.8%) and on the soil (54.0%) (Figure 6), suggesting an equal contribution of soil and leaves to airborne communities. In accordance with H3, microbial community in the near-surface atmosphere were more heterogeneous than the phyllosphere and soil bacterial communities (Figure 7) (Permutest: F-value = 60.449, P-value = 0.001***).

Table 3. Permutational multivariate analyses of variance (PERMANOVA) of compositional dissimilarities among bacterial communities of air, leaves and soil. Compositional dissimilarity among samples was estimated using the binary Jaccard distance. The total number of sequences per sample (sequencing depth, SD) was introduced as the first explanatory variable in the model.

	d.f.	F-value	P-value	R2
Air				
Sequencing depth	1	1.1248	0.0478	0.01431
Land cover	1	1.4304	0.0002	0.01820
Season	1	1.6512	0.0001	0.02101
Season x Land cover	1	1.4024	0.0002	0.01784
Leaf				
Sequencing depth	1	7.1351	<0.0001	0.05663
Land cover	1	5.9272	<0.0001	0.04704
Season	1	13.0421	<0.0001	0.10351
Season x Land cover	1	4.8877	<0.0001	0.03879
Soil				
Sequencing depth	1	12.0411	<0.0001	0.07412
Land cover	1	7.0727	<0.0001	0.04353
Season	1	31.1486	<0.0001	0.19173
Season x Land cover	1	5.2010	<0.0001	0.03201

Figure 5. Compositional dissimilarities among bacterial communities of air, leaf and soil samples, estimated using Jaccard binary distance and represented with PCoA plots for (A) all samples, (B) samples collected in summer and (C) autumn. Bacterial community composition differed significantly among microbial habitats, land cover and season (Table 2).

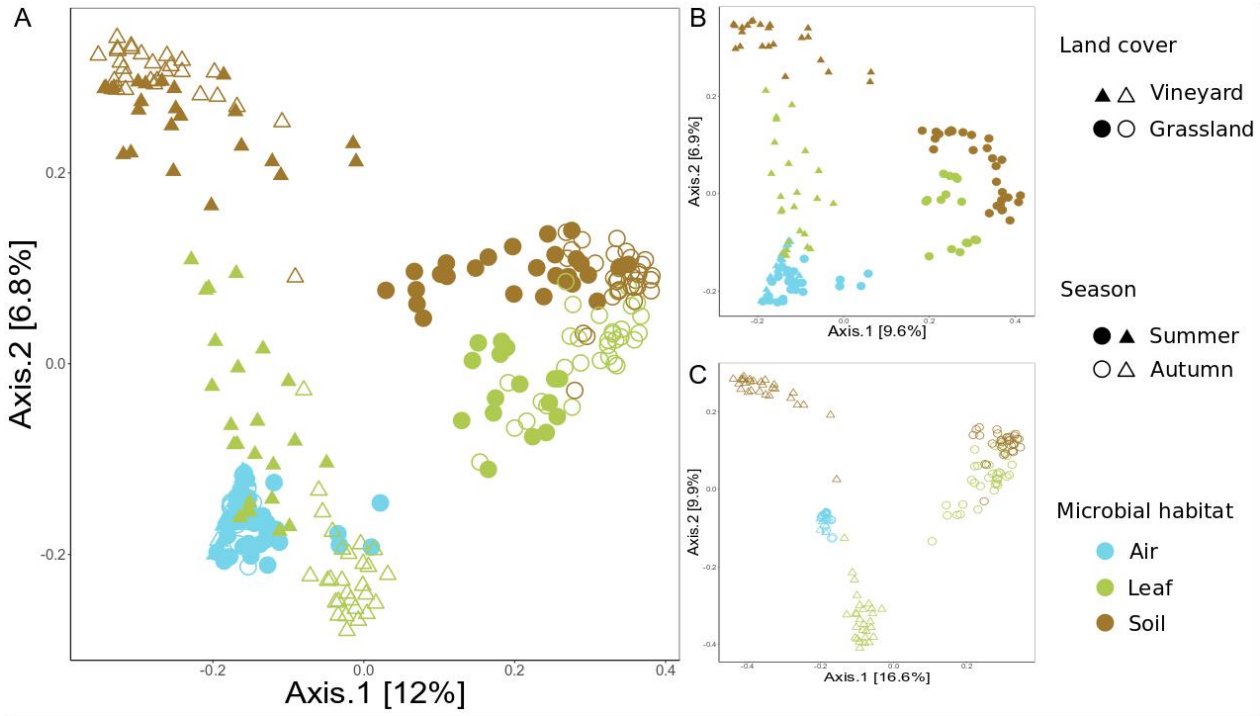


Figure 6. Venn diagram displaying the number of unique and shared taxa between the air, leaf surface and soil. Bacterial ASV were summed between each sample type for all sampling campaigns.

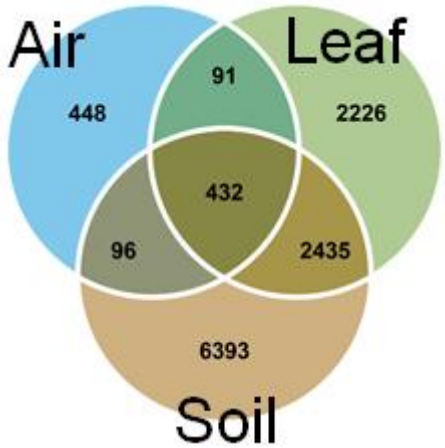
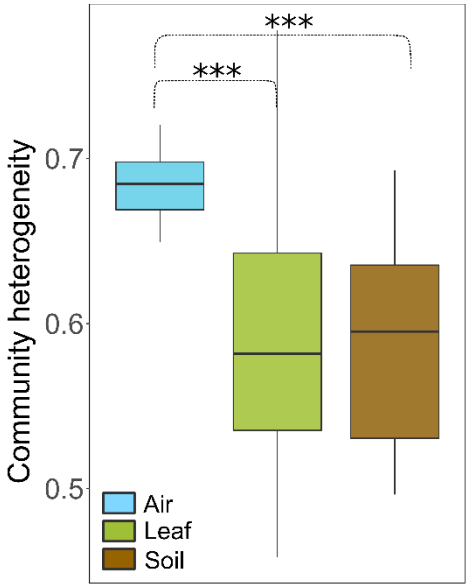


Figure 7. Bacterial community heterogeneity across microbial habitats. Distance from centroids was calculated using Jaccard binary distance. Microbial community composition differed significantly across microbial habitats.



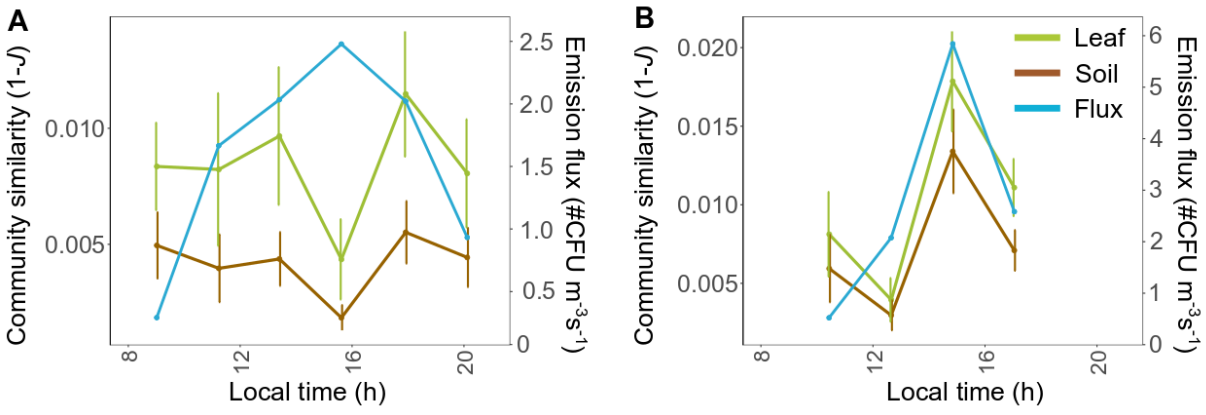
Daily dynamics of airborne bacterial communities

In both the July sampling campaign in the vineyard and the October one in grassland, airborne bacterial communities shared more species with the phyllosphere communities than with the upper soil communities, all along the day (Figure 8). In accordance to H4, the community similarity between airborne and phyllosphere communities, and between air and soil communities, appeared influenced by bacterial fluxes over grassland in autumn but not over vineyard in summer (Table 4; Figure 8). Over the grassland, the bacterial flux showed a strikingly similar trend to the community similarities in bacterial composition between the atmosphere and the biosphere, with a strong peak in the afternoon followed by a decrease (Figure 8B). In the vineyard, the overall trend was similar between the bacterial fluxes and the similarities in bacterial composition, with a morning increase and a late afternoon decrease (Figure 8A). However, the drop in similarity that is visible in the mid-afternoon does not reflect the behaviour of the flux, which exhibits a maximum at this time of the day. Overall, this set of observations is supported by GLM model (Table 4): the atmosphere-biosphere similarity is controlled by the bacterial fluxes but also by the type of vegetation cover.

Table 4. Generalized Linear Model (GLM) of the proportion of bacterial taxa shared between the air and the environmental sources (phyllosphere and soil). Compositional similarities were investigated for air samples paired with putative environmental sources (leaf and soil) for each sampling time over a vineyard in summer and grassland in autumn. The community similarity was defined as 1-J where J is the binary Jaccard distance, and the associated standard error. The effects of bacterial flux (F), environmental sources (leaf versus soil, ES), their interaction were tested for the most complete sampling campaign (Vineyard in summer and grassland in autumn).

	d.f.	Chisq	P-value
Grassland autumn			
Bacterial flux (F)	1	69.68	<0.0001***
Environmental sources (ES)	1	10.53	<0.001**
F x ES	1	0.012	0.91
Vineyard summer			
Bacterial flux (F)	1	1.96	0.16
Environmental sources (ES)	1	44.59	<0.001**
F x ES	1	0.021	0.88

Figure 8. Compositional similarity between airborne bacterial communities, environmental sources (leaf and soil) and bacterial fluxes over the day. Compositional similarities were investigated for air samples paired with leaf (green lines) and soil (brown lines) samples over a vineyard in summer and a grassland in autumn. Curves represent the average of community similarities between each sample pair, estimated as 1-*J* where *J* is the binary Jaccard distance, and the associated standard error. Bacterial fluxes (blue lines) were averaged over two-hour time steps.



Discussion

The concentration ranges of airborne bacteria during the day (20 and 80 CFU m^{-3}) are in line with those observed above various crops (see for example the review by Burrows & Butler, 2009). The day of grassland sampling in autumn was characterized by a persistent fog during the whole morning, with low wind velocity. In such conditions, the stable nighttime conditions tend to favor accumulation of various constituents (such as CO_2 for example) in the lowest layers of the atmosphere, before they are dispersed whenever the radiative forcing or the wind velocity reach sufficiently large values. This is the likely cause for the abrupt drop in air bacterial concentration observed in the morning.

The net emission flux observed in all sampling campaigns was in line with the literature: Most flux values shown by Carotenuto et al. (2017) laid between 0 and 20 CFU m^{-2} s^{-1}. Moreover, a curvilinear polynomial fit provided a maximum of about 5 CFU m^{-2} s^{-1} in the study of Lighthart & Shaffer (1994) over sparse vegetation in a desert location, while Chen *et al.* (2001) obtained about 10 CFU m^{-2} s^{-1} at an urban site (but their curve was based on a very small data set). Both studies exhibited a high run-to-run variability, which was not observed in our study. The increased bacterial fluxes observed at noon (solar time) over the vineyard in summer is in agreement with the curve estimated by Lighthart & Shaffer (1994). However, our study revealed no bacterial flux maximum in the middle of the day. The bacterial flux showed opposite trends in autumn, exhibiting a progressive increase over the day. This slow increase in the morning is probably due the fog that limited surface heating, and the wind velocity that started to increase only after 12:00, suggesting that microclimatic conditions greatly influence bacterial fluxes in the near-surface atmosphere.

The comparison of culture-dependent and independent methods during one hour of air sampling yielded contrasted results: the latter (MiSeq) detected a greater bacterial diversity, which is in accordance with the low percentage (1%) of bacterial richness often recovered (Bunge, 2009). As mentioned by Yoo *et al.* (2017), large differences observed between these methods may be linked to the incapacity of various bacterial taxa to colonize specific growth conditions provided by the agar media and the loss of microorganisms viability due to stress induced during aerosolization. These results emphasize the limitations of cultured-based methods and the advantages of choosing culture-independent ones. However, it should be noted that the analyses of rarefaction curves revealed a sequencing depth that was too shallow in most air samples to correctly estimate specific richness. These results illustrate the current challenges in establishing a standard protocol for reliable estimation of the atmospheric microbial diversity (Ferguson *et al.*, 2019). In this study, the

air collection was sampled over a very short time window and under low airflow rate. As a result, very few bacteria were impacted on filters, which may explain the unsaturated rarefaction curves in air samples. This also explains the very low diversity found in the air, which contrasts with previous studies on bioaerosol diversity in the near-surface atmosphere (Brodie *et al.*, 2007; Bowers *et al.*, 2013). In addition, the comparison of microbial communities between the atmosphere and the biosphere is still challenging, as the sampling unit differ between air, leaf surface and soil samples (i.e. m^3, cm^2 and mg).

Airborne bacterial communities were dominated by *Actinobacteria* (30.6%), *Alphaproteobacteria* (24.3%) and *Bacilli* (12.0%). These three classes are ubiquitous, often found in the outdoor air or in association with plants and soil (Després *et al.*, 2012; Bulgarelli *et al.*, 2013; Baldrian, 2017). The proportion of *Actinobacteria* and *Bacilli* classes was greater in air samples than in leaves and soils. Many of the taxa belonging to these classes are Gram-positive that form spores for aerial transport (Després *et al.*, 2007). In addition, *Bacilli* taxa are often found in the atmosphere (Harrison *et al.*, 2005; Fang *et al.*, 2014; Han *et al.*, 2019; Yoo *et al.*, 2019) and are known to thrive in hostile environments. They can produce spores that maintain their cellular viability from extreme temperatures, UV radiation and hydrophobic conditions over long periods of time (Yoo *et al.*, 2019 and references therein). It has been previously discussed that their presence in the atmosphere may reflect their high resilience to constraining atmospheric conditions, suggesting that they may be transported over long distances (Yoo *et al.*, 2019). In comparison to air bacterial communities, leaves contained a higher proportion of *Alphaproteobacteria* (55.1%) and soils contained a higher proportion of *Gammaproteobacteria* (25.0%). *Alphaproteobacteria* represents one of the largest group in the bacteria domain, which is often more abundant in plants and soils. This is likely due to a selection of this class by edaphic factors and plant host traits (Bulgarelli *et al.*, 2013). Whatever the type of terrestrial compartment (air, vegetation and soil), bacterial communities were dominated by aerobic and non-aerobic chemoheterotrophs, which suggests that bacteria present in the air, leaf surface and soil depend on the availability of organic compounds.

The airborne communities were characterized by genera associated to all kinds of microbial habitats ranging from human, plant and soil to hostile environments. Most of these genera have previously been described in association with plants or soil and three of them were found in leaf and soil samples (*Cutibacterium* sp., *Methylobacterium* sp., *Hymenobacter* sp. and *Frigoribacterium* sp.). In our study, *Cutibacterium* was also present on the leaf surface of vines, supporting the hypothesis that this species is associated with vines (Campisano *et al.*, 2014). Moreover, many human-associated pathogens were highly abundant in the air, indicating that human presence may

influence the regional species pool in the atmosphere. All precautions were taken to insure that manipulation in the field and in the laboratory did not contaminate our samples. However, these results may also be a consequence of the greater information availability on human pathogens, as many studies on airborne bacteria were not designed to study a wide range of species, but only to detect specific and often pathogenic species (Després *et al.*, 2012). Together these results corroborate the common assumption that the atmosphere carries bacteria that can come from various sources.

The analyses of beta-diversity within each microbial habitat demonstrate that the bacterial community drivers vary according to the microbial habitat. The analysis of beta-diversity dispersion indicated that airborne composition was very heterogeneous and the PERMANOVA test indicated that this composition varied marginally between land cover types and seasons compared to leaf and soil associated communities. Altogether, our results suggest that the near-surface atmosphere represents a highly diverse taxonomic and functional pool of bacterial taxa. At the regional scale, we found that the bacterial communities inhabiting the leaves may be as important as soil communities in determining the bacterial composition of the near-surface atmosphere. PCoA plots revealed that the epiphytic communities associated with vine leaves were similar to the atmospheric bacterial communities for both sampled sites, implying that agricultural practices drive the airborne composition at the regional scale. These observations are consistent with previous research demonstrating the effect of agriculture on atmospheric microbial composition (Lighthart & Shaffer, 1994; Wei *et al.*, 2019). Moreover, PCoA plots emphasized a seasonal shift between airborne communities and the microbial composition of vine leaves. The higher differences in composition between microbial habitats in autumn days (as compared to summer days) supports the hypothesis that during the summer season, the main source of bacteria in the atmosphere are plant-associated microbiota (Bertolini *et al.*, 2013). However, the bacterial fluxes measured over the sampling sites are not larger over the vineyard in summer. It is therefore likely that the contribution of vine leaves to the bacterial composition of the airborne communities may occur at larger spatial scales.

The analysis of airborne communities and their biotic environment within a day of sampling indicates that bacterial exchanges between the atmosphere and the biosphere vary throughout the day. The leaf turned out to be systematically closer to the atmospheric composition than the soil. The atmosphere-biosphere similarities followed the same trends for leaf and soil comparison over the days but the temporal patterns were not identical for the vineyard and grassland, with a decrease in community similarities between the atmosphere and the biosphere over the vineyard

between 14:00 and 16:00 on a summer day. These results contrast with the peak in bacterial emissions observed during the warm part of sunny days, which should lead to bacteria aerosolization and therefore increase the similarity of communities between the atmosphere and the biosphere. In autumn, the composition of bacterial communities between the atmosphere and the biosphere was more similar, suggesting that the bacterial emission during that day was the main factor controlling the composition of bacteria in the atmosphere. The climatic conditions between these two days of sampling were highly distinct (the grassland sampling day had begun with several hours of intense fog), suggesting that solar radiation, relative humidity and wind velocity strongly influence the emission of bacteria at the local level.

Conclusion

No previous study has attempted to measure, simultaneously, the fluxes and the composition of bacterial communities in the near-surface atmosphere. Our study employed an innovative bioaerosol capture approach using the flux-gradient method to measure the emission fluxes of bacteria to the atmosphere and identify bacterial genera through two sequencing approaches. It revealed that airborne bacterial communities represent a heterogeneous pool of taxa that hardly fluctuates between the season and sites in the same region. Such limited temporal and spatial variations may be linked to a constant contribution of the surrounding vegetation. Indeed, as the Gironde department is covered with a large area of vines representing more than 120,000 hectares, it is characterized by a great number of vineyard crops and by the microbial communities associated with vine plants. Therefore, the nature of plant species seems to be decisive in determining the atmospheric microbial composition. However, additional measures, including a wider range of ecosystems such as forests, should be performed to better understand the origin of microbial emissions.

The evolution of bioaerosols and their effects on biogeochemical cycles in the context of climate change are among the most uncertain predictions (Fröhlich-Nowoisky *et al.*, 2016). Although there is a history of research investigating the role of microbial communities in water cycle and atmosphere chemistry, little is known about the sources of microorganisms and the mechanisms that affect their emissions (Jones & Harrison, 2004; Burrows & Butler, 2009). Recent studies on bioprecipitations involving ice nuclei accumulation in clouds have highlighted potential feedback effects of microorganisms on rainfall, suggesting that changes in microbial composition at the regional scale can alter precipitation patterns in a warming climate (Morris *et al.*, 2014a). In this

context, identifying and quantifying the strength of microbial sources and the type of microorganisms they bring to the atmosphere may be of great interest in determining the drivers of these feedback effects.

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Author contributions

YB had the original idea for the project and coordinated the bacterial flux measurements and analyses. CV coordinated the barcoding and metabarcoding analyses. TF, CV, JM, DG, FD and YB performed the sampling campaigns. JM, DG and YB developped and set up the measurement mast and collected the flux data. FD performed the bacterial cultures. TF, CV, JFA and CL processed the samples and developped the molecular biology protocols. JFA performed all extractions and amplifications. EC performed the sequencing and demultiplexing. TF performed the bioinformatic and statistical analyses of the barcoding and metabarcoding data and wrote the first draft or the article. All authors contributed to the final version of the manuscript.

References

Amato P, Joly M, Besaury L, Oudart A, Taib N, Deguillaume L, Delort A, Debroas D, Mone I. **2017**. Active microorganisms thrive among extremely diverse communities in cloud water. *PLoS One* **3**: 1–22.

Baldrian P. **2017**. Forest microbiome: Diversity, complexity and dynamics. *FEMS Microbiology*

Reviews **41**: 109–130.

Bálint M, Schmidt PA, Sharma R, Thines M, Schmitt I. **2014**. An Illumina metabarcoding pipeline for fungi. *Ecology and Evolution* **4**: 2642–2653.

Barberán A, Ladau J, Leff JW, Pollard KS, Menninger HL, Dunn RR, Fierer N. **2015**. Continental-scale distributions of dust-associated bacteria and fungi. *Proceedings of the National Academy of Sciences* **112**: 5756–5761.

Barret M, Briand M, Bonneau S, Préveaux A, Valière S, Bouchez O, Hunault G, Simoneau P, Jacquesa MA. **2015**. Emergence shapes the structure of the seed microbiota. *Applied and Environmental Microbiology* **81**: 1257–1266.

Bauer H, Giebl H, Hitzenberger R, Kasper-giebl A, Reischl G, Zibuschka F, Puxbaum H. **2003**. Airborne bacteria as cloud condensation nuclei. *Journal of Geophysical Research Atmospheres* **108**: 1–5.

Bernardeau M, Vernoux JP, Henri-Dubernet S, Guéguen M. **2008**. Safety assessment of dairy microorganisms: The Lactobacillus genus. *International Journal of Food Microbiology* **126**: 278–285.

Bertolini V, Gandolfi I, Ambrosini R, Bestetti G, Innocente E, Rampazzo G, Franzetti A. **2013**. Temporal variability and effect of environmental variables on airborne bacterial communities in an urban area of Northern Italy. *Applied Microbiology and Biotechnology* **97**: 6561–6570.

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White JSS. **2009**. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology and Evolution* **24**: 127–135.

Bowers RM, Clements N, Emerson JB, Wiedinmyer C, Hannigan MP, Fierer N. **2013**. Seasonal variability in bacterial and fungal diversity of the near-surface atmosphere. *Environmental Science and Technology* **47**: 12097–12106.

Bowers RM, Mcletchie S, Knight R, Fierer N. **2010**. Spatial variability in airborne bacterial communities across land-use types and their relationship to the bacterial communities of potential source environments. *The ISME Journal* **5**: 601–612.

Brodie EL, Desantis TZ, Parker JPM, Zubietta IX, Piceno YM, Andersen GL. **2007**. Urban aerosols harbor diverse and dynamic bacterial populations. *Proceedings of the National Academy of Sciences* **104**: 299–304.

Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P. **2013**. Structure and Functions of the Bacterial Microbiota of Plants. *Annual Review of Plant Biology* **64**: 807–838.

Bunge J. **2009**. Uncultivated Microorganisms. *Springer Science & Business Media*

Burrows SM, Butler T. **2009**. Bacteria in the global atmosphere – Part 2 : Modeling of emissions and transport between different ecosystems. *Atmospheric Chemistry and Physics* **9**: 9281–9297.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. **2016**. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**: 581–583.

Campisano A, Ometto L, Compant S, Pancher M, Antonielli L, Yousaf S, Varotto C, Anfora G, Pertot I, Sessitsch A, *et al.* 2014. Interkingdom transfer of the acne-causing agent, propionibacterium acnes, from human to grapevine. *Molecular Biology and Evolution* **31**: 1059–1065.

Carotenuto F, Georgiadis T, Gioli B, Leyronas C, Morris CE, Nardino M, Wohlfahrt G, Miglietta F. 2017. Measurements and modeling of surface–atmosphere exchange of microorganisms in Mediterranean grassland*. Atmospheric Chemistry and Physics* **17**, 14919–14936.

Cernava T, Chen X, Krug L, Li H, Yang M, Berg G. **2019**. The tea leaf microbiome shows specific responses to chemical pesticides and biocontrol applications. *Science of the Total Environment* **667**: 33–40.

Collins MD, Hoyles L, Foster G, Falsen E. **2004**. Corynebacterium caspium sp. nov., from a Caspian seal (Phoca caspica). *International Journal of Systematic and Evolutionary Microbiology* **54**: 925–928.

Compant S, Samad A, Faist H, Sessitsch A. **2019**. A review on the plant microbiome : Ecology , functions , and emerging trends in microbial application. *Journal of Advanced Research journal* **9**: 29–37.

Copeland JK, Yuan L, Layeghifard M, Wang PW, Guttman DS. **2015**. Seasonal community succession of the phyllosphere microbiome. *Molecular Plant-Microbe Interactions* **28**: 274–285.

Chelius MK, Triplett EW. **2001**. The diversity of archaea and bacteria in association with the roots of Zea mays L. *Microbial Ecology* **41**: 252–263.

Chen M, Jin L, Sun Z, Lu J, Wang Q, Hu Q, Sun R, Li Z. **2001**. Concentration and flux of bioaerosol and environmental factors. *Progress in Natural Science* **11**: 686–687.

Compant S, Samad A, Faist H, Sessitsch A. **2019**. A review on the plant microbiome : Ecology, functions, and emerging trends in microbial application. *Journal of Advanced Research* **19**: 29–37.

Després V, Huffman JA, Burrows SM, Hoose C, Safatov A, Buryak G, Fröhlich-nowoisky J, Andreae M, Pöschl U, Jaenicke R, *et al.* 2012. Primary biological aerosol particles in the atmosphere : a review. *TellusB* **64**: 15598.

Després VR, Nowoisky JF, Klose M, Conrad R, Andreae MO, Pöschl U, Després VR, Nowoisky JF, Klose M, Conrad R, *et al.* 2007. Characterization of primary biogenic aerosol particles in urban, rural, and high-alpine air by DNA sequence and restriction fragment analysis of ribosomal RNA genes. *Biogeosciences* **4**: 1127-1141.

Ferguson RMW, Garcia-Alcega S, Coulon F, Dumbrell AJ, Whitby C, Colbeck I. **2019**. Bioaerosol biomonitoring: Sampling optimization for molecular microbial ecology. *Molecular Ecology Resources* **19**: 672–690.

Foster ZSL, Sharpton TJ, Grünwald NJ. **2017**. Metacoder: An R package for visualization and manipulation of community taxonomic diversity data. *PLoS Computational Biology* **13**: 1–15.

Friesen ML, Porter SS, Stark SC, von Wettberg EJ, Sachs JL, Martinez-Romero E. **2011**. Microbially Mediated Plant Functional Traits. *Annual Review of Ecology, Evolution, and Systematics* **42**: 23–46.

Fröhlich-Nowoisky J, Kampf CJ, Weber B, Huffman JA, Pï¿½hlker C, Andreae MO, Lang-Yona N, Burrows SM, Gunthe SS, Elbert W, *et al.* 2016. Bioaerosols in the Earth system: Climate, health, and ecosystem interactions. *Atmospheric Research* **182**: 346–376.

Galan M, Razzauti M, Bard E, Bernard M, Brouat C, Charbonnel N, Dehne-Garcia A, Loiseau A, Tatard C, Tamisier L, *et al.* 2016. 16S rRNA Amplicon Sequencing for Epidemiological Surveys of Bacteria in Wildlife. *mSystems* **1**: e00032-16.

Garratt JR. 1992. The atmospheric boundary layer. Cambridge University Press, 316 p.

Gu J, Ford T, Mitchell R. **2011**. Microbial degradation of materials: general processes. In: Uhlig's corrosion handbook. Wiley, New York, 351–363.

Han Y, Yang K, Yang T, Zhang M, Li L. **2019**. Bioaerosols emission and exposure risk of a wastewater treatment plant with A 2 O treatment process. *Ecotoxicology and Environmental Safety* **169**: 161–168.

Hardoim PR, van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M,

Sessitsch A. **2015**. The Hidden World within Plants: Ecological and Evolutionary Considerations for Defining Functioning of Microbial Endophytes. *Microbiology and Molecular Biology Reviews* **79**: 293–320.

Harrison RM, Jones AM, Biggins PDE, Pomeroy N, Cox CS, Kidd SP, Hobman JL, Brown NL, Beswick A. **2005**. Climate factors influencing bacterial count in background air samples. *International Journal of Biometeorology* **49**: 167–178.

Hothorn T, Bretz F, Westfall P. **2008**. Simultaneous inference in general parametric models. *Biometrical Journal* **50**: 346–363.

Innerebner G, Knief C, Vorholt JA. **2011**. Protection of Arabidopsis thaliana against leaf-pathogenic Pseudomonas syringae by Sphingomonas strains in a controlled model system. *Applied and Environmental Microbiology* **77**: 3202–3210.

Ivanova EG, Doronina N V., Shepelyakovskaya AO, Laman AG, Brovko FA, Trotsenko YA. **2000**. Facultative and obligate aerobic methylobacteria synthesize cytokinins. *Microbiology* **69**: 646–651.

Ivanova EG, Doronina N V., Trotsenko YA. **2001**. Aerobic methylobacteria are capable of synthesizing auxins. *Microbiology* **70**: 392–397.

Ivanova EG, Fedorov DN, Doronina N V., Trotsenko YA. **2006**. Production of vitamin B12 in aerobic methylotrophic bacteria. *Microbiology* **75**: 494–496.

Jaccard P. **1901**. Étude comparative de la distribution florale dans une portion des Alpes et du Jura. *Bulletin de la Société Vaudoise des Sciences Naturelles* **37**: 547–579.

Jones AM, Harrison RM. **2004**. The effects of meteorological factors on atmospheric bioaerosol concentrations - A review. *Science of the Total Environment* **326**: 151–180.

Joung YS, Ge Z, Buie CR. **2017**. Bioaerosol generation by raindrops on soil. *Nature Communications* **8**: 1–10.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, *et al.* 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* **28**: 1647–1649.

Krishna MP, Mohan M. **2017**. Litter decomposition in forest ecosystems: a review. *Energy, Ecology and Environment* **2**: 236–249.

Laforest-Lapointe I, Messier C, Kembela SW. **2017**. Tree Leaf Bacterial Community Structure and Diversity Differ along a Gradient of Urban Intensity. *mSystems* **2**: 1–16.

Lange JL, Thorne PS, Lynch N. **1997**. Application of Flow Cytometry and Fluorescent *In Situ* Hybridization for Assessment of Exposures to Airborne Bacteria. *Applied Environmental Microbiology* **63**: 1557–1563.

Lee YB, Lorenz N, Dick LK, Dick RP. **2007**. Cold Storage and Pretreatment Incubation Effects on Soil Microbial Properties. *Soil Science Society of America Journal* **71**: 1299.

Lighthart B, Shaffer BT. **1994**. Bacterial flux from chaparral into the atmosphere in mid-summer at a high desert location. *Atmospheric Environment* **28**: 1267–1274.

Lighthart B, Shaffer BT. **1995**. Airborne bacteria in the atmospheric surface layer: Temporal distribution above a grass seed field. *Applied and Environmental Microbiology* **61**: 1492–1496.

Lindemann J, Constantinidou HA, Barchet WR, Upper CD. **1982**. Plants as sources of airborne bacteria, including ice nucleation-active bacteria. *Applied and Environmental Microbiology* **44**: 1059–1063.

Lindow SE, Brandl MT. **2003**. Microbiology of the Phyllosphere. *Applied and Environmental Microbiology* **69**: 1875–1883.

Louca S, Jacques SMS, Pires APF, Leal JS, Srivastava DS, Parfrey LW, Farjalla VF, Doebeli M. **2016**. High taxonomic variability despite stable functional structure across microbial communities. *Nature Ecology & Evolution* **1**: 0015.

Lymperopoulou DS, Adams RI, Lindow SE. **2016**. Contribution of vegetation to the microbial composition of nearby outdoor air. *Applied and Environmental Microbiology* **82**: 3822–3833.

Lyons JA, Bemis DA, Bryant MJ, Kania SA, Abd-Eldaim M, Newman SJ. **2009**. Isolation of Propionibacterium acnes from a case of placentitis and abortion in a cow. *Journal of Veterinary Diagnostic Investigation* **21**: 274–277.

Manceau C, Horvais A. **1997**. Assessment of genetic diversity among strains of Pseudomonas syringae by PCR-restriction fragment length polymorphism analysis of rRNA operons with special emphasis on P. *syringae* pv. tomato. *Applied and Environmental Microbiology* **63**: 498–505.

Martin M. **2011**. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet journal* **17**: 2803–2809.

McMurdie PJ, Holmes S. **2013**. Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE* **8**: e61217.

McMurdie PJ, Holmes S. **2014**. Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. *PLoS Computational Biology* **10**.

Mhuireach G, Johnson BR, Altrichter AE, Ladau J, Meadow JF, Pollard KS, Green JL. **2016**. Urban greenness influences airborne bacterial community composition. *Science of the Total Environment* **571**: 680–687.

Morris CE, Conen F, Alex Huffman J, Phillips V, Pöschl U, Sands DC. **2014a**. Bioprecipitation: A feedback cycle linking Earth history, ecosystem dynamics and land use through biological ice nucleators in the atmosphere. *Global Change Biology* **20**: 341–351.

Morris CE, Leyronas C, Nicot PC. **2014b**. Movement of Bioaerosols in the Atmosphere and the Consequences for Climate and Microbial Evolution. *Aerosol Science: Technology and Applications*: 393–415.

Mortazavi, R., Hayes, C. T., & Ariya, P. A. 2008. Ice nucleation activity of bacteria isolated from snow compared with organic and inorganic substrates. **5**: 373–381.

Nisbet M, Briggs S, Ellis-Pegler R, Thomas M, Holland D. **2007**. Propionibacterium acnes: An under-appreciated cause of post-neurosurgical infection. *Journal of Antimicrobial Chemotherapy* **60**: 1097–1103.

Niu B, Paulson JN, Zheng X, Kolter R. **2017**. Simplified and representative bacterial community of maize roots. *Proceedings of the National Academy of Sciences of the United States of America* **114**: E2450–E2459.

Ottesen AR, González Peña A, White JR, Pettengill JB, Li C, Allard S, Rideout S, Allard M, Hill T, Evans P, *et al.* 2013. Baseline survey of the anatomical microbial ecology of an important food plant: Solanum lycopersicum (tomato). *BMC Microbiology* **13**.

Ottesen AR, White JR, Skaltsas DN, Newell MJ, Walsh CS. **2009**. Impact of organic and conventional management on the phyllosphere microbial ecology of an apple crop. *Journal of Food Protection* **72**: 2321–2325.

Oksanen AJ, Kindt R, Legendre P, Hara BO, Simpson GL, Stevens MHH, Wagner H. **2008**. The vegan Package; Community Ecology Package (Version 1.15-1).

Perazzolli M, Antonielli L, Storari M, Puopolo G, Pancher M, Giovannini O, Pindo M, Pertot I.

2014. Resilience of the natural phyllosphere microbiota of the grapevine to chemical and biological pesticides. *Applied and Environmental Microbiology* **80**: 3585–3596.

Qiu B, Al K, Pena-Diaz AM, Athwal GS, Drosdowech D, Faber KJ, Burton JP, O'Gorman DB. **2018**. Cutibacterium acnes and the shoulder microbiome. *Journal of Shoulder and Elbow Surgery* **27**: 1734–1739.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. **2013**. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research* **41**: 590–596.

Redford AJ, Bowers RM, Knight R, Linhart Y, Fierer N. **2010**. The ecology of the phyllosphere: Geographic and phylogenetic variability in the distribution of bacteria on tree leaves. *Environmental Microbiology* **12**: 2885–2893.

Roselló G, Bonaterra A, Francés J, Montesinos L, Badosa E, Montesinos E. **2013**. Biological control of fire blight of apple and pear with antagonistic Lactobacillus plantarum. *European Journal of Plant Pathology* **137**: 621–633.

Ryu J, Madhaiyan M, Poonguzhali S, Yim W, Indiragandhi P, Kim K, Anandham R, Yun J, Kim KH, Sa T. **2006**. Plant growth substances produced by methylobacterium spp. and their effect on tomato (lycopersicon esculentum L.) and red pepper (capsicum annuum L.) growth. *Journal of Microbiology and Biotechnology* **16**: 1622–1628.

Salter SJ, Cox MJ, Turek EM, Calus ST, Cookson WO, Moffatt MF, Turner P, Parkhill J, Loman NJ, Walker AW. **2014**. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biology* **12**: 87.

Simpson EH. **1949**. Measurement of diversity. *Nature* **163**: 688.

Sixt BS, Siegl A, Müller C, Watzka M, Wultsch A, Tziotis D, Montanaro J, Richter A, Schmitt-Kopplin P, Horn M. **2013**. Metabolic Features of Protochlamydia amoebophila Elementary Bodies - A Link between Activity and Infectivity in Chlamydiae. *PLoS Pathogens* **9**.

Striluk ML, Aho K, Weber CF. **2017**. The effect of season and terrestrial biome on the abundance of bacteria with plant growth-promoting traits in the lower atmosphere. *Aerobiologia* **33**: 137–149.

Trotsenko YA, Ivanova EG, Doronina N V. 2001. Aerobic methylotrophic bacteria as phytosymbionts. *Mikrobiologiya* **70**: 725–736.

Turner TR, James EK, Poole PS, Gilbert J, Meyer F, Jansson J, Gordon J, Pace N, Tiedje J, Ley R, *et al.* 2013. The plant microbiome. *Genome Biology* **14**: 209.

Upper CD, Hirano SS. **1991**. Aerial dispersal of bacteria. *Biotechnology (Reading, Mass.)* **15**: 75–93.

Vacher C, Hampe A, Porté AJ, Sauer U, Compant S, Morris CE. **2016**. The Phyllosphere: Microbial Jungle at the Plant–Climate Interface. *Annual Review of Ecology, Evolution, and Systematics* **47**: 1–24.

Vogel C, Bodenhausen N, Gruissem W, Vorholt JA. **2016**. The Arabidopsis leaf transcriptome reveals distinct but also overlapping responses to colonization by phyllosphere commensals and pathogen infection with impact on plant health. *New phytologist* **212**: 192–207.

Vorholt JA. **2012**. Microbial life in the phyllosphere. *Nature Reviews Microbiology* **10**: 828–840.

Wang Q, Garrity GM, Tiedje JM, Cole JR. **2007**. Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* **73**: 5261–5267.

Wei M, Xu C, Xu X, Zhu C, Li J, Lv G. **2019**. Size distribution of bioaerosols from biomass burning emissions: Characteristics of bacterial and fungal communities in submicron (PM 1.0) and fine (PM 2.5) particles. *Ecotoxicology and Environmental Safety* **171**: 37–46.

Wolf R, El-Haddad I, Slowik JG, Dällenbach K, Bruns E, Vasilescu J, Baltensperger U, Prévôt ASH. **2017**. Contribution of bacteria-like particles to PM2.5 aerosol in urban and rural environments. *Atmospheric Environment* **160**: 97–106.

Womack AM, Bohannan BJM, Green JL. **2010**. Biodiversity and biogeography of the atmosphere. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**: 3645–3653.

Yang K, Li L, Wang Y, Xue S, Han Y, Liu J. **2019**. Airborne bacteria in a wastewater treatment plant: Emission characterization, source analysis and health risk assessment. *Water Research* **149**: 596–606.

Yoo K, Han I, Ko KS, Lee TK, Yoo H, Khan MI, Tiedje JM, Park J. **2019**. Bacillus-Dominant Airborne Bacterial Communities Identified During Asian Dust Events. *Microbial Ecology* **78**: 677–687.

Yoo K, Lee TK, Choi EJ, Yang J, Shukla SK, Hwang S il, Park J. **2017**. Molecular approaches for the detection and monitoring of microbial communities in bioaerosols: A review. *Journal of Environmental Sciences (China)* **51**: 234–247.

V Conclusion et perspectives

Les travaux de cette thèse ont permis d'identifier et de quantifier l'effet de plusieurs facteurs biotiques et abiotiques sur l'assemblage et la transmission des communautés microbiennes associées aux parties aériennes des arbres. Le chapitre II montre qu'une partie de cette variation est expliquée par l'effet de la sélection via les traits foliaires de l'hôte tandis que les chapitres III et IV soulignent l'importance de la transmission verticale et horizontale et donc de la dispersion des micro-organismes dans les processus d'assemblage des communautés microbiennes. Le chapitre III suggère également que les interactions biotiques représentent une composante clé dans les processus d'assemblage du mycobiote des graines.

La variation de traits foliaires est à la fois le fruit de la variation du génotype de l'hôte et de sa plasticité phénotypique, cette dernière représentant l'acclimatation de l'hôte aux conditions environnementales (Schlichting, 1986 ; Al Hayek et al. 2014). Les variations de composition microbienne expliquée par les traits foliaires dans le chapitre II représentent les variations du phénotype de l'hôte qui est contrôlé par son génotype et son environnement. Ces résultats sont en accord avec des comparaisons de communautés microbiennes réalisées entre arbres de différentes espèces (Kembel & Mueller, 2014; Kembel *et al.*, 2014; Laforest-Lapointe *et al.*, 2016a) ou entre différents sites géographiques (Kraft & Ackerly, 2010; Redford *et al.*, 2010) et indiquent que des variations de traits foliaires au sein d'une même population d'arbre peuvent conduire à une différenciation dans la structure des communautés microbiennes qui leur sont associés. La variation des traits foliaires étant directement influencée par les conditions microclimatiques incluant le gradient de lumière, de température et d'humidité à l'intérieur de la canopée (Niinemets, 2016), il est donc possible que l'effet des traits foliaires sur la composition du microbiote reflète l'impact direct du microclimat sur les micro-organismes. En effet, il a été démontré que plusieurs facteurs climatiques tels que la température, les précipitations, le vent et le rayonnement solaire jouent un rôle important dans la colonisation de la phyllosphère (Kinkel, 1997 ; Aydogan et al. 2018 ; Gomes et al. 2018).

Les résultats du chapitre II indiquent également que la composition des communautés épiphytes et endophytes diffère, suggérant que les pressions de sélection diffèrent à l'échelle de la feuille. En effet, les épiphytes et les endophytes de la phyllosphère occupent deux microenvironnements distincts et sont donc contrôlées par différents facteurs: les épiphytes sont en contact avec l'environnement extérieur et dépendent des nutriments provenant de l'atmosphère et des exsudats

des feuilles (Vorholt et al. 2011 ; Vacher et al. 2016), tandis que les endophytes sont en contact avec l'environnement interne de la plante et dépendent des nutriments contenus dans les tissus hôtes (Clay et al. 2002). Les plantes sont donc plus en mesure de contrôler la colonisation microbienne à l'intérieur de leurs tissus que sur celle des surfaces extérieures (Santamaria et al. 2005). Ainsi plusieurs études ont montré que le phénotype des plantes affecte de manière systématique la diversité et composition microbienne des endophytes (Lau et al. 2013 ; Peršoh et al. 2013 ; Yao et al. 2019) tandis que son effet est réduit chez les épiphytes (Flessa et al. 2012 ; Fonseca-García et al. 2016).

La comparaison des communautés bactériennes et fongiques dans le chapitre II indique que les processus responsables de l'assemblage des communautés microbiennes diffèrent entre groupes taxonomiques. La composition fongique des feuilles variait fortement entre arbres tandis que la composition bactérienne était plus influencée par la date d'échantillonnage. Les travaux de Coleman-Derr (2016) et Li (2018) sur la composition des communautés microbiennes associées aux feuilles indiquent que les communautés fongiques sont plus influencées par l'origine et l'identité de l'hôte contrairement aux communautés bactériennes qui sont principalement influencées par le compartiment végétal. Notre étude indique que ces conclusions sont également valables *in situ* pour les feuilles d'arbres forestiers. Ces différences sont probablement liées à une dispersion limitée et une dépendance pour l'hôte plus forte chez les communautés fongiques que chez les communautés bactériennes. La dispersion passive des spores fongiques se produit fréquemment à de courtes distances : la plupart des spores ne se dispersant pas à plus de quelques mètres (Norros *et al.*, 2012). De plus, les champignons consomment plus directement et plus efficacement les nutriments des plantes (De Boer *et al.*, 2005; Šťursová *et al.*, 2012), ainsi les champignons peuvent être plus facilement influencés par des facteurs biotiques en raison de leur association étroite avec des espèces végétales (Lange *et al.*, 2014; Urbanová *et al.*, 2015). La composition des communautés bactériennes variait plus fortement au cours de la saison végétative montrant une homogénéisation à l'intérieur de la canopée. Ces variations suggèrent que contrairement aux communautés fongiques, les bactéries ne sont que faiblement influencées par les filtres environnementaux au début de la saison végétative et que la dispersion opèrant au cours de la saison végétative conduit à une homogénéisation dans la canopée. Ces observations sont confortées par les travaux de Maignien (2014) sur *Arabidopsis thaliana* qui montrent que le début de la colonisation des feuilles par les bactéries est caractérisée par des événements stochastiques et que la trajectoire des communautés est ensuite influencée par l'effet de la dispersion et de la sélection par l'hôte.

Les analyses du chapitre III suggèrent également que la sélection - par le biais des filtres environnementaux abiotiques, du génotype et phénotype de l'hôte et des interactions biotiques entre micro-organismes - influence de manière significative la composition du microbiote des graines. Les précédentes études réalisées sur le mycobiote des graines indiquent que les pressions de sélection de la plante hôte ne sont pas un facteur majeur dans l'assemblage des communautés tandis que les filtres environnementaux prédominent sur les graines de plantes cultivées (Barret *et al.*, 2015; Klaedtke *et al.*, 2016). Nos résultats suggèrent que le génotype et le phénotype de l'hôte sont importants dans l'assemblage des communautés en milieu naturel. Cependant nos analyses indiquent également que les pressions de sélection n'opèrent pas directement sur la graine mais plutôt sur le mycobiote qui l'entoure soulignant ainsi l'importance de la dispersion dans l'assemblage des communautés fongiques associés aux graines. Ce constat concorde avec les patrons spatiaux des communautés fongiques entre sites et entre arbres mentionnés auparavant. Ainsi le mycobiote des graines est influencé par le mycobiote de l'arbre et le mycobiote de son environnement eux-mêmes contrôlés par des processus déterministes et stochastiques qui opèrent à de plus grandes échelles spatiales. De plus, les inférences de réseau fongique ont révélé une association positive entre le pathogène foliaire *Taphrina carpini* et son mycoparasite *Cladosporium delicatulum* suggérant que la sélection opère aussi par le biais des interactions biotiques entre micro-organismes et que les graines servent de disperseurs aussi bien pour les pathogènes des plantes que pour leurs parasites (Ewald, 1989; Feldman *et al.*, 2008).

Les résultats du chapitre IV démontrent également que la dispersion des micro-organismes via l'atmosphère représente un processus important dans la dispersion du microbiote foliaire. Il a été montré que le nombre moyen de flux d'émission de bactéries et de spores fongiques sur les régions continentales est de l'ordre de ~102 m-2 s-1 (Lindemann *et al.*, 1982; Lighthart & Shaffer, 1994; Burrows & Butler, 2009; Burrows *et al.*, 2009; Crawford *et al.*, 2014), ce qui reflète un échange intense et rapide de matière biologique et d'informations génétiques entre l'atmosphère et la biosphère. De plus Lindemann (1982) a montré que le type de couverture végétale influence le taux d'émissions mais aussi la diversité des micro-organismes qui peuvent potentiellement être émis dans l'atmosphère. Nos analyses confirment les résultats de la littérature : les bactéries au cours de la journée sont systématiquement émises et non déposées sur les feuilles et la composition de ces flux est similaire au microbiote foliaire de la vigne qui est l'espèce dominante à l'échelle régionale. La composition bactérienne dans l'atmosphère était moins diverse et plus hétérogène que les communautés bactériennes associées à la phyllosphère et au sol. Ces résultats suggèrent donc que la

composition atmosphérique représente une petite fraction de bactéries capables d'être émise dans l'atmosphère et provenant du type de couvert végétale dominant à l'échelle régionale.

Effets potentiels du microbiote de la canopée sur le fonctionnement de la plante et de l'environnement

L'ensemble des résultats de cette thèse suggère que les communautés foliaires sont impliquées dans le cycle des nutriments et de l'eau et dans le cycle de vie des arbres forestiers. Le chapitre II indique que les feuilles du hêtre sont colonisées par différentes espèces fongiques capables de modifier le cycle des nutriments, la croissance et la survie des plantes comme les champignons saprotrophes, les pathogènes potentiels des plantes et leurs mycoparasites. La faible abondance des bactéries fixatrices d'azote ne permet pas de conclure quant aux effets relatifs de ces dernières sur le cycle des nutriments. Ces analyses diffèrent des résultats trouvés dans plusieurs études montrant que certaines bactéries fixatrices d'azote atmosphérique étaient présentes chez différentes espèces tropicales (Fürnkranz *et al.*, 2008; Reed *et al.*, 2011) et tempérées (Rico *et al.*, 2014; Doty *et al.*, 2016; Moyes *et al.*, 2016b). Néanmoins, les taux mesurés de fixation de l'azote dans la phyllosphère varient considérablement entre feuilles et entre espèces hôtes (Fürnkranz *et al.*, 2008; Reed *et al.*, 2011). De plus, en forêt tempérée, les quantités d'azote fixées dans la phyllosphère sont généralement considérablement inférieures à celle des forêts tropicales (Freiberg, 1998), ce qui peut expliquer l'absence de ces espèces dans les feuilles du hêtre. En revanche l'analyse de la composition fonctionnelle fongique a permis de mettre en évidence l'augmentation de l'abondance relative des saprotrophes en bas de la canopée et au cours de la saison végétative. Ces variations peuvent être dues à un plus grand nombre de feuilles sénescentes en bas de la canopée et en fin de saison végétative (Yasumura *et al.*, 2002; Brouwer *et al.*, 2012) qui favorise l'établissement des champignons consommateurs de matière organique morte. Enfin, l'effet de la position à l'intérieur de la canopée persiste pendant les deux mois de décomposition des feuilles, ce qui suggère que la composition initiale des communautés microbiennes à l'intérieur de la canopée peut influencer la succession microbienne pendant la décomposition des feuilles.

Le chapitre III met en évidence l'effet du phénotype étendu, définie comme la diversité et la composition des communautés microbiennes associées à l'hôte (Whitham *et al.*, 2006), dans la composition du mycobiote des graines en forêt. La transmission de micro-organismes de génération en génération peut entraîner une diminution des attaques de pathogènes et ainsi favoriser la croissance et le développement des plantes (Mitter *et al.*, 2017; Nelson *et al.*, 2018). Cependant

plusieurs études montrent également que les pathogènes des plantes peuvent utiliser les graines comme moyen de dispersion (Augspurger & Kelly, 1984; Tancos *et al.*, 2013; Barret *et al.*, 2016) et ainsi influencer la répartition et la diversité des arbres en forêts (Gilbert, 2002; Bradley *et al.*, 2008). Dans notre étude, la transmission de micro-organismes de l'arbre mère à ses descendants semblerait impliquer des partenaires bénéfiques comme délétères chez le chêne sessile. Il semblerait donc que l'effet du mycobiote des arbres peut avoir ainsi un effet bénéfique ou délétère pour les graines déterminant la croissance et la survie des graines.

