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Role of environmental plasticity and adaptation of nurse species from the subalpine and oromediterranean zones of the Pyrenees and the Mount-Lebanon for alpine communities structure

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Titre : Rôle de la plasticité environnementale et de l’adaptation d’espèces nurses des étages subalpins et oroméditerranéens des Pyrénées et du Mont-Liban pour la structure des communautés alpines

Résumé : Les plantes en coussins sont connues pour leur capacité fondatrice de nouvel habitat pour les autres espèces. Par ailleurs, des études ont montré que la variation morphologique au sein d’espèces fondatrices peut induire une variation des effets compétiteurs sur les espèces subordonnées, ce qui peut affecter la composition des communautés. Cette thèse a pour objectif d’étudier les conséquences d’une variation intraspécifique de deux espèces fondatrices (Festuca gautieri et Onobrychis cornuta) occupant des micro-environnements contrastés dans deux systèmes montagneux [les Pyrénées (France) et le Mont-Liban (Liban), respectivement] sur les communautés végétales associées (subalpines et oroméditerranéennes, respectivement). Nous avons évalué par des approches descriptives et/ou expérimentales (jardins expérimentaux, transplantations réciproques) les contributions de la génétique et de la plasticité à la variation morphologique entre deux phénotypes de coussins (dense et lâche) pour chaque espèce fondatrice, et à leurs effets contrastés sur les espèces subordonnées. Nous avons également quantifié les effets rétroactifs de la communauté pour la reproduction des espèces fondatrices. Nos résultats montrent une contribution à la fois de la génétique et de la plasticité à la variation phénotypique. La base génétique des variations morphologiques entre les phénotypes a induit des différences héréditaires d’effets compétiteurs sur les espèces subordonnées, tout en contrecarrant l’augmentation de la compétition avec la diminution du stress – le résultat dominant dans la littérature sur les systèmes subalpins. Nous avons aussi trouvé des effets rétroactifs négatifs des espèces subordonnées pour l’espèce fondatrice, avec une diminution de la production de fleurs (par les coussins) due au nombre croissant d’espèces subordonnées. La diversité des espèces subordonnées était plus élevée dans les conditions environnementales favorables que dans les stressantes. Par conséquent, nous avons conclu que les effets génétiques surmontent les effets environnementaux, limitant la compétition dans les milieux favorables, maintenant ainsi une plus grande diversité dans ces milieux que dans les milieux stressants.

Mots clés : jardin expérimental, compétition, facilitation, effets rétroactifs, Festuca gautieri, génétique, interactions entre plantes, Onobrychis cornuta, phénotype, plasticité, transplantation réciproque.

Title : Roles of environmental plasticity and adaptation of nurse species from the subalpine and oromediterranean zones of the Pyrenees and the Mount-Lebanon for alpine communities structure

Abstract : Alpine cushion plants are foundation species known for their nursing ability. Moreover, studies have shown that morphological variation in foundation species can trigger variation in competitive effects on subordinate species, likely to affect community composition. We investigated the consequences of intraspecific variation within two alpine cushion species (Festuca gautieri and Onobrychis cornuta) across heterogeneous environments in two mountain ranges [the Pyrenees (France) and Mount-Lebanon (Lebanon), respectively] for the associated plant communities (subalpine and oromediterranean, respectively). We assessed with observational and/or experimental (common-gardens, reciprocal transplantation experiments) approaches the relative contribution of genetics and plasticity to the morphological variation between two cushion phenotypes (tight and loose) of the foundation species, and to their differential effects on subordinate species. Community feedbacks were also quantified. Our results show that both genetics and plasticity contributed to the phenotypic variation. The genetic basis of the morphological differences between phenotypes induced heritable differences in competitive effects on subordinate species, but however counteracted the general increase in competition with decreasing stress dominantly found in the literature on subalpine systems. We also found negative feedbacks of subordinates on foundation species fitness, with higher cover of subordinate species reducing the cushions flower production. Subordinate species diversity was higher in benign than in stressed environmental conditions. Consequently, we concluded that genetic effects overcome the environmental effects by limiting competition in benign physical conditions, thus maintaining a higher diversity in benign than stressed conditions.

Keywords : common-garden, competition, facilitation, feedback, Festuca gautieri, genetics, Onobrychis cornuta, phenotype, plant-plant interactions, plasticity, reciprocal transplantation.

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Titre : Rôle de la plasticité environnementale et de l’adaptation d’espèces nurses des étages subalpins et oroméditerranéens des Pyrénées et du Mont-Liban pour la structure des communautés alpines

Résumé

Le sujet de cette thèse s’intègre dans une thématique émergente en interface entre l’écologie des communautés et la biologie évolutive: la génétique des communautés (étude des interactions génétiques entre les espèces et l’environnement abiotique dans les communautés). La plupart des études de génétique des communautés ont mis l'accent sur les interactions entre plusieurs niveaux trophiques, tandis que les interactions au même niveau trophique, et plus particulièrement les interactions entre plantes, restent faiblement étudiées sous une perspective évolutive. De plus, la plupart des études de génétique des communautés limitées aux plantes ont principalement porté sur les interactions négatives (i.e. compétition). Cependant, ces dernières années, l'intérêt pour des questions évolutives associées à des interactions positives (i.e. facilitation) entre les plantes a augmenté. La facilitation entre plantes joue un rôle considérable dans les environnements contraints tels que les milieux alpins, augmentant la diversité et structurant les communautés. Ces milieux sont souvent dominés par des plantes en forme de coussins. Les plantes en coussins sont connues pour leur capacité fondatrice de nouvel habitat pour les autres espèces. Elles dominent souvent les écosystèmes subalpin et alpin. Leur forme hémisphérique, leur petite taille et leur architecture plus ou moins compacte les rendent d’efficaces pièges de chaleur et d'eau. Ainsi, ces plantes jouent un rôle-clé dans les communautés, hébergeant plusieurs espèces au sein de leur canopée, maintenant ainsi la diversité dans des conditions environnementales difficiles. Des études ont montré que la variation morphologique au sein d’espèces fondatrices peut induire une variation des effets compétiteurs de ces espèces sur les espèces subordonnées, ce qui peut affecter la composition des communautés.

Cette thèse a pour but d’étudier les conséquences d’une variation intraspécifique de deux espèces fondatrices - *Festuca gautieri* et *Onobrychis cornuta* - occupant des

micro-environnements contrastés dans deux systèmes montagneux - les Pyrénées (France) et le Mont-Liban (Liban), respectivement - sur les communautés végétales associées (subalpines et oroméditerranéennes, respectivement). Dans les deux systèmes, chacune des deux espèces fondatrices présente deux phénotypes: un phénotype « dense » avec une morphologie compacte dominant les habitats en topographie convexe, et un phénotype « lâche » avec une morphologie peu compacte dominant des habitats en topographie concave. Dans un contexte évolutif, il est important d'évaluer la différenciation génétique au sein des espèces fondatrices afin d'augmenter les chances d'avoir des conséquences au niveau de la communauté. Ainsi, le premier objectif de cette thèse est d'évaluer la base génétique des différences phénotypiques observées au sein de chacune des espèces fondatrices étudiées. De plus, dans les deux systèmes, des différences d'association avec des espèces subordonnées ont été observées entre les phénotypes (pour chaque espèce fondatrice), avec très peu d'espèces subordonnées associées au phénotype « dense » et plusieurs espèces subordonnées associées au phénotype « lâche ». Cela a conduit à l'hypothèse qu'il existe des différences dans les effets entre les deux phénotypes sur les espèces subordonnées, avec le phénotype « dense » étant compétiteur et le phénotype « lâche » étant facilitateur. Ainsi, le deuxième objectif est d'évaluer les différences entre les effets des phénotypes sur les espèces subordonnées, et d'évaluer la contribution de la génétique et de la variation de l'environnement (par exemple, le stress provoqué par la sécheresse et la perturbation provoquée par le pâturage) aux variations des effets phénotypiques. De plus, des patrons contrastés de production de fleurs ont été observé entre les phénotypes avec le phénotype « dense » (hébergeant très peu d'espèces subordonnées) ayant une forte production de fleurs, et le phénotype « lâche » (hébergeant plusieurs espèces subordonnées) ayant une faible production de fleurs. Ceci suggère que le fait d'héberger des espèces subordonnées peut avoir des conséquences pour les espèces fondatrices. Ainsi le troisième objectif de cette thèse est d'évaluer les effets de rétroaction des espèces subordonnées sur les espèces fondatrices.

Les principales questions résumant les objectifs de cette thèse sont les suivantes:

(1) Quelles sont les contributions relatives de la génétique et de la plasticité aux différences phénotypiques au sein des espèces fondatrices?

(2) Existe-t-il des différences d'effets entre les phénotypes (de chaque espèce fondatrice étudiée) sur les espèces subordonnées ? Si oui, ces effets sont-ils héréditaires?
(3) Ces effets sont-ils influencés par des changements dans les conditions environnementales (stress provoqué par la sécheresse et perturbation provoquée par le pâturage)?
(4) Les effets rétroactifs des espèces subordonnées affectent-ils la *fitness* des espèces fondatrices ?

Nous avons évalué par des approches descriptives et/ou expérimentales (jardins expérimentaux, transplantations réciproques) les contributions de la génétique et de la plasticité à la variation morphologique entre les deux phénotypes de coussins (dense et lâche) pour chaque espèce fondatrice, et à leurs effets contrastés sur les espèces subordonnées. Nous avons également quantifié les effets rétroactifs de la communauté pour la reproduction des espèces fondatrices.

Nos résultats montrent une contribution à la fois de la génétique et de la plasticité à la variation phénotypique. La base génétique des variations morphologiques entre les phénotypes a induit des différences héréditaires d'effets compétiteurs sur les espèces subordonnées, tout en contrecarrant l'augmentation de la compétition avec la diminution du stress – le résultat dominant dans la littérature sur les systèmes subalpins. Nous avons aussi trouvé des effets rétroactifs négatifs des espèces subordonnées pour l'espèce fondatrice, avec une diminution de la production de fleurs (par les coussins) due au nombre croissant d'espèces subordonnées. La diversité des espèces subordonnées était plus élevée dans les conditions environnementales favorables que dans les conditions stressantes. Par conséquent, nous avons conclu que les effets génétiques surmontent les effets environnementaux, limitant la compétition dans les milieux favorables, maintenant ainsi une plus grande diversité dans ces milieux que dans les milieux stressants.

À la lumière des résultats de cette étude exploratoire, d'autres expérimentations pourraient révéler de nouvelles idées qui aident à comprendre la complexité des interactions entre plantes. Avec les progrès techniques de la biologie moléculaire, il serait important d'étudier le mécanisme moléculaire qui sous-tend la variation phénotypique au sein de chacune des espèces modèles, non seulement pour contre-vérifier leur déterminisme génétique, mais surtout pour étudier l'histoire de cette différenciation et la divergence génétique entre les deux phénotypes.

To my family

&

To the one who is always there for me*

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Jean-Paul, I am tremendously thankful for you and your family. You were my first contact in Bordeaux back in 2010 when I first sent you an email asking if you knew about any Ph.D. propositions and you helped me getting in contact with Richard. Later on, I discovered that this was not the first time you helped me, when Carla told me that it was you who provided the statistical help in my Master’s thesis. So thanks for all of that my friend, I wish you all the success and happiness in your future, whether it is France or in Lebanon. **Max**, thank you for the help you provided in the shadehouse and in correcting my English mistakes in my articles, I really appreciate it. **Lilian** (also known as Pithivier), you are one of the people I appreciate most in the Lab. Thanks for all the help you gave me whether it was statistical advice or a ride to buy materials for my field experiments. **Yoann**, thank you for all the help you provided in the field and for your contribution to my first article. **Matt**, you introduced me to the ∫©∆˙☺√ç≈ß˜π∑ software that is R. I am very thankful for all the time you spent explaining to me how to use this awesome and life-facilitating program. **Hassan**, I appreciate all our chitchats, especially on religion and political issues. Thanks to **Nadège** and **Nastasia** for adding a feminine touch to our Ph.D. student’s office, long-time known as the “boys room”! Also, thanks to **Pauline**, **Elsa** and **Hanan** (from the “girls room”) for their encouragements. I also acknowledge **Regis** for his technical support (sorry for being very demanding sometimes), **Chantal and Gaëlle** for their encouragements, and mostly **Sylvia** for never rejecting a demand and being always there when I needed help whether to book a flight or to sign a purchase order. Also, I would like to thank my trainees, **Etienne**, **Clément**, **Florène** and **Guillaume** for their help in the field. I would not have been able to accomplish this work without the support of all my friends: **Gio**, **Nat**, **Nicos**, **Omz**, **Rabab**, **Jad**, **Adib**, **Badr**, **Karmen**, **Pia**, **Jinane**, **Joyce**, **Christelle**, **Nabil**, **Zouna**, **Khouloud**, and many others (sorry if your name is not mentioned, it just means that I don’t love you! Kidding).

For my biggest Fans, my parents, **Elias** (also known as ‘Dad’), **Tackla** (known as ‘Mom’), **Lara** and **Nataly** (my sisters). I am very fortunate for having you as family. All of this would not have been possible if it was not for all your care and support (Mom thanks for all the food you always sent, and Dad thanks for the Arak[1]). Thanks for all your sacrifices and love, all of which made me who I am today. I love you all.

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[1] A Lebanese alcohol.

Last but not least, to **Rita**, my friend, my love and my future wife (this is not a proposal, not yet). Much have we overcome to get to where we are today! Thank you for your love, your encouragements and your patience (which was not an easy task, especially during my dissertation writing these last few months). You were always there for me (it’s you I meant in the `dedication*`) when I needed someone to rely on. I hope our adventure will continue through the years and I wish nothing but the best for ~~you~~ us.

“Wisdom ceases to be wisdom when it becomes too proud to weep, too grave to laugh, and too selfish to seek other than itself”

Gibran Khalil Gibran

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DISSERTATION PLAN

The dissertation is subdivided into six chapters. In the **first chapter**, I present an overview of the topics in the literature that were tackled in this Ph.D.: the extended effects of a genetic variation within a species on the community, biotic interactions (competition and facilitation), gradients of stress and disturbance, and local adaptation. In the **second chapter**, I present the study sites and the experimental designs set up in order to answer the main questions of my Ph.D. In the **third chapter** (first article, article published in *Oecologia*), I present experiments in which my colleagues and I investigate the genetic vs. phenotypic plasticity basis of differences in cushion morphological traits of a foundation species, *Festuca gautieri*, and the differences in competitive effects between two contrasting phenotypes of this foundation species on its subordinate species, and quantify community feedbacks on the foundation species fitness. In the **fourth chapter** (second article, submitted to *Journal of Ecology*) I present experiments in which my colleagues and I quantify the plasticity of the competitive / facilitative effects of the two phenotypes of *Festuca gautieri*, evaluate the genetic difference in competitive / facilitative effects between these phenotypes in the field (in natural conditions), and finally check if the genetic effects vary with the changes in micro-environmental conditions (existence of a Genetic x Environment interaction). In the **fifth chapter** (third article, accepted in *Journal of Vegetation Science*), I present a study in which my colleagues and I evaluate the contribution of environmental effects by quantifying the plasticity of phenotypic community effects of a foundation legume shrub, *Onobrychis cornuta*, across exposure (North vs. South) and grazing conditions (Grazed vs. Ungrazed) in a subalpine xerophytic community of western Mount-Lebanon. In the **sixth chapter**, I conclude with a general synthesis on all the results of these experiments answering my questions and propose perspectives for future studies. I end the dissertation with a list of the extensive bibliography used in this Ph.D.

CHAPTER ONE: LITERATURE OVERVIEW

1- Bridging ‘community ecology’ to ‘evolutionary biology’: the emergence of ‘community genetics’

Community genetics[2] is a growing field within biology seeking to link community ecology[3] to evolutionary biology[4], which disciplines are usually studied relatively separately (Johnson & Stinchcombe 2007). The term ‘community genetics’ was first suggested by Jim Collins and was later introduced to scientific literature by Janis Antonovics (Antonovics 1992; Collins 2003) who proposed that community genetics investigates “evolutionary genetic processes (e.g. natural selection, genetic drifts, mutation, gene flow…) that occur among interacting populations in communities”. In a simplified manner, community genetics studies the effects of intraspecific genetic variability on community structure. Researches have shown that genetic variation within a single species, especially a foundation[5] (*sensu* Ellison *et al.* 2005) or a keystone[6] (*sensu* Power *et al.* 1996) species, can have significant effects at the community level, thus, beyond the population level only traditionally assessed by geneticists. The impact of a genetic variation within a plant species has been shown to operate across trophic levels such as for arthropod communities (Fritz & Price 1988; Johnson & Agrawal 2005; Whitham *et al*. 2006; Bailey *et al*. 2009; Johnson *et al*. 2009), microbes and fungi (Whitham *et al*. 2003; Bailey *et al*. 2009), plant pollinators (Genung *et al*. 2012), and herbivores (Genung *et al*. 2011), but also within trophic levels – among plants (Iason *et al*. 2005; Pakeman *et al*. 2006; Genung *et al*. 2011, 2012; Gibson *et al*. 2012; Bailey *et al*. 2014).

Whitham *et al*. (2006) proposed that a full community genetics study should include three fundamental premises: extended phenotype[7] (community and ecosystem phenotypes), community heritability, and community feedbacks. They argued that the ‘traditional’ phenotype of an individual (which represents the observable characteristics of its genotype’s expression in its environment) has predictable effects not only at the population level but extends to affect its community and ecosystem (see Figure 1).

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[2] The study of genetic interactions that occur between species and their abiotic environment in complex communities (Whitham *et al*. 2006)

[3] A subfield of ecology that examines how interactions among species and their environment affect the abundance, distribution and diversity of species within communities” (Johnson & Stinchcombe 2007) and therefore community structure

[4] A subfield of biology that “considers genetic variation and the mechanisms that result in genetic and phenotypic changes within populations” (Johnson & Stinchcombe 2007), and thus study the evolutionary processes that produce diversity.

[5] A single species that defines much of the structure of a community by creating locally stable conditions for other species, and by modulating and stabilizing fundamental ecosystem processes (Ellison *et al*. 2005).

[6] A species whose effect is large, and disproportionately large relative to its abundance (Power *et al*. 1996).

[7] The effects of genes at levels higher than the population (Dawkins 1982; Whitham *et al*. 2003, 2006).

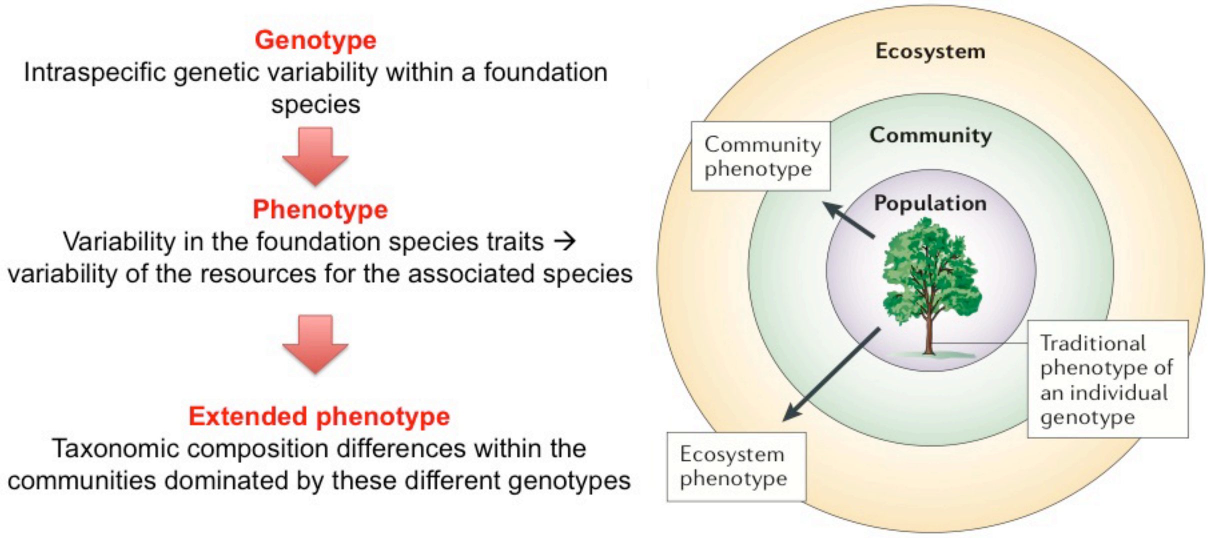

Figure 1. The extended phenotype traced from the genetic variability within a foundation species to the community and the ecosystem levels. The effects of a genetic variability within a foundation species cascade at levels higher than the population, and thus affect community structure and ecosystem processes (adapted from Whitham *et al*. 2006).

Whitham *et al*. (2003) present an example that shows the extended effects of a genetic variation within *Pinus edulis*, a dominant species[8] of pinyon-juniper woodlands in the USA. *P. edulis* presents variation (which has both a genetic and an environmental component) in resistance to a moth – *Dioryctria albovitella* – that, at larval stage, feeds in the bark of terminal shoots causing dieback of the branches. The moth has minimal impact on its host (independently of the tree's genotype) under normal environmental conditions, and thus both *P. edulis* types – moth-resistant and moth-susceptible – have a conical form. But, in drought and oligotrophic soil conditions, the impact of the insect on the moth-susceptible trees increases turning them into shrubs (Figure 2), decreasing the production of female cones, which in turn will affect seed dispersal by birds and rodents (in the absence of the moth, seed dispersal happens mainly by birds over long distances; in the presence of the moth, seed dispersal happens mainly by rodents over short distances). Therefore, the resistance to moth controls the outcome of competitive interactions among birds and rodents for seeds, which in turn feed back on the tree itself, determining the distance of its seed dispersal (local *versus* long-distance). This genetic variability of *P. edulis* in resistance to moths also affects

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[8] Species that dominate community biomass and have total impacts that are large, but not disproportionate to their biomass (Power *et al*. 1996).

microbial and fungi communities (Figure 3), thus, showing that a variation within a single species can have consequences on the community structure of a multitude of species.

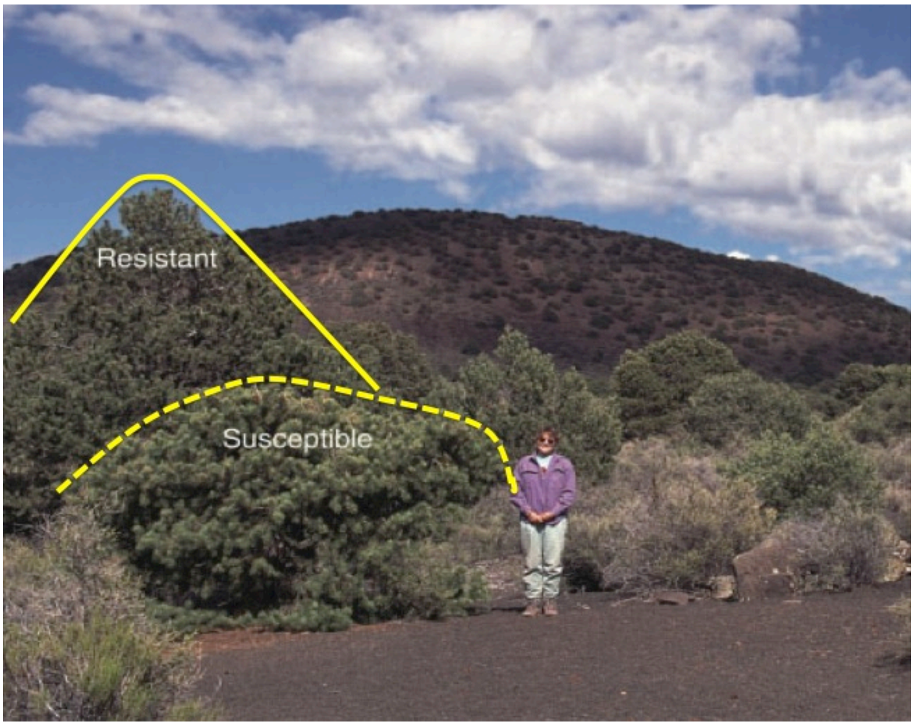

Figure 2. Photo showing two morphotypes of *Pinus edulis* trees induced by a genetic variation in resistance to a stem-boring moth, *Dioructria albovitella.* In presence of the insect and in conditions of high soil nutrient and moisture stress (black cinder soil here), moth-susceptible trees turn into flat-topped shrubs (dashed yellow line on the photo) and reduce female cone production while moth-resistant trees stay unchanged (conical form with normal female cone production – straight yellow line on the photo; adapted from Brown *et al*. 2001, Photo courtesy of Thomas G. Whitham).

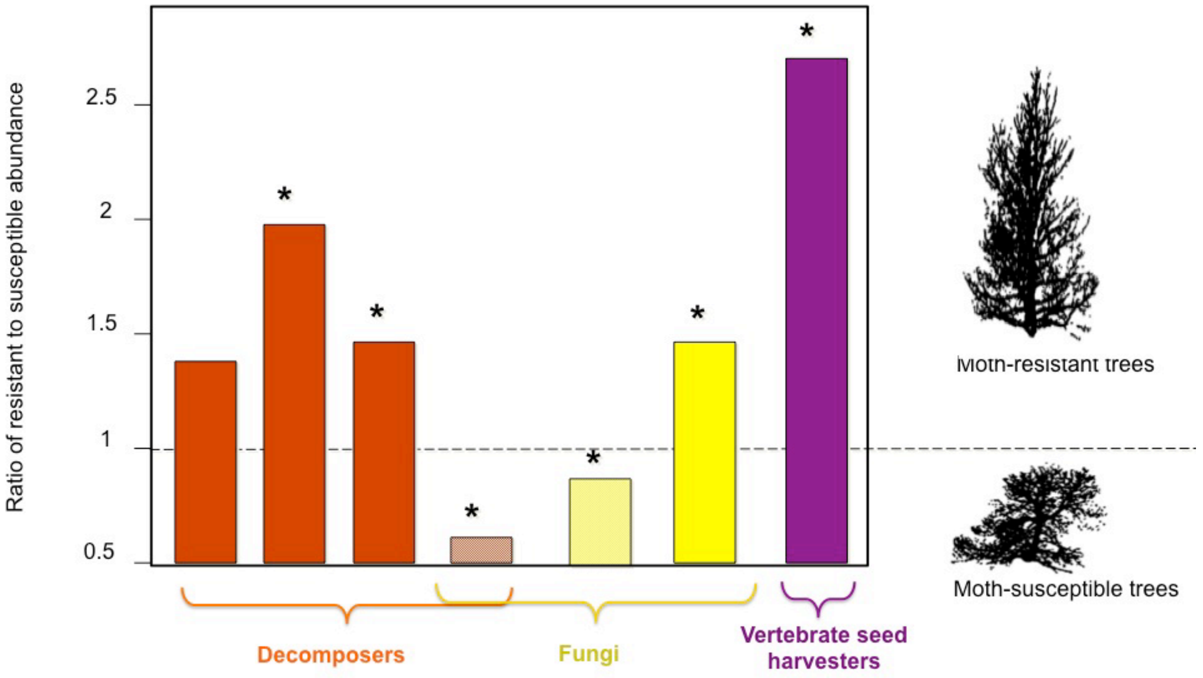

Figure 3. Effect of a genetic variation within *Pinus edulis* in resistance to *Dioructria albovitella* (a stem-boring moth) on the taxonomic and functional composition of communities associated to *Pinus edulis*. The graph shows the ratio of the abundance of decomposers, fungi and vertebrate seed harvesters on resistant trees relative to susceptible trees. Bars with values > 1 indicate greater abundance on resistant trees than on susceptible trees; bars with values < 1 indicate greater abundance on susceptible trees than on resistant trees. An asterisk above the bar denotes a statistically significant difference at $P < 0.05$ (adapted from Whitham *et al*. 2003).

Many studies have shown feedback relationships between foundation plants and their associated communities (Holzapfel & Mahall 1999; Whitham *et al*. 2003, 2006; Lankau & Strauss 2007; Michalet *et al*. 2011; Cranston *et al*. 2012; Schöb *et al*. 2014). In the same review mentioned before (Whitham *et al*. 2006), the authors present a study on a foundation poplar species with two phenotypes varying in their foliar concentration in condensed tannin (low *versus* high), which has a genetic basis. Poplars with low condensed tannin were selected and fell by beavers (Figure 4a). With time, the abundance of standing poplars with high foliar condensed tannin increased while the abundance of those with low foliar condensed tannin decreased (Figure 4b). Since leaves fall mostly beneath their mother tree, and since high levels of condensed tannin were related to the decrease in nutrient release by decomposition and to the decrease in nitrogen mineralization (Figure 4c) by constraining microbial activity, poplars with high foliar condensed tannins levels could affect nutrients and nitrogen availability. Thus, these trees will be pushed to invest more energy in fine-root production (Figure 4d) as a compensatory response to their need for nutrient acquisition, which could feed back to affect the performance of the poplars themselves.

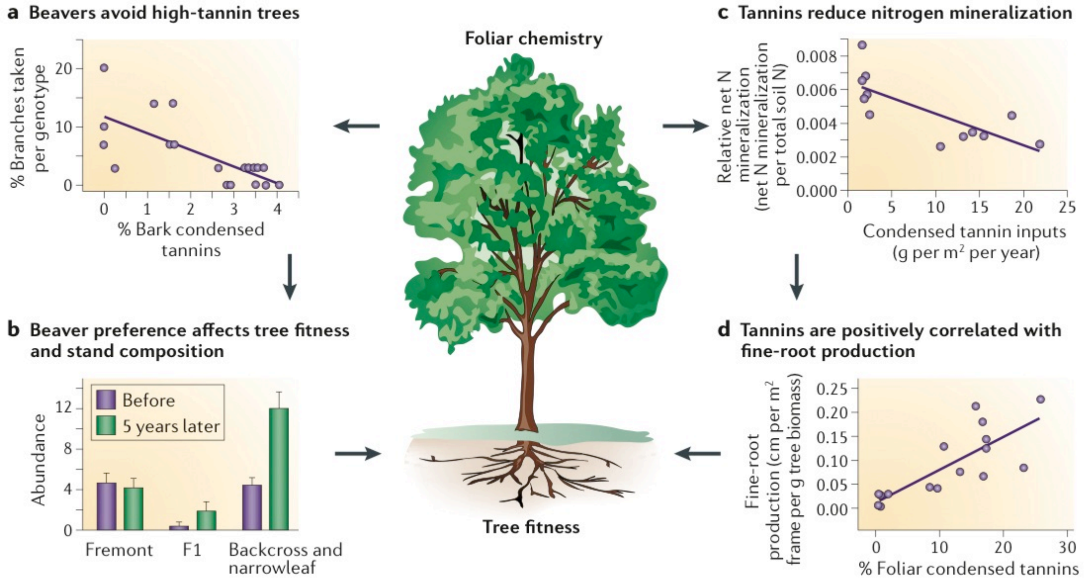

Figure 4. Selection pressures that are exerted on foundation species can affect interactions with other species, which in turn might feed back to affect the fitness[9] of the individual that produced the phenotype. Here we show how the condensed tannin phenotype in the poplar could affect the foraging of an important herbivore, nutrient cycling and nutrient acquisition. Panels **a,b** show that the beaver *Castor canadensis* is an important agent of natural selection in which interactions with a foundation tree species could affect many other species that depend on the tree for their survival. Beavers selectively fell trees low in condensed tannins ($r^2 = 0.52$, $P < 0.001$), which in turn affects the fitness of different tree genotypes and cross types. After 5 years of selective felling of trees, cross types that were high in condensed tannins (backcross hybrids and *Populus angustifolia*) had nearly tripled in abundance, whereas the cross type lowest in condensed tannins (*Populus fremontii*) had significantly declined in abundance, and the cross type intermediate in condensed tannins (F1 hybrids) showed an intermediate increase in abundance (whole-model ANOVA test, change in cross types; $F = 15.66$, $P < 0.0001$). Panels **c,d** illustrate a potentially important feedback loop that presumably interacts through the microbial community to affect the tree's performance. Panel **c** suggests that an increased concentration of condensed tannins in leaves of individual trees can inhibit the microbially mediated process of nitrogen mineralization ($r^2 = 0.65$, $P < 0.003$). In turn, variation in soil nutrients could feed

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[9] The contribution of the genes of an individual to the next generation, usually approximated through measuring survival and reproductive success (Savolainen *et al*. 2013).

back to affect the tree’s investment into fine-root production to forage for limiting nutrients (panel **d**; $r^2 = 0.60$, $P < 0.001$), which can affect tree performance (adapted from Whitham *et al*. 2006).

2- Community assembly: from genes to communities

Community assembly rules (Diamond 1975) are a set of debatable rules in ecology (Rule 1: Forbidden species combinations, and Rule 2: Reduce niche overlap, Diamond 1975). These rules highlight the role of competition[10] in determining the patterns of assemblage composition. Keddy (1992) proposed that the role of community assembly rules is not limited only to the assembly of species within a community, but integrates all factors responsible for the occurrence of a species within the community. He states that: “*The process of constructing communities from species pools is in many ways analogous to the processes of evolution through natural selection. Habitats serve as filters for genotypes, with the least suited genotypes being filtered out, and the best suited surviving to reproduce. In the case of assembly rules, habitats are again serving as filters. However, in this case, the filters operate on traits*[11] *and eliminate those sets of traits which are unsuitable to that environment. The species which comprise the community are those which survive the filter*”.

Thus, species (or individuals) face different filters (stochastic, abiotic and biotic) before being able to participate to the final composition of a community (Figure 5). The stochastic filter acts on the initial species pool to form a regional then a local species pool; here, aleatory processes define species presence. The local species pool is than simultaneously subjected to the abiotic and biotic filters. The abiotic filter (i.e. environmental factors: temperature, soil water content, salinity…) acts on a regional and local scale. Species that tolerate the regional or local abiotic conditions can cross this filter, and ecologically similar species are filtered within the same fundamental niche (Lavorel & Garnier 2002). Thus, the abiotic filter acts in a way to reduce trait variability between species – trait convergence. The biotic filter (i.e. biotic interactions) acts on the individual scale in a way to eliminate similar species, and thus participate to the increase in trait variability between the species of a community – trait divergence (Weiher *et al*. 1998). This filter incorporates all kinds of

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[10] A negative biotic interaction occurring between individuals of the same species (intra-specific) or between species of the same trophic levels (inter-specific), in which the performance (survival, growth or fecundity) of one is lowered by the presence of the other.

[11] A trait is a distinct variant of a phenotypic character of an organism that may be inherited, environmentally determined or a combination of the two.

interactions between two species (e.g. competition, facilitation, allelopathy[12], herbivory, predation, pollination...).

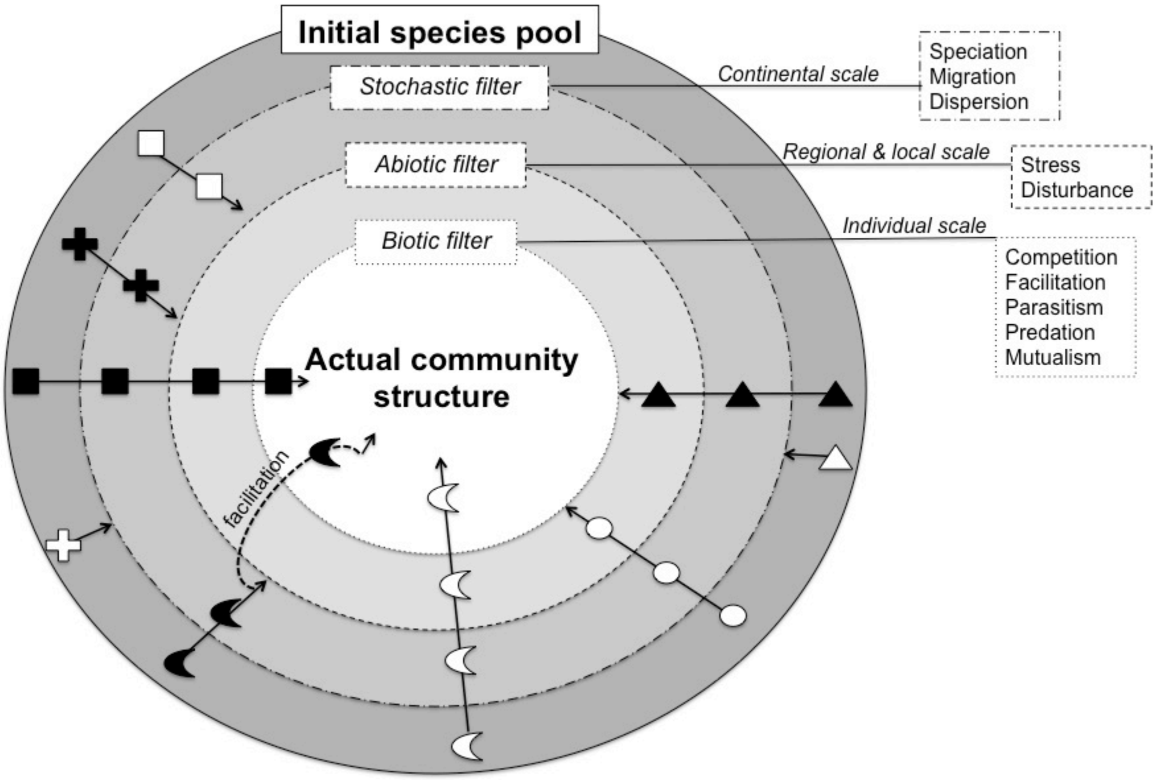

Figure 5. The main filters that structure a plant community. Each species is represented by a different geometrical form and color. Species cross simultaneously (from outside to inside) the stochastic filter, the abiotic filter and the biotic filter. The spatial scale, at which each of the filter operate, decreases from the initial species pool to the final community composition (adapted from Keddy 1992; Zobel 1997; Díaz *et al*. 1999; Lortie *et al*. 2004)

However, Pärtel *et al*. (2011) argued that the term "species pool" should only be used to refer to species that can potentially occupy a particular habitat due to suitable local ecological conditions. They also emphasised that studies comparing species diversity of different ecosystems, regions or taxonomic groups should consider not only the observed local diversity, but also the "dark diversity" (i.e. species that are currently absent from a site but which belong to its species pool). Recently, de Bello *et al*. (2012) re-evaluated the role of biotic processes in generating trait divergence between the species of a community. They showed that biotic processes such as competition could lead to both trait divergence (through the exclusion of similar species – niche differentiation) and convergence (through exclusion

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[12] A biological phenomenon - negative interaction - by which an organism produces biochemicals that influence the growth, survival, and reproduction of other organisms.

of dissimilar species – weaker competitor exclusion). Thus abiotic and biotic processes can produce similar patterns of traits diversity, and separating them cannot be done by comparing the trait diversity observed within communities to patterns of randomly generated communities based on sampling within a region. Instead, de Bello *et al.* (2012) proposed a framework, the “functional species pool”, in which they separated abiotic and biotic processes and distinguished opposing biotic effects (convergence and divergences) on community assembly (see Figure 6). A valuable point in their approach is the incorporation of the dark diversity within the species pool.

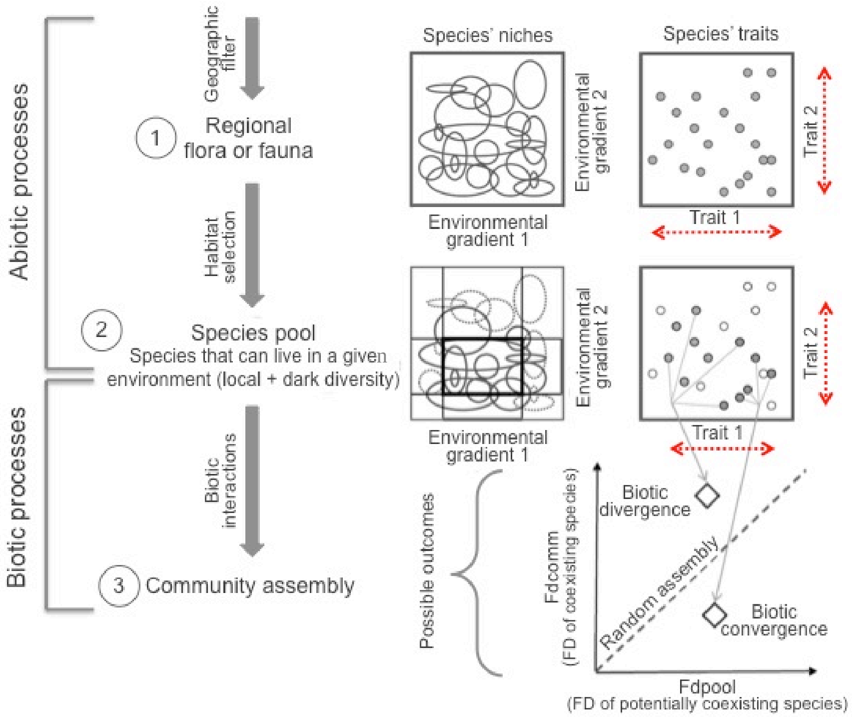

Figure 6. Given a particular regional flora or fauna, that was formed according to different geographical and historical filters (1), habitat selection will filter out species whose environmental preference falls outside the range of environmental conditions available in a given site, thus creating a convergence in trait values (e.g., compare trait ranges – red dashed arrows – between 1 and 2). This convergence is removed when using the functional species pool approach. The test for assessing the relevance of biotic interactions on community assembly in each single site (3) is performed by comparing the functional diversity[13] in the sampled community (FDcomm) with the functional diversity expected within the corresponding functional species pool (FDpool; species that potentially

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[13] The extent of trait differences between species (de Bello *et al*. 2012).

coexist - filled grey circles). The deviance from a 1:1 relationship (i.e., random assembly[14]) between FDcomm and FDpool corresponds to prevailing biotic assembly processes (FDcomm > FDpool biotic divergence vs. FDcomm < FDpool indicating biotic convergence). Adapted from de Bello *et al*. 2012.

2.1- Overview on biotic interactions and abiotic factors (stress and disturbance)

For long time, ecologists have tried to come up with a generalised principle that holds across the natural world (Graham & Duda 2011). In their work on island biogeography, MacArthur & Wislon (1967) proposed one of the first models, the r/K selection model, seeking to explain and predict species distribution (initially designed to be applicable to all living beings). The r/K model was based on environmental stability to predict species selection. The r-selected species (or r-strategists) do best in unpredictable/disturbed environments; they are characterised by a rapid growth, early maturity, but poor competitive ability. The K-selected species (or K-strategists) do well in more predictable/stable environments; they are characterised by a slow growth rate (long life-span), late maturity, but high competitive ability. Although drawbacks of the r/K theory have been pointed out (Wilbur *et al*. 1974; Barbault 1987; Kuno 1991), it is still widely used as "*enough people have found it a useful framework in which to interpret their observations* [and thus,] *it must contain an element of truth*" (Stearns 1992). In particular, this model is well adapted for understanding tree species functional strategies and forest successions, in particular in benign physical conditions (Michalet *et al*. 2008). Also, Michalet *et al*. (2011) used this model to contrast the two different phenotypes of the alpine foundation species *Geum rossii* in northern Arizona (USA). Later on, more detailed models emerged and included gradients of stress[15] and disturbance[16].

Early in the seventies, Grime (1973) presented a model showing a unimodal relationship between species density[17] and the intensity of stress (and site productivity) or disturbance (e.g. grazing, burning, flooding), commonly known as the "humped-back model" (Figure 7). Later on, this model was considered to be universal by plant ecologists (but see

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[14] Approach by which the functional diversity observed within communities is compared to the functional diversity simulated in randomly generated communities based on sampling within a region (de Bello *et al*. 2012).

[15] External constraints limiting plant growth and community productivity (e.g. salinity, light, nutrient content; Grime 1973).

[16] Mechanisms limiting plant biomass by causing its destruction (Grime 1973). It can be abiotic (e.g. fire, flood, avalanche) or biotic (e.g. grazing), and is usually considered unpredictable.

[17] Number of species in a defined area.

Adler *et al*. 2011; Fridley *et al*. 2012). The principle of this model is that in stable conditions (in absence of disturbance) species diversity is low in most productive (resource-rich) environments where stress is low and competition is high due to the abundance of competitive (tall fast-growing) species. In contrast, on the other side of the hump, with the increase in stressful conditions species density decreases due to the decrease in productivity as environmental conditions become too harsh even for stress-tolerant species to persist (left curve in Figure 7). When considering disturbance only in productive communities (without stress), a similar pattern is observed. At low disturbance, species density is low due to competitive exclusion by the abundant competitive species. With the increase of disturbance, ruderal species (i.e. fast-growing short-lived species), which are well adapted to disturbance (Grime 1974), progressively replace competitive species that poorly tolerate disturbance. Species density reaches its highest value at intermediate levels of disturbance, where competitive and ruderal species co-occur. This is similar to the “Intermediate Disturbance Hypothesis” (Connell 1978). At high levels of disturbance, species density decreases as the abiotic constraint become extreme for any species to exist.

The universality of Grime’s “humped-back model” was held true, until Waide *et al*. (1999) doubted of its scale-dependency, which was later confirmed by Mittelbach *et al*. (2001) who, however, found it to be the dominant pattern for plants, especially at local to landscape scales. Subsequently, Gillman & Wright (2006) performed a survey on 159 productivity-plant species richness relationships from 131 published studies and concluded that positive relationships were the exclusive form of relationships at continental to global extents, and that unimodal (humped-back) relationships were more likely to occur at small spatial (local) scales. Moreover, Pärtel *et al*. (2007) argue that the productivity-diversity humped-back relationship is not universal, as it is valid in temperate regions but not in tropical ones where positive relationships are more common. Later on, based on an intercontinental data set (from 48 herbaceous-dominated plant communities on five continents), Adler *et al*. (2011) challenged the concept of the humped-back model of plant diversity, doubting of its utility by showing no consistent general relationship between productivity and species richness at local, regional or global scales. However, Adler *et al*.’s work was criticised by Fridley *et al*. (2012) who showed that the data used was not exactly representative, mostly because it lacked sufficient high-productivity sites and excluded anthropogenic sites for no scientific reasons; by including high-productivity sites (e.g. salt marshes, herbaceous floodplains…), Adler *et al*.’s data would have revealed a pattern consistent with the humped-back model (Fridley *et al*. 2012), i.e. a decrease in richness at high productivity levels.

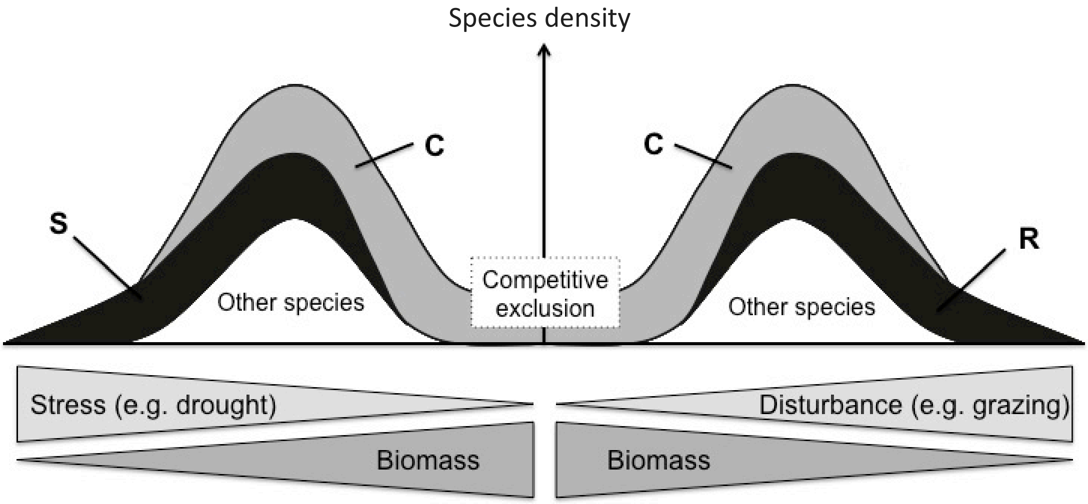

Figure 7. Diagrams representing Grime's humped-shape relationship between species density and stress/biomass (left panel), and between species density and disturbance/biomass (right panel). The vertical axe also represents an index of competitive exclusion. Species diversity is highest at intermediate stress and disturbance levels where competitive exclusion is at its peak. The letters represent: C: competitive species (or ecotypes), S: stress-tolerant species – species of high resistance to stress, R: ruderal species – species with a rapid seedling establishment and growth (adapted from Grime 1973).

2.2- Facilitation: a long-time forgotten interaction due to the predominance of competition

Until the mid-nineties, ecological theories and models have considered only negative interactions (e.g. Grime 1973; Connell 1978; Huston 1979; Tilman 1980, 1982), even though positive interactions (i.e. facilitation) have been reported in experimental studies [Niering *et al*. 1963 and Turner *et al*. 1966 in Callaway 2007; Hunter & Aarssen 1988] and ecological theories (Clements 1916). This is because negative interactions (e.g. competition or interference) were thought to be the main biotic filter structuring plant communities (Goldberg & Barton 1992). However, two schools of thoughts regarding the strength of competition along productivity gradients [in plant community ecology] emerged over the time. This divergence in thoughts was known as the "Grime-Tilman debate". Grime considered that [aboveground] competition decreases from high to low levels of productivity, and interactions vanish under stressful conditions (Grime 1973, 1977). In contrast, Tilman founded his "resource-ratio" theory by arguing that when productivity decreases, competition for limiting resources switches from aboveground to belowground, and thus competition is held constant (Tilman 1980, 1982). Both theories have gained significant attention in the field of plant ecology. Grace (1991) argues that the "Grime-Tilman debate" is due to differences in the definitions of some terms used by each of these authors (e.g. 'competition', for Grime, it is the capacity for resource capture and the mechanism by which a plant suppresses the fitness of a neighbour; for Tilman, competitive success is the ability to draw resources to a low level and to tolerate those low levels – to have a low equilibrium resource requirement). Welden & Slauson (1986) tried resolving this 'debate' by clarifying the difference between the intensity and the importance of competition (see Box 1 for the definition of 'competition intensity' and 'competition importance'). Competition importance has been proposed to explain Grime's (1973) theory on competition, whereas intensity explains Tilman's theory – competition intensity stays constant along the productivity gradient, but switches from aboveground to belowground in unproductive environments – (Welden & Slauson 1986; Grace 1991).

Until recently, negative interaction have been the primary concern of studies in plant community genetics (e.g. Whitlock *et al*. 2007; Lankau & Strauss 2007; Johnson *et al*. 2008; Bossdorf *et al*. 2009; Silvertown *et al*. 2009; Genung *et al*. 2011). However, [as already said] facilitation has been found in theoretical and experimental studies (Clements 1916; Hunter & Aarssen 1988). For instance, Clements (1916) argues that plants themselves cause succession to occur by improving site factors (e.g. light capture by leaves, production of detritus, water and nutrient uptake, nitrogen fixation), which allows the establishment of plants of the next succession stage. This means that plants of one stage directly 'facilitate' plants of the next succession stage. Though, the little attention given to facilitation and the predominance of competition for a long time in research fields such as ecology is likely because facilitation could go undetected, as it appears weaker than competitive mechanisms (Gross 2008).

Box 1. 'Competition intensity' *versus* 'Competition importance'

Welden & Slauson (1986) state that:

*"The **intensity of competition** is a physiological concept, related to the well-being of individual organisms but only indirectly and conditionally to their fitness, and even more indirectly to the evolution of populations and the structure of communities. **The importance of competition** is primarily an ecological and evolutionary concept, related directly to the ecology and fitness of individuals but only indirectly to their physiological states. The intensity of competition is not necessarily correlated with the intensities of predation, disturbance, abiotic stress, or other ecological processes. The importance of competition is necessarily relative to the importances of other processes. Intensity refers primarily to the processes of present competition, whereas importance refers primarily to the products of past competition".*

In other words:

Intensity refers to the absolute impact of neighbouring plants on a target plant (negative for competition and positive for facilitation).

Importance is the contribution of biotic interactions relative to other environmental processes such as stress and disturbance (*sensu* Grime 1973) to the change in the performance of a target plant.

Nevertheless, interest in facilitation in ecological studies has significantly increased over the past two decades, leading Bertness & Callaway (1994) to come up with the Stress Gradient Hypothesis (SGH; Figure 8). The SGH predicts that the outcome of plant-plant interactions extends from competition at intermediate levels of stress and disturbance (under favourable conditions) to facilitation at both extremes of these two gradients (Bertness & Callaway 1994; Brooker & Callaghan 1998). At high levels of stress, facilitation is due to habitat amelioration by stress-tolerant species leading to the extension of the realised niche of [stress-intolerant] competitive species (Bruno *et al*. 2003). At high levels of disturbance, facilitation is indirect by the means of associational defences (e.g. associational defences against herbivores, Rousset & Lepart 2000; Milchunas & Noy-Meir 2002; Rebollo *et al*. 2002; Baraza *et al*. 2006; Smit *et al*. 2007). Because of the differences in the methods followed (observational *versus* experimental; Maestre *et al*. 2005) and the complexity added by factors like the variation in the nature (resource *versus* non-resource) and the length of the gradients considered (Maestre *et al*. 2005; Lortie & Callaway 2006; Brooker *et al*. 2008), the chosen estimator of performance (Maestre *et al*. 2005; Brooker *et al*. 2008; Gómez-Aparicio *et al*. 2008) and the diverse characteristics (chemical and physical, Baraza *et al*. 2006) and strategies (competitive *versus* stress-tolerant) of both the nurse[18] and the beneficiary[19] species involved (Liancourt *et al*. 2005; Michalet 2007; Maestre *et al*. 2009), the outcomes of plant-plant interaction studies in the literature were ambiguous and conflicting. Many experimental studies have supported the predictions of the SGH (Callaway *et al*. 2002; Liancourt *et al*. 2005; Schiffers & Tielbörger 2006) while others contradicted it and found competition to be important under high stress levels (Maestre & Cortina 2004).

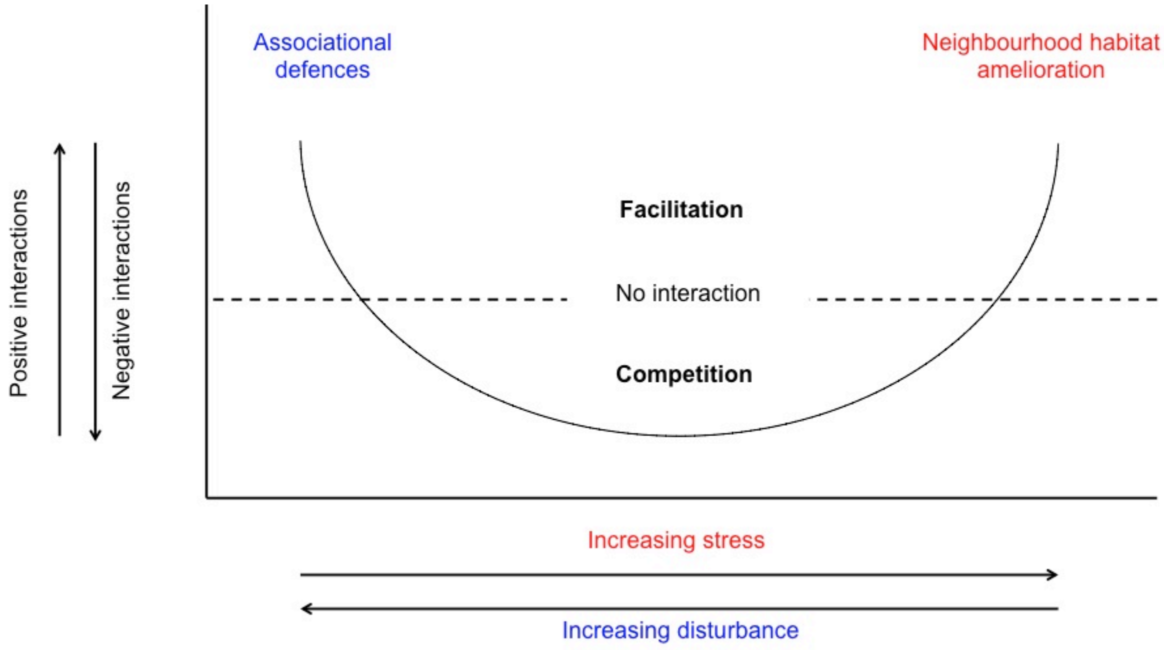

Figure 8. The Stress Gradient Hypothesis (SGH): the shift in biotic interactions along stress and disturbance gradients. Facilitation increases by neighbourhood habitat amelioration with the increase in stressful conditions (in red), and by associational defences with the increase in physical disturbance (in blue; adapted from Bertness & Callaway 1994).

Hacker & Gaines (1997) suggested that at intermediate levels of stress and

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[18] In the context of facilitation, a 'nurse' or facilitator species is the species that ameliorates the environmental conditions.

[19] In the context of facilitation, a beneficiary is the 'nursed' or facilitated species that benefit from the amelioration of the environmental conditions by the nurse species.

disturbance, facilitator species that might normally be competitively excluded are released from competition and therefore enhance species diversity from intermediate to very high levels of stress and disturbance. Later on, Michalet *et al*. (2006) presented a revision of Grime's humped-back model by integrating facilitation to it (Figure 9; for models integrating facilitation to ecological theory, also see Bruno *et al*. 2003 and Lortie *et al*. 2004). According to them, the model remains unchanged in productive environments where diversity is low due to competitive exclusion (part A1 in Figure 9). In conditions of low environmental severity (part A2 in Figure 9), competition is gradually replaced by facilitation, thus increasing diversity by expanding the realized niche of stress-intolerant competitive (stress-intolerant) species. Diversity reaches its maximum at intermediate levels of environmental severity where species of the three strategy types (competitive, ruderal and stress-tolerant species) co-occur. From intermediate to high environmental severity levels (part B1 in Figure 9), facilitation decreases for competitive and then for stress-tolerant species, thus decreasing diversity. At very high environmental severity levels, facilitation 'collapses' as environmental conditions become too harsh for the 'nursing' plants to facilitate other plants (due to a decrease in the size of the nurse plants; see also Forey *et al*. 2010; Le Bagousse-Pinguet *et al*. 2013; Michalet *et al*. 2014a). Additionally, some authors proposed that at the most severe conditions, all interactions (positive and negative) collapse as both competitive and facilitative species are weakened (Malkinson & Tilbörger 2010), consistent with the results of Maalouf *et al*. (2012).

The inclusion of positive interactions into ecological theories proved that facilitative interactions also have strong effects on community and ecosystem properties, including structure, productivity and stability (Mulder et al. 2001; Michalet *et al*. 2006; Callaway 2007; Brooker *et al*. 2008; Butterfield *et al*. 2013; Le Bagousse-Pinguet *et al*. 2014a), specifically in severe environments. Thus, it is not surprising to see the growing number of studies of facilitative interactions among plants in an evolutionary perspective (Valiente-Banuet *et al*. 2006; Crutsinger *et al*. 2010, 2013 ; Liancourt & Tielbörger 2011; Michalet *et al*. 2011; Thorpe *et al*. 2011; Butterfield *et al*. 2013; Bailey *et al*. 2014; Le Bagousse-Pinguet *et al*. 2014a).

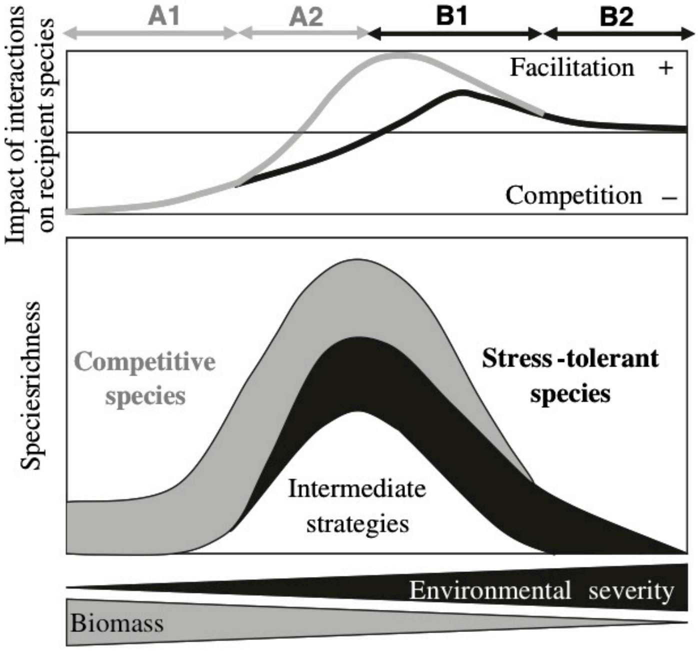

Figure 9. Michalet *et al*.'s (2006) inclusion of facilitation into Grime's humped-back model of the relationship between species richness and both biomass and environmental severity (adapted from Grime 1973). Lower panel: variation of species richness along two opposite gradients of environmental severity (stress and disturbance) and biomass. Upper panel: the average type of net interactions (the sum of positive and negative interactions between neighbours) received by competitive stress-intolerant competitive species (grey curve) and stress-tolerant species (black curve). In the parts A1 and B2 of the graph only one curve is drawn, because only one of these two types of strategies occurs in the communities (as is consistent with the lower panel).

2.3- Measuring biotic interactions

Positive and negative interactions act simultaneously in nature (Holmgren *et al*. 1997; Maestre *et al*. 2003). Usually, the net result of an interaction is measured by comparing the performances (generally survival and growth) of target individuals in presence and absence of a neighbour. Competition occurs when the target performance is better in absence rather than in presence of a neighbour; facilitation occurs in the opposite case. Thus, measuring plant-plant interactions requires experimental approaches. 'Neighbour removal' is the commonly used method. Other methods, such as comparing the response of a target species in natural open areas near the neighbour, have also been used particularly in dry ecosystems or alpine communities. Michalet (2006) stressed the importance of choosing the method to quantify biotic interactions, as it can affect the outcome of the experiment, specifically in arid environments. Usually, target plants used in such experiments are phytometers randomly collected at the site. Weigelt & Jolliffe (2003) present 50 indices that help calculating the net effect of an interaction. It is now widely accepted that the Relative Interaction Index (RII, Armas *et al*. 2004) offers the most consistent results for calculating interaction intensity (see chapters 3, 4 or 5 for details on RII calculation); it can be used to measure multispecific interactions at the community level (Armas *et al*. 2004).

3- Evolution, natural selection and adaptation

In *The Origin of Species* (1859), Charles Darwin presents evidence of evolution. He argues that all living beings are descendants of an earlier species (shared a common ancestral parent at some point in their history), and explains the existence of evolution by proposing the mechanism of natural selection, which is an important process - but not the only one - by which evolution takes place within a population of organisms. The forces of natural selection act on the apparent characters (phenotype) of an organism; however, only when natural selection acts on heritable characters – selecting the genotype expressing 'the most appropriate' phenotype to 'fit' its environment – evolution can occur (Whitham *et al*. 2003), eventually leading to new species or ecotypes (Grassein *et* al. 2010). As said above, natural selection is not the only mechanism of evolution but one of the processes that leads to it. Since the time when Charles Darwin and Alfred Russel Wallace first came up with *The Theory of Evolution*, the growing knowledge has led to the 'evolution' of this theory giving birth to the *Modern Synthesis* (or *Modern Evolutionary Synthesis*) theory [Julien Huxley in *Evolution: The Modern Synthesis* (1942)]. This theory recognizes several possible mechanisms of evolution other than natural selection, such as genetic drift (or allelic drift), mutations, and migration, which also play a role in the evolution of new species. Also, phenotypic plasticity is believed to be an evolutionary adaptation to environmental variation (Sultan 1995), allowing individuals to change their phenotypes in order to 'fit' a new environment.