Après leur émission dans l'atmosphère, les particules de bioaérosols peuvent interagir avec d'autres aérosols et gaz présent dans l'atmosphère et peuvent constitués des noyaux de condensation et de glaciation impliquées dans la formation des nuages et des précipitations (Morris et al. 2014 ; Fröhlich-Nowoisky et al. 2016 ; Delort et al. 2018). L'émission constante de bactéries des couverts végétaux vers l'atmosphère au cours de la journée observée dans le chapitre IV suggère que le microbiote foliaire, et tout particulièrement celui de la vigne, participe à la composition en bioaérosols de l'atmosphère. Il est donc possible qu'une partie des micro-organismes impliqués dans le cycle de l'eau proviennent des vignes en Nouvelle-Aquitaine.

Limites et perspectives

Bien que les techniques de séquençage à haut débit ont permis l'identification de la fraction non cultivable des communautés microbiennes (Chakravorty *et al.*, 2007; Sibley *et al.*, 2012), le séquençage du gène bactérien 16S et fongique ITS ne font pas de distinction entre les cellules microbiennes mortes et vivantes. Ainsi la composition des communautés microbiennes étudiée rend compte non seulement des membres actifs de la communauté mais aussi des membres passés. Du fait de l'immense diversité des communautés microbiennes à l'échelle de la planète, la grande partie des séquences bactériennes 16S et fongiques ITS ne sont pas assignées à une espèce microbienne. Des bases de données telles que FAPROTAX et FUNGuild permettent actuellement d'assigner des fonctions aux bactéries et champignons identifiés dans la communauté. Néanmoins ces bases de données, tout comme les bases de données taxonomiques, sont incomplètes et ne permettent l'identification que d'une fraction du microbiote. De plus, certaines séquences microbiennes peuvent être assignées à plusieurs fonctions, leurs proportions ne sommant pas à 1, elles ne peuvent être analysées comme des fréquences. Une multitude d'autres techniques permettent actuellement d'étudier la distribution, l'activité et les fonctions assurées par les communautés microbiennes telles que l'analyse de protéines (protéomique) et de l'ARN

(transcriptomique) ou le marquage des séquences via hybridation *in situ* de sondes (FISH). De plus des outils tels que l'Omnilog permettent d'estimer l'effet de la nature et la quantité de la ressource sur la croissance et les interactions biotiques entre micro-organismes. Des expériences sur un grand nombre de feuilles provenant d'une espèce modèle tel qu'*Arabidopsis thaliana* combinant plusieurs de ces techniques permettent actuellement de mettre en évidence la complexité du microbiote et son effet sur le fonctionnement de la plante (Pochon *et al.*, 2013; Maignien *et al.*, 2014; Ryffel *et al.*, 2016; Vogel *et al.*, 2016; Gimenez-Ibanez *et al.*, 2017; Duran *et al.*, 2018; Peredo & Simmons, 2018). De telles approches interdisciplinaires sur arbre forestier permettraient de mieux comprendre l'assemblage des communautés microbiennes qui lui sont associées et leur rôle dans sa croissance et sa survie.

Plusieurs expériences complémentaires permettraient de confirmer les rôles potentiels des communautés microbiennes sur le fonctionnement des plantes et des écosystèmes identifiés lors de cette thèse. Tout d'abord l'effet du microclimat et du génotype de l'hôte sur la composition fonctionnelle des communautés microbiennes permettrait de modéliser l'effet du changement climatique sur l'évolution du rôle des micro-organismes dans la canopée. Concernant le microbiote des graines, notre étude démontre que le mycobiote de l'arbre explique une importante fraction du mycobiote de ses descendants mais ne permet pas d'identifier la voie par laquelle ce microbiote est transmis. Des études récentes démontrent que la transmission verticale de génération en génération est variable chez les espèces cultivées et dépend de plusieurs facteurs tels que la sélection de l'hôte ou la dérive écologique (Afkhami *et al.*, 2015; Rezki *et al.*, 2018). L'étude de l'assemblage des communautés microbiennes des graines associées aux arbres forestiers est encore très peu connue et de plus importants efforts sont nécessaires pour mieux comprendre les processus d'assemblage des communautés dans ces systèmes. Plusieurs expériences *in situ* et en conditions contrôlées permettraient de mesurer la variabilité de l'effet maternel sur la composition des communautés sur plusieurs espèces forestières dans différentes conditions climatiques et l'effet de ce microbiote sur la germination et la croissance des descendants. Enfin les mesures de flux bactériens au cours de la journée et leur composition pourraient être réalisées sur une gamme d'habitats forestiers et non-forestiers plus large et lors de plusieurs journées avant, pendant et après la saison végétative. Ces mesures couplées à des mesures climatiques permettraient de mettre en évidence d'une part l'effet du climat sur l'émission de bioaérosols et d'autre part les effets feed-back des bioaérosols sur les conditions climatiques.

En conclusion, les résultats de cette thèse mettent en évidence l'importance des pressions de sélection exercées par la plante hôte et l'environnement et de la dispersion via la transmission

verticale et horizontale des micro-organismes sur l'assemblage du microbiote foliaire sur l'assemblage du microbiote foliaire. Cette dernière joue un rôle important dans l'établissement et la prolifération des micro-organismes associés à la plante hôte mais est souvent omise dans la littérature (Whipps *et al.*, 2008; Bulgarelli *et al.*, 2013). Une étude combinant des analyses d'écologie moléculaire des organes aériens et souterrains de la plante et du sol, des mesures de flux microbiens en-dessous et au-dessus de la canopée de plusieurs arbres forestiers au cours de la saison végétative et des mesures de microclimat et d'écophysiologie végétale permettraient de distinguer le rôle de la sélection et de la dispersion dans l'assemblage des communautés à l'échelle de la forêt.

Abarenkov K, Nilsson RH, Larsson K-H, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E, Pennanen T, *et al.* 2018. The UNITE database for molecular identification of fungi – recent updates and future perspectives. *New Phytologist* **219**: 5.

Afkhami ME, Rudgers JA, Afkhami ME, Rudgers JA. **2015**. Symbiosis Lost : Imperfect Vertical Transmission of Fungal Endophytes in Grasses. **172**: 405–416.

Agler MT, Ruhe J, Kroll S, Morhenn C, Kim ST, Weigel D, Kemen EM. **2016**. Microbial Hub Taxa Link Host and Abiotic Factors to Plant Microbiome Variation. *PLoS Biology* **14**: 1–31.

Agrios GN. **1997**. Control of Plant Diseases. In: Plant Pathology. San Diego, 200–216.

Ahn JH, Kim BC, Kim SJ, Lee GH, Song J, Kwon SW, Weon HY. **2015**. Sphingomonas parvus sp. nov. isolated from a ginseng-cultivated soil. *Journal of Microbiology* **53**: 673–677.

Aleklett K, Hart M, Shade A. **2014**. The microbial ecology of flowers: An emerging frontier in phyllosphere research1. *Botany* **92**: 253–266.

Amato P, Joly M, Besaury L, Oudart A, Taib N, Deguillaume L, Delort A, Debroas D, Mone I. **2017**. Active microorganisms thrive among extremely diverse communities in cloud water. *PLoS ONE* **3**: 1–22.

Amend AS, Cobian GM, Laruson AJ, Remple K, Tucker SJ, Poff KE, Antaky C, Boraks A, Jones CA, Kuehu D, *et al.* 2019. Phytobiomes are compositionally nested from the ground up. *PeerJ* **2019**: 1–18.

Aneja M, Gianfagna TJ, Hebbar PK. **2006**. Trichoderma harzianum produces nonanoic acid, an inhibitor of spore germination and mycelial growth of two cacao pathogens. *Physiological and Molecular Plant Pathology* **67**: 304–307.

Ariya PA, Amyot M. **2004**. New Directions: The role of bioaerosols in atmospheric chemistry and physics. *Atmospheric Environment* **38**: 1231–1232.

Arnold AE, Mejia LC, Kyllo D, Rojas EI, Maynard Z, Robbins N, Herre EA. **2003**. Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences* **100**: 15649–15654.

Arrigoni E, Antonielli L, Pindo M, Pertot I, Perazzolli M. **2018**. Tissue age and plant genotype affect the microbiota of apple and pear bark. *Microbiological Research* **211**: 57–68.

Atamna-Ismaeel N, Finkel O, Glaser F, von Mering C, Vorholt JA, Koblížek M, Belkin S, Béjà O. **2012a**. Bacterial anoxygenic photosynthesis on plant leaf surfaces. *Environmental Microbiology Reports* **4**: 209–216.

Atamna-Ismaeel N, Finkel OM, Glaser F, Sharon I, Schneider R, Post AF, Spudich JL, von Mering C, Vorholt JA, Iluz D, *et al.* 2012b. Microbial rhodopsins on leaf surfaces of terrestrial plants. *Environmental Microbiology* **14**: 140–146.

Augspurger CK, Kelly CK. **1984**. Pathogen mortality of tropical tree seedlings: experimental studies of the effects of dispersal distance, seedling denisty, and light conditions. *Oecologia.* **61**: 211–217.

Aydogan EL, Moser G, Müller C, Kämpfer P, Glaeser SP. **2018**. Long-term warming shifts the composition of bacterial communities in the phyllosphere of Galium album in a permanent grassland field-experiment. *Frontiers in Microbiology* **9**: 1–17.

Badri D V., Weir TL, van der Lelie D, Vivanco JM. **2009**. Rhizosphere chemical dialogues: plant-microbe interactions. *Current Opinion in Biotechnology* **20**: 642–650.

Bailey MJ, Lilley AK, Timms-Wilson TM, Spencer-Phillips PTN. **2006**. Microbial ecology of aerial plant surfaces. *Microbial Ecology of Aerial Plant Surfaces*: 1–321.

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. **2006**. the Role of Root Exudates in Rhizosphere Interactions With Plants and Other Organisms. *Annual Review of Plant Biology* **57**: 233–266.

Baker KF, Smith SH. **1966**. Dynamics of seed transmission of plan pathogens. *Annual Review of Phytopathology* **4**: 311 – 332.

Baldotto LEB, Olivares FL. **2008**. Phylloepiphytic interaction between bacteria and different plant species in a tropical agricultural system. *Canadian Journal of Microbiology* **54**: 918–931.

Baldrian P. **2017**. Forest microbiome: Diversity, complexity and dynamics. *FEMS Microbiology Reviews* **41**: 109–130.

Balint-Kurti P, Simmons SJ, Blum JE, Ballaré CL, Stapleton AE. **2010**. Maize leaf epiphytic bacteria diversity patterns are genetically correlated with resistance to fungal pathogen infection. *Molecular Plant-Microbe Interactions* **23**: 473–484.

Balint M, Bahram M, Eren AM, Faust K, Fuhrman JA, Lindahl B, O'Hara RB, Ï¿½pik M, Sogin ML, Unterseher M, *et al.* 2016. Millions of reads, thousands of taxa: Microbial community structure and associations analyzed via marker genesa. *FEMS Microbiology Reviews* **40**: 686–700.

Bálint M, Schmidt PA, Sharma R, Thines M, Schmitt I. **2014**. An Illumina metabarcoding pipeline for fungi. *Ecology and Evolution* **4**: 2642–2653.

Barbeta A, Jones SP, Clavé L, Wingate L, Gimeno TE, Fréjaville B, Wohl S, Ogee J. **2019**. Unexplained hydrogen isotope offsets complicate the identification and quantification of tree water sources in a riparian forest. *Hydrology and Earth System Sciences* **23**: 2129–2146.

Barbi F, Bragalini C, Vallon L, Prudent E, Dubost A, Fraissinet-Tachet L, Marmeisse R, Luis P. **2014**. PCR primers to study the diversity of expressed fungal genes encoding lignocellulolytic enzymes in soils using high-throughput sequencing. *PLoS ONE* **9**: 1–22.

Barret M, Briand M, Bonneau S, Préveaux A, Valière S, Bouchez O, Hunault G, Simoneau P, Jacquesa MA. **2015**. Emergence shapes the structure of the seed microbiota. *Applied and Environmental Microbiology* **81**: 1257–1266.

Barret M, Guimbaud JF, Darrasse A, Jacques MA. **2016**. Plant microbiota affects seed transmission of phytopathogenic microorganisms. *Molecular plant pathology* **17**: 791–795.

Bartlett DH. **2009**. Microbial life in the trenches. *Marine Technology Society Journal* **43**: 128–131.

Bashan Y, Holguin G. **1998**. Proposal for the division of plant growth-promoting rhizobacteria into two classifications: Biocontrol-PGPB (plant growth-promoting bacteria) and PGPB. *Soil Biology and Biochemistry* **30**: 1225–1228.

Bassas-Galia M, Nogales B, Arias S, Rohde M, Timmis KN, Molinari G. **2012**. Plant original Massilia isolates producing polyhydroxybutyrate, including one exhibiting high yields from glycerol. *Journal of Applied Microbiology* **112**: 443–454.

Bauer H, Giebl H, Hitzenberger R, Kasper-giebl A, Reischl G, Zibuschka F, Puxbaum H. **2003**.

Airborne bacteria as cloud condensation nuclei. **108**: 1–5.

Beattie GA, Lindow SE. **1995**. The Secret Life of Foliar Bacterial Pathogens on Leaves. *Annual Review of Phytopathology* **33**: 145–172.

Beattie GA, Marcell LM. **2002**. Effect of alterations in cuticular wax biosynthesis on the physicochemical properties and topography of maize leaf surfaces. *Plant, Cell and Environment* **25**: 1–16.

Beckers B, De Beeck MO, Weyens N, Boerjan W, Vangronsveld J. **2017**. Structural variability and niche differentiation in the rhizosphere and endosphere bacterial microbiome of field-grown poplar trees. *Microbiome* **5**: 1–17.

Begerow D, Nilsson H, Unterseher M, Maier W. **2010**. Current state and perspectives of fungal DNA barcoding and rapid identification procedures. *Applied Microbiology and Biotechnology* **87**: 99–108.

Bent E. **2006**. *Induced Systemic ResistanceMediated by PlantGrowth-Promoting Rhizobacteria(PGPR) and Fungi (PGPF)*.

Bentley BL, Carpenter EJ. **1984**. Direct transfer of newly-fixed nitrogen from free-living epiphyllous microorganisms to their host plant. *Oecologia* **63**: 52–56.

Berg G, Raaijmakers JM. **2018**. Saving seed microbiomes. *ISME Journal* **12**: 1167–1170.

Bertaux J, Schmid M, Hutzler P, Hartmann A, Garbaye J, Frey-Klett P. **2005**. Occurrence and distribution of endobacteria in the plant-associated mycelium of the ectomycorrhizal fungus Laccaria bicolor S238N. *Environmental Microbiology* **7**: 1786–1795.

Bever JD, Mangan SA, Alexander HM. **2015**. Maintenance of Plant Species Diversity by Pathogens. *Annual Review of Ecology, Evolution, and Systematics* **46**: 305–325.

Bjorn D. Lindahl Johanna Boberg, Susan E. Trumbore, KI, Peter Hogberg Roger D. Finlay JS. **2007**. Spatial separation of litter decomposition and mycorrhizal nitrogen \nuptake in a boreal forest. *New Phytologist*: 611–620.

Blaser MJ, Cardon ZG, Cho MK, Dangl JL, Donohue TJ, Green JL, Knight R, Maxon ME, Northen TR, Pollard KS, *et al.* 2016. Toward a predictive understanding of earth's microbiomes to address 21st century challenges. *mBio* **7**: 1–16.

Bodenhausen N, Horton MW, Bergelson J. **2013**. Bacterial Communities Associated with the Leaves and the Roots of Arabidopsis thaliana. *PLoS ONE* **8**.

De Boer W, Folman LB, Summerbell RC, Boddy L. **2005**. Living in a fungal world: Impact of fungi on soil bacterial niche development. *FEMS Microbiology Reviews* **29**: 795–811.

Boller T, Felix G. **2009**. A Renaissance of Elicitors: Perception of Microbe-Associated Molecular Patterns and Danger Signals by Pattern-Recognition Receptors. *Annual Review of Plant Biology* **60**: 379–406.

Brader G, Compant S, Vescio K, Mitter B, Trognitz F, Ma L-J, Sessitsch A. **2017**. Ecology and Genomic Insights into Plant-Pathogenic and Plant-Nonpathogenic Endophytes. *Annual Review of Phytopathology* **55**: 61–83.

Bradley DJ, Gilbert GS, Martiny JBH. **2008**. Pathogens promote plant diversity through a compensatory response. *Ecology Letters* **11**: 461–469.

Bray JR, Gorham E. **1964**. Litter Production in Forests of the World†. *Advances in Ecological Research* **2**: 101–157.

Bright M, Bulgheresi S. **2010**. A complex journey: Transmission of microbial symbionts. *Nature Reviews Microbiology* **8**: 218–230.

Bringel F, Couée I. **2015**. Pivotal roles of phyllosphere microorganisms at the interface between plant functioning and atmospheric trace gas dynamics. *Frontiers in Microbiology* **6**: 1–14.

Brouwer B, Ziolkowska A, Bagard M, Keech O, Gardeström P. **2012**. The impact of light intensity on shade-induced leaf senescence. *Plant, Cell and Environment* **35**: 1084–1098.

Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P. **2013**. Structure and Functions of the Bacterial Microbiota of Plants. *Annual Review of Plant Biology* **64**: 807–838.

Burch AY, Zeisler V, Yokota K, Schreiber L, Lindow SE. **2014**. The hygroscopic biosurfactant syringafactin produced by Pseudomonas syringae enhances fitness on leaf surfaces during fluctuating humidity. *Environmental Microbiology* **16**: 2086–2098.

Burrows SM, Butler T. **2009**. Bacteria in the global atmosphere – PartPart 2 : Modeling of emissions and transport between different ecosystems. : 9281–9297.

Burrows SM, Elbert W, Lawrence MG. **2009**. Bacteria in the global atmosphere – Part 1: Review

and synthesis of literature data for different ecosystems. *Atmospheric Chemistry and Physics*: 9263–9280.

Busse HJ, Denner EB, Buczolits S, Salkinoja-Salonen M, Bennasar A, Kämpfer P. **2003**. Sphingomonas aurantiaca sp . nov ., Sphingomonas aerolata sp . nov . and Sphingomonas faeni sp . nov ., air- and dustborne and Antarctic , orange- pigmented , psychrotolerant bacteria , and emended description of the genus Sphingomonas. *International journal of systematic and evolutionary microbiology* **53**: 1253–1260.

Callahan BJ, McMurdie PJ, Holmes SP. **2017**. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME Journal* **11**: 2639–2643.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. **2016**. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**: 581–583.

Campisano A, Ometto L, Compant S, Pancher M, Antonielli L, Yousaf S, Varotto C, Anfora G, Pertot I, Sessitsch A, *et al.* 2014. Interkingdom transfer of the acne-causing agent, propionibacterium acnes, from human to grapevine. *Molecular Biology and Evolution* **31**: 1059–1065.

Cánovas FM, Cañas RA, de la Torre FN, Pascual MB, Castro-Rodríguez V, Avila C. **2018**. Nitrogen metabolism and biomass production in forest trees. *Frontiers in Plant Science* **9**: 1–9.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman F d, Costello elizabeth K, Fierer N, Peña antonio G, Goodrich JK, Gordon JI, *et al.* 2010. QIIME allows analysis of high- throughput community sequencing data. *Nature Publishing Group* **7**: 335–336.

Cappelletti. **2018**. Update on the biopesticide regulation and development of protein-based products for the biocontrol of crop diseases.

Carrell AA, Frank AC. **2014**. Pinus flexilis and Picea engelmannii share a simple and consistent needle endophyte microbiota with a potential role in nitrogen fixation. *Frontiers in Microbiology* **5**: 1–11.

Cernava T, Chen X, Krug L, Li H, Yang M, Berg G. **2019**. The tea leaf microbiome shows specific responses to chemical pesticides and biocontrol applications. *Science of the Total Environment* **667**: 33–40.

Chakravorty S, Helb D, Burday M, Connell N, Alland D. **2007**. A detailed analysis of 16S ribosomal

RNA gene segments for the diagnosis of pathogenic bacteria. *Journal of Microbiological Methods* **69**: 330–339.

Champlot S, Berthelot C, Pruvost M, Andrew Bennett E, Grange T, Geigl EM. **2010**. An efficient multistrategy DNA decontamination procedure of PCR reagents for hypersensitive PCR applications. *PLoS ONE* **5**.

Chi Z, Wang F, Chi Z, Yue L, Liu G, Zhang T. **2009**. Bioproducts from Aureobasidium pullulans, a biotechnologically important yeast. *Applied Microbiology and Biotechnology* **82**: 793–804.

Clay K, Schardl C. **2002a**. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *American Naturalist* **160**.

Clay K, Schardl C. **2002b**. Evolutionary Origins and Ecological Consequences of Endophyte Symbiosis with Grasses. *The American Naturalist* **160**: S99–S127.

Coble AP, Vanderwall B, Mau A, Cavaleri MA. **2016**. How vertical patterns in leaf traits shift seasonally and the implications for modeling canopy photosynthesis in a temperate deciduous forest. *Tree Physiology* **36**: 1077–1091.

Coleman-Derr D, Desgarennes D, Fonseca-Garcia C, Gross S, Clingenpeel S, Woyke T, North G, Visel A, Partida-Martinez LP, Tringe SG. **2016**. Plant compartment and biogeography affect microbiome composition in cultivated and native Agave species. *New Phytologist* **209**: 798–811.

Compant S, Samad A, Faist H, Sessitsch A. **2019**. A review on the plant microbiome : Ecology , functions , and emerging trends in microbial application. *Journal of Advanced Research journal* **9**: 29–37.

Copeland JK, Yuan L, Layeghifard M, Wang PW, Guttman DS. **2015**. Seasonal community succession of the phyllosphere microbiome. *Molecular Plant-Microbe Interactions* **28**: 274–285.

Cordier T, Robin C, Capdevielle X, Fabreguettes O, Desprez-Loustau ML, Vacher C. **2012**. The composition of phyllosphere fungal assemblages of European beech (fagus sylvatica) varies significantly along an elevation gradient. *New Phytologist* **196**: 510–519.

Costello EK, Stagaman K, Dethlefsen L, Bohannan BJM, Relman DA. **2012**. The application of ecological theory toward an understanding of the human microbiome. *Science* **336**: 1255–1262.

Crawford I, Robinson NH, Flynn MJ, Foot VE, Gallagher MW, Huffman JA, Stanley WR, Kaye PH. **2014**. Characterisation of bioaerosol emissions from a Colorado pine forest: Results from the

beachon-rombas experiment. *Atmospheric Chemistry and Physics* **14**: 8559–8578.

Cregger MA, Veach AM, Yang ZK, Crouch MJ, Vilgalys R, Tuskan GA, Schadt CW. **2018**. The Populus holobiont: Dissecting the effects of plant niches and genotype on the microbiome. *Microbiome* **6**: 1–14.

Crowther TW, Stanton DWG, Thomas SM, A'Bear AD, Hiscox J, Jones TH, Voříšková J, Baldrian P, Boddy L. **2013**. Top-down control of soil fungal community composition by a globally distributed keystone consumer. *Ecology* **94**: 2518–2528.

Cruaud P, Vigneron A, Lucchetti-Miganeh C, Ciron PE, Godfroy A, Cambon-Bonavita MA. **2014**. Influence of DNA extraction method, 16S rRNA targeted hypervariable regions, and sample origin on microbial diversity detected by 454 pyrosequencing in marine chemosynthetic ecosystems. *Applied and Environmental Microbiology* **80**: 4626–4639.

D'Amore R, Ijaz UZ, Schirmer M, Kenny JG, Gregory R, Darby AC, Shakya M, Podar M, Quince C, Hall N. **2016**. A comprehensive benchmarking study of protocols and sequencing platforms for 16S rRNA community profiling. *BMC Genomics* **17**.

Darlington AB, Dat JF, Dixon MA. **2001**. The biofiltration of indoor air: Air flux and temperature influences the removal of toluene, ethylbenzene, and xylene. *Environmental Science and Technology* **35**: 240–246.

Das A, Varma A. **2009**. Symbiosis: The Art of Living. In: Symbiotic Fungi Principles and Practice. 1–28.

Delmotte N, Knief C, Chaffron S, Innerebner G, Roschitzki B, Schlapbach R, Von Mering C, Vorholt JA. **2009**. Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 16428–16433.

Després V, Huffman JA, Burrows SM, Hoose C, Safatov A, Buryak G, Fröhlich-nowoisky J, Andreae M, Pöschl U, Jaenicke R, *et al.* 2012. Primary biological aerosol particles in the atmosphere : a review. *Tellus* **0889**.

Didion M, Frey B, Rogiers N, Thürig E. **2014**. Validating tree litter decomposition in the Yasso07 carbon model. *Ecological Modelling* **291**: 58–68.

Domke GM, Perry CH, Walters BF, Woodall CW, Russell MB, Smith JE. **2016**. Estimating litter

carbon stocks on forest land in the United States. *Science of the Total Environment* **557–558**: 469–478.

Doty SL, Sher AW, Fleck ND, Khorasani M, Bumgarner RE, Khan Z, Ko AWK, Kim SH, DeLuca TH. **2016**. Variable nitrogen fixation in wild Populus. *PLoS ONE* **11**: 1–22.

Douglas AE. **2009**. The microbial dimension in insect nutritional ecology. *Functional Ecology* **23**: 38–47.

Douglas AE, Werren JH. **2016**. Holes in the hologenome: Why host-microbe symbioses are not holobionts. *mBio* **7**: 1–7.

Duran P, Thiergart T, Garrido-Oter R, Agler M, Kemen E, Schulze-Lefert P, Hacquard S. **2018**. Microbial interkingdom interactions in roots promote Arabidopsis survival. *bioRxiv*: 354167.

Edgar RC, Flyvbjerg H. **2015**. Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics* **31**: 3476–3482.

Eichlerová I, Homolka L, Žifčáková L, Lisá L, Dobiášová P, Baldrian P. **2015**. Enzymatic systems involved in decomposition reflects the ecology and taxonomy of saprotrophic fungi. *Fungal Ecology* **13**: 10–22.

Ekblad A, Wallander H, Godbold DL, Cruz C, Johnson D, Baldrian P, Björk RG, Epron D, Kieliszewska-Rokicka B, Kjøller R, *et al.* 2013. The production and turnover of extramatrical mycelium of ectomycorrhizal fungi in forest soils: Role in carbon cycling. *Plant and Soil* **366**: 1–27.

Elad Y. **1996**. Mechanisms involved in the biological control of Botrytis cinerea incited diseases. *European Journal of Plant Pathology* **102**: 719–732.

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE. **2007**. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters* **10**: 1135–1142.

Escudié F, Auer L, Bernard M, Mariadassou M, Cauquil L, Vidal K, Maman S, Hernandez-Raquet G, Combes S, Pascal G. **2018**. FROGS: Find, Rapidly, OTUs with Galaxy Solution. *Bioinformatics* **34**: 1287–1294.

Ewald PW. **1989**. Transmission modes and evolution of the parasitism-mutualism continuum. *Annals of the New York Academy of Sciences* **503**: 295–306.

Fadrosh DW, Ma B, Gajer P, Sengamalay N, Ott S, Brotman RM, Ravel J. **2014**. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the IlluminaMiSeqplatform. *Microbiome* **2**: 6.

Faeth SH. **2002**. Are endophytic fungi defensive plant mutualists? *Oikos* **98**: 25–36.

Fang Z, Gong C, Ouyang Z, Liu P, Sun L, Wang X. **2014**. Characteristic and Concentration Distribution of Culturable Airborne Bacteria in Residential Environments in Beijing , China. : 943–953.

Fargione J, Tilman D, Dybzinski R, Lambers JHR, Clark C, Harpole WS, Knops JMH, Reich PB, Loreau M. **2007**. From selection to complementarity: Shifts in the causes of biodiversity-productivity relationships in a long-term biodiversity experiment. *Proceedings of the Royal Society B: Biological Sciences* **274**: 871–876.

Farré-Armengol G, Filella I, Llusia J, Peñuelas J. **2016**. Bidirectional Interaction between Phyllospheric Microbiotas and Plant Volatile Emissions. *Trends in Plant Science* **21**: 854–860.

Faust K, Raes J. **2012**. Microbial interactions: From networks to models. *Nature Reviews Microbiology* **10**: 538–550.

Fehsenfeld F, Calvert J, Fall R, Goldan P, Guenther AB, Hewitt CN, Lamb B, Trainer M, Westberg H. **1992**. Emissions of volatile organic compounds from vegetation and the implications ofr atmospheric chemistry. *Global biogeochemical cylces* **6**: 389–430.

Feldman TS, Brien HEO, Arnold AE. **2008**. Moths that Vector a Plant Pathogen also Transport Endophytic Fungi and Mycoparasitic Antagonists. *Microbial Ecology* **56**: 742–750.

Fenner M, Thompson K. **2005**. *The ecology of seeds*.

Field CB, Behrenfeld MJ, Randerson JT, Falkowski P. **1998**. Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science* **281**: 237–240.

Fioretto A, Papa S, Pellegrino A, Fuggi A. **2007**. Decomposition dynamics of Myrtus communis and Quercus ilex leaf litter: Mass loss, microbial activity and quality change. *Applied Soil Ecology* **36**: 32–40.

Flemming HC, Wuertz S. **2019**. Bacteria and archaea on Earth and their abundance in biofilms. *Nature Reviews Microbiology* **17**: 247–260.

Fliegerova K, Tapio I, Bonin A, Mrazek J, Callegari ML, Bani P, Bayat A, Vilkki J, Kopečný J, Shingfield KJ, *et al.* 2014. Effect of DNA extraction and sample preservation method on rumen bacterial population. *Anaerobe* **29**: 80–84.

Forchetti G, Masciarelli O, Alemano S, Alvarez D, Abdala G. 2007. Endophytic bacteria in sunflower (Helianthus annuus L.): Isolation, characterization, and production of jasmonates and abscisic acid in culture medium. *Applied Microbiology and Biotechnology* **76**: 1145–1152.

Foster KR, Schluter J, Coyte KZ, Rakoff-Nahoum S. 2017. The evolution of the host microbiome as an ecosystem on a leash. *Nature* **548**: 43–51.

Franc GD, Demott PJ. 1998. Cloud activation characteristics of airborne Erwinia carotovora cells. *Journal of Applied Meteorology* **37**: 1293–1300.

Frank A, Saldierna Guzmán J, Shay J. 2017. Transmission of Bacterial Endophytes. *Microorganisms* **5**: 70.

Franzetti A, Gandolfi I, Bestetti G, Padoa Schioppa E, Canedoli C, Brambilla D, Cappelletti D, Sebastiani B, Federici E, Papacchini M, *et al.* 2020. Plant-microorganisms interaction promotes removal of air pollutants in Milan (Italy) urban area. *Journal of Hazardous Materials* **384**.

Freiberg E. 1998. Microclimatic parameters influencing nitrogen fixation in the phyllosphere in a Costa Rican premontane rain forest. *Oecologia* **117**: 9–18.

Freilich S, Zarecki R, Eilam O, Segal ES, Henry CS, Kupiec M, Gophna U, Sharan R, Ruppin E. 2011. Competitive and cooperative metabolic interactions in bacterial communities. *Nature Communications* **2**.

Friesen ML, Porter SS, Stark SC, von Wettberg EJ, Sachs JL, Martinez-Romero E. 2011. Microbially Mediated Plant Functional Traits. *Annual Review of Ecology, Evolution, and Systematics* **42**: 23–46.

Fröhlich-Nowoisky J, Kampf CJ, Weber B, Huffman JA, Pï¿½hlker C, Andreae MO, Lang-Yona N, Burrows SM, Gunthe SS, Elbert W, *et al.* 2016. Bioaerosols in the Earth system: Climate, health, and ecosystem interactions. *Atmospheric Research* **182**: 346–376.

Fukasawa Y, Osono T, Takeda H. 2009. Dynamics of physicochemical properties and occurrence of fungal fruit bodies during decomposition of coarse woody debris of Fagus crenata. *Journal of Forest Research* **14**: 20–29.

Fukasawa Y, Osono T, Takeda H. **2011**. Wood decomposing abilities of diverse lignicolous fungi on nondecayed and decayed beech wood. *Mycologia* **103**: 474–482.

Fürnkranz M, Wanek W, Richter A, Abell G, Rasche F, Sessitsch A. **2008**. Nitrogen fixation by phyllosphere bacteria associated with higher plants and their colonizing epiphytes of a tropical lowland rainforest of Costa Rica. *ISME Journal* **2**: 561–570.

Galan M, Razzauti M, Bard E, Bernard M, Brouat C, Charbonnel N, Dehne-Garcia A, Loiseau A, Tatard C, Tamisier L, *et al.* 2016. 16S rRNA Amplicon Sequencing for Epidemiological Surveys of Bacteria in Wildlife. *mSystems* **1**: e00032-16.

Gallart M, Adair KL, Love J, Meason DF, Clinton PW, Xue J, Turnbull MH. **2018**. Host Genotype and Nitrogen Form Shape the Root Microbiome of Pinus radiata. *Microbial Ecology* **75**: 419–433.

Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, *et al.* 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry 70:*: 153–226.

Gesheva V, Vasileva-Tonkova E. **2012**. Production of enzymes and antimicrobial compounds by halophilic Antarctic Nocardioides sp. grown on different carbon sources. *World Journal of Microbiology and Biotechnology* **28**: 2069–2076.

Gevers D, Knight R, Petrosino JF, Huang K, McGuire AL, Birren BW, Nelson KE, White O, Methé BA, Huttenhower C. **2012**. The Human Microbiome Project: A Community Resource for the Healthy Human Microbiome. *PLoS Biology* **10**: 6–10.

Ghyselinck J, Pfeiffer S, Heylen K, Sessitsch A, De Vos P. **2013**. The effect of primer choice and short read sequences on the outcome of 16S rRNA gene based diversity studies. *PloS one* **8**: 1–14.

Gilbert GS. **2002**. Evolutionary ecology of plant diseases in natural ecosystems. *Annual Review of Phytopathology* **40**: 13–43.

Gilbert JA, Neufeld JD. **2014**. Life in a World without Microbes. *PLoS Biology* **12**: 1–3.

Gimenez-Ibanez S, Boter M, Ortigosa A, Garcï¿½a-Casado G, Chini A, Lewsey MG, Ecker JR, Ntoukakis V, Solano R. **2017**. JAZ2 controls stomata dynamics during bacterial invasion. *New Phytologist* **213**: 1378–1392.

Goodwin S, McPherson JD, McCombie WR. **2016**. Coming of age: Ten years of next-generation sequencing technologies. *Nature Reviews Genetics* **17**: 333–351.

Graham JH, Colburn GC, Chung KR, Cubero J. **2012**. Protection of citrus roots against infection by Phytophthora spp. by hypovirulent P. nicotianae is not related to induction of systemic acquired resistance. *Plant and Soil* **358**: 39–49.

Greenstone MH, Weber DC, Coudron TA, Payton ME, Hu JS. **2012**. Removing external DNA contamination from arthropod predators destined for molecular gut-content analysis. *Molecular Ecology Resources* **12**: 464–469.

Großkopf T, Soyer OS. **2014**. Synthetic microbial communities. *Current Opinion in Microbiology* **18**: 72–77.

Gundel PE, Rudgers JA, Ghersa CM. **2011**. Incorporating the process of vertical transmission into understanding of host-symbiont dynamics. *Oikos* **120**: 1121–1128.

Gundel PE, Rudgers JA, Whitney KD. **2017**. Vertically transmitted symbionts as mechanisms of transgenerational effects. *American Journal of Botany* **104**: 787–792.

Hacquard S, Garrido-Oter R, González A, Spaepen S, Ackermann G, Lebeis S, McHardy AC, Dangl JL, Knight R, Ley R, *et al.* 2015. Microbiota and host nutrition across plant and animal kingdoms. *Cell Host and Microbe* **17**: 603–616.

Haga DI, Iannone R, Wheeler MJ, Mason R, Polishchuk EA, Fetch T, Van Der Kamp BJ, McKendry IG, Bertram AK. **2013**. Ice nucleation properties of rust and bunt fungal spores and their transport to high altitudes, where they can cause heterogeneous freezing. *Journal of Geophysical Research Atmospheres* **118**: 7260–7272.

Hahn K, Christensen M. **2004**. Dead Wood in European Forest Reserves – A Reference for Forest Management. In: Monitoring and Indicators of Forest Biodiversity in Europe – From Ideas to Operationality. 181.

Hania W Ben, Godbane R, Postec A, Hamdi M, Ollivier B, Fardeau M. **2019**. Defluviitoga tunisiensis gen. nov., sp. nov., a thermophilic bacterium isolated from a mesothermic and anaerobic whey digester. *International Journal of Systematic and Evolutionary Microbiology* **62**: 1377–1382.

Hardoim PR, Hardoim CCP, van Overbeek LS, van Elsas JD. **2012**. Dynamics of seed-borne rice endophytes on early plant growth stages. *PLoS ONE* **7**.

Hardoim PR, van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M, Sessitsch A. **2015**. The Hidden World within Plants: Ecological and Evolutionary Considerations for

Defining Functioning of Microbial Endophytes. *Microbiology and Molecular Biology Reviews* **79**: 293–320.

Haritash AK, Kaushik CP. **2009**. Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A review. **169**: 1–15.

Harmon ME, Franklin JF, Swanson FJ, Sollins P, Gregory S V., Lattin JD, Anderson NH, Cline SP, Aumen NG, Sedell JR, *et al.* 1986. Ecology of Coarse Woody Debris in Temperate Ecosystems. *Advances in Ecological Research* **34**: 59–234.

Harrison JG, Forister ML, Parchman TL, Koch GW. **2016**. Vertical stratification of the foliar fungal community in the world's tallest trees. *American Journal of Botany* **103**: 2087–2095.

Hassani MA, Durán P, Hacquard S. **2018**. Microbial interactions within the plant holobiont. : 1–17.

Heather JM, Chain B. **2016**. The sequence of sequencers: The history of sequencing DNA. *Genomics* **107**: 1–8.

Hebert PDN, Cywinska A, Ball SL, DeWaard JR. **2003**. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences* **270**: 313–321.

Heilmann-Clausen J, Christensen M. **2005**. Wood-inhabiting macrofungi in Danish beech-forests - Conflicting diversity patterns and their implications in a conservation perspective. *Biological Conservation* **122**: 633–642.

Heywood VH. **1969**. Scanning electron microscopy in the study of plant materials. *Micron (1969)* **1**: 1–14.

Hodgson S, de Cates C, Hodgson J, Morley NJ, Sutton BC, Gange AC. **2014**. Vertical transmission of fungal endophytes is widespread in forbs. *Ecology and Evolution* **4**: 1199–1208.

Högberg MN, Högberg P. **2002**. Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. *New Phytologist* **154**: 791–795.

Högberg P, Plamboeck AH, Taylor AFS, Fransson PMA. **1999**. Natural 13 C abundance reveals trophic status of fungi and host-origin of carbon in mycorrhizal fungi in mixed forests. *Proceedings of the National Academy of Sciences of the United States of America* **96**: 8534–8539.

Huffman JA, Prenni AJ, Demott PJ, Pöhlker C, Mason RH, Robinson NH, Fröhlich-Nowoisky J,

Tobo Y, Després VR, Garcia E, *et al.* 2013. High concentrations of biological aerosol particles and ice nuclei during and after rain. *Atmospheric Chemistry and Physics* **13**: 6151–6164.

Iguchi H, Sato I, Sakakibara M, Yurimoto H, Sakai Y. **2012**. Distribution of methanotrophs in the phyllosphere. *Bioscience, Biotechnology and Biochemistry* **76**: 1580–1583.

Innerebner G, Knief C, Vorholt JA. **2011**. Protection of Arabidopsis thaliana against leaf-pathogenic Pseudomonas syringae by Sphingomonas strains in a controlled model system. *Applied and Environmental Microbiology* **77**: 3202–3210.

Ivanova EG, Doronina N V., Shepelyakovskaya AO, Laman AG, Brovko FA, Trotsenko YA. **2000**. Facultative and obligate aerobic methylobacteria synthesize cytokinins. *Microbiology* **69**: 646–651.

Ivanova EG, Doronina N V., Trotsenko YA. **2001**. Aerobic methylobacteria are capable of synthesizing auxins. *Microbiology* **70**: 392–397.

Ivanova EG, Fedorov DN, Doronina N V., Trotsenko YA. **2006**. Production of vitamin B12 in aerobic methylotrophic bacteria. *Microbiology* **75**: 494–496.

Johnston SR, Boddy L, Weightman AJ. **2016**. Bacteria in decomposing wood and their interactions with wood-decay fungi. *FEMS microbiology ecology* **92**: 1–12.

Jumpponen A, Jones KL. **2009**. Massively parallel 454 sequencing indicates hyperdiverse fungal communities in temperate Quercus macrocarpa phyllosphere. *New Phytologist* **184**: 438–448.

Junker RR, Tholl D. **2013**. Volatile Organic Compound Mediated Interactions at the Plant-Microbe Interface. *Journal of Chemical Ecology* **39**: 810–825.

Kämpfer P, Rainey FA, Andersson MA, Nurmiaho Lassila E-L, Ulrych U, Busse H., Weiss N, Mikkola R, Salkinoja-Salonen M. **2019**. Frigoribacterium faeni gen . nov ., sp . nov ., a novel psychrophilic genus of the family Microbacteriaceae. : 355–363.

Kanters C, Anderson IC, Johnson D. **2015**. Chewing up the wood-wide web: Selective grazing on ectomycorrhizal fungi by collembola. *Forests* **6**: 2560–2570.

Karamanoli K, Menkissoglu-Spiroudi U, Bosabalidis AM, Vokou D, Constantinidou HIA. **2005**. Bacterial colonization of the phyllosphere of nineteen plant species and antimicrobial activity of their leaf secondary metabolites against leaf associated bacteria. *Chemoecology* **15**: 59–67.

Kawaguchi K, Yurimoto H, Oku M, Sakai Y. **2011**. Yeast methylotrophy and autophagy in a

methanol-oscillating environment on growing Arabidopsis thaliana leaves. *PLoS ONE* **6**: 1–9.

Kellner H, Vandenbol M. **2010**. Fungi unearthed: Transcripts encoding lignocellulolytic and chitinolytic enzymes in forest soil. *PLoS ONE* **5**.

Kembel SW, Mueller RC. **2014**. Plant traits and taxonomy drive host associations in tropical phyllosphere fungal communities [1]. *Botany* **92**: 303–311.

Kembel SW, O'Connor TK, Arnold HK, Hubbell SP, Wright SJ, Green JL. **2014**. Relationships between phyllosphere bacterial communities and plant functional traits in a neotropical forest. *Proceedings of the National Academy of Sciences* **111**: 13715–13720.

Kemen E, Kemen AC, Agler MT, Kemen E. **2015**. Host – microbe and microbe – microbe interactions in the evolution of obligate plant parasitism. *New Phytologist*: 1–18.

De Kempeneer L, Sercu B, Vanbrabant W, Van Langenhove H, Verstraete W. **2004**. Bioaugmentation of the phyllosphere for the removal of toluene from indoor air. *Applied Microbiology and Biotechnology* **64**: 284–288.

Kesselmeier J, Staudt M. **1999**. An Overview on Emission, Physiology and Ecology.pdf. *Journal of Atmospheric Chemistry* **33**: 23–88.

Khan AL, Waqas M, Kang SM, Al-Harrasi A, Hussain J, Al-Rawahi A, Al-Khiziri S, Ullah I, Ali L, Jung HY, *et al.* 2014. Bacterial endophyte Sphingomonas sp. LK11 produces gibberellins and IAA and promotes tomato plant growth. *Journal of Microbiology* **52**: 689–695.

Kielak AM, Scheublin TR, Mendes LW, van Veen JA, Kuramae EE. **2016**. Bacterial community succession in pine-wood decomposition. *Frontiers in Microbiology* **7**: 1–12.

Kiers ET, Heijden MGA Van Der. **2006**. Mutualistic stability in the arbuscular mycorrhizal symbiosis: Exploring hypothesis of evolutionary cooperation. *Ecology* **87**: 1627–1636.

Klaedtke S, Jacques MA, Raggi L, Préveaux A, Bonneau S, Negri V, Chable V, Barret M. **2016**. Terroir is a key driver of seed-associated microbial assemblages. *Environmental microbiology* **18**: 1792–1804.

Klassen JL, Foght JM. **2011**. Characterization of Hymenobacter isolates from Victoria Upper Glacier, Antarctica reveals five new species and substantial non-vertical evolution within this genus. *Extremophiles* **15**: 45–57.

Kloepper J, Schroth M. 1978. Plant growth-promoting rhizobacteria on radishes. IV international conference on plant pathogenic bacteria Article. *Reads*: 879.

Knief C, Chaffron S, Stark M, Innerebner G. **2012**. Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. *The ISME Journal*: 1378–1390.

Knoch TR, Faeth SH, Arnott DL. **1993**. Endophytic fungi alter foraging and dispersal by desert seed-harvesting ants. *Oecologia* **95**: 470–473.

Knoll D, Schreiber L. **2000**. Plant-microbe interactions: Wetting of ivy (Hedera helix L.) leaf surfaces in relation to colonization by epiphytic microorganisms. *Microbial Ecology* **40**: 33–42.

Konopka A. **2009**. What is microbial community ecology. *ISME Journal* **3**: 1223–1230.

Koskella B, Brockhurst MA. **2014**. Bacteria-phage coevolution as a driver of ecological and evolutionary processes in microbial communities. *FEMS Microbiology Reviews* **38**: 916–931.

Kraft NJB, Ackerly DD. **2010**. Functional trait and phylogenetic tests of community assembly across spatial scales in an Amazonian forest. *Ecological Monographs* **80**: 401–422.

Krishna MP, Mohan M. **2017**. Litter decomposition in forest ecosystems: a review. *Energy, Ecology and Environment* **2**: 236–249.

L H. **1904**. Über neuere Erfahrungen und Probleme aufdem Gebiete der Bodenbakteriologie unter besonderer Berücksichtigung der Gründüngung und Brache. : 59–78.

Lafferty doty S. **2017**. *Functional Importance of the Plant Microbiome*.

Laforest-Lapointe I. **2017**. Exploring the temperate tree leaf microbiome: From natural forests to controlled experiments and urban environments.

Laforest-Lapointe I, Messier C, Kembel SW. **2016a**. Host species identity, site and time drive temperate tree phyllosphere bacterial community structure. *Microbiome* **4**: 1–10.

Laforest-Lapointe I, Messier C, Kembel SW. **2016b**. Tree phyllosphere bacterial communities: exploring the magnitude of intra- and inter-individual variation among host species. *PeerJ* **4**: 1–18.

Laforest-Lapointe I, Paquette A, Messier C, Kembel SW. **2017**. Leaf bacterial diversity mediates plant diversity and ecosystem function relationships. *Nature* **546**: 145–147.

Lambais MR, Crowley DE, Cury JC, Büll RC, Rodrigues RR. **2006**. Bacterial diversity in tree canopies of the Atlantic Forest. *Science* **312**: 1917.

Lange M, Habekost M, Eisenhauer N, Roscher C, Bessler H, Engels C, Oelmann Y, Scheu S, Wilcke W, Schulze ED, *et al.* 2014. Biotic and abiotic properties mediating plant diversity effects on soil microbial communities in an experimental grassland. *PLoS ONE* **9**.

Larsson J. **2018**. eulerr: Area-Proportional Euler Diagrams with Ellipses.

Last FT. **1955**. Seasonal incidence of Sporobolomyces on cereal leaves. *Transactions of the British Mycological Society*: 221–239.

Lauber CL, Hamady M, Knight R, Fierer N. **2009**. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology* **75**: 5111–5120.

Lavoie M, Mack MC, Hiers JK, Pokswinski S. **2012**. The effect of restoration treatments on the spatial variability of soil processes under longleaf pine trees. *Forests* **3**: 591–604.

LeBauer, D; Treseder K. **2008**. Nitrogen Limitation of Net Primary Productivity. *Ecology* **89**: 371–379.

Lebeis SL. **2015**. Greater than the sum of their parts: Characterizing plant microbiomes at the community-level. *Current Opinion in Plant Biology* **24**: 82–86.

Leck MA, Parker VT, Simpson RL. **2008**. Seedling ecology and evolution. *Seedling Ecology and Evolution*: 1–514.

Lecours PB, Veillette M, Marsolais D, Duchaine C. **2012**. Characterization of bioaerosols from dairy barns: Reconstructing the puzzle of occupational respiratory diseases by using molecular approaches. *Applied and Environmental Microbiology* **78**: 3242–3248.

Lee YM, Kim EH, Lee HK, Hong SG. **2014**. Biodiversity and physiological characteristics of Antarctic and Arctic lichens-associated bacteria. *World Journal of Microbiology and Biotechnology* **30**: 2711–2721.

Lee SD, Lee DW, Ko Y. **2011**. Marmoricola korecus sp. nov. *International Journal of Systematic and Evolutionary Microbiology* **61**: 1628–1631.

Lee HS, Madhaiyan M, Kim CW, Choi SJ, Chung KY, Sa TM. **2006**. Physiological enhancement of early growth of rice seedlings (Oryza sativa L.) by production of phytohormone of N2-fixing methylotrophic isolates. *Biology and Fertility of Soils* **42**: 402–408.

Leff JW, Del Tredici P, Friedman WE, Fierer N. **2015**. Spatial structuring of bacterial communities within individual Ginkgo biloba trees. *Environmental Microbiology* **17**: 2352–2361.

Lemanceau P, Barret M, Mazurier S, Mondy S, Pivato B, Fort T, Vacher C. **2017**. *Plant Communication With Associated Microbiota in the Spermosphere, Rhizosphere and Phyllosphere*. Elsevier Ltd.

Lemons A, Clay K, Rudgers JA. **2005**. Connecting plant-microbial interactions above and belowground: A fungal endophyte affects decomposition. *Oecologia* **145**: 595–604.

Leveau JHJ, Lindow SE. **2001**. Appetite of an epiphyte: Quantitative monitoring of bacterial sugar consumption in the phyllosphere. *Proceedings of the National Academy of Sciences of the United States of America* **98**: 3446–3453.

Li Y, Wu X, Chen T, Wang W, Liu G, Zhang W, Li S, Wang M, Zhao C, Zhou H, *et al.* 2018. Plant phenotypic traits eventually shape its microbiota: A common garden test. *Frontiers in Microbiology* **9**: 1–13.

Lighthart B, Shaffer BT. **1994**. Bacterial flux from chaparral into the atmosphere in mid-summer at a high desert location. *Atmospheric Environment* **28**: 1267–1274.

Lindahl BD, Nilsson RH, Tedersoo L. **2013**. Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide - Lindahl - 2013 - New Phytologist - Wiley Online Library. *New …*.

Lindemann J, Constantinidou HA, Barchet WR, Upper CD. **1982**. Plants as sources of airborne bacteria, including ice nucleation-active bacteria. *Applied and Environmental Microbiology* **44**: 1059–1063.

Lindow SE, Brandl MT. **2003**. Microbiology of the Phyllosphere. *Applied and Environmental Microbiology* **69**: 1875–1883.

Links MG, Demeke T, Gräfenhan T, Hill JE, Hemmingsen SM, Dumonceaux TJ. **2014**. Simultaneous profiling of seed-associated bacteria and fungi reveals antagonistic interactions between microorganisms within a shared epiphytic microbiome on Triticum and Brassica seeds. *New Phytologist* **202**: 542–553.

Liu Y, Zuo S, Zou Y, Wang J, Song W. **2012**. Investigation on diversity and population succession dynamics of indigenous bacteria of the maize spermosphere. *World Journal of Microbiology and*

Biotechnology **28**: 391–396.

Lladó S, López-Mondéjar R, Baldrian P. **2017**. Forest Soil Bacteria: Diversity, Involvement in Ecosystem Processes, and Response to Global Change. *Microbiology and Molecular Biology Reviews* **81**: 1–27.