Evolution implicates two related phenomena: adaptation[20] and speciation[21]. Over the course of time, species struggle to cope with their changing environment, which requires them to modify their phenotypes in ways that permit them to succeed and persist in their

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[20] A modification in structure or behaviour, often hereditary, by which a species or individual improves its condition in relationship to its environment.

[21] An evolutionary process by which new species arise.

environment. These changes in one species can result in the emergence of two or more new species, leading to the multiplication of the number of species.

3.1- Local adaptation: the contribution of genetics and plasticity

Local adaptation is defined as “a process whereby natural selection increases the frequency of traits within a population that enhance the survival or reproductive success of individuals expressing them” (Taylor 1991). In other words, it is an adaptive variation in response to local changes in environmental conditions. Generally, local adaptation is more important for immobile organisms (e.g. plants) than mobile organisms (e.g. animals). This is because, when local conditions become stressful, mobile organisms are often capable to migrate to a more suitable environment, whereas immobile organisms have to increase physiological tolerance or phenotypic plasticity in order to cope with the changes and survive (Bradshaw 1972). Plants can be locally adapted either through genetic variation (e.g. genetic mutation, gene flow, genetic recombination, genetic drift, migration; Kawecki & Ebert 2004) or by phenotypic plasticity[22] (Sultan 1995), which is important for short-term responses to environmental change as far as it helps species to persist for a longer period during which evolutionary adaptation may occur (Pratt & Mooney 2013). When no other forces and constraints occur, local adaptation is expressed in improved fitness of each genotype (or deme) in its local habitat than genotypes from other habitats (Kawecki & Ebert 2004). Nevertheless, species performance and distribution is in part determined by their interactions with other species within their community (Brooker & Callaghan 1998).

3.2- Detecting local adaptation: reciprocal transplant and common-garden experiments

A way to straightforwardly study local adaptation is throughout reciprocal transplant experiments (Joshi *et al*. 2001; Kawecki & Ebert 2004; Ågren & Schemske 2012; Bennington *et al*. 2012). This approach involves assessing the performance of individuals of at least two different genotypes (or phenotypes) by reciprocally raising them in home and away sites. As argued by Kawecki & Ebert (2004), from the viewpoint of local adaptation, a home-site advantage must be evaluated between ‘local’ *versus* ‘immigrant’ genotypes (or phenotypes) and not between a given genotype at ‘home’ and ‘away’ (which is a reaction norm[23] used for assessing plasticity, Pigliucci *et al*. 2006; Vitasse *et al*. 2010). When applicable in the field, this approach is highly relevant as it allows assessing the performance of the differing

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[22] The capacity of a genotype to change its expression in response to environmental variation.

[23] The set of values of a character expressed by an organism in response to different environments (*sensu* Nijhout 2003).

genotypes (or phenotypes) under natural environmental conditions that are difficult or even impossible to artificially reproduce in laboratory. However, an approach of reciprocal transplant with adult individuals is sometimes technically, ethically or legally impossible (Kawecki & Ebert 2004; García-Fernández *et al*. 2013). In these situations, greenhouse and common-garden[24] experiments offer an opportunity to assess the performance of different genotypes (or phenotypes) under controlled environmental conditions, thus excluding confounding effects that cannot be taken into account in the field. Additionally, such experimental approaches are useful as they allow separating the effects of plasticity from those of genetic differences (Clausen *et al*. 1940; Roach & Wulff 1987; Schmid & Dolt 1994).

4- Thesis objectives

The main scope of this Ph.D. is to examine the community-level consequences of a phenotypic differentiation within two foundation species – *Festuca gautieri* (from the Poaceae family) in the French Pyrenees and *Onobrychis cornuta* (from the Fabaceae family) in Mount-Lebanon (Lebanon) – of the subalpine and oromediterranean communities, respectively.

In both systems, each species presents two phenotypes: a 'tight' phenotype with a compact morphology dominating convex topographic habitats, and a 'loose' phenotype with open stem morphology dominating concave topographic habitats. In an evolutionary context, it is important to assess the genetic differentiation within foundations species, due to the increased chances of having consequences at the community level (Whitham *et al*. 2006; Gibson *et al*. 2012). Thus, my first objective was to assess the genetic basis of the observed phenotypic differences within each of the studied foundation species.

In both systems, contrasting associational patterns with subordinate[25] species were observed between the phenotypes (for each foundation species), with the tight phenotype associated with few subordinate species and the loose phenotype associated with a high cover of subordinate species. This lead to the hypothesis that there are differences in effects between the two phenotypes on their subordinate species, with the tight phenotype being a competitor

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[24] An experimental approach involving planting individuals at the same field site so that all individuals experience the same environmental conditions. Observed differences in the phenotypes among plants are assumed to be than environmentally based (Whitham *et al*. 2006).

[25] In a community including a dominant species (i.e. species found frequently with high relative cover), subordinate species (or simply, subordinates) are species found frequently, but with low relative cover (*sensu* Grime 1998).

and the loose one being a facilitator. Thus, my second objective was to assess the differences across phenotypes in effects on subordinate species, and to evaluate the contribution of genetics and the environmental variation (e.g. drought stress and grazing disturbance) to changes in phenotype effects.

Additionally, contrasting flower production was observed between tight and loose phenotypes with tight phenotypes (associated with very few subordinate species) having a high flower production and loose phenotypes (associated with a high cover of subordinate species) having a low flower production. This suggests that harbouring subordinate species may have consequences for the foundation species. Thus my third objective was to assess the feedback effects of subordinate species on the foundation species.

The following are the main questions resuming my thesis objectives:

1- What are the relative contributions of genetics and plasticity to the phenotypic differences within foundation species? **[Chapter 3]**
2- Do the differing phenotypes have contrasting effects on subordinates? If so, are these effects heritable? **[Chapters 3 and 4]**
3- Are these effects influenced by changes in environmental conditions (drought stress and grazing disturbance)? **[Chapters 3, 4, 5]**
4- Do community feedbacks affect foundation species fitness? **[Chapter 3]**

CHAPTER TWO: STUDY SITES, MODEL SPECIES AND EXPERIMENTAL DESIGNS

My Ph.D. study is implemented in two mountainous ranges, the Pyrenees (Pyrénées-Atlantiques, France) and the Mount-Lebanon (Lebanon). In the following parts of this chapter, I present the two study sites and model species in each mountainous range, and a description of my experimental designs.

1- The study site in the Pyrenees, the model species (*Festuca gautieri* subsp. *scoparia* Hackel & Kerner), and the experimental designs

1.1- Study site and model species

The Pyrénées-Atlantiques is a department in Aquitaine region, southwest of France. It takes its name from the Atlantic Ocean and the Pyrenees chain, which crosses it from the Col d'Aubisque in the East to the mouth of the Bidassoa River in the West. In this department, the Pyrenees culminates at 2884 m (Pic du Midi d'Ossau). Cattle, sheep, pigs and horses farming are a major human activity in the Pyrenees; the beginning of summer season is marked by the 'summer transhumance', the migration of the herds from the valley to the mountain.

I carried out my research on the northern side of the Pyrenees, 100 km from the Atlantic Ocean, at La Pierre Saint-Martin pass (42°58'N, 0°45'W, altitude: 1744 m a.s.l.; Figure 10). Climate is temperate oceanic with the highest rainfall occurring in winter (mean annual precipitation is 2850 mm). Mean temperature ranges between -2°C in winter and 12°C in summer (Soum-Couy, 42°58'N, 0°43'W, altitude: 2150 m, Météo France). The bedrock is calcareous, mainly limestone with contrasting soil depths depending on topography (Le Bagousse-Pinguet *et al*. 2014b). The vegetation form is calcareous grassland, characterised by short herbaceous plant communities dominated by a cushion-forming foundation species, *Festuca gautieri*. Similar to other cold-temperate calcareous grasslands in Europe, the subalpine grasslands on calcareous substrates in the Pyrenees are ecosystems with a rich diversity of plant species (Sebastiá 2004).

1.2- Model species

My model species, *Festuca gautieri* subsp. *scoparia* Hackel & Kerner, the 'Bearskin fescue', is a dwarf grass with a circular to elliptic cushion-like form. It has bright green leaves with yellowish-green inflorescences flowering from July to August. At my site, this species dominates the steep scree slopes (Figures 9 and 10), most probably because of its low grazing tolerance. It is endemic to the Pyrenees and plays a fundamental ecological role, as it is a foundation species (i.e. species that structures a community by creating locally stable conditions, Ellison *et al*. 2005) typical of subalpine[26] [and alpine] grasslands[27] on rocky calcareous soils (Saule 1991). The habitat dominated by *F. gautieri* came under the terms “Pelouse pyrénéennes à *Festuca gautieri* (36.434)” in the CORINE biotopes code and “Pelouses calcaires alpines et subalpines (6170)” in the EU code (Annex 1 of the Directive Habitat). Cushion-like plants are best adapted to alpine habitats (Körner 1995) and mostly common in cool and nutrient poor environments, especially on bare soils and in open vegetation in exposed habitats (Körner 2003). These forms of plants are known for their ability to create a favourable microenvironment for other species (plants and/or animals) as they attenuate the extreme environmental temperatures and increase soil moisture within their canopy (Arroyo *et al*. 2003; Körner 2003; Cavieres *et al*. 2006). However, this depends on the morphology of the cushion, as cushions of the same species may present morphological differences (Michalet *et al*. 2011). In my study system, two distinct cushion phenotypes, tight and loose, occur under contrasting topographic and soil conditions (Le Bagousse-Pinguet *et al*. 2014b) with contrasting associations with other species of the community. The tight phenotype, characterised by compact stem morphology, dominates convex topographies with shallow stony (stone cover = 73.6 ± 3.7%, n = 30) and relatively dry soils (soil volumetric water content measured with a theta-probe - ML3X - Delta-T Devices, Cambridge, UK - 3 days after a rain event: 13.10 ± 1.28, n = 15) and shelters few if any other species. The loose phenotype, characterised by loose stem morphology with open area within its canopy sheltering many subordinate species dominates concave topographies with relatively deeper and less stony (stone cover = 53.0 ± 3.4%, n = 30, result of the t-test: $P < 0.001$) and relatively wet soils (soil volumetric water content: 27.73 ± 1.74, n = 15, result of the t-test: $P < 0.001$). Cushions with intermediate traits are also observed in intermediate ecological conditions, but were much less frequent that the two extreme phenotypes (cushion frequency measured in ten 10-m-long transects, 10.4% for intermediate cushions vs. 66.7% for tight phenotypes and 22.9% for loose phenotypes; cushion classification in the field was based on a leaf pungency index varying from 0: not pungent, to 5: very pungent; details on methods are provided in the methods part of chapter 3, article published in *Oecologia*). The scale of habitat heterogeneity is less than one meter, which allows both phenotypes to exist side-by-side (Figure 12). Frequent subordinate species of the community are *Agrostis capillaris* L., *Galium*

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[26] The subalpine zone is the zone of plants just below the tree line. In the Pyrenees, the subalpine vegetation zone ranges from 1700-1900 to 2300-2500 m in altitude. When ascending, it follows the Mountainous zone and precedes the Alpine zone.

[27] Grasslands are areas where the dominant vegetation is grass (Poaceae).

pumilum Murray, *Campanula rotundifolia* L., *Alchemilla conjuncta* Bab., *Lotus corniculatus* L., *Festuca rubra* L. and *Trifolium pratense* L.

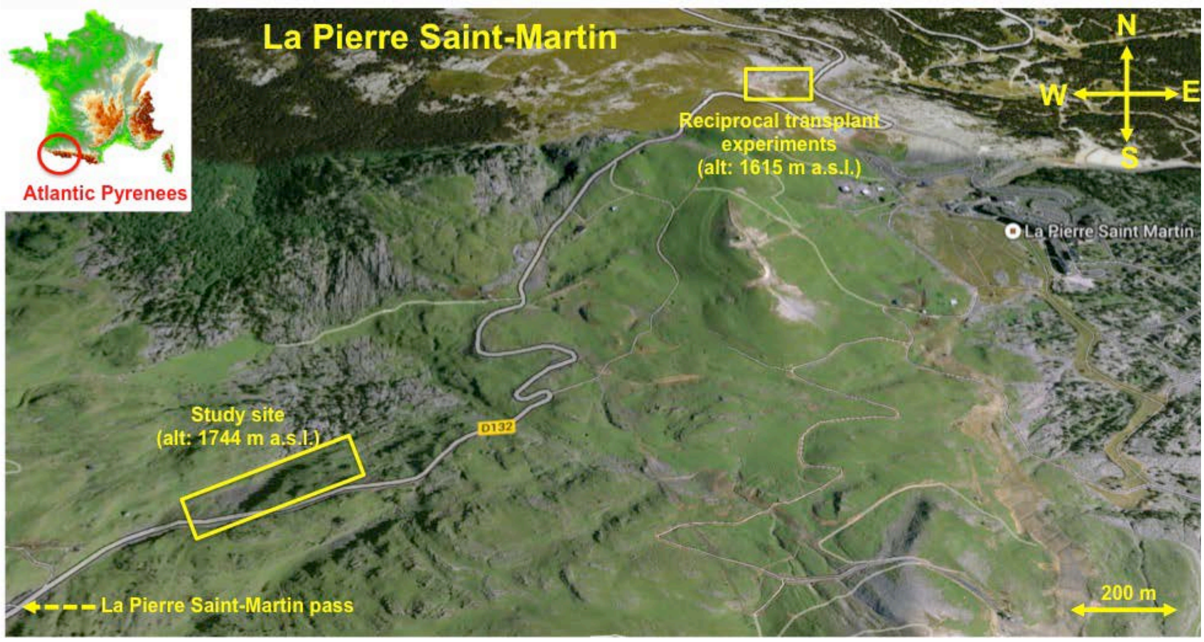

Figure 10. Location of the study site (yellow rectangle on the left; also see Figure 11) and of the reciprocal transplant experimental gardens (yellow rectangle on the right) at La Pierre Saint-Martin in the Pyrenees (42°58'N, 0°45'W). The picture was captured using Google Earth 7.1.2.2041.

1.3- Experimental designs

1.3.1- What are the relative contributions of genetics and plasticity to the phenotypic differences within *F. gautieri*? [Chapters 3 and 4]

I used a trait-based approach, a powerful tool allowing visualizing and comparing patterns (Keddy 1992; Garnier & Navas 2012), in a way to understand the morphological differences between *F. gautieri* phenotypes and the differing effects of these phenotypes on other species of the community. In order to answer my first main question of this thesis, I first adopted an observational approach in order to assess field differences in cushion traits between the phenotypes. In July 2012, I measured maximum leaf length, cushion penetration (an index of cushion compactness and interference), leaf thickness and number of inflorescences on 60 cushions (30 tight and 30 loose) in their natural habitats (42°58'N, 0°45'W, altitude: 1744 m a.s.l., La Pierre Saint-Martin, see Figure 9). In parallel, a shadehouse study was set up in order to experimentally assess the contribution of genetics and plasticity in changing traits among phenotypes and habitats. The shadehouse was located at the INRA station of Cestas-Pierroton, France (44°44'N, 0°46'W; 60 m a.s.l.). Three hundred replicates of each phenotype were grown from April 2011 till September 2013 in contrasting environmental conditions mimicking the two natural habitats (convex topography with shallow, stony and relatively dry soil for the tight cushion vs. concave topography with deep, less stony and wet soil for the loose cushion). The 300 replicates were obtained from 15 mature and discrete cushions (hereafter genotypes) that were collected on site in November 2010, each separated into 20 standardised tillers (leaves: 5-10, roots: 5 cm). Each tiller was transplanted in a separate pot, and all pots were randomly placed on benches. In 2011 and 2012, from April to September, a watering treatment was applied with 10 replicates of each treatment combination (phenotype x watering treatment) by irrigating half of the pots with one litre of tap water (Watered pots) three times a week and the other half once a week only (Dry pots). This was done to assess the potential plastic responses of phenotypes to the occurrence of a weak drought stress. At the beginning and the end of each growing season (April and October respectively), I recorded survival, maximum leaf length, cushion penetration, leaf thickness, cushion surface and leaf density (more details on methods are provided in chapter 3, article published in *Oecologia*). Additionally, in order to reinforce my results and to evaluate the adaptation of each phenotype to its habitat, I set up a reciprocal transplant experiment at La Pierre Saint-Martin (42°58'N, 0°45'W, altitude: 1615 m a.s.l., see Figure 9). In July 2013, I established two experimental gardens in contrasting topographic positions. The first garden (convex garden hereafter) was set up in a convex topographic position on shallow, stony and relatively dry soil simulating the tight cushion's natural microhabitat. The second garden (concave garden hereafter) was set up in a concave topographic position on deep, less stony and relatively wet soil simulating the loose cushion's natural microhabitat (Figure 13). The two gardens were 50 m apart. In each garden, I randomly transplanted 40 mature cushion replicates of each *F. gautieri* phenotype (50 cm distant from each other), representing 8 different genotypes with 5 replicates each (all transplanted cushions were grown from tillers for 2 years within the shadehouse at Cestas-Pierroton). Before planting out, resident vegetation was manually eliminated to limit competition. The gardens were fenced with metallic nets to prevent herbivory (more details on methods can be found in chapter 4).

Figure 11. *Festuca gautieri* cushions dominating steep (40°) North-facing scree slopes at the study site (altitude: 1744 m, La Pierre Saint-Martin, Atlantic Pyrenees, France). Photo taken in July 2012 by Patrick AL Hayek.

Figure 12. The two phenotypes of *Festuca gautieri* in their natural habitats at La Pierre Saint-Martin (Atlantic Pyrenees, France): tight cushion in rocky convex topography and loose cushion in concave topography. Photo taken in July 2014 by Patrick Al Hayek.

Figure 13. Two photos showing the two gardens (convex and concave) of the reciprocal transplant experiment at La Pierre Saint-Martin (Atlantic-Pyrenees, France). Photo (a) was taken in July 2012 by Clément Lalait, photo (b) was taken in July 2014 by Patrick Al Hayek.

1.3.2- Do the differing phenotypes have contrasting effects on the subordinate species? Are these effects heritable? [Chapters 3 and 4]

I set up three experiments to evaluate the competitive and/or facilitative ability (effect and response) of both *F. gautieri* phenotypes: in their natural environment (in the field), in the shadehouse and in the reciprocal transplant gardens. I used adult tight and loose cushions of *F. gautieri* to assess their competitive/facilitative effects, and three target individuals, *Agrostis capillaris* and the two phenotypes of *F. gautieri* to also assess their competitive/facilitative response.

In the field, a cushion removal experiment was set up (Al Hayek *et al*. 2014). In June 2011, 5 sites distant of at least 100 m were selected in a northern slope. In each site, we selected 6 discrete cushions of each *F. gautieri* phenotype that include one discrete individual of *A. capillaris*. For half of the cushions (3 tight and 3 loose in each site), we removed the aboveground parts of *F. gautieri* cushions within a circular area of 15 cm in diameter centred on the *A. capillaris* individual. Then, we transplanted one individual (tiller) of each *F. gautieri* phenotype at 5 cm from *A. capillaris* (this was done within removed and control cushions; Figure 14). In July 2012, we recorded survival, height, leaf number and biomass of all target individuals.

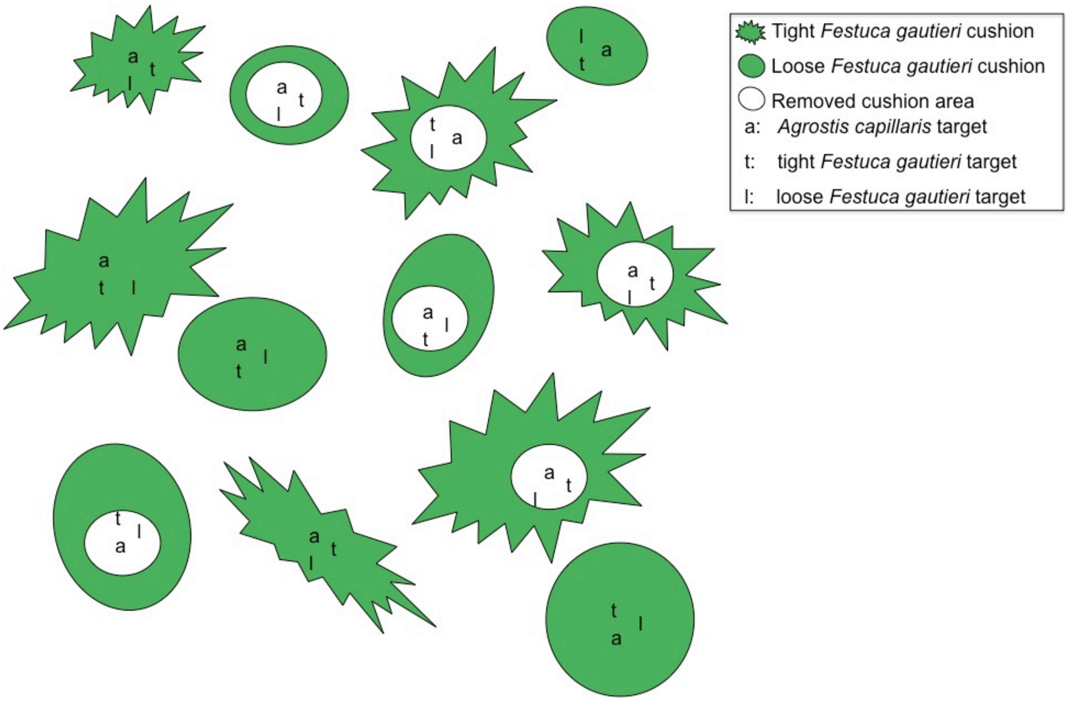

Figure 14. A schematic representation of the cushion removal experiment I conducted on site from June 2011 to July 2012. In this figure I represent one of the five sites of this experiment.

In the shadehouse, I recorded survival and biomass for the same three target species (*A. capillaris*, tight *F. gautieri*, and loose *F. gautieri*; Target treatment) grown in pots including a tight or loose cushion or no cushion (Neighbour treatment), with 36 replicates (6 replicates of 6 cushion genotypes) per treatment combination. All pots (with and without cushion) were randomly placed on benches (for field and shadehouse experiments, more details concerning methods can be found in the third section of the methods in chapter 3, article published in *Oecologia*).

In the reciprocal transplant gardens, in July 2013 I set up a competition experiment by transplanting the same three target individuals (*A. capillaris*, tight *F. gautieri*, and loose *F. gautieri*; Target treatment) within the 40 cushions of each *F. gautieri* phenotype and in 40 open areas (Neighbour treatment) between the cushions in each garden (Garden treatment). In September 2013 (after summer season), I recorded targets survival, growth (height and total leaf number), and in June 2014 I recorded survival but not growth as very few targets succeeded to survive and thus I did not have a sufficient number of replicates to perform statistical analyses.

In July 2014, I carried out a floristic survey within the cushions and the defined open areas and recorded species richness and abundance (for the reciprocal transplant competition experiment, more details concerning methods can be found in the third section of the methods in chapter 4).

1.3.3- Do community feedbacks affect *F. gautieri* cushion fitness? [Chapter 3]

This issue was addressed with both observational and experimental approaches. In July 2012, my colleagues and I counted the number of inflorescences of 60 cushions (30 tight and 30 loose). In June 2011, we randomly selected 40 loose cushions on site and removed all subordinate species growing within a circular area of 20 cm in diameter in half of the selected loose cushions. In late July 2012, I counted the number of inflorescences produced by all cushions within the delimited circular plot.

2- The study site in the Mount-Lebanon, the model species (*Onobrychis cornuta* (L.) Desv.) and the experimental design

2.1- Study site

The Mount-Lebanon is the highest Lebanese mountainous range extending along 170 km parallel to the Mediterranean coast (oriented NNE-SSW) and 25 to 30 km wide. It culminates at 3083 m a.s.l. (‘Qurnat as Sawda’, the highest peak of the Middle East). Its slopes are relatively steep due to the short distance over which the topography changes (exceeding 3000 m elevation within 60 km horizontal distance). The range receives a considerable precipitation, with more than 2000 mm on the highest summits (most of it is snow). In the high summit dominating the Mount-Lebanon range is the oromediterranean vegetation belt (starting altitude: 1900 - 2000 m). It is dominated by xerophytic dwarf thorny vegetation mostly from the Fabaceae (*Astragalus* spp. and *Onobrychis cornuta*) and Plumbaginaceae (*Acantholimon* spp.) families (Quézel & Médail 2003) and *Juniperus excelsa*, the only tree present at this altitude. This vegetation type, located above the current timberline, results from centuries of important grazing by domestic sheep and goats, after deforestation of *Cedrus libani* and *Juniperus excelsa* forests.

My study site was located in the central part of Mount-Lebanon, on the western external slopes, 20 km east from the Mediterranean Sea, at Ouyoun El Simane - Kfardebian (33°59’N, 35°51’E, altitude: 2000 m a.s.l.). Climate is Mediterranean with very high precipitation in winter and very low during summer (950 mm and 10 mm, respectively, with 1720 mm of annual rainfall). Mean temperatures are 2°C in winter and 16°C in summer.

2.2- Model species

My model species, *Onobrychis cornuta* (L.) Desv., the ‘horned sainfoin’, is a spiny shrub forming flattened circular to elliptic cushions with highly entangled branches and many axillary peduncles differentiated into rigid sterile or flowering thorns exceeding the short green leaves. Fertile stems hold pubescent spineless pods of 6-8 mm long, and 10-15 mm bright purple-pink flowers (Tohmé & Tohmé 2014) blossoming from May to July and constituting a source of nectar for pollinator insects. Although the species has low palatability due to its small leaves and spines (Díaz et al. 2001), it may provide forage for grazing animals such as cattle and sheep, in the absence of more palatable species (Shahriary *et al*. 2012). At my study site, *O. cornuta* is the dominant shrub acting as foundation species (Ellison *et al*. 2005) sheltering most subordinate species of the community. Two different phenotypes, tight and loose (with no intermediate phenotype, Figure 15), with contrasting associations with subordinate species, occur in two contrasting topographic positions and soil conditions. Tight cushions, characterized by dense stem morphology, dominate convex topographies on shallow stony soils (stone cover = 47.56 ± 2.38 %, n = 80). Loose cushions, characterized by loose stem morphology with open areas within their canopies, dominate concave topographies on relatively deeper and less stony soils (stone cover = 37.13 ± 2.45 %, n = 80, *t*-test: <0.01). Contrasting patterns of association with other plant species along with contrasting cushion flower productions were observed. Tight cushions have a low cover of other species and a very high flower production, whereas loose cushions have a high cover of other species and a low flower production, suggesting a probable reproductive cost for loose ecotypes for hosting other species (Michalet *et al*. 2011; Schöb *et al*. 2014). The community includes up to 60 species. Most frequent subordinates of the community are *Bromus tomentellus* Boiss., *Festuca pinifoli*a (Hack.), *Alyssum condensatum* Boiss, *Asyneuma rigidum* (Wild.) Grossh subsp. *sinai*, *Festuca* sp., *Asperula setosa* Jaub. & Spach., *Cruciata pedemontana* (Bell.) and *Prunus prostrata* Labill.

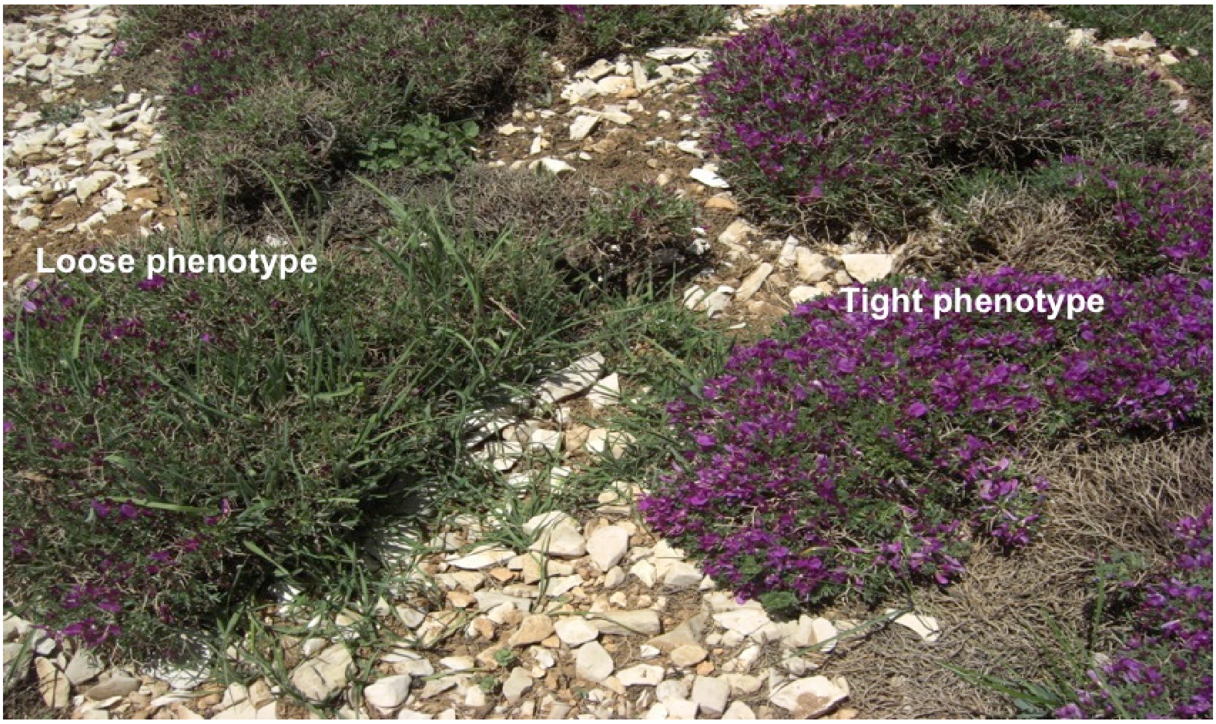

Figure 15. The two cushion phenotypes of *Onobrychis cornuta*: the loose phenotype with few flowers sheltering many subordinate species (on the left) and the tight phenotype with high number of flowers but no subordinate species (on the right). Photo taken at Ouyoun El Simane – Kfardebian (Mount-Lebanon, Lebanon) in June 2010 by Patrick Al Hayek.

2.3- Experimental design

2.3.1- Are the differences in effects between *O. cornuta* phenotypes (and thus differences in associations with subordinate species) affected by changes in environmental conditions (drought stress and grazing disturbance)? [Chapter 5]

In June 2012, I recorded cushion surface, height, penetration and flower number on 20 randomly selected cushions of each phenotype (tight and loose, Phenotype treatment) in two exposures (north and south, Exposure treatment) in two grazing conditions (grazed and ungrazed, Grazing treatment; Figures 16, 17 and 18). I also recorded the number of individuals of all vascular species present in each cushion and in a paired open area (open area near the cushion in the same microtopographic position, with a surface similar to the paired cushion).

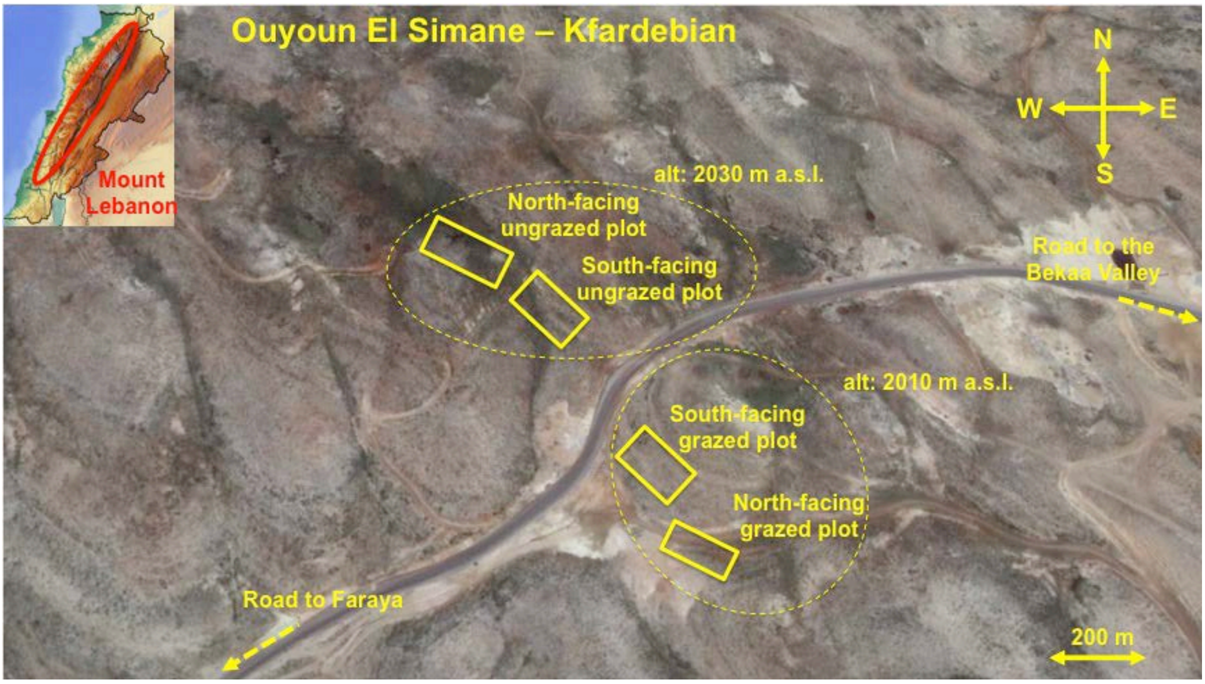

Figure 16. Location of the grazed (low dashed circle) and ungrazed (high dashed circle) plots at Ouyoun El Simane – Kfardebian in Mount-Lebanon (33°59'N, 35°51'E). The picture also shows the location on the North- and South-facing plots within each of the grazed and ungrazed areas. The picture was captured using Google Earth 7.1.2.2041.

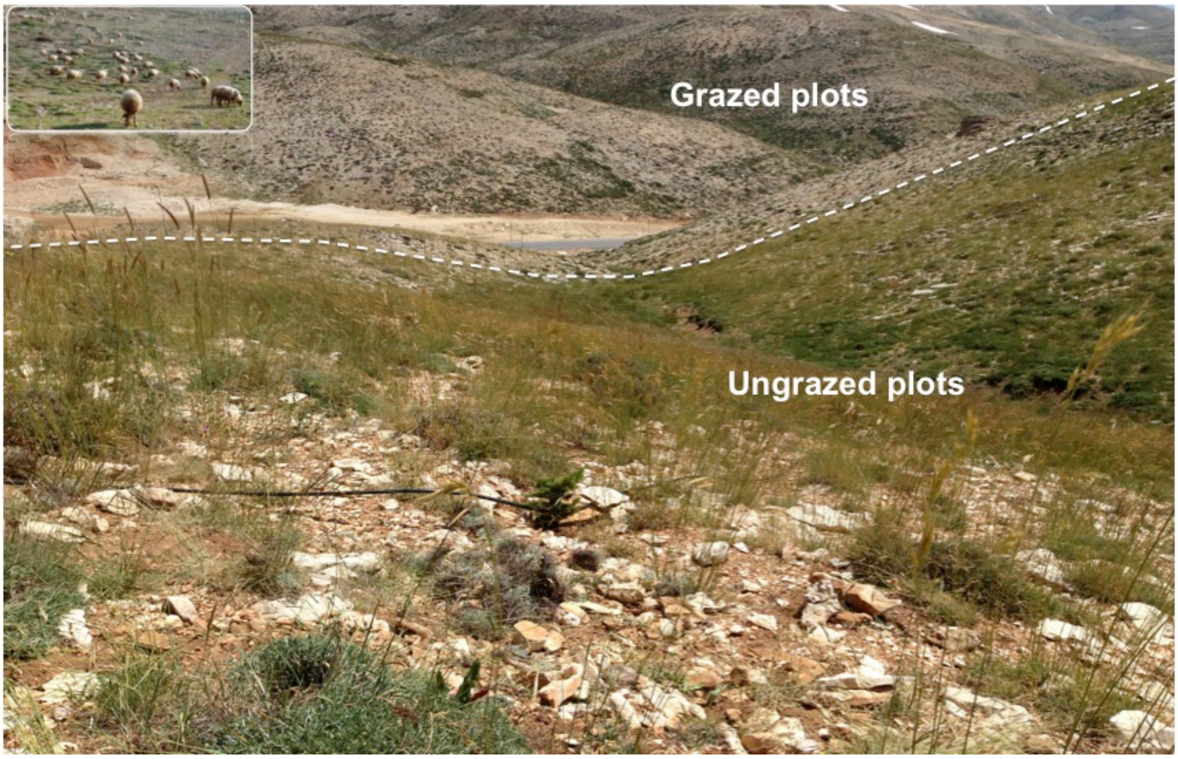

Figure 17. The grazed (upper part) and the ungrazed (lower part) plots. The dominating Poaceae is *Bromus tomentellus*. Photo taken at Ouyoun El Simane – Kfardebian (33°59'N, 35°51'E, Mount-Lebanon, Lebanon) in June 2013 by Patrick Al Hayek.

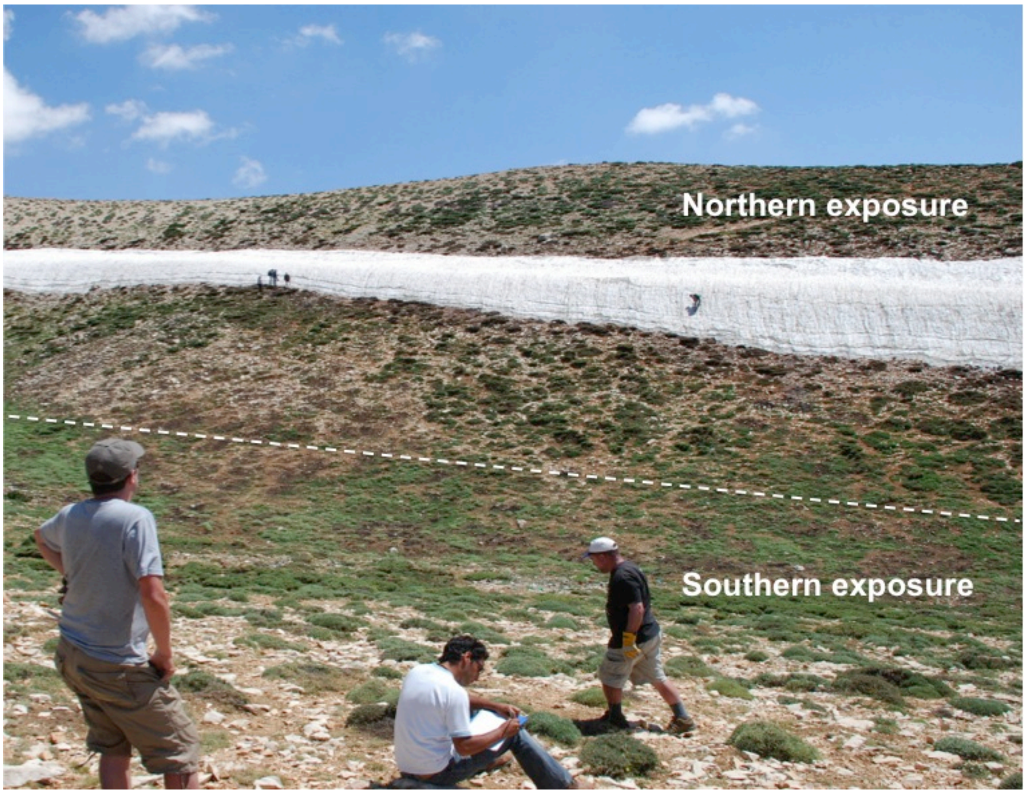

Figure 18. The North-facing (upper part) and the South-facing (lower part) grazed plots. Photo taken at Ouyoun El Simane – Kfardebian (33°59’N, 35°51’E, Mount-Lebanon, Lebanon) in June 2012 by Magda Bou Dagher-Kharrat.

CHAPTER THREE:

Phenotypic differentiation within a foundation grass species correlates with species richness in a subalpine community

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ABSTRACT

Few studies have examined consequences of ecotypic differentiation within alpine foundation species for community diversity and their feedbacks for the foundation species' fitness. Additionally, no study has quantified ecotypic differences in competitive effects in the field and in controlled conditions to disentangle genetic from plasticity effects in foundation/subordinate species interactions. We focused on a subalpine community of the French Pyrenees including two phenotypes of a cushion-forming species, *Festuca gautieri*: tight cushions in dry convex outcrops, and loose cushions (exhibiting high subordinate species richness) in wet concave slopes. We assessed, with field and shadehouse experiments, the genetic vs. plasticity basis of differences in: (1) cushion traits and (2) competitive effects on subordinates, and (3) quantified community feedbacks on foundation species' fitness. We found that trait differences across habitats had both genetic and plasticity bases, with stronger contribution of the latter. Field results showed higher competition within loose than tight phenotypes. In contrast, shadehouse results showed higher competitive ability for tight phenotypes. However, as changes in interactions across habitats were due to environmental effects without changes in cushion effects, we argue that heritable and plastic changes in competitive effects maintain high subordinate species diversity through decreasing competition. We showed high reproduction cost for loose cushions when hosting subordinates highlighting the occurrence of community feedbacks. These results suggest that phenotypic differentiation within foundation species may cascade on subordinate species diversity through heritable and plastic changes in the foundation species' competitive effects, and that community feedbacks may affect foundation species' fitness.

Keywords

Biotic interactions · Competition · Phenotypic plasticity · Facilitation · Feedback effects

INTRODUCTION

The evolutionary processes of local adaptation and eco- logical speciation in alpine environments have been largely addressed through the study of genetic differentiation in adaptive traits, which is likely because of the steepness of the environmental gradients (Billings 1974; Körner 2003; Choler et al. 2004). Many mountain ranges have provided numerous examples of ecologically differentiated plant taxa at a very small scale of environmental variation, in particular along topographic and soil gradients (Choler and Michalet 2002; Michalet et al. 2011; Lekberg et al. 2012; Liancourt et al. 2013). For example, Liancourt et al. (2013) showed, in the Mongolian steppe, differences in stress tolerance within the dominant grass species *Festuca lenensis* along a topographic gradient with a more stress-tolerant phenotype occurring in steep and dry upper slopes and a less stress-tolerant phenotype occurring in wetter and more productive lower slopes. Heterogeneous soil environments are considered particularly prone to sympatric phenotypic differentiation, probably due to increased reproductive isolation (Sambatti and Rice 2006; Lekberg et al. 2012).

Of particular interest, from an evolutionary ecology perspective, is genetic differentiation within foundation species (Ellison et al. 2005), because of increased chances of consequences at the community and ecosystem levels (Whitham et al. 2006; Gibson et al. 2012). Genetic effects have been shown to translate at higher complexity levels through biotic interactions (review by Whitham et al. 2006), as demonstrated by many studies of interactions across trophic levels. In contrast, studies of interactions within trophic levels (e.g. within plant communities) remain scarce (Bailey et al. 2009; but see Adams et al. 2011; Michalet et al. 2011; Gibson et al. 2012) compared with across trophic-level studies, likely due to the diffuse nature of plant–plant interactions and the rarity of feed- back effects (Bronstein 2009; Michalet et al. 2011; Schöb et al. 2014). Plant community genetic studies have mainly focused on competition or allelopathy (Lankau and Strauss 2007; Bossdorf et al. 2009; Silvertown et al. 2009), while evolutionary questions associated with positive interactions among plants (i.e. facilitation) such as nurse plant effects remain less documented (but see Valiente-Banuet et al. 2006; Liancourt and Tielbörger 2011; Michalet et al. 2011; Thorpe et al. 2011; Butterfield et al. 2013). Facilitative interactions are known to have strong effects on community and ecosystem properties, including diversity, structure, productivity and stability (Michalet et al. 2006; Callaway 2007; Brooker et al. 2008; Le Bagousse-Pinguet et al. 2014a). Thus, there is a high potential for exploring how phenotypic differentiation within foundation plants may affect community structure through facilitative interactions, and in particular along local soil gradients known to be particularly prone to genetic differentiation.

Michalet et al. (2011) showed that variation in competitive and facilitative effects among phenotypes of *Geum rossii* had strong consequences on species diversity for alpine communities in Northern Arizona, and that beneficiary species had feedback effects acting as selective pressure for the cushion plant. In highly physically disturbed concave volcanic slopes, they observed a phenotype of *G. rossii* characterised by loose stem morphology and strong facilitative effects on other species, and in adjacent more stable convex slopes they found a competitive phenotype, characterised by dense stem morphology. However, because the very low growth rate of this alpine cushion plant did not allow them to manipulate adults in reciprocal transplantations or in common garden experiments, Michalet et al. (2011) only compared the effects of each phenotype on other species in their own environment. Thus, they could not clearly separate heritable from environmental effects in the foundation/beneficiary interactions, and thus fully address the genetic basis of the processes driving changes in community diversity and composition. Indeed, an increase in facilitation from stable convex slopes to disturbed concave slopes may be due to either genetically based variation in nurse effects, or to nurse trait plasticity induced by changing environments, as well as direct environmental effects on target beneficiary species (Michalet et al. 2014).

We conducted our study in subalpine grassland com- munities of the French Pyrenees. Alpine communities are particularly prone to positive interactions (Callaway et al. 2002), thus are suitable systems with which to explore how phenotypic differentiation within foundation plant species may affect community structure through facilitative interactions. Competition and facilitation co-occur in the studied system along a natural soil moisture gradient and are directly related to species richness (Le Bagousse-Pinguet et al. 2014b). Also, phenotypic variation within a foundation grass species (*Festuca gautieri* subsp. *scoparia* Hackel and Kerner) has been observed along a soil moisture gradient (Le Bagousse-Pinguet et al. 2014b). A ‘tight’ phenotype with dense cushions with short stiff leaves occurs on dry soils in convex topographical positions (high-stress habitat), and a ‘loose’ phenotype with cushions characterised by open stem morphology and long soft leaves occurs on wetter soils in concave topographies (low-stress habitat). Le Bagousse-Pinguet et al. (2014b) showed that the tight *F. gautieri* phenotype was more drought tolerant than the loose phenotype. In the same subalpine system they transplanted tillers of each phenotype along a complex gradient of soil water availability. They showed that the probability of survival of the loose phenotype was reduced by over 50 % when soil moisture decreased from 40 to 15 %, whereas there was no change in survival of the tight phenotype. Considered together, the contrasting habitat distribution and experimental evidence suggest that the two phenotypes correspond to sympatric ecotypes. Both phenotypes can reach an adult size in a very short time in a shadehouse, allowing an assessment of a potential heritable component of differences in competitive and facilitative effects.

Here, we assess whether changes in subordinate species diversity among habitats and phenotypes are driven by heritable changes in foundation/subordinate species interactions, and thus, evaluate the genetic basis of the ecological processes acting in this subalpine community. Additionally, we assess the potential evolutionary feedbacks of these changing interactions by quantifying the effects of subordinates on the phenotypes' fitness. Specifically, we aimed to answer these main questions, each related to one of the three fundamental premises of a full community genetics perspective (Whitham et al. 2006):

. What are the relative contributions of genetic differentiation and phenotypic plasticity in the morphological trait divergences?

. To what extent do heritable differences in foundation species' morphological traits between phenotypes contribute to changing cushion effects on subordinate species across habitats?

. Do subordinate species have feedback effects on the fitness of the foundation species?

Variation in phenotypic traits was first measured both in the field and in a shadehouse in order to assess the heritability of field phenotypic variation. In the shadehouse, morphological traits of both phenotypes were measured under contrasting water stress conditions mimicking natural environmental variation, in order to also quantify the contribution of plasticity in changing traits among phenotypes and environments (see Online Resource 1 for the design). Second, we quantified changes in foundation/subordinate species interactions among phenotypes, both across habitats in the field and in the shadehouse in constant environmental conditions, in order to assess the likely heritability of changes in interactions (Online Resource 1). Third, the potential feedback effects of subordinate species on the cushion species' fitness were quantified in the field (Online Resource 1).

MATERIALS AND METHODS

Study system and target species

The experimental site is located in the western Pyrenees, 100 km from the Atlantic ocean at La Pierre Saint-Martin pass (42°58′N, 0°45′W, altitude 1,744 m a.s.l., Pyrénées-Atlantiques, France). The climate is temperate oceanic; the mean winter temperature is −2.2 °C and the mean summer temperature is 11.5 °C; the mean annual precipitation is 2,850 mm with the highest rainfall occurring in winter. *Festuca gautieri* is a very distinctive dwarf grass forming circular to elliptic cushions of thin bright green leaves with yellowish-green inflorescences. Flowering occurs from July to August. It is a foundation species typical of subalpine and alpine grasslands on rocky calcareous soils in the entire Pyrenees chain (Saule 1991). This species only dominates grasslands on steep scree slopes under low grazing conditions in the studied communities, likely because of its low grazing tolerance. Two distinct phenotypes of cushions occur in the system, under contrasting topographical and soil conditions (Le Bagousse-Pinguetet al. 2014b). A tight phenotype dominates con- vex topographical habitats with shallow, stony (stone cover = 73.6 ± 3.7 %, *n* = 30) and relatively dry soils [soil volumetric water content measured with a ML3X ThetaProbe (Delta-T Devices, Cambridge, UK) 3 days after a rain event, 13.10 ± 1.28, *n* = 15]. A loose phenotype dominates concave topographical habitats with deep, less stony (stone cover = 53.0 ± 3.4 %, *n* = 30, *t*-test $P < 0.001$) and relatively wet soils (soil volumetric water content, 27.73 ± 1.74, *n* = 15, *t*-test $P < 0.001$). The scale of habitat heterogeneity was less than 1 m, which allows both phenotypes to exist side by side. Cushions with inter- mediate traits are also observed in intermediate ecological conditions, but they are less frequent than the two extreme phenotypes [cushion frequency measured in ten 10-m-long transects, 10.4 % for intermediate cushions vs. 66.7 % for tight phenotypes and 22.9 % for loose phenotypes; our field phenotype classification was based on a leaf pungency index varying between 0 (not pungent) and 5 (very pungent); see Online Resource 2]. We also observed contrasting patterns of association with other species along with contrasting flower productions of the cushions. Loose phenotypes had a high cover of other species and a low flower production, whereas tight phenotypes had a low cover of other species and a very high flower production, suggesting a potential reproductive cost for loose phenotypes (see Online Resource 1, 2 for the method). Most frequent subordinate species (i.e. species associated with a community dominant and with a lower abundance) of the community were *Agrostis capillaris* L., *Galium pumilum* Murray, *Campanula rotundifolia* L., *Alchemilla conjuncta* Bab., *Lotus corniculatus* L., *Festuca rubra* L. and *Trifolium pratense* L.

Differences in cushion traits between *F. gautieri* phenotypes

In order to quantify field variation in cushion traits across phenotypes and habitats, we measured maximum leaf length, cushion penetration [an index of cushion compact- ness and interference (R. M. Callaway, unpublished data)], leaf thickness and number of inflorescences on a total of 60 cushions (30 tight and 30 loose phenotypes) from our site (see Online Resource 1). In July 2012, cushion penetration was measured by loosely introducing a metal ruler vertically within the cushion. Leaf thickness was measured to a precision of 0.01 mm using a digital micrometer (TESTWELL, Saint-Ouen, France). Measurements were done on three fresh leaves per cushion, and on the middle length of the leaves. Number of inflorescences was counted on the whole cushion surface.

A shadehouse experiment was set up to quantify the contribution of heritability and plasticity in changing traits among phenotypes and environments (see Online Resource 1). The shadehouse was located at the INRA station of Cestas-Pierroton, France (44°44′N, 0°46′W; 60 m a.s.l.). Replicates of the two phenotypes were grown for 26 months in contrasting environmental conditions mimicking the two natural habitats. In November 2010, we collected 15 *F. gautieri* cushions (hereafter ‘genotypes’) of each phenotype at our site. We chose mature and discrete cushion individuals in order to increase the probability of selecting genetically different cushions. Each cushion was planted in a 4-L pot with a soil-less mix of peat moss, perlite, and gravels in a 1:1:0.5 proportion. Pots were stored during the first winter in an unheated greenhouse. In late April 2011, i.e. at the beginning of the treatments, the experiment was set up in a shadehouse transmitting 50 % of light without intercepting precipitation, protecting plants from direct sunlight and air desiccation. Each cushion was separated into 20 individual tillers of from five to ten leaves. Each tiller was standardised by cutting the roots to a length of 5 cm and planted into a separate pot (total $n = 600$). Pots were then randomly placed on benches within the shadehouse before treatments.

We applied a watering treatment with ten replicates of each genotype in each treatment combination (phenotype × watering) to assess the potential plastic responses of phenotypes to varying soil moisture conditions. It is important to note that our goal was not to exactly mimic the environmental conditions of the natural habitats of each phenotype, but rather to assess the response of the two phenotypes to the occurrence of a weak drought stress. The watering treatment was applied in 2011 and 2012 from late April to late September and in 2013 from late April to the end of the experiment (late June 2013) by irrigating half of the pots with 1 L of tap water (watered pots) three times a week and the other half once a week only (dry pots). Survival and five cushion traits were recorded for all pots at the beginning and the end of each growing season (in April and October—intermediate measurements) in 2011 and 2012, and at the end of the experiment, late June 2013 (final measurement). As survival was very high in all treatments, only final growth measurements are presented here. Maximum leaf length, cushion penetration and cushion leaf thickness were measured as in the field. We also quantified cushion surface simplified as an elliptic surface by measuring cushion length and width, and cushion leaf density by counting all leaves within a circular ring of 6 cm^2. Cushion surface is a proxy of cushion growth rate as all cushion individuals had a similar size at the beginning of the experiment and there was very low leaf mortality during the experiment, even during winter (P. Al Hayek, personal observation).

Differences in cushion effects between *F. gautieri* phenotypes

A field cushion-removal experiment was conducted to quantify the potential variation in cushion facilitative and/ or competitive effects on subordinate species across phenotypes and habitats (Online Resource 1). Three target species with contrasting drought-tolerance abilities and habitat distributions were used to encompass a large and representative panel of possible responses to neighbours (Brooker et al. 2008). We chose two drought-intolerant target species, i.e. the loose fescue phenotype itself and *A. capillaris* (its most frequent subordinate species), and a drought-tolerant target, the tight fescue phenotype itself. Both fescue phenotypes were chosen as targets to also assess responses to neighbours, since both the effect of a neighbour on a tar- get individual and the response of a target individual to the effect of a neighbour are important components of species competitive/facilitative abilities (Goldberg 1990; Liancourt et al. 2009; Le Bagousse-Pinguet et al. 2013). We used naturally occurring individuals for *A. capillaris* and trans- plants for the two *F. gautieri* phenotypes. In June 2011, we selected five sites in a northern slope with a distance of at least 100 m between sites. In each site, six cushions of each phenotype that included one discrete individual of *A. capillaris* were selected. For half of the cushions (three tight and three loose at each site), we removed by hand the aboveground parts of *F. gautieri* cushions within a circular area of 15 cm in diameter centred on the target individual of *A. capillaris*. Then, one individual of each *F. gautieri* phenotype was transplanted at 5 cm from the *A. capillaris* individual, both within removed and control cushions of both phenotypes. Transplanted individuals of both *F.*

gautieri phenotypes were randomly collected at the site in at least ten cushions of each phenotype. Each transplanted individual had from five to ten leaves. All target individuals were tagged with metal rings. Thus, there were five replicates of each combination of the three treatments (cushion phenotype, removal and target species). In late July 2012, we recorded survival, measured height and leaf number of all target individuals. All target individuals were harvested for aboveground biomass measurements. Harvested target individuals were dried for 2 days at 70 °C and weighed. Survival was expressed in percentages (0, 33, 66 or 100 %) per treatment combination (cushion phenotype and removal) and per site, and growth data were averaged per treatment combination and per site before statistical analyses.

To quantify heritable differences in competitive/facilitative effects and responses among phenotypes, a pot experiment was set up in the shadehouse, from early April to late November 2012 (Online Resource 1). We manipulated cushion presence and phenotypes (neighbour treatment) and target identity. The neighbour treatment was applied by planting targets either within a tight cushion phenotype or within a loose cushion phenotype, or alone without a cushion. We used six different genotypes of each cushion phenotype with six replicates per cushion genotype and one cushion per pot (a total of 36 pots per phenotype plus 36 pots with no cushion). Pots were randomly distributed on benches in the shadehouse at the beginning of the experiment and watered three times a week. The 72 cushions were prepared before the experiment by growing them in separate pots from small tillers of from five to ten leaves to a cushion size of 15 cm in diameter between late April 2011 and early April 2012. As in the field competition experiment, we planted the three different targets—the tight and the loose phenotype of *F. gautieri*, and *A. capillaris*—in each pot. In each with-neighbour pot, the three target individuals were planted within the cushion canopy with an equal distance between them (5 cm). For the two fescue phenotypes transplanted as targets within and without cushions, we used six different genotypes per phenotype with six replicates per genotype. A replicate of target genotype was planted in each cushion genotype. This genotypic sampling was done in order to maximise differences in competitive ability within phenotypes, but was not used as a statistical treatment because of insufficient replication. Survival was recorded at the end of the experiment in late November 2012. All target individuals were harvested for aboveground biomass measurements. Harvested plants were dried for 2 days at 70 °C and weighed. Survival was expressed in percentages (0, 16.7, 33.3, 50, 66.7, 83.4 or 100 %) per target genotype and treatment combination. Biomass measurements were averaged per target genotype in each treatment combination before statistical analyses. In order to quantify responses of the three target species to both cushion phenotype effects, we calculated the relative interaction index (RII) for survival and biomass performances, following Armas et al. (2004):

$$\mathrm{RII} = \frac{P_{\text{+neighbour}} - P_{-\text{neighbour}}}{P_{\text{+neighbour}} + P_{-\text{neighbour}}}$$

where $P_{\text{+neighbour}}$ and $P_{-\text{neighbour}}$ represent target performances (survival and biomass) in the presence and absence of cushions, respectively. This index is symmetrical around zero (no significant interaction), and has defined limits between –1 and +1. Negative values indicate competition whereas positive values reflect facilitation.

Community feedbacks

We assessed the potential cost of hosting subordinate species for *F. gautieri* cushions by conducting a removal of subordinate species experiment to test the hypothesis that subordinate species reduce the inflorescence production of loose cushions. In late June 2011, we randomly selected 40loose cushions and removed all subordinate species growing within a circular area of 20 cm in diameter (surface 314 cm^2) in half of the selected loose cushions. In late June 2012, regrowth of subordinate species was again removed in the 20 loose treated cushions. In late July 2012, we counted the number of inflorescences produced by all cushions within the delimited circular plot.

Statistical analyses

To analyse field differences in cushion traits among phenotypes (tight and loose), we conducted a Student's *t*-test with cushion phenotypes as treatment and the four field traits as response variables. Differences in cushion traits in the shadehouse experiment were analysed using a two- way ANOVA model with phenotype and watering as treatments and the five cushion traits as the response variables. For the removal of *F. gautieri* field experiment and for each target species separately, we conducted a two-way ANOVA with phenotype and removal as treatments, and percentage of target survival and leaf number as dependent variables. For the shadehouse competition experiment, we conducted separate analyses per target species for survival and growth data, and one analysis grouping target species for RII data. We conducted one-way ANOVAs (followed by Tukey tests when necessary) on target percentage of survival and biomass with neighbour presence and identity as treatment (no neighbour, tight phenotype and loose phenotype), and a two-way ANOVA on the RII for survival and aboveground biomass with cushion phenotype and target as treatments. We used one-sample *t*-tests to detect significant deviation of RII values from zero (i.e. no cushion effect on target performance). For the removal of subordinate species experiment we compared the inflorescence production of control and removed loose cushions with a Student's *t*-test. Dependent variables were checked for normality and log (cushion traits and target growth) or arcsine root transformed (target survival) before parametric tests. All statistical analyses were done using R (R Development Core Team 2012).

RESULTS

Differences in field traits between the two cushion phenotypes were highly significant for all measured traits ($P < 0.001$; Table 1). Loose phenotypes had 25 % longer leaves, and 100 % higher cushion penetration than tight phenotypes, whereas the latter had ten times more inflorescences and 20 % thicker leaves than the former.

In the shadehouse, after two growing seasons, *F. gautieri* maximum leaf length, cushion penetration, cushion surface and cushion leaf density were highly significantly affected by both the phenotype and watering treatments (Fig. 1a, b, d, e, respectively).

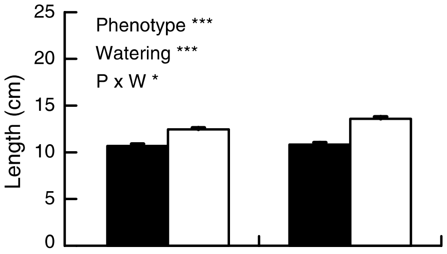
Table 1. Mean trait values of the two phenotypes (tight and loose) of *Festuca gautieri* measured in the field ($n = 30$) and significance of phenotype effect determined by *t*-tests

Variables	Trait values		Phenotype effect
	Tight (SE)	Loose (SE)	*P*
Maximum leaf length (cm)	5.99 (0.31)	7.58 (0.23)	*<0.001*
Cushion penetration (cm)	4.25 (0.17)	8.05 (0.33)	*<0.001*
Number of inflorescences (inflorescences cm^{-2})	0.18 (0.03)	0.02 (0.01)	*<0.001*
Leaf thickness (mm)	0.48 (0.01)	0.40 (0.01)	*<0.001*

Significant effects are indicated in *italic*

Leaf thickness was highly affected by the phenotype treatment only (Fig. 1c), but for all other traits the effect size was stronger for the watering treatment than for the phenotype treatment. For example, leaves were 5 % longer for loose phenotypes compared to tight ones, whereas watered cushions had 17 % longer leaves than dry ones. Penetration was 15 % higher in loose cushions than in tight ones, but 25 % higher in watered cushions than in dry ones. Similarly, cushion surface was 8.5 % larger for loose than for tight cushions, but 20 % larger for watered than dry cushions. Leaf density was 9 % higher for loose cushions than for tight ones, whereas watered cushions had12 % higher leaf density than dry ones. In contrast with the other four traits, tight cushions had 7 % thicker leaves than loose cushions, but there was no difference between watered and dry cushions. Nevertheless, there was a significant phenotype × watering interaction ($P < 0.05$) for cushion leaf length, penetration and surface, and this interaction was even highly significant for leaf density ($P < 0.001$). This interaction was due to a higher effect of watering on those traits for the loose cushions than for the tight ones, showing that loose cushions were more plastic than tight ones. Even so, this interaction was not observed for leaf thickness (Fig. 1c). To summarise, trait differences between phenotypes were explained by both genetic and plasticity effects, but with a stronger contribution of the latter.