López-Mondéjar R, Voříšková J, Větrovský T, Baldrian P. **2015**. The bacterial community inhabiting temperate deciduous forests is vertically stratified and undergoes seasonal dynamics. *Soil Biology and Biochemistry* **87**: 43–50.

Lopez-Velasco G, Carder PA, Welbaum GE, Ponder MA. **2013**. Diversity of the spinach (Spinacia oleracea) spermosphere and phyllosphere bacterial communities. *FEMS Microbiology Letters* **346**: 146–154.

Louca S, Jacques SMS, Pires APF, Leal JS, Srivastava DS, Parfrey LW, Farjalla VF, Doebeli M. **2016**. High taxonomic variability despite stable functional structure across microbial communities. *Nature Ecology & Evolution* **1**: 0015.

Lugtenberg B, Kamilova F, Lugtenberg B, Kamilova F. **2015**. Plant-Growth-Promoting Rhizobacteria Plant-Growth-Promoting Rhizobacteria.

Lynn M, Fester R. **1991**. *Symbiogenesis and Symbionticism*.

Lyons JA, Bemis DA, Bryant MJ, Kania SA, Abd-Eldaim M, Newman SJ. **2009**. Isolation of Propionibacterium acnes from a case of placentitis and abortion in a cow. *Journal of Veterinary Diagnostic Investigation* **21**: 274–277.

Maignien L, DeForce EA, Chafee ME, Murat Eren A, Simmons SL. **2014**. Ecological succession and stochastic variation in the assembly of Arabidopsis thaliana phyllosphere communities. *mBio* **5**.

Malfanova N, Lugtenberg BJJ, Berg G. **2013**. Bacterial Endophytes: Who and Where, and What are they doing there? *Molecular Microbial Ecology of the Rhizosphere* **1**: 391–403.

Manirajan BA, Maisinger C, Ratering S, Rusch V, Schwiertz A, Cardinale M, Schnell S. **2018**. Diversity, specificity, co-occurrence and hub taxa of the bacterial–fungal pollen microbiome. *FEMS Microbiology Ecology*: 1–11.

Marchesi JR, Ravel J. **2015**. The vocabulary of microbiome research: a proposal. *Microbiome* **3**: 1–3.

Martinez-romero E. **2006**. Dinitrogen-Fixing Prokaryotes. *Prokaryotes*: 793–817.

Maude RB. **1996**. *Seedborne Diseases and Their Control: Principles and Practice*.

Maus I, Gabriela K, Wibberg D, Winkler A, Stolze Y, König H, Pühler A, Schlüter A. **2015**. Complete genome sequence of the strain Defluviitoga tunisiensis L3 , isolated from a thermophilic , production-scale biogas plant. *Journal of Biotechnology* **203**: 17–18.

McDonald D, Price MN, Goodrich J, Nawrocki EP, Desantis TZ, Probst A, Andersen GL, Knight R, Hugenholtz P. **2012**. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME Journal* **6**: 610–618.

Melotto M, Underwood W, He SY. **2008**. Role of Stomata in Plant Innate Immunity and Foliar Bacterial Diseases. *Annual Review of Phytopathology* **46**: 101–122.

Mercier J, Lindow SE. **2000**. Role of leaf surface sugars in colonization of plants by bacterial epiphytes. *Applied and Environmental Microbiology* **66**: 369–374.

Miliūtė I, Buzaitė O. **2011**. IAA production and other plant growth promoting traits of endophytic bacteria from apple tree. *Biologija* **57**: 98–102.

Mitter B, Pfaffenbichler N, Flavell R, Compant S, Antonielli L, Petric A, Berninger T, Naveed M, Sheibani-Tezerji R, Maltzahn G von, *et al.* 2017. A new approach to modify plant microbiomes and traits by introducing beneficial bacteria at flowering into progeny seeds. *Frontiers in Microbiology* **8**.

Miura T, Sánchez R, Castañeda LE, Godoy K, Barbosa O. **2019**. Shared and unique features of bacterial communities in native forest and vineyard phyllosphere. *Ecology and Evolution* **9**: 3295–3305.

Monier JM, Lindow SE. **2003**. Differential survival of solitary and aggregated bacterial cells promotes aggregate formation on leaf surfaces. *Proceedings of the National Academy of Sciences of the United States of America* **100**: 15977–15982.

Morris CE, Conen F, Alex Huffman J, Phillips V, Pöschl U, Sands DC. **2014a**. Bioprecipitation: A feedback cycle linking Earth history, ecosystem dynamics and land use through biological ice nucleators in the atmosphere. *Global Change Biology* **20**: 341–351.

Morris CE, Leyronas C, Nicot PC. **2014b**. Movement of Bioaerosols in the Atmosphere and the Consequences for Climate and Microbial Evolution. *Aerosol Science: Technology and Applications*: 393–415.

Morris CE, National I, Recherche D. **2001**. Phyllosphere. : 1–8.

Morris CE, Sands DC, Glaux C, Samsatly J, Asaad S, Moukahel AR, Gonçalves FLT, Bigg EK. **2013**. Urediospores of rust fungi are ice nucleation active at >-10 °c and harbor ice nucleation active bacteria. *Atmospheric Chemistry and Physics* **13**: 4223–4233.

Moyes AB, Kueppers LM, Pett-Ridge J, Carper DL, Vandehey N, O'Neil J, Frank AC. **2016a**. Evidence for foliar endophytic nitrogen fixation in a widely distributed subalpine conifer. *New Phytologist* **210**: 657–668.

Moyes AB, Kueppers LM, Pett-Ridge J, Carper DL, Vandehey N, O'Neil J, Frank AC. **2016b**. Evidence for foliar endophytic nitrogen fixation in a widely distributed subalpine conifer. *New Phytologist* **210**: 657–668.

Müller T, Ruppel S. **2014**. Progress in cultivation-independent phyllosphere microbiology. *FEMS Microbiology Ecology* **87**: 2–17.

Müller DB, Vogel C, Bai Y, Vorholt JA. **2016**. The Plant Microbiota: Systems-Level Insights and Perspectives. *Annual Review of Genetics* **50**: 211–234.

Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. **1986**. Specific enzymatic amplification of DNA in vitro: The polymerase chain reaction. : 11.

Munkvold GP. **2009**. Seed Pathology Progress in Academia and Industry. *Annual Review of Phytopathology* **47**: 285–311.

Nakazato T, Ohta T, Bono H. **2013**. Experimental Design-Based Functional Mining and Characterization of High-Throughput Sequencing Data in the Sequence Read Archive. *PLoS ONE* **8**.

Naveed M, Mitter B, Reichenauer TG, Wieczorek K, Sessitsch A. **2014**. Increased drought stress resilience of maize through endophytic colonization by Burkholderia phytofirmans PsJN and Enterobacter sp. FD17. *Environmental and Experimental Botany* **97**: 30–39.

Nelson EB. **2004**. Microbial Dynamics and Interactions in the Spermosphere. *Annual Review of Phytopathology* **42**: 271–309.

Nelson EB. **2017**. The seed microbiome: Origins, interactions, and impacts. *Plant and Soil* **422**: 7–34.

Nelson EB, Simoneau P, Barret M, Mitter B, Compant S. **2018**. The soil, the seed, the microbes

and the plant. *Plant Soil* **422**: 1–5.

Nemergut DR, Schmidt SK, Fukami T, O'Neill SP, Bilinski TM, Stanish LF, Knelman JE, Darcy JL, Lynch RC, Wickey P, *et al.* 2013. Patterns and Processes of Microbial Community Assembly. *Microbiology and Molecular Biology Reviews* **77**: 342–356.

Neubauer M, Sourek J, Ryc M, Bohacek J, Mara M, Mnukova J. 1991. Corynebacterium accolens sp . nov ., a Gram-Positive Rod Exhibiting Satellitism , from Clinical Material. **51**: 46–51.

Newton AC, Gravouil C, Fountaine JM. 2010. Managing the ecology of foliar pathogens: Ecological tolerance in crops. *Annals of Applied Biology* **157**: 343–359.

Nguyen SN, Flores A, Talamantes D, Dar F, Valdez A, Schwans J, Berlemont R. 2019. GeneHunt for rapid domain-specific annotation of glycoside hydrolases. *Scientific Reports* **9**: 1–10.

Nguyen NH, Smith D, Peay K, Kennedy P. 2014. Parsing ecological signal from noise in next generation amplicon sequencing. *New p*.

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG. 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology* **20**: 241–248.

Nilakanta H, Drews KL, Firrell S, Foulkes MA, Jablonski KA. 2014. A review of software for analyzing molecular Sequences. *BMC Research Notes* **7**.

Nilsson RH, Anslan S, Bahram M, Wurzbacher C, Baldrian P, Tedersoo L. 2019. Mycobiome diversity: high-throughput sequencing and identification of fungi. *Nature Reviews Microbiology* **17**: 95–109.

Nisbet M, Briggs S, Ellis-Pegler R, Thomas M, Holland D. 2007. Propionibacterium acnes: An under-appreciated cause of post-neurosurgical infection. *Journal of Antimicrobial Chemotherapy* **60**: 1097–1103.

Niu B, Paulson JN, Zheng X, Kolter R. 2017. Simplified and representative bacterial community of maize roots. *Proceedings of the National Academy of Sciences of the United States of America* **114**: E2450–E2459.

Norros V, Penttilä R, Suominen M, Ovaskainen O. 2012. Dispersal may limit the occurrence of specialist wood decay fungi already at small spatial scales. *Oikos* **121**: 961–974.

O'Brien HE, Parrent JL, Jackson JA, Moncalvo JM, Vilgalys R. **2005**. Fungal community analysis by large-scale sequencing of environmental samples. *Applied and Environmental Microbiology* **71**: 5544–5550.

Ofek M, Hadar Y, Minz D. **2011**. Colonization of cucumber seeds by bacteria during germination. *Environmental Microbiology* **13**: 2794–2807.

Oksanen AJ, Kindt R, Legendre P, Hara BO, Simpson GL, Stevens MHH, Wagner H. **2008**. The vegan Package; Community Ecology Package (Version 1.15-1).

Osono T. **2006**. Role of phyllosphere fungi of forest trees in the development of decomposer fungal communities and decomposition processes of leaf litter. *Canadian Journal of Microbiology* **52**: 701–716.

Osono T, Mori A. **2003**. Colonization of Japanese beech leaves by phyllosphere fungi. *Mycoscience* **44**: 437–441.

Osono T, Mori A. **2004**. Distribution of phyllosphere fungi within the canopy of giant dogwood. *Mycoscience* **45**: 161–168.

Osono T, Takeda H. **2001**. Organic chemical and nutrient dynamics in decomposing beech leaf litter in relation to fungal ingrowth and succession during 3-year decomposition processes in a cool temperate deciduous forest in Japan. *Ecological Research* **16**: 649–670.

Ottesen AR, White JR, Skaltsas DN, Newell MJ, Walsh CS. **2009**. Impact of organic and conventional management on the phyllosphere microbial ecology of an apple crop. *Journal of Food Protection* **72**: 2321–2325.

Ouayjan A, Hampe A. **2018**. Extensive sib-mating in a refugial population of beech (Fagus sylvatica) growing along a lowland river. *Forest Ecology and Management* **407**: 66–74.

Pascazio S, Crecchio C, Ricciuti P, Palese AM, Xiloyannis C, Sofo A. **2015**. Phyllosphere and carposphere bacterial communities in olive plants subjected to different cultural practices. *International Journal of Plant Biology* **6**: 15–19.

Pauvert C, Buée M, Laval V, Edel-Hermann V, Fauchery L, Gautier A, Lesur I, Vallance J, Vacher C. **2019**. Bioinformatics matters: The accuracy of plant and soil fungal community data is highly dependent on the metabarcoding pipeline. *Fungal Ecology* **41**: 23–33.

Penuelas J, Llusia J. **2001**. The complexity of factors driving volatile organic compound emissions

by plants. *Biologia plantarum* **44**: 481–487.

Peredo EL, Simmons SL. **2018**. Leaf-FISH: Microscale imaging of bacterial taxa on phyllosphere. *Frontiers in Microbiology* **8**: 1–14.

Pereira SIA, Castro PML. **2014**. Diversity and characterization of culturable bacterial endophytes from Zea mays and their potential as plant growth-promoting agents in metal-degraded soils. : 14110–14123.

Perry DA. **2009**. Forest Ecosystems. *Forest Ecosystems*.

Peršoh D. **2013a**. Factors shaping community structure of endophytic fungi-evidence from the Pinus-Viscum-system. *Fungal Diversity* **60**: 55–69.

Peršoh D. **2013b**. Factors shaping community structure of endophytic fungi-evidence from the Pinus-Viscum-system. *Fungal Diversity* **60**: 55–69.

Pettersson BMF, Behra PRK, Manduva S, Das S, Dasgupta S, Bhattacharya A, Kempf A, Grund E, Rm K, Hj K. **2014**. Draft Genome Sequence of Saccharopolyspora rectivirgula. *Genome Announc* **2**: 5–6.

Plummer E, Twin J. **2015**. A Comparison of Three Bioinformatics Pipelines for the Analysis of Preterm Gut Microbiota using 16S rRNA Gene Sequencing Data. *Journal of Proteomics & Bioinformatics* **8**: 283–291.

Pochon S, Simoneau P, Pigné S, Balidas S, Bataillé-Simoneau N, Campion C, Jaspard E, Calmes B, Hamon B, Berruyer R, *et al.* 2013. Dehydrin-like Proteins in the Necrotrophic Fungus Alternaria brassicicola Have a Role in Plant Pathogenesis and Stress Response. *PLoS ONE* **8**.

Pollock J, Glendinning L, Wisedchanwet T, Watson M. **2018**. The Madness of Microbiome : Attempting To Find Consensus. *Applied and Environmental Microbiology* **84**: 1–12.

Pöschl U, Martin ST, Sinha B, Chen Q, Gunthe SS, Huffman JA, Borrmann S, Farmer DK, Garland RM, Helas G, *et al.* 2010. Rainforest Aerosols as Biogenic Nuclei of Clouds and Precipitation in the Amazon. *Science* **329**: 1513–1517.

Purahong W, Kapturska D, Pecyna MJ, Schulz E, Schloter M, Buscot F, Hofrichter M, Kruğer D. **2014**. Influence of different forest system management practices on leaf litter decomposition rates, nutrient dynamics and the activity of ligninolytic enzymes: A case study from Central European forests. *PLoS ONE* **9**: 1–11.

Purahong W, Wubet T, Lentendu G, Schloter M, Pecyna MJ, Kapturska D, Hofrichter M, Krüger D, Buscot F. **2016**. Life in leaf litter: novel insights into community dynamics of bacteria and fungi during litter decomposition. *Molecular ecology* **25**: 4059–4074.

Pusey PL, Stockwell VO, Reardon CL, Smits THM, Duffy B. **2011**. Antibiosis activity of Pantoea agglomerans biocontrol strain E325 against Erwinia amylovora on apple flower stigmas. *Phytopathology* **101**: 1234–1241.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. **2013**. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research* **41**: 590–596.

Quatrini R, Johnson DB. **2018**. Microbiomes in extremely acidic environments: functionalities and interactions that allow survival and growth of prokaryotes at low pH. *Current Opinion in Microbiology* **43**: 139–147.

Radulovic NS, Blagojevic PD, Stojanovic-Radic ZZ, Stojanovic NM. **2013**. Antimicrobial Plant Metabolites: Structural Diversity and Mechanism of Action. *Current Medicinal Chemistry* **20**: 932–952.

Rajala T, Peltoniemi M, Hantula J, Mäkipää R, Pennanen T. **2011**. RNA reveals a succession of active fungi during the decay of Norway spruce logs. *Fungal Ecology* **4**: 437–448.

Rasmussen TT. **2000**. Resident aerobic microbiota of the adult human nasal cavity.

Rastogi G, Coaker GL, Leveau JHJ. **2013**. New insights into the structure and function of phyllosphere microbiota through high-throughput molecular approaches. *FEMS Microbiology Letters* **348**: 1–10.

Reading NC, Sperandio V. **2006**. Quorum sensing: The many languages of bacteria. *FEMS Microbiology Letters* **254**: 1–11.

Redford AJ, Bowers RM, Knight R, Linhart Y, Fierer N. **2010**. The ecology of the phyllosphere: Geographic and phylogenetic variability in the distribution of bacteria on tree leaves. *Environmental Microbiology* **12**: 2885–2893.

Reed SC, Cleveland CC, Townsend AR. **2011**. Functional Ecology of Free-Living Nitrogen Fixation: A Contemporary Perspective. *Annual Review of Ecology, Evolution, and Systematics* **42**: 489–512.

Reisberg EE, Hildebrandt U, Riederer M, Hentschel U. **2013**. Distinct phyllosphere bacterial

communities on Arabidopsis wax mutant leaves. *PLoS ONE* **8**.

Rennie EA, Turgeon R. **2009**. A comprehensive picture of phloem loading strategies. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 14162–14167.

Requena N, Perez-solis E, Azcon-aguilar C, Jeffries P, Barea J. **2001**. Management of Indigenous Plant-Microbe Symbioses Aids Restoration of Desertified Ecosystems. *Applied and environmental microbiology* **67**: 495–498.

Rezki S, Campion C, Simoneau P, Jacques MA, Shade A, Barret M. **2018**. Assembly of seed-associated microbial communities within and across successive plant generations. *Plant and Soil* **422**: 67–79.

Rico L, Ogaya R, Terradas J, Peñuelas J. **2014**. Community structures of N2-fixing bacteria associated with the phyllosphere of a Holm oak forest and their response to drought. *Plant Biology* **16**: 586–593.

Riegel P, Heller R, Prevost G, Jehl F, Monteil H. **2019**. Corynebacterium durum sp . nov ., from Human Clinical SDecimens. : 1107–1111.

Rissanen AJ, Kurhela E, Aho T, Oittinen T, Tiirola M. **2010**. Storage of environmental samples for guaranteeing nucleic acid yields for molecular microbiological studies. *Applied Microbiology and Biotechnology* **88**: 977–984.

Robertson GP, Paul EA. **2000**. Decomposition and Soil Organic Matter Dynamics. *Methods in Ecosystem Science*: 104–116.

Rodriguez RJ, White JF, Arnold a E, Redman RS. **2009**. Fungal endophytes: diversity and functional roles. *New phytologist* **182**: 314–330.

Rognes T, Flouri T, Nichols B, Quince C, Mahé F. **2016**. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* **4**: e2584.

Roh SW, Quan Z, Nam Y, Chang H, Kim K, Kim M, Im W, Jin L, Kim S, Lee S, *et al.* 2019. Pedobacter agri sp . nov ., from soil. : 1640–1643.

Rosado BHP, Almeida LC, Alves LF, Lambais MR, Oliveira RS. **2018**. The importance of phyllosphere on plant functional ecology: A phyllo trait manifesto. *New Phytologist*.

Rubin BER, Gibbons SM, Kennedy S, Hampton-Marcell J, Owens S, Gilbert JA. **2013**.

Investigating the Impact of Storage Conditions on Microbial Community Composition in Soil Samples. *PLoS ONE* **8**: 1–9.

Ruinen J. **1956**. Occurrence of Beijerinckia species in the 'phyllosphere'. *Nature* **177**: 220–221.

Ryffel F, Helfrich EJN, Kiefer P, Peyriga L, Portais JC, Piel J, Vorholt JA. **2016**. Metabolic footprint of epiphytic bacteria on Arabidopsis thaliana leaves. *ISME Journal* **10**: 632–643.

Ryu J, Madhaiyan M, Poonguzhali S, Yim W, Indiragandhi P, Kim K, Anandham R, Yun J, Kim KH, Sa T. **2006**. Plant growth substances produced by methylobacterium spp. and their effect on tomato (lycopersicon esculentum L.) and red pepper (capsicum annuum L.) growth. *Journal of Microbiology and Biotechnology* **16**: 1622–1628.

Sastry KS. **2013**. *Seed-borne Plant Virus Diseases*.

Sayer EJ, Tanner EVJ, Lacey AL. **2006**. Effects of litter manipulation on early-stage decomposition and meso-arthropod abundance in a tropical moist forest. *Forest Ecology and Management* **229**: 285–293.

Schlechter RO, Miebach M, Remus-emsermann MNP. **2019**. Driving factors of epiphytic bacterial communities : A review. *Journal of Advanced Research* **19**: 57–65.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, *et al.* 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* **75**: 7537–7541.

Schmidt MWI, Torn MS, Abiven S, Dittmar T, Guggenberger G, Janssens IA, Kleber M, Kögel-Knabner I, Lehmann J, Manning DAC, *et al.* 2011. Persistence of soil organic matter as an ecosystem property. *Nature* **478**: 49–56.

Schneider T, Keiblinger KM, Schmid E, Sterflinger-Gleixner K, Ellersdorfer G, Roschitzki B, Richter A, Eberl L, Zechmeister-Boltenstern S, Riedel K. **2012**. Who is who in litter decomposition Metaproteomics reveals major microbial players and their biogeochemical functions. *ISME Journal* **6**: 1749–1762.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Bolchacova E, Voigt K, Crous PW, *et al.* 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences* **109**:

6241–6246.

Schröter D, Wolters V, De Ruiter PC. **2003**. C and N mineralisation in the decomposer food webs of a European forest transect. *Oikos* **102**: 294–308.

Serna-Chavez HM, Fierer N, Van Bodegom PM. **2013**. Global drivers and patterns of microbial abundance in soil. *Global Ecology and Biogeography* **22**: 1162–1172.

Sessitsch A, Reiter B, Berg G. **2004**. Endophytic bacterial communities of field-grown potato plants and their plant-growth-promoting and antagonistic abilities. **249**: 239–249.

Shade A, Jacques MA, Barret M. **2017**. Ecological patterns of seed microbiome diversity, transmission, and assembly. *Current Opinion in Microbiology* **37**: 15–22.

Shivlata L, Satyanarayana T. **2015**. Thermophilic and alkaliphilic Actinobacteria : biology and potential applications. **6**: 1–29.

Sibley CD, Peirano G, Church DL. **2012**. Molecular methods for pathogen and microbial community detection and characterization: Current and potential application in diagnostic microbiology. *Infection, Genetics and Evolution* **12**: 505–521.

Singh D, Mathur SB. **2004**. *Histopathology of Seed-Borne Infections*. CRC Press.

Sittenfeld A, Uribe-lorío L, Mora M, Nielsen V, Arrieta G, Janzen DH. **2002**. Does a polyphagous caterpillar have the same gut microbiota when feeding on different species of food plants ? **50**: 547–560.

Šnajdr J, Cajthaml T, Valášková V, Merhautová V, Petránková M, Spetz P, Leppänen K, Baldrian P. **2011**. Transformation of Quercus petraea litter: Successive changes in litter chemistry are reflected in differential enzyme activity and changes in the microbial community composition. *FEMS Microbiology Ecology* **75**: 291–303.

Staley JT, Irgens RL, Brenner DJ. **2019**. Enhydrobacter aerasaccus gen. nov. , sp. nov. a Gas-Vacuolated, Facultatively Anaerobic, Heterotrophic Rod. *International journal of systematic bacteriology* **37**: 289–291.

Stokland JN, Jonsson BG, Information C. **2012**. Biodiversity in Dead Wood. *Cambridge University Press*.

Stone BWG, Jackson CR. **2019**. Canopy position is a stronger determinant of bacterial community

composition and diversity than environmental disturbance in the phyllosphere. : 1–11.

Stout JD. **1960**. Bacteria of soil and pasture leaves at Claudelands Showgrounds. *New Zealand Journal of Agricultural Research* **3**: 413–430.

Štursová M, Žifčáková L, Leigh MB, Burgess R, Baldrian P. **2012**. Cellulose utilization in forest litter and soil: Identification of bacterial and fungal decomposers. *FEMS Microbiology Ecology* **80**: 735–746.

Su S, Chen M, Teng C, Jiang S, Zhang C, Lin M, Zhang W. **2014**. Hymenobacter kanuolensis sp. nov., a novel radiation-resistant bacterium. *International Journal of Systematic and Evolutionary Microbiology* **64**: 2108–2112.

Sullivant DJO, Gara FO. **1992**. Traits of Fluorescent Pseudomonas spp . Involved in Suppression of Plant Root Pathogens. *Microbiological Reviews* **56**: 662–676.

Sun H, Terhonen E, Kovalchuk A, Tuovila H, Chen H, Oghenekaro AO, Heinonsalo J, Kohler A, Kasanen R, Vasander H, *et al.* 2016. Dominant tree species and soil type affect the fungal community structure in a boreal peatland forest. *Applied and Environmental Microbiology* **82**: 2632–2643.

Sy A, Timmers ACJ, Knief C, Vorholt JA. **2005**. Methylotrophic metabolism is advantageous for Methylobacterium extorquens during colonization of Medicago truncatula under competitive conditions. *Applied and Environmental Microbiology* **71**: 7245–7252.

Taberlet P, Coissac E. **2012**. Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology* **21**: 2045–2050.

Talbot JM, Bruns TD, Taylor JW, Smith DP, Branco S, Glassman SI, Erlandson S, Vilgalys R, Liao HL, Smith ME, *et al.* 2014. Endemism and functional convergence across the North American soil mycobiome. *Proceedings of the National Academy of Sciences of the United States of America* **111**: 6341–6346.

Tanaka Y, Sano T, Tamaoki M, Nakajima N, Kondo N, Hasezawa S. **2006**. Cytokinin and auxin inhibit abscisic acid-induced stomatal closure by enhancing ethylene production in Arabidopsis. *Journal of Experimental Botany* **57**: 2259–2266.

Tancos MA, Chalupowicz L, Barash I, Manulis-sasson S, Smart D. **2013**. Tomato Fruit and Seed Colonization by Clavibacter michiganensis subsp . michiganensis through External and Internal

Routes. **79**: 6948–6957.

Tedersoo L, Drenkhan R, Anslan S, Morales-Rodriguez C, Cleary M. **2019**. High-throughput identification and diagnostics of pathogens and pests: Overview and practical recommendations. *Molecular Ecology Resources* **19**: 47–76.

Terhonen E, Blumenstein K, Kovalchuk A. **2019**. Forest Tree Microbiomes and Associated Fungal Endophytes : Functional Roles and Impact on Forest Health. : 1–32.

Thines M. **2014**. Phylogeny and evolution of plant pathogenic oomycetes-a global overview. *European Journal of Plant Pathology* **138**: 431–447.

Thompson LR, Sanders JG, McDonald D, Amir A, Ladau J, Locey KJ, Prill RJ, Tripathi A, Gibbons SM, Ackermann G, *et al.* 2017. A communal catalogue reveals Earth's multiscale microbial diversity. *Nature* **551**: 457–463.

Tlaskal V, Vorıskov J, Baldrian P. **2016**. Bacterial succession on decomposing leaf litter exhibits a specific occurrence pattern of cellulolytic taxa and potential decomposers of fungal mycelia. *FEMS Microbiology Ecology* **92**: 1–10.

Trotsenko YA, Ivanova EG, Doronina N V. 2001. Aerobic methylotrophic bacteria as phytosymbionts. *Mikrobiologiya* **70**: 725–736.

Truyens S, Weyens N, Cuypers A, Vangronsveld J. **2015**. Bacterial seed endophytes: Genera, vertical transmission and interaction with plants. *Environmental Microbiology Reports* **7**: 40–50.

Tsavkelova EA, Klimova SY, Cherdyntseva TA, Netrusov AI. **2006**. Microbial producers of plant growth stimulators and their practical use: A review. *Applied Biochemistry and Microbiology* **42**: 117–126.

Tukey HB. **1970**. The Leaching of Substances from Plants. *Annual Review of Plant Physiology* **21**: 305–324.

Turner TR, James EK, Poole PS, Gilbert J, Meyer F, Jansson J, Gordon J, Pace N, Tiedje J, Ley R, *et al.* 2013. The plant microbiome. *Genome Biology* **14**: 209.

Unterseher M, Peršoh D, Schnittler M. **2013**. Leaf-inhabiting endophytic fungi of European Beech (Fagus sylvatica L.) co-occur in leaf litter but are rare on decaying wood of the same host. *Fungal Diversity* **60**: 43–54.

Urbanová M, Šnajdr J, Baldrian P. **2015**. Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. *Soil Biology and Biochemistry* **84**: 53–64.

Vacher C, Cordier T, Vallance J. **2016a**. Phyllosphere Fungal Communities Differentiate More Thoroughly than Bacterial Communities Along an Elevation Gradient. *Microbial Ecology*: 1–3.

Vacher C, Hampe A, Porté AJ, Sauer U, Compant S, Morris CE. **2016b**. The Phyllosphere: Microbial Jungle at the Plant–Climate Interface. *Annual Review of Ecology, Evolution, and Systematics* **47**: 1–24.

Vala V, Baldrian P, Herinkova J. **2008**. Soil Biology & Biochemistry Spatial variability of enzyme activities and microbial biomass in the upper layers of Quercus petraea forest soil. **40**: 2068–2075.

Valverde A, Peix A, Rivas R, Velázquez E, Salazar S, Santa-Regina I, Rodríguez-Barrueco C, Igual JM. **2008**. Paenibacillus castaneae sp. nov., isolaled from the phyllosphere of Castanea sativa Miller. *International Journal of Systematic and Evolutionary Microbiology* **58**: 2560–2564.

Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A. **2015**. The importance of the microbiome of the plant holobiont. *New Phytologist* **206**: 1196–1206.

Vayssier-Taussat M, Albina E, Citti C, Cosson JF, Jacques MA, Lebrun MH, Le Loir Y, Ogliastro M, Petit MA, Roumagnac P, *et al.* 2014. Shifting the paradigm from pathogens to pathobiome new concepts in the light of meta-omics. *Frontiers in Cellular and Infection Microbiology* **5**: 1–7.

Verma VC, Gange AC. **2014**. *Advances in endophytic research*.

Vitousek PM, Cassman K, Cleveland C, Crews T, Field CB, Grimm NB, Howarth RW, Marino R, Martinelli L, Rastetter EB, *et al.* 2002. Towards an ecological understanding of biological nitrogen fixation. *Biogeochemistry* **57–58**: 1–45.

Vogel C, Bodenhausen N, Gruissem W, Vorholt JA. **2016**. The Arabidopsis leaf transcriptome reveals distinct but also overlapping responses to colonization by phyllosphere commensals and pathogen infection with impact on plant health. *New phytologist* **212**: 192–207.

Vorholt JA. **2012**. Microbial life in the phyllosphere. *Nature Reviews Microbiology* **10**: 828–840.

Voriskova J, Baldrian P. **2013**. Fungal community on decomposing leaf litter undergoes rapid successional changes. *ISME Journal* **7**: 477–486.

Waight K, Pinyakong O, Luepromchai E. **2007**. Degradation of phenanthrene on plant leaves by phyllosphere bacteria. *Journal of General and Applied Microbiology* **53**: 265–272.

Van Der Wal A, Leveau JHJ. **2011**. Modelling sugar diffusion across plant leaf cuticles: The effect of free water on substrate availability to phyllosphere bacteria. *Environmental Microbiology* **13**: 792–797.

Wellner S, Lodders N, Kämpfer P. **2011**. Diversity and biogeography of selected phyllosphere bacteria with special emphasis on Methylobacterium spp. *Systematic and Applied Microbiology* **34**: 621–630.

Whipps JM, Hand P, Pink D, Bending GD. **2008**. Phyllosphere microbiology with special reference to diversity and plant genotype. *Journal of Applied Microbiology* **105**: 1744–1755.

Whitham TG, Bailey JK, Schweitzer| JA, Shuster SM, Bangert RK, LeRoy CJ, Lonsdorf E V., Allan GJ, DiFazio SP, Potts BM, *et al.* 2006. A framework for community and ecosystem genetics : from genes to ecosystems. *Nature Reviews Genetics* **7**: 510–523.

Wilson M, Savka MA, Hwang I, Farrand SK, Lindow SE. **1995**. Altered epiphytic colonization of mannityl opine-producing transgenic tobacco plants by a mannityl opine-catabolizing strain of Pseudomonas syringae. *Applied and Environmental Microbiology* **61**: 2151–2158.

Witzell J, Martín JA. **2018**. Endophytes of Forest Health. In: Endophytes of Forest Trees. 261–282.

Womack AM, Bohannan BJM, Green JL. **2010**. Biodiversity and biogeography of the atmosphere. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**: 3645–3653.

Wong SCY, Poon RWS, Chen JHK, Tse H, Lo JYC, Ng T, Au JCK, Tse CWS. Corynebacterium kroppenstedtii Is an Emerging Cause of Mastitis Especially in Patients With Psychiatric Illness on Antipsychotic Medication. : 1–4.

Yang B, Wang Y, Qian PY. **2016**. Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. *BMC Bioinformatics* **17**: 1–8.

Yao H, Sun X, He C, Maitra P, Li XC, Guo LD. **2019**. Phyllosphere epiphytic and endophytic fungal community and network structures differ in a tropical mangrove ecosystem. *Microbiome* **7**: 1–15.

Yarza P, Yilmaz P, Pruesse E, Glöckner FO, Ludwig W, Schleifer KH, Whitman WB, Euzéby J, Amann R, Rosselló-Móra R. **2014**. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nature Reviews Microbiology* **12**: 635–645.

Yasumura Y, Hikosaka K, Matsui K, Hirose T. **2002**. Leaf-level nitrogen-use efficiency of canopy and understorey species in a beech forest. *Functional Ecology* **16**: 826–834.

Zeng Q, Liu Y, Zhang H, An S. **2019**. Fast bacterial succession associated with the decomposition of Quercus wutaishanica litter on the Loess Plateau. *Biogeochemistry* **144**: 119–131.

Zhang J, Kobert K, Flouri T, Stamatakis A. **2014**. PEAR: A fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* **30**: 614–620.

Zilber-Rosenberg I, Rosenberg E. **2008**. Role of microorganisms in the evolution of animals and plants: The hologenome theory of evolution. *FEMS Microbiology Reviews* **32**: 723–735.

Zipfel C. **2009**. Early molecular events in PAMP-triggered immunity. *Current Opinion in Plant Biology* **12**: 414–420.

Références

Abarenkov K, Nilsson RH, Larsson K-H, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E, Pennanen T, *et al.* 2018. The UNITE database for molecular identification of fungi – recent updates and future perspectives. *New Phytologist* **219**: 5.

Afkhami ME, Rudgers JA, Afkhami ME, Rudgers JA. **2015**. Symbiosis Lost : Imperfect Vertical Transmission of Fungal Endophytes in Grasses. **172**: 405–416.

Agler MT, Ruhe J, Kroll S, Morhenn C, Kim ST, Weigel D, Kemen EM. **2016**. Microbial Hub Taxa Link Host and Abiotic Factors to Plant Microbiome Variation. *PLoS Biology* **14**: 1–31.

Agrios GN. **1997**. Control of Plant Diseases. In: Plant Pathology. San Diego, 200–216.

Ahn JH, Kim BC, Kim SJ, Lee GH, Song J, Kwon SW, Weon HY. **2015**. Sphingomonas parvus sp. nov. isolated from a ginseng-cultivated soil. *Journal of Microbiology* **53**: 673–677.

Aleklett K, Hart M, Shade A. **2014**. The microbial ecology of flowers: An emerging frontier in phyllosphere research1. *Botany* **92**: 253–266.

Amato P, Joly M, Besaury L, Oudart A, Taib N, Deguillaume L, Delort A, Debroas D, Mone I. **2017**. Active microorganisms thrive among extremely diverse communities in cloud water. *PLoS ONE* **3**: 1–22.

Amend AS, Cobian GM, Laruson AJ, Remple K, Tucker SJ, Poff KE, Antaky C, Boraks A, Jones CA, Kuehu D, *et al.* 2019. Phytobiomes are compositionally nested from the ground up. *PeerJ* **2019**: 1–

18.

Aneja M, Gianfagna TJ, Hebbar PK. **2006**. Trichoderma harzianum produces nonanoic acid, an inhibitor of spore germination and mycelial growth of two cacao pathogens. *Physiological and Molecular Plant Pathology* **67**: 304–307.

Ariya PA, Amyot M. **2004**. New Directions: The role of bioaerosols in atmospheric chemistry and physics. *Atmospheric Environment* **38**: 1231–1232.

Arnold AE, Mejia LC, Kyllo D, Rojas EI, Maynard Z, Robbins N, Herre EA. **2003**. Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences* **100**: 15649–15654.

Arrigoni E, Antonielli L, Pindo M, Pertot I, Perazzolli M. **2018**. Tissue age and plant genotype affect the microbiota of apple and pear bark. *Microbiological Research* **211**: 57–68.

Atamna-Ismaeel N, Finkel O, Glaser F, von Mering C, Vorholt JA, Koblížek M, Belkin S, Béjà O. **2012a**. Bacterial anoxygenic photosynthesis on plant leaf surfaces. *Environmental Microbiology Reports* **4**: 209–216.

Atamna-Ismaeel N, Finkel OM, Glaser F, Sharon I, Schneider R, Post AF, Spudich JL, von Mering C, Vorholt JA, Iluz D, *et al.* 2012b. Microbial rhodopsins on leaf surfaces of terrestrial plants. *Environmental Microbiology* **14**: 140–146.

Augspurger CK, Kelly CK. **1984**. Pathogen mortality of tropical tree seedlings: experimental studies of the effects of dispersal distance, seedling denisty, and light conditions. *Oecologia.* **61**: 211–217.

Aydogan EL, Moser G, Müller C, Kämpfer P, Glaeser SP. **2018**. Long-term warming shifts the composition of bacterial communities in the phyllosphere of Galium album in a permanent grassland field-experiment. *Frontiers in Microbiology* **9**: 1–17.

Badri D V., Weir TL, van der Lelie D, Vivanco JM. **2009**. Rhizosphere chemical dialogues: plant-microbe interactions. *Current Opinion in Biotechnology* **20**: 642–650.

Bailey MJ, Lilley AK, Timms-Wilson TM, Spencer-Phillips PTN. **2006**. Microbial ecology of aerial plant surfaces. *Microbial Ecology of Aerial Plant Surfaces*: 1–321.

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM. **2006**. the Role of Root Exudates in Rhizosphere Interactions With Plants and Other Organisms. *Annual Review of Plant Biology* **57**: 233–266.

Baker KF, Smith SH. **1966**. Dynamics of seed transmission of plan pathogens. *Annual Review of Phytopathology* **4**: 311 – 332.

Baldotto LEB, Olivares FL. **2008**. Phylloepiphytic interaction between bacteria and different plant species in a tropical agricultural system. *Canadian Journal of Microbiology* **54**: 918–931.

Baldrian P. **2017**. Forest microbiome: Diversity, complexity and dynamics. *FEMS Microbiology Reviews* **41**: 109–130.

Balint-Kurti P, Simmons SJ, Blum JE, Ballaré CL, Stapleton AE. **2010**. Maize leaf epiphytic bacteria diversity patterns are genetically correlated with resistance to fungal pathogen infection. *Molecular Plant-Microbe Interactions* **23**: 473–484.

Balint M, Bahram M, Eren AM, Faust K, Fuhrman JA, Lindahl B, O'Hara RB, Ï¿½pik M, Sogin ML, Unterseher M, *et al.* 2016. Millions of reads, thousands of taxa: Microbial community structure and associations analyzed via marker genesa. *FEMS Microbiology Reviews* **40**: 686–700.

Bálint M, Schmidt PA, Sharma R, Thines M, Schmitt I. **2014**. An Illumina metabarcoding pipeline for fungi. *Ecology and Evolution* **4**: 2642–2653.

Barbeta A, Jones SP, Clavé L, Wingate L, Gimeno TE, Fréjaville B, Wohl S, Ogee J. **2019**. Unexplained hydrogen isotope offsets complicate the identification and quantification of tree water sources in a riparian forest. *Hydrology and Earth System Sciences* **23**: 2129–2146.

Barbi F, Bragalini C, Vallon L, Prudent E, Dubost A, Fraissinet-Tachet L, Marmeisse R, Luis P. **2014**. PCR primers to study the diversity of expressed fungal genes encoding lignocellulolytic enzymes in soils using high-throughput sequencing. *PLoS ONE* **9**: 1–22.

Barret M, Briand M, Bonneau S, Préveaux A, Valière S, Bouchez O, Hunault G, Simoneau P, Jacquesa MA. **2015**. Emergence shapes the structure of the seed microbiota. *Applied and Environmental Microbiology* **81**: 1257–1266.

Barret M, Guimbaud JF, Darrasse A, Jacques MA. **2016**. Plant microbiota affects seed transmission of phytopathogenic microorganisms. *Molecular plant pathology* **17**: 791–795.

Bartlett DH. **2009**. Microbial life in the trenches. *Marine Technology Society Journal* **43**: 128–131.

Bashan Y, Holguin G. **1998**. Proposal for the division of plant growth-promoting rhizobacteria into two classifications: Biocontrol-PGPB (plant growth-promoting bacteria) and PGPB. *Soil Biology and Biochemistry* **30**: 1225–1228.

Bassas-Galia M, Nogales B, Arias S, Rohde M, Timmis KN, Molinari G. **2012**. Plant original Massilia isolates producing polyhydroxybutyrate, including one exhibiting high yields from glycerol. *Journal of Applied Microbiology* **112**: 443–454.

Bauer H, Giebl H, Hitzenberger R, Kasper-giebl A, Reischl G, Zibuschka F, Puxbaum H. **2003**. Airborne bacteria as cloud condensation nuclei. **108**: 1–5.

Beattie GA, Lindow SE. **1995**. The Secret Life of Foliar Bacterial Pathogens on Leaves. *Annual Review of Phytopathology* **33**: 145–172.

Beattie GA, Marcell LM. **2002**. Effect of alterations in cuticular wax biosynthesis on the physicochemical properties and topography of maize leaf surfaces. *Plant, Cell and Environment* **25**: 1–16.

Beckers B, De Beeck MO, Weyens N, Boerjan W, Vangronsveld J. **2017**. Structural variability and niche differentiation in the rhizosphere and endosphere bacterial microbiome of field-grown poplar trees. *Microbiome* **5**: 1–17.

Begerow D, Nilsson H, Unterseher M, Maier W. **2010**. Current state and perspectives of fungal DNA barcoding and rapid identification procedures. *Applied Microbiology and Biotechnology* **87**: 99–108.

Bent E. **2006**. *Induced Systemic ResistanceMediated by PlantGrowth-Promoting Rhizobacteria(PGPR) and Fungi (PGPF)*.

Bentley BL, Carpenter EJ. **1984**. Direct transfer of newly-fixed nitrogen from free-living epiphyllous microorganisms to their host plant. *Oecologia* **63**: 52–56.

Berg G, Raaijmakers JM. **2018**. Saving seed microbiomes. *ISME Journal* **12**: 1167–1170.

Bertaux J, Schmid M, Hutzler P, Hartmann A, Garbaye J, Frey-Klett P. **2005**. Occurrence and distribution of endobacteria in the plant-associated mycelium of the ectomycorrhizal fungus Laccaria bicolor S238N. *Environmental Microbiology* **7**: 1786–1795.

Bever JD, Mangan SA, Alexander HM. **2015**. Maintenance of Plant Species Diversity by Pathogens. *Annual Review of Ecology, Evolution, and Systematics* **46**: 305–325.

Bjorn D. Lindahl Johanna Boberg, Susan E. Trumbore, KI, Peter Hogberg Roger D. Finlay JS. **2007**. Spatial separation of litter decomposition and mycorrhizal nitrogen \nuptake in a boreal forest. *New Phytologist*: 611–620.

Blaser MJ, Cardon ZG, Cho MK, Dangl JL, Donohue TJ, Green JL, Knight R, Maxon ME, Northen TR, Pollard KS, *et al.* 2016. Toward a predictive understanding of earth's microbiomes to address 21st century challenges. *mBio* **7**: 1–16.

Bodenhausen N, Horton MW, Bergelson J. **2013**. Bacterial Communities Associated with the Leaves and the Roots of Arabidopsis thaliana. *PLoS ONE* **8**.

De Boer W, Folman LB, Summerbell RC, Boddy L. **2005**. Living in a fungal world: Impact of fungi on soil bacterial niche development. *FEMS Microbiology Reviews* **29**: 795–811.

Boller T, Felix G. **2009**. A Renaissance of Elicitors: Perception of Microbe-Associated Molecular Patterns and Danger Signals by Pattern-Recognition Receptors. *Annual Review of Plant Biology* **60**: 379–406.

Brader G, Compant S, Vescio K, Mitter B, Trognitz F, Ma L-J, Sessitsch A. **2017**. Ecology and Genomic Insights into Plant-Pathogenic and Plant-Nonpathogenic Endophytes. *Annual Review of Phytopathology* **55**: 61–83.

Bradley DJ, Gilbert GS, Martiny JBH. **2008**. Pathogens promote plant diversity through a compensatory response. *Ecology Letters* **11**: 461–469.

Bray JR, Gorham E. **1964**. Litter Production in Forests of the World†. *Advances in Ecological Research* **2**: 101–157.

Bright M, Bulgheresi S. **2010**. A complex journey: Transmission of microbial symbionts. *Nature Reviews Microbiology* **8**: 218–230.

Bringel F, Couée I. **2015**. Pivotal roles of phyllosphere microorganisms at the interface between plant functioning and atmospheric trace gas dynamics. *Frontiers in Microbiology* **6**: 1–14.

Brouwer B, Ziolkowska A, Bagard M, Keech O, Gardeström P. **2012**. The impact of light intensity on shade-induced leaf senescence. *Plant, Cell and Environment* **35**: 1084–1098.

Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P. **2013**. Structure and Functions of the Bacterial Microbiota of Plants. *Annual Review of Plant Biology* **64**: 807–838.

Burch AY, Zeisler V, Yokota K, Schreiber L, Lindow SE. **2014**. The hygroscopic biosurfactant syringafactin produced by Pseudomonas syringae enhances fitness on leaf surfaces during fluctuating humidity. *Environmental Microbiology* **16**: 2086–2098.

Burrows SM, Butler T. **2009**. Bacteria in the global atmosphere – PartPart 2 : Modeling of emissions and transport between different ecosystems. : 9281–9297.

Burrows SM, Elbert W, Lawrence MG. **2009**. Bacteria in the global atmosphere – Part 1: Review and synthesis of literature data for different ecosystems. *Atmospheric Chemistry and Physics*: 9263–9280.

Busse HJ, Denner EB, Buczolits S, Salkinoja-Salonen M, Bennasar A, Kämpfer P. **2003**. Sphingomonas aurantiaca sp . nov ., Sphingomonas aerolata sp . nov . and Sphingomonas faeni sp . nov ., air- and dustborne and Antarctic , orange- pigmented , psychrotolerant bacteria , and emended description of the genus Sphingomonas. *International journal of systematic and evolutionary microbiology* **53**: 1253–1260.

Callahan BJ, McMurdie PJ, Holmes SP. **2017**. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME Journal* **11**: 2639–2643.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. **2016**. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**: 581–583.

Campisano A, Ometto L, Compant S, Pancher M, Antonielli L, Yousaf S, Varotto C, Anfora G, Pertot I, Sessitsch A, *et al.* 2014. Interkingdom transfer of the acne-causing agent, propionibacterium acnes, from human to grapevine. *Molecular Biology and Evolution* **31**: 1059–1065.

Cánovas FM, Cañas RA, de la Torre FN, Pascual MB, Castro-Rodríguez V, Avila C. **2018**. Nitrogen metabolism and biomass production in forest trees. *Frontiers in Plant Science* **9**: 1–9.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman F d, Costello elizabeth K, Fierer N, Peña antonio G, Goodrich JK, Gordon JI, *et al.* 2010. QIIME allows analysis of high- throughput community sequencing data. *Nature Publishing Group* **7**: 335–336.

Cappelletti. **2018**. Update on the biopesticide regulation and development of protein-based products for the biocontrol of crop diseases.

Carrell AA, Frank AC. **2014**. Pinus flexilis and Picea engelmannii share a simple and consistent needle endophyte microbiota with a potential role in nitrogen fixation. *Frontiers in Microbiology* **5**: 1–11.

Cernava T, Chen X, Krug L, Li H, Yang M, Berg G. **2019**. The tea leaf microbiome shows specific

responses to chemical pesticides and biocontrol applications. *Science of the Total Environment* **667**: 33–40.

Chakravorty S, Helb D, Burday M, Connell N, Alland D. **2007**. A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. *Journal of Microbiological Methods* **69**: 330–339.

Champlot S, Berthelot C, Pruvost M, Andrew Bennett E, Grange T, Geigl EM. **2010**. An efficient multistrategy DNA decontamination procedure of PCR reagents for hypersensitive PCR applications. *PLoS ONE* **5**.

Chi Z, Wang F, Chi Z, Yue L, Liu G, Zhang T. **2009**. Bioproducts from Aureobasidium pullulans, a biotechnologically important yeast. *Applied Microbiology and Biotechnology* **82**: 793–804.

Clay K, Schardl C. **2002a**. Evolutionary origins and ecological consequences of endophyte symbiosis with grasses. *American Naturalist* **160**.

Clay K, Schardl C. **2002b**. Evolutionary Origins and Ecological Consequences of Endophyte Symbiosis with Grasses. *The American Naturalist* **160**: S99–S127.

Coble AP, Vanderwall B, Mau A, Cavaleri MA. **2016**. How vertical patterns in leaf traits shift seasonally and the implications for modeling canopy photosynthesis in a temperate deciduous forest. *Tree Physiology* **36**: 1077–1091.

Coleman-Derr D, Desgarennes D, Fonseca-Garcia C, Gross S, Clingenpeel S, Woyke T, North G, Visel A, Partida-Martinez LP, Tringe SG. **2016**. Plant compartment and biogeography affect microbiome composition in cultivated and native Agave species. *New Phytologist* **209**: 798–811.

Compant S, Samad A, Faist H, Sessitsch A. **2019**. A review on the plant microbiome : Ecology , functions , and emerging trends in microbial application. *Journal of Advanced Research journal* **9**: 29–37.

Copeland JK, Yuan L, Layeghifard M, Wang PW, Guttman DS. **2015**. Seasonal community succession of the phyllosphere microbiome. *Molecular Plant-Microbe Interactions* **28**: 274–285.

Cordier T, Robin C, Capdevielle X, Fabreguettes O, Desprez-Loustau ML, Vacher C. **2012**. The composition of phyllosphere fungal assemblages of European beech (fagus sylvatica) varies significantly along an elevation gradient. *New Phytologist* **196**: 510–519.

Costello EK, Stagaman K, Dethlefsen L, Bohannan BJM, Relman DA. **2012**. The application of

ecological theory toward an understanding of the human microbiome. *Science* **336**: 1255–1262.

Crawford I, Robinson NH, Flynn MJ, Foot VE, Gallagher MW, Huffman JA, Stanley WR, Kaye PH. **2014**. Characterisation of bioaerosol emissions from a Colorado pine forest: Results from the beachon-rombas experiment. *Atmospheric Chemistry and Physics* **14**: 8559–8578.

Cregger MA, Veach AM, Yang ZK, Crouch MJ, Vilgalys R, Tuskan GA, Schadt CW. **2018**. The Populus holobiont: Dissecting the effects of plant niches and genotype on the microbiome. *Microbiome* **6**: 1–14.

Crowther TW, Stanton DWG, Thomas SM, A'Bear AD, Hiscox J, Jones TH, Voříšková J, Baldrian P, Boddy L. **2013**. Top-down control of soil fungal community composition by a globally distributed keystone consumer. *Ecology* **94**: 2518–2528.

Cruaud P, Vigneron A, Lucchetti-Miganeh C, Ciron PE, Godfroy A, Cambon-Bonavita MA. **2014**. Influence of DNA extraction method, 16S rRNA targeted hypervariable regions, and sample origin on microbial diversity detected by 454 pyrosequencing in marine chemosynthetic ecosystems. *Applied and Environmental Microbiology* **80**: 4626–4639.