(a) Maximum leaf length



(b) Cushion penetration

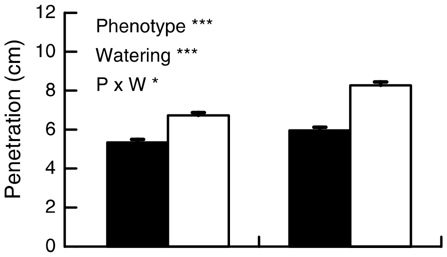


Fig. 1a–e Shadehouse trait measurements of tight and loose phenotypes of *Festuca gautieri*, with and without watering. Data shown are means + SE ($n_{\text{loose-watered}}$ and $n_{\text{tight-watered}} = 15$, $n_{\text{loose-dry}} = 13$, $n_{\text{tight-dry}} = 14$). Significant results of two-way ANOVAs on the effects of phenotype (*P*), watering (*W*) and their interactions are shown for each trait. *$P < 0.05$, ***$P < 0.001$

In the field cushion-removal experiment, target survival was higher in the loose cushion habitat than in the tight one for both *A. capillaris* and the loose target fescue (phenotype effect; Fig. 2a, e). The phenotype effect was very strong for *A. capillaris*, with survival in the tight cushion habitat three times lower than in the loose one. The tight fescue target survived less in the loose cushion habitat than in the tight cushion, and also less with neighbours than without neighbours (significant phenotype and removal effects; Fig. 2c). Additionally, there was a significant phenotype × removal interaction because the negative effect of neighbours on the tight fescue target was much stronger in the loose cushion habitat than in the tight one (Fig. 2c). For target leaf number there was a marginally significant higher performance in the loose cushion habitat than in the tight one for *A. capillaris* (Fig. 2b), but no differences among habitats for both tight and loose fescue targets (Fig. 2d, f, respectively). For *A. capillaris* there was also a significant removal effect and marginally significant phenotype × removal interaction. Competition was higher for *A. capillaris* in the loose cushion habitat than in the tight one, with a much higher leaf number without neighbours in the former than in the latter but no difference with neighbours across habitats (Fig. 2b). To summarise, these results show that the tight cushion habitat was more stressful for *A. capillaris*, and that the loose cushion habitat was more competitive for both the tight fescue and *A. capillaris* targets.

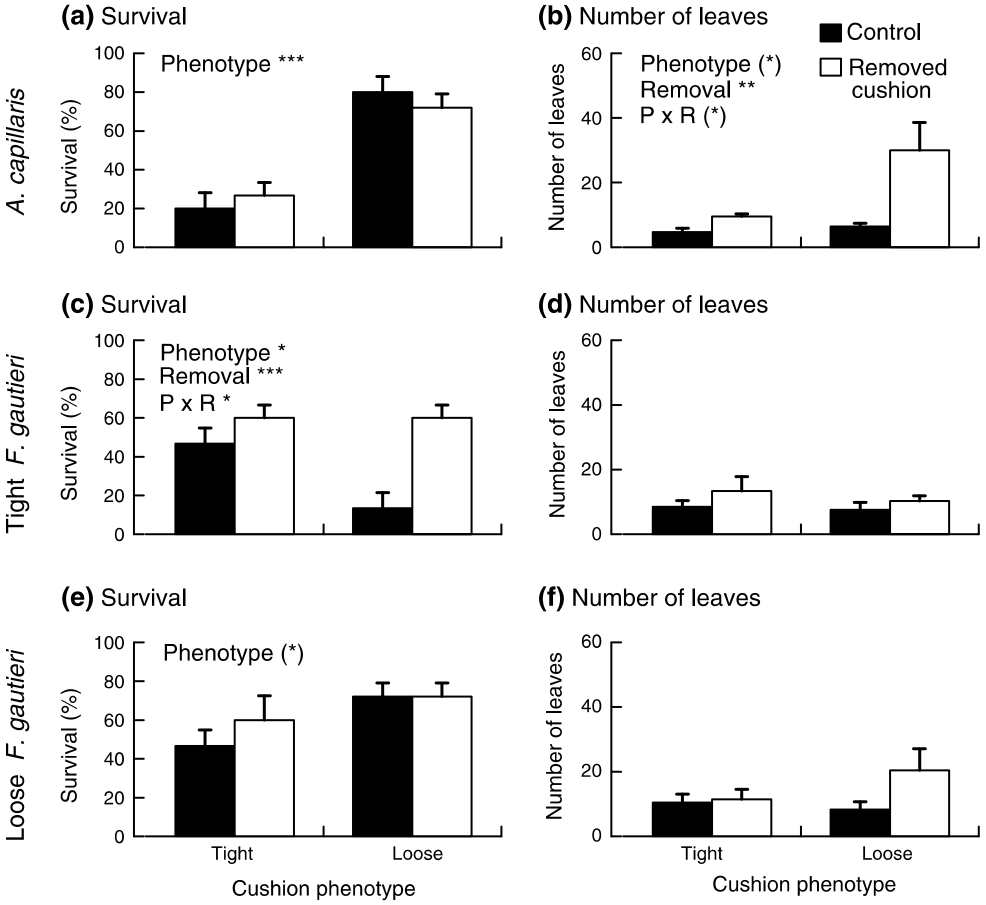


Fig. 2 Effect of cushion removal, survival and leaf number of **a**, **b** *Agrostis capillaris*, **c**, **d** tight *F. gautieri* phenotype, **e**, **f** loose *F. gautieri* phenotype in the habitats of the two phenotypes of *F. gautieri*. Data shown are means + SE (*n* = 5). Significant results of two-way ANOVAs on the effects of phenotype (*P*), removal (*R*) and their interactions are indicated. (*)*P* < 0.1, **P* < 0.05, ***P* < 0.01, ****P* < 0.001

In the shadehouse competition experiment, there was a significant neighbour effect for the survival of the loose fescue target with a higher survival without neighbour than with neighbour (Fig. 3e). Additionally, RII results showed that competition for survival was more intense within the tight fescue cushions than within the loose ones (significant cushion phenotype effect; Fig. 4a). There were also significant differences in competitive responses among the three targets (highly significant target effect for RII survival). The loose fescue phenotype was the worst response competitor for survival and there were no significant differences between the two other targets [Tukey test for the target effect–*A. capillaris* (a), tight *F. gautieri* (a), loose *F. gautieri* (b); Fig. 4a]. However, this effect was mostly found in the tight fescue cushions as shown by the marginally significant cushion phenotype × target interaction (Fig. 4a). For biomass there was a significant neighbour effect for the three targets due to a strong decrease in target biomass when grown within cushions (Fig. 3b, d, f). However, in contrast to survival, the worst response competitor was *A. capillaris*, which had the lowest RII values (target effect; Fig. 4b) and there were no differences in competitive effects between both fescue phenotypes. To summarise differences in competitive abilities between the two fescue phenotypes, results on survival showed that the tight phenotype was the strongest effect competitor and the loose phenotype the weakest response competitor.

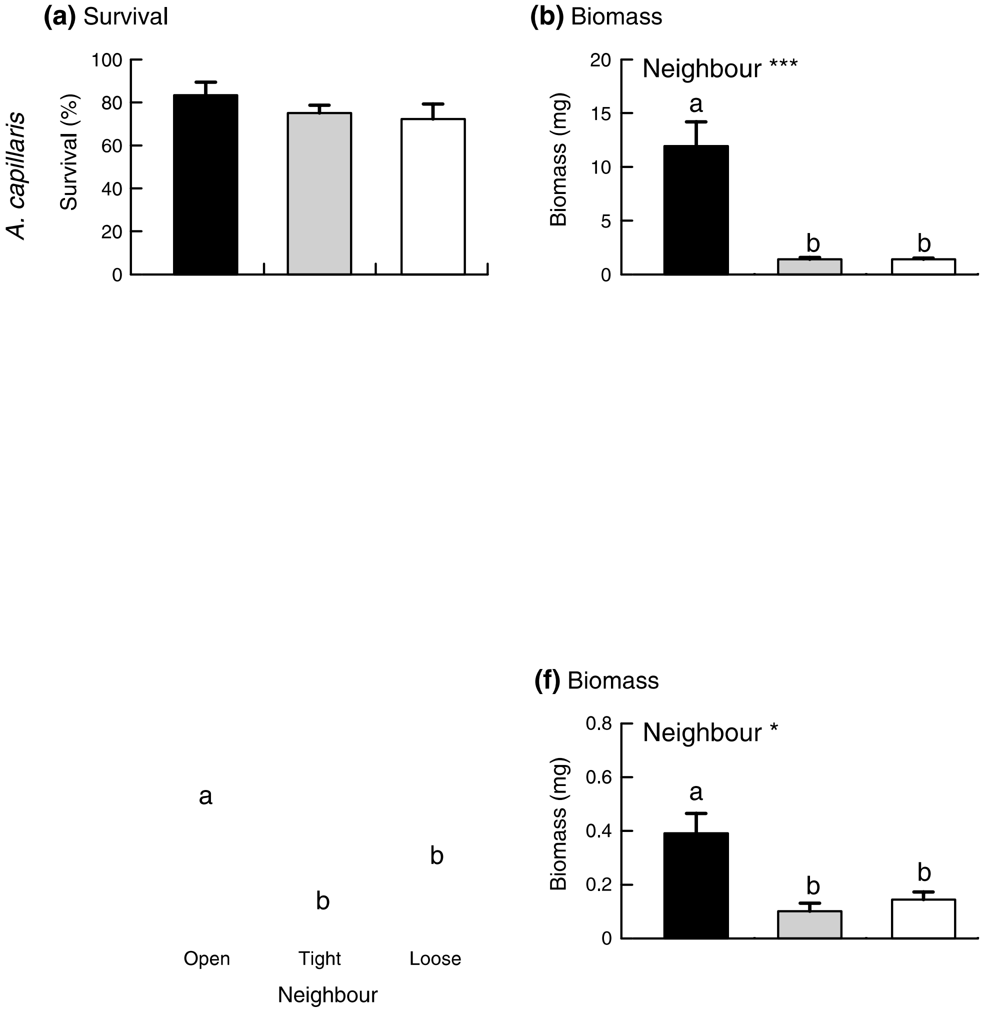
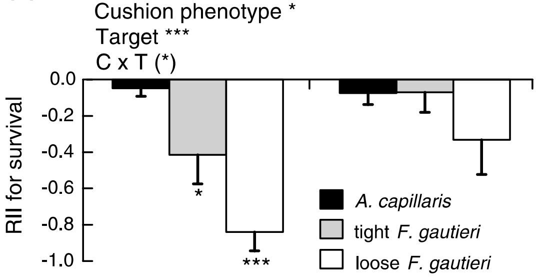


Fig. 3 Survival and biomass **a**, **b** *A. capillaris*, **c**, **d** tight *F. gautieri*, and **e**, **f** loose *F. gautieri* targets in the three neighbouring conditions: no neighbour (*Open*), within tight *F. gautieri* cushions (*Tight*), and within loose *F. gautieri* cushion of the shadehouse competition experiment (*Loose*). Data shown are means + SE (*n* = 6). Results of Tukey tests are shown at the *top* of *error bars* when a significant neighbour effect occurs. Significant results of one-way ANOVAs on neighbouring effects are indicated. *P < 0.05, **P < 0.01, ***P < 0.001

The removal of subordinate species within loose cushions evidenced a substantial cost of hosting other species for loose cushions, because loose cushions with subordinate species removed produced almost twice as many inflorescences as controls (0.42 ± 0.09 and 0.23 ± 0.05 inflorescences cm^{-2}, respectively; $P = 0.04$). However, loose cushions with subordinate species removed produced 75 % less inflorescences than tight cushions (1.84 ± 0.25 inflorescences cm^{-2} for tight cushions; $P = 0.002$).

(a) RII survival



(b) RII biomass

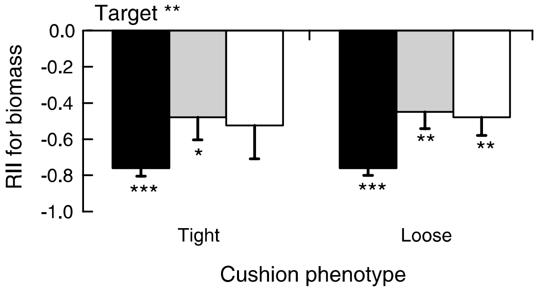


Fig. 4 Relative interaction index (RII) for **a** survival (RII_{survival}) and **b** biomass (RII_{Biomass}) of the three targets (*A. capillaris,* tight *F. gautieri* and loose *F. gautieri*) calculated for both cushion phenotypes as neighbours. Data shown are means + SE ($n = 6$). Significant results of two-way ANOVAs on cushion phenotype (*C*), target (*T*), and their interactions are indicated *above panels* and results of one-sample *t*-tests are shown *below error bars.* (*)$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

DISCUSSION

Our shadehouse trait measurements showed that field morphological differences between phenotypes had both genetic and phenotypic plasticity bases, with an overall higher contribution of the latter. Our shadehouse competition experiment showed that heritable differences in cushion traits drove contrasting interactive effects and responses, with the highest competitive ability for the tight fescue phenotype. However, our field competition experiment provided opposite results, with higher competition found in the loose (low-stress habitat) rather than in the tight habitat (high-stress habitat). Together, these results suggest that field differences in cushion traits and interactions with other species have both genetic and phenotypic plasticity bases, but that heritable interactions contribute to changes in subordinate species diversity across habitats in a complex way. Finally, our removal of subordinate species experiment showed that loose phenotypes pay a cost for hosting other species.

Phenotypic differentiation within *Festuca gautieri* cushions

The five measured traits remained significantly different between both *F. gautieri* cushion phenotypes at the end of the 2-year shadehouse experiment, suggesting that intraspecific morphological variation observed across natural habitats may have partly a genetic basis. This is consistent with the experiment of Le Bagousse-Pinguet et al. (2014b) who found that young transplants of the two phenotypes of *F. gautieri* had contrasting survival responses along a soil moisture gradient occurring in the same field system. Both genetic variability and phenotypic plasticity are known to explain trait variation in heterogeneous environments (Pigliucci 2001; Byars et al. 2007; Grassein et al. 2010) and we acknowledge that reciprocal transplant experiments conducted in field conditions are necessary to reach straightforward conclusions on the heritability of adult trait differences suggested by our shadehouse experiment. Although common gardens are commonly used to experimentally separate genetic from phenotypic plasticity effects (Clausen et al. 1940; Schmid and Dolt 1994), maternal effects may persist in short-term experiments (Schmid and Dolt 1994). We used cuttings of *F. gautieri* in our shadehouse experiments, which did not clearly allow the ruling out of maternal effects. However, maternal effects on offspring phenotypes - which are generally more prevalent in juvenile life history stages relative to adult life history stages (Roach and Wulff 1987) - are likely to be reduced by selecting mature individuals (Ravenscroft et al. 2014). Thus, trait differences among phenotypes are likely in part due to genetic effects.

In our shadehouse experiment, watering also had a highly significant effect for all traits except leaf thickness, with watered cushions having longer leaves, and higher penetration, surface and leaf density than dry ones. Thus phenotypic plasticity also contributes to the observed morphological variation across habitats. Additionally, the significant phenotype × watering interaction for maximum leaf length, cushion penetration, surface and leaf density showed that loose cushions are more plastic than tight ones. Interestingly, genetic and phenotypic plasticity effects acted in the same direction, with overall the strongest trait differences observed between dried tight phenotypes and watered loose phenotypes. Additional heritable and environmental effects were also found by Vitasse et al. (2009) in the Pyrenees mountain range for phenological traits of an oak and an ash species. In contrast, they found counteracting heritable and environmental effects for a beech species.

Effects of *F. gautieri* phenotypes on subordinate species and consequences for diversity

The shadehouse trait measurements and competitive experiment suggest that a heritable component may also explain differences in competitive ability among phenotypes. Tight cushions had a higher competitive ability (higher competitive effect - likely through interference mechanisms - and response) than loose cushions under the same environmental conditions. Contrasting penetration between tight and loose cushions is mainly related to higher leaf thickness and stiffness. Thus tight, thick and stiff leaves of tight cushions can impede the recruitment of other species. Previous studies have shown that phenotypic architectural variation in foundation species may induce contrasting competitive and facilitative effects on other plant species (Callaway et al. 1991; Pugnaire et al. 1996; Rudgers and Maron 2003; Michalet et al. 2011). Our results are consistent with Michalet et al. (2011) who showed that heritable differences in the tightness of two phenotypes of the alpine cushion species *Geum rossii* were related to differences in their effects on subordinate species, with also a higher competitive effect for the tight than for the loose phenotypes. Similarly, Callaway et al. (1991) have shown in Californian oak savannah that differences in root density among phenotypes of *Quercus douglasii* induced contrasting interference effects on their herbaceous understories. However, to our knowledge, our study is the first to show differences in competitive effects among different phenotypes of a foundation species in controlled similar environmental conditions.

Because we did not mimic field variation in water availability in our shadehouse competition experiment, we were not able to directly assess the plasticity of competitive effects across phenotypes' habitats. However, results of the shadehouse trait measurements allow us to predict that with increasing water availability from the tight phenotype's habitat to the loose one, the competitive effect ability of both phenotypes should decrease since both showed an increase in cushion penetration with watering. This effect should be even stronger for the loose phenotype because it showed higher trait plasticity than the tight one. With watering, cushions had softer leaves allowing a higher cushion penetration and thus a decrease in interference effect.

The results of the two shadehouse experiments suggest the prediction that in the field, the highest cushion competitive effect should be observed for the tight phenotype in its dry habitat and the lowest for the loose phenotype in its wet habitat, and thus, that competition should likely decrease from the former to the latter habitat. However, our field competition experiment provided an opposite result. Competition significantly increased for the survival of tight fescue targets only, and weakly increased for leaf number only for *A. capillaris*. Additionally, we did not find any facilitation in this experiment for these two response variables. An increase in competition from the dry convex habitat of the tight phenotype towards the wet concave habitat of the loose phenotype is consistent with Grime (1974) and the stress gradient hypothesis (Bertness and Callaway 1994), models that predict an increase in competition with decreasing stress. Thus, should we conclude that varying interactions and species richness across habitats in natural conditions are not influenced by heritable and plastic differences in competitive effects among phenotypes?

In order to answer this crucial question, we need to dis- entangle changes in cushion effects from changes in target responses across habitats. Michalet et al. (2014) have recently proposed a conceptual framework to partition net interactions along stress gradients. They showed that the switch from competition to facilitation that has been recurrently demonstrated from subalpine to alpine communities (e.g. Callaway et al. 2002) was due to two different processes that needed to be disentangled. The increase in competition from low alpine to subalpine communities is due to a neighbour trait effect, defined as a decrease in the target response with neighbours with decreasing stress due to an increase in the competitive effect of the neighbours [consistent with Grime's (1974) model]. In contrast, the increase in facilitation from low alpine to high alpine communities is due to an environmental severity effect, defined as a decrease in the performance of the target species without neighbours with increasing stress, without changes in the facilitative effect of the neighbours. In our field experiment conducted in subalpine conditions we showed that the increase in competition from the stressful convex habitat of the tight phenotype towards the more benign concave habitat of the loose phenotype was either due to neighbour trait or environmental severity effects depending on the target species and the response variable. For leaf number, for which there was only a marginally significant increase in competition from tight (high stress) to loose (low stress) cushion habitats for *A. capillaris,* this increase in competition was due to an environmental severity effect (see Fig. 2b), with no changes in the three targets performance with neighbours. For survival, the increase in competition was significant only for the tight fescue target and was due to a neighbour trait effect, whereas for the two other targets performance with and without neighbours increased towards the loose cushion habitat, due to decreasing environmental severity (i.e. evidence of an environmental severity effect). Thus, we conclude that for the two target species naturally abundant in the loose cushion habitat, where species richness was the highest within cushions, there were only environmental severity effects. This result is not consistent with Grime's (1974) model and the meta-analysis of Michalet et al. (2014), which predicted or showed, respectively, that an increase in competition in subalpine habitats with decreasing stress is due to an increase in the negative effect of the dominant competitor (i.e. a neighbour trait effect). We argue that competitive effects did not increase in the field from tight to loose cushion habitats because of both the counteracting heritable and plastic effects that were evidenced in the two shadehouse experiments (decrease in competitive effects due to phenotypes and watering). In other words, in the absence of these heritable and plastic effects inherent to this foundation species, competition should have increased more strongly from the stressful habitat of the tight phenotype to the benign habitat of the loose phenotype, consistent with Grime's (1974) competition theory and the meta-analysis of Michalet et al. (2014). However, this hypothesis remains to be tested in reciprocal transplant experiments including neighbour removal. Finally, the decrease in competition due to these genetic and plastic effects suggested by our results likely contribute to the high species richness occurring in the loose cushions.

Feedback effects of the subordinate species on the foundation species' fitness

We found evidence of a cost of hosting other species for *F. gautieri*. The removal of subordinate species within loose cushions significantly increased the flower production of *F. gautieri*. Loose cushions in which we removed subordinate species produced twice the number of inflorescences as control loose cushions. Negative feedback effects of subordinate species have been found on water status, growth, and reproductive output of their host nurse shrub *Ambrosia dumosa* in the Mojave Desert (Holzapfel and Mahall 1999). Negative feedback effects of recipient species have also been found on the reproductive output of nurse cushion plants *G. rossii* (Michalet et al. 2011) and *Silene acaulis* (Cranston et al. 2012). In contrast, in semi-arid Spain, Pugnaire et al. (1996) found a benefit for the nurse shrub *Retama* *sphaerocarpa* of hosting *Marrubium vulgare*. Shrubs hosting the forb had higher biomass and nitrogen contents in branches than shrubs alone. In an evolutionary context, it is crucial to quantify not only the costs but also the benefits of hosting other species (Bronstein 2009). Using an individual-based model, Michalet et al. (2011) showed that the inclusion of a benefit of hosting other species strongly increased the selective pressure of feedback effects for the alpine cushion foundation species' genetic differentiation. However, like in this study, they did not find evidence for this benefit that was only hypothetical in their model. However, a benefit may exist for other traits than flowering output, which was not explored in both studies. For example, Schöb et al. (2014) found in an intercontinental study assessing feedback effects of alpine beneficiary species on their benefactors that the richness of cushion-associated species had positive effects on seed density, although the dominant effect was parasitic with a decline in cushion seed production with increasing cover of beneficiaries.

We showed in our study that phenotypic differences in cushion traits within the foundation cushion grass species, *F. gautieri*, had both heritable and environmental bases. We also showed that these trait divergences induce contrasting competitive effects for other species that counterbalanced the strong increase in competition, which has been recurrently shown to exist from dry to mesic subalpine habitats. Thus, heritable and plastic effects strongly contribute to maintain high subordinate community diversity through decreasing competition. The high diversity of other species within cushions had feedback effects on the fitness of the loose cushion. Further experiments are needed to also eventually detect a benefit for loose cushions to hosting other species in order to test the hypothesis that these contrasting competitive effects have evolutionary consequences for the foundation grass species. Our study brought additional sup- port to the emerging perspective that diffuse within-trophic level biotic interactions might have important evolutionary consequences at the community level.

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CZ.1.07/2.3.00/30.0006) realized through the EU Education for Competitiveness Operational Program. This project was funded by the European Social Fund and the Czech State Budget. We acknowledge the Experimental Unit of INRA-Pierroton (Cestas, France) for hosting the shadehouse at the Site de recherche Forêt-Bois Bordeaux-Aquitaine, and Mr Pierre Casabonne, mayor of Arette, for giving us permission to carry out experiments on Arette's community lands. We are grateful to Guillaume Caro, Ghassen Chaieb, Clément Lalait, Audrey Lefrançois, Etienne Mansa, Claire Meziere, Florène-Diane Richard, Coralie Rieu, Maximilien Larter and Quentin Sanz for their help in the field and/ or the shadehouse. We also thank two anonymous reviewers for their constructive suggestions. Maximilien Larter revised the English. The experiments comply with the current laws of France, in which the experiments were performed.

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Online Resource: Electronic Supplementary Material (ESM)

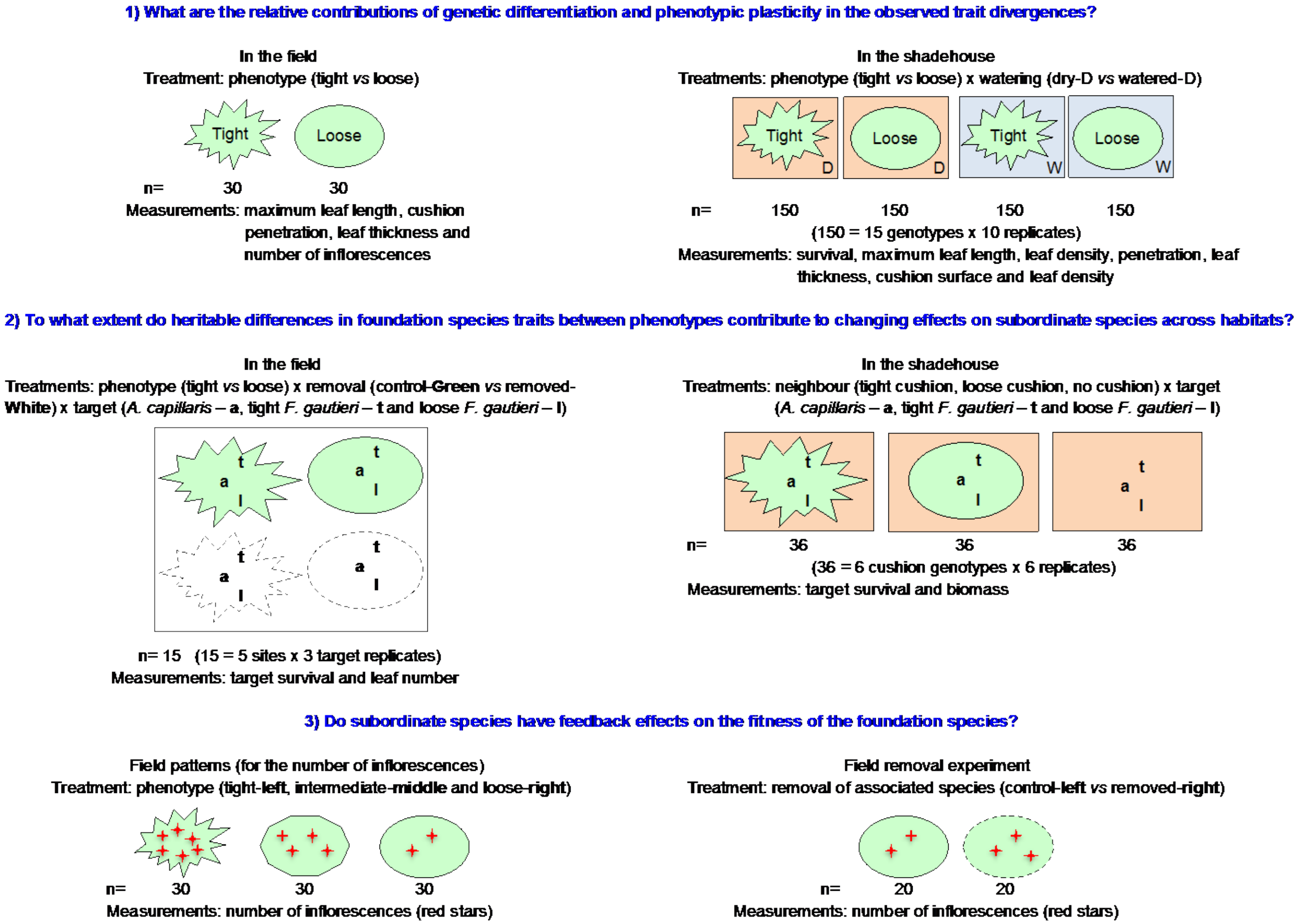

Fig. S1 Summary of our experimental design with the different field and shadehouse surveys or experiments realised in order to answer to our three main questions

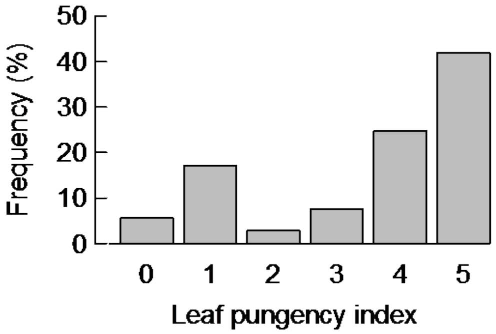

Fig. S2 Leaf pungency frequency distribution of *F. gautieri* cushions measured in ten 10-meter long transects; the leaf pungency index varies between 0 (not pungent) and 5 (highly pungent) with 0-1 values for loose *F. gautieri* phenotypes, 2-3 for intermediate phenotypes, and 4-5 for tight phenotypes

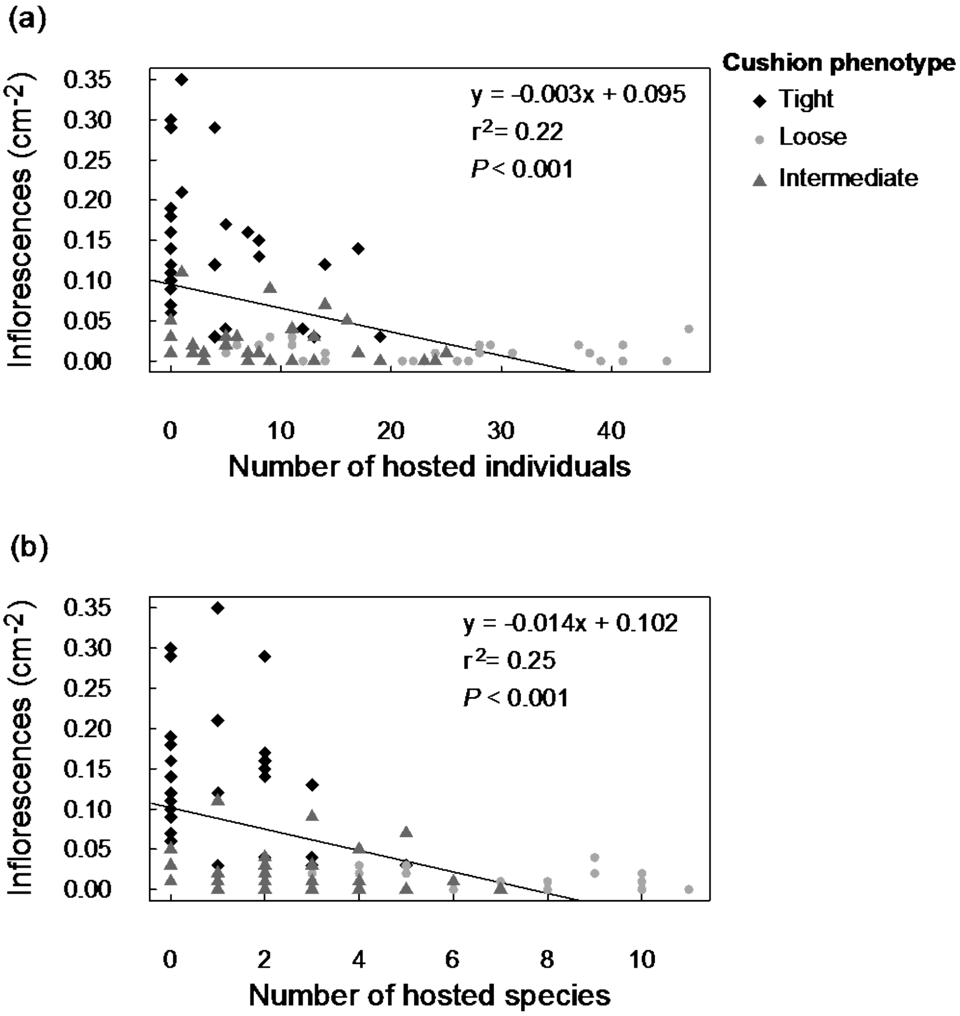

Figure S3 Relationship between the number of inflorescences of *F. gautieri* and a) the number of individuals of hosted species, b) the number of hosted species. The number of inflorescences is expressed per cm^2 of cushion area and the number of individuals and of species per sampling area (i.e., 314 cm^2)

CHAPTER FOUR:

Disentangling the heritable and plastic components of the competitive and facilitative effects of two contrasting phenotypes of a foundation species

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Summary

- Differences in effects between phenotypes of foundation species on dependent species have been reported, but no study has separated their genetic and plastic components. In a subalpine community of the French Pyrenees, we observed two phenotypes of *Festuca gautieri*: tight cushions in dry convex outcrops with few subordinate species, and loose cushions in wet concave slopes with many subordinate species, suggesting differences in effects of the two phenotypes on subordinates.
- We studied in two common-gardens with adult cushion reciprocal transplantations the responses of the two phenotypes to changes in environmental conditions, thus assessing the contribution of genetics and plasticity to the phenotypic variation. We also assessed the plasticity and genetic bases of their contrasting effects with a target cross-transplantation experiment, and quantifying the recolonisation of resident plants within the reciprocally transplanted cushions.
- We found that morphological differences between cushions had both genetic and plasticity bases. The two competition experiments showed genetic-based increase in competitive effects from loose to tight cushions. This was counteracted by plasticity effects, which decreased competition from the benign to the stressful garden.
- We conclude that genetic effects overcome plasticity effects resulting in higher diversity in the loose phenotype from the benign habitats.