D'Amore R, Ijaz UZ, Schirmer M, Kenny JG, Gregory R, Darby AC, Shakya M, Podar M, Quince C, Hall N. **2016**. A comprehensive benchmarking study of protocols and sequencing platforms for 16S rRNA community profiling. *BMC Genomics* **17**.

Darlington AB, Dat JF, Dixon MA. **2001**. The biofiltration of indoor air: Air flux and temperature influences the removal of toluene, ethylbenzene, and xylene. *Environmental Science and Technology* **35**: 240–246.

Das A, Varma A. **2009**. Symbiosis: The Art of Living. In: Symbiotic Fungi Principles and Practice. 1–28.

Delmotte N, Knief C, Chaffron S, Innerebner G, Roschitzki B, Schlapbach R, Von Mering C, Vorholt JA. **2009**. Community proteogenomics reveals insights into the physiology of phyllosphere bacteria. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 16428–16433.

Després V, Huffman JA, Burrows SM, Hoose C, Safatov A, Buryak G, Fröhlich-nowoisky J, Andreae M, Pöschl U, Jaenicke R, *et al.* 2012. Primary biological aerosol particles in the atmosphere : a review. *Tellus* **0889**.

Didion M, Frey B, Rogiers N, Thürig E. **2014**. Validating tree litter decomposition in the Yasso07 carbon model. *Ecological Modelling* **291**: 58–68.

Domke GM, Perry CH, Walters BF, Woodall CW, Russell MB, Smith JE. **2016**. Estimating litter carbon stocks on forest land in the United States. *Science of the Total Environment* **557–558**: 469–478.

Doty SL, Sher AW, Fleck ND, Khorasani M, Bumgarner RE, Khan Z, Ko AWK, Kim SH, DeLuca TH. **2016**. Variable nitrogen fixation in wild Populus. *PLoS ONE* **11**: 1–22.

Douglas AE. **2009**. The microbial dimension in insect nutritional ecology. *Functional Ecology* **23**: 38–47.

Douglas AE, Werren JH. **2016**. Holes in the hologenome: Why host-microbe symbioses are not holobionts. *mBio* **7**: 1–7.

Duran P, Thiergart T, Garrido-Oter R, Agler M, Kemen E, Schulze-Lefert P, Hacquard S. **2018**. Microbial interkingdom interactions in roots promote Arabidopsis survival. *bioRxiv*: 354167.

Edgar RC, Flyvbjerg H. **2015**. Error filtering, pair assembly and error correction for next-generation sequencing reads. *Bioinformatics* **31**: 3476–3482.

Eichlerová I, Homolka L, Žifčáková L, Lisá L, Dobiášová P, Baldrian P. **2015**. Enzymatic systems involved in decomposition reflects the ecology and taxonomy of saprotrophic fungi. *Fungal Ecology* **13**: 10–22.

Ekblad A, Wallander H, Godbold DL, Cruz C, Johnson D, Baldrian P, Björk RG, Epron D, Kieliszewska-Rokicka B, Kjøller R, *et al.* 2013. The production and turnover of extramatrical mycelium of ectomycorrhizal fungi in forest soils: Role in carbon cycling. *Plant and Soil* **366**: 1–27.

Elad Y. **1996**. Mechanisms involved in the biological control of Botrytis cinerea incited diseases. *European Journal of Plant Pathology* **102**: 719–732.

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE. **2007**. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters* **10**: 1135–1142.

Escudié F, Auer L, Bernard M, Mariadassou M, Cauquil L, Vidal K, Maman S, Hernandez-Raquet G, Combes S, Pascal G. **2018**. FROGS: Find, Rapidly, OTUs with Galaxy Solution. *Bioinformatics* **34**: 1287–1294.

Ewald PW. **1989**. Transmission modes and evolution of the parasitism-mutualism continuum. *Annals of the New York Academy of Sciences* **503**: 295–306.

Fadrosh DW, Ma B, Gajer P, Sengamalay N, Ott S, Brotman RM, Ravel J. **2014**. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the IlluminaMiSeqplatform. *Microbiome* **2**: 6.

Faeth SH. **2002**. Are endophytic fungi defensive plant mutualists? *Oikos* **98**: 25–36.

Fang Z, Gong C, Ouyang Z, Liu P, Sun L, Wang X. **2014**. Characteristic and Concentration Distribution of Culturable Airborne Bacteria in Residential Environments in Beijing , China. : 943–953.

Fargione J, Tilman D, Dybzinski R, Lambers JHR, Clark C, Harpole WS, Knops JMH, Reich PB, Loreau M. **2007**. From selection to complementarity: Shifts in the causes of biodiversity-productivity relationships in a long-term biodiversity experiment. *Proceedings of the Royal Society B: Biological Sciences* **274**: 871–876.

Farré-Armengol G, Filella I, Llusia J, Peñuelas J. **2016**. Bidirectional Interaction between Phyllospheric Microbiotas and Plant Volatile Emissions. *Trends in Plant Science* **21**: 854–860.

Faust K, Raes J. **2012**. Microbial interactions: From networks to models. *Nature Reviews Microbiology* **10**: 538–550.

Fehsenfeld F, Calvert J, Fall R, Goldan P, Guenther AB, Hewitt CN, Lamb B, Trainer M, Westberg H. **1992**. Emissions of volatile organic compounds from vegetation and the implications ofr atmospheric chemistry. *Global biogeochemical cylces* **6**: 389–430.

Feldman TS, Brien HEO, Arnold AE. **2008**. Moths that Vector a Plant Pathogen also Transport Endophytic Fungi and Mycoparasitic Antagonists. *Microbial Ecology* **56**: 742–750.

Fenner M, Thompson K. **2005**. *The ecology of seeds*.

Field CB, Behrenfeld MJ, Randerson JT, Falkowski P. **1998**. Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science* **281**: 237–240.

Fioretto A, Papa S, Pellegrino A, Fuggi A. **2007**. Decomposition dynamics of Myrtus communis and Quercus ilex leaf litter: Mass loss, microbial activity and quality change. *Applied Soil Ecology* **36**: 32–40.

Flemming HC, Wuertz S. **2019**. Bacteria and archaea on Earth and their abundance in biofilms. *Nature Reviews Microbiology* **17**: 247–260.

Fliegerova K, Tapio I, Bonin A, Mrazek J, Callegari ML, Bani P, Bayat A, Vilkki J, Kopečný J, Shingfield KJ, *et al.* 2014. Effect of DNA extraction and sample preservation method on rumen bacterial population. *Anaerobe* **29**: 80–84.

Forchetti G, Masciarelli O, Alemano S, Alvarez D, Abdala G. **2007**. Endophytic bacteria in sunflower (Helianthus annuus L.): Isolation, characterization, and production of jasmonates and abscisic acid in culture medium. *Applied Microbiology and Biotechnology* **76**: 1145–1152.

Foster KR, Schluter J, Coyte KZ, Rakoff-Nahoum S. **2017**. The evolution of the host microbiome as an ecosystem on a leash. *Nature* **548**: 43–51.

Franc GD, Demott PJ. **1998**. Cloud activation characteristics of airborne Erwinia carotovora cells. *Journal of Applied Meteorology* **37**: 1293–1300.

Frank A, Saldierna Guzmán J, Shay J. **2017**. Transmission of Bacterial Endophytes. *Microorganisms* **5**: 70.

Franzetti A, Gandolfi I, Bestetti G, Padoa Schioppa E, Canedoli C, Brambilla D, Cappelletti D, Sebastiani B, Federici E, Papacchini M, *et al.* 2020. Plant-microorganisms interaction promotes removal of air pollutants in Milan (Italy) urban area. *Journal of Hazardous Materials* **384**.

Freiberg E. **1998**. Microclimatic parameters influencing nitrogen fixation in the phyllosphere in a Costa Rican premontane rain forest. *Oecologia* **117**: 9–18.

Freilich S, Zarecki R, Eilam O, Segal ES, Henry CS, Kupiec M, Gophna U, Sharan R, Ruppin E. **2011**. Competitive and cooperative metabolic interactions in bacterial communities. *Nature Communications* **2**.

Friesen ML, Porter SS, Stark SC, von Wettberg EJ, Sachs JL, Martinez-Romero E. **2011**. Microbially Mediated Plant Functional Traits. *Annual Review of Ecology, Evolution, and Systematics* **42**: 23–46.

Fröhlich-Nowoisky J, Kampf CJ, Weber B, Huffman JA, Pï¿½hlker C, Andreae MO, Lang-Yona N, Burrows SM, Gunthe SS, Elbert W, *et al.* 2016. Bioaerosols in the Earth system: Climate, health, and ecosystem interactions. *Atmospheric Research* **182**: 346–376.

Fukasawa Y, Osono T, Takeda H. **2009**. Dynamics of physicochemical properties and occurrence

of fungal fruit bodies during decomposition of coarse woody debris of Fagus crenata. *Journal of Forest Research* **14**: 20–29.

Fukasawa Y, Osono T, Takeda H. **2011**. Wood decomposing abilities of diverse lignicolous fungi on nondecayed and decayed beech wood. *Mycologia* **103**: 474–482.

Fürnkranz M, Wanek W, Richter A, Abell G, Rasche F, Sessitsch A. **2008**. Nitrogen fixation by phyllosphere bacteria associated with higher plants and their colonizing epiphytes of a tropical lowland rainforest of Costa Rica. *ISME Journal* **2**: 561–570.

Galan M, Razzauti M, Bard E, Bernard M, Brouat C, Charbonnel N, Dehne-Garcia A, Loiseau A, Tatard C, Tamisier L, *et al.* 2016. 16S rRNA Amplicon Sequencing for Epidemiological Surveys of Bacteria in Wildlife. *mSystems* **1**: e00032-16.

Gallart M, Adair KL, Love J, Meason DF, Clinton PW, Xue J, Turnbull MH. **2018**. Host Genotype and Nitrogen Form Shape the Root Microbiome of Pinus radiata. *Microbial Ecology* **75**: 419–433.

Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, *et al.* 2004. Nitrogen cycles: past, present, and future. *Biogeochemistry 70:*: 153–226.

Gesheva V, Vasileva-Tonkova E. **2012**. Production of enzymes and antimicrobial compounds by halophilic Antarctic Nocardioides sp. grown on different carbon sources. *World Journal of Microbiology and Biotechnology* **28**: 2069–2076.

Gevers D, Knight R, Petrosino JF, Huang K, McGuire AL, Birren BW, Nelson KE, White O, Methé BA, Huttenhower C. **2012**. The Human Microbiome Project: A Community Resource for the Healthy Human Microbiome. *PLoS Biology* **10**: 6–10.

Ghyselinck J, Pfeiffer S, Heylen K, Sessitsch A, De Vos P. **2013**. The effect of primer choice and short read sequences on the outcome of 16S rRNA gene based diversity studies. *PloS one* **8**: 1–14.

Gilbert GS. **2002**. Evolutionary ecology of plant diseases in natural ecosystems. *Annual Review of Phytopathology* **40**: 13–43.

Gilbert JA, Neufeld JD. **2014**. Life in a World without Microbes. *PLoS Biology* **12**: 1–3.

Gimenez-Ibanez S, Boter M, Ortigosa A, Garcï¿½a-Casado G, Chini A, Lewsey MG, Ecker JR, Ntoukakis V, Solano R. **2017**. JAZ2 controls stomata dynamics during bacterial invasion. *New Phytologist* **213**: 1378–1392.

Goodwin S, McPherson JD, McCombie WR. **2016**. Coming of age: Ten years of next-generation sequencing technologies. *Nature Reviews Genetics* **17**: 333–351.

Graham JH, Colburn GC, Chung KR, Cubero J. **2012**. Protection of citrus roots against infection by Phytophthora spp. by hypovirulent P. nicotianae is not related to induction of systemic acquired resistance. *Plant and Soil* **358**: 39–49.

Greenstone MH, Weber DC, Coudron TA, Payton ME, Hu JS. **2012**. Removing external DNA contamination from arthropod predators destined for molecular gut-content analysis. *Molecular Ecology Resources* **12**: 464–469.

Großkopf T, Soyer OS. **2014**. Synthetic microbial communities. *Current Opinion in Microbiology* **18**: 72–77.

Gundel PE, Rudgers JA, Ghersa CM. **2011**. Incorporating the process of vertical transmission into understanding of host-symbiont dynamics. *Oikos* **120**: 1121–1128.

Gundel PE, Rudgers JA, Whitney KD. **2017**. Vertically transmitted symbionts as mechanisms of transgenerational effects. *American Journal of Botany* **104**: 787–792.

Hacquard S, Garrido-Oter R, González A, Spaepen S, Ackermann G, Lebeis S, McHardy AC, Dangl JL, Knight R, Ley R, *et al.* 2015. Microbiota and host nutrition across plant and animal kingdoms. *Cell Host and Microbe* **17**: 603–616.

Haga DI, Iannone R, Wheeler MJ, Mason R, Polishchuk EA, Fetch T, Van Der Kamp BJ, McKendry IG, Bertram AK. **2013**. Ice nucleation properties of rust and bunt fungal spores and their transport to high altitudes, where they can cause heterogeneous freezing. *Journal of Geophysical Research Atmospheres* **118**: 7260–7272.

Hahn K, Christensen M. **2004**. Dead Wood in European Forest Reserves – A Reference for Forest Management. In: Monitoring and Indicators of Forest Biodiversity in Europe – From Ideas to Operationality. 181.

Hania W Ben, Godbane R, Postec A, Hamdi M, Ollivier B, Fardeau M. **2019**. Defluviitoga tunisiensis gen. nov., sp. nov., a thermophilic bacterium isolated from a mesothermic and anaerobic whey digester. *International Journal of Systematic and Evolutionary Microbiology* **62**: 1377–1382.

Hardoim PR, Hardoim CCP, van Overbeek LS, van Elsas JD. **2012**. Dynamics of seed-borne rice endophytes on early plant growth stages. *PLoS ONE* **7**.

Hardoim PR, van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M, Sessitsch A. **2015**. The Hidden World within Plants: Ecological and Evolutionary Considerations for Defining Functioning of Microbial Endophytes. *Microbiology and Molecular Biology Reviews* **79**: 293–320.

Haritash AK, Kaushik CP. **2009**. Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A review. **169**: 1–15.

Harmon ME, Franklin JF, Swanson FJ, Sollins P, Gregory S V., Lattin JD, Anderson NH, Cline SP, Aumen NG, Sedell JR, *et al.* 1986. Ecology of Coarse Woody Debris in Temperate Ecosystems. *Advances in Ecological Research* **34**: 59–234.

Harrison JG, Forister ML, Parchman TL, Koch GW. **2016**. Vertical stratification of the foliar fungal community in the world's tallest trees. *American Journal of Botany* **103**: 2087–2095.

Hassani MA, Durán P, Hacquard S. **2018**. Microbial interactions within the plant holobiont. : 1–17.

Heather JM, Chain B. **2016**. The sequence of sequencers: The history of sequencing DNA. *Genomics* **107**: 1–8.

Hebert PDN, Cywinska A, Ball SL, DeWaard JR. **2003**. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences* **270**: 313–321.

Heilmann-Clausen J, Christensen M. **2005**. Wood-inhabiting macrofungi in Danish beech-forests - Conflicting diversity patterns and their implications in a conservation perspective. *Biological Conservation* **122**: 633–642.

Heywood VH. **1969**. Scanning electron microscopy in the study of plant materials. *Micron (1969)* **1**: 1–14.

Hodgson S, de Cates C, Hodgson J, Morley NJ, Sutton BC, Gange AC. **2014**. Vertical transmission of fungal endophytes is widespread in forbs. *Ecology and Evolution* **4**: 1199–1208.

Högberg MN, Högberg P. **2002**. Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. *New Phytologist* **154**: 791–795.

Högberg P, Plamboeck AH, Taylor AFS, Fransson PMA. **1999**. Natural 13 C abundance reveals trophic status of fungi and host-origin of carbon in mycorrhizal fungi in mixed forests. *Proceedings of the National Academy of Sciences of the United States of America* **96**: 8534–8539.

Huffman JA, Prenni AJ, Demott PJ, Pöhlker C, Mason RH, Robinson NH, Fröhlich-Nowoisky J, Tobo Y, Després VR, Garcia E, *et al.* 2013. High concentrations of biological aerosol particles and ice nuclei during and after rain. *Atmospheric Chemistry and Physics* **13**: 6151–6164.

Iguchi H, Sato I, Sakakibara M, Yurimoto H, Sakai Y. **2012**. Distribution of methanotrophs in the phyllosphere. *Bioscience, Biotechnology and Biochemistry* **76**: 1580–1583.

Innerebner G, Knief C, Vorholt JA. **2011**. Protection of Arabidopsis thaliana against leaf-pathogenic Pseudomonas syringae by Sphingomonas strains in a controlled model system. *Applied and Environmental Microbiology* **77**: 3202–3210.

Ivanova EG, Doronina N V., Shepelyakovskaya AO, Laman AG, Brovko FA, Trotsenko YA. **2000**. Facultative and obligate aerobic methylobacteria synthesize cytokinins. *Microbiology* **69**: 646–651.

Ivanova EG, Doronina N V., Trotsenko YA. **2001**. Aerobic methylobacteria are capable of synthesizing auxins. *Microbiology* **70**: 392–397.

Ivanova EG, Fedorov DN, Doronina N V., Trotsenko YA. **2006**. Production of vitamin B12 in aerobic methylotrophic bacteria. *Microbiology* **75**: 494–496.

Johnston SR, Boddy L, Weightman AJ. **2016**. Bacteria in decomposing wood and their interactions with wood-decay fungi. *FEMS microbiology ecology* **92**: 1–12.

Jumpponen A, Jones KL. **2009**. Massively parallel 454 sequencing indicates hyperdiverse fungal communities in temperate Quercus macrocarpa phyllosphere. *New Phytologist* **184**: 438–448.

Junker RR, Tholl D. **2013**. Volatile Organic Compound Mediated Interactions at the Plant-Microbe Interface. *Journal of Chemical Ecology* **39**: 810–825.

Kämpfer P, Rainey FA, Andersson MA, Nurmiaho Lassila E-L, Ulrych U, Busse H., Weiss N, Mikkola R, Salkinoja-Salonen M. **2019**. Frigoribacterium faeni gen . nov ., sp . nov ., a novel psychrophilic genus of the family Microbacteriaceae. : 355–363.

Kanters C, Anderson IC, Johnson D. **2015**. Chewing up the wood-wide web: Selective grazing on ectomycorrhizal fungi by collembola. *Forests* **6**: 2560–2570.

Karamanoli K, Menkissoglu-Spiroudi U, Bosabalidis AM, Vokou D, Constantinidou HIA. **2005**. Bacterial colonization of the phyllosphere of nineteen plant species and antimicrobial activity of their leaf secondary metabolites against leaf associated bacteria. *Chemoecology* **15**: 59–67.

Kawaguchi K, Yurimoto H, Oku M, Sakai Y. **2011**. Yeast methylotrophy and autophagy in a methanol-oscillating environment on growing Arabidopsis thaliana leaves. *PLoS ONE* **6**: 1–9.

Kellner H, Vandenbol M. **2010**. Fungi unearthed: Transcripts encoding lignocellulolytic and chitinolytic enzymes in forest soil. *PLoS ONE* **5**.

Kembel SW, Mueller RC. **2014**. Plant traits and taxonomy drive host associations in tropical phyllosphere fungal communities [1]. *Botany* **92**: 303–311.

Kembel SW, O'Connor TK, Arnold HK, Hubbell SP, Wright SJ, Green JL. **2014**. Relationships between phyllosphere bacterial communities and plant functional traits in a neotropical forest. *Proceedings of the National Academy of Sciences* **111**: 13715–13720.

Kemen E, Kemen AC, Agler MT, Kemen E. **2015**. Host – microbe and microbe – microbe interactions in the evolution of obligate plant parasitism. *New Phytologist*: 1–18.

De Kempeneer L, Sercu B, Vanbrabant W, Van Langenhove H, Verstraete W. **2004**. Bioaugmentation of the phyllosphere for the removal of toluene from indoor air. *Applied Microbiology and Biotechnology* **64**: 284–288.

Kesselmeier J, Staudt M. **1999**. An Overview on Emission, Physiology and Ecology.pdf. *Journal of Atmospheric Chemistry* **33**: 23–88.

Khan AL, Waqas M, Kang SM, Al-Harrasi A, Hussain J, Al-Rawahi A, Al-Khiziri S, Ullah I, Ali L, Jung HY, *et al.* 2014. Bacterial endophyte Sphingomonas sp. LK11 produces gibberellins and IAA and promotes tomato plant growth. *Journal of Microbiology* **52**: 689–695.

Kielak AM, Scheublin TR, Mendes LW, van Veen JA, Kuramae EE. **2016**. Bacterial community succession in pine-wood decomposition. *Frontiers in Microbiology* **7**: 1–12.

Kiers ET, Heijden MGA Van Der. **2006**. Mutualistic stability in the arbuscular mycorrhizal symbiosis: Exploring hypothesis of evolutionary cooperation. *Ecology* **87**: 1627–1636.

Klaedtke S, Jacques MA, Raggi L, Préveaux A, Bonneau S, Negri V, Chable V, Barret M. **2016**. Terroir is a key driver of seed-associated microbial assemblages. *Environmental microbiology* **18**: 1792–1804.

Klassen JL, Foght JM. **2011**. Characterization of Hymenobacter isolates from Victoria Upper Glacier, Antarctica reveals five new species and substantial non-vertical evolution within this genus. *Extremophiles* **15**: 45–57.

Kloepper J, Schroth M. 1978. Plant growth-promoting rhizobacteria on radishes. IV international conference on plant pathogenic bacteria Article. *Reads*: 879.

Knief C, Chaffron S, Stark M, Innerebner G. **2012**. Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. *The ISME Journal*: 1378–1390.

Knoch TR, Faeth SH, Arnott DL. **1993**. Endophytic fungi alter foraging and dispersal by desert seed-harvesting ants. *Oecologia* **95**: 470–473.

Knoll D, Schreiber L. **2000**. Plant-microbe interactions: Wetting of ivy (Hedera helix L.) leaf surfaces in relation to colonization by epiphytic microorganisms. *Microbial Ecology* **40**: 33–42.

Konopka A. **2009**. What is microbial community ecology. *ISME Journal* **3**: 1223–1230.

Koskella B, Brockhurst MA. **2014**. Bacteria-phage coevolution as a driver of ecological and evolutionary processes in microbial communities. *FEMS Microbiology Reviews* **38**: 916–931.

Kraft NJB, Ackerly DD. **2010**. Functional trait and phylogenetic tests of community assembly across spatial scales in an Amazonian forest. *Ecological Monographs* **80**: 401–422.

Krishna MP, Mohan M. **2017**. Litter decomposition in forest ecosystems: a review. *Energy, Ecology and Environment* **2**: 236–249.

L H. **1904**. Über neuere Erfahrungen und Probleme aufdem Gebiete der Bodenbakteriologie unter besonderer Berücksichtigung der Gründüngung und Brache. : 59–78.

Lafferty doty S. **2017**. *Functional Importance of the Plant Microbiome*.

Laforest-Lapointe I. **2017**. Exploring the temperate tree leaf microbiome: From natural forests to controlled experiments and urban environments.

Laforest-Lapointe I, Messier C, Kembel SW. **2016a**. Host species identity, site and time drive temperate tree phyllosphere bacterial community structure. *Microbiome* **4**: 1–10.

Laforest-Lapointe I, Messier C, Kembel SW. **2016b**. Tree phyllosphere bacterial communities: exploring the magnitude of intra- and inter-individual variation among host species. *PeerJ* **4**: 1–18.

Laforest-Lapointe I, Paquette A, Messier C, Kembel SW. **2017**. Leaf bacterial diversity mediates plant diversity and ecosystem function relationships. *Nature* **546**: 145–147.

Lambais MR, Crowley DE, Cury JC, Büll RC, Rodrigues RR. **2006**. Bacterial diversity in tree canopies of the Atlantic Forest. *Science* **312**: 1917.

Lange M, Habekost M, Eisenhauer N, Roscher C, Bessler H, Engels C, Oelmann Y, Scheu S, Wilcke W, Schulze ED, *et al.* 2014. Biotic and abiotic properties mediating plant diversity effects on soil microbial communities in an experimental grassland. *PLoS ONE* **9**.

Larsson J. **2018**. eulerr: Area-Proportional Euler Diagrams with Ellipses.

Last FT. **1955**. Seasonal incidence of Sporobolomyces on cereal leaves. *Transactions of the British Mycological Society*: 221–239.

Lauber CL, Hamady M, Knight R, Fierer N. **2009**. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology* **75**: 5111–5120.

Lavoie M, Mack MC, Hiers JK, Pokswinski S. **2012**. The effect of restoration treatments on the spatial variability of soil processes under longleaf pine trees. *Forests* **3**: 591–604.

LeBauer, D; Treseder K. **2008**. Nitrogen Limitation of Net Primary Productivity. *Ecology* **89**: 371–379.

Lebeis SL. **2015**. Greater than the sum of their parts: Characterizing plant microbiomes at the community-level. *Current Opinion in Plant Biology* **24**: 82–86.

Leck MA, Parker VT, Simpson RL. **2008**. Seedling ecology and evolution. *Seedling Ecology and Evolution*: 1–514.

Lecours PB, Veillette M, Marsolais D, Duchaine C. **2012**. Characterization of bioaerosols from dairy barns: Reconstructing the puzzle of occupational respiratory diseases by using molecular approaches. *Applied and Environmental Microbiology* **78**: 3242–3248.

Lee YM, Kim EH, Lee HK, Hong SG. **2014**. Biodiversity and physiological characteristics of Antarctic and Arctic lichens-associated bacteria. *World Journal of Microbiology and Biotechnology* **30**: 2711–2721.

Lee SD, Lee DW, Ko Y. **2011**. Marmoricola korecus sp. nov. *International Journal of Systematic and Evolutionary Microbiology* **61**: 1628–1631.

Lee HS, Madhaiyan M, Kim CW, Choi SJ, Chung KY, Sa TM. **2006**. Physiological enhancement of early growth of rice seedlings (Oryza sativa L.) by production of phytohormone of N2-fixing methylotrophic isolates. *Biology and Fertility of Soils* **42**: 402–408.

Leff JW, Del Tredici P, Friedman WE, Fierer N. **2015**. Spatial structuring of bacterial communities within individual Ginkgo biloba trees. *Environmental Microbiology* **17**: 2352–2361.

Lemanceau P, Barret M, Mazurier S, Mondy S, Pivato B, Fort T, Vacher C. **2017**. *Plant Communication With Associated Microbiota in the Spermosphere, Rhizosphere and Phyllosphere*. Elsevier Ltd.

Lemons A, Clay K, Rudgers JA. **2005**. Connecting plant-microbial interactions above and belowground: A fungal endophyte affects decomposition. *Oecologia* **145**: 595–604.

Leveau JHJ, Lindow SE. **2001**. Appetite of an epiphyte: Quantitative monitoring of bacterial sugar consumption in the phyllosphere. *Proceedings of the National Academy of Sciences of the United States of America* **98**: 3446–3453.

Li Y, Wu X, Chen T, Wang W, Liu G, Zhang W, Li S, Wang M, Zhao C, Zhou H, *et al.* 2018. Plant phenotypic traits eventually shape its microbiota: A common garden test. *Frontiers in Microbiology* **9**: 1–13.

Lighthart B, Shaffer BT. **1994**. Bacterial flux from chaparral into the atmosphere in mid-summer at a high desert location. *Atmospheric Environment* **28**: 1267–1274.

Lindahl BD, Nilsson RH, Tedersoo L. **2013**. Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide - Lindahl - 2013 - New Phytologist - Wiley Online Library. *New …*.

Lindemann J, Constantinidou HA, Barchet WR, Upper CD. **1982**. Plants as sources of airborne bacteria, including ice nucleation-active bacteria. *Applied and Environmental Microbiology* **44**: 1059–1063.

Lindow SE, Brandl MT. **2003**. Microbiology of the Phyllosphere. *Applied and Environmental Microbiology* **69**: 1875–1883.

Links MG, Demeke T, Gräfenhan T, Hill JE, Hemmingsen SM, Dumonceaux TJ. **2014**. Simultaneous profiling of seed-associated bacteria and fungi reveals antagonistic interactions between microorganisms within a shared epiphytic microbiome on Triticum and Brassica seeds. *New Phytologist* **202**: 542–553.

Liu Y, Zuo S, Zou Y, Wang J, Song W. **2012**. Investigation on diversity and population succession dynamics of indigenous bacteria of the maize spermosphere. *World Journal of Microbiology and*

Biotechnology **28**: 391–396.

Lladó S, López-Mondéjar R, Baldrian P. **2017**. Forest Soil Bacteria: Diversity, Involvement in Ecosystem Processes, and Response to Global Change. *Microbiology and Molecular Biology Reviews* **81**: 1–27.

López-Mondéjar R, Voříšková J, Větrovský T, Baldrian P. **2015**. The bacterial community inhabiting temperate deciduous forests is vertically stratified and undergoes seasonal dynamics. *Soil Biology and Biochemistry* **87**: 43–50.

Lopez-Velasco G, Carder PA, Welbaum GE, Ponder MA. **2013**. Diversity of the spinach (Spinacia oleracea) spermosphere and phyllosphere bacterial communities. *FEMS Microbiology Letters* **346**: 146–154.

Louca S, Jacques SMS, Pires APF, Leal JS, Srivastava DS, Parfrey LW, Farjalla VF, Doebeli M. **2016**. High taxonomic variability despite stable functional structure across microbial communities. *Nature Ecology & Evolution* **1**: 0015.

Lugtenberg B, Kamilova F, Lugtenberg B, Kamilova F. **2015**. Plant-Growth-Promoting Rhizobacteria Plant-Growth-Promoting Rhizobacteria.

Lynn M, Fester R. **1991**. *Symbiogenesis and Symbionticism*.

Lyons JA, Bemis DA, Bryant MJ, Kania SA, Abd-Eldaim M, Newman SJ. **2009**. Isolation of Propionibacterium acnes from a case of placentitis and abortion in a cow. *Journal of Veterinary Diagnostic Investigation* **21**: 274–277.

Maignien L, DeForce EA, Chafee ME, Murat Eren A, Simmons SL. **2014**. Ecological succession and stochastic variation in the assembly of Arabidopsis thaliana phyllosphere communities. *mBio* **5**.

Malfanova N, Lugtenberg BJJ, Berg G. **2013**. Bacterial Endophytes: Who and Where, and What are they doing there? *Molecular Microbial Ecology of the Rhizosphere* **1**: 391–403.

Manirajan BA, Maisinger C, Ratering S, Rusch V, Schwiertz A, Cardinale M, Schnell S. **2018**. Diversity, specificity, co-occurrence and hub taxa of the bacterial–fungal pollen microbiome. *FEMS Microbiology Ecology*: 1–11.

Marchesi JR, Ravel J. **2015**. The vocabulary of microbiome research: a proposal. *Microbiome* **3**: 1–3.

Martinez-romero E. **2006**. Dinitrogen-Fixing Prokaryotes. *Prokaryotes*: 793–817.

Maude RB. **1996**. *Seedborne Diseases and Their Control: Principles and Practice*.

Maus I, Gabriela K, Wibberg D, Winkler A, Stolze Y, König H, Pühler A, Schlüter A. **2015**. Complete genome sequence of the strain Defluviitoga tunisiensis L3 , isolated from a thermophilic , production-scale biogas plant. *Journal of Biotechnology* **203**: 17–18.

McDonald D, Price MN, Goodrich J, Nawrocki EP, Desantis TZ, Probst A, Andersen GL, Knight R, Hugenholtz P. **2012**. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME Journal* **6**: 610–618.

Melotto M, Underwood W, He SY. **2008**. Role of Stomata in Plant Innate Immunity and Foliar Bacterial Diseases. *Annual Review of Phytopathology* **46**: 101–122.

Mercier J, Lindow SE. **2000**. Role of leaf surface sugars in colonization of plants by bacterial epiphytes. *Applied and Environmental Microbiology* **66**: 369–374.

Miliūtė I, Buzaitė O. **2011**. IAA production and other plant growth promoting traits of endophytic bacteria from apple tree. *Biologija* **57**: 98–102.

Mitter B, Pfaffenbichler N, Flavell R, Compant S, Antonielli L, Petric A, Berninger T, Naveed M, Sheibani-Tezerji R, Maltzahn G von, *et al.* 2017. A new approach to modify plant microbiomes and traits by introducing beneficial bacteria at flowering into progeny seeds. *Frontiers in Microbiology* **8**.

Miura T, Sánchez R, Castañeda LE, Godoy K, Barbosa O. **2019**. Shared and unique features of bacterial communities in native forest and vineyard phyllosphere. *Ecology and Evolution* **9**: 3295–3305.

Monier JM, Lindow SE. **2003**. Differential survival of solitary and aggregated bacterial cells promotes aggregate formation on leaf surfaces. *Proceedings of the National Academy of Sciences of the United States of America* **100**: 15977–15982.

Morris CE, Conen F, Alex Huffman J, Phillips V, Pöschl U, Sands DC. **2014a**. Bioprecipitation: A feedback cycle linking Earth history, ecosystem dynamics and land use through biological ice nucleators in the atmosphere. *Global Change Biology* **20**: 341–351.

Morris CE, Leyronas C, Nicot PC. **2014b**. Movement of Bioaerosols in the Atmosphere and the Consequences for Climate and Microbial Evolution. *Aerosol Science: Technology and Applications*: 393–415.

Morris CE, National I, Recherche D. **2001**. Phyllosphere. : 1–8.

Morris CE, Sands DC, Glaux C, Samsatly J, Asaad S, Moukahel AR, Gonçalves FLT, Bigg EK. **2013**. Urediospores of rust fungi are ice nucleation active at >-10 °c and harbor ice nucleation active bacteria. *Atmospheric Chemistry and Physics* **13**: 4223–4233.

Moyes AB, Kueppers LM, Pett-Ridge J, Carper DL, Vandehey N, O'Neil J, Frank AC. **2016a**. Evidence for foliar endophytic nitrogen fixation in a widely distributed subalpine conifer. *New Phytologist* **210**: 657–668.

Moyes AB, Kueppers LM, Pett-Ridge J, Carper DL, Vandehey N, O'Neil J, Frank AC. **2016b**. Evidence for foliar endophytic nitrogen fixation in a widely distributed subalpine conifer. *New Phytologist* **210**: 657–668.

Müller T, Ruppel S. **2014**. Progress in cultivation-independent phyllosphere microbiology. *FEMS Microbiology Ecology* **87**: 2–17.

Müller DB, Vogel C, Bai Y, Vorholt JA. **2016**. The Plant Microbiota: Systems-Level Insights and Perspectives. *Annual Review of Genetics* **50**: 211–234.

Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. **1986**. Specific enzymatic amplification of DNA in vitro: The polymerase chain reaction. : 11.

Munkvold GP. **2009**. Seed Pathology Progress in Academia and Industry. *Annual Review of Phytopathology* **47**: 285–311.

Nakazato T, Ohta T, Bono H. **2013**. Experimental Design-Based Functional Mining and Characterization of High-Throughput Sequencing Data in the Sequence Read Archive. *PLoS ONE* **8**.

Naveed M, Mitter B, Reichenauer TG, Wieczorek K, Sessitsch A. **2014**. Increased drought stress resilience of maize through endophytic colonization by Burkholderia phytofirmans PsJN and Enterobacter sp. FD17. *Environmental and Experimental Botany* **97**: 30–39.

Nelson EB. **2004**. Microbial Dynamics and Interactions in the Spermosphere. *Annual Review of Phytopathology* **42**: 271–309.

Nelson EB. **2017**. The seed microbiome: Origins, interactions, and impacts. *Plant and Soil* **422**: 7–34.

Nelson EB, Simoneau P, Barret M, Mitter B, Compant S. **2018**. The soil, the seed, the microbes

and the plant. *Plant Soil* **422**: 1–5.

Nemergut DR, Schmidt SK, Fukami T, O'Neill SP, Bilinski TM, Stanish LF, Knelman JE, Darcy JL, Lynch RC, Wickey P, *et al.* 2013. Patterns and Processes of Microbial Community Assembly. *Microbiology and Molecular Biology Reviews* **77**: 342–356.

Neubauer M, Sourek J, Ryc M, Bohacek J, Mara M, Mnukova J. 1991. Corynebacterium accolens sp . nov ., a Gram-Positive Rod Exhibiting Satellitism , from Clinical Material. **51**: 46–51.

Newton AC, Gravouil C, Fountaine JM. 2010. Managing the ecology of foliar pathogens: Ecological tolerance in crops. *Annals of Applied Biology* **157**: 343–359.

Nguyen SN, Flores A, Talamantes D, Dar F, Valdez A, Schwans J, Berlemont R. 2019. GeneHunt for rapid domain-specific annotation of glycoside hydrolases. *Scientific Reports* **9**: 1–10.

Nguyen NH, Smith D, Peay K, Kennedy P. 2014. Parsing ecological signal from noise in next generation amplicon sequencing. *New p*.

Nguyen NH, Song Z, Bates ST, Branco S, Tedersoo L, Menke J, Schilling JS, Kennedy PG. 2016. FUNGuild: an open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology* **20**: 241–248.

Nilakanta H, Drews KL, Firrell S, Foulkes MA, Jablonski KA. 2014. A review of software for analyzing molecular Sequences. *BMC Research Notes* **7**.

Nilsson RH, Anslan S, Bahram M, Wurzbacher C, Baldrian P, Tedersoo L. 2019. Mycobiome diversity: high-throughput sequencing and identification of fungi. *Nature Reviews Microbiology* **17**: 95–109.

Nisbet M, Briggs S, Ellis-Pegler R, Thomas M, Holland D. 2007. Propionibacterium acnes: An under-appreciated cause of post-neurosurgical infection. *Journal of Antimicrobial Chemotherapy* **60**: 1097–1103.

Niu B, Paulson JN, Zheng X, Kolter R. 2017. Simplified and representative bacterial community of maize roots. *Proceedings of the National Academy of Sciences of the United States of America* **114**: E2450–E2459.

Norros V, Penttilä R, Suominen M, Ovaskainen O. 2012. Dispersal may limit the occurrence of specialist wood decay fungi already at small spatial scales. *Oikos* **121**: 961–974.

O'Brien HE, Parrent JL, Jackson JA, Moncalvo JM, Vilgalys R. **2005**. Fungal community analysis by large-scale sequencing of environmental samples. *Applied and Environmental Microbiology* **71**: 5544–5550.

Ofek M, Hadar Y, Minz D. **2011**. Colonization of cucumber seeds by bacteria during germination. *Environmental Microbiology* **13**: 2794–2807.

Oksanen AJ, Kindt R, Legendre P, Hara BO, Simpson GL, Stevens MHH, Wagner H. **2008**. The vegan Package; Community Ecology Package (Version 1.15-1).

Osono T. **2006**. Role of phyllosphere fungi of forest trees in the development of decomposer fungal communities and decomposition processes of leaf litter. *Canadian Journal of Microbiology* **52**: 701–716.

Osono T, Mori A. **2003**. Colonization of Japanese beech leaves by phyllosphere fungi. *Mycoscience* **44**: 437–441.

Osono T, Mori A. **2004**. Distribution of phyllosphere fungi within the canopy of giant dogwood. *Mycoscience* **45**: 161–168.

Osono T, Takeda H. **2001**. Organic chemical and nutrient dynamics in decomposing beech leaf litter in relation to fungal ingrowth and succession during 3-year decomposition processes in a cool temperate deciduous forest in Japan. *Ecological Research* **16**: 649–670.

Ottesen AR, White JR, Skaltsas DN, Newell MJ, Walsh CS. **2009**. Impact of organic and conventional management on the phyllosphere microbial ecology of an apple crop. *Journal of Food Protection* **72**: 2321–2325.

Ouayjan A, Hampe A. **2018**. Extensive sib-mating in a refugial population of beech (Fagus sylvatica) growing along a lowland river. *Forest Ecology and Management* **407**: 66–74.

Pascazio S, Crecchio C, Ricciuti P, Palese AM, Xiloyannis C, Sofo A. **2015**. Phyllosphere and carposphere bacterial communities in olive plants subjected to different cultural practices. *International Journal of Plant Biology* **6**: 15–19.

Pauvert C, Buée M, Laval V, Edel-Hermann V, Fauchery L, Gautier A, Lesur I, Vallance J, Vacher C. **2019**. Bioinformatics matters: The accuracy of plant and soil fungal community data is highly dependent on the metabarcoding pipeline. *Fungal Ecology* **41**: 23–33.

Penuelas J, Llusia J. **2001**. The complexity of factors driving volatile organic compound emissions

by plants. *Biologia plantarum* **44**: 481–487.

Peredo EL, Simmons SL. **2018**. Leaf-FISH: Microscale imaging of bacterial taxa on phyllosphere. *Frontiers in Microbiology* **8**: 1–14.

Pereira SIA, Castro PML. **2014**. Diversity and characterization of culturable bacterial endophytes from Zea mays and their potential as plant growth-promoting agents in metal-degraded soils. : 14110–14123.

Perry DA. **2009**. Forest Ecosystems. *Forest Ecosystems*.

Peršoh D. **2013a**. Factors shaping community structure of endophytic fungi-evidence from the Pinus-Viscum-system. *Fungal Diversity* **60**: 55–69.

Peršoh D. **2013b**. Factors shaping community structure of endophytic fungi-evidence from the Pinus-Viscum-system. *Fungal Diversity* **60**: 55–69.

Pettersson BMF, Behra PRK, Manduva S, Das S, Dasgupta S, Bhattacharya A, Kempf A, Grund E, Rm K, Hj K. **2014**. Draft Genome Sequence of Saccharopolyspora rectivirgula. *Genome Announc* **2**: 5–6.

Plummer E, Twin J. **2015**. A Comparison of Three Bioinformatics Pipelines for the Analysis of Preterm Gut Microbiota using 16S rRNA Gene Sequencing Data. *Journal of Proteomics & Bioinformatics* **8**: 283–291.

Pochon S, Simoneau P, Pigné S, Balidas S, Bataillé-Simoneau N, Campion C, Jaspard E, Calmes B, Hamon B, Berruyer R, *et al.* 2013. Dehydrin-like Proteins in the Necrotrophic Fungus Alternaria brassicicola Have a Role in Plant Pathogenesis and Stress Response. *PLoS ONE* **8**.

Pollock J, Glendinning L, Wisedchanwet T, Watson M. **2018**. The Madness of Microbiome : Attempting To Find Consensus. *Applied and Environmental Microbiology* **84**: 1–12.

Pöschl U, Martin ST, Sinha B, Chen Q, Gunthe SS, Huffman JA, Borrmann S, Farmer DK, Garland RM, Helas G, *et al.* 2010. Rainforest Aerosols as Biogenic Nuclei of Clouds and Precipitation in the Amazon. *Science* **329**: 1513–1517.

Purahong W, Kapturska D, Pecyna MJ, Schulz E, Schloter M, Buscot F, Hofrichter M, Kruğer D. **2014**. Influence of different forest system management practices on leaf litter decomposition rates, nutrient dynamics and the activity of ligninolytic enzymes: A case study from Central European forests. *PLoS ONE* **9**: 1–11.

Purahong W, Wubet T, Lentendu G, Schloter M, Pecyna MJ, Kapturska D, Hofrichter M, Krüger D, Buscot F. **2016**. Life in leaf litter: novel insights into community dynamics of bacteria and fungi during litter decomposition. *Molecular ecology* **25**: 4059–4074.

Pusey PL, Stockwell VO, Reardon CL, Smits THM, Duffy B. **2011**. Antibiosis activity of Pantoea agglomerans biocontrol strain E325 against Erwinia amylovora on apple flower stigmas. *Phytopathology* **101**: 1234–1241.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. **2013**. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Research* **41**: 590–596.

Quatrini R, Johnson DB. **2018**. Microbiomes in extremely acidic environments: functionalities and interactions that allow survival and growth of prokaryotes at low pH. *Current Opinion in Microbiology* **43**: 139–147.

Radulovic NS, Blagojevic PD, Stojanovic-Radic ZZ, Stojanovic NM. **2013**. Antimicrobial Plant Metabolites: Structural Diversity and Mechanism of Action. *Current Medicinal Chemistry* **20**: 932–952.

Rajala T, Peltoniemi M, Hantula J, Mäkipää R, Pennanen T. **2011**. RNA reveals a succession of active fungi during the decay of Norway spruce logs. *Fungal Ecology* **4**: 437–448.

Rasmussen TT. **2000**. Resident aerobic microbiota of the adult human nasal cavity.

Rastogi G, Coaker GL, Leveau JHJ. **2013**. New insights into the structure and function of phyllosphere microbiota through high-throughput molecular approaches. *FEMS Microbiology Letters* **348**: 1–10.

Reading NC, Sperandio V. **2006**. Quorum sensing: The many languages of bacteria. *FEMS Microbiology Letters* **254**: 1–11.

Redford AJ, Bowers RM, Knight R, Linhart Y, Fierer N. **2010**. The ecology of the phyllosphere: Geographic and phylogenetic variability in the distribution of bacteria on tree leaves. *Environmental Microbiology* **12**: 2885–2893.

Reed SC, Cleveland CC, Townsend AR. **2011**. Functional Ecology of Free-Living Nitrogen Fixation: A Contemporary Perspective. *Annual Review of Ecology, Evolution, and Systematics* **42**: 489–512.

Reisberg EE, Hildebrandt U, Riederer M, Hentschel U. **2013**. Distinct phyllosphere bacterial

communities on Arabidopsis wax mutant leaves. *PLoS ONE* **8**.

Rennie EA, Turgeon R. **2009**. A comprehensive picture of phloem loading strategies. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 14162–14167.

Requena N, Perez-solis E, Azcon-aguilar C, Jeffries P, Barea J. **2001**. Management of Indigenous Plant-Microbe Symbioses Aids Restoration of Desertified Ecosystems. *Applied and environmental microbiology* **67**: 495–498.

Rezki S, Campion C, Simoneau P, Jacques MA, Shade A, Barret M. **2018**. Assembly of seed-associated microbial communities within and across successive plant generations. *Plant and Soil* **422**: 67–79.

Rico L, Ogaya R, Terradas J, Peñuelas J. **2014**. Community structures of N2-fixing bacteria associated with the phyllosphere of a Holm oak forest and their response to drought. *Plant Biology* **16**: 586–593.

Riegel P, Heller R, Prevost G, Jehl F, Monteil H. **2019**. Corynebacterium durum sp . nov ., from Human Clinical SDecimens. : 1107–1111.

Rissanen AJ, Kurhela E, Aho T, Oittinen T, Tiirola M. **2010**. Storage of environmental samples for guaranteeing nucleic acid yields for molecular microbiological studies. *Applied Microbiology and Biotechnology* **88**: 977–984.

Robertson GP, Paul EA. **2000**. Decomposition and Soil Organic Matter Dynamics. *Methods in Ecosystem Science*: 104–116.

Rodriguez RJ, White JF, Arnold a E, Redman RS. **2009**. Fungal endophytes: diversity and functional roles. *New phytologist* **182**: 314–330.

Rognes T, Flouri T, Nichols B, Quince C, Mahé F. **2016**. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* **4**: e2584.

Roh SW, Quan Z, Nam Y, Chang H, Kim K, Kim M, Im W, Jin L, Kim S, Lee S, *et al.* 2019. Pedobacter agri sp . nov ., from soil. : 1640–1643.

Rosado BHP, Almeida LC, Alves LF, Lambais MR, Oliveira RS. **2018**. The importance of phyllosphere on plant functional ecology: A phyllo trait manifesto. *New Phytologist*.

Rubin BER, Gibbons SM, Kennedy S, Hampton-Marcell J, Owens S, Gilbert JA. **2013**.

Investigating the Impact of Storage Conditions on Microbial Community Composition in Soil Samples. *PLoS ONE* **8**: 1–9.

Ruinen J. **1956**. Occurrence of Beijerinckia species in the 'phyllosphere'. *Nature* **177**: 220–221.

Ryffel F, Helfrich EJN, Kiefer P, Peyriga L, Portais JC, Piel J, Vorholt JA. **2016**. Metabolic footprint of epiphytic bacteria on Arabidopsis thaliana leaves. *ISME Journal* **10**: 632–643.

Ryu J, Madhaiyan M, Poonguzhali S, Yim W, Indiragandhi P, Kim K, Anandham R, Yun J, Kim KH, Sa T. **2006**. Plant growth substances produced by methylobacterium spp. and their effect on tomato (lycopersicon esculentum L.) and red pepper (capsicum annuum L.) growth. *Journal of Microbiology and Biotechnology* **16**: 1622–1628.

Sastry KS. **2013**. *Seed-borne Plant Virus Diseases*.

Sayer EJ, Tanner EVJ, Lacey AL. **2006**. Effects of litter manipulation on early-stage decomposition and meso-arthropod abundance in a tropical moist forest. *Forest Ecology and Management* **229**: 285–293.

Schlechter RO, Miebach M, Remus-emsermann MNP. **2019**. Driving factors of epiphytic bacterial communities : A review. *Journal of Advanced Research* **19**: 57–65.

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, *et al.* 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* **75**: 7537–7541.

Schmidt MWI, Torn MS, Abiven S, Dittmar T, Guggenberger G, Janssens IA, Kleber M, Kögel-Knabner I, Lehmann J, Manning DAC, *et al.* 2011. Persistence of soil organic matter as an ecosystem property. *Nature* **478**: 49–56.

Schneider T, Keiblinger KM, Schmid E, Sterflinger-Gleixner K, Ellersdorfer G, Roschitzki B, Richter A, Eberl L, Zechmeister-Boltenstern S, Riedel K. **2012**. Who is who in litter decomposition Metaproteomics reveals major microbial players and their biogeochemical functions. *ISME Journal* **6**: 1749–1762.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Bolchacova E, Voigt K, Crous PW, *et al.* 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences* **109**:

6241–6246.

Schröter D, Wolters V, De Ruiter PC. **2003**. C and N mineralisation in the decomposer food webs of a European forest transect. *Oikos* **102**: 294–308.

Serna-Chavez HM, Fierer N, Van Bodegom PM. **2013**. Global drivers and patterns of microbial abundance in soil. *Global Ecology and Biogeography* **22**: 1162–1172.

Sessitsch A, Reiter B, Berg G. **2004**. Endophytic bacterial communities of field-grown potato plants and their plant-growth-promoting and antagonistic abilities. **249**: 239–249.

Shade A, Jacques MA, Barret M. **2017**. Ecological patterns of seed microbiome diversity, transmission, and assembly. *Current Opinion in Microbiology* **37**: 15–22.

Shivlata L, Satyanarayana T. **2015**. Thermophilic and alkaliphilic Actinobacteria : biology and potential applications. **6**: 1–29.

Sibley CD, Peirano G, Church DL. **2012**. Molecular methods for pathogen and microbial community detection and characterization: Current and potential application in diagnostic microbiology. *Infection, Genetics and Evolution* **12**: 505–521.

Singh D, Mathur SB. **2004**. *Histopathology of Seed-Borne Infections*. CRC Press.

Sittenfeld A, Uribe-lorío L, Mora M, Nielsen V, Arrieta G, Janzen DH. **2002**. Does a polyphagous caterpillar have the same gut microbiota when feeding on different species of food plants ? **50**: 547–560.

Šnajdr J, Cajthaml T, Valášková V, Merhautová V, Petránková M, Spetz P, Leppänen K, Baldrian P. **2011**. Transformation of Quercus petraea litter: Successive changes in litter chemistry are reflected in differential enzyme activity and changes in the microbial community composition. *FEMS Microbiology Ecology* **75**: 291–303.