Key words: common-garden, competitive effect, *Festuca gautieri* (Bearskin fescue), foundation species, genetics, phenotypes, plasticity, reciprocal transplantation

Introduction

Since the call for a new field inquiry bridging the gap between community ecology and evolutionary biology in a way “to emphasize the analyses of evolutionary genetic processes that occur among interacting populations in communities” (Antonovics, 1992; see also Wilson, 1976), research in community genetics provided evidence that genetic variation within one species, particularly a foundation (Ellison *et al*., 2005), may have cascading effects at the community and ecosystem levels through biotic interactions (Antonovics, 1992; Whitham *et al*., 2003, 2006, 2008; Pakeman *et al*., 2006; Johnson & Stinchcombe, 2007; Gibson *et al*., 2012; Bailey *et al*., 2014). As primary producers, plants are the primary resource in most terrestrial food webs (Chapin & Eviner, 2012); thus they are potentially perfect organisms for assessing the consequences of a genetic or phenotypic variation within a focal species on its community. Yet, studies of interactions within plant communities (i.e. plant-plant interactions) have been minimally explored in an evolutionary context (Lankau & Straus, 2007; Michalet *et al*., 2011; Gibson *et al*., 2012) compared to interactions across trophic levels such as species-specific mutualistic or parasitic interactions (Bailey *et al*., 2009; Bronstein, 2009; Adams *et al*., 2011; Crutsinger *et al*., 2014) probably because their evolutionary consequences are hindered by the diffuse nature of plant-plant interactions.

Steep slopes and topographically complex landscapes in mountainous regions, such as the alpine and subalpine systems, are known to generate heterogeneous (micro)-environmental conditions and habitat variation over short-scale distances. Plant-plant interactions have been widely studied in alpine and subalpine systems and are known to strongly vary across these contrasting environments, with competition dominating in the subalpine and in concave topographies on deep soils and facilitation in alpine and convex topographies on shallow soils (Choler *et al*., 2001; Callaway *et al*., 2002; Cavieres & Badano, 2009; Michalet *et al*., 2014), consistent to the Stress Gradient Hypothesis (hereafter SGH, Bertness & Callaway, 1994; Brooker & Callaghan, 1998). Facilitation has also been shown to contribute to the diversity of alpine systems (Cavieres *et al*., 2014), consistent to ecological theory (Michalet *et al*., 2006). Alpine systems are also particularly suitable for exploring ecologically differentiated plant species over a small-scale environmental variation induced by the relatively rugged topography (Choler *et al*., 2004; Liancourt *et al*., 2013) as topographic heterogeneity may induce sympatric genetic differentiation within species (Sambatti & Rice, 2006; Lekberg *et al*., 2012).

Phenotypic differentiation within a plant species may have contrasting effects on dependent species, due to their varying traits (Lankau & Strauss, 2007; Crutsinger *et al*., 2010; Michalet *et al*., 2011; Liancourt *et al*., 2013). Most cases of changes in effects of differing phenotypes on dependent species have been evidenced in studies where the different phenotypes occur in similar environmental conditions (Callaway *et al.*, 1991; Crutsinger *et al.*, 2010). For example, Callaway *et al.* (1991) showed in Californian oak savannah that two phenotypes of *Quercus douglasii* differing in surface root density induce contrasting interference effects on their herbaceous understories. Similarly, Crutsinger *et al.* (2010) showed contrasting facilitative and competitive effects of erect and prostrate phenotypes of *Baccharis pilularis*, respectively, under constant environmental conditions in coastal Californian dunes. Though, most phenotypic differentiation has been shown to occur across environmentally contrasting conditions, particularly across topography and/or soil gradients (Choler & Michalet, 2002; Michalet *et al.*, 2011; Lekberg *et al.*, 2012; Liancourt *et al.*, 2013). Intraspecific morphological trait variation in heterogeneous environments can be explained by genetic variability and/or phenotypic plasticity (Pigliucci, 2001; Byars et *al.*, 2007; Grassein *et al.*, 2010). Reciprocal transplant experiments (Joshi *et al.*, 2001; Kawecki & Ebert, 2004; Ågren & Schemske, 2012; Bennington *et al.*, 2012) help revealing local adaptation, and thus the heritability of trait differences, even over short range of environmental variation and despite the occurrence of gene flow (Byars *et al.*, 2007; Gonzalo-Turpin & Hazard, 2009). However, differentiating with cross-transplantation competitive experiments the contribution of genetic and plasticity in driving differences in phenotypic effects across contrasting environmental conditions is not an easy task in natural alpine systems. In such hard physical conditions most nurse species have too low growth-rate to attain a sufficient size for assessing their effects on other species (Liancourt *et al.*, 2009). For example, Michalet *et al.* (2011) found contrasting phenotypic effects of the alpine foundation species *Geum rossii* on other species in a field experiment conducted across natural habitats in northern Arizona. They also evidenced the genetic basis of phenotypic traits differences in a common-garden. However, they could not straightforwardly conclude on the genetic basis of changes in effects on other species across habitats because they could not separate the confounding effects of varying environmental conditions (i.e. plasticity effects) in a cross-transplantation competitive experiment with this slow-growing species.

In a subalpine system of the French Pyrenees, Al Hayek *et al.* (2014) observed two different phenotypes of *Festuca* L. *gautieri* (Hack.) K. Richt. with contrasting patterns of association with dependent species in two microtopographic conditions: a 'tight' phenotype with stiff leaves in dry outcrops with few if any other species within its canopy, and a 'loose' phenotype with soft leaves in wet concave slopes hosting many other species. In a low elevation shadehouse experiment, they showed both the heritability of trait differentiation and the stronger competitive effect of the tight phenotype as compared to the loose phenotype. However, in a field experiment in the same subalpine system, they found an opposite result with a slightly higher competition in the habitat of the loose phenotype than in the habitat of the tight phenotype. They concluded that genetic effects counteracted the increase in competition from the stressful convex habitat to the more productive one predicted by ecological theory (Grime, 1973; Bertness & Callaway, 1994) and evidenced by several authors in subalpine and alpine systems (Choler *et al.*, 2001; Cavieres & Badano, 2009; Callaway *et al.*, 2002; Michalet *et al.*, 2014). They argued that genetic effects contribute to maintain diversity in this subalpine system through decreasing competition in the predicted highly competitive and species-poor concave habitat. However, Michalet *et al*. (2014) have shown for alpine and subalpine communities that an increase in competition from a stressful habitat to a more benign one might be due to either an increase in the target performance growing without neighbours (defined as strict environmental severity effect) or an increase in the target performance growing within neighbours (i.e. neighbour trait effect), and thus eventually to the plasticity of competitive effects in absence of interspecific turnover of nurse species. Al Hayek *et al*. (2014) could not separate these two effects in their study because they did not quantify the plasticity of the competitive effects (changes in effects of a single genotype on dependent species along an environmental gradient). Moreover, the genetic basis of the differences in phenotypic traits and facilitative/competitive effects was not assessed in the natural environment of the cushions but in a low-elevation shadehouse (with no physical stress), which is particularly problematic for assessing facilitation known to occur in stressful conditions (Bertness & Callaway, 1994).

Thus, in this study conducted in the same system formerly explored by Al Hayek *et al*. (2014) we separated the relative contribution of genetic differentiation and phenotypic plasticity to changes in phenotypic traits and competitive/facilitative effects across habitats, using two subalpine common-gardens mimicking the natural habitats of the two phenotypes of *F. gautieri*. Our approach is novel as intraspecific variation in foundation species effects on dependent species has been minimally explored through reciprocal transplant experiments. First, we transplanted adult cushions of the two phenotypes in both gardens and measured their morphological traits to assess the genetic and plasticity bases of changes in phenotypic traits between phenotypes across natural habitats. We, then, transplanted three target species (*Agrostis capillaris* L., tight *F. gautieri* and loose *F. gautieri*) within the reciprocally transplanted cushions and outside cushions (open area) to assess the genetic and plasticity bases of their differences in competitive and facilitative effects across natural habitats. Additionally, as our ultimate goal was to assess the ecological consequences of phenotypic differentiation for the diversity of this subalpine system, we counted the number of naturally re-established subordinate species (species richness) in all cushions and open areas between cushions in both gardens. This latter survey should allow us to assess the relevance of our target competition experiment for community richness.

Materials and Methods

Study site and target species

The experimental site is located in the western Pyrenees, 100 km from the Atlantic Ocean at La Pierre Saint-Martin pass (42°58′N, 0°45′W, altitude: 1744 m a.s.l., Pyrénées-Atlantiques, France). Climate is temperate oceanic with a mean winter temperature of -2.2°C and a mean summer temperature of 11.5°C; mean annual precipitation is 2850 mm with the highest rainfall occurring in winter. Our target species, *Festuca* L. *gautieri* (Hack.) K. Richt., is a dwarf grass with a circular to elliptic cushion-like form with thin bright green leaves and yellowish-green inflorescences flowering from July to August. It is a foundation species typical of subalpine and alpine grasslands on rocky calcareous soils in the entire Pyrenees (Saule, 1991). Two contrasting phenotypes of *F. gautieri* cushions occur in the system across contrasting topographic and soil conditions (Le Bagousse-Pinguet *et al*., 2014; Al Hayek *et al*., 2014). A tight phenotype dominates convex topographic habitats with shallow, stony and relatively dry soils, and a loose phenotype dominates concave topographic habitats with deep, less stony and relatively wet soils (Al Hayek *et al*., 2014). Tight phenotypes have been shown to be more drought-tolerant than loose ones (Le Bagousse-Pinguet *et al*., 2014). Both phenotypes may occur side-by-side, as the scale of habitat heterogeneity is less than one meter. Moreover, cushions with intermediate traits occur in intermediate ecological conditions, but they are much less frequent than the two extreme phenotypes (Al Hayek *et al*., 2014). Inflorescences cover is high for tight phenotypes, intermediate for intermediate phenotypes and low for loose phenotypes (Al Hayek *et al*., 2014). Loose phenotypes have a higher subordinate species richness and abundance than intermediate or tight phenotypes [n = 30, one-way Analysis Of Variance (ANOVA): $P < 0.001$, Fig. 1 and Supporting information Table S1]. Most frequent species of the community are *Agrostis capillaris* L., *Galium pumilum* Murray, *Campanula rotundifolia* L., *Alchemilla conjuncta* Bab., *Lotus corniculatus* L., *Festuca rubra* L. and *Trifolium pratense* L.

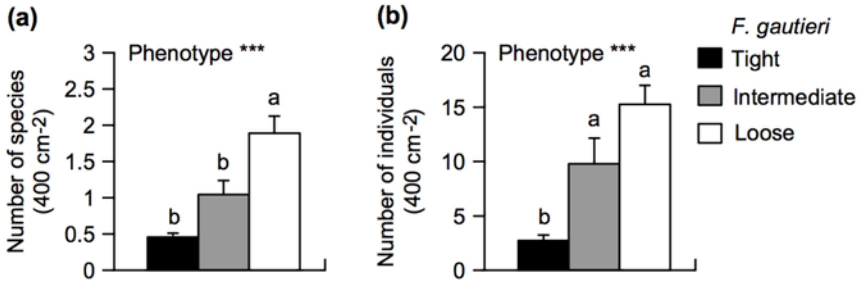

Fig. 1 Means + SE (*n* = 30) of species richness (a) and abundance (b) of subordinate plant species within tight, intermediate and loose cushions of *Festuca gautieri* in their natural habitats. Significant results of one-way ANOVAs on the effect of Phenotype are indicated: ***, $P < 0.001$. Results of Tukey tests for significant Phenotype effect are shown on top of error bars.

Reciprocal cushion transplant experiment

In order to assess the heritability and plasticity of cushion traits in natural conditions, we set up a reciprocal transplant experiment in two experimental gardens mimicking the environmental conditions of the natural habitats of the two phenotypes. On November 2010, we collected eight *F. gautieri* cushions (hereafter genotypes) of each phenotype, tight and loose, at our site. We chose distant, adult and discrete cushion individuals in order to maximise chances of selecting genetically different cushions. Each cushion was separated into ten tillers of 5-10 leaves which were grown for 32 months in a greenhouse [see Al Hayek *et al*. (2014) for detailed methodology]. Early July 2013, when the cushions had attained an adult size (approximately 20 cm in diameter) we transplanted them in the two experimental gardens at our site. The first garden (convex garden hereafter) was set up in a convex topographic position on shallow, stony and relatively dry soil [soil volumetric water content measured with a ML3X ThetaPorbe (Delta-T Devices, Cambridge, UK) 3 days after a rain event, 7.13 ± 1.57, *n* = 25] mimicking the tight cushion's natural microhabitat. The second garden (concave garden hereafter) was set up in a concave topographic position on deep, less stony and relatively wet soil (soil volumetric water content, 30.43 ± 0.85, *n* = 25) mimicking the loose cushion's natural microhabitat. The two gardens were 50 m apart, with a surface of 46 m^2 each. In each garden, we transplanted five adult cushions of each genotype of each phenotype (Phenotype treatment; total cushions per garden: *n* = 80). The 80 cushions were randomly transplanted, 50 cm distant from each other and tagged with metal rings. All cushions were watered once after transplantation. Before planting out, resident vegetation was manually eliminated, and a mulching canvas, permeable to water and air, was spread out to inhibit the regeneration of resident vegetation. New recruits or regrowth of resident vegetation was removed again in September 2013 after the growing season. Circular holes of 30 cm in diameter were made in the mulching canvas in order to transplant the cushions, and two paired holes were made in open areas near each cushion. One of the paired open areas was used in the competition experiments; the other one was used to assess the effects of cushions on the natural re-establishment of subordinate species. The gardens were fenced with metallic nets (height: 1 m – mesh: 10 cm x 10 cm) to prevent herbivory.

In July 2014, we measured cushion surface, maximum leaf length, cushion penetration (an index of cushion compactness and interference, Al Hayek *et al*., 2014), leaf thickness and leaf density (five morphological traits) on both tight and loose cushions (Phenotype treatment) in both gardens. All morphological traits were averaged per cushion genotype in each treatment combination (Garden x Phenotype for morphological traits, and Garden x Neighbour for naturally re-established species richness and abundance).

Reciprocal transplant competition experiment

In order to assess the relative contribution of the genetic and plastic components to the variation in competitive and facilitative abilities (effect and response) among *F. gautieri* phenotypes, we set up a reciprocal transplant competition experiment in the same gardens in July 2013. We manipulated cushion presence and phenotype (Neighbour treatment), microhabitat (Garden treatment) and target identity (Target treatment). The Neighbour treatment was applied by planting targets either within a tight or a loose cushion, or alone without cushion (open area). For the Target treatment, in order to cover a representative panel of possible responses to neighbouring cushions (Brooker *et al*., 2008), we used two drought-intolerant target species, i.e. the loose *F. gautieri* phenotype itself and *A. capillaris* (the most frequent subordinate species), and a drought-tolerant target, the tight *F. gautieri* phenotype itself. We used both *F. gautieri* phenotypes as targets in order to assess not only their effects but also their responses to neighbours, as both effect and response are important components in determining a species competitive and facilitative abilities (Goldberg, 1990; Liancourt *et al*., 2009; Le Bagousse-Pinguet *et al.,* 2013). We planted the three target individuals (one tight and one loose *F. gautieri*, and one *A. capillaris*) within each cushion's canopy with an equal distance of 10 cm between them, and in the paired open area near the cushion (in the holes previously made in the mulching canvas). As transplanted targets remained very small during the whole experiment, this distance was sufficient to avoid any interaction between target individuals within a single cushion. We used five different genotypes per target with eight replicates per genotype. A replicate of target genotype was planted in each cushion genotype. This genotypic sampling was applied in order to maximize differences in competitive ability within phenotypes, but was not used as a statistical treatment due to insufficient replication. Transplanted targets were randomly collected at the site in five distant and discrete cushions for each *F. gautieri* phenotype and in five distant and discrete patches for *A. capillaris*. Targets had from 5 to10 leaves. All targets were tagged with metal rings. Beginning of August 2013, dead targets were replaced.

In September 2013 (after summer season) we recorded survival and total leaf number for each target. We also recorded survival in June 2014 but did not measure growth (total leaf number) since there was very low survival in some treatments. Survival was expressed in percentages (0, 12.5, 25, 37.5, 50, 62.5, 75, 87.5 or 100%) calculated for all targets (regardless their genotype) transplanted within the same cushion genotype, then averaged per treatment combination (Garden-Neighbour-Target) before statistical analyses. Total leaf number measurements were also averaged per cushion genotype in each treatment combination.

Survey of naturally re-established plant species

In July 2014 we recorded the number of naturally re-established plant species (richness) and their abundance within all the cushions and in 40 randomly selected paired open areas (Neighbour treatment) in both gardens (Garden treatment), using quadrats of 20 x 20 cm, in order to quantify their responses to the effects of both cushion phenotypes. These naturally re-established plant species (resident vegetation) were lastly eliminated at the beginning of autumn 2013 (to limit competition with our transplanted subordinate species and cushions). Species richness and abundance were expressed per 400 cm^2 (mean cushion size) due to differences in cushion surfaces and then averaged per cushion genotype in each treatment combination (Garden-Neighbour) before statistical analyses.

Calculation of biotic interaction index

We quantified the net effect of cushion phenotypes on transplanted targets and on naturally re-established resident species in each treatment combination using the Relative Interaction Index (RII, Armas *et al.*, 2004) for target survival ($RII_{survival}$) and total leaf number ($RII_{total\ leaf\ number}$) for the transplanted targets, and RII for species richness ($RII_{Richness}$) and abundance ($RII_{Abundance}$) for the naturally re-established species with:

$RII = (P_{+neighbour} - P_{-neighbour}) / (P_{+neighbour} + P_{-neighbour})$

where $P_{+neighbour}$ and $P_{-neighbour}$ represent target species performances (survival and total leaf number) in the presence and absence of a neighbouring cushion, respectively. This index is symmetrical around zero (no significant interaction) and has defined limits between -1 and +1. Negative values indicate competition whereas positive values reflect facilitation.

Data analysis

Differences in cushion traits (cushion surface, maximum leaf length, cushion penetration, leaf thickness and leaf density) were analysed using a two-way ANOVA (followed by Tukey tests when necessary) with Garden and Phenotype as treatments. For the reciprocal transplant competition experiment, we conducted separate analyses per target species for survival and growth data, and one analysis grouping target species for RII data. Differences in survival and total leaf number of transplanted targets were analysed using a two-way ANOVA model (followed by Tukey tests when necessary) with Neighbour and Garden as treatments and survival and total leaf number as response variables. Differences in cushions net effects on target survival and growth were analysed using a three-way ANOVA model with Garden, Phenotype and Target as treatments, and $RII_{survival}$ and $RII_{total\ leaf\ number}$ as response variables. Differences in re-established plant species richness and abundance were analysed using a two-way ANOVA (followed by Tukey tests when necessary) with Garden and Neighbour as treatments, and species richness and abundance as response variables. Richness and abundance data were treated with ANOVA models instead of Generalised Linear Models (GLM), as they were not discrete but continuous, resulting from averaging per cushion genotype in each treatment combination (see above). Differences in cushions net effects on re-established plant species richness and abundance were analysed using a two-way ANOVA model with Garden and Phenotype as treatments, and $RII_{Richness}$ and $RII_{Abundance}$ as response variables. One sample *t*-tests were used to analyse significant deviation of RII values from zero, which represents no cushion effect.

All dependent variables were checked for normality and log- (cushion traits, target growth, and re-established plant species richness and abundance) or arcsineroot-transformed (target survival) before parametric tests. All statistical analyses were done using R (R Development Core Team, 2013).

Results

Reciprocal cushion transplant experiment

After one growing season, *F. gautieri* cushion traits were significantly affected by both the Phenotype and Garden treatments (Fig. 2 and Table S2). Cushions were 100% bigger in the concave than convex garden (Garden effect: $P < 0.001$). However, the increase in cushion surface from the convex to the concave garden was higher for loose than tight cushions, and thus, tight cushions were 10% larger than loose cushions in the convex garden but 15% smaller in the concave garden (G x P effect: $P < 0.05$, Fig. 2a). Cushion leaves were 40% longer in the concave than convex garden (Garden effect on maximum leaf length: $P < 0.001$) and 17% longer for loose than tight cushions (Phenotype effect: $P < 0.01$, Fig.2b). Cushions penetration was 40% higher in the concave than convex garden (Garden effect: $P < 0.001$), and 25% higher for loose cushions than for tight cushions (Phenotype effect: $P < 0.001$, Fig. 2c). Leaves were 5% thicker in the convex than concave garden (Garden effect: $P < 0.05$) and 15% thicker for tight than loose cushions (Phenotype effect: $P < 0.001$, Fig. 2d). Cushions had overall a higher leaf density in the convex than concave garden (Garden effect: $P < 0.001$), but this effect differed among phenotypes (G x P effect: $P < 0.001$, Fig. 2e): tight cushion had 15% more leaves per unit area than loose cushions in the convex garden but a converse effect was observed in the concave garden.

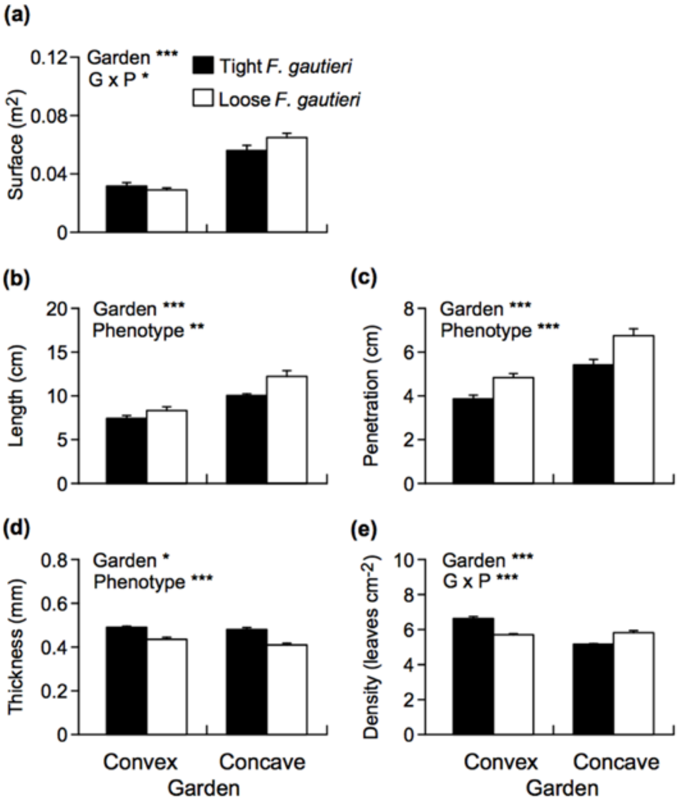

Fig. 2 Means + SE ($n = 8$) of cushion surface (a), maximum leaf length (b), cushion penetration (c), leaf thickness (d) and leaf density (e) of tight and loose *Festuca gautieri* cushion phenotypes in the convex and concave gardens. Significant results of two-way ANOVAs on the effects of Garden (G), Phenotype (P) and their interaction are indicated: *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$.

Reciprocal transplant competition experiment

In September 2013, after summer season, there was a high survival for *A. capillaris,* which was affected by neither the Garden and Neighbour treatments nor their interaction (Fig. S1a and Table S3). In contrast the two other target species had a much lower survival, in particular in the open of the convex garden (significant G x N interactions, Fig. S1b,c). Thus, RII results showed an overall switch from facilitation in the convex garden to competition in the concave garden (Garden effect: $P < 0.001$, Fig. S2, also see Table S4), and in particular for the two fescue targets (significant G x T interaction, $P < 0.05$). In contrast, differences in effects of the two phenotypes were marginally significant at this first date (Phenotype effect: $P < 0.1$). In June 2014, one year after the beginning of the experiment, survival of *A. capillaris* was significantly higher without neighbours than within tight or loose cushions (Neighbour effect: $P < 0.001$, Fig. S3a, also see Table S5), whereas survival of the tight fescue target was significantly higher without neighbour and within loose cushions than within tight cushions [Neighbour effect: $P < 0.01$, Tukey results: open area (a), tight *F. gautieri* cushion (b), loose *F. gautieri* cushion (a), Fig. S3b]. For the loose fescue target, survival was higher in the convex rather than the concave garden (Garden effect: $P < 0.05$, Fig. S3c). Thus, RII results showed that competition for survival was stronger in the concave than the convex garden (Garden effect: $P < 0.01$, Fig. 3 and Table S6) but the most significant effect was the Phenotype effect, with an overall increase in RII from tight to loose cushions (Phenotype effect: $P < 0.001$, Fig. 3). There were also significant differences in competitive responses for survival between targets [Target effect: $P < 0.001$, Tukey results: *A. capillaris* (ab), tight *F. gautieri* target (b), loose *F. gautieri* target (a)] as the loose fescue target was the strongest response competitor and the tight fescue target the weakest. We also found a significant Garden x Phenotype x Target interaction (G x P x T, $P < 0.05$) due to a strong shift from positive to negative RII from the convex to the concave garden, only observed for the tight fescue target within the loose cushions (Fig. 3). This latter result shows the stronger plasticity of the effect of the loose cushion as compared to the tight one, as well as the lower competitive response of the tight fescue target as compared to the two other target species.

For growth, in September 2013, there was a highly significant Neighbour effect for *A. capillaris* as there were more leaves without than within both cushion types (Neighbour effect: $P < 0.001$, Fig. S4a, also see Table S7), in particular in the concave garden (significant G x N interaction: $P < 0.05$). We also found a significant G x N interaction for the loose fescue target ($P < 0.05$, Fig. S4c) as there were more leaves in the open area and the loose *F. gautieri* cushions than within tight cushions in the concave garden but no differences in the convex garden (see Tukey results in Fig. S4c). In contrast, there was no significant effect of any treatment for the tight *F. gautieri* (Fig. S4b). RII total leaf number results showed significant differences in targets competitive response, with *A. capillaris* being the weakest response competitor [Target effect: $P < 0.001$, Tukey results: *A. capillaris* (b), tight *F. gautieri* (a), loose *F. gautieri* (a), Fig. 4, also see Table S8]. There was an increase in competition, or a decrease in facilitation, from the convex to the concave garden (Garden effect: $P < 0.05$) and no Phenotype effect at this date. We also found a significant G x T interaction ($P < 0.05$) because there was a decrease in RII from the convex to the concave garden for both *A. capillaris* and the loose *F. gautieri* targets but not change for the tight *F. gautieri* target. Specifically, the loose fescue target was facilitated only in the convex garden and competition increased for *A. capillaris* in the concave garden (Fig. 4).

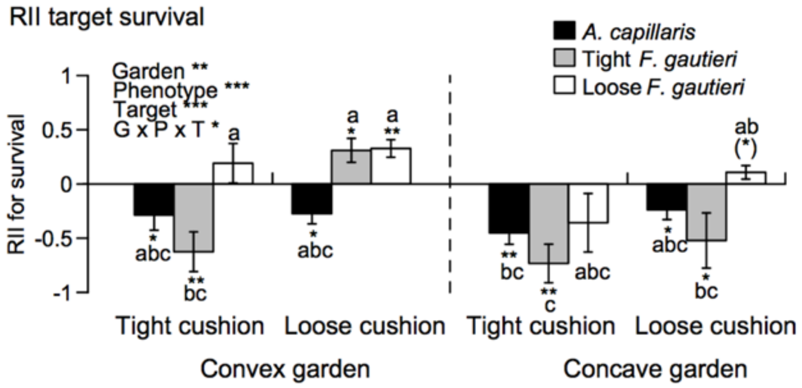

Fig. 3 Means ± SE (n = 8) of RII$_{\text{survival}}$ (June 2014) of the three targets (*Agrostis capillaris*, the tight *Festuca gautieri* and the loose *Festuca gautieri*) calculated for both cushion phenotypes in convex and concave gardens. Significant results of the three-way ANOVA on the effects of the Garden (G), Phenotype (P), Target (T) treatments and their interactions are indicated. Tukey tests are shown on top of error bars when a significant Garden x Phenotype x Target (G x P x T) interaction is shown. Also, results of one sample *t*-tests are shown above error bars: (*), $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

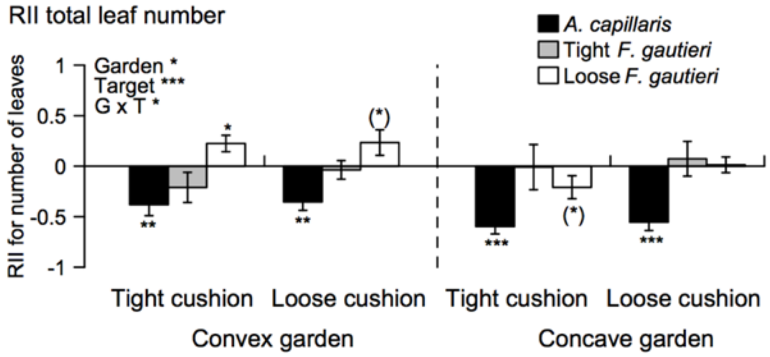

Fig. 4 Means ± SE (n = 8) of RII$_{\text{total leaf number}}$ of the three targets (*Agrostis capillaris*, the tight *Festuca gautieri* and the loose *Festuca gautieri*) calculated for both cushion phenotypes in the convex and concave gardens. Significant results of the three-way ANOVA on the effects of the Garden (G), Phenotype (P), Target (T) treatments and their interactions are indicated, and results of one sample *t*-tests are shown above error bars: (*), $P < 0.1$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Re-establishment of subordinate species

The natural re-establishment of subordinate species within tight and loose cushions (in both gardens) was significantly affected by the Garden and Phenotype treatments (Fig. 5 and Table

S9). For species richness there was a significant Garden effect ($P < 0.001$, Fig. 5a) with a higher richness in the convex than concave garden. Additionally, RII results showed that competition for species richness was significantly higher in the concave than convex garden (Garden effect: $P < 0.001$, Fig. 5c), but there was a tendency for this effect to be stronger for the tight than loose phenotype (marginally significant G x P interaction and highly significant competition within the tight phenotype in the concave garden, see result of the sample *t*-tests in Fig. 5c). For abundance, there was a significant Neighbour effect ($P < 0.05$, Fig. 5b), as individuals re-established more within loose than tight cushions with intermediate values in the open [Tukey results for the neighbour effect: open area (ab), tight *F. gautieri* (b) and loose *F. gautieri* (a)]. Thus, there was a significant Phenotype effect on $\mathrm{RII}_{\mathrm{Abundance}}$ ($P < 0.01$, Fig. 5d) with tight cushions being highly competitive and loose ones neutral or weakly facilitative. Additionally there was a significant Garden effect ($P < 0.05$) as $\mathrm{RII}_{\mathrm{Abundance}}$ became less negative for the tight cushion and shifted from negative to positive for the loose cushion from the convex to the concave garden.