Staley JT, Irgens RL, Brenner DJ. **2019**. Enhydrobacter aerasaccus gen. nov. , sp. nov. a Gas-Vacuolated, Facultatively Anaerobic, Heterotrophic Rod. *International journal of systematic bacteriology* **37**: 289–291.

Stokland JN, Jonsson BG, Information C. **2012**. Biodiversity in Dead Wood. *Cambridge University Press*.

Stone BWG, Jackson CR. **2019**. Canopy position is a stronger determinant of bacterial community

composition and diversity than environmental disturbance in the phyllosphere. : 1–11.

Stout JD. **1960**. Bacteria of soil and pasture leaves at Claudelands Showgrounds. *New Zealand Journal of Agricultural Research* **3**: 413–430.

Štursová M, Žifčáková L, Leigh MB, Burgess R, Baldrian P. **2012**. Cellulose utilization in forest litter and soil: Identification of bacterial and fungal decomposers. *FEMS Microbiology Ecology* **80**: 735–746.

Su S, Chen M, Teng C, Jiang S, Zhang C, Lin M, Zhang W. **2014**. Hymenobacter kanuolensis sp. nov., a novel radiation-resistant bacterium. *International Journal of Systematic and Evolutionary Microbiology* **64**: 2108–2112.

Sullivant DJO, Gara FO. **1992**. Traits of Fluorescent Pseudomonas spp . Involved in Suppression of Plant Root Pathogens. *Microbiological Reviews* **56**: 662–676.

Sun H, Terhonen E, Kovalchuk A, Tuovila H, Chen H, Oghenekaro AO, Heinonsalo J, Kohler A, Kasanen R, Vasander H, *et al.* 2016. Dominant tree species and soil type affect the fungal community structure in a boreal peatland forest. *Applied and Environmental Microbiology* **82**: 2632–2643.

Sy A, Timmers ACJ, Knief C, Vorholt JA. **2005**. Methylotrophic metabolism is advantageous for Methylobacterium extorquens during colonization of Medicago truncatula under competitive conditions. *Applied and Environmental Microbiology* **71**: 7245–7252.

Taberlet P, Coissac E. **2012**. Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology* **21**: 2045–2050.

Talbot JM, Bruns TD, Taylor JW, Smith DP, Branco S, Glassman SI, Erlandson S, Vilgalys R, Liao HL, Smith ME, *et al.* 2014. Endemism and functional convergence across the North American soil mycobiome. *Proceedings of the National Academy of Sciences of the United States of America* **111**: 6341–6346.

Tanaka Y, Sano T, Tamaoki M, Nakajima N, Kondo N, Hasezawa S. **2006**. Cytokinin and auxin inhibit abscisic acid-induced stomatal closure by enhancing ethylene production in Arabidopsis. *Journal of Experimental Botany* **57**: 2259–2266.

Tancos MA, Chalupowicz L, Barash I, Manulis-sasson S, Smart D. **2013**. Tomato Fruit and Seed Colonization by Clavibacter michiganensis subsp . michiganensis through External and Internal

Routes. **79**: 6948–6957.

Tedersoo L, Drenkhan R, Anslan S, Morales-Rodriguez C, Cleary M. **2019**. High-throughput identification and diagnostics of pathogens and pests: Overview and practical recommendations. *Molecular Ecology Resources* **19**: 47–76.

Terhonen E, Blumenstein K, Kovalchuk A. **2019**. Forest Tree Microbiomes and Associated Fungal Endophytes : Functional Roles and Impact on Forest Health. : 1–32.

Thines M. **2014**. Phylogeny and evolution of plant pathogenic oomycetes-a global overview. *European Journal of Plant Pathology* **138**: 431–447.

Thompson LR, Sanders JG, McDonald D, Amir A, Ladau J, Locey KJ, Prill RJ, Tripathi A, Gibbons SM, Ackermann G, *et al.* 2017. A communal catalogue reveals Earth's multiscale microbial diversity. *Nature* **551**: 457–463.

Tlaskal V, Vorıskov J, Baldrian P. **2016**. Bacterial succession on decomposing leaf litter exhibits a specific occurrence pattern of cellulolytic taxa and potential decomposers of fungal mycelia. *FEMS Microbiology Ecology* **92**: 1–10.

Trotsenko YA, Ivanova EG, Doronina N V. 2001. Aerobic methylotrophic bacteria as phytosymbionts. *Mikrobiologiya* **70**: 725–736.

Truyens S, Weyens N, Cuypers A, Vangronsveld J. **2015**. Bacterial seed endophytes: Genera, vertical transmission and interaction with plants. *Environmental Microbiology Reports* **7**: 40–50.

Tsavkelova EA, Klimova SY, Cherdyntseva TA, Netrusov AI. **2006**. Microbial producers of plant growth stimulators and their practical use: A review. *Applied Biochemistry and Microbiology* **42**: 117–126.

Tukey HB. **1970**. The Leaching of Substances from Plants. *Annual Review of Plant Physiology* **21**: 305–324.

Turner TR, James EK, Poole PS, Gilbert J, Meyer F, Jansson J, Gordon J, Pace N, Tiedje J, Ley R, *et al.* 2013. The plant microbiome. *Genome Biology* **14**: 209.

Unterseher M, Peršoh D, Schnittler M. **2013**. Leaf-inhabiting endophytic fungi of European Beech (Fagus sylvatica L.) co-occur in leaf litter but are rare on decaying wood of the same host. *Fungal Diversity* **60**: 43–54.

Urbanová M, Šnajdr J, Baldrian P. **2015**. Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. *Soil Biology and Biochemistry* **84**: 53–64.

Vacher C, Cordier T, Vallance J. **2016a**. Phyllosphere Fungal Communities Differentiate More Thoroughly than Bacterial Communities Along an Elevation Gradient. *Microbial Ecology*: 1–3.

Vacher C, Hampe A, Porté AJ, Sauer U, Compant S, Morris CE. **2016b**. The Phyllosphere: Microbial Jungle at the Plant–Climate Interface. *Annual Review of Ecology, Evolution, and Systematics* **47**: 1–24.

Vala V, Baldrian P, Herinkova J. **2008**. Soil Biology & Biochemistry Spatial variability of enzyme activities and microbial biomass in the upper layers of Quercus petraea forest soil. **40**: 2068–2075.

Valverde A, Peix A, Rivas R, Velázquez E, Salazar S, Santa-Regina I, Rodríguez-Barrueco C, Igual JM. **2008**. Paenibacillus castaneae sp. nov., isolaled from the phyllosphere of Castanea sativa Miller. *International Journal of Systematic and Evolutionary Microbiology* **58**: 2560–2564.

Vandenkoornhuyse P, Quaiser A, Duhamel M, Le Van A, Dufresne A. **2015**. The importance of the microbiome of the plant holobiont. *New Phytologist* **206**: 1196–1206.

Vayssier-Taussat M, Albina E, Citti C, Cosson JF, Jacques MA, Lebrun MH, Le Loir Y, Ogliastro M, Petit MA, Roumagnac P, *et al.* 2014. Shifting the paradigm from pathogens to pathobiome new concepts in the light of meta-omics. *Frontiers in Cellular and Infection Microbiology* **5**: 1–7.

Verma VC, Gange AC. **2014**. *Advances in endophytic research*.

Vitousek PM, Cassman K, Cleveland C, Crews T, Field CB, Grimm NB, Howarth RW, Marino R, Martinelli L, Rastetter EB, *et al.* 2002. Towards an ecological understanding of biological nitrogen fixation. *Biogeochemistry* **57–58**: 1–45.

Vogel C, Bodenhausen N, Gruissem W, Vorholt JA. **2016**. The Arabidopsis leaf transcriptome reveals distinct but also overlapping responses to colonization by phyllosphere commensals and pathogen infection with impact on plant health. *New phytologist* **212**: 192–207.

Vorholt JA. **2012**. Microbial life in the phyllosphere. *Nature Reviews Microbiology* **10**: 828–840.

Voriskova J, Baldrian P. **2013**. Fungal community on decomposing leaf litter undergoes rapid successional changes. *ISME Journal* **7**: 477–486.

Waight K, Pinyakong O, Luepromchai E. **2007**. Degradation of phenanthrene on plant leaves by phyllosphere bacteria. *Journal of General and Applied Microbiology* **53**: 265–272.

Van Der Wal A, Leveau JHJ. **2011**. Modelling sugar diffusion across plant leaf cuticles: The effect of free water on substrate availability to phyllosphere bacteria. *Environmental Microbiology* **13**: 792–797.

Wellner S, Lodders N, Kämpfer P. **2011**. Diversity and biogeography of selected phyllosphere bacteria with special emphasis on Methylobacterium spp. *Systematic and Applied Microbiology* **34**: 621–630.

Whipps JM, Hand P, Pink D, Bending GD. **2008**. Phyllosphere microbiology with special reference to diversity and plant genotype. *Journal of Applied Microbiology* **105**: 1744–1755.

Whitham TG, Bailey JK, Schweitzer| JA, Shuster SM, Bangert RK, LeRoy CJ, Lonsdorf E V., Allan GJ, DiFazio SP, Potts BM, *et al.* 2006. A framework for community and ecosystem genetics : from genes to ecosystems. *Nature Reviews Genetics* **7**: 510–523.

Wilson M, Savka MA, Hwang I, Farrand SK, Lindow SE. **1995**. Altered epiphytic colonization of mannityl opine-producing transgenic tobacco plants by a mannityl opine-catabolizing strain of Pseudomonas syringae. *Applied and Environmental Microbiology* **61**: 2151–2158.

Witzell J, Martín JA. **2018**. Endophytes of Forest Health. In: Endophytes of Forest Trees. 261–282.

Womack AM, Bohannan BJM, Green JL. **2010**. Biodiversity and biogeography of the atmosphere. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**: 3645–3653.

Wong SCY, Poon RWS, Chen JHK, Tse H, Lo JYC, Ng T, Au JCK, Tse CWS. Corynebacterium kroppenstedtii Is an Emerging Cause of Mastitis Especially in Patients With Psychiatric Illness on Antipsychotic Medication. : 1–4.

Yang B, Wang Y, Qian PY. **2016**. Sensitivity and correlation of hypervariable regions in 16S rRNA genes in phylogenetic analysis. *BMC Bioinformatics* **17**: 1–8.

Yao H, Sun X, He C, Maitra P, Li XC, Guo LD. **2019**. Phyllosphere epiphytic and endophytic fungal community and network structures differ in a tropical mangrove ecosystem. *Microbiome* **7**: 1–15.

Yarza P, Yilmaz P, Pruesse E, Glöckner FO, Ludwig W, Schleifer KH, Whitman WB, Euzéby J, Amann R, Rosselló-Móra R. **2014**. Uniting the classification of cultured and uncultured bacteria and archaea using 16S rRNA gene sequences. *Nature Reviews Microbiology* **12**: 635–645.

Yasumura Y, Hikosaka K, Matsui K, Hirose T. **2002**. Leaf-level nitrogen-use efficiency of canopy and understorey species in a beech forest. *Functional Ecology* **16**: 826–834.

Zeng Q, Liu Y, Zhang H, An S. **2019**. Fast bacterial succession associated with the decomposition of Quercus wutaishanica litter on the Loess Plateau. *Biogeochemistry* **144**: 119–131.

Zhang J, Kobert K, Flouri T, Stamatakis A. **2014**. PEAR: A fast and accurate Illumina Paired-End reAd mergeR. *Bioinformatics* **30**: 614–620.

Zilber-Rosenberg I, Rosenberg E. **2008**. Role of microorganisms in the evolution of animals and plants: The hologenome theory of evolution. *FEMS Microbiology Reviews* **32**: 723–735.

Zipfel C. **2009**. Early molecular events in PAMP-triggered immunity. *Current Opinion in Plant Biology* **12**: 414–420.

VI Annexes

Annexe 1: Microbial association networks give relevant insights into plant pathobiomes

Pauvert Charlie[1], Fort Tania[1], Calonnec Agnès[2], Faivre d'Arcier Julie[2], Chancerel Emilie[1], Massot Marie[1], Bohan David A.[3], Chiquet Julien[4], Robin Stéphane[4], Vallance Jessica[2], Vacher Corinne[1]

1. UMR BIOGECO, INRA, Bordeaux
2. UMR SAVE, INRA, Bordeaux
3. UMR Agroécologie, INRA, Dijon
4. UMR MIA, INRA, Paris

Abstract

Plant growth and health depend on their associations with a large number of microorganisms that interact with each other. The networks of interactions between plant pathogens and other microorganisms, termed pathobiomes, regulate disease development. Our aim was to investigate whether microbial association networks inferred from metabarcoding data after correcting for environmental effects contain signals of ecological interactions, despite methodological biases associated to sampling and molecular biology. Grapevine (*Vitis vinifera*) and the causal agent of powdery mildew (*Erysiphe necator*) were used as a model system. We used Poisson Lognormal Models to highlight direct associations among fungal species on grapevine leaves, and tested some of these associations using co-cultures and text-mining. Our results suggested that the abundance of *E. necator* covaried with that of 23 other fungal species, forming its putative pathobiome. None of these associations were known as interactions in the literature, but one of them was confirmed by our experiments. The yeast *Buckleyzyma aurantiaca* altered pathogen growth rate and morphology, in line with the inferred negative association. Another association, involving two yeast species, was also supported by co-cultures. Moreover, our results showed that the foliar fungal community was robust to invasion by *E. necator* but significantly influenced by the cropping system. This latter could hence be used to promote beneficial microbial interactions highlighted by microbial networks. Together, these findings indicate that microbial networks, when properly inferred, provide plausible hypotheses of ecological interactions that can be useful to develop microbiome-based strategies for crop protection.

Keywords: Anna Karenina principle, Biocontrol, Community ecology, Microbial networks, Network inference, Pathobiome.

Introduction

Plant growth and health depend on their associations with a large number of microorganisms that interact with each other (Hassani et al. 2018; Vannier et al. 2019). Among all these microorganisms, those that interact with pathogens and regulate diseases are particularly important and have recently been defined as the plant's pathobiome (Vayssier-Taussat et al. 2014; Brader et al. 2017; Bass et al. 2019). Some pathobiome members form a barrier that limits pathogen development through direct antagonistic interactions (Arnold et al. 2003; Kemen 2014; Durán et al. 2018; Li et al. 2019), while others can prime the plant immune system (Vogel et al. 2016; Lee et al. 2017; Hacquard et al. 2017). In turn, a successful pathogen invasion can disrupt the plant microbiota, triggering, in some cases, more heterogeneity (Zaneveld et al. 2017). This increase in heterogeneity among infected microbiotas is often termed the Anna Karenina principle, based on the first sentence of Leo Tolstoy's book: "Happy families are all alike; every unhappy family is unhappy in its own way". In microbiology, the Anna Karenina principle means: "all healthy microbiomes are similar; each dysbiotic microbiome is dysbiotic in its own way" (Zaneveld et al. 2017).

Deciphering microbial interactions within pathobiomes, and determining what environmental factors shape those interactions, is a step towards plant health protection. A better understanding of plant pathobiomes will allow us to reduce agriculture reliance on chemical pesticides, through the discovery of novel biocontrol agents (Poudel et al. 2016) and cultural practices fostering the protective microbiota (Hartman et al. 2018). To reach this aim, research at the interface between plant pathology, microbial community ecology, culturomics, metagenomics and big data analytics is needed. Metabarcoding approaches can for instance be used to describe culturable and unculturable microorganisms associated with plants (Nilsson et al. 2018; Abdelfattah et al. 2018), provided that rigorous methods are used from sampling to bioinformatics (Pauvert et al. 2019a, Zinger et al. 2019). Network inference methods can then be applied to reconstruct microbial association networks from microbial community data (Faust and Raes 2012, Vacher et al. 2016; Layeghifard et al. 2017). These networks, in which nodes correspond to microbial taxa and links to direct statistical associations between their sequence counts, can be interpreted as hypotheses of microbial interactions (Jakuschkin et al. 2016; Poudel et al. 2016; Carr et al. 2019). Microbiology experiments can then be performed to validate interaction hypotheses (*e.g.* Lima-Mendez et al. 2015; Biswas et al. 2016; Wang et al. 2017; Das et al. 2018; Durán et al. 2018; Tipton et al. 2018).

However, the application of network inference methods to pathobiome research is still in its infancy. Several methodological issues must be overcome to generate robust hypotheses of microbial interactions from metabarcoding data (Pauvert et al. 2019b), because statistical

associations between sequence counts do not directly reflect ecological interactions (*e.g.* competition, parasitism) between microorganisms (Weiss et al. 2016; Derocles et al. 2018; Röttgers and Faust 2018). For instance, the compositional nature of metabarcoding data induces statistical associations between sequence counts that are not related to any ecological process (Friedman and Alm 2012; Gloor et al. 2017). Moreover, environmental filtering can generate statistical associations between microbial taxa abundances, that are not triggered by ecological interactions but environmental variations (Berry and Widder 2014; Vacher et al. 2016; Derocles et al. 2018; Röttgers and Faust 2018). Several recent methods of network inference deal with these two issues: HSMC (Ovaskainen et al. 2017), PLN (Chiquet et al. 2017; 2018), FlashWeave (Tackmann et al. 2018) and MAGMA (Cougoul et al. 2019b). Their relevance to pathobiome research remains to be demonstrated.

The aim of this study was to deepen our knowledge of plant pathobiomes by testing the following hypotheses: (H1) successful infection events destabilize plant-associated microbial communities and increase their heterogeneity (Anna Karenina principle), (H2) interactions among microorganisms within pathobiomes can be detected by inferring microbial networks from metabarcoding data and environmental covariates, and (H3) cropping systems influence the abundance of microorganisms forming pathobiomes. These hypotheses were tested using Grapevine (*Vitis vinifera*) and the causal agent of powdery mildew (*Erysiphe necator*; Armijo et al. 2016, Gadoury et al. 2012), as a model system. To maximize the diversity of grapevine microbial communities and thus the possibility of discovering microbial antagonists of this fungal pathogen, the experiment was conducted in an untreated vineyard. Powdery mildew was inoculated on several vines at the beginning of the growing season, to ensure the production of abundant and evenly distributed inoculum. Under these conditions, spatial variations in the level of secondary infections were likely to be caused by variations in the barrier effect of the pathobiome, rather than variations in the abundance of the inoculum. The fungal component of the pathobiome of *Erysiphe necator* was then analyzed by combining metabarcoding approaches, network inference methods and co-culture experiments. The habitat of the fungal species belonging to the pathobiome was characterized by analyzing fungal communities of bark, ground cover and upper soil, in addition to that of vine leaves. Their response to inter-row cover cropping was also assessed.

Materials and methods

Study site and sampling design

The study was conducted in an experimental vineyard located near Bordeaux (INRA, Villenave d'Ornon, France; 44°47'24.0"N 0°34'33.6"W; Figure 1A). This experimental vineyard was planted in 1991 with *Vitis vinifera* L. cv. Merlot grafted onto 101-14 rootstock, at a density of 5350 vines ha^{-1} (1.7m x 1.1m). The study was performed on a sub-plot of 5 almost untreated rows (Figure 1B) where, since 2006, few chemical treatments had been applied in case of strong epidemics (Table S1). In this sub-plot, two cropping systems (Figure 1C) were compared: (i) perennial cover crop in the inter-rows (i.e. cover crop: CC) and (ii) chemical weed control with glyphosate (i.e. no cover crop: NCC). Eight experimental units were considered across the sub-plot: four units in NCC areas and four units in CC areas, an experimental unit being defined as a group of 5 adjacent vines in the same row (Figure 1D).

In 2016, each experimental unit was artificially inoculated with the powdery mildew agent *Erysiphe necator* to ensure a uniform intensity of primary infection. Inoculation was performed on April 22, on the central vine of each experimental unit (Figure 1E). The inoculum consisted in a monoconidial isolate collected in a greenhouse in INRA precincts (Villenave d'Ornon, Bordeaux, France) that year (strain S16). Leaves were then regularly checked for powdery mildew colonies. The first symptoms appeared on May 5 for the inoculated leaves, and around May 20 for other leaves. Leaf age was monitored in all experimental units based on biweekly records of newly grown leaves. In addition, a weather station located on the edge of the vineyard allowed us to monitor local variations in air temperature and humidity throughout the experiment.

Grapevine leaves were sampled on three dates: 40 (June 1), 62 (June 23) and 77 (July 5) days post-inoculation (Figure 1F). Four infected (I) and four non-infected (NI) leaves were collected in each experimental unit on each date (Figure 1G). A total of 192 leaves, corresponding to 8 leaves x 3 dates x 8 experimental units, were thus collected. Sampling dates were chosen according to current knowledge on powdery mildew cycle (Calonnec et al. 2006, 2009). All sampled leaves were approximately 20-day old and had received secondary inoculum before the onset of ontogenic resistance (21-28 days see Gadoury et al. 2012). The percentage of lower leaf surface covered with powdery mildew was visually assessed for each infected leaf collected. The position of each sampled leaf within the canopy was recorded as well as its distance to potential environmental sources of microorganisms. Three distances were measured: distance to the ground, distance to the cordons and distance to the artificially inoculated leaf on the central vine of the experimental unit.

Sampled leaves were placed in individual sterile plastic bags (Whirl-Pak®, USA) and taken to the lab in a cooler with ice. Foliar tissues were sampled on the day of collection in the sterile environment of a MICROBIO electric burner (MSEI, France). Tissues were collected from three tissue types of leaf

blade: the healthy zones of non-infected (HNI) and infected (HI) leaves, and the center of disease spots of infected leaves (DI) (Figure 1E). For each leaf, two foliar discs of 6 mm diameter were collected in each zone and placed together in a collection microtube of a 96-well plate (QIAGEN), with two autoclaved glass beads. The processed leaves were kept on ice in individual closed plastic bags to avoid water loss. Leaf fresh weight and surface were then measured using the WinFOLIA® software (Regent Instrument, Canada). Dry weight was measured after drying the leaves in an oven at 65°C for 72h.

On the last date of sampling (July 5), additional environmental samples were collected in each experimental unit. Two old leaves (approximately 70 days old) were collected from the central vine of each experimental unit, close to the place where the inoculation had been performed, and placed in a sterile plastic bag (Whirl-Pak®, USA). The inoculated leaf was collected if it was still attached to the vine. Small pieces of bark (including mosses and lichens) were collected on three vines of each experimental unit and stored in 50mL sterile Falcon tubes. Fragments of ground cover (including upper soil) were also collected beneath three vines of each experimental unit and stored in 50mL sterile Falcon tubes.

Finally, the sub-plot phytosanitary status and canopy vigor were assessed on July 11. The severity (% of lower leaf surface infected) of four diseases, *i.e.* powdery mildew (*E. necator*), downy mildew (*Plasmopara viticola*), black rot (*Guignardia bidwellii*) and grape erineum mite (*Colomerus vitis*), was visually evaluated on 468 leaves randomly chosen within the 8 experimental units (12 leaves per vine). The vigor of the vines was defined as the product of the number of shoots per vine by the number of leaves on the longest shoot.

DNA extraction and Illumina MiSeq sequencing

Leaf discs were cold ground at 1500 rpm with the Geno/Grinder® three times for 1 min and twice for 30 s, with manual shaking between each grinding step. Plates were then centrifuged for 1 min at 6200 rpm. Total DNA was then extracted using the DNeasy Plant Mini Kit (Qiagen, USA) following the manufacturer's instructions, except that DNA extracts were eluted twice with 50 µl of elution buffer (10 mM Tris-Cl, 0.5 mM EDTA; pH 9.0). The ITS1 region of the fungal ITS rDNA gene (Schoch et al. 2012) was amplified using the primers ITS1F-ITS2 (White *et al.*, 1990; Gardes and Bruns, 1993). To avoid a two-stage PCR protocol, each primer contained the Illumina adaptor sequence and a tag (ITS1F: 5'-CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTxxxxxxxxxxxxCTTGGTCATTTAGAGGAAGTAA-3'; ITS2: 5'-

AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTxxxxxxxxxxxxGCTG CGTTCTTCATCGATGC-3', where "x" is the 12 nucleotides tag). The PCR mixture (20 µL of final volume) consisted of 10 µL of 2X QIAGEN Multiplex PCR Master Mix (1X final), 2 µL each of the forward and reverse primers (1 µM), 4 µl of water, 1 µL of 10 $mg.ml^{-1}$ BSA and 1 µL of DNA template. PCR cycling reactions were conducted on a Veriti 96-well Thermal Cycler (Applied Biosystems) using the following conditions: initial denaturation at 95 °C for 15 min followed by 35 cycles at 94°C for 30 s, 57°C for 90 s, 72°C for 90 s with final extension of 72 °C for 10 min. ITS1 amplification was confirmed by electrophoresis on a 2% agarose gel. Each PCR plate contained one negative extraction control, one negative PCR control, and one positive control. The negative extraction control corresponded to a microtube left empty on the collection plate. The negative PCR controls were represented by PCR mix without any DNA template. The positive PCR controls were composed of an equimolar mixture of the DNA of two marine fungal strains --*Candida oceani* and *Yamadazyma barbieri*-- (Burgaud et al. 2011, 2016), kindly provided by Dr. Gaétan Burgaud (LUBEM EA 3882 – ESIAB, Plouzané, France). These strains were chosen as positive controls as they were unlikely to be found in the grapevine samples. PCR products were purified, quantified (Quant-it PicoGreen dsDNA assay kit ; Thermo Fisher Scientific) and equimolarly pooled (Hamilton Microlab STAR robot). Average size fragment was checked using Tapestation instrument (Agilent Technologies). Libraries were sequenced on the Miseq Instrument (Illumina) with the reagent kit v2 (500-cycles). Sequence demultiplexing (with exact index search) was performed at the PGTB sequencing facility (Genome Transcriptome Facility of Bordeaux, Pierroton, France) using DoubleTagDemultiplexer.

Environmental samples collected on the last sampling date (old leaves, bark and ground cover samples) were processed separately following the protocols described in Fort et al. (2019). They were manually ground in liquid nitrogen, amplified using a two-step PCR and then sequenced on a separate MiSeq run at the GetPlaGe sequencing facility (Toulouse, France).

Fungal DNA quantification by ddPCR™

Fungal DNA was quantified with digital droplet PCR assays (ddPCR™; Hindson et al. 2011) using the universal fungal primer pair ITS1F (5'-TCCGTAGGTGAACCTGCGG-3') and 5.8S (5'-CGCTGCGTTCTTCATCG-3'), developed and validated by Fierer et al. (2005). Assays were carried out with the QX200 Droplet Digital PCR (ddPCR™) System from Bio-Rad at the PGTB sequencing facility (Pierroton, France). The PCR reactions were carried out in a final volume of 20 µl using the ddPCR™ EvaGreen Supermix (Bio-Rad, USA). The reaction mix consisted of 10 µl of 2X EvaGreen Supermix, 2.5 µL of each primer at 1.2 µM and 3 µL of DNA template or ultrapure water in the negative control.

The mix containing the sample was partitioned into droplets with the QX200 Droplet Generator and then transferred to 96-well PCR plates. A thermocycling protocol [95 °C × 5 min; 40 cycles of (95 °C × 30 s, 53 °C × 1 min 30 s), 4 °C × 5 min, 90 °C × 5 min] was undertaken in a Bio-Rad C1000 (Bio-Rad, USA). QX200 droplet reader analyzed each droplet individually to detect the fluorescence signal. The number of copies of the target DNA sequence per µl of sample was determined from the number of positive droplets (out of an average of ~20k droplets per sample) estimated from fluorescence signals of both samples and negative controls using the Umbrella procedure implemented in R (Jacobs et al. 2017). Total fungal abundance was then obtained by multiplying the obtained concentration by the mix volume and after adjusting for the 1/100 dilution of the DNA extract.

Bioinformatic analysis

We used DADA2 v1.8 (Callahan et al. 2016) to describe fungal communities in terms of Amplicon Sequence Variants (ASVs, Callahan et al. 2017) because bioinformatic approaches based on DADA2 were shown to recover accurately the composition of an artificial community of 189 fungal strains (Pauvert et al. 2019). We followed the DADA2 ITS workflow (https://benjjneb.github.io/dada2/ITS_workflow.html) except on the read assembly step. Only forward reads in which the primer sequence was found exactly by cutadapt (Martin 2011) were processed with DADA2. Quality filtering retained sequences with less than one expected error and longer than 50 bp. ASVs were subsequently inferred for each sample. Chimeric sequences were then identified using the consensus method of the *removeBimeras* function. ASVs identical in sequence but not in length were finally combined with the *collapseNoMismatch* function. Taxonomic assignments were then performed using the RDP classifier (Wang et al. 2007) implemented in DADA2 and trained with the latest UNITE database (UNITE Community 2019). ASV table and taxonomic assignments were imported in R through the phyloseq package (McMurdie et al. 2013). Only ASVs assigned to a fungal phylum were kept. Positive and negative controls were then used to remove contaminants (Galan et al. 2016). The two ASVs corresponding to the marine strains contained in the positive controls (*Candida oceani* and *Yamadazyma barbieri*) were identified after aligning the ASV sequences with the corresponding Genbank reference sequences (*C. oceani* KY102240 and *Y. barbieri* LT547714) using a similarity threshold of 100% with *usearch_global* function of VSEARCH (Rognes et al. 2016). The cross-contamination threshold (T_{CC}) was defined as the maximal number of sequences of each ASV found in negative or positive control samples. The false-assignment threshold (T_{FA}) was defined as $T_{FA} = N \times R_{fa}$ where R_{fa} is the highest sequence count of a positive control strain in a non-control sample, divided by the total number of sequences of the

strain in the whole run and N is the total number of sequences of each ASV. ASVs were removed from all samples where they harbored fewer sequences than either threshold (T_{FA} or T_{CC}) using a custom script (https://gist.github.com/cpauvert/1ba6a97b01ea6cde4398a8d531fa62f9). The ASV table was finally aggregated at the species level and ASVs that could not be assigned at the species level were removed.

Hypothesis testing

Before testing the three hypotheses, we checked that the foliar tissue samples considered as infected based on visual symptom assessment, had a higher fungal load and a higher relative abundance of *Erysiphe necator* than samples considered as healthy. Variations in fungal total abundance (log-transformed) and in *Erysiphe necator* sequence counts (CLR-transformed) were analyzed using linear regressions performed with the *lm* function (R core Team 2018). The models had cropping system (CC or NCC), sampling date (40, 62 or 77 dpi), tissue type (HI, HNI or DI) and their interactions as fixed effects. ANOVA *F*-tests were used to assess the significance of the fixed effects and post-hoc pairwise comparisons were performed for the significant interactions using Tukey's adjustment method with the emmeans R package (Lenth 2018).

- *Test of H1: Successful infection events destabilize plant-associated microbial communities and increase their heterogeneity (Anna Karenina principle)*

To test H1, we first investigated whether infection altered the composition of foliar fungal communities, using a permutational analysis of variance (PERMANOVA; Anderson et al. 2001) performed with the *adonis* function of the vegan R package (Oksanen et al. 2018). The model had tissue type, cropping system, sampling date and their interactions as fixed effects. The analysis was performed using CLR-transformed data, as advised by Gloor et al. (2017). A total of 128 CLR-transformed species × samples matrices were generated using the aldex.clr function of ALDEx2 (Fernandes et al. 2014) and averaged. Euclidean distances among samples of the average matrix were then used to test differences in community composition among conditions. These analyses were performed twice, with and without removing the ASV corresponding to the pathogen *Erysiphe necator*.

Then, we investigated whether compositional heterogeneity was larger among infected samples than among healthy ones, using tests of homogeneity of multivariate dispersions (Anderson 2006) performed with the *betadisper* function of the R vegan package (Oksanen et al. 2018). Compositional heterogeneity within a group of samples (*i.e.* dispersion) was measured using the distances between each sample and the centroid of its group. Dispersion was calculated for every combination of

sampling date and tissue type. Differences in dispersion among groups were tested using ANOVA *F*-tests. Post-hoc pairwise comparisons were performed with Tukey's adjustment method with the emmeans R package (Lenth 2018).

- *Test of H2: Interactions among microorganisms within pathobiomes can be detected by inferring microbial networks from metabarcoding data and environmental covariates*

Species included in the network reconstruction were selected based on their prevalence rather than their relative abundance (Röttjers & Faust, 2018). Based on the theoretical work of Cougoul et al. (2019a), we chose to include only species present in more than 20% of the samples of any experimental unit (Methods S1). To test H2, we first inferred the foliar microbial network from metabarcoding data after correcting for environmental effects, and then tested some of the associations using co-culture experiments and text-mining. Joint variations in sequence counts between fungal species were modelled with the Poisson Lognormal Model (PLN, Aitchison and Ho 1989) to account for uneven sequencing depth among samples and for environmental covariates potentially explaining variations in the sequence counts per fungal species across the dataset. Associations between species were then inferred with the network variant of the PLN model implemented in the PLNmodels R package (Chiquet et al, 2018). This PLNnetwork variant applies a sparsity-inducing penalty on the inverse covariance matrix in the latent Gaussian layer of the PLN model to select direct statistical associations between species, in the same spirit as in the graphical-Lasso. The extended BIC criteria (Chen and Chen 2008) was used to perform model selection among a 40-size grid of penalties (Chiquet et al. 2017; 2018). The stability of each association of the BIC network was then defined as its selection frequency in the bootstrap subsamples of the StARS procedure (Liu et al. 2010). Covariates included several foliar traits and climate variables known to influence fungal development: leaf age, leaf water content, specific leaf area and average vapor pressure deficit experienced by the leaf since unfolding (Table S2). These covariates were introduced in the model to get rid of fungal species associations triggered by similar habitat requirements. The distance between the sampled leaf and environmental sources of microorganisms (old leaves, bark, ground cover) was also included as a covariate to get rid of fungal associations triggered by joint colonization events. Only the distance between the sampled leaf and old leaves was introduced in the model as all three distances were significantly correlated (Fig. S1). To account for additional, non-measured environmental variations associated with the experimental design, we also included the three main factors of the experiment (sampling date, experimental unit and tissue type) as covariates. Finally, sequencing depth (log-transformed) was included as an offset in the model to get rid of spurious associations triggered by compositionality.

A subset of the fungal network was then tested using co-cultures, to determine whether the statistical associations among fungal species were triggered by ecological interactions. Co-cultures investigated the interactions among eight yeast strains, and between these eight yeast strains and one strain of the pathogen *Erysiphe necator*. The eight yeast strains were chosen among the most abundant fungal species found on grapevine leaves. They were all present in the inferred fungal network. They were also available in the CBS collection (Westerdjik Fungal Biodiversity Institute, Utrecht, The Netherlands) and could grow on PDA (Potato Dextrose Agar) at 25°C: *Buckleyzyma aurantiaca* CBS 8074, *Cystofilobasidium macerans* CBS 9032, *Dioszegia hungarica* CBS 7091, *Filobasidium oeirense* CBS 8681, *Filobasidium wieringae* CBS 1937, *Udeniomyces pyricola* CBS 6754, *Sporobolomyces roseus* CBS 486 and *Vishniacozyma victoriae* CBS 9000. Strains were revived following the CBS instructions and further maintained in collection on MEA and PDA media [Malt Extract Agar (Malt 15 $g.l^{-1}$, Agar 20 $g.l^{-1}$); Potato Dextrose Agar, Biokar diagnostics, France (Potato extract 4 $g.l^{-1}$, Glucose 20 $g.l^{-1}$, Agar 15 $g.l^{-1}$)]. To produce inoculum for the co-culture experiments, yeast strains were grown in liquid ME medium (Malt Extract, 15 $g.l^{-1}$) at 22°C for 48h. The number of yeast cells was then count using a hemacytometer before being homogenized at the same concentration. *E. necator* strain S19 was isolated in a greenhouse in INRA precincts (Villenave d'Ornon, Bordeaux, France) in 2019. As it is an obligate biotroph parasite of grapevine, its preservation was carried out every 2 weeks on sterilized grapevine leaves by blowing spores from sporulating leaves (approx. 1000 conidia per cm^2 of leaf). Inoculated leaves were then incubated for 10–14 days at 22 °C with a 16 h day / 8 h night photoperiod following the procedure of Dufour et al. (2013).

To investigate whether the statistical associations among the yeast species were triggered by ecological interactions, the eight yeast strains were confronted with each other using spot-on-lawn assay (Polonelli et Morace, 1986; Addis et al., 2001). Inoculations were performed on MEA medium buffered at pH 4.5 with 0.5 M phosphate-citrate buffer (Heard and Fleet, 1987). Approximately 10^5 $cfu.ml^{-1}$ of each strain was suspended in 15 ml of sterile MEA medium (pH 4.5) maintained at 45°C and then poured into sterile Petri dishes. For each strain, five replicates were made, totalizing 40 plates. Each of the eight strains was then drop-inoculated (50 µl at 10^5 $cfu.ml^{-1}$) (adapted from Polonelli and Morace, 1986) onto the surface of each of the Petri dishes prepared as described above. Once the drops dried, the plates were sealed with Parafilm® and incubated at 22°C. Yeast growth was visually assessed 4 and 7 days after inoculation. We considered that the MEA-included strain inhibited (or promoted) the growth of a drop-inoculated strain, if the latter had a reduced (or increased) growth relative to its own confrontation.

To investigate whether the statistical associations between *E. necator* and the yeast species were triggered by ecological interactions, liquid yeast cultures incubated in ME for 48h were centrifuged at 5000 rpm and 4°C for 15 min. Supernatant (liquid medium) and pellet (yeast cells) were then separated. Pellets were resuspended in sterile distilled water at a concentration of 10^6 cfu.ml^{-1}. Powdery mildew strain S19 was inoculated under sterile conditions on decontaminated grape leaf discs (eight 2-cm discs per plate, each coming from a different leaf). Both components, *i.e.* supernatants and resuspended pellets, were tested against *E. necator* in a preventive assay (applied 24h before *E. necator*) and a curative one (applied 6h after *E. necator*). Negative controls were treated either with sterile distilled water or sterile ME respectively in the pellet and supernatant conditions. BTH (S-methyl benzo [1,2,3]thiadiazole-7-carbothioate, Bion®, 50WG, Syngenta) at a concentration of 0.1% active ingredient was used as a positive control (Dufour et al. 2013). A total of 320 foliar discs were inoculated corresponding to 8 foliar discs per plate x 10 plates (8 plates, one for each yeast strain + 1 negative control + 1 positive control) x 2 assays (preventive and curative) x 2 components tested (medium supernatant and yeast cells). After incubation for 12 days at 22°C with a 16 h day/8 h night photoperiod, powdery mildew development was evaluated on each foliar disc by assessing visually the infected leaf area (ILA, in%). The presence of altered conidial chains was also monitored. Infected leaf areas were modelled for each strain using linear regressions. Differences in pathogen growth among strains were estimated relative to the negative control using *trt.vs.ctrl* contrasts in emmeans (Lenth 2018). The number of discs with altered conidia in the presence of a yeast strain or BTH was compared to that of the negative control using a Fisher exact test with the alternative = “greater” option.

In addition, the subset of the fungal network involving *E. necator* (i.e. its putative pathobiome) was checked for in the literature using a custom R script (Methods S2). First, every pair of fungal species belonging to the pathobiome was replicated using obligate and anamorph synonyms of species names, queried from the MycoBank webservice (Robert et al. 2013). For each pair, co-occurrences of species names were then searched for in the Scopus database (that includes article titles, abstracts, keywords and references) using its API through the rscopus R package (Muschelli 2019). The corresponding articles were read carefully to search for experimental evidence of ecological interaction between the selected species.

- *Test of H3: Cropping systems influence the abundance of microorganisms forming pathobiomes.*

To test H3, we investigated whether the fungal species belonging to the putative pathobiome of *E. necator* differed in abundance between CC and NCC cropping systems. ALDEx2 (Fernandes et al. 2014) was used to detect species differentially abundant between systems as this method is suitable for compositional data (Gloor et al. 2017) and outputs very few false-positives with default values (Thorsen et al. 2016). Species with a false discovery rate below 0.1 after Benjamini-Hochberg adjustment were considered as differentially abundant. To better understand the ecology of the fungal species belonging to the pathobiome of *E. necator*, we also searched for their presence in the environmental samples. Finally, to assess more broadly the effects of cropping systems, we compared grapevine vigor and disease severity between CC and NCC experimental units using t-tests.

Results

Grapevine foliar fungal communities were composed of 4148 fungal ASVs (totalizing 10 195 266 sequences), among which 1454 could be assigned at the species level (totalizing 7 276 628 sequences). These ASVs corresponded to 547 fungal species (306 Ascomycetes and 241 Basidiomycetes). The pathogen *Erysiphe necator* was among the ten most abundant species (Table S3). The relative abundance of *E. necator* was significantly higher in tissues considered as infected based on visual assessments (DI), than in tissues considered as healthy (HI and HNI), except on the last sampling date (Table 1A and Figure 2A). On the last sampling date, the pathogen was highly abundant in all tissue types (Figure 2A). The sequences assigned to *E. necator* represented 39%, 39.6% and 65.5% of all the fungal sequences on the three sampling dates, respectively. The total fungal abundance also varied significantly with tissue type and sampling date (Table 1B). As expected, the total fungal abundance was higher in infected tissues, except on the last sampling date (Figure S2).

Infection does not destabilize the microbial community (no Anna Karenina effect)

In contrast to H1, infection did not trigger any significant change in fungal community composition (Table 1C). Fungal community composition did not differ among healthy and infected tissues, but it differed between cropping systems and, to a larger extend, between sampling dates (Table 1C and Figure S3). While there was a significant difference in community heterogeneity among healthy and infected tissues (ANOVA: $df = 8$; $F = 37.7$; $p < 0.01$), the heterogeneity was not higher among infected samples than among healthy ones (Figure 2B). It was even lower in infected samples on the

first sampling date (Figure 2B). The same results were obtained when *E. necator* was removed from the fungal community data (not shown), confirming the absence of Anna Karenina effect.

Inferred microbial networks give relevant insights into the pathobiome

The fungal association network inferred from metabarcoding data and environmental covariates using the sparse PLN model was composed of 61 fungal species and 702 statistical associations (Figure 3A). Within this network, *E. necator* was negatively associated with 15 species and positively associated with 8 species (Figure 4). All associations, except two, were robust to subsampling (stability over 0.5; Table S4). These 23 species are hereafter considered as the putative pathobiome of *E. necator*.

The 8 yeast species selected for microbiology experiments were linked by 7 positive associations in the PLN network (Figure 3B). In contrast, spot-on-lawn experiments (Figure 3C) revealed 3 growth-promoting interactions and 12 growth-inhibiting interactions (Figure 3D) among the 7 yeast strains that grew in the experimental conditions (all strains but *D. hungarica*). The statistical association network (Figure 3B) and the *in vitro* interaction network (Figure 3D) shared two links, one between *C. macerans* and *U. pyricola* and another between *F. oeirense* and *C. macerans*. The positive association between *C. macerans* and *U. pyricola* was upheld by the spot-on-lawn assay as *U. pyricola* enhanced the growth of *C. macerans*. In contrast, the link between *F. oeirense* and *C. macerans* was found as a positive association with PLNnetwork, while the growth of *C. macerans* was inhibited by *F. oeirense* in the spot-on-lawn assay.

Among the 8 yeast species selected for microbiology experiments, 3 were part of the putative pathobiome of *E. necator*: *B. aurantiaca*, *F. wieringae* and *V. victoriae* (Figure 4). The three species were negatively associated with the pathogen in the PLN network (Figure 4). Microbiology experiments supported the antagonistic effect of *B. aurantiaca* on *E. necator*. The supernatant of *B. aurantiaca* centrifuged liquid culture, when applied as a curative treatment, reduced the growth of *E. necator* by about 30% (Table 2) and significantly increased the number of collapsed conidia (Table 3 and Figure 5). The pellet of *U. pyricola* culture also inhibited *E. necator* growth when applied as a curative treatment (Table 2) but there was no association between the two species in the PLN network.

Finally, literature-mining identified 9 abstracts in which *E. necator* and at least one species of its putative pathobiome were mentioned (Table S5). None of these articles provided experimental evidence of an interaction between *E. necator* and the species found in the abstracts.

Overall, network inference provided a list of 15 fungal species that might be antagonists of *E. necator* (Figure 4). Literature mining showed that these interactions have never been studied experimentally so far. We tested experimentally three of these putative interactions and validated one of them, involving the yeast species *B. aurantiaca* (Tables 2, 3 and Figure 5). Hence, in accordance with H2, interactions among microorganisms within the pathobiome can be detected by inferring microbial networks from metabarcoding data and environmental covariates.

Cropping systems impact the pathobiome

Differential abundance analyses revealed that 25 fungal species were favored by cover cropping (Table S6). Among them, 9 fungal species belonged to the putative pathobiome of *E. necator* and were all putative antagonists (Figure 4). The yeast *B. aurantiaca* was part of the species favored by cover cropping. Six of the putative antagonists favored by cover cropping, including *B. aurantiaca*, were generalist fungal species found in grapevine leaves but also on bark and ground cover (Figure 4). These findings indicate that cropping systems do modulate pathobiomes, in accordance with H3. Interestingly, only the pathogen *E. necator* was favored by the absence of cover crop according to differential abundance analyses (Table S6), a pattern which might be accounted for by the drastic increase in vine vigor in the absence of cover crop (Figure S4A). However, visual disease severity assessment performed mid-July did not confirm the higher abundance of *E. necator* in the absence of cover crop (Figure S4B).

Discussion

Dealing with pathogens is a matter of host survival, and in the case of crops, it also concerns the species that depend on them, including human beings. Among the range of disease control strategies is the exploitation of ecological interactions between pathogenic microorganisms and microorganisms that inhabit the host plant (Brader et al. 2017; Koskella et al. 2017). These interactions, termed the pathobiome (Vayssier-Taussat et al. 2014), were studied here using a metabarcoding approach, with grapevine powdery mildew (*Erysiphe necator*; Gadoury et al. 2012, Armijo et al. 2016) as a model system. Fungal communities sampled from visually healthy and infected foliar zones did not differ in terms of composition, contrasting with the results obtained for oak powdery mildew (*Erysiphe alphitoides*; Jakuschkin et al. 2016). Furthermore, we had no evidence of the presumed Anna Karenina principle, which predicts more heterogeneous microbial

communities following infection (Zaneveld et al. 2017). These findings parallel other studies on plant pathogens (Suda et al. 2009; Agler et al. 2016; Rezki et al. 2016; Koskella et al. 2017; Zhang et al. 2019), suggesting that the Anna Karenina principle is not the rule for plants. To discover fungal species with potential antagonistic activity against the pathogen, we sampled hosts in both healthy and diseased conditions (Poudel et al. 2016; Ellis 2017) and used a new statistical approach, the PLNnetwork variant of the PLN model family (Chiquet et al. 2017; 2018), which allowed us to take into account environmental variability. We were able to generate new hypotheses in the form of a network of associations between microorganisms and some of these hypotheses were tested using co-cultures. Interestingly, the yeast *Buckleyzyma aurantiaca* was found to reduce pathogen colonization and even alter the structure of asexual reproduction organs, the conidia. These findings upheld a negative association between the abundances of *B. aurantiaca* and *E. necator* across foliar samples collected in the field, which makes this yeast a potential biocontrol candidate deserving further investigations. Moreover, our results revealed that *B. aurantiaca* and eight other putative antagonists of *E. necator* were favored by cover cropping, suggesting that cropping systems can be used to favor protective plant microbiota. Finally, another positive association, involving the yeast species *Cystofilobasidium macerans* and *Udeniomyces pyricola*, was also supported by co-cultures. These findings prove that network inference methods can detect relevant signals of interactions between microorganisms and be useful tools for the development of microbiome-based crop protection strategies.

Although informative, all these results are only a glimpse of the complex processes that occur *in planta* and *in natura*. The destructive sampling of foliar tissues prevented us to monitor fungal communities continuously over time and to generate time series that could better inform us on microbial interactions (*e.g.* Carr et al. 2019). We relied on cross-sectional data with a large number of samples instead. In this study, 276 samples were used to reconstruct the fungal network, which is in line with the recent recommendation by Hirano and Takemoto (2019) of at least 200 samples per network, and the inferred associations were robust. To complete this association network, future studies will have to collect data on bacteria, oomycetes, viruses and even insect communities because inter-kingdom interactions influence the structure and dynamics microbial networks (Jakuschkin et al. 2016; Tipton et al. 2017; Durán et al., 2018). This additional information will also improve the inference of fungal associations because missing species can generate indirect associations (*e.g.* Li et al. 2016). Prior information on interactions gathered from literature could also be used to improve network inference (Lo and Marculescu 2017).

In conclusion, microbial networks are no silver bullet but like any other methods provide an insight into the complex processes at play in ecosystems. The field of metabarcoding approaches is now mature enough to be aware of its pitfalls (e.g. Zinger et al. 2019). We suggest that the difficult hurdles to overcome should not prevent microbial network inferences but rather be an incentive for cautious interpretations and additional experiments in line with recent recommendations (Armitage et al. 2019; Carr et al. 2019). Better insights into microbial ecosystems may not necessarily require more metabarcoding samples but more complementary evidence (Widder et al. 2016).

References

Abdelfattah A, Malacrinò A, Wisniewski M, Cacciola SO, Schena L, 2018. Metabarcoding: A powerful tool to investigate microbial communities and shape future plant protection strategies. *Biological Control*, Metagenomics and the Science of Biological Control **120**: 1–10.

Addis E, Fleet GH, Cox JM, Kolak D, Leung T, 2001. The growth, properties and interactions of yeasts and bacteria associated with the maturation of Camembert and blue-veined cheeses. *International Journal of Food Microbiology* **69**: 25–36.

Agler MT, Ruhe J, Kroll S, Morhenn C, Kim S-T, Weigel D, Kemen EM, 2016. Microbial Hub Taxa Link Host and Abiotic Factors to Plant Microbiome Variation. *PLOS Biology* **14**: e1002352.

Aitchison J, Ho CH, 1989. The multivariate Poisson-log normal distribution. *Biometrika* **76**: 643–653.

Anderson MJ, 2006. Distance-Based Tests for Homogeneity of Multivariate Dispersions. *Biometrics* **62**: 245–253.

Anderson MJ, 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* **26**: 32–46.

Andrews JH, Kinkel LL, Berbee FM, Nordheim EV, 1987. Fungi, leaves, and the theory of island biogeography. *Microbial Ecology* **14**: 277–290.

Armijo G, Schlechter R, Agurto M, Muñoz D, Nuñez C, Arce-Johnson P, 2016. Grapevine Pathogenic Microorganisms: Understanding Infection Strategies and Host Response Scenarios. *Frontiers in Plant Science* **7**: 382.

Armitage DW, Jones SE, 2019. How sample heterogeneity can obscure the signal of microbial interactions. *The ISME Journal*. Online ahead of print.

Arnold AE, Mejía LC, Kyllo D, Rojas EI, Maynard Z, Robbins N, Herre EA, 2003. Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences* **100**: 15649–15654.

Bálint M, Bahram M, Eren AM, Faust K, Fuhrman JA, Lindahl B, O'Hara RB, Öpik M, Sogin ML, Unterseher M, Tedersoo L, 2016. Millions of reads, thousands of taxa: microbial community structure and associations analyzed via marker genes. *FEMS Microbiology Reviews* **40**: 686–700.

Bálint M, Bartha L, O'Hara RB, Olson MS, Otte J, Pfenninger M, Robertson AL, Tiffin P, Schmitt I, 2015. Relocation, high-latitude warming and host genetic identity shape the foliar fungal microbiome of poplars. *Molecular Ecology* **24**: 235–248.

Bass D, Stentiford GD, Wang H-C, Koskella B, Tyler CR, 2019. The Pathobiome in Animal and Plant Diseases. *Trends in Ecology & Evolution*. Online ahead of print.

Berry D, Widder S, 2014. Deciphering microbial interactions and detecting keystone species with co-occurrence networks. *Frontiers in Microbiology* **5**: 219.

Biswas S, Mcdonald M, Lundberg DS, Dangl JL, Jojic V, 2016. Learning microbial interaction networks from metagenomic count data. *Journal of Computational Biology* **23**: 526–535.

Brader G, Compant S, Vescio K, Mitter B, Trognitz F, Ma L-J, Sessitsch A, 2017. Ecology and Genomic Insights on Plant-Pathogenic and -Nonpathogenic Endophytes. *Annual Review of Phytopathology* **55**: 61–83.

Bradley DJ, Gilbert GS, Parker IM, 2003. Susceptibility of clover species to fungal infection: the interaction of leaf surface traits and environment. *American Journal of Botany* **90**: 857–864.

Brem S, Rast DM, Ruffner HP, 1986. Partitioning of photosynthate in leaves of Vitis vinifera infected with Uncinula necator or Plasmopora viticola. *Physiological and Molecular Plant Pathology* **29**: 285–291.

Brown JH, Bedrick EJ, Ernest SKM, Cartron J-LE, Kelly JF, 2004. Constraints on Negative Relationships: Mathematical Causes and Ecological Consequences, in: The Nature of Scientific Evidence: Statistical, Philosophical, and Empirical Considerations (Eds Mark L. Taper and Subhash R. Lele). University of Chicago Press, pp. 298–323.

Bruez E, Vallance J, Gerbore J, Lecomte P, Costa J-PD, Guerin-Dubrana L, Rey P, 2014. Analyses of the Temporal Dynamics of Fungal Communities Colonizing the Healthy Wood Tissues of Esca Leaf-Symptomatic and Asymptomatic Vines. *PLOS ONE* **9**: e95928.

Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P, 2013. Structure and Functions of the Bacterial Microbiota of Plants. *Annual Review of Plant Biology* **64**: 807–838.

Burgaud G, Arzur D, Sampaio JP, Barbier G, 2011. Candida oceani sp. nov., a novel yeast isolated from a Mid-Atlantic Ridge hydrothermal vent (−2300 meters). *Antonie van Leeuwenhoek* **100**: 75–82.

Burgaud G, Coton M, Jacques N, Debaets S, Maciel NOP, Rosa CA, Gadanho M, Sampaio JP, Casaregola S, 2016. Yamadazyma barbieri f.a. sp. nov., an ascomycetous anamorphic yeast isolated from a Mid-Atlantic Ridge hydrothermal site (−2300 m) and marine coastal waters. *International Journal of Systematic and Evolutionary Microbiology* **66**: 3600–3606.

Callahan BJ, McMurdie PJ, Holmes SP, 2017. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *The ISME Journal* **11**: 2639–2643.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP, 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**: 581–583.

Calonnec A, Cartolaro P, Chadœuf J, 2009. Highlighting Features of Spatiotemporal Spread of Powdery Mildew Epidemics in the Vineyard Using Statistical Modeling on Field Experimental Data. *Phytopathology* **99**: 411–422.

Calonnec A, Cartolaro P, Delière L, Chadœuf J, 2006. Powdery mildew on grapevine: the date of primary contamination affects disease development on leaves and damage on grape. *Bulletin OILB/SROP* **29**: 67–73.

Calonnec A, Jolivet J, Vivin P, Schnee S, 2018. Pathogenicity Traits Correlate With the Susceptible Vitis vinifera Leaf Physiology Transition in the Biotroph Fungus Erysiphe necator: An Adaptation to Plant Ontogenic Resistance. *Frontiers in Plant Science* **9**: 1808.

Carr A, Diener C, Baliga NS, Gibbons SM, 2019. Use and abuse of correlation analyses in microbial ecology. *The ISME Journal*. Online ahead of print.

Carroll JE, Wilcox WF, 2003. Effects of Humidity on the Development of Grapevine Powdery Mildew. *Phytopathology* **93**: 1137–1144.

Caubel J, Launay M, Lannou C, Brisson N, 2012. Generic response functions to simulate climate-based processes in models for the development of airborne fungal crop pathogens. *Ecological Modelling* **242**: 92–104.

Chang H-X, Noel ZA, Sang H, Chilvers MI, 2018. Annotation resource of tandem repeat-containing secretory proteins in sixty fungi. *Fungal Genetics and Biology* **119**: 7–19.

Chen J, Chen Z, 2008. Extended Bayesian information criteria for model selection with large model spaces. *Biometrika* **95**: 759–771.

Chiquet J, Mariadassou M, Robin S, 2019. Variational inference for sparse network reconstruction from count data. *Proceedings of the 36th International Conference on Machine Learning (ICLM'19)*.

Chiquet J, Mariadassou M, Robin S, 2018. Variational inference for probabilistic Poisson PCA. *Annals of Applied Statististics* 12: 2674–2698.

Copeland JK, Yuan L, Layeghifard M, Wang PW, Guttman DS, 2015. Seasonal Community Succession of the Phyllosphere Microbiome. *Molecular Plant-Microbe Interactions* **28**: 274–285.

Cordier T, Robin C, Capdevielle X, Fabreguettes O, Desprez-Loustau M-L, Vacher C, 2012. The composition of phyllosphere fungal assemblages of European beech (Fagus *sylvatica*) varies significantly along an elevation gradient. *New Phytologist* **196**: 510–519.

Cougoul A, Bailly X, Vourc'h G, Gasqui P, 2019a. Rarity of microbial species: In search of reliable associations. *PLOS ONE* **14**: e0200458.

Cougoul A, Bailly X, Wit EC, 2019b. MAGMA: inference of sparse microbial association networks. *bioRxiv* 538579.

Crandall SG, Gilbert GS, 2017. Meteorological factors associated with abundance of airborne fungal spores over natural vegetation. *Atmospheric Environment* **162**: 87–99.

Das P, Ji B, Kovatcheva-Datchary P, Bäckhed F, Nielsen J, 2018. In vitro co-cultures of human gut bacterial species as predicted from co-occurrence network analysis. *PLOS ONE* **13**: e0195161.

Derocles SAP, Bohan DA, Dumbrell AJ, Kitson JJN, Massol F, Pauvert C, Plantegenest M, Vacher C, Evans DM, 2018. Chapter One - Biomonitoring for the 21st Century: Integrating Next-Generation Sequencing Into Ecological Network Analysis, in: Bohan, D.A., Dumbrell, A.J., Woodward, G., Jackson, M. (Eds.), Advances in Ecological Research, Next Generation Biomonitoring: Part 1. Academic Press, pp. 1–62.

Dufour MC, Lambert C, Bouscaut J, Mérillon JM, Corio-Costet MF, 2013. Benzothiadiazole-primed defence responses and enhanced differential expression of defence genes in *Vitis vinifera* infected with biotrophic pathogens *Erysiphe necator* and *Plasmopara viticola:* Elicitation and grapevine responses to mildews. *Plant Pathology* **62**: 370–382.

Durán P, Thiergart T, Garrido-Oter R, Agler M, Kemen E, Schulze-Lefert P, Hacquard S, 2018. Microbial Interkingdom Interactions in Roots Promote Arabidopsis Survival. *Cell* **175**: 973-983.e14.

Ellis JG, 2017. Can Plant Microbiome Studies Lead to Effective Biocontrol of Plant Diseases? *Molecular Plant-Microbe Interactions* **30**: 190–193.

Emerson JB, Adams RI, Román CMB, Brooks B, Coil DA, Dahlhausen K, Ganz HH, Hartmann EM, Hsu T, Justice NB, Paulino-Lima IG, Luongo JC, Lymperopoulou DS, Gomez-Silvan C, Rothschild-Mancinelli B, Balk M, Huttenhower C, Nocker A, Vaishampayan P, Rothschild LJ, 2017. Schrödinger's microbes: Tools for distinguishing the living from the dead in microbial ecosystems. *Microbiome* **5**:86.

Eschen R, Hunt S, Mykura C, Gange AC, Sutton BC, 2010. The foliar endophytic fungal community composition in Cirsium arvense is affected by mycorrhizal colonization and soil nutrient content. *Fungal Biology* **114**: 991–998.

Faust K, Raes J, 2012. Microbial interactions: from networks to models. *Nature Reviews Microbiology* **10**: 538–550.

Fernandes AD, Reid JN, Macklaim JM, McMurrough TA, Edgell DR, Gloor GB, 2014. Unifying the analysis of high-throughput sequencing datasets: characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. *Microbiome* **2**: 15.

Fierer N, Jackson JA, Vilgalys R, Jackson RB, 2005. Assessment of Soil Microbial Community Structure by Use of Taxon-Specific Quantitative PCR Assays. *Applied and Environmental Microbiology* **71**: 4117–4120.

Fort T, Pauvert C, Zanne AE, Ovaskainen O, Caignard T, Barret M, Compant S, Hampe A, Delzon S, Vacher C, 2019. Maternal effects and environmental filtering shape seed fungal communities in oak trees. *bioRxiv* 691121.

Friedman J, Alm EJ, 2012. Inferring correlation networks from genomic survey data. *PLoS Computational Biology* **8**: e1002687.

Gadoury DM, Cadle-Davidson L, Wilcox WF, Dry IB, Seem RC, Milgroom MG, 2012. Grapevine powdery mildew (Erysiphe necator): a fascinating system for the study of the biology, ecology and epidemiology of an obligate biotroph. *Molecular Plant Pathology* **13**: 1–16.

Galan M, Razzauti M, Bard E, Bernard M, Brouat C, Charbonnel N, Dehne-Garcia A, Loiseau A, Tatard C, Tamisier L, Vayssier-Taussat M, Vignes H, Cosson J-F, 2016. 16S rRNA Amplicon Sequencing for Epidemiological Surveys of Bacteria in Wildlife. *mSystems* **1**: e00032-16.

Gardes M, Bruns TD, 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. *Molecular Ecology* **2**: 113–118.

Gloor GB, Macklaim JM, Pawlowsky-Glahn V, Egozcue JJ, 2017. Microbiome Datasets Are Compositional: And This Is Not Optional. *Frontiers in Microbiology* **8**: 2224.

Hacquard S, Spaepen S, Garrido-Oter R, Schulze-Lefert P, 2017. Interplay Between Innate Immunity and the Plant Microbiota. *Annual Review of Phytopathology* **55**: 565–589.

Harrison JG, Beltran LP, Buerkle CA, Cook D, Gardner DR, Parchman TL, Forister ML, 2019. A suite of rare microbes interacts with a dominant, heritable, fungal endophyte to influence plant trait expression. *bioRxiv* 608729.

Hartman K, van der Heijden MGA, Wittwer RA, Banerjee S, Walser J-C, Schlaeppi K, 2018. Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. *Microbiome* **6**: 14.

Hassani MA, Durán P, Hacquard S, 2018. Microbial interactions within the plant holobiont. *Microbiome* **6**: 58.

Heard GM, Fleet GH, 1987. Occurrence and Growth of Killer Yeasts during Wine Fermentation. *Applied and Environmental Microbiology* **53**: 2171–2174.

Hernandez A, Cooke DT, Lewis M, Clarkson DT, 1997. Fungicides and sterol-deficient mutants of Ustilago maydis: plasma membrane physico-chemical characteristics do not explain growth inhibition. *Microbiology* **143**: 3165–3174.

Hindson BJ, Ness KD, Masquelier DA, Belgrader P, Heredia NJ, Makarewicz AJ, Bright IJ, Lucero MY, Hiddessen AL, Legler TC, Kitano TK, Hodel MR, Petersen JF, Wyatt PW, Steenblock ER, Shah PH, Bousse LJ, Troup CB, Mellen JC, Wittmann DK, Erndt NG, Cauley TH, Koehler RT, So AP, Dube S, Rose KA, Montesclaros L, Wang S, Stumbo DP, Hodges SP, Romine S, Milanovich FP, White HE, Regan JF, Karlin-Neumann GA, Hindson CM, Saxonov S, Colston BW, 2011. High-Throughput Droplet Digital PCR System for Absolute Quantitation of DNA Copy Number. *Analytical Chemistry* **83**: 8604–8610.

Hirano H, Takemoto K, 2019. Difficulty in inferring microbial community structure based on co-occurrence network approaches. *BMC Bioinformatics* **20**: 329.

Itagaki K, Shibuya T, 2017. Differences in early hyphal development of Podosphaera xanthii on Cucumis sativus leaves acclimatized to high or low relative humidity. *Botany* **96**: 67–71.

Jacobs BKM, Goetghebeur E, Vandesompele J, De Ganck A, Nijs N, Beckers A, Papazova N, Roosens NH, Clement L, 2017. Model-Based Classification for Digital PCR: Your Umbrella for Rain. *Analytical Chemistry* **89**: 4461–4467.

Jakuschkin B, Fievet V, Schwaller L, Fort T, Robin C, Vacher C, 2016. Deciphering the Pathobiome: Intra- and Interkingdom Interactions Involving the Pathogen *Erysiphe alphitoides*. *Microbial Ecology* **72**: 870–880.

Kemen E, 2014. Microbe–microbe interactions determine oomycete and fungal host colonization. *Current Opinion in Plant Biology* **20**: 75–81.

Kiss L, 1998. Natural occurrence of ampelomyces intracellular mycoparasites in mycelia of powdery mildew fungi. *New Phytologist* **140**: 709–714.

Koskella B, Meaden S, Crowther WJ, Leimu R, Metcalf CJE, 2017. A signature of tree health? Shifts in the microbiome and the ecological drivers of horse chestnut bleeding canker disease. *New Phytologist* **215**: 737–746.

Kües U, Khonsuntia W, Subba S, 2018. Complex fungi. *Fungal Biology Reviews* **32**: 205–218.

Kurtz ZD, Müller CL, Miraldi ER, Littman DR, Blaser MJ, Bonneau RA, 2015. Sparse and Compositionally Robust Inference of Microbial Ecological Networks. *PLOS Computational Biology* **11**: e1004226.

Layeghifard M, Hwang DM, Guttman DS, 2017. Disentangling Interactions in the Microbiome: A Network Perspective. *Trends in Microbiology* **25**: 217–228.

Lee G, Lee S-H, Kim KM, Ryu C-M, 2017. Foliar application of the leaf-colonizing yeast Pseudozyma churashimaensis elicits systemic defense of pepper against bacterial and viral pathogens. *Scientific Reports* **7**: 39432.

Lenth R, 2018. Emmeans: estimated marginal means, aka least-squares means.

Li C, Lim KMK, Chng KR, Nagarajan N, 2016. Predicting microbial interactions through computational approaches. *Methods* **102**: 12–19.

Li M, Wei Z, Wang J, Jousset A, Friman V, Xu Y, Shen Q, Pommier T, 2019. Facilitation promotes invasions in plant-associated microbial communities. *Ecology Letters* **22**: 149–158.

Lima-Mendez G, Faust K, Henry N, Decelle J, Colin S, Carcillo F, Chaffron S, Ignacio-Espinosa JC, Roux S, Vincent F, Bittner L, Darzi Y, Wang J, Audic S, Berline L, Bontempi G, Cabello AM, Coppola L, Cornejo-Castillo FM, d'Ovidio F, Meester LD, Ferrera I, Garet-Delmas M-J, Guidi L, Lara E, Pesant S, Royo-Llonch M, Salazar G, Sánchez P, Sebastian M, Souffreau C, Dimier C, Picheral M, Searson S, Kandels-Lewis S, Coordinators TO, Gorsky G, Not F, Ogata H, Speich S, Stemmann L, Weissenbach J, Wincker P, Acinas SG, Sunagawa S, Bork P, Sullivan MB, Karsenti E, Bowler C, Vargas C de, Raes J, 2015. Determinants of community structure in the global plankton interactome. *Science* **348**: 1262073.

Liu H, Roeder K, Wasserman L, 2010. Stability Approach to Regularization Selection (StARS) for High Dimensional Graphical Models, in: Proceedings of the 23rd International Conference on Neural Information Processing Systems - Volume 2, NIPS'10. Curran Associates Inc., USA, pp. 1432–1440.

Lo C, Marculescu R, 2017. MPLasso: Inferring microbial association networks using prior microbial knowledge. *PLOS Computational Biology* **13**: e1005915.

López-González RC, Gómez-Cornelio S, De la Rosa-García SC, Garrido E, Oropeza-Mariano O, Heil M, Partida-Martínez LP, 2017. The age of lima bean leaves influences the richness and diversity of the endophytic fungal community, but not the antagonistic effect of endophytes against Colletotrichum lindemuthianum. *Fungal Ecology* **26**: 1–10.

Lundberg DS, Lebeis SL, Paredes SH, Yourstone S, Gehring J, Malfatti S, Tremblay J, Engelbrektson A, Kunin V, Rio TG del, Edgar RC, Eickhorst T, Ley RE, Hugenholtz P, Tringe SG, Dangl JL, 2012. Defining the core *Arabidopsis thaliana* root microbiome. *Nature* **488**: 86–90.

Lynch MDJ, Neufeld JD, 2015. Ecology and exploration of the rare biosphere. *Nature Reviews Microbiology* **13**: 217–229.

Martin M, 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* **17**: 10.

McLaren MR, Callahan BJ, 2018. In Nature, There Is Only Diversity. *mBio* **9**: e02149-17.

McMurdie PJ, Holmes S, 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLOS ONE* **8**: e61217.

Miura T, Sánchez R, Castañeda LE, Godoy K, Barbosa O, 2017. Is microbial terroir related to geographic distance between vineyards? *Environmental Microbiology Reports* **9**: 742–749.

Mueller GM, Bills GF, Foster MS (Eds.), 2004. Biodiversity of fungi: inventory and monitoring methods. Elsevier, Amsterdam ; Boston.

Muschelli J, 2019. rscopus: Scopus Database "API" Interface.

Mysyakina IS, Funtikova NS, 2007. The role of sterols in morphogenetic processes and dimorphism in fungi. *Microbiology* **76**: 1–13.

Nezhad AS, Geitmann A, 2013. The cellular mechanics of an invasive lifestyle. *Journal of Experimental Botany* **64**: 4709–4728.

Nilsson RH, Anslan S, Bahram M, Wurzbacher C, Baldrian P, Tedersoo L, 2019. Mycobiome diversity: high-throughput sequencing and identification of fungi. *Nature Reviews Microbiology* **17**: 95–109.

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H, 2018. vegan: Community Ecology Package.

Osono T, 2008. Endophytic and epiphytic phyllosphere fungi of Camellia japonica: seasonal and leaf age-dependent variations. *Mycologia* **100**: 387–391.

Ovaskainen O, Tikhonov G, Norberg A, Blanchet FG, Duan L, Dunson D, Roslin T, Abrego N, 2017. How to make more out of community data? A conceptual framework and its implementation as models and software. *Ecology Letters* **20**: 561–576.

Panstruga R, Kuhn H, 2019. Mutual interplay between phytopathogenic powdery mildew fungi and other microorganisms. *Molecular Plant Pathology* **20**: 463–470.

Pauvert C, Buée M, Laval V, Edel-Hermann V, Fauchery L, Gautier A, Lesur I, Vallance J, Vacher C, 2019a. Bioinformatics matters: The accuracy of plant and soil fungal community data is highly dependent on the metabarcoding pipeline. *Fungal Ecology* **41**: 23–33.

Pauvert C, Vallance J, Deliere L, Buee M, Vacher C, 2019b. Microbial networks inferred from metabarcoding data lack replicability: consequences for next-generation biomonitoring. *bioRxiv* 642199.

Pinto C, Pinho D, Sousa S, Pinheiro M, Egas C, Gomes AC, 2014. Unravelling the diversity of grapevine microbiome. *PLoS One* **9**: e85622.

Polonelli L, Morace G, 1986. Reevaluation of the yeast killer phenomenon. *Journal of Clinical Microbiology* **24**: 866–869.

Poudel R, Jumpponen A, Schlatter DC, Paulitz TC, Gardener BBM, Kinkel LL, Garrett KA, 2016. Microbiome Networks: A Systems Framework for Identifying Candidate Microbial Assemblages for Disease Management. *Phytopathology* **106**: 1083–1096.

Proulx SR, Promislow DEL, Phillips PC, 2005. Network thinking in ecology and evolution. *Trends in Ecology & Evolution*, SPECIAL ISSUE: BUMPER BOOK REVIEW **20**: 345–353.

R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rezki S, Campion C, Iacomi-Vasilescu B, Preveaux A, Toualbia Y, Bonneau S, Briand M, Laurent E, Hunault G, Simoneau P, Jacques M-A, Barret M, 2016. Differences in stability of seed-associated microbial assemblages in response to invasion by phytopathogenic microorganisms. *PeerJ* **4**: e1923.

Robert V, Vu D, Amor ABH, Wiele N van de, Brouwer C, Jabas B, Szoke S, Dridi A, Triki M, Daoud S ben, Chouchen O, Vaas L, Cock A de, Stalpers JA, Stalpers D, Verkley GJM, Groenewald M, Santos FB dos, Stegehuis G, Li W, Wu L, Zhang R, Ma J, Zhou M, Gorjón SP, Eurwilaichitr L, Ingsriswang S, Hansen K, Schoch C, Robbertse B, Irinyi L, Meyer W, Cardinali G, Hawksworth DL, Taylor JW, Crous PW, 2013. MycoBank gearing up for new horizons. *IMA Fungus* **4**: 371.

Rognes T, Flouri T, Nichols B, Quince C, Mahé F, 2016. VSEARCH: a versatile open source tool for metagenomics. *PeerJ* **4**: e2584.

Röttjers L, Faust K, 2018. From hairballs to hypotheses–biological insights from microbial networks. *FEMS Microbiology Reviews* **42**: 761–780.

Sanchez-Azofeifa A, Oki Y, Wilson Fernandes G, Ball RA, Gamon J, 2012. Relationships between endophyte diversity and leaf optical properties. *Trees* **26**: 291–299.

Sapkota R, Knorr K, Jørgensen LN, O'Hanlon KA, Nicolaisen M, 2015. Host genotype is an important determinant of the cereal phyllosphere mycobiome. *New Phytologist* **207**: 1134–1144.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Consortium FB, List FBCA, Bolchacova E, Voigt K, Crous PW, Miller AN, Wingfield MJ, Aime MC, An K-D, Bai F-Y, Barreto RW, Begerow D, Bergeron M-J, Blackwell M, Boekhout T, Bogale M, Boonyuen N, Burgaz AR, Buyck B, Cai L, Cai Q, Cardinali G, Chaverri P, Coppins BJ, Crespo A, Cubas P, Cummings C, Damm U, Beer ZW de, Hoog GS de, Del-Prado R, Dentinger B, Diéguez-Uribeondo J, Divakar PK, Douglas B, Dueñas M, Duong TA, Eberhardt U, Edwards JE, Elshahed MS, Fliegerova K, Furtado M, García MA, Ge Z-W, Griffith GW, Griffiths K, Groenewald JZ, Groenewald M, Grube M, Gryzenhout M, Guo L-D, Hagen F, Hambleton S, Hamelin RC, Hansen K, Harrold P, Heller G, Herrera C, Hirayama K, Hirooka Y, Ho H-M, Hoffmann K, Hofstetter V, Högnabba F, Hollingsworth PM, Hong S-B, Hosaka K, Houbraken J, Hughes K, Huhtinen S, Hyde KD, James T, Johnson EM, Johnson JE, Johnston PR, Jones EBG, Kelly LJ, Kirk PM, Knapp DG, Kõljalg U, Kovács GM, Kurtzman CP, Landvik S, Leavitt SD, Liggenstoffer AS, Liimatainen K, Lombard L, Luangsa-ard JJ, Lumbsch HT, Maganti H, Maharachchikumbura SSN, Martin MP, May TW, McTaggart AR, Methven AS, Meyer W, Moncalvo J-M, Mongkolsamrit S, Nagy LG, Nilsson RH, Niskanen T, Nyilasi I, Okada G, Okane I, Olariaga I, Otte J, Papp T, Park D, Petkovits T, Pino-Bodas R, Quaedvlieg W, Raja HA, Redecker D, Rintoul TL, Ruibal C, Sarmiento-Ramírez JM, Schmitt I, Schüßler A, Shearer C, Sotome K, Stefani FOP, Stenroos S, Stielow B, Stockinger H, Suetrong S, Suh S-O, Sung G-H, Suzuki M, Tanaka K, Tedersoo L, Telleria MT, Tretter E, Untereiner WA, Urbina H, Vágvölgyi C, Vialle A, Vu TD, Walther G, Wang Q-M, Wang Y, Weir BS, Weiß M, White MM, Xu J, Yahr R, Yang ZL, Yurkov A, Zamora J-C, Zhang N, Zhuang W-Y, Schindel D, 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences* **109**: 6241–6246.

Setati ME, Jacobson D, Andong U-C, Bauer F, 2012. The Vineyard Yeast Microbiome, a Mixed Model Microbial Map. *PLoS ONE* **7**: e52609.

Suda W, Nagasaki A, Shishido M, 2009. Powdery Mildew-Infection Changes Bacterial Community Composition in the Phyllosphere. *Microbes and Environments* **24**: 217–223.

Tabata J, De Moraes CM, Mescher MC, 2011. Olfactory Cues from Plants Infected by Powdery Mildew Guide Foraging by a Mycophagous Ladybird Beetle. *PLoS ONE* **6**: e23799.

Tackmann J, Rodrigues JFM, Mering C von, 2018. Rapid inference of direct interactions in large-scale ecological networks from heterogeneous microbial sequencing data. *bioRxiv* 390195.

Talley SM, Coley PD, Kursar TA, 2002. The effects of weather on fungal abundance and richness among 25 communities in the Intermountain West. *BMC Ecology* **2**: 7.

Thorsen J, Brejnrod A, Mortensen M, Rasmussen MA, Stokholm J, Al-Soud WA, Sørensen S, Bisgaard H, Waage J, 2016. Large-scale benchmarking reveals false discoveries and count transformation

sensitivity in 16S rRNA gene amplicon data analysis methods used in microbiome studies. *Microbiome* **4**:.

Tipton L, Müller CL, Kurtz ZD, Huang L, Kleerup E, Morris A, Bonneau R, Ghedin E, 2018. Fungi stabilize connectivity in the lung and skin microbial ecosystems. *Microbiome* **6**: 12.

Toome M, Heinsoo K, Luik A, 2010. Relation between leaf rust (Melampsora epitea) severity and the specific leaf area in short rotation coppice willows. *European Journal of Plant Pathology* **126**: 583–588.

UNITE Community, 2019. UNITE general FASTA release for Fungi 2.

Vacher C, Tamaddoni-Nezhad A, Kamenova S, Peyrard N, Moalic Y, Sabbadin R, Schwaller L, Chiquet J, Smith MA, Vallance J, Fievet V, Jakuschkin B, Bohan DA, 2016. Learning Ecological Networks from Next-Generation Sequencing Data, in: Advances in Ecological Research. pp. 1–39.

Valdés-Gómez H, Gary C, Cartolaro P, Lolas-Caneo M, Calonnec A, 2011. Powdery mildew development is positively influenced by grapevine vegetative growth induced by different soil management strategies. *Crop Protection* **30**: 1168–1177.

Vannier N, Agler M, Hacquard S, 2019. Microbiota-mediated disease resistance in plants. *PLOS Pathogens* **15**: e1007740.

Vayssier-Taussat M, Albina E, Citti C, Cosson J-F, Jacques M-A, Lebrun M-H, Le Loir Y, Ogliastro M, Petit M-A, Roumagnac P, Candresse T, 2014. Shifting the paradigm from pathogens to pathobiome: new concepts in the light of meta-omics. *Frontiers in Cellular and Infection Microbiology* **4**: 29.

Vogel C, Bodenhausen N, Gruissem W, Vorholt JA, 2016. The Arabidopsis leaf transcriptome reveals distinct but also overlapping responses to colonization by phyllosphere commensals and pathogen infection with impact on plant health. *New Phytologist* **212**: 192–207.

Wang H, Wei Z, Mei L, Gu J, Yin Suisui, Faust K, Raes J, Deng Y, Wang Y, Shen Q, Yin Shixue, 2017. Combined use of network inference tools identifies ecologically meaningful bacterial associations in a paddy soil. *Soil Biology and Biochemistry* **105**: 227–235.

Wang Q, Garrity GM, Tiedje JM, Cole JR, 2007. Naïve Bayesian Classifier for Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy. *Applied and Environmental Microbiology* **73**: 5261–5267.

Weete JD, Abril M, Blackwell M, 2010. Phylogenetic Distribution of Fungal Sterols. *PLoS ONE* **5**: e10899.

Weiss S, Van Treuren W, Lozupone C, Faust K, Friedman J, Deng Y, Xia LC, Xu ZZ, Ursell L, Alm EJ, Birmingham A, Cram JA, Fuhrman JA, Raes J, Sun F, Zhou J, Knight R, 2016. Correlation detection strategies in microbial data sets vary widely in sensitivity and precision. *The ISME Journal* **10**: 1669–1681.

Whitaker BK, Bakker MG, 2019. Bacterial endophyte antagonism toward a fungal pathogen in vitro does not predict protection in live plant tissue. *FEMS Microbiology Ecology* **95**:.

White TJ, Bruns T, Lee S, Taylor J, 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications* 315–322.

Widder S, Allen RJ, Pfeiffer T, Curtis TP, Wiuf C, Sloan WT, Cordero OX, Brown SP, Momeni B, Shou W, Kettle H, Flint HJ, Haas AF, Laroche B, Kreft J-U, Rainey PB, Freilich S, Schuster S, Milferstedt K, van der Meer JR, Groβkopf T, Huisman J, Free A, Picioreanu C, Quince C, Klapper I, Labarthe S, Smets BF, Wang H, Isaac Newton Institute Fellows, Soyer OS, 2016. Challenges in microbial ecology: building predictive understanding of community function and dynamics. *The ISME Journal* **10**: 2557–2568.

Zaneveld JR, McMinds R, Vega Thurber R, 2017. Stress and stability: applying the Anna Karenina principle to animal microbiomes. *Nature Microbiology* **2**: 17121.

Zhang Z, Kong X, Jin D, Yu H, Zhu X, Su X, Wang P, Zhang R, Jia M, Deng Y, 2019. Euonymus japonicus phyllosphere microbiome is significantly changed by powdery mildew. *Archives of Microbiology* **201**: 1099–1109.

Zinger L, Bonin A, Alsos IG, Bálint M, Bik H, Boyer F, Chariton AA, Creer S, Coissac E, Deagle BE, Barba MD, Dickie IA, Dumbrell AJ, Ficetola GF, Fierer N, Fumagalli L, Gilbert MTP, Jarman S, Jumpponen A, Kauserud H, Orlando L, Pansu J, Pawlowski J, Tedersoo L, Thomsen PF, Willerslev E, Taberlet P, 2019. DNA metabarcoding—Need for robust experimental designs to draw sound ecological conclusions. *Molecular Ecology* **28**: 1857–1862.

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management of the experimental site was partly funded by the AFB (French Agency for Biodiversity) within the DEPHY network.

Authors contribution

JV and AC performed inoculations. JV, CV and AC performed leaf monitoring and sampling. JV and CV processed the sampled leaves. TF and JFA performed the extractions and amplifications. EC performed the sequencing. CP and MM quantified fungal abundance using ddPCR. JV and CP performed the microbiology experiments. CP performed the bioinformatic and statistical analyses. SR and JC provided methods and advice on network inference. CV and DB had the original idea for the project. CV and JV coordinated all stages of the experiment. CP wrote the first draft of the manuscript, and CV and JV made a major contribution to the writing of the final draft. All authors revised the manuscript.

Figures and Tables

Figure 1 – Experimental design. The study took place in an experimental vineyard located near Bordeaux, France (A), in a sub-plot of 5 slightly treated vine rows (B) with two cropping systems differing in the presence or not of cover crop (CC *versus* NCC) in the inter-rows (C). Sampling occurred in eight experimental units each composed of five adjacent vines (underlined in green) (D). The causal agent of grape powdery mildew, *Erysiphe necato*r, was inoculated on the central vine of each experimental unit (E) and we performed three sampling campaigns 40, 62 and 77 days post inoculation (F). For each campaign, leaves with and without visible symptoms were collected to analyze healthy foliar tissues and foliar tissue from diseased spots (G).

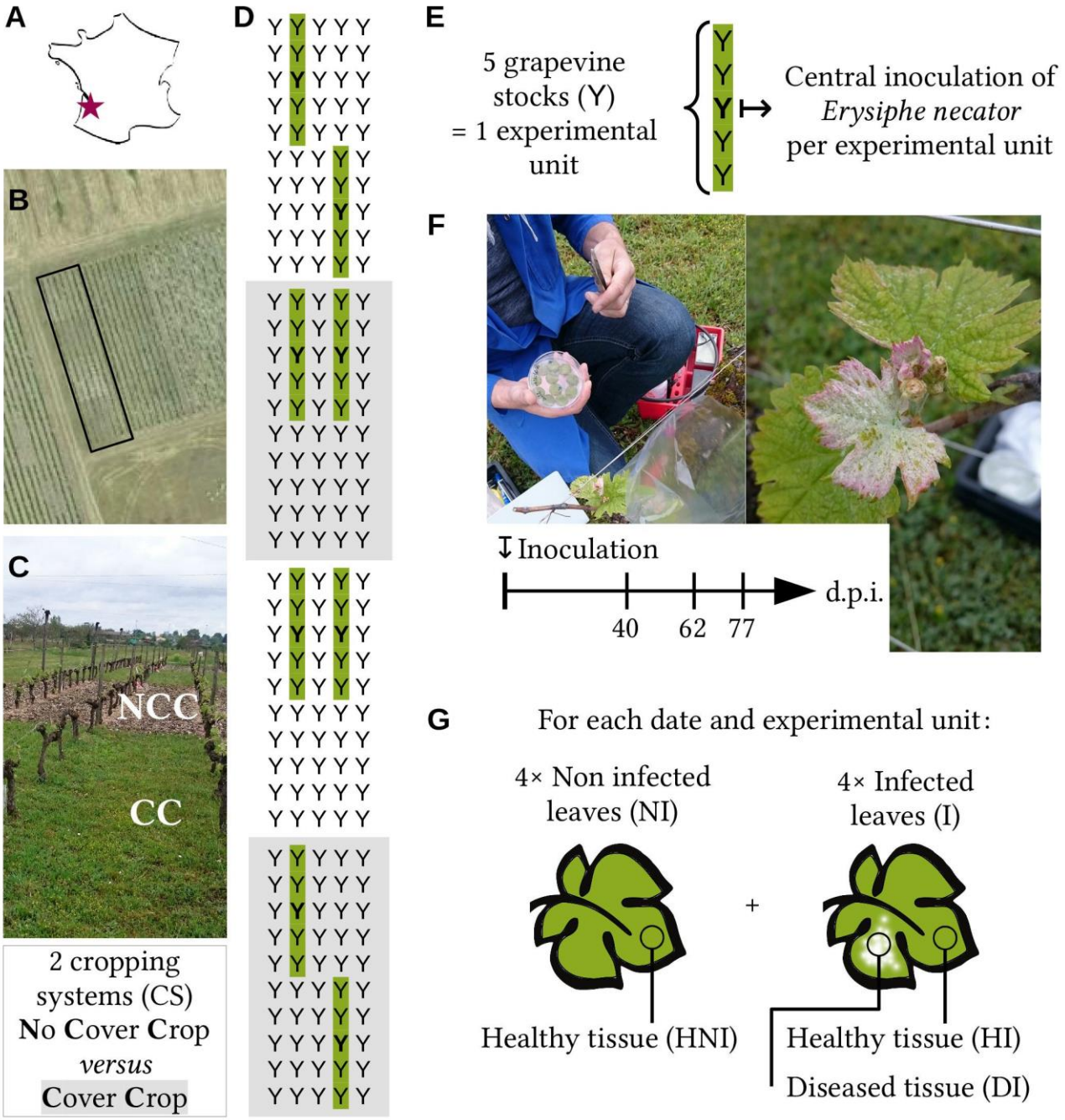

Figure 2 – Variations in (A) pathogen abundance and (B) fungal community heterogeneity among tissue types over time. Tissue types consisted in Healthy tissue in Non Infected leaves (HNI), Healthy tissue in Infected leaves (HI), and Diseased tissue in Infected leaves (DI).

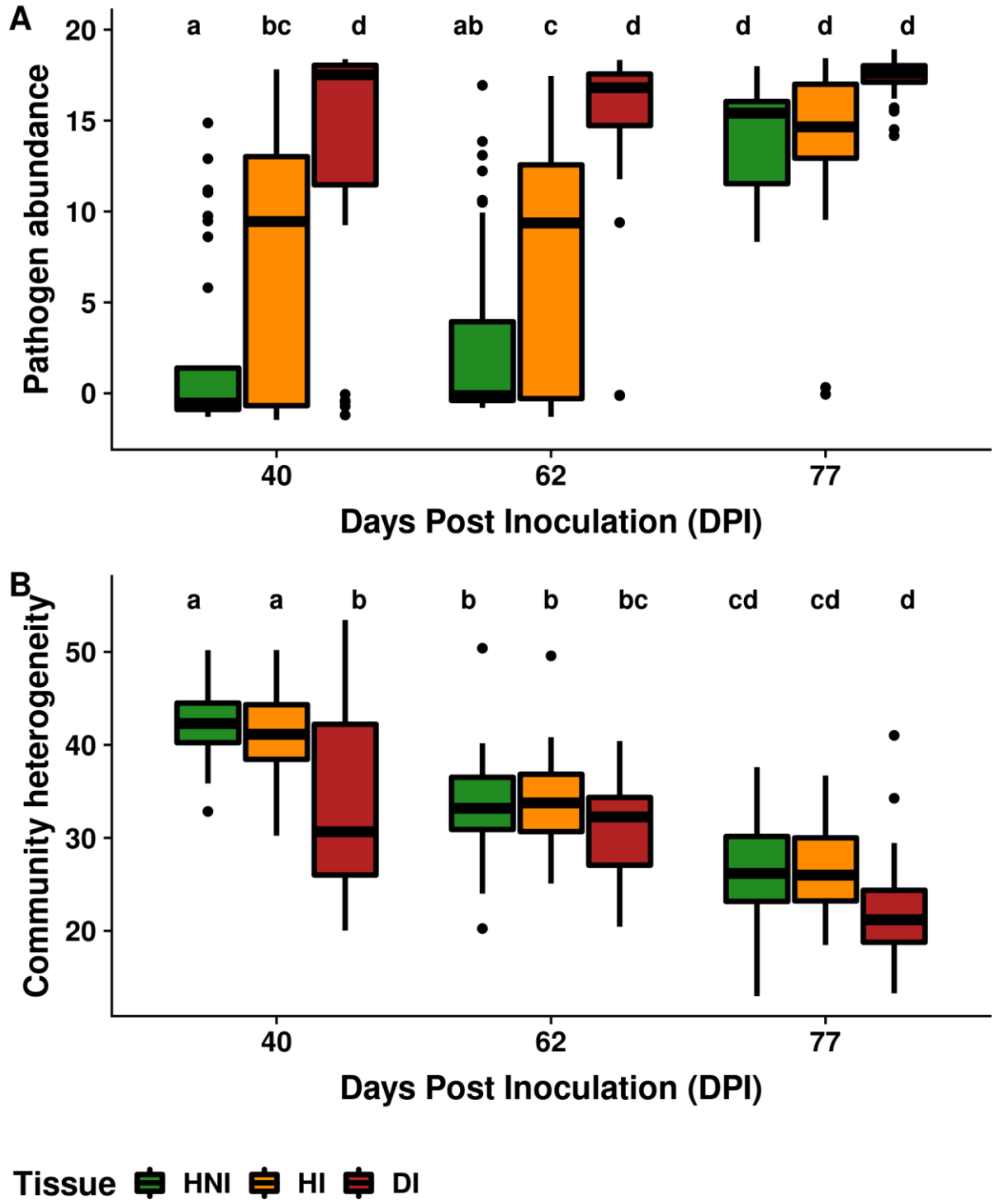

Figure 3 – Association network versus *in vitro* interaction network among eight yeast species. First, an association network (A) was obtained with PLN from metabarcoding data and environmental covariates. The node corresponding to the pathogen *Erysiphe necator* is labelled *En*. A subset of the association network involving 8 yeast species was extracted. It consisted only in positive associations (B). We confronted this network with spot-on-lawn experiments (C) where a single yeast species was seeded in a Petri dish and further inoculated by spots of 8 yeast species to evaluate their interaction. Growth-promoting and -inhibiting interactions were represented as an interaction network (D). The yeast species consisted in *Buckleyzyma aurantiaca* (B), *Cystofilobasidium macerans* (C), *Dioszegia hungarica* (D), *Filobasidium oeirense* (Fo), *Filobasidium wieringae* (Fw), *Udeniomyces pyricola* (P), *Sporobolomyces roseus* (S) and *Vishniacozyma victoriae* (V).

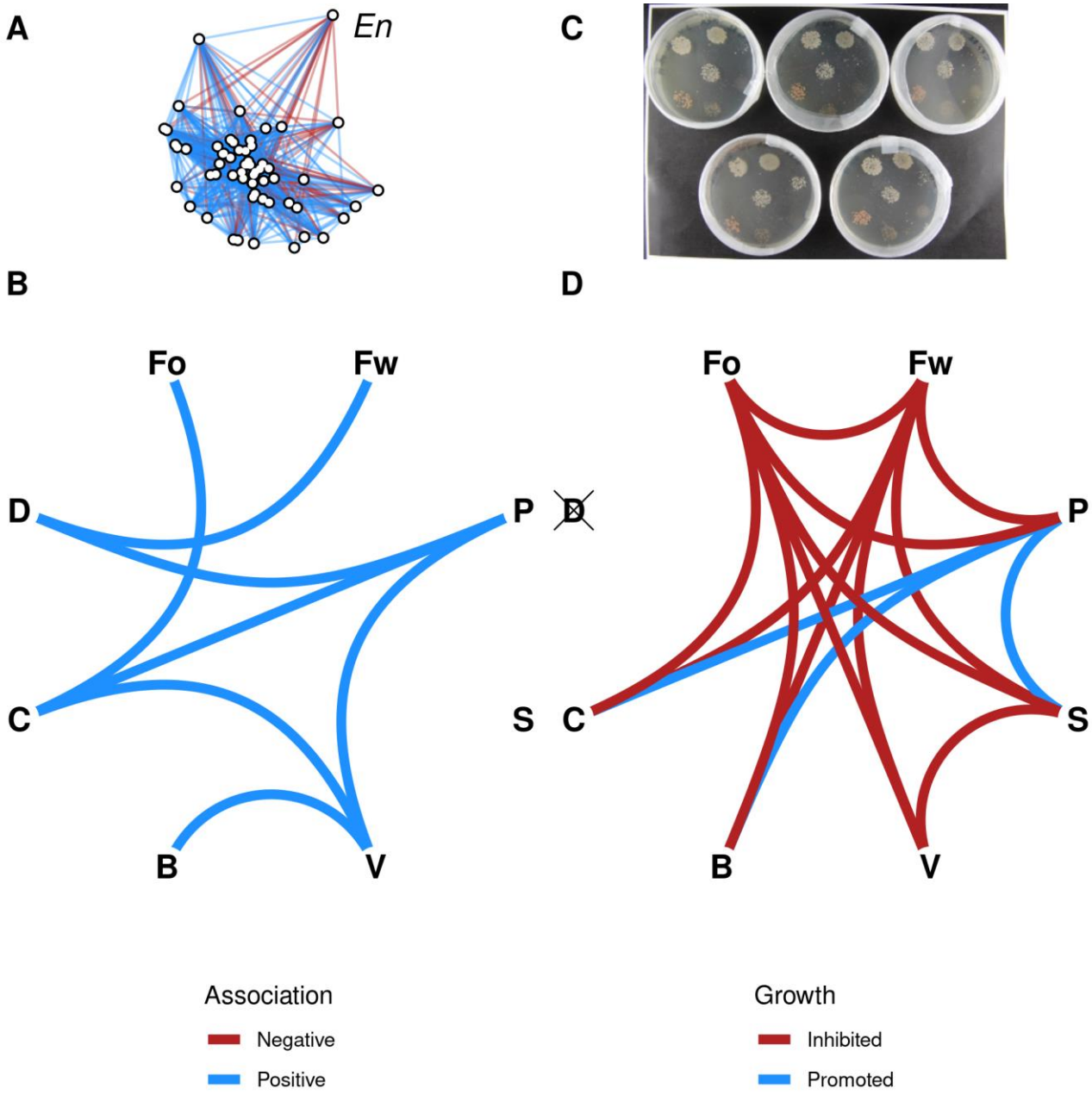

Figure 4 – Putative pathobiome of the grapevine powdery mildew agent, *Erysiphe necator.* The subset of the association network (Figure 3A) involving the pathogen was extracted. It consisted of 25 species with positive or negative associations with the pathogen. Node shape indicate whether these species were significantly favored by cover cropping (black circles), by the absence of cover (white circle) or neither (cross). Green, yellow and brown circles indicate whether the species were detected in other microbial environments (old leaves, ground cover and bark, respectively).

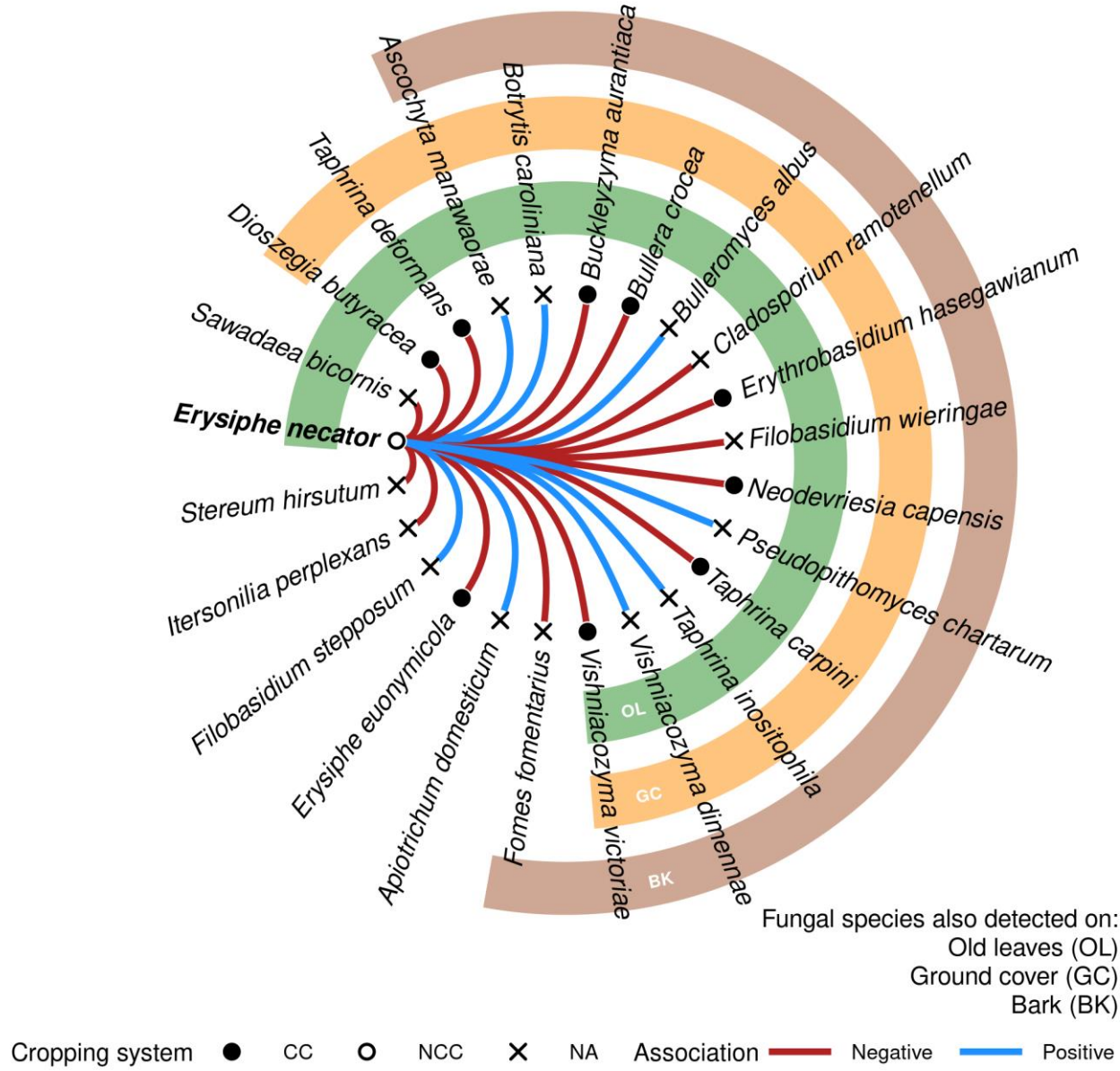

Figure 5 - Pictures of *E. necator* conidia in the *E. necator*-yeast strains confrontation test. Unaltered conidial chains on control foliar discs treated with sterile distilled water (A), collapsed conidia on foliar discs treated with *B. aurantiaca* in the preventive assay (B).

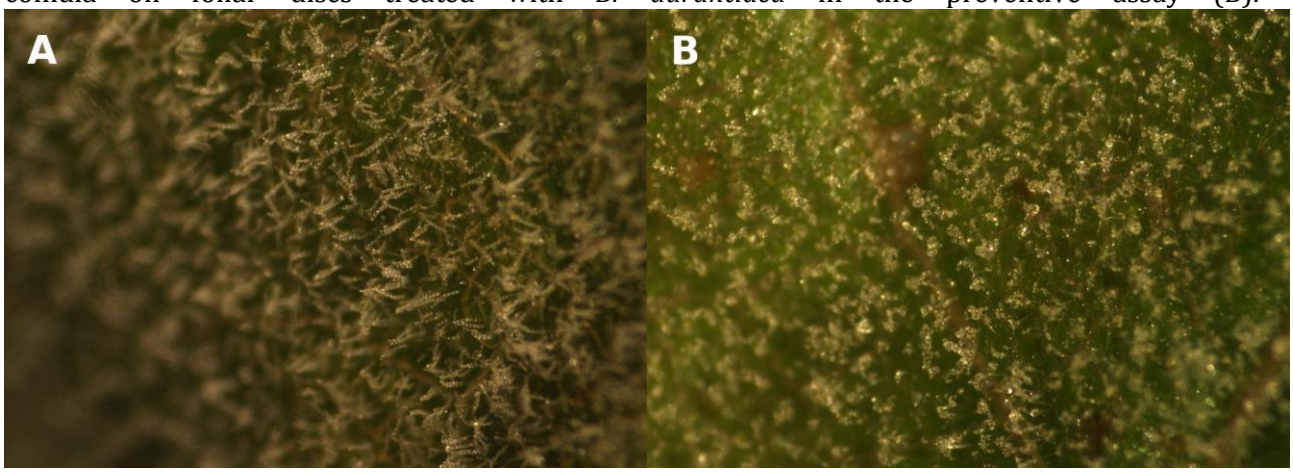

Table 1 – Effects of date, cropping system (CS) and tissue type (TT) on pathogen abundance (A), total fungal load (B) and community composition (C). Significant effects ($p < 0.05$) are in bold.

		A. Pathogen abundance			B. Total fungal abundance			C. Fungal community composition		
Variable	Df	F	Pr(>F)		F	Pr(>F)		F	R2	Pr(>F)
Date	**2**	**55.29**	**1.00E-20**		**54.27**	**2.10E-20**		**5.1**	**0.0336**	**0.001**
CS	1	0.1	7.50E-01		0.76	3.90E-01		**1.5**	**0.005**	**0.021**
TT	**2**	**70.82**	**3.10E-25**		**50.87**	**2.40E-19**		1	0.0068	0.377
Date × CS	2	0.29	7.50E-01		1.42	2.40E-01		**9.7**	**0.0642**	**0.001**
Date × TT	**4**	**6.89**	**2.80E-05**		**17.9**	**5.50E-13**		1.1	0.0148	0.13
CS × TT	2	1.08	3.40E-01		0.34	7.10E-01		1.2	0.0079	0.114
Date × CS × TT	4	1.11	3.50E-01		1.72	1.50E-01		1.1	0.0148	0.13
Residuals	258				NA	NA		NA	0.8529	NA
Total	275							NA	1	NA

Table 2 – Percent increase in *Erysiphe necator* (*En*) growth triggered by preventive or curative treatments with yeast strains or exposure to benzothiadiazole (BTH). Significant effects of the treatments ($p < 0.05$) are in bold.