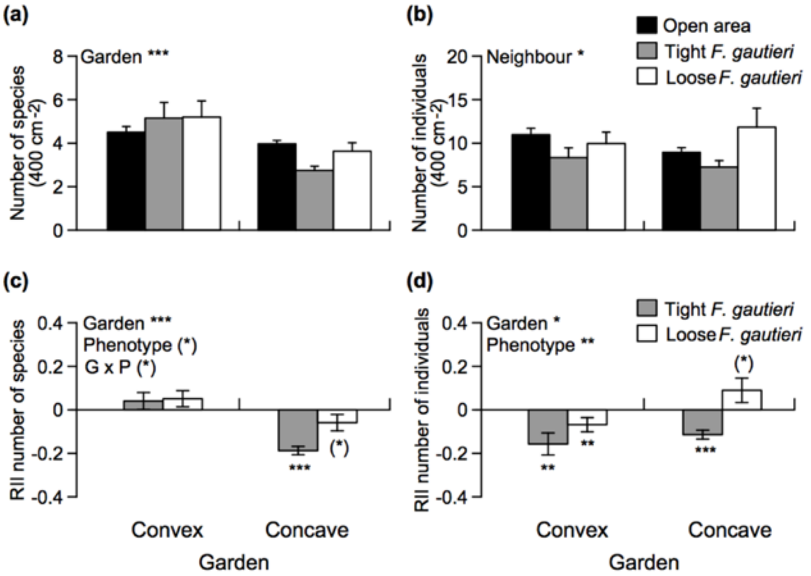

Fig. 5 Upper panels: means + SE ($n = 8$) of species richness (a) and abundance (b) of naturally re-established plant species in open areas and within tight and loose cushions of *Festuca gautieri* in the convex and concave gardens. Significant results of the two-way ANOVAs on the effects of the Garden, Neighbour treatments and their interaction are indicated: *, $P < 0.01$; ***, $P < 0.001$. Lower panels: mean ± SE ($n = 8$) of $\mathrm{RII}_{\mathrm{Richness}}$ (c) and $\mathrm{RII}_{\mathrm{Abundance}}$ (d) of naturally re-established plant species for the two *Festuca gautieri* cushion phenotypes in convex and concave gardens. Significant results of the two-way ANOVAs on the effects of the Garden (G), Phenotype (P) treatments and their interaction are indicated, and significant results of one sample *t*-tests on RII values are shown above (or below) error bars: (*), $P < 0.1$; **, $P < 0.01$; ***, $P < 0.001$.

Discussion

We aimed to assess the genetic and plasticity bases of the differences in morphological traits of the two phenotypes of *F. gautieri* and of their effects on subordinate species. Our results indicated that both genetics and plasticity account for differences in phenotypic traits and in effects on subordinate species. However, both results from our transplant competition experiments and survey of naturally re-established subordinate species showed that genetic and plastic effects act in opposite directions with increasing stress. Genetic effects are in line with an increase in competition with increasing stress, whereas plastic effects are in line with a decrease in competition with increasing stress.

Our reciprocal cushion transplant experiment indicated considerable variation in morphological traits between the tight and loose *F. gautieri* phenotypes. Tight cushions were more compact (lower penetration) with shorter and thicker leaves than loose ones. The pattern was consistent across topographical position (Garden treatment) four years after field collection suggesting that a genetic component for the intraspecific morphological differences observed across natural habitats very likely exists. This is consistent with the experiment of Al Hayek *et al*. (2014) who found that differences in morphological traits between transplants of both *F. gautieri* phenotypes were maintained two years after being grown under the same environmental conditions. Other authors have shown genetic-based morphological variations within plant species [e.g. Rowland (2001) between four populations of *Populus deltoides*, Crutsinger *et al*. (2010) for erect and prostrate *Baccharis pilularis* phenotypes, Michalet *et al*. (2011) for tight and loose *Geum rossii* phenotypes]. Moreover, tight and loose cushions differed significantly in their optimal environmental conditions for two growth traits, surface and leaf density. We found higher performance for these traits for tight than loose cushions in the convex garden (high stress) and *vice versa* in the concave garden (low stress). This suggests a phenotypic specialisation and thus an adaptation of tight cushions to high stress habitat, and loose cushions to low stress habitat, consistent to their natural distribution. The higher increase in cushion surface from the convex to the concave garden observed for loose cushions than for tight ones also shows the higher plasticity of the former. This highlights the occurrence of differences in functional strategies among phenotypes, the loose phenotype being more exploitative with long and thin leaves and the tight one more conservative with short and thick leaves (Grime, 1974; Tilman, 1982; Liancourt *et al*., 2005), consistent with the results of Michalet *et al*. (2011) for the alpine cushion species *Geum rossii*. On the other hand, varying environmental conditions across gardens had a significant effect on all morphological traits, with cushions in the concave garden having longer leaves, larger surface and higher penetration, and cushions in the convex garden having ticker leaves and higher leaf density. Thus, variation in morphological traits across natural habitats is also in part due to plasticity effects. To summarise, both genetic and plastic effects explained the morphological trait differences between *F. gautieri* phenotypes. This is in accordance with other studies that showed the implication of both genetic and plastic effects in determining morphological traits (Bresson *et al.*, 2010; Michalet *et al.*, 2011).

Results of our competition experiment also showed that both genetic and plastic effects account for differences in competitive and facilitative abilities between *F. gautieri* phenotypes. The Stress-Gradient-Hypothesis predicts that competition should shift to facilitation when increasing stress (Bertness & Callaway, 1994; Brooker & Callaghan, 1998), as demonstrated by several experimental studies (Choler *et al.*, 2001; Callaway *et al.*, 2002; Schiffers & Tielbörger, 2006; but see Maestre & Cortina, 2004). Consistently, cushion competitive effects decreased from the concave to the convex garden for both target survival and leaf number. For survival the phenotype effect was even stronger than the garden effect, and increased through time with only a marginally significant effect in September 2013 but a highly significant effect at the end of the experiment in July 2014. For growth there was no significant phenotype effect, likely because measurements could only be analysed in September 2013 due to the too low target survival at the end of the experiment. These results suggest that differences in effects on dependent species across phenotypes have established through time; as the cushions grew (most cushions almost doubled in size between the two dates, particularly the loose phenotype, P. Al Hayek, pers. obs.), their effects were settled and tight cushions became significantly more competitive than loose ones in both gardens in July 2014. This asserts our prediction of higher competitive effects for tight than loose *F. gautieri* phenotypes, and proves the heritability of these effects. Tight cushions had higher competitive effects than loose ones, probably due to lower penetration (related to higher leaf thickness, stiffness and leaf density in the convex garden) inhibiting target species within the cushion's canopy. Contrasted effects of different phenotypes of foundation species on subordinate species, have been evidenced in several studies (Callaway *et al.*, 1991; Pugnaire *et al.*, 1996; Rudgers & Maron, 2003; Crutsinger *et al.*, 2010; Michalet *et al.*, 2011; Cranston *et al.*, 2012; Schöb *et al.*, 2013; Al Hayek *et al.*, 2014). Our results are consistent with Michalet *et al.* (2011) who showed, contrasting competitive and facilitative effects of genetic-based tight and loose phenotypes of *Geum rossii*, respectively. Similar results were found by Crutsinger *et al.* (2010) in a common garden experiment for the coastal dune shrub *Baccharis pilularis*, with contrasting competitive and facilitative effects of erect and prostrate phenotypes, respectively.

However, the genetic-based increase in competition from loose to tight cushions shows that genetic effects are in line with an increase in competition with increasing stress and thus, in contrast to plasticity effects, contradicts Grime's (1974) model and the SGH (Bertness & Callaway, 1994). In contrast, this is consistent with other theoretical models proposing either the occurrence of high competition in nutrient-poor soils (Tilman, 1982) or even an increase in competition with decreasing resource availability (MacArthur & Wilson, 1967; Taylor *et al.*, 1990). This alternative prediction has been supported by several experiments, in particular in dry environments (Davis *et al.*, 1998; Tielbörger & Kadmon, 2000; Maestre & Cortina, 2004; Saccone *et al.*, 2009).

We also found a difference in plasticity of cushion effects (i.e. changes in cushion effects across gardens) between tight and loose phenotypes. For survival, there was a shift from facilitation to competition within loose cushions from the convex (high stress) to the concave (low stress) garden for the tight fescue target, while no such effect was observed in tight cushions for any target species (Fig. 3). The stronger plasticity of loose cushions effects, as compared to tight ones, is consistent with their higher growth rate and lower stress-tolerance shown by our traits measurements, the results of Le Bagousse-Pinguet *et al.* (2014) and their contrasting natural distributions along environmental gradients. Thus, the loose cushion with its exploitative strategy, higher plasticity and higher competitive ability in conditions of low stress (concave topography) is more representative of the Grime's (1974) competitor, whereas the tight cushion with its conservative strategy and higher competitive ability in stressful conditions is more representative of the Tilman's (1982) nutrient competitor or the K-strategy of MacArthur & Wilson (1967). Similar differences in functional strategy were described by Michalet *et al.* (2011) for the two phenotypes of the alpine plant *Geum rossii*.

Our assessment of richness and abundance of naturally re-established subordinate species brought additional evidence that differences in competitive and facilitative effects among *F. gautieri* phenotypes have both genetic and plasticity bases. As in the transplant competition experiment, we found a genetically-driven increase in competition from the loose to the tight phenotype for both richness and abundance. Plasticity effects were also consistent with those of the transplant competition experiment for richness, with an increase in competition from the dry to the wet garden. Thus, our results overall confirm those of the transplant competition experiment, and thus, ensure their relevance for understanding interactions occurring in natural habitats. However, there were interesting differences between the patterns of resident species re-establishment and the richness and abundance of dependent species within the two natural habitats.

For the loose phenotype, the number of the re-established subordinate species (richness) in the concave garden was twice as high as the number of subordinate species occurring in loose cushions in their natural habitat, and their abundance was similar (see Figs 1 and 5a,b). In contrast, for the tight phenotype, both the re-established subordinate species richness and abundance were much higher in the convex garden than in the natural habitat (ten times higher for richness and four times for abundance, see Figs 1 and 5a,b). This suggests that the effects of the loose cushions are almost completely established and similarly expressed as in their natural habitat, whereas the effects of the tight cushions are far from being established, and, thus, not identically expressed as in their natural habitat. Several studies comparing the short-term effect of an experimental treatment to a similar change in the same factor across natural gradients have observed such discrepancy, in particular for functional traits. For example, Sandel *et al.* (2010) have shown that increasing water availability in experimental conditions in the American prairie induces a decrease in seed size and an increase in leaf nitrogen content, whereas converse patterns are observed along natural gradient of water availability in the same system. They argued that the short-term effects observed in experimental conditions were due to the response of fast-growing ruderals, likely to be replaced by more conservative species through time. Similarly, we argue that the lower growth rate of the conservative tight phenotype as compared to the exploitative loose one may explain why the competitive effects of the former were not completely established in the convex garden, in contrast to the latter in the concave garden, thanks to its higher plasticity. Additionally, K-strategy species such as the tight phenotypes are known to acquire their competitive ability through time, in contrast to exploitative species such as the Grime’s competitor, which wins competition thanks to their higher plasticity (Liancourt *et al.*, 2009).

To conclude, our results showed that both genetics and plasticity play a role in the variation of the competitive effects between tight and loose *F. gautieri* phenotypes, but that genetic and plastic effects act in opposite directions, with the former enhancing competition and the latter decreasing it with increasing stress. But in order to assess the ecological consequences of phenotypic differentiation for the diversity of this subalpine system, it is worth trying to evaluate which of the genetic and plastic components have a higher effect on diversity. Diversity is thought to increase in semi-stressful environments due to the decrease in competition (Grime, 1973) and to an increase in facilitation (Hacker & Gaines, 1997; Michalet *et al.*, 2006), and again to decrease at extreme stress level due to the collapse of facilitation (Michalet *et al.*, 2006) and the too stressful conditions for most plants (Grime, 1974), as shown by Le Bagousse-Pinguet *et al.* (2014) in the same subalpine system. Thus, as the habitat of the tight phenotype is located in semi-stressful conditions (Le Bagousse-Pinguet *et al.*, 2014) we may predict that diversity should increase from the natural habitat of loose *F. gautieri* cushions (low stress and high competition) to that of the tight ones (high stress), as plasticity effects reduce competition. However, the opposite pattern is observed in our system, as diversity decreased from loose to tight cushion habitats. We argue that this is most probably due to the genetically-driven higher competitive effect of tight cushions in the high stress habitat, which counteracts plasticity effects. Thus, for diversity we conclude that genetic effects overcome plasticity effects.

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Supporting Information

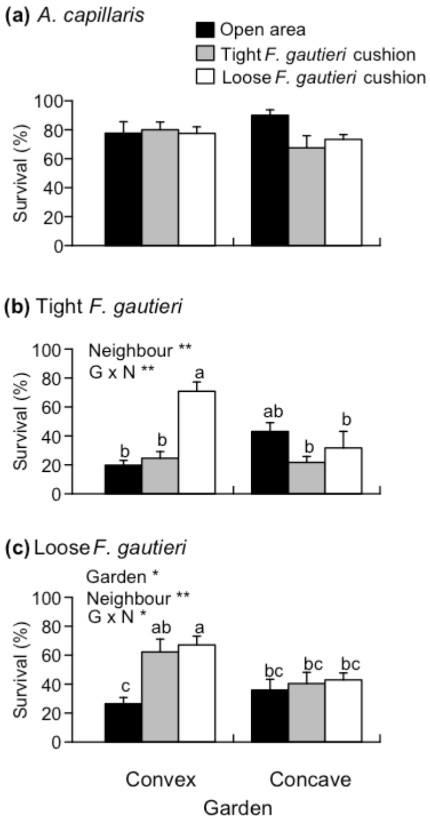

Fig. S1 Means + SE (*n* = 8) of survival (September 2013) of *A. capillaris* (a), the tight *F. gautieri* (b) and the loose *F. gautieri* targets (c) in the three neighbouring conditions (open area: no neighbour, within tight *F. gautieri* cushions and within loose *F. gautieri* cushions) in the convex and concave gardens. Significant results of the two-way ANOVAs on the effects of the Garden (G), Neighbour (N) treatments and their interactions are indicated: *, $P < 0.05$; **, $P < 0.01$.

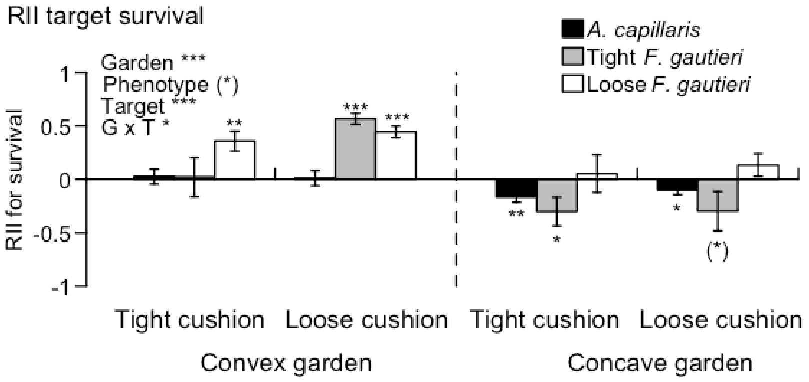

Fig. S2 Means ± SE (*n* = 8) of $\text{RII}_{\text{survival}}$ (September 2013) of the three targets (*A. capillaris*, the tight *F. gautieri* and the loose *F. gautieri*) calculated for both cushion phenotypes in the convex and concave gardens. Significant results of the three-way ANOVA on the effects of the Garden (G), Phenotype (P), Target (T) treatments and their interactions are indicated. Results of one sample *t*-tests are shown above error bars: (*), *P* < 0.1; *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001.

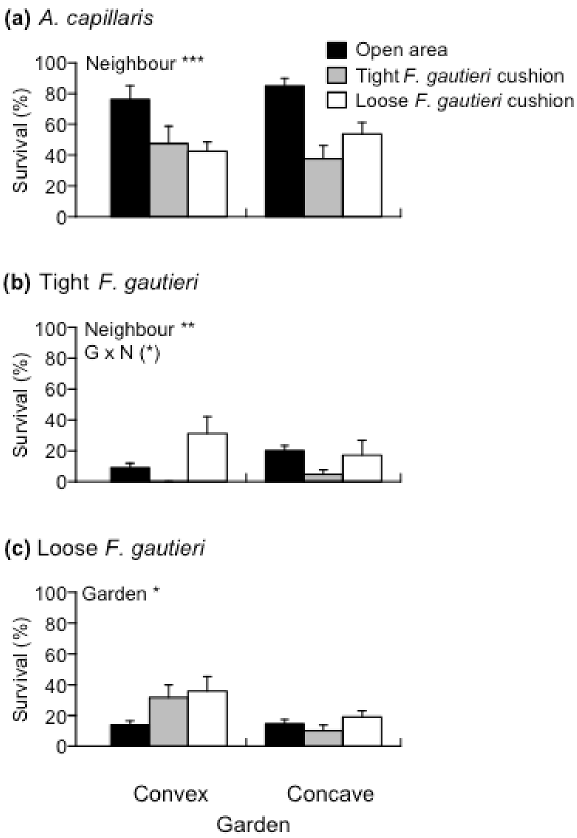

Fig. S3 Means + SE (n = 8) of survival (June 2014) of *A. capillaris* (a), the tight *F. gautieri* (b) and the loose *F. gautieri* targets (c) in the three neighbouring conditions (open area: no neighbour, within tight *F. gautieri* cushions and within loose *F. gautieri* cushions) in the convex and concave gardens. Significant results of the two-way ANOVAs on the effects of the Garden (G), Neighbour (N) treatments and their interactions are indicated: (*), $P < 0.1$; *, $P < 0.05$; ***, $P < 0.001$.

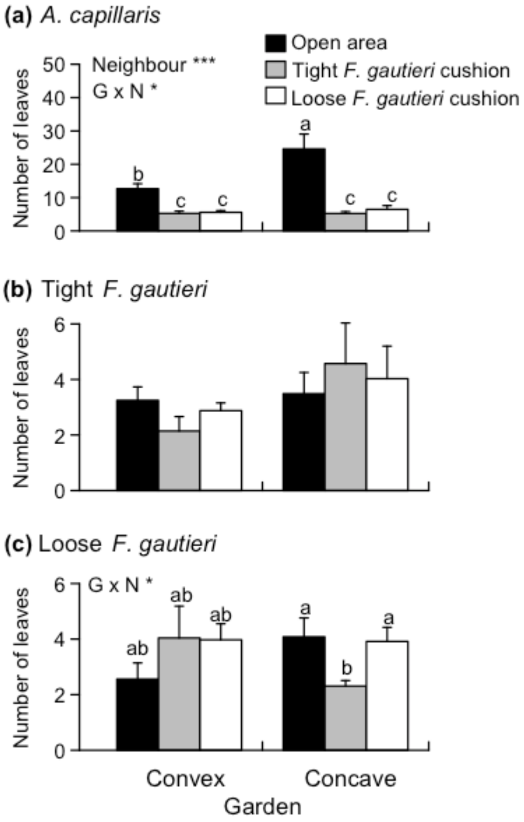

Fig. S4 Total leaf number (September 2013) of the three target species transplanted within and outside *Festuca gautieri* cushions. Means + SE (*n* = 8) of total leaf number in September 2013 of *A. capillaris* (a), the tight *F. gautieri* (b) and the loose *F. gautieri* targets (c) in the three neighbouring conditions (open area: no neighbour, within tight *F. gautieri* cushions and within loose *F. gautieri* cushions) in the convex and concave gardens. Significant results of the two-way ANOVAs on the effects of the Garden (G), Neighbour (N) treatments and their interaction are indicated: *, $P < 0.05$; ***, $P < 0.001$. Results of Tukey tests are shown on top of error bars when a significant Garden x Neighbour (G x N) interaction is shown.

Table S1 Results of the one-way ANOVAs on the effect of the Phenotype treatment on species richness and abundance of subordinate plant species in the natural habitats of *F. gautieri* cushions. Significant results are shown in bold. ***, $P < 0.001$

		Species richness		Abundance	
	df	*F*	*P*	*F*	*P*
Phenotype	2	18.88	**<0.001 *** **	19.54	**<0.001 *** **
Error	75				

Table S2 Results of the two-way ANOVAs on the effects of the Garden (G), Phenotype (P) treatments and their interaction (G x P) on cushion surface, leaf length, cushion penetration, leaf thickness and leaf density of *F. gautieri* cushions. Significant results are shown in bold. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

		Cushion surface		Leaf length		Cushion penetration	
	df	*F*	*P*	*F*	*P*	*F*	*P*
Garden	1	125.73	**<0.001 *** **	56.76	**<0.001 *** **	54.22	**<0.001 *** **
Phenotype	1	1.36	0.253	12.76	**0.001 ** **	24.09	**<0.001 *** **
G x P	1	4.64	**0.040 * **	2.24	0.146	0.61	0.443
Error	28						

		Leaf thickness		Leaf density	
	df	*F*	*P*	*F*	*P*
Garden	1	4.96	**0.034 * **	54.32	**<0.001 *** **
Phenotype	1	61.61	**<0.001 *** **	2.11	0.158
G x P	1	0.98	0.330	75.53	**<0.001 *** **
Error	28				

Table S3 Results of the two-way ANOVAs on the effects of the Garden (G), Neighbour (N) treatments and their interaction on survival (September 2013) of *A. capillaris*, the tight *F. gautieri* and the loose *F. gautieri* targets. Significant results are shown in bold. *, $P < 0.05$; **, $P < 0.01$

		A. capillaris		Tight *F. gautieri*		Loose *F. gautieri*	
	df	*F*	*P*	*F*	*P*	*F*	*P*
Garden	1	0.04	0.840	1.04	0.314	4.82	**0.034 * **
Neighbour	2	2.15	0.129	7.05	**0.002 ** **	6.07	**0.005 ** **
G x N	2	1.38	0.264	7.30	**0.002 ** **	3.49	**0.040 * **
Error	42						

Table S4 Results of the three-way ANOVA on the effects of the Garden (G), Phenotype (P), Target (T) treatments and their interactions on $RII_{survival}$ (September 2013) of the three targets (*A. capillaris*, the tight *F. gautieri* and the loose *F. gautieri*). Significant results are shown in bold. (*), *P* < 01; *, *P* < 0.05; ***, *P* < 0.001

	$RII_{survival}$		
	df	*F*	*P*
Garden	1	28.44	**<0.001 *****
Phenotype	1	3.74	**0.056 (*)**
Target	2	8.07	**<0.001 *****
G x P	1	1.44	0.233
G x T	2	3.87	**0.025 ***
P x T	2	1.32	0.273
G x P x T	2	2.16	0.121
Error	84		

Table S5 Results of the two-way ANOVAs on the effects of the Garden (G), Neighbour (N) treatments and their interaction on survival (June 2014) of *A. capillaris,* the tight *F. gautieri* and the loose *F. gautieri* targets. Significant results are shown in bold. (*), *P* < 0.1; *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001

	A. capillaris			Tight *F. gautieri*		Loose *F. gautieri*	
	df	*F*	*P*	*F*	*P*	*F*	*P*
Garden	1	0.17	0.686	0.21	0.649	4.44	**0.041 ***
Neighbour	2	13.80	**<0.001 *****	6.89	**0.003 ****	1.68	0.199
G x N	2	0.65	0.528	2.68	**0.080 (*)**	1.48	0.240
Error	42						

Table S6 Results of the three-way ANOVA on the effects of the Garden (G), Phenotype (P), Target (T) treatments and their interactions on $\mathrm{RII}_{\mathrm{survival}}$ (June 2014) of the three targets (*A. capillaris*, the tight *F. gautieri* and the loose *F. gautieri*). Significant results are shown in bold. *, *P* < 0.05; **, *P* < 0.01; ***, *P* < 0.001

	$\mathrm{RII}_{\mathrm{survival}}$		
	df	*F*	*P*
Garden	1	11.02	**0.001 ****
Phenotype	1	12.73	**<0.001 *****
Target	2	9.44	**<0.001 *****
G x P	1	0.12	0.727
G x T	2	1.79	0.174
P x T	2	2.10	0.129
G x P x T	2	3.23	**0.044 ***
Error	84		

Table S7 Results of the two-way ANOVAs on the effects of the Garden (G), Neighbour (N) treatments and their interaction on total leaf number of *A. capillaris*, the tight *F. gautieri* and the loose *F. gautieri* targets. Significant results are shown in bold. *, *P* < 0.05 ; ***, *P* < 0.001

		A. capillaris		Tight *F. gautieri*		Loose *F. gautieri*	
	df	*F*	*P*	*F*	*P*	*F*	*P*
Garden	1	1.33	0.255	1.36	0.250	0.28	0.599
Neighbour	2	43.32	**<0.001 *****	0.38	0.687	0.84	0.437
G x N	2	4.21	**0.022 ***	1.31	0.281	4.13	**0.023 ***
Error	42						

Table S8 Results of the three-way ANOVA on the effects of the Garden (G), Phenotype (P), Target (T) treatments and their interactions on $\mathrm{RII}_{\text{total leaf number}}$ (September 2013) of the three targets (*A. capillaris*, the tight *F. gautieri* and the loose *F. gautieri*). Significant results are shown in bold. *, *P* < 0.05; ***, *P* < 0.001

		$\mathrm{RII}_{\text{total leaf number}}$	
	df	*F*	*P*
Garden	1	4.18	**0.044 ***
Phenotype	1	1.82	0.182
Target	2	23.76	**<0.001 *****
G x P	1	0.13	0.722
G x T	2	4.23	**0.018 ***
P x T	2	0.19	0.830
G x P x T	2	0.42	0.661
Error	80		

Table S9 Results of the two-way ANOVAs on the effects of a) Garden (G), Neighbour (N) treatments and their interaction on species richness and abundance of naturally re-established plant species, and b) Garden (G), Phenotype (P) treatments and their interaction on $\mathrm{RII}_{\text{richness}}$ and $\mathrm{RII}_{\text{abundance}}$ of naturally re-established plant species. Significant results are shown in bold. (*), *P* < 0.1; *, *P* < 0.05; ***, *P* < 0.001

a)		Species richness		Abundance	
	df	*F*	*P*	*F*	*P*
Garden	1	15.10	**<0.001 *****	0.17	0.683
Neighbour	2	0.49	0.613	3.35	**0.045 ***
G x N	2	1.97	0.152	1.38	0.264
Error	42				
b)		$\mathrm{RII}_{\text{richness}}$		$\mathrm{RII}_{\text{abundance}}$	
	df	*F*	*P*	*F*	*P*
Garden	1	24.20	**<0.001 *****	5.62	**0.025 ***
Phenotype	1	4.09	**0.053 (*)**	11.9	**0.002 ***
G x P	1	2.95	**0.097 (*)**	1.84	0.186
Error	28				

CHAPTER FIVE:

Differential effects of contrasting phenotypes of a foundation legume shrub drive plant–plant interactions in a Mediterranean mountain

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Summary

Questions: Are variable effects of different phenotypes of foundation plant species on subordinates across contrasting habitat conditions (with varying stress and disturbance levels) due to either varying environmental conditions or heritable differences in traits between phenotypes? To evaluate the contribution of environmental effects we quantified the effects of contrasting phenotypes of a foundation legume shrub on their subordinate species across exposure (drought stress) and grazing conditions.

Location: A sub-alpine xerophytic community of western Mount Lebanon, Lebanon.

Methods: For two phenotypes of the spiny cushion shrub *Onobrychis cornuta*, a facilitative phenotype that occurs in concave topographies (mesic soil) and a competitive phenotype that occurs in convex topographies (xeric soil), we quantified cushion traits, environmental conditions and subordinate plant species abundances (within and outside the cushions) for the two phenotypes in northern (low stress) and southern (high stress) exposures, and with and without grazing. Relative interaction index (RII) for subordinate species richness and abundance was calculated in the eight treatment combinations and a correspondence analysis (CA) was conducted on species composition.

Results: Drought stress exacerbated phenotypic effects in southern exposure, with loose phenotypes being more facilitative and tight phenotypes more competitive than in northern exposure. This was related to both changes in cushion traits of the two phenotypes and to an increase in the pool of subordinate species sensitive to cushion effects. In contrast, grazing increased cushion positive effects of both phenotypes through the occurrence of indirect facilitation, with loose phenotypes becoming more facilitative and tight phenotypes less competitive. This was due both to changes in cushion traits of the two phenotypes and to their shared spiny phenotype limiting grazing effects.

Conclusions: Because increasing stress from northern to southern exposure did not increase competitive effects of either phenotype, and because the cessation of grazing did not cancel out differences in facilitative effects between phenotypes, neither drought stress nor grazing disturbance appeared to be the main drivers of the observed phenotypic effects on subordinate species across habitats. We conclude that differences in phenotypic effects of this legume shrub are very likely due to heritable differences in traits between phenotypes.

Keywords: Community phenotypes; Competition; Cushion plants; Exposure; Facilitation; Foundation species; Grazing; Indirect facilitation; Mount Lebanon; *Onobrychis cornuta*.

Nomenclature: Euro+Med (2006-): Euro+Med PlantBase - the information resource for Euro-Mediterranean plant diversity. Published on the Internet http://ww2.bgbm.org/EuroPlusMed/ [March 2014].

Abbreviations: ANOVA = Analysis of Variance; CA = Correspondence Analysis; RII = Relative Interaction Index.

Introduction

Plant–plant interactions (competition and facilitation) have been an important topic of interest in the community ecology literature for several decades (Grime 1974; Tilman 1982; Bertness & Callaway 1994; Bruno et al. 2003; Brooker et al. 2008). Most studies have focused on changes in interactions along environmental gradients, with several models proposed in the literature (Grime 1974; Tilman 1982; Bertness & Callaway 1994; Maestre & Cortina 2004; Michalet et al. 2006; Holmgren & Scheffer 2010). In dry ecosystems there is a debate on the likely occurrence of a shift from competition to facilitation with increasing drought, as originally proposed by Bertness & Callaway (1994), i.e. the stress gradient hypothesis (Maestre et al. 2005; Lortie & Callaway 2006; He et al. 2013; He & Bertness 2014; Michalet et al. 2014). Herbivory pressure also alters plant–plant interactions, decreasing competition (Grime 1974) and potentially increasing indirect facilitation processes (Bertness & Callaway 1994). The interaction between grazing and drought has recently been assessed, in particular in the context of global change, with most studies showing the occurrence of a collapse of interactions (i.e. decline and loss of all interactions) under both constraints (Kéfi et al. 2007; Smit et al. 2009; Soliveres et al. 2011; Maalouf et al. 2012; Verwijmeren et al. 2013; Le Bagousse et al. 2014).

However, there is a high contingency in biotic interactions (Chamberlain et al. 2014), and the identity of the interacting species has been shown to be as important as environmental conditions (Michalet 2007; Maestre et al. 2009; Soliveres & Maestre 2014). Facilitation studies have highlighted the importance of the functional strategy of the beneficiary species (Choler et al. 2001; Liancourt et al. 2005; Michalet et al. 2006; Forey et al. 2010; Gross et al. 2010; Butterfield & Briggs 2011) and of the dominant benefactor or 'nurse' species (Michalet 2007; Gross et al. 2008; Gomez-Aparicio 2009; Maestre et al. 2009; Pugnaire et al. 2011).