	Preventive treatment				Curative treatment			
	Pellet		Supernatant		Pellet		Supernatant	
Yeast strain	*En* growth (in %)	p-value	*En* growth (in %)	p-value	*En* growth (in %)	p-value	*En* growth (in %)	p-value
Control (BTH)	**-98.62**	**0**	**-90.62**	**0**	**-85.62**	**1.70E-14**	**-86.25**	**0**
B. aurantiaca	-1.87	0.33	2.25	0.99	-19.63	0.18	**-31.25**	**0**
U. pyricola	-1	0.85	6	0.83	**-32.5**	**0**	-3.75	0.97
V. victoriae	-0.37	0.99	7.63	0.67	-10	0.79	3.88	0.97
D. hungarica	0.25	1	0.63	1	-5	0.98	-10.62	0.44
F. wieringae	0.5	0.98	6.38	0.8	-10.63	0.75	-1.87	1
S. roseus	0.88	0.9	0.13	1	-6.88	0.93	-5.25	0.91
F. oeirense	1.13	0.78	-4.5	0.93	-19.38	0.19	-0.87	1
C. macerans	1.38	0.64	6.25	0.81	-10.63	0.75	5	0.93

Table 3 - Effect of yeast strains on the conidia of *E. necator*. The number of foliar discs (out of 8) with altered conidia were counted in different co-culture conditions. Bold entries indicate significant differences in the number of altered conidia relative to the negative control using Fisher exact test. No conidia were observed with the positive control (benzothiadiazole) which was thus not included.

	Preventive treatment				Curative treatment			
	Pellet		Supernatant		Pellet		Supernatant	
Yeast strain	Altered conidia	p-value	Altered conidia	p-value	Altered conidia	p-value	Altered conidia	p-value
V. victoriae	4	0.141	4	0.141	1	0.7667	1	0.7667
B. aurantica	3	0.2846	5	0.0594	5	0.0594	**8**	**0.0007**
D. hungarica	1	0.7667	1	0.7667	4	0.141	0	1
U. pyricola	1	0.7667	0	1	5	0.0594	0	1
S. roseus	1	0.7667	1	0.7667	5	0.0594	1	0.7667
C. macerans	0	1	2	0.5	3	0.2846	0	1
F. oeirense	0	1	5	0.0594	3	0.2846	0	1
F. wieringae	0	1	0	1	3	0.2846	1	0.7667
Negative control	1		1		1		1	

Supplementary figures and tables

Figure S1 - Relationships among the distances between sampled leaves and the closest environmental sources of microorganisms. The distances (D, in cm) to old inoculated leaves, bark, and ground cover were measured. Spearman's rank correlation and test are presented (* p<0.05; **p<0.01; ***p<0.001).

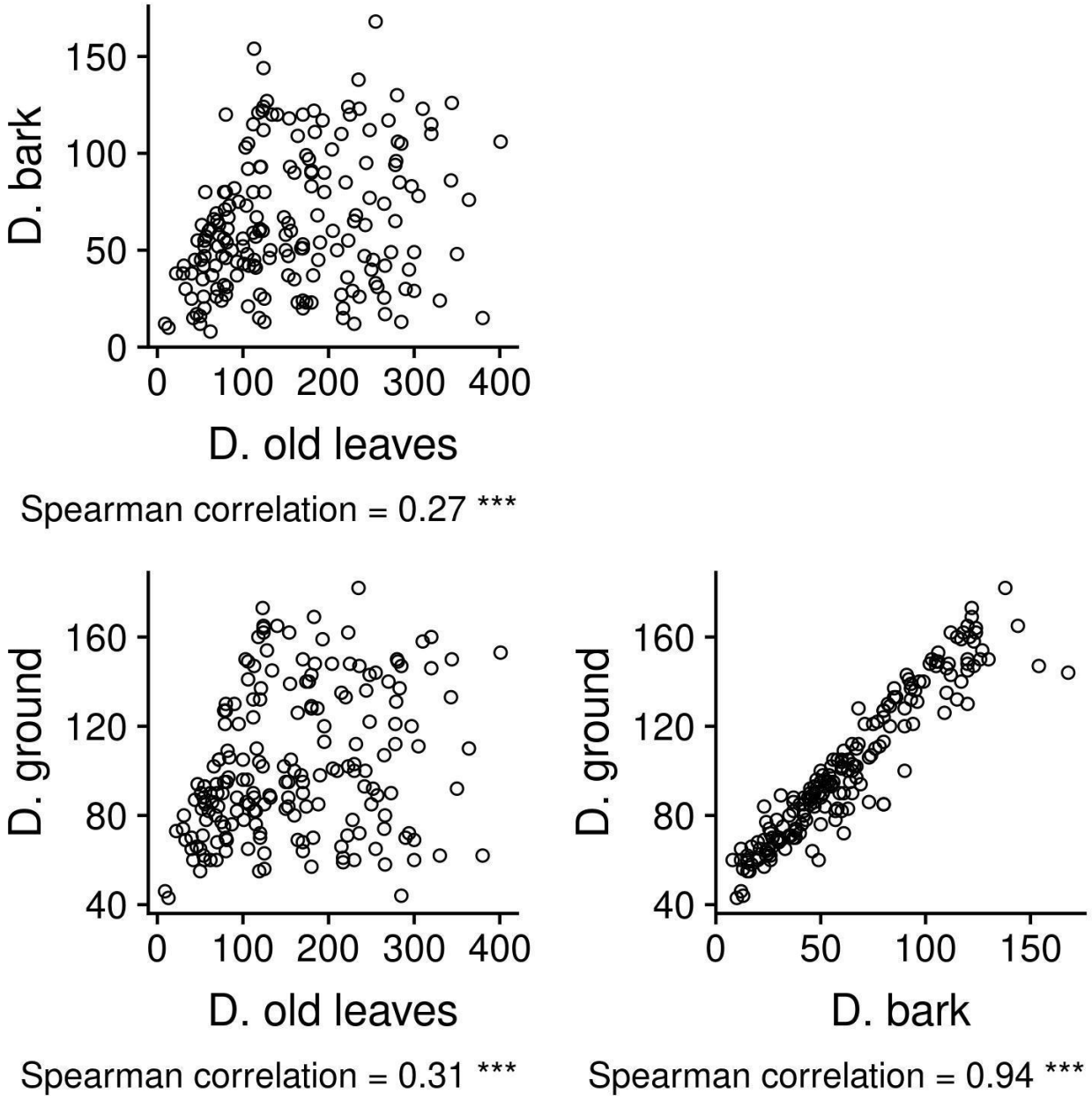

Figure S2 – Variations in fungal total abundance among tissue types over time. Different letters indicate significant post-hoc comparisons. Tissue type consisted in Healthy tissue in Non Infected leaves (HNI), Healthy tissue in Infected leaves (HI), and Diseased tissue in Infected leaves (DI).

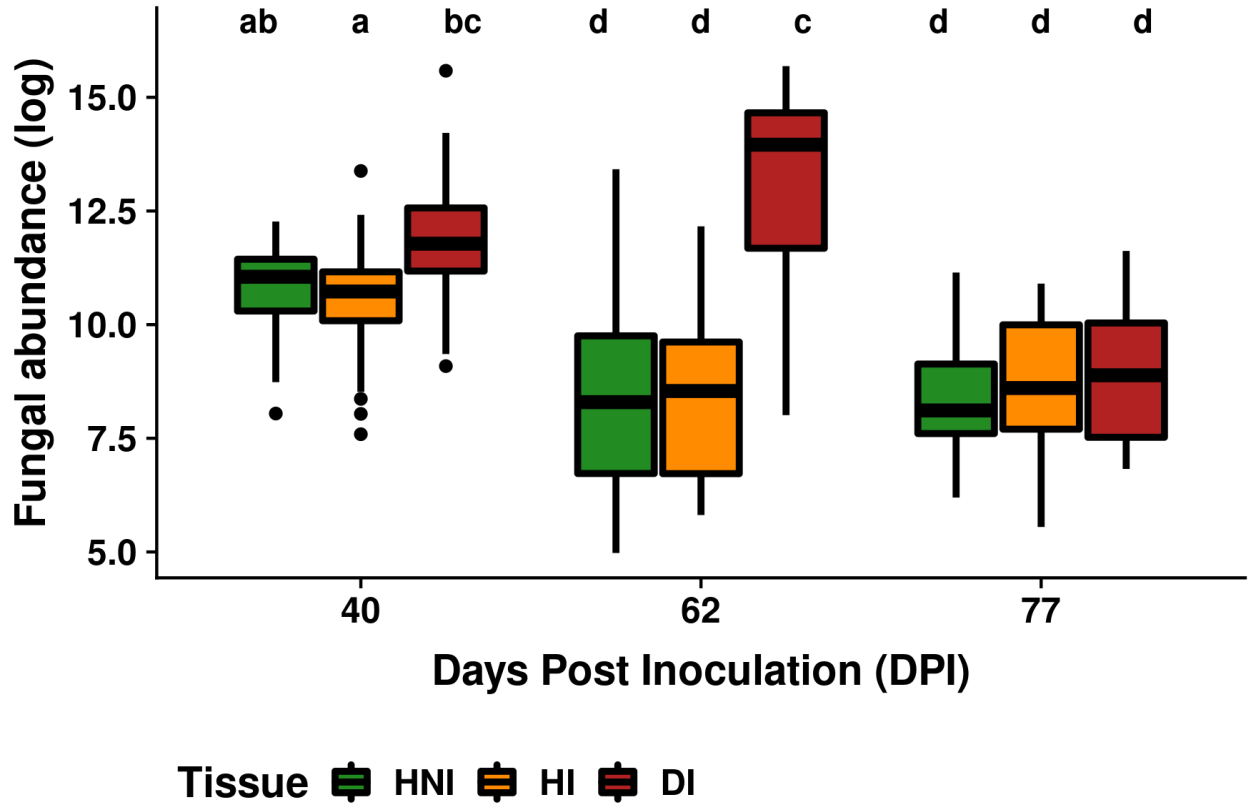

Figure S3 - Fungal community compositions depending on the sampling date, the inter-row cropping system and the foliar tissue analyzed. The first two axes of the Principal Component Analysis (PCA) were used to represent the dissimilarities in composition, from left to right, among samples across sampling dates (in days post inoculation, DPI), cropping systems (Cover crop, CC, and no cover crop, NCC) and tissue types (Healthy tissue in Non Infected leaves, HNI, Healthy tissue in Infected leaves, HI, and Diseased tissue in Infected leaves, DI). PCA was performed from the Euclidean distance on clr-transformed sequence counts.

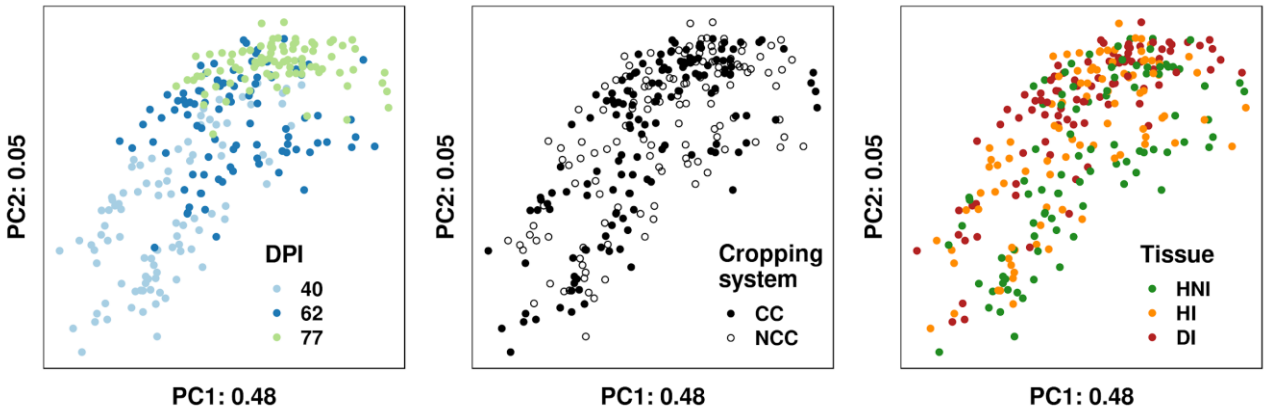

Figure S4 - Canopy vigor and disease pressure indexes per cropping system (CS), i.e. with (CC) or without cover crop (NCC). Vigor was estimated on ~20 grapevines per cropping system (A). Grapevine diseases severity were monitored on 468 leaves randomly distributed among the two cropping systems (B) (* p<0.05; **p<0.01; ***p<0.001).

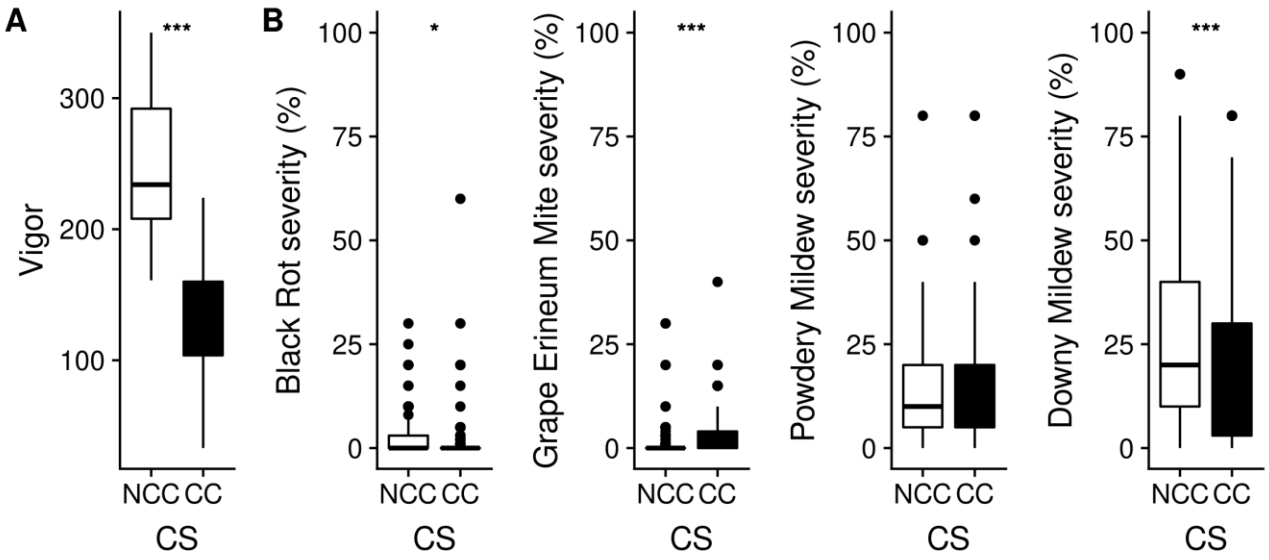

Table S1 - Pesticides (active compounds) used on the sub-plot since 2006 according to the pest targeted. D: Downy mildew (*Plasmopara viticola*); P: Powdery mildew (*Erysiphe necator*), E: European grapevine moth (*Lobesia botrana*) and S: Flavescence dorée vector (*Scaphoideus titanus*). Applications were done in the 5 rows between May and July 2006-2012.

Pesticide active compounds	Pest	Year (date)
Chlorpyriphos-ethyl	T, S	2006 (7/3); 2009 (5/20, 7/6)
Copper compounds	D	2008 (7/30)
Fosetyl + Folpet	D	2008 (7/21)
Lambda-cyhalothrin	S	2009 (6/16)
Mancozeb + Cymoxanil	D	2006 (5/23, 6/9); 2007 (6/11); 2008 (5/28, 6/6, 6/26); 2009 (6/26); 2011 (5/19); 2012 (6/22, 7/2)
Quinoxyfen	P	2008 (6/6); 2011 (5/19)
Spiroxamin	P	2008 (6/26, 7/21)

Table S2 - List of covariates included in the network inference. Covariate fluctuations can be tied positively (↗), negatively (↘) to their effect on foliar fungi or without a definite sign (∝). Bold entries indicate references concerning our biological models *Vitis vinifera* or *Erysiphe necator* (EN). The VPD relates to the gap between a water saturated air to the actual air given the temperature (see Cordier et al. 2012 for more details on the function *f*).

Type	Covariates	Definition	Effects on fungi	References
Foliar traits	Leaf water content (LWC)	1 – (D/F) with fresh (F) and dry (D) leaf weight	↗ with endophytic fungal richness	Sanchez-Azofeifa et al. 2012
			∝ the occurrence of endophytes	Eschen et al., 2010
	Leaf age (LA)	Days since leaf unfolding	↗ with endophytic fungal richness	Osono 2008
			↗ with endophytic fungal richness and number of isolates	López-González et al. 2017
			↘ the susceptibility to EN	Gadoury et al. 2012; Calonnec et al. 2018
	Specific Leaf Area (SLA)	S/D with leaf surface (S)	↘ the development of mildew and rust pathogens	Itagaki and Shibuya 2017; Toome et al. 2011
			↘ the establishment of endophytes and hence ∝ fungal abundance	Nezhad and Geitmann 2013
			S ↗ collected water and hence ∝ the infection susceptibility	Bradley et al. 2003
			S ↗ with the log number of individuals fungi	Andrews et al. 1987
Climatic variables	Vapor Pressure Deficit (VPD)	*f*(RH x T) function of relative humidity (RH) and temperature (T)	RH x T ∝ with spore density	Crandall and Gilbert 2017
			↘ with fungal richness and abundance	Talley et al. 2002
			↘ with the incidence and severity of EN.	Carrol and Wilcox 2003
Dispersal	Distance	Distances to microbial sources	**↘ with microbial invasion**	Miura et al. 2017
Experimental design	Sampling date		**∝ the fungal community composition**	This study; Pinto et al. 2014; Fort et al. 2016
	Experimental unit		**∝ the fungal community composition**	This study; Setati et al. 2012
	Tissue type		**∝ the fungal community composition**	This study; Bruez et al. 2014

Table S3 - Most abundant species in grapevine foliar fungal communities in the sampled foliar tissues. The relative abundances (RA, in %) and ranks of species were calculated for all leaf samples (TOTAL; n = 276) and for samples collected from healthy zones of non-infected (HNI; *n* = 93) and infected (HI; *n* = 92) leaves, and the center of disease spots of infected leaves (DI; *n* = 91).

Species	TOTAL		HNI		HI		DI	
	Rank	RA	Rank	RA	Rank	RA	Rank	RA
Erysiphe necator	1	35.1	3	14.9	1	22	1	68.9
Mycosphaerella tassiana	2	17.8	1	26.2	2	20.2	3	7
Filobasidium wieringae	3	13.9	2	15.5	3	19.8	4	6.2
Sporobolomyces roseus	4	9.2	4	9.9	4	10.3	2	7.4
Udeniomyces pyricola	5	4.4	5	6.3	5	5.3	5	1.6
Vishniacozyma victoriae	6	1.9	7	1.9	6	2.6	6	1.2
Erysiphe euonymicola	7	1.6	6	2.3	7	1.7	8	0.6
Dioszegia hungarica	8	1.2	8	1.6	8	1.3	9	0.6
Itersonilia pannonica	9	1	10	1.2	9	1.2	7	0.7
Symmetrospora coprosmae	10	0.8	11	1.2	11	1	16	0.2

Table S4 - List of the species associated with *Erysiphe necator* (Figure 4). Associations are described with the partial correlation provided by PLN and with the stability, which is the fraction of bootstrap subsamples that contained this association. The detection of theses species in other microbial environments (ME) such as old leaves (OL), ground cover (GC) or bark (BK) is indicated or left blank if undetected. The most favourable cropping system it indicated as CS (*i.e.* cover crop CC or no cover crop NCC), when a significant abundance between the cropping systems is observed, and left blank otherwise.

Species	Correlation	Stability	Other ME	CS
Dioszegia butyracea	-0.087	0.96	OL / GC	CC
Cladosporium ramotenellum	-0.074	0.94	OL / GC / BK	
Sawadaea bicornis	-0.071	0.96	OL	
Taphrina carpini	-0.059	0.96	OL / GC / BK	CC
Stereum hirsutum	-0.043	0.84		
Buckleyzyma aurantiaca	-0.035	0.82	OL / GC / BK	CC
Erythrobasidium hasegawianum	-0.03	0.76	OL / GC / BK	CC
Taphrina deformans	-0.02	0.56	OL / GC	CC
Erysiphe euonymicola	-0.015	0.58		CC
Fomes fomentarius	-0.011	0.6	BK	
Bullera crocea	-0.011	0.68	OL / GC / BK	CC
Filobasidium wieringae	-0.01	0.52	OL / GC / BK	
Neodevriesia capensis	-0.01	0.38	OL / GC / BK	CC
Vishniacozyma victoriae	-0.005	0.62	OL / GC / BK	CC
Itersonilia perplexans	-0.003	0.38		
Ascochyta manawaorae	0.011	0.52	OL / GC / BK	
Taphrina inositophila	0.017	0.68	OL / GC / BK	
Vishniacozyma dimennae	0.02	0.64	OL / GC / BK	

Botrytis caroliniana	0.035	0.86	OL / GC / BK	
Apiotrichum domesticum	0.038	0.8		
Filobasidium stepposum	0.055	1		
Bulleromyces albus	0.149	1	OL / GC / BK	
Pseudopithomyces chartarum	0.158	1	OL / GC / BK	

Table S5 – Literature mining of the pathobiome. The following papers were reported by the text-mining approach because both the pathogen *Erysiphe necator* and the name of the potential antagonists presented in the Figure 4 were found in abstracts, titles, keywords or references of the Scopus database. This search also included fungal synonyms through a custom script (Methods S2).

Pathogen	Potential antagonist searched for	References
Erysiphe necator	*Erythrobasidium hasegawianum*	Mueller, G. M., Bills, G. F., & Foster, M. S. (Eds.). (2004). *Biodiversity of fungi: inventory and monitoring methods*. Amsterdam ; Boston: Elsevier.
Erysiphe necator	*Fomes fomentarius*	Tabata, J., De Moraes, C. M., & Mescher, M. C. (2011). Olfactory Cues from Plants Infected by Powdery Mildew Guide Foraging by a Mycophagous Ladybird Beetle. *PLoS ONE*, *6*(8), e23799. doi: 10.1371/journal.pone.0023799
Erysiphe necator	*Sawadaea bicornis*	Kiss, L. (1998). Natural occurrence of ampelomyces intracellular mycoparasites in mycelia of powdery mildew fungi. *New Phytologist*, *140*(4), 709–714. doi: 10.1046/j.1469-8137.1998.00316.x
Erysiphe necator	*Taphrina deformans*	Chang, H.-X., Noel, Z. A., Sang, H., & Chilvers, M. I. (2018). Annotation resource of tandem repeat-containing secretory proteins in sixty fungi. *Fungal Genetics and Biology*, *119*, 7–19. doi: 10.1016/j.fgb.2018.07.004
Erysiphe necator	*Taphrina deformans*	Kües, U., Khonsuntia, W., & Subba, S. (2018). Complex fungi. *Fungal Biology Reviews*, *32*(4), 205–218. doi: 10.1016/j.fbr.2018.08.001
Erysiphe necator	*Taphrina deformans*	Caubel, J., Launay, M., Lannou, C., & Brisson, N. (2012). Generic response functions to simulate climate-based processes in models for the development of airborne fungal crop pathogens. *Ecological Modelling*, *242*, 92–104. doi: 10.1016/j.ecolmodel.2012.05.012
Erysiphe necator	*Taphrina deformans*	Weete, J. D., Abril, M., & Blackwell, M. (2010). Phylogenetic Distribution of Fungal Sterols. *PLoS ONE*, *5*(5), e10899. doi: 10.1371/journal.pone.0010899
Erysiphe necator	*Taphrina deformans*	Mysyakina, I. S., & Funtikova, N. S. (2007). The role of sterols in morphogenetic processes and

		dimorphism in fungi. *Microbiology*, *76*(1), 1–13. doi: 10.1134/S0026261707010018
Erysiphe necator	*Taphrina deformans*	Hernandez, A., Cooke, D. T., Lewis, M., & Clarkson, D. T. (1997). Fungicides and sterol-deficient mutants of Ustilago maydis: plasma membrane physico-chemical characteristics do not explain growth inhibition. *Microbiology*, *143*(10), 3165–3174. doi: 10.1099/00221287-143-10-3165

Table S6 - List of the species differentially abundant between cropping systems. Species were assigned to a preferred cropping system: either no cover crop (NCC) or cover crop (CC) depending on the sign of the effect size generated by ALDEx2. Selection of the species was based on the false discovery rate (FDR) after Benjamini-Hochberg correction using the default threshold of ALDEX2 (FDR < 0.1).

Species	CS	Effect size	FDR (BH)
Erysiphe necator	NCC	0.45	2.80E-07
Udeniomyces pyricola	CC	-0.17	6.90E-02
Taphrina caerulescens	CC	-0.17	7.10E-02
Blumeria graminis	CC	-0.18	6.10E-02
Symmetrospora gracilis	CC	-0.21	4.00E-02
Filobasidium oeirense	CC	-0.22	9.30E-02
Articulospora proliferata	CC	-0.25	2.60E-02
Gelidatrema spencermartinsiae	CC	-0.26	3.10E-02
Dioszegia butyracea	CC	-0.26	3.50E-02
Erythrobasidium hasegawianum	CC	-0.26	1.30E-02
Limonomyces culmigenus	CC	-0.27	1.60E-02
Vishniacozyma victoriae	CC	-0.3	4.90E-04
Dioszegia hungarica	CC	-0.3	4.80E-04
Neodevriesia capensis	CC	-0.3	3.00E-02
Taphrina deformans	CC	-0.31	1.60E-03
Bullera crocea	CC	-0.32	6.10E-03
Erysiphe euonymicola	CC	-0.39	1.70E-07
Buckleyzyma aurantiaca	CC	-0.46	2.30E-05
Curvibasidium cygneicollum	CC	-0.46	5.60E-05
Mycosphaerella tassiana	CC	-0.51	6.80E-08
Taphrina carpini	CC	-0.51	7.20E-06
Cystofilobasidium macerans	CC	-0.55	5.10E-07

Mycosphaerella punctiformis	CC	-0.57	3.50E-07
Angustimassarina acerina	CC	-0.62	1.70E-08
Itersonilia pannonica	CC	-0.74	5.40E-12

Methods S1

Species selection needs to be done prior to network inference (Röttgers and Faust 2018). However, there is reluctance to filter out infrequent species, or rare, that could be specialists or possess an important role (Lynch and Neufeld 2015; Harrison et al. 2019). Röttgers and Faust (2018) have highlighted the importance of removing infrequent ASV prior to network inference, as they can cause spurious associations due to matching zeros. Others have previously indicated the removal of infrequent species but without consensus on the prevalence threshold at which to keep species: present in at least 20% of the samples (Berry and Widder 2014), at least in 2 (Durán et al., 2018), 5 (Poudel et al. 2016) or 10 samples (Agler et al. 2016). Indeed, the more sparsity in the ASV table, the less reliable are the inferred networks and no more than 50% of zeroes was suggested (Weiss et al. 2016). A recent study did not trim species but suggest instead that associations between pairs of rare species should not be inferred but those between more abundant species and rare species could be (Cougoul et al. 2019a). They provide a test based on the prevalence of the two species to determine whether the association could be inferred and their approach improved the inference of networks according to simulations (Cougoul et al. 2019a).

However, the method we used to infer the network at the time did not allow for discarding associations *a priori* and left us with the option of trimming species. But we decided to evaluate the testability of the associations to guide us for species trimming. Moreover, we performed the species selection for each experimental unit (Figure 1D) separately to ensure that both ubiquitous and locally-distributed species were kept.

Therefore, we assessed the testability of every ASV pairs in the table for different minimum values of ASV prevalence. We started from 1 sample minimum and increased to the maximum number of samples containing two ASVs. We used the fraction of testable pairs over the total number of pairs as a trade-off criterium to identify the threshold. Keeping species in the network inference if they were detected in 20% of the samples considered, seemed to well balance in each experimental unit (Figure 1) the testability of associations and the number of species. The final network inference was conducted with the ASV table of all samples but only with the species present in at least 20% of the samples of any experimental unit.

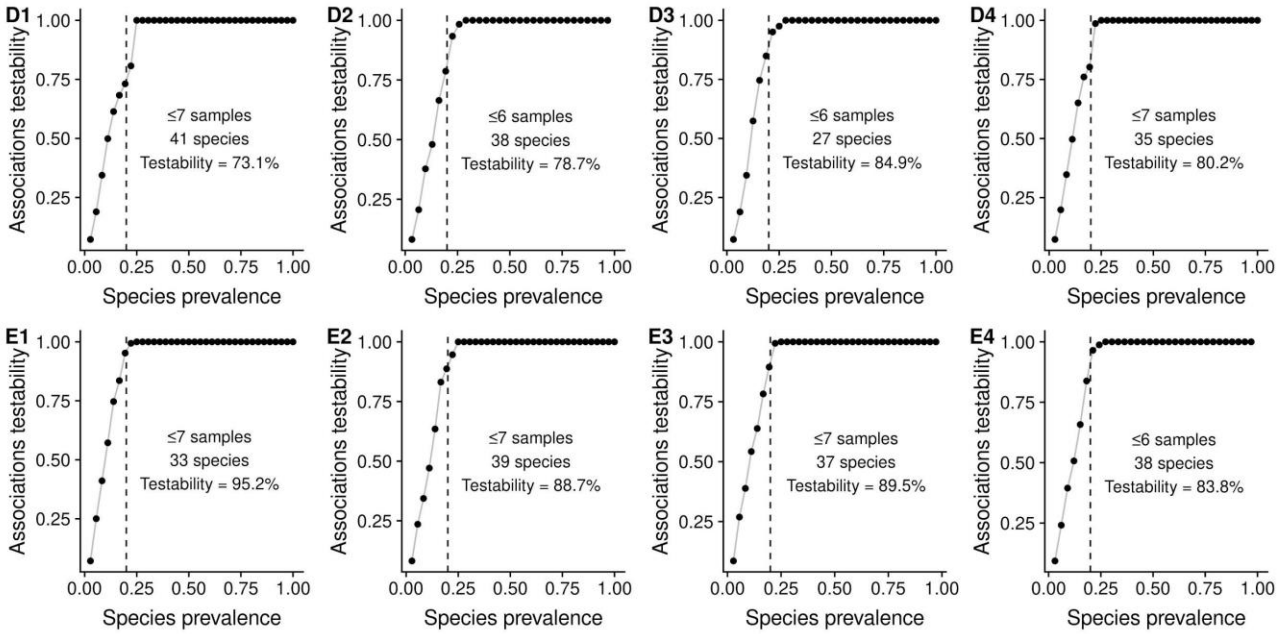

Figure 1 – Fraction of testable associations depending on species prevalence in each experimental unit. The dashed vertical line indicates the species prevalence threshold of 20%, which appeared as a good trade-off between testability and species number. Several metrics are provided (minimum number of samples, final number of species and testability value) for this threshold.

Methods S2 - R code for searching the literature for the associations in the pathobiome

```
### TEXT-MINING OF PUTATIVE ASSOCIATIONS WITH SYNONYMS SEARCH
### Charlie Pauvert
### 2019-05-31

### SYNONYMS SEARCH

# Extracting fungal synonyms from MycoBank (unpublished)
# based on the API documented here
# http://www.mycobank.org/Services/
#  Generic/Help.aspx?s=searchservice
get_synonyms<-function(species){
  require(xml2)
  base_url<-paste("http://www.mycobank.org/",
                  "Services/Generic/SearchService.svc/",
                  "rest/xml?layout=14682616000000161&filter=name CONTAINS")
  # Sanitize species name and url
  url<-paste0(base_url,' "',gsub("_"," ",species),"") # Add the quotes!
  url<-gsub(" ","%20", url)
  message("Checking for ", species)
  # Fetch XML from MycoBank
  x<-read_xml(url)
  # Get the obligate synonyms
  oblig<-xml_text(xml_find_first(x,"//obligatesynonyms_pt_"))
  anamo<-xml_text(xml_find_first(x,"//anamorph_pt_"))
  # Extract the name
  f<-function(x) gsub(".*<Name>([A-Z][a-z]+ [a-z]+)</Name>.*","\\1",x)
  # Output
  c("CurrentName"=gsub("_"," ",species),
    "ObligateSyn"=f(oblig),
    "AnamorphSyn"=f(anamo)
  )
}
# List of lists of synonyms! Empty strings when unavailable
# The fungal name can have space or underscore

(examples.syn<-lapply(
            c("Erysiphe_necator","Mycosphaerella tassiana"), get_synonyms))
# [[1]]
# CurrentName        ObligateSyn        AnamorphSyn
# "Erysiphe necator" "Uncinula necator"    "Oidium tuckeri"
#
# [[2]]
# CurrentName                ObligateSyn                AnamorphSyn
# "Mycosphaerella tassiana"      "Davidiella tassiana" "Helmisporium vesiculosum"

# Function to restore the current name if looking at a synonym
get_current<-function(sp,synonyms){
  # Find in which sublists the species belongs
  i<-sapply(synonyms, function(bar) sp %in% bar)
  # and get its name
  synonyms[i][[1]][["CurrentName"]]
}
get_current("Oidium tuckeri",examples.syn)
# [1] "Erysiphe necator"
```

```
### TEXT-MINING OF PUTATIVE ASSOCIATIONS
# Construct a List of lists of synonyms!
# Empty strings when unavailable
patho.syn<-lapply(V(patho.net)$name, get_synonyms)
# Reduce to a single vector
patho.syn.vec<-Reduce(c,patho.syn) %>% unique() %>% .[.!=""]
# Generate pairs between (Erysiphe necator and its synonyms) AND the others
patho.pairs<-expand.grid(patho.syn.vec[1:3],patho.syn.vec[-c(1:3)],
                         KEEP.OUT.ATTRS = F,stringsAsFactors = F)

# Basd on rscopus library
# to be installed using devtools::install_github("muschellij2/rscopus")
library(rscopus)
rscopus::set_api_key("INSERT SCOPUS API KEY HERE")
# Needs a data.frame Var1 Var2 with all the combinations
#  of species wanted to be searched for
text_cooccurrence<-function(pair){
  # Abbreviate the species name like E. necator
  i<-function(x) gsub("([A-Z])[a-z]+ ([a-z]+)","\\1. \\2",x)
  # Build query
  query<-paste0('ALL("',pair$Var1,'" OR "',i(pair$Var1),'") ',
                'AND ALL("',pair$Var2,'" OR "',i(pair$Var2),'")')
  message("Querying ",pair$Var1," vs. ",pair$Var2)
  r_search <- scopus_search(query = query,view = "STANDARD",
                  field = "dc:title,prism:doi",verbose = F,
                  max_count = 25,count = 25)
  gen_entries_to_df(r_search$entries)$df
}
# Apply the search function to all pairs constructed earlier
patho.tm.scopus<-adply(patho.pairs,1, text_cooccurrence)
# Rename back the species to current name to build a summary of the search
patho.tm.scopus %>%
  mutate(
    Var1 = gsub(" ","_",sapply(Var1,get_current,synonyms = patho.syn)),
    Var2 = gsub(" ","_",sapply(Var2,get_current,synonyms = patho.syn))
    ) %>% group_by(Var1,Var2) %>%
    dplyr::summarise(  n = length(na.omit(unique(`prism:doi`)))) %>%
  ungroup() %>% filter(n > 0) %>% data.frame()
```

Annexe 2: Different patterns in leaf-associated viromes and mycobiomes of wild plant populations between cultivated and natural ecosystems

Yuxin Ma[1], Tania Fort[2], Armelle Marais[1], Marie Lefebvre[1], Sébastien Theil[1,3], Corinne Vacher[2] and Thierry Candresse[1]*

[1] UMR 1332 BFP, INRA, Univ. Bordeaux, CS20032, 33882 Villenave d'Ornon cedex, France

[2] UMR BioGeCo, INRA, Univ.Bordeaux, 33615 Pessac, France

Abstract

Plants are colonized by complex microbial communities (bacteria, fungi, viruses and other microorganisms) that affect plant growth and survival as well as ecosystem functions. However, research on leaf-associated fungal and viral communities, especially in wild plants, has been so far fairly limited. Using metagenomic approaches to characterize the plant core microbiome, we assessed the richness and composition of leaf-associated fungal and viral communities from complex pools of herbaceous wild plants collected in cultivated and natural ecosystems. We identified 161 fungal families and 18 viral families comprising 249 RNA dependent RNA polymerase-based operational taxonomy units (RdRp OTUs) from the leaf samples. Fungal culturomics captured ca. 9% of the fungal diversity but there was virtually no correlation between the plant samples virome and that from fungal cultures. Mycobiome and, more markedly, virome composition showed a strong site specificity. The mycobiomes were more diverse in unmanaged sites while the plant associated viromes showed a higher family-level richness in cultivated sites, suggesting that mycobiome and virome are shaped by different drivers. Further efforts will be needed to confirm these trends in other settings and to begin to unravel the drivers contributing to the structuring of plant-associated fungal and viral populations.

Keywords: Mycobiome, mycovirus, phytovirus, plant, virome

Introduction

Plant leaves represent one of the largest microbial habitats on Earth (Morris, 2001) and harbor hyperdiverse microbial communities including bacteria, archaea, fungi, yeasts and viruses (Lindow & Brandl, 2003; Vorholt, 2012; Koskella, 2013; Vacher *et al.*, 2016). These microbial communities influence plant health (Arnold *et al.*, 2003; Hacquard *et al.*, 2017; Saleem *et al.*, 2017), plant nitrogen nutrition (Fürnkranz *et al.*, 2008; Moyes *et al.*, 2016; Doty, 2017), ecosystem primary productivity (Laforest-Lapointe *et al.*, 2017) and biogeochemical cycles (Osono, 2006; Morris *et al.*, 2014; Bringel & Couée, 2015). Despite their potential importance for natural and managed ecosystems, our knowledge of leaf microbial communities remains very limited to date, compared to that of root and rhizosphere communities. Experimental studies are needed to better understand the functions of leaf microbial communities (Rosado *et al.*, 2018) and predict their dynamics in response to global change (Laforest-Lapointe & Whitaker, 2019). Viruses and fungi, in particular, should be better integrated in future studies of the leaf microbiome (Laforest-Lapointe & Whitaker, 2019).

Leaf-associated viruses include viruses of plants (phytoviruses), viruses of fungi (mycoviruses), and viruses of bacteria and archaea (phages). A large fraction of plant-associated viruses in wild plants are double-stranded RNA (dsRNA) viruses infecting either plants (named persistent viruses) or fungi (named mycoviruses) or possibly both. A recurrent question in plant-associated virome analysis, in particular when it comes to dsRNA viruses, concerns the identity of the host(s) of the identified viruses (Roossinck, 2015a), so that the proportion of phytoviruses to mycoviruses in plant-associated viromes is still unknown (Roossinck, 2015a).

Phytovirus diversity has been grossly underestimated so far, as highlighted recently by high throughput sequencing (HTS)-based metagenomic studies (Roossinck *et al.*, 2010; Rosario & Breitbart, 2011; Roossinck, 2012; Shi *et al.*, 2016). For example, the newly released 2018b ICTV taxonomy lists a total of 4958 virus species, however only about 1337 of them (~27.0%) are plant viruses (Siddell *et al.*, 2019). This percentage is to be compared with the ca. 5500 known mammalian species contrasted with the estimated ca. 400.000 described plant species and ca. 2.000 new plant species described each year. One of the reasons for this situation is that viruses have been traditionally thought as pathogens though many are beneficial to their hosts, providing essential functions in some cases and conditionally beneficial functions in others (Roossinck, 2011). For example, several acute plant viruses confer conditional mutualism by enhancing drought tolerance in plants (Xu *et al.*, 2008). The studies has been biased and largely focusing on viruses causing visible symptoms in economically important crops (Wren *et al.*, 2006) which comprise only a minute fraction of all plant species. In addition, there is now

evidence that only a small fraction of viruses are associated with obvious disease (Roossinck, 2005). Taken together, these biases suggest that there exists a huge gap in our understanding of plant virus diversity, evolution and ecology.

To bridge this gap, some recent studies focused on virus populations of wild plant populations (Thapa *et al.*, 2015; Bernardo *et al.*, 2018; Susi *et al.*, 2019), which are likely to represent reservoirs for both known viruses and novel, emerging agents (Anderson *et al.*, 2004; Cooper & Jones, 2006; Elena *et al.*, 2014; Stobbe & Roossinck, 2014; McLeish *et al.*, 2019). These studies revealed that virus infection is quite common and often asymptomatic in wild plants (Roossinck, 2012), unfortunately, wild plant populations has been longtime neglected. Therefore to adjust this bias, wild plants can be an important research object for the exploration of viral diversity at plant individual, population, species or even ecosystem scales.

Both mycobiomes and viromes are shaped by abiotic and biotic environmental factors. In woody perennials, for example, Jumpponen and Jones's studies showed that phyllosphere fungal communities in temperate *Quercus macrocarpa* appear distinct between trees in urban and nonurban environments, possibly as a consequence of geographic distance, air pollution, human management etc. (Jumpponen & Jones, 2009; Jumpponen & Jones, 2010). Phyllosphere fungal richness of *Quercus ilex* in a mixed Mediterranean forest increased with summer season and long-term drought (Penuelas *et al.*, 2012) while in *Fagus sylvatica* mycobiome composition was significantly correlated with elevation gradient and microclimate (Cordier *et al.*, 2012). More recently, host genotype has been shown to be an important determinant of phyllosphere mycobiomes for a wide range of cereal (Sapkota *et al.*, 2015). The study of the wheat canopy mycobiome showed geographical location as a major factor along with leaf position, growth stage and cultivar identity (Sapkota *et al.*, 2017). In our literature survey, so far there are very few studies focusing on wild herbaceous plants associated mycobiomes. Virus communities may influence microbial communities by modulating microbial population size, diversity, metabolic output, and gene flow (Brum *et al.*, 2015). However, the study of phytoviruses at the community scale across ecosystems, and of the potential drivers shaping the communities was only initiated very recently (Roossinck, 2015b; Thapa *et al.*, 2015). The environmental factors determining plant virus community composition in both cultivated and natural ecosystems thus remain largely unknown (Malmstrom *et al.*, 2011). One recent study investigated virome composition in 6 native plant species from 20 sites over 4 years to test the effects of host identity, location, and sampling year (Thapa *et al.*, 2015). The results showed that only host species identity was significantly correlated with virome composition. Recent results also suggest that some viral families are more adapted to cultivated ecosystems, while others are more adapted to natural

ecosystems (Bernardo *et al.*, 2018). A few other studies focusing on specific plant or virus species showed that latitude, climate and seasonality were correlated with variations in virome composition (Coutts & Jones, 2002; Cadle-Davidson & Bergstrom, 2004; Seabloom *et al.*, 2010).

Plant-associated fungi and viruses interact but in most cases, the output of the interaction on the phenotype and fitness of the fungal partner remains unknown. Many mycoviruses induce no obvious infection phenotype in their fungal host, they appear to be latent. On the other hand, some mycoviruses have been shown to impact positively or negatively (hypovirulence) the fitness and pathogenicity of their fungal host(s) (Nuss, 2008; Ghabrial *et al.*, 2015). The interactions between phytoviruses and plant-associated fungi are even more poorly described. There is however some circumstantial evidence that fungi-plant interactions might impact plant-phytovirus ones. For example, the mycorhizal fungal species *Piriformospora indica* can interfere with pepino mosaic virus (PepMV) accumulation in tomato apical shoots, depending on the light intensity (Fakhro *et al.*, 2010), while tomato colonization by PepMV can be inhibited by *Verticillium* spp. or by the oomycete *Pythium aphanidermatum* (Spence *et al.*, 2006; Schwarz *et al.*, 2010). Conversely, virus occurrence and symptomatology were increased by arbuscular mycorrhizal fungi in tobacco (Shaul *et al.*, 1999).

The aim of the present study was to deepen the knowledge of viral and fungal communities associated with wild plants, by combining metagenomics and culture-based approaches. We collected wild plant leaves in both cultivated and natural ecosystems to determine whether (1) ecosystem management jointly influences the composition of fungal and viral communities, and whether (2) these communities are richer in natural ecosystems than in cultivated ecosystems. These hypotheses were tested using four subsets of leaf-associated microbial communities, namely the phytovirome (encompassing all viruses recovered by metagenomics), the mycovirome (all viruses derived from fungal cultures), the mycobiome (all fungi recovered by meta-barcoding from leaves) and the mycoculturome (all fungi recovered by culturomics).

Materials and Methods

Study sites and sampling design

Herbaceous wild plants and weeds were sampled in 2017 in four sites in southwest France (Table S1). Two sites (VO and CT) were cultivated, horticultural agroecosystems in which vegetable crops were grown. The VO site harbored a large range of crops, including lettuce, spinach, pepper, turnip, while the CT site mostly had carrots. Two other sites (INRA and SP) were natural, dry grasslands (Table S1). In each site, a total of ca. 200 individual plants representing the locally most abundant species were collected (Table S1). The number of

sampled plant species ranged from 29 (SP site) to 40 (VO site) with an identical number of individuals for each plant species. Crop plants were not sampled in the cultivated sites. Individual plants were selected at random but plants with necrotic tissues or with insect infestation were not collected. Plants were identified in the field by botanists and subsequently stored in a cool ice chest before being brought back and processed in the laboratory.

Leaf processing for phytovirome and mycobiome analysis

For each site, 4 multi-species pools of ca. 50 plants were assembled from the ca. 200 collected plants. All individual plants for a given species were allocated to the same pool, so that the 4 pools of a given site did not share any plant species (Fig. 1). For virome description, the multi-species pools were assembled using 0.1 g fresh leaf blade of each individual plant (ca. 5 g in total). For mycobiome description, the multi-species pools were assembled using 0.5 mg of leaf blade dessicated over anhydrous calcium chloride dry weight (ca. 25 mg).

Fungi culturing for mycoculturome and mycovirome analysis

In order to culture out fungi from the plant samples collected at each sampling site, a dilution strategy (Unterseher & Schnittler, 2009) was used. For each site, the sampled 200 individual plants were divided in ca. 10 multi-species pools (with no plant species shared between pools) (Fig. 1). Pools were assembled from ca. 20 leaf fragments of 1 cm^2 each individual plant) which were added to sterile Erlenmeyer flasks containing 15 ml sterile water with 0.1% Tween 20 (Fig. 1). The flasks were then incubated at room temperature on an orbital shaker for 20 minutes before filtering with sterile gauze. Based on pilot experiments, the filtered solution was then serially diluted 10, 100 and 1000 times and 500 µl aliquots used to inoculate respectively 10 plates of malt agar (MA) and of potato dextrose agar (PDA) containing 0.025% chloramphenicol. Plates were incubated at 22°C and observed regularly for development of fungal colonies. All developing fungal colonies were isolated from the original plates and transferred to new petri dishes (4 isolates per plate) containing culture media covered by cellophane in order to facilitate the final collection of mycelia. Grown mycelia (ca. 3.5 cm in diameter) were recovered, transferred to plastic tubes and lyophilized. For dsRNA extraction for mycovirome characterization mycelia were assembled in pools (ca. 1 mg dry weight per mycelium for a total of ca. 0.48-0.64 g dry weight per pool) while for DNA extraction for mycobiome analysis, the mycelia were assembled in pools of ca. 250 colonies (ca. 0.1 mg dry weight per mycelium for a total of 25 mg per pool) (Fig. 1).

Double stranded RNAs extraction, whole genome amplification (WGA) and Illumina sequencing for phytovirome and mycovirome analysis

Double stranded RNAs (dsRNAs) were extracted from each plant and fungal pool by two rounds of CF11 cellulose chromatography as described by Marais *et al.* (2018). A blank control using only reagents was prepared in parallel with every extraction. For conversion to cDNA and random amplification of dsRNAs, 3 µl of purified dsRNAs were denatured at 99°C for 5 min and submitted to a reverse transcription step initiated by a mixture of primers consisting of 1 mM dT18 and 2 mM PcDNA12 (5' TGTGTTGGGTGTGTTTGGN$_{12}$ 3') using the SuperscriptII Reverse Transcriptase according to the manufacturer's instructions (Invitrogen). A random whole genome amplification (WGA) was performed using the obtained complementary DNAs (cDNAs), allowing at the same time the tagging of pools from the same site with a specific site-level multiplex identifier (MID) adapter (Marais *et al.*, 2018). PCR products were purified using the MinElute PCR Purification Kit (QIAGEN). Finally, for dsRNA plant samples, equal amounts of PCR products obtained from each of the 4 plant pools corresponding to a sampling site were assembled in one library integrating the 200 sampled plants. The same pooling strategy was performed on dsRNAs of fungal cultures for mycovirome analysis, therefore a library integrating all the fungi corresponding to a sampling site was prepared (Fig. 1). A minimum of two independent amplifications with different site-level MID tags were performed for each pool and the libraries were prepared as described above. The prepared libraries, including negative controls (buffer-only libraries) for plant samples and cultured fungi dsRNA extractions (Fig. 1) and were sequenced in multiplexed format (2×150 bp) on an Illumina HiSeq 3000 system at the GenoToul platform (INRA Toulouse, France).

Bioinformatics for phytovirome and mycovirome analysis: reads cleaning, assembly, contigs annotation and Operational Taxonomic Units (OTU) identification

Virome analysis was performed using the virAnnot pipeline (Lefebvre *et al.*, 2019). More precisely, the raw sequence reads were first demultiplexed and the MID tags removed using the *cutadapt* tool (Martin, 2011). To reduce the cross-talk between samples caused by index hopping (Illumina, 2017; van der Valk *et al.*, 2019), only paired-end reads with identical MID tags identified in both members of the pair were retained for the next steps. In some cases, to compensate for uneven sequencing depth between libraries, a normalization step was performed by pooling the sequences derived from different replicates and randomly subsampling libraries size to the same depth using the seqtk tool (https://github.com/lh3/seqtk). Therefore, one final phytovirome for each site were generated (Fig. 1).The clean paired-end reads were *de novo* assembled into contigs using the IDBA-UD

assembler (https://academic.oup.com/bioinformatics/article/28/11/1420/266973). Contigs were annotated using BlastN and BlastX against the non-redundant nucleotide (nt) and protein (nr) Genbank databases with a conservative e-value cut-off of 10^{-4}. A clustering approach (Lefebvre *et al*., 2019) was used to define operational taxonomy units, following the strategy highlighted by (Simmonds, 2015). Briefly, a search for RNA-dependent RNA polymerase (RdRp) conserved motifs was performed on all contigs and those encoding a viral RdRp were retrieved and aligned with reference sequences. Distance matrices computed with the ETE3 toolkit (Huerta-Cepas *et al.*, 2016) were used to cluster into a single OTU all contigs diverging by less than 10%. This 10% threshold was shown to generate, in many viral families, OTUs that are a relatively good approximation of taxonomic species (Lefebvre *et al.*, 2019). This allowed us to generate an OTU table indicating, for each sampling site, the presence/absence and the number of reads corresponding to each OTU.