How phenotypic differentiation within dominant species may alter plant–plant interactions has been less well assessed (but see Callaway et al. 1991; Crutsinger et al. 2010), although community genetics studies have shown that phenotypic differentiation within foundation species (i.e. a species that structures a community by ameliorating abiotic stress and creating locally stable conditions for other species, and by modulating and stabilizing fundamental ecosystem processes; Ellison et al. 2005) has the potential to affect community structure through changes in effects on subordinate species (i.e. a species associated with the dominant foundation species; Whitham et al. 2006; Michalet et al. 2011). Additionally, very few studies have compared the relative contribution of environmental conditions and foundation plant species phenotype as drivers of the foundation species effect on their subordinate species.

The strongest evidence of phenotypic effects on subordinate species has been revealed in systems where the varying phenotypes of a foundation species occur in similar environmental conditions (Callaway et al. 1991; Crutsinger et al. 2010). However, phenotypic differentiation within foundation species may also occur in different environ- mental conditions, in particular across topography and/or soil gradients (Choler & Michalet 2002; Michalet et al. 2011; Lekberg et al. 2012; Liancourt et al. 2013). Although both have been acknowledged to be important, the relative contribution of phenotypic differentiation and environmental conditions to foundation species effects on neighbours is poorly studied.

One way to straightforwardly assess the relative contribution of heritable phenotypic variation and of different environmental conditions is to quantify the interactions with subordinate plant species of transplanted individuals of different phenotypes in contrasting environments. However, in most systems, such as the alpine community of Michalet et al. (2011), this experimental test is almost impossible even on a long-time scale because of both the very low growth rate of the often long-lived foundation species and the necessity to have adult transplants to really assess the contrasting effects of different phenotypes (Liancourt et al. 2009; Le Bagousse-Pinguet et al. 2013). We suggest that an alternative method for at least qualitatively assessing the contribution of varying environmental conditions on the contrasting effects of different foundation plant species phenotypes is an assessment of the effects of such phenotypes on subordinate species across contrasting environmental conditions (see also Callaway et al. 1991). For example, an assessment of the effects of different phenotypes in two contrasting (local) conditions of environ- mental severity may be an appropriate test to decipher the contribution of both drivers. Specifically, if changes in interactions across phenotypes are mostly environmentally driven, then an increase in environmental severity should induce a similar increase in facilitation (or decrease in competition) irrespective of phenotypes. On the other hand, if the facilitation observed in a more fertile concave habitat by a known facilitative phenotype is higher than that observed in a convex habitat by a known competitive phenotype, and if this is due to increasing herbivory pressure (indirect facilitation) in the former, then a cessation of herbivory should cancel the facilitation observed in this habitat and thus the difference in phenotypic effects between habitats.

We assessed this issue in a sub-alpine system from Lebanon, with cushion-like dwarf thorny shrubs as foundation species, in particular the legume shrub *Onobrychis cornuta* (L.) Desv. We observed two different phenotypes of *O. cornuta*, a tight phenotype with entangled stems forming compact cushions occurring on dry soils with convex topographies, and a loose phenotype with an open stem morphology occurring on mesic soils with concave topographies. Contrasting associational patterns with subordinate species along with contrasting flower production of the two phenotypes were also observed (Figs S1, S2).

Our main goal is to assess the contribution of environmental variation (stress and disturbance by grazing) and two contrasting phenotypes in driving changes in the association of *O. cornuta* with other species. Specifically, we aim to quantify differences in competitive and facilitative effects of the two foundation species cushion phenotypes (tight and loose), and to answer the following questions: (1) are these effects altered by varying environmental stress with exposure; and (2) are these effects affected by grazing disturbance? To answer these questions, we assessed differences in cushion traits, environmental characteristics, and composition, abundance and richness of subordinate species, in contrasting exposure and grazing conditions. These measurements were conducted in the habitats of the two phenotypes, both within and outside cushions. In our study, competition appears to be highest in the most stressful conditions (in dry outcrops for the tight phenotype) and facilitation highest in the most mesic ones (in concave slopes for the loose phenotype). Thus, if environmental stress is the main driver of changes in phenotypic effects across habitats, we predict that with increasing stress from northern to southern exposure, competition should increase for the tight phenotype and facilitation decrease for the loose one, consistent with Maestre & Cortina (2004). Additionally, if grazing disturbance is the main driver of changes in phenotypic effects habitats, through differences in grazing pressure habitats due to grazers' preferences, we predict that differences in effects on other species among phenotypes should decrease with the cessation of grazing disturbance.

Methods

Study site and target species

The research was carried out on the western side of Mount-Lebanon, 20 km East from the Mediterranean Sea, at Ouyoun El Simane - Kfardebian (33°59' N, 35°51' E, altitude: 2000 m a.s.l., Mount-Lebanon, Lebanon). Climate is Mediterranean with very high precipitation in winter (mainly snow) and very low during summer (950 mm and 10 mm, respectively, with 1720 mm of annual rainfall). Mean temperatures are 2°C in winter and 16°C in summer.

At this elevation on Mount-Lebanon and in general in the Middle East the dominant vegetation type is an oromediterranean open xerophytic community dominated by several spiny shrubs, mostly from the Fabaceae (*Astragalus* spp. and *O. cornuta*) and Plumbaginaceae (*Acantholimon* spp.; Quézel & Médail 2003). This vegetation type, located above the current timberline, results from centuries of grazing by domestic sheep (*Ovis aries*) and goats (*Capra hircus*), following deforestation of *Cedrus libani* and *Juniperus excelsa* forests. Our model species, *O. cornuta* (L.) Desv., the horned sainfoin, is common at high elevations from Lebanon in the Middle East to Kyrgyzstan and Pakistan in Central Asia. It is a spiny shrub forming flattened circular to elliptic cushions with highly entangled branches and many axillary peduncles differentiated into rigid sterile or flowering thorns exceeding short green leaves. Fertile stems hold pubescent spineless pods of 6- to 8-mm long, and 10-15 mm bright purple-pink flowers (Tohmé & Tohmé 2014), blooming from May to July and providing nectar for insects. Although the species has low palatability due to its small leaves and spines (Diaz et al. 2001), it may provide forage for grazing animals such as sheep in the absence of more palatable species (Shahriary et al. 2012). Shepherds often burn old cushions (P. Al Hayek pers. obs.).

At our study site, *O. cornuta* is the dominant spiny shrub, acting as a foundation species hosting most other species of the community (Ellison et al. 2005). Two different phenotypes differing in their associations with other species occur in two different topographic positions and soil conditions (Figs S1 and S2). In convex topographies and on shallow stony soils (stone cover = 47.56 ± 2.38%, n = 80) the dominant phenotype is a tight compact cushion characterized by dense stem morphology. In concave topographies and on deeper and less stony soils (stone cover = 37.13 ± 2.45%, n = 80), the dominant phenotype is a loose cushion characterized by loose stem morphology with open areas within its canopy. We observed contrasting patterns of association with other plant species along with contrasting cushion flower productions. Tight phenotypes have a low cover of other species and a very high flower production, whereas loose phenotypes have a high cover of other species and a low flower production, suggesting a probable reproductive cost for loose phenotypes for hosting other species (Michalet et al. 2011; Schöb et al. 2014). The community includes up to 60 species, the most common among them are *Bromus tomentellus* Boiss., *Festuca pinifolia* (Hack.), *Alyssum condensatum* Boiss, *Asyneuma rigidum* (Wild.) Grossh subsp. *sinai*, *Festuca* sp., *Asperula setosa* Jaub. & Spach., *Cruciata pedemontana* (Bell.) and *Prunus prostrata* Labill.

Data collection

In order to answer our two main questions, for both phenotypes we measured cushion traits and environmental conditions, and quantified subordinate species composition in two contrasting exposures and grazing conditions. The ungrazed plots were located in a protected area of 450 000 m^2 fenced in 2008, and the grazed plots no further than 500 m outside the fenced area. The grazed and ungrazed areas were located at the same elevation, on the same calcareous rock, and both included contrasting exposures. Mount Lebanon is formed of very similar rolling hills of calcareous rocks, thus minimizing the problem of pseudo-replication inherent to designs including large fenced and unfenced areas. In each of the two grazing treatments, and in both northern and southern exposures, we randomly selected 20 cushions of each phenotype and 20 paired open plots (total: 320 plots). The 20 pairs of each phenotype were selected on a minimum of five hills per grazing condition. Each open plot was located at a very short distance (<2 m) from its paired cushion and in the microtopographic position; the areas of the open plots were delimited using a flexible wire to occupy a similar area as the paired cushion. We used the term 'Microsite' for each pair of cushion-paired open plots. For the 160 selected cushions we measured, in June 2012, cushion surface, height, penetration (an index of cushion compactness and interference; Al Hayek et al. 2014), stem number and flower number. Penetration was measured to a precision of 1 mm by loosely introducing a metal ruler vertically within the cushion. Stems and flowers were counted within 100 cm^2 of cushion area. In order to assess the environmental conditions of each cushion, we recorded stone cover at the soil surface and the degree convexity of the cushions' microhabitats using five classes (from 1: very concave, to 5: very convex). We recorded the number of individuals of all vascular species present in the cushions and the paired open areas, and calculated species richness for both plots. To quantify the net effect of each cushion individual on other species in each combination of the states of our three factors (exposure, grazing, phenotype), we calculated the relative interaction index (RII) for species richness (number of species; $RII_{Richness}$) and for abundance (number of individuals of all species; $RII_{Abundance}$) following Armas et al. (2004):

$RII = (P_{cushion} - P_{open})/(P_{cushion} + P_{open})$

where $P_{cushion}$ and P_{open} represent the species richness (number of species) or abundance (number of individuals of other species) within the cushion and the open paired plots, respectively. This index is symmetrical around zero (no significant interaction), and has defined limits between -1 and +1; negative values indicate competition whereas positive values reflect facilitation.

Data analysis

Differences in cushion traits and environmental conditions were analysed using a three-way ANOVA model (followed by Tukey tests when necessary) with Grazing, Exposure and Phenotype as factors, and the five cushion traits (cushion surface, height, penetration, stem number and flower number) and two environmental variables (soil convexity and stone cover) as dependent variables. Differences in net effects of cushions on species richness and abundance were analysed using a three-way ANOVA model with Grazing, Exposure and Phenotype as factors, and $\mathrm{RII}_{\mathrm{Richness}}$ and $\mathrm{RII}_{\mathrm{Abundance}}$ as response variables. One sample *t*-tests were used to analyse significant deviation of RII values from zero, which represents no cushion effect.

In order to assess the effects of the Grazing, Exposure, Phenotype and Neighbour treatments on species composition, we conducted a correspondence analysis (CA) on the 320 plots. We excluded 21 (out of 76) species with a frequency below five in the whole data set (and three plots with no species), in order to avoid rare species effects. To test if treatments significantly affected species composition, a mixed model ANOVA was performed on CA relevée scores, separately for each CA axis, with Grazing, Exposure and Phenotype treated as fixed effects, and Neighbour nested within Microsite as the random effects structure. We also conducted a cluster analysis on CA species scores in order to identify groups of species with similar responses in the CA. In order to assess the effect of the four treatments on the abundances of the species of each cluster group, a mixed model ANOVA was performed on the pooled species abundance of each cluster group, with Grazing, Exposure and Phenotype treated as fixed effects, and Neighbour nested within Microsite treated as the random effects. Dependent variables were checked for normality and log-transformed before ANOVAs. All statistical analyses were done using R (R Foundation for Statistical Computing, Vienna, AT).

Results

Morphological differences between tight and loose phenotypes of *O. cornuta* were highly significant for the five measured traits (phenotype effect, $P < 0.001$; Table S1). Tight phenotypes were larger, higher, tighter (lower penetration and higher stem density) and had more flowers than loose phenotypes (Fig. 1, Table S1). Additionally, for both cushion surface and height, there was a significant Grazing x Phenotype interaction, because grazing decreased both traits for tight phenotypes but not for loose ones (Fig. 1a,b). Cushion surface and height decreased with grazing for southern-exposed phenotypes, but not for northern-exposed phenotypes (significant and marginally significant Grazing x Exposure interaction, respectively; Fig. 1a,b). There was a significant Exposure x Phenotype interaction on cushion penetration due to a higher difference between phenotypes in southern vs. northern exposure (Fig. 1c). There was a highly significant Exposure effect on number of flowers due to higher flower number in northern than southern exposures, and this difference was highest for grazed tight phenotypes and the lowest for the ungrazed loose phenotype (marginally significant

Grazing x Exposure x Phenotype interaction; Fig. 1d).

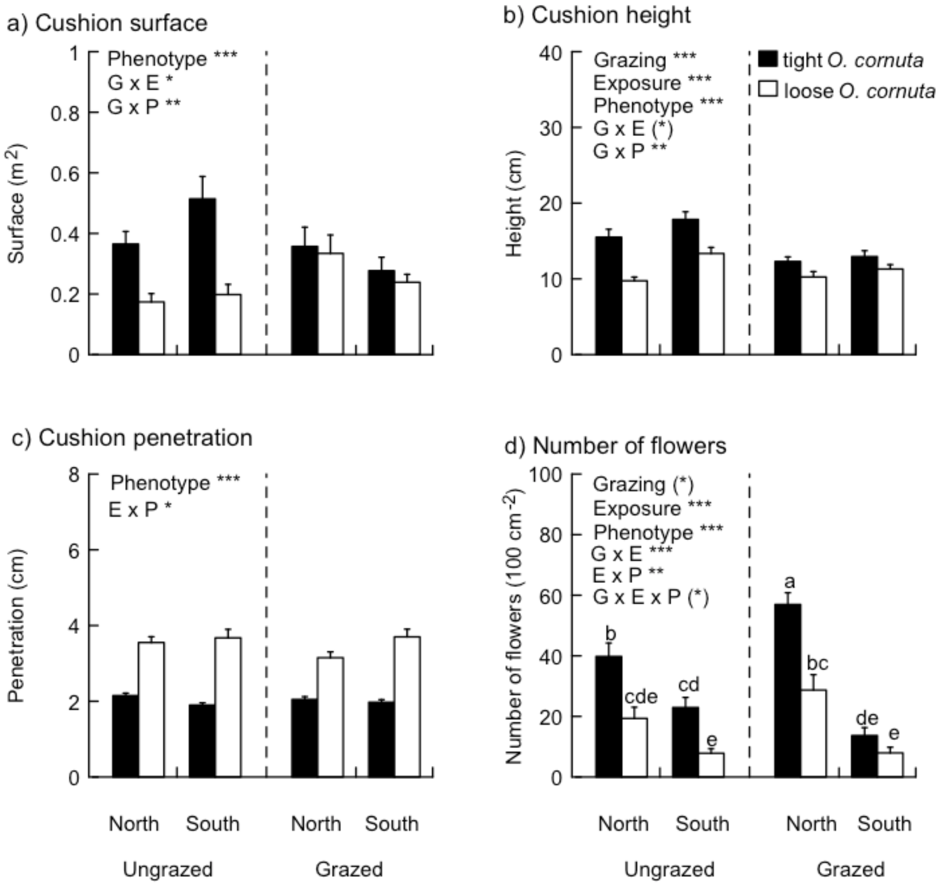

Fig. 1. Means ± SE (n = 20) of cushion surface (a), cushion height (b), cushion penetration (c) and number of flowers (d) of tight and loose phenotypes of *O. cornuta* under ungrazed and grazed conditions in northern and southern exposures. Significant results of three-way ANOVAs on the effects of Grazing (G), Exposure (E), Phenotype (P) and their interactions are shown in the upper left part of each panel: (*): $P < 0.1$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

The $\mathrm{RII}_{\mathrm{Richness}}$ values were overall weakly positive, except for tight phenotypes from southern ungrazed habitats, which had a negative $\mathrm{RII}_{\mathrm{Richness}}$ (Fig. 2a). There was a significant Exposure x Phenotype interaction (Table S2) due to a decrease in $\mathrm{RII}_{\mathrm{Richness}}$ for tight phenotypes from northern to southern exposures, but no change for loose phenotypes. Additionally, there was a marginally significant Grazing effect due to an overall increase in RII leading to significant RII in all grazed habitats except south-exposed tight ones. For $\mathrm{RII}_{\mathrm{Abundance}}$ there was a highly significant Phenotype effect, with highly positive RII for loose phenotypes but not for tight ones (Fig. 2b, Table S2). $\mathrm{RII}_{\mathrm{Abundance}}$ was significantly negative for ungrazed tight phenotypes from southern exposures (one sample *t*-test; Fig. 2b).

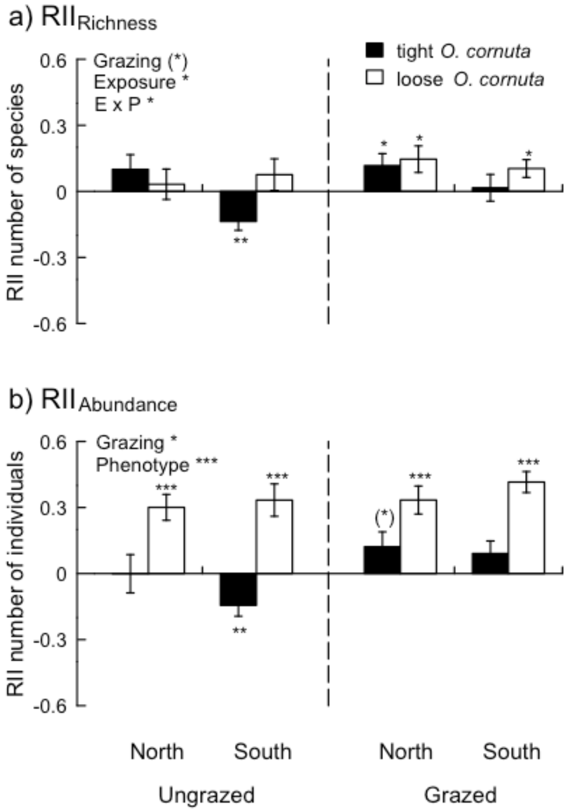

Fig. 2. Means ± SE (n = 20) of $\mathrm{RII}_{\mathrm{Richness}}$ (a) and $\mathrm{RII}_{\mathrm{Abundance}}$ (b) of subordinate plant species for the two phenotypes under ungrazed and grazed conditions in northern and southern exposures. Significant results of three-way ANOVAs on Grazing (G), Exposure (E), Phenotype (P), and their interactions are shown in the upper left part of each panel, and significant results of one sample *t*-tests on RII values are shown above error bars: (*): $P < 0.1$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

The CA revealed important differences in species com- position related to the Exposure, Grazing, Phenotype and Neighbour treatments. CA axis 1 was primarily influenced by Exposure and secondarily by Grazing (P < 0.001 for both treatments; Fig. 3a, Table S3). All south-exposed plots were located in the positive half of CA axis 1 and all north-exposed plots in the negative half, while within each exposure group, grazed plots had higher scores than ungrazed ones. There was also a significant Grazing x Exposure x Phenotype interaction on CA axis 1, with plots from loose phenotype habitats having higher scores than those from the tight phenotype, but only in the presence of grazing and in northern exposures. The second main source of variation in species composition, observed on CA axis 2, was due to additive effects of the Phenotype and Neighbour treatments, with the highest scores observed for loose phenotype plots and the lowest for open plots of the tight phenotype habitats (Fig. 3a, Table

S3). There was also a significant Grazing x Exposure interaction on CA axis 2, with grazed plots having lower scores than un- grazed ones in northern exposures only.

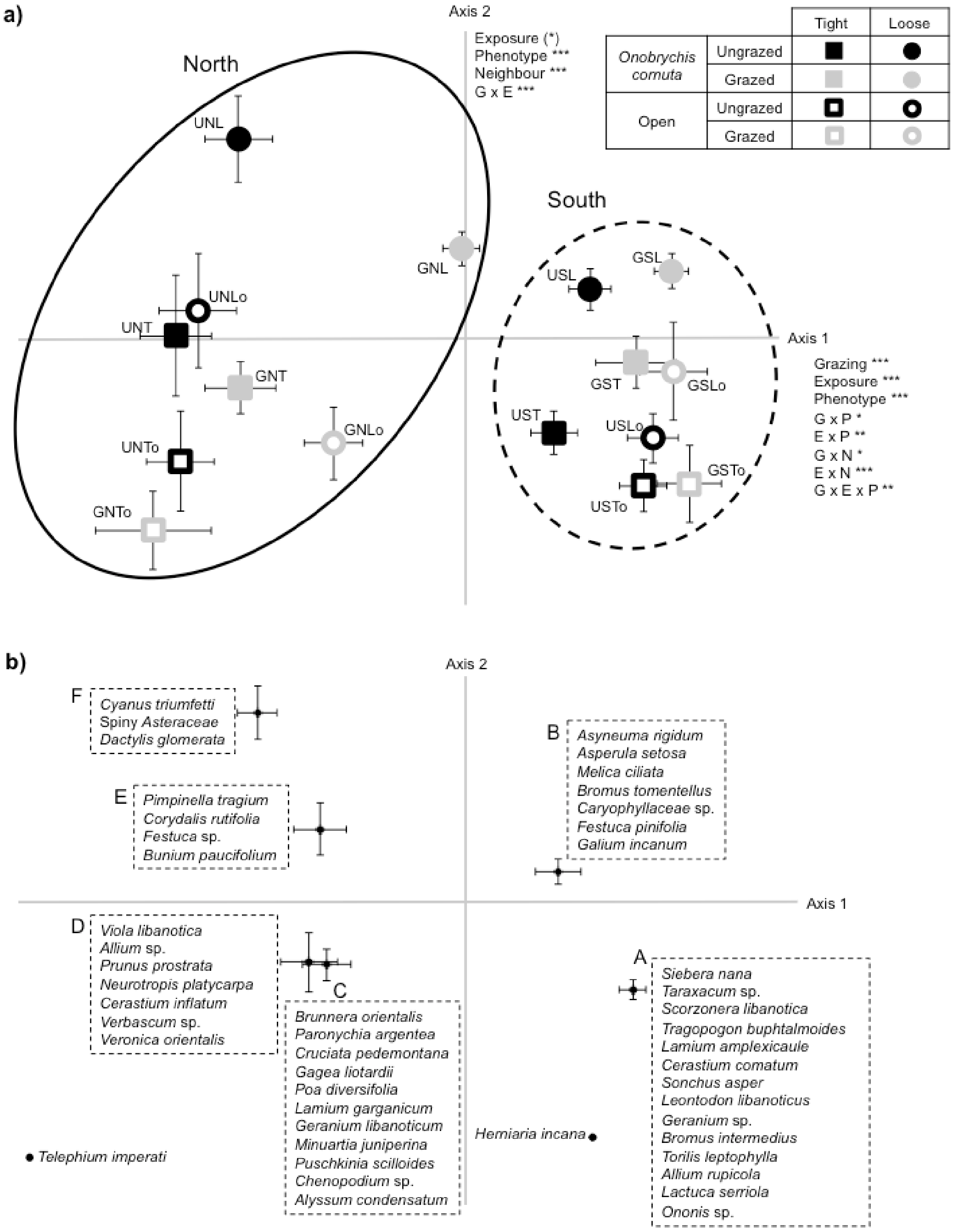

Fig. 3. CA diagrams of relevés (a) and species (b). The relevés diagram represents the mean relevés scores ± SE of the four treatment-combinations (treatment-combination codes: U: ungrazed; G: grazed; N: north; S: south; T: tight; L: loose; o: open). Results of four-way ANOVAs on the effects of Grazing (G), Exposure (E), Phenotype (P), Neighbour (N) and their interactions are shown below axis 1 and at the right of axis 2: (*): $P < 0.1$; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$. (b) Mean CA1 and CA2 scores ± SE are displayed for species cluster group (A, B, C, D, E and F).

Six main groups were generated from the cluster analysis conducted on CA species scores (Figs 3b and S3, Table S4). Groups A and B included species more frequent in southern exposures (e.g. *Bromus intermedius*, *Bromus tomentellus*, *Festuca pinifolia*, *Lamium amplexicaule*; positive scores on CA 1 axis), whereas groups D, E and F included species more frequent in northern exposures (e.g. *Corydalis rutifolia*, *Cyanus triumfetti*, *Dactylis glomerata*, *Pimpinella tragium*, *Prunus prostrata*, *Viola libanotica*; negative scores on CA 1 axis). Additionally, species from groups B, E and F (e.g. *Bromus tomentellus*, *Corydalis rutifolia*, *Cyanus triumfetti*, *Dactylis glomerata*, *Festuca pinifolia* and *Pimpinella tragium*) were more frequent within rather than away from cushions (positive scores on CA 2 axis), with species from the two former groups occurring mostly in loose phenotypes, whereas those from the latter were equally frequent in both cushion phenotypes (Figs 3b and S3, Table S4). In contrast, species from group A (e.g. *Bromus intermedius* and *Lamium amplexicaule*) were more frequent outside than inside cushions and species from group D (e.g. *Prunus prostrata* and *Viola libanotica*) were more frequent in tight- rather than loose-phenotype habitats (negative scores on CA 2 axis). Finally, species from group C (e.g. *Brunnera orientalis*, *Gagea liotardii* and *Lamium garganicum* subsp. *striatum*) were more frequent in north-exposed open plots in ungrazed conditions rather than south-exposed cushions in grazed plots (significant Grazing x Exposure and Grazing x Neighbour interactions; Table S4). Species from the two southern exposure groups had contrasting life-history traits: group A (open plots) including 14 short-sized annual species (mostly Asteraceae with a ruderal strategy), and group B (loose phenotypes) comprising seven perennials, and among them two very abundant bunch grass species (*B. tomentellus* and *F. pinifolia*). Species from the northern exposure groups were less different than species from the two southern exposure groups. Group F (within cushions) included tall species from grazed nitrogen-enriched sites, among them *Cyanus triumfetti* and *D. glomerata*, group E (loose cushions) mostly small geophytes (*P. tragium*, *C. rutifolia*, *Bunium paucifolium*) and group D (tight phenotype habitats) a mix of annuals and perennials, among them the creeping shrub *P. prostrata*. Finally, group C included the tallest subordinate species, among them several perennials (*Brunnera orientalis*, *Geranium libanoticum*, *Minuartia juniperina* and *Lamium garganicum* subsp. *striatum*).

Discussion

We found that differences in phenotypic effects varied in intensity depending on the performance variable (species richness or abundance) of the subordinate species, and were significantly affected by variation in environmental conditions. With increasing drought stress from northern to southern exposure, both competition and facilitation increased for the tight and loose phenotypes, respectively, thus exacerbating differences in phenotypic effects. Moreover, the cessation of grazing did not decrease differences in phenotypic effects, but rather increased negative effects for both phenotypes (i.e. increased competition for the tight phenotype, and decreased facilitation for the loose phenotype). These results, and the strong differences in traits among phenotypes (e.g. cushion penetration; Fig. 1c), suggest that differences in effects of the two phenotypes on subordinate species across habitats are not driven by changes in environmental conditions, but rather by other effects, among which are possible heritable differences between phenotypes. This is an important novel result, as most plant–plant interaction studies have focused on the importance of environmental conditions in changing competition and facilitation intensity or importance across habitats, neglecting the likely contribution of heritable differences between phenotypes. The most important phenotypic effect on subordinate species was found for abundance, with high facilitation in loose phenotypes and no significant or negative interaction in tight phenotypes. Over all exposure and grazing conditions, in the loose phenotype habitats the abundance of subordinate species was much higher in cushions than in surrounding open areas, whereas there were no such differences between cushions and open areas in the tight phenotype habitats. In contrast, there were no phenotypic effects on subordinate species richness. There were up to twice as many species within loose compared to tight phenotypes but this difference was due to habitat conditions, with presumably more favourable conditions in the loose phenotype habitats, and not to cushion effects because the same pattern was observed in the open plots (data not shown). Results of the CA showed that phenotypic effects on community composition were weak, and that most variation in community composition was explained by environmental conditions, i.e. exposure and grazing. However, there were subtle differences on CA axis 2 due to increasing abundance in the loose phenotypes for some species, depending on exposure conditions (group E in northern exposure and B in southern exposure, and in particular the dominant *B. tomentellus*). Since most phenotypic effects were observed for species abundance but not for community composition, there were no true community phenotypes (*sensu* Whitham al. 2003) within our system. Most evidence of community phenotypes has come from interactions across trophic levels, and in particular for plant–insect herbivore interactions, where specific plant genotypes are known to host particular herbivores (e.g. Crutsinger et al. 2006). In the case of plant–plant interactions, the occurrence of true community phenotypes seems to be more likely when phenotypic effects are due to contrasting leaf chemistry (Pakeman et al. 2006; Adams et al. 2011) rather than to contrasting plant architectures (Callaway et al. 1991; Crutsinger et al. 2010; Michalet et al. 2011). Our results are consistent with those of previous studies because differences in competitive and facilitative effects between the two phenotypes of *O. cornuta* were highly correlated to differences in cushion architecture (with larger, higher and tighter cushions for tight than loose phenotypes), and we found weak differences in subordinate species composition between phenotypes (i.e. community phenotypes), but only for species abundances. The negative effect of tight phenotypes on subordinate species were likely more due to interference mechanisms than to resource competition, as shown by Michalet et al. (2011) in northern Arizona (USA) for the alpine foundation cushion species *Geum rossii*.

Drought stress related to exposure enhanced facilitative and competitive effects on subordinate species of loose and tight phenotypes, respectively, both for species richness and abundance (Fig. 2). Two different processes may explain this enhancement of phenotypic effects in southern exposure. First, the CA results showed that the number of subordinate species sensitive to the effects of the two phenotypes was much higher in southern-exposed plots. In southern exposures, there were 14 annuals (species group A) negatively affected by both cushion phenotypes, and seven perennials (species group B and in particular the abundance of *B. tomentellus*) positively affected by the loose phenotypes, whereas in northern-exposed plots only four perennials (species group E) were positively affected by loose phenotypes and no species were negatively affected by cushions. Thus, in south-exposed plots, 21 species were sensitive to the contrasting effects of the two phenotypes vs. only four species in northern exposure. Second, changes in cushion traits with exposure certainly contributed to exacerbating phenotypic effects in southern expo- sure. Loose phenotypes were less tight (higher penetration) and both phenotypes had a higher height in southern- compared to northern-exposed plots (Fig. 1). The decrease in cushion height from southern to northern exposure might be due to increasing snow cover duration in the less sunny northern exposure, which effect is known to negatively affect woody species (Kudo & Ito 1992; Nykänen et al. 1997; Michalet et al. 2002). Taller cushions in southern exposure might have stronger effects on subordinate species than smaller northern cushions, as shown by Le Bagousse-Pinguet et al. (2013) in coastal dunes for the nurse shrub *Helichrysum stoechas* subjected to contrasting fertilization levels.

To summarize, increasing stress in our system from northern to southern exposure strongly exacerbated phenotypic effects, likely because of both increasing the pool of sensitive subordinate species and enhancing facilitative and competitive traits of the cushions. We acknowledge that differences in soils across microhabitats of the two phenotypes are not solely related to water availability but also to contrasting soil fertilities. However, because with increasing drought stress from northern to southern exposures loose phenotypes became more facilitative and tight phenotypes more competitive, the contrasting phenotypic effects of the two phenotypes are unlikely driven by changes in drought stress across phenotypes' habitats. Indeed, this alternative hypothesis would have been sup- ported if the effects of both phenotypes had changed in the same direction with increasing drought stress. Specifically, in our system where competition is highest in the most stressful habitat, consistent with Maestre & Cortina (2004), competitive effects should have increased for both phenotypes from northern to southern exposure to support this hypothesis.

For both subordinate species richness and abundance, grazing increased the facilitative effects of the loose phenotypes and reduced