DNA extractions, ITS1 and ITS2 amplification and Illumina sequencing for mycobiome and mycoculturome analysis

Total DNA was extracted from each multi-species pool of plants and cultured fungi (Fig. 1) using two different kits, the PowerSoil® DNA Isolation Kit (MO BIO) and the DNeasy Plant Mini Kit (QIAGEN), according to the instructions of the kits. The internal transcribed spacer 1 and 2 regions (ITS1 and ITS2) were first amplified according to (Op De Beeck *et al.*, 2014) using primer pairs ITS1F/ITS2 and ITS86F/ITS4, respectively, tailed with Illumina adaptors (https://web.uri.edu/gsc/files/16s-metagenomic-library-prep-guide-15044223-b.pdf) in our lab.The following steps were all performed by the PGTB Facility (INRA – Pierroton, France) including library preparation, sequencing and demultiplexing. In detail, the first PCR products were added adapters throng a second PCR, then the PCR products were purified using platform-specific SPRI magnetic beads (1X ratio) and quantified using Quant-it dsDNA Assay kit. The libraries were pooled equimolarly with a Hamilton Microlab STAR robot and sequenced on an Illumina MiSeq platform using the MiSeq Reagent Kit v3 (PE 2 x 300 bp). Sequences were cut to 250 bp and demultiplexed with exact index search.

Bioinformatics for mycobiome and mycoculturome analysis

Metabarcoding datasets for ITS1 and ITS2 were similarly processed. The ITS primers were first removed, and the sequences were then filtered, trimmed, merged and chimeras removed using the open-source software package DADA2 ITS Pipeline Workflow (1.8) (https://benjjneb.github.io/dada2/ITS_workflow.html) running in R (Callahan *et al.*, 2016a) with parameters in detail described in supporting information Methods S1 and S2. ASV

taxonomic assignments were subsequently conducted with the RDP classifier (Wang *et al.*, 2007) embedded within DADA2 and trained with the UNITE general FASTA release for Fungi-version 18.11.2018 (https://dx.doi.org/10.15156/BIO/786343) (UNITE, 2019). The ASV, taxonomy tables and sample metadata tables were integrated into one phyloseq object (Methods S3). Only fungal ASVs were retained for further analyses (McMurdie & Holmes, 2013; Callahan *et al.*, 2016b). The decontamination procedure of Galan *et al* (2016), specifically Tcc threshold value, was used in the present study for correcting of cross contamination since no positive control was included.

In order to be able to compare the fungal community richness and diversity between plant pools, the ASV tables were resampled to the minimum sequencing depth observed in the datasets (corresponding to 23270 reads/sample for ITS1 and 15297 reads/sample for ITS2) using the '*rarefy_even_depth*' function in the Phyloseq package in R (McMurdie & Holmes, 2013) (Methods S4).

Alpha and beta diversity analyses

Alpha diversity analyses and visualization of mycobiome community composition were performed in R using the Phyloseq package (McMurdie & Holmes, 2013) for data import and richness metrics calculation and using the ggplot2 package for visualization (Wickham, 2016) (Methods S5). The alpha-diversity variations among samples were analyzed using Generalized Linear (Mixed) Models including both ecological factors (Ecosystem type, region, sampling season) and methodological factors (DNA extraction kit, sequencing depth, number of plant species). Principal coordinates analysis (PCoA) (Ramette, 2007) were performed on distance matrices estimated with the binary Jaccard index (Hamers *et al.*, 1989) method and the Principal component analysis (PCA) was performed based on CLR-transformed counts data. in R (Methods S5). Permutational analyses of variance (PERMANOVA), using the *adonis* function (Anderson, 2001; McArdle & Anderson, 2001) were used to estimate the effects of various factors (sampling site, ecosystem type, region, extraction kit…) on mycobiome composition (Methods S5). Given that a better performance was obtained with the ITS1 amplicons (larger number of filtered reads, richer ASV mycobiomes), only the ITS1 information was subsequently analyzed in detail. On the other hand, a comparable but slightly better performance was obtained with PowerSoil® DNA Isolation Kit (MO BIO) (2 samples with no initial amplification using the DNeasy Plant Mini Kit) so that the reads corresponding to the ITS1 amplicons obtained from DNA extracted with PowerSoil® DNA Isolation Kit were used for the

downstream analysis. Overall, for each site, four mycobiomes, one phytovirome, one mycoculturome, and one mycovirome were analyzed (Fig. 1).

Results

Phytovirome diversity and compositional variation in different environments

Overall, more viral reads and viral contigs were detected for the CT site followed in turn by the VO, SP and INRA sites (Table 1). The ratio of dsRNA/ssRNA reads ranged from 0.7 to 1.6 except for the SP site where it reached a high value of 19.6 possibly linked to a saturation of the amplification libraries by reads from *Endornaviridae* members (81.9%) (Table S3). Few reads annotated as retro-transcribing viruses were discovered only from the VO and CT sites while virus-associated RNAs reads were only detected for the INRA site. Besides the SP site, *Endornaviridae* also accounted for a large proportion of the CT virome (30.9%), followed by *Alphaflexiviridae* (17.4%) (Table S3). Based on a Blast-based annotation, a total of 17 viral families were discovered from these four sites with respectively 15 and 14 families for VO and CT, but only 11 and 7 for SP and INRA. The family-level richness of the virome thus appears to be higher for the cultivated than for the unmanaged sites, an observation associated with the absence of several single-stranded RNA (ssRNA) virus families (*Bromoviridae*, *Secoviridae*, *Virgaviridae* and *Benyviridae*) and of the *Caulimoviridae* pararetroviruses from the unmanaged sites (Table S3). On the contrary, double-stranded RNA (dsRNA) virus families *Partitiviridae* and *Totiviridae* and ssRNA families *Endornaviridae*, *Alphaflexiviridae* and *Tombusviridae* were present in all four sites (Table S3).

A viral RdRp clustering approach was used to define operational taxonomic units (OTUs) at a level close to ICTV species using the virAnnot pipeline (Lefebvre *et al*., 2019). In total, 190 viral RdRp OTUs were identified (Table S4) representing 16 viral families. Respectively 73, 50, 55 and 26 OTUs representing 13, 12, 9 and 5 viral families were respectively discovered from the VO, CT, SP and INRA sites, confirming the higher viral family richness in the cultivated sites (Table 1 and Fig. 2a). The lower number of families identified for each site results from the constraint that any virus for which the RdRp core-encoding region is missing (due to incomplete genome coverage) will not be considered by this approach. The OTU-based analysis therefore provides a lower bound of viral diversity while allowing to analyze the virome at a rank closer to taxonomic species. Double-stranded viruses OTUs account for a larger proportion (54.0% to 70.9%) than ssRNA viruses OTUs (21.8% to 28.0%), except for the INRA site in which the situation was reversed (42.3% ssRNA OTUs vs 30.8% dsRNA ones). Between 7.3% and 26.9% of OTUs could not be annotated by Blast at family level, depending on the site (Fig. 2b). Overall, and as already seen in other phytovirome studies, a large fraction of the detected OTUs (83.7%

to 96.3%) putatively correspond to novel viruses since no RdRp-encoding sequence in Genbank fullfiled the identity criterion (>=90% nt or aa identity) to be included in the corresponding OTU (Table 1 and Fig. 2b). The majority of the OTUs for which a Genbank counterpart could be identified correspond to ssRNA viruses (Table S4 and Fig. 2b).

Alpha diversity of fungal communities from mycobiome libraries in different sampling sites

Taking only into account the 16 ITS1 libraries extracted with Powersoil kit for the 4 sampling sites, a total of 1188 unique ASVs were discovered, comprising 4 phyla, 21 classes, 161 families and 247 genera. Of those unique ASVs, 361 ASVs appear to correspond to unknown/novel ASVs (at genus level) (Table S5). Fungal communities in each library were dominated by *Ascomycota* and *Basidiomycota,* with a relative abundance of 53.2%+/-21.0% and 46.4%+/-20.8%, respectively. At class level, *Dothideomycetes* and *Tremellomycetes we*re dominant ones with a relative abundance of 40.2%+/-19.1% and 39.6%+/-20.6% (Table S6).

Richer and more diverse fungal communities were observed for the unmanaged SP and INRA sampling sites (Fig. 2c) with an average of 191.3+/-54.8 and 252.3+/-34.3 ASVs per library, respectively, and with a total of 483 and 639 unique ASVs, respectively (Table S7). This translates in higher fungal diversity in these sites (Fig. 2d) with average Shannon indexes of 3.72+/-0.33 and 3.86+/-0.15, respectively, as compared to values of 3.0+/-0.41 and 2.72+/-0.41 for the cultivated VO and CT sampling sites (Table S7). This difference in fungal diversity cannot be ascribed to a higher diversity of the sampled plant species since fewer plant species were sampled in the unmanaged sites than in the cultivated ones [respectively 29 (SP) and 33 (INRA) sampled plant species as compared to 34 (CT) and 40 (VO).

Mycobiome composition: largely consistent within ecosystem whatever sampled host plants but specific between sampling sites

For each sampling site, 4 different plant pools were analyzed, which assemble different plant species. It is thus possible to use our data to compare the mycobiome of different plant species growing together at the same sampling site. The analysis of Venn diagrams of fungal ASVs within each sampling site show that a significant proportion of the ASVs detected are shared between plant pools with 31.1% to 42.9% (on average 35.5%+/-5.2%) of ASVs shared between at least two pools and a core of on average 12.4% +/- 4.8% of ASVs shared between all pools of a sampling site (extremes 9.2-19.3%) (Fig. S1). For these core ASVs of each site (31, 45, 53 and 59 ASVs for VO, SP, CT and INRA, respectively, Fig. S1), a significant proportion (55.3%) are shared between at least two sites, suggesting they correspond to broadly distributed fungal

taxa. The 14 most common ASVs shared between all the 16 tested libraries are annotated as *Alternaria infectoria*, *Bensingtonia* sp., *Botrytis caroliniana*, *Cystofilobasidium macerans*, *Epicoccum nigrum*, *Filobasidium stepposum*, *Filobasidium wieringae*, *Holtermanniella wattica*, *Mycosphaerella tassiana*, *Sporobolomyces roseus*, *Stemphylium* sp., *Symmetrospora coprosmae*, *Vishniacozyma carnescens*, *Vishniacozyma victoriae* (Table S5).

Subsequently, the compositional dissimilarities between pools were quantified using a Jaccard metric calculated on presence/absence (binary) data of ASVs (Table S5). Principal coordinates analysis (PCoA) and hierarchical clustering analysis (HCA) on the distance matrixes revealed that fungal communities from pools of a sampling site (that therefore share no common plant species) are more closely related than pools from different sites (Fig. 3), so that the composition difference is strongly correlated with the sampling site (ANOSIM test: R=0.89, p =1E-04) (Table S8 and Fig. 3). Secondly, factors such as ecosystem type (cultivated/natural) and geolocation (Bordeaux/Bergerac) also contribute to the composition dissimilarity with respectively R =0.52, p =4E-04 and R=0.48, p = 3E-04 (Table S8 and Fig. 3). The contributions of factors causing the compositional difference were also tested by ADONIS statistics, providing essentially similar results in particular for sampling site contribution (R^2 =0.37, p =1E-04), followed by ecosystem type and geolocation (R^2 >0.13, p < 5E-04) (Table S8).

Mycoculturome and mycovirome diversity analysis following a culturomics approach

Several families of dsRNA viruses have members with either plant or fungal hosts, so that it is not easy to decide whether the agents detected are *bona-fide* plant-infecting viruses or infect fungi associated with the plant samples analyzed. The situation is further complicated by recent reports of cross-kingdom transmission (Andika *et al.*, 2017; Nerva *et al.*, 2017). In an effort to begin to address this complex question, we characterized the mycobiome and mycovirome of fungal populations that had been cultured from the plant populations sampled in our 4 study sites.

From 480 to 1270 fungal colonies were obtained for each sampling site through a culturomics approach (Table S9). Using the cultured fungal pools thus obtained, fungal metabarcoding and dsRNA-based virome analyses were then performed in the same fashion as for the plant pool samples. As expected and despite the relatively large number of cultivated colonies involved in these experiments, the cultivated fungal ASV output data shows that only a small fraction of the ASVs identified from the plant samples were identified among the cultivated fungi ASVs (4.8% to 13.8%, average 9.0% +/- 3.9%) (Table S9 and Fig. S2). Although a significant fraction of the cultivated fungi ASVs were not detected by the metabarcoding performed on the plant samples

(15.4% to 42.9%) an even larger fraction had already been detected from the plant samples (57.1 to 84.6%, average 67.5% +/- 12.6%) (Table S9 and Fig. S2).

In order to maximize the ability to detect shared viruses between the plant- and fungus-associated viromes the non-normalized datasets were used. Remarkably, the viromes obtained from the cultivated fungal pools were almost completely different from the viromes obtained from the plant pools. In total, based on the Blast annotation, the mycoviromes collectively comprised 14 viral families (7 ssRNA families and 7 dsRNA families, Table S10). *Totiviridae*, together with *Chrysoviridae*, *Endornaviridae*, *Alphaflexiviridae* and *Partitiviridae* were detected from all the mycoviromes and phytoviromes. On the other hand, a range of families were only detected from the cultured fungi, including the *Gammaflexiviridae*, *Hypoviridae*, *Tymoviridae*, *Narnaviridae*, *Fusarividae and Birnaviridae*. Also contrasting with the phytovirome data, the mycovirome of the INRA site proved not less diverse than at other sites with 8 families discovered (Table S10). At the more precise viral RdRp OTU level, although a large fraction of the fungal ASVs in cultured fungal pools are shared with the plant samples mycobiomes (average 67.5% +/- 12.6%, see above), the viromes from the two types of samples were found almost totally different, with only 2 OTUs shared for the CT site (out of a total of 29), while no shared OTU could be detected in the other 3 sites out of a total of 54 viral OTUs detected from the corresponding fungal cultures (Fig. S2). The reciprocal mapping of the reads of one virome type against the contigs of the other type confirmed that only a very minor fraction of agents is shared between the plant and fungal cultures derived viromes (data not shown).

Discussion

While the sampled host plant species certainly affects the fungal community, a core mycobiome was shared, for a given site, between plant pools which gather different plant species. For each site, 31.1% to 42.9% of the mycobiome ASVs were present in at least two pools (Fig. S1), the corresponding core mycobiome representing a "signature" of the sampling site but also containing some widespread ASVs also represented in the mycobiome of other sampling sites. This core mycobiome, in particular site-specific ASVs, explains the clustering and PCoA analysis that group together different plant pools from a given site and unambiguously separate them from other sampling sites (Fig. 3). Theses variations are potentially associated with their mutual environments. As reviewed in Vacher *et al.* (2016), environmental conditions have been recognized to significantly affect the assemblage of phyllosphere fungi such as elevation, landscape, climatic condition of a continent and across latitudes, and season.

The comparison of the aggregated mycobiomes from different sites showed that leaf-associated mycobiomes are consistently richer in natural than in cultivated sampling sites (Fig. 2c, 2d).

Even if cultivated plants were not sampled in the results reported here, they represent a high proportion of the plant biomass at the cultivated sampling sites and the lower diversity of their mycobiome (Compant *et al.*, 2019) may have impacted that of the weeds and wild plants growing nearby. Another hypothesis could be that fungicide treatments applied in cultivated sites may have reduced fungal diversity on the sampled plants. These two hypotheses are, by the way, not mutually exclusive.

Remarkably, a different picture emerged from phytovirome analysis in that more viral families were found from cultivated ecosystems than from unmanaged sampling sites (Table 1). This result parallels that of Bernardo *et al*. (2018) who also observed a higher family level virus diversity in cultivated areas. The results are less clear-cut when considering viral richness as estimated by the number of OTUs, which represent a proxy to viral species (Table 1). While diversity at the INRA unmanaged site was low and that at the VO cultivated site high, comparable and intermediate numbers of OTUs were observed in the other two CT and SP sites (CT cultivated, SP unmanaged). The finding of a lower viral richness for sites with a higher mycobiome diversity suggests that virome and mycobiome richness may not be influenced by the same drivers. Differences in dispersion mechanisms between fungi and viruses or the contrasted impact of fungicide treatments in mycobiomes and viromes are certainly among potential driver candidates. Domestication and cultivation, by reducing biodiversity have been suggested to be responsible for increased viral infections in cultivated ecosystems (Roossinck & García-Arenal, 2015). Such an effect may also have contributed to the results reported here if spill-over of frequent infections in crops contributes a significant share of the virome of weeds/wild plants growing side by side with the crops.

A study of Thapa *et al.* (2015) has demonstrated for a few selected plant species in an unmanaged ecosystem that host species played a significant effect on virome composition as compared to location and sampling time. The results reported here show extremely high site specificity of the phytoviromes, with a high fraction of 93.2% of viral OTUs solely detected in one of the study sites, to be compared with the corresponding values of 74.7% and 55% respectively for the mycobiome ASVs and the sampled plant species (Fig. S3). Under our experimental conditions, the virome therefore appears to be more site-specific than either the mycobiome or the sampled plant populations. It should however be considered that this observation is likely only valid for viruses present at a high frequency in the sampled plant populations. Indeed, with only 5-7 individual plants sampled per plant species, the ability to detect viruses with a low, less than 10% prevalence in the sampled species, would have been limited. It is therefore possible that deeper sampling of each plant species, involving more

numerous individual plants may provide in the future a different picture by allowing to take into account low prevalence viruses.

Fungal culturomics of plant leaves have made clear that *in vitro* culture-based approaches grossly underestimate fungal diversity (Roossinck, 2015a), and the results reported here are in line with this general observation. Indeed, only 4.8% to 13.8% of fungal ASVs were recovered here as fungal cultures (Table S10). However, it is noteworthy that the culturomics provided a significant fraction of cultivated fungi ASVs (15.4% to 42.9%, Fig. S2 and Table S9) or of viral OTUs that were not detected during the direct analysis of plant samples, highlighting the incompleteness of these efforts. The analyzed phytoviromes and mycoviromes, although derived from the same initial samples proved remarkably different. In particular a range of viral families we specifically detected from the mycoviromes: *Gammaflexiviridae* (all four sites), *Hypoviridae* and *Narnaviridae* (3 sites), *Fusariviridae* (2 sites), *Birnaviridae* and *Tymoviridae* (1 site) (Table S10). Similarly there was almost no congruence between phytoviromes and mycoviromes at the OTU level (Fig. S2). These results are in contrast with some observations, in particular those reported by Al Rwahnih *et al.* (2011) in which a limited culturomics effort, involving only 11 fungal colonies, allowed to demonstrate a mycovirus status for 5 of the 25 (20%) viruses identified in a grapevine virome. While the culturomics effort reported here is 2 to 3 orders of magnitude higher, the proportion of matched OTUs is at least one order of magnitude lower. One possible hypothesis to explain these differences may be linked to the pooling strategy used here which, while allowing the analysis of many more individual samples, may favor the detection of highly prevalent or high concentration viruses. In this respect, further efforts are clearly needed to better understand the links between the mycovirome and the plant-associated virome.

The results presented here provide a large scale parallel analysis of the virome, mycovirome and mycobiome associated with complex plant populations in cultivated and unmanaged ecosystems. While the results obtained confirm a higher viral family richness in cultivated environments (Bernardo *et al.*, 2018), they suggest that mycobiome and virome might be under the influence of different drivers, an observation that clearly deserves further confirmatory efforts.

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Author Contribution

Thierry Candresse and Armelle Marais designed the experiments and supervised the progress, Corinne Vacher provided many useful suggestion for ITS sequencing. Yuxin Ma performed the molecular experiments, data analysis and interpretation. Tania Fort provided R scripts and contributed to the data processing of fungal ITS datasets. YM and TC wrote the manuscript, TF and CV with other authors provided critical reading of this manuscript and its further improvement. Marie Lefebvre and Sébastien Theil developed the virAnnot pipeline and performed the viral sequence processing and OTU annotation.

References

Al Rwahnih M, Daubert S, Úrbez-Torres JR, Cordero F, Rowhani A. 2011. Deep sequencing evidence from single grapevine plants reveals a virome dominated by mycoviruses. *Archives of Virology* **156**(3): 397-403.

Anderson MJ. 2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* **26**(1): 32-46.

Anderson PK, Cunningham AA, Patel NG, Morales FJ, Epstein PR, Daszak P. 2004. Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. *Trends Ecol Evol* **19**(10): 535-544.

Andika IB, Wei S, Cao C, Salaipeth L, Kondo H, Sun L. 2017. Phytopathogenic fungus hosts a plant virus: A naturally occurring cross-kingdom viral infection. *Proceedings of the National Academy of Sciences of the United States of America* **114**(46): 12267-12272.

Arnold AE, Mejía LC, Kyllo D, Rojas EI, Maynard Z, Robbins N, Herre EA. 2003. Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences* **100**(26): 15649-15654.

Bernardo P, Charles-Dominique T, Barakat M, Ortet P, Fernandez E, Filloux D, Hartnady P, Rebelo TA, Cousins SR, Mesleard F, et al. 2018. Geometagenomics illuminates the impact of agriculture on the distribution and prevalence of plant viruses at the ecosystem scale. *The ISME journal* **12**(1): 173-184.

Bringel F, Couée I. 2015. Pivotal roles of phyllosphere microorganisms at the interface between plant functioning and atmospheric trace gas dynamics. *Frontiers in Microbiology* **6**: 486-486.

Brum JR, Ignacio-Espinoza JC, Roux S, Doulcier G, Acinas SG, Alberti A, Chaffron S, Cruaud C, de Vargas C, Gasol JM, et al. 2015. Patterns and ecological drivers of ocean viral communities. *Science* **348**(6237): 1261498.

Cadle-Davidson L, Bergstrom G. 2004. The effects of postplanting environment on the incidence of soilborne viral diseases in winter cereals. *Phytopathology* **94**(5): 527-534.

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP. 2016a. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods* **13**: 581.

Callahan BJ, Sankaran K, Fukuyama J, McMurdie P, Holmes S. 2016b. Bioconductor workflow for microbiome data analysis: from raw reads to community analyses [version 1; peer review: 3 approved]. *F1000Research* **5**(1492).

Clarke KR. 1993. Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* **18**(1): 117-143.

Compant S, Samad A, Faist H, Sessitsch A. 2019. A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. *Journal of Advanced Research*.

Cooper I, Jones RAC 2006. Wild Plants and Viruses: Under-Investigated Ecosystems. *Advances in Virus Research*: Academic Press, 1-47.

Cordier T, Robin C, Capdevielle X, Fabreguettes O, Desprez-Loustau ML, Vacher C. 2012. The composition of phyllosphere fungal assemblages of E uropean beech (F agus sylvatica) varies significantly along an elevation gradient. *New Phytologist* **196**(2): 510-519.

Coutts B, Jones R. 2002. Temporal dynamics of spread of four viruses within mixed species perennial pastures. *Annals of Applied Biology* **140**(1): 37-52.

Doty SL 2017. Endophytic N-Fixation: Controversy and a Path Forward. *Functional Importance of the Plant Microbiome*: Springer, 7-20.

Elena SF, Fraile A, García-Arenal F 2014. Evolution and emergence of plant viruses. *Advances in Virus Research*: Elsevier, 161-191.

Fakhro A, Andrade-Linares DR, von Bargen S, Bandte M, Büttner C, Grosch R, Schwarz D, Franken P. 2010. Impact of Piriformospora indica on tomato growth and on interaction with fungal and viral pathogens. *Mycorrhiza* **20**(3): 191-200.

Fürnkranz M, Wanek W, Richter A, Abell G, Rasche F, Sessitsch A. 2008. Nitrogen fixation by phyllosphere bacteria associated with higher plants and their colonizing epiphytes of a tropical lowland rainforest of Costa Rica. *The ISME journal* **2**(5): 561.

Galan M, Razzauti M, Bard E, Bernard M, Brouat C, Charbonnel N, Dehne-Garcia A, Loiseau A, Tatard C, Tamisier L. 2016. 16S rRNA amplicon sequencing for epidemiological surveys of bacteria in wildlife. *MSystems* **1**(4): e00032-00016.

Ghabrial SA, Caston JR, Jiang D, Nibert ML, Suzuki N. 2015. 50-plus years of fungal viruses. *Virology* **479-480**: 356-368.

Hacquard S, Spaepen S, Garrido-Oter R, Schulze-Lefert P. 2017. Interplay Between Innate Immunity and the Plant Microbiota. *Annual Review of Phytopathology* **55**: 565-589.

Hamers L, Hemeryck Y, Herweyers G, Janssen M, Keters H, Rousseau R, Vanhoutte A. 1989. Similarity measures in scientometric research: the Jaccard index versus Salton's cosine formula. *Inf. Process. Manage.* **25**(3): 315-318.

Huerta-Cepas J, Bork P, Serra F. 2016. ETE 3: Reconstruction, Analysis, and Visualization of Phylogenomic Data. *Mol Biol Evol* **33**(6): 1635-1638.

Illumina. 2017. Effects of index misassignment on multiplexing and downstream analysis.

Jumpponen A, Jones K. 2010. Seasonally dynamic fungal communities in the Quercus macrocarpa phyllosphere differ between urban and nonurban environments. *New Phytologist* **186**(2): 496-513.

Jumpponen A, Jones KL. 2009. Massively parallel 454 sequencing indicates hyperdiverse fungal communities in temperate Quercus macrocarpa phyllosphere. *New Phytologist* **184**(2): 438-448.

Koskella B. 2013. Phage-mediated selection on microbiota of a long-lived host. *Current Biology* **23**(13): 1256-1260.

Laforest-Lapointe I, Paquette A, Messier C, Kembel SW. 2017. Leaf bacterial diversity mediates plant diversity and ecosystem function relationships. *Nature* **546**(7656): 145-147.

Laforest-Lapointe I, Whitaker BK. 2019. Decrypting the phyllosphere microbiota: progress and challenges. *American Journal of Botany* **106**(2): 171-173.

Lefebvre M, Theil S, Ma Y, Candresse T. 2019. The VirAnnot Pipeline: A Resource for Automated Viral Diversity Estimation and Operational Taxonomy Units Assignation for Virome Sequencing Data. *Phytobiomes Journal*: PBIOMES-07-19-0037-A.

Lindow SE, Brandl MT. 2003. Microbiology of the phyllosphere. *Applied and Environmental Microbiology* **69**(4): 1875-1883.

Malmstrom CM, Melcher U, Bosque-Pérez NA. 2011. The expanding field of plant virus ecology: historical foundations, knowledge gaps, and research directions. *Virus Research* **159**(2): 84-94.

Marais A, Faure C, Bergey B, Candresse T. 2018. Viral Double-Stranded RNAs (dsRNAs) from Plants: Alternative Nucleic Acid Substrates for High-Throughput Sequencing. *Methods Mol Biol* **1746**: 45-53.

Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. journal* **17**(1): 10-12.

McArdle BH, Anderson MJ. 2001. Fitting Multivariate Models to Community Data: A Comment on Distance-Based Redundancy Analysis. *Ecology* **82**(1): 290-297.

McLeish MJ, Fraile A, García-Arenal F. 2019. Evolution of plant–virus interactions: host range and virus emergence. *Current Opinion in Virology* **34**: 50-55.

McMurdie PJ, Holmes S. 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PloS One* **8**(4): e61217.

Morris CE. 2001. Phyllosphere. *e LS*.

Morris CE, Conen F, Alex Huffman J, Phillips V, Pöschl U, Sands DC. 2014. Bioprecipitation: a feedback cycle linking Earth history, ecosystem dynamics and land use through biological ice nucleators in the atmosphere. *Global Change Biology* **20**(2): 341-351.

Moyes AB, Kueppers LM, Pett-Ridge J, Carper DL, Vandehey N, O'Neil J, Frank AC. 2016. Evidence for foliar endophytic nitrogen fixation in a widely distributed subalpine conifer. *New Phytologist* **210**(2): 657-668.

Nerva L, Varese GC, Falk BW, Turina M. 2017. Mycoviruses of an endophytic fungus can replicate in plant cells: evolutionary implications. *Scientific Reports* **7**(1): 1908.

Nuss D. 2008. Hypoviruses.

Op De Beeck M, Lievens B, Busschaert P, Declerck S, Vangronsveld J, Colpaert JV. 2014. Comparison and validation of some ITS primer pairs useful for fungal metabarcoding studies. *PloS One* **9**(6): e97629.

Osono T. 2006. Role of phyllosphere fungi of forest trees in the development of decomposer fungal communities and decomposition processes of leaf litter. *Canadian Journal of Microbiology* **52**(8): 701-716.

Penuelas J, Rico L, Ogaya R, Jump A, Terradas J. 2012. Summer season and long-term drought increase the richness of bacteria and fungi in the foliar phyllosphere of Quercus ilex in a mixed Mediterranean forest. *Plant Biology* **14**(4): 565-575.

Ramette A. 2007. Multivariate analyses in microbial ecology. *FEMS Microbiology Ecology* **62**(2): 142-160.

Roossinck MJ. 2005. Symbiosis versus competition in plant virus evolution. *Nature Reviews: Microbiology* **3**(12): 917-924.

Roossinck MJ. 2011. The good viruses: viral mutualistic symbioses. *Nature Reviews Microbiology* **9**(2): 99-108.

Roossinck MJ. 2012. Plant Virus Metagenomics: Biodiversity and Ecology. *Annual Review of Genetics, Vol 46* **46**: 359-369.

Roossinck MJ. 2015a. Metagenomics of plant and fungal viruses reveals an abundance of persistent lifestyles. *Frontiers in Microbiology* **5**.

Roossinck MJ. 2015b. Plants, viruses and the environment: ecology and mutualism. *Virology* **479**: 271-277.

Roossinck MJ, García-Arenal F. 2015. Ecosystem simplification, biodiversity loss and plant virus emergence. *Current Opinion in Virology* **10**: 56-62.

Roossinck MJ, Saha P, Wiley GB, Quan J, White JD, Lai H, Chavarria F, Shen GA, Roe BA. 2010. Ecogenomics: using massively parallel pyrosequencing to understand virus ecology. *Molecular Ecology* **19**: 81-88.

Rosado BH, Almeida LC, Alves LF, Lambais MR, Oliveira RS. 2018. The importance of phyllosphere on plant functional ecology: a phyllo trait manifesto. *New Phytologist* **219**(4): 1145-1149.

Rosario K, Breitbart M. 2011. Exploring the viral world through metagenomics. *Current Opinion in Virology* **1**(4): 289-297.

Saleem M, Meckes N, Pervaiz ZH, Traw MB. 2017. Microbial Interactions in the Phyllosphere Increase Plant Performance under Herbivore Biotic Stress. *Frontiers in Microbiology* **8**(41).

Sapkota R, Jørgensen LN, Nicolaisen M. 2017. Spatiotemporal Variation and Networks in the Mycobiome of the Wheat Canopy. *Frontiers in plant science* **8**: 1357-1357.

Sapkota R, Knorr K, Jørgensen LN, O'Hanlon KA, Nicolaisen M. 2015. Host genotype is an important determinant of the cereal phyllosphere mycobiome. *New Phytologist* **207**(4): 1134-1144.

Schwarz D, Beuch U, Bandte M, Fakhro A, Büttner C, Obermeier C. 2010. Spread and interaction of Pepino mosaic virus (PepMV) and Pythium aphanidermatum in a closed nutrient solution recirculation system: effects on tomato growth and yield. *Plant Pathology* **59**(3): 443-452.

Seabloom EW, Borer ET, Mitchell CE, Power AG. 2010. Viral diversity and prevalence gradients in North American Pacific Coast grasslands. *Ecology* **91**(3): 721-732.

Shaul O, Galili S, Volpin H, Ginzberg I, Elad Y, Chet I, Kapulnik Y. 1999. Mycorrhiza-induced changes in disease severity and PR protein expression in tobacco leaves. *Molecular Plant-Microbe Interactions* **12**(11): 1000-1007.

Shi M, Lin X-D, Tian J-H, Chen L-J, Chen X, Li C-X, Qin X-C, Li J, Cao J-P, Eden J-S, et al. 2016. Redefining the invertebrate RNA virosphere. *Nature* **540**: 539.

Siddell SG, Walker PJ, Lefkowitz EJ, Mushegian AR, Adams MJ, Dutilh BE, Gorbalenya AE, Harrach B, Harrison RL, Junglen S, et al. 2019. Additional changes to taxonomy ratified in a special vote by the International Committee on Taxonomy of Viruses (October 2018). *Archives of Virology* **164**(3): 943-946.

Simmonds P. 2015. Methods for virus classification and the challenge of incorporating metagenomic sequence data. *Journal of General Virology* **96**(Pt 6): 1193-1206.

Spence N, Basham J, Mumford R, Hayman G, Edmondson R, Jones D. 2006. Effect of Pepino mosaic virus on the yield and quality of glasshouse-grown tomatoes in the UK. *Plant Pathology* **55**(5): 595-606.

Stobbe AH, Roossinck MJ. 2014. Plant virus metagenomics: what we know and why we need to know more. *Frontiers in plant science* **5**(150).

Susi H, Filloux D, Frilander MJ, Roumagnac P, Laine AL. 2019. Diverse and variable virus communities in wild plant populations revealed by metagenomic tools. *PeerJ* **7**: e6140.

Thapa V, McGlinn DJ, Melcher U, Palmer MW, Roossinck MJ. 2015. Determinants of taxonomic composition of plant viruses at the Nature Conservancy's Tallgrass Prairie Preserve, Oklahoma. *Virus Evolution* **1**(1).

UNITE C. 2019. UNITE general FASTA release for Fungi. Version 18.11.2018. . *UNITE Community.* .

Unterseher M, Schnittler M. 2009. Dilution-to-extinction cultivation of leaf-inhabiting endophytic fungi in beech (Fagus sylvatica L.)--different cultivation techniques influence fungal biodiversity assessment. *Mycological Research* **113**(5): 645-654.

Vacher C, Hampe A, Porté AJ, Sauer U, Compant S, Morris CE. 2016. The Phyllosphere: Microbial Jungle at the Plant–Climate Interface. *Annual Review of Ecology, Evolution, and Systematics* **47**(1): 1-24.

van der Valk T, Vezzi F, Ormestad M, Dalén L, Guschanski K. 2019. Index hopping on the Illumina HiseqX platform and its consequences for ancient DNA studies. *Molecular Ecology Resources*.

Vorholt JA. 2012. Microbial life in the phyllosphere. *Nature Reviews Microbiology* **10**: 828.

Wang Q, Garrity GM, Tiedje JM, Cole JR. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology* **73**(16): 5261-5267.

Wickham H. 2016. *ggplot2: Elegant Graphics for Data Analysis*: Springer-Verlag New York.

Wren JD, Roossinck MJ, Nelson RS, Scheets K, Palmer MW, Melcher U. 2006. Plant Virus Biodiversity and Ecology. *PLoS Biology* **4**(3): e80.

Xu P, Chen F, Mannas JP, Feldman T, Sumner LW, Roossinck MJ. 2008. Virus infection improves drought tolerance. *New Phytologist* **180**(4): 911-921.

Figures

Figure 1. Schematic representation of the sample processing and sequencing strategies (metagenomics and culturomics) for viral communities from plants samples and fungal cultures (phytovirome and mycovirome) and for fungal communities from plant samples and fungal cultures (mycobiome and mycoculturome) analyses.

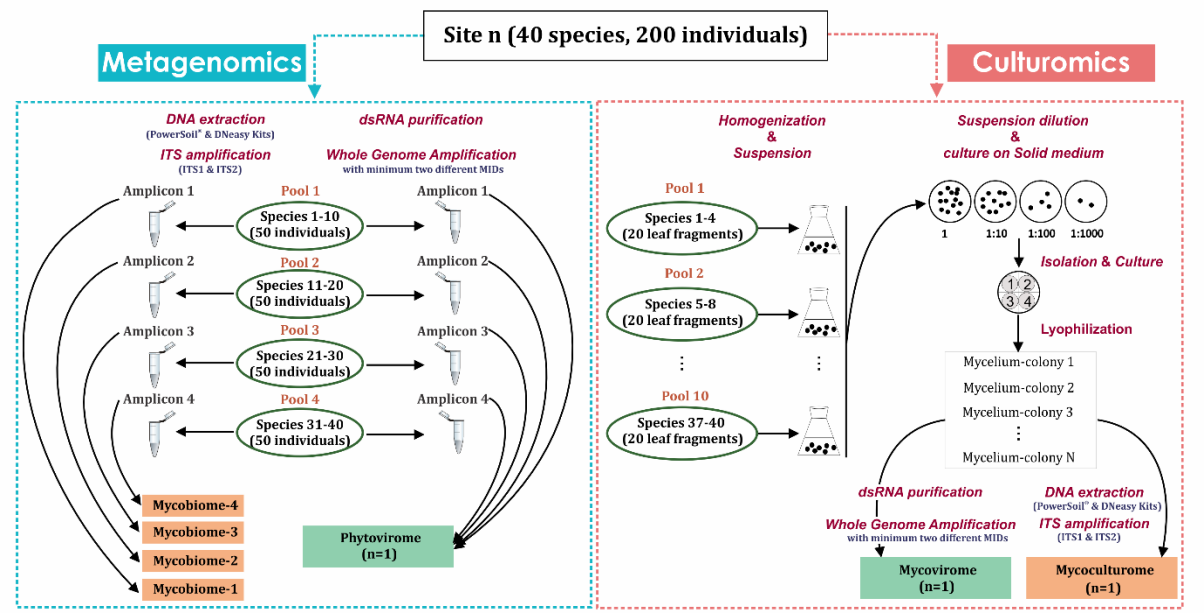


Figure 2. RdRp Operational Taxonomy Units (OTUs) virome composition and known/novel status of RdRp OTUs at each sampling site. (**A**) Virome composition based on family level OTUs annotation. (**B**) Pie charts showing the proportion of ssRNA, dsRNA and unclassified OTUs. Colors separate in each group the known viral OTUs (in green) for which a RdRp with >=90% identity was identified in Genbank and the potentially novel viral OTUs (in grey). Box plots illustrating fungal community richness and diversity in plant pools from cultivated or unmanaged sampling sites reflected by (**C**) the number of detected amplicon sequence variants (ASVs) and (**D**) Shannon diversity index calculated using read numbers as a proxy to individual ASV prevalence.

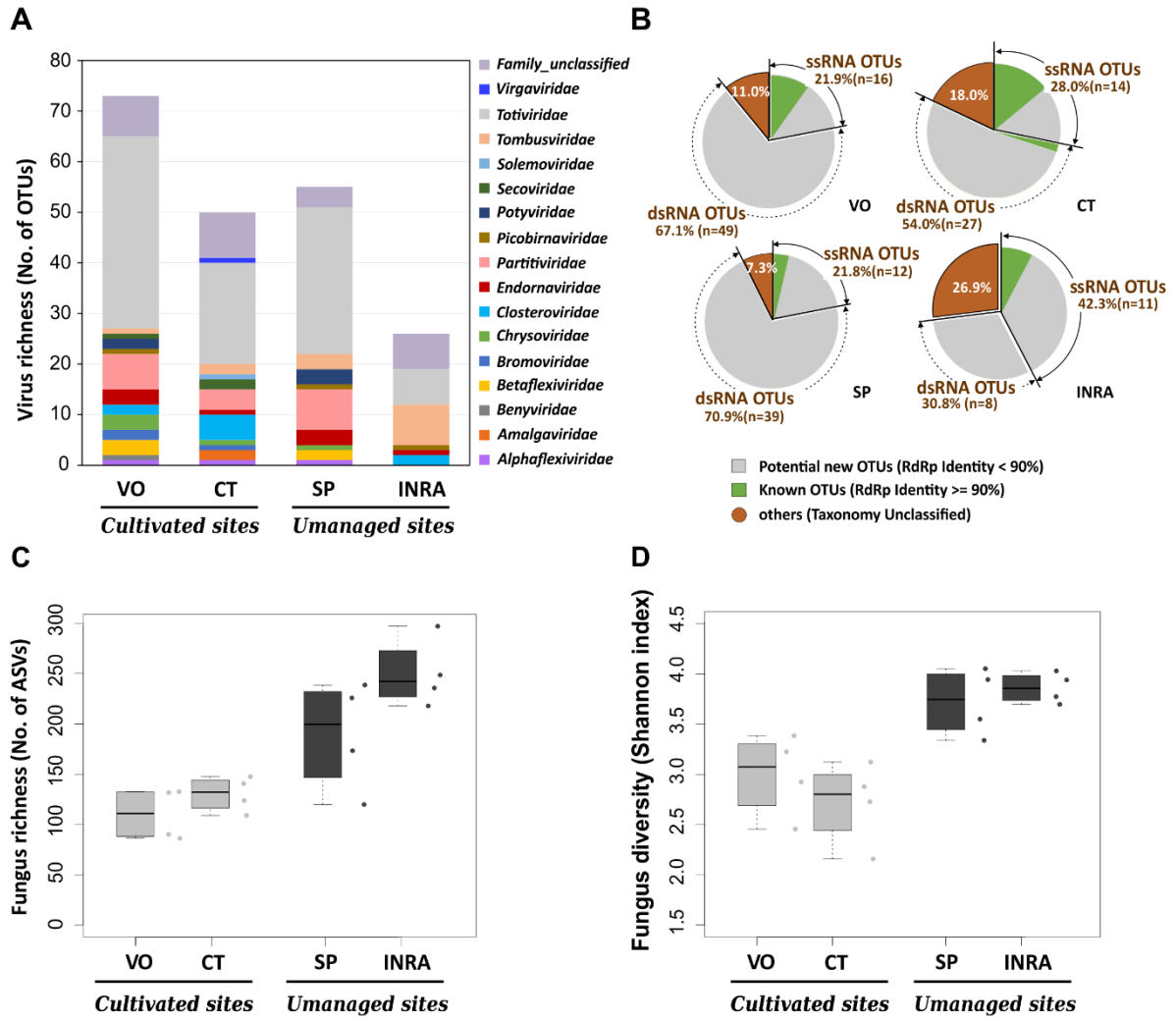
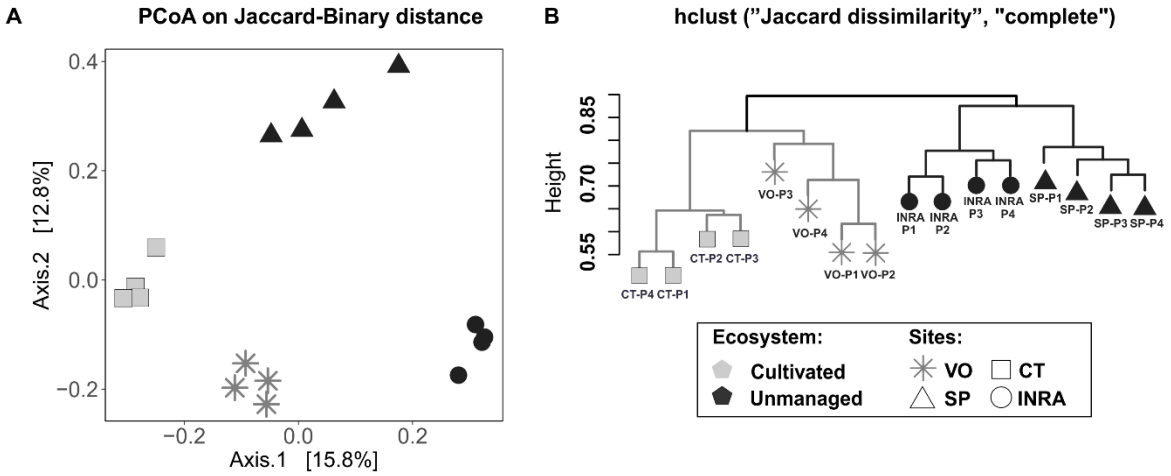


Figure 3. Principal coordinates analysis (PCoA) and hierarchical clustering analysis of mycobiome compositions for independent plant pools coming from the same or from different sampling sites. Plant pools from the same site do not contain shared plant species. (A) PCoA (A) and dendrogram (B) calculated using the Jaccard-Binary distance based on presence/absence of amplicon sequence variants (ASVs) for each library. Different shapes indicates the plant pools from a specific sampling sites (CT, INRA, SP, VO). The shapes are colored according to the sampling site status (cultivated or unmanaged).



Tables

Table 1. Main characteristics of the plant-associated viromes from different sampling sites

Site	**VO**	**CT**	**SP**	**INRA**
Ecosystem_type	***Cultivated***	***Cultivated***	***Unmanaged***	***Unmanaged***
Plant_species	*40*	*33*	*29*	*34*
Blast annotation				
Reads in viral contigs	289019	651841	234088	106501
% reads in viral contigs	22.8%	51.4%	18.4%	8.4%
dsRNA / ssRNA viruses reads	1.6	1.1	19.6	0.7
Viral families	15	14	11	7
RdRp-OTU classification				
Total no. of OTUs	73	50	55	26
Viral families	13	12	9	5
Percent OTUs with Genbank RdRp identity >=90%	10.0%	16.3%	3.7%	12.0%
Percent OTUs with Genbank RdRp identity <90%	90.0%	83.7%	96.3%	88.0%

Supporting Information

The supplementary tables and methods are available at https://doi.org/10.15454/WFFFD8

Table S1 Individual plant samples and species for phytovirome and mycobiome analysis.

Table S2 Phytoviromes and mycoviromes from different sites with information on HTS output.

Table S3 Number of reads integrated in contigs belonging to different viral families as determined by Blast-based annotation.

Table S4 Viral RdRp OTUs identified from the VO, CT, SP and INRA sampling sites.

Table S5 Identified amplicon sequence variants (ASVs) with their taxonomic assignation and the corresponding number of reads identified in each library.

Table S6 Relative abundance of fungal phyla and classes in each library.

Table S7 Number of ITS1 ASVs detected and diversity indices.

Table S8 ANOSIM and ADONIS test for potential factors affecting mycobiome composition.

Table S9 Comparison of fungal richness reflected by the statistics of fungal ASVs calculated using non-normalized data between plant mycobiomes and fungal culturomes.

Table S10 Viral families detected by Blast annotation of contigs from plant or cultured fungal samples for the four different study sites.

Methods S1 R script for ITS1 data processing based on the DADA2 pipeline.

Methods S2 R script for ITS2 data processing based on the DADA2 pipeline.

Methods S3 R script for formatting ITS1 and ITS2 and generating *phyloseq* objects.

Methods S4 R script for normalization of ITS1 and ITS2 data.

Methods S5 R script for diversity analysis of ITS1 and ITS2 data

Fig. S1 Venn diagrams showing the shared fungal ASVs between different plant pools from the same sampling site.

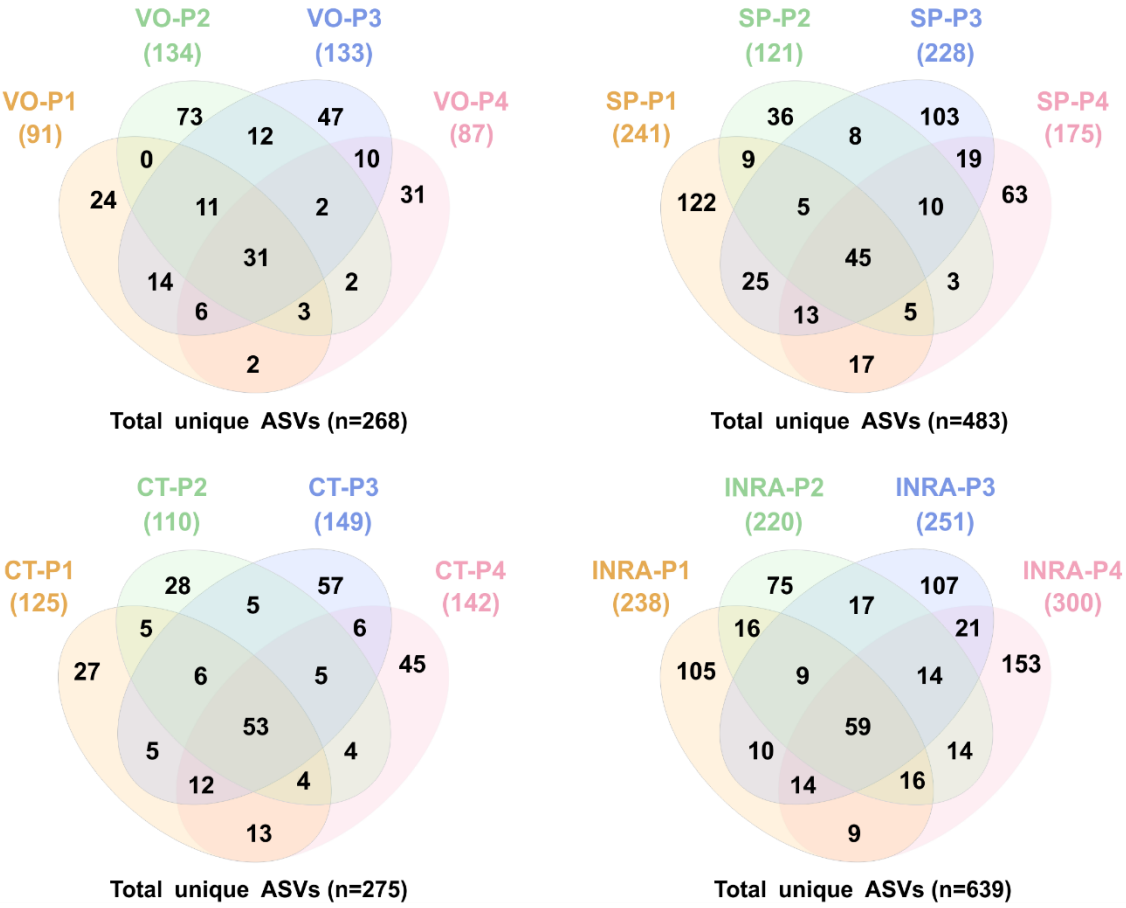


Fig. S2 Scaled Venn diagrams showing the shared fungal ASVs/shared viral OTUs between the mycobiomes/viromes of plants pools and the cultured fungal pools obtained from the plant pools from different sampling sites.

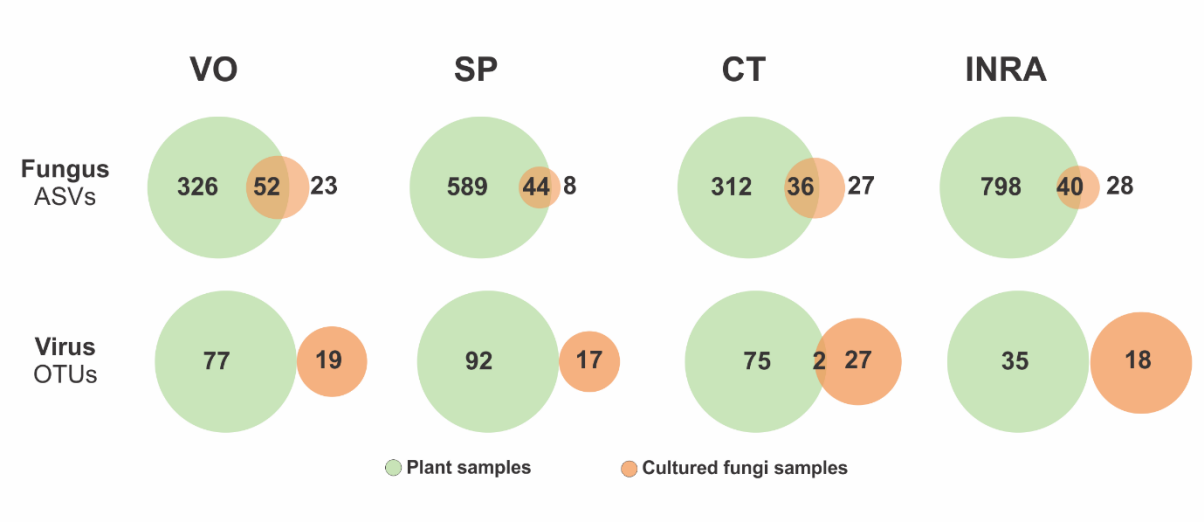


Fig. S3 Bar plot showing the frequency of sampled plant species, detected fungal ASVs and detected viral OTUs between the four study sites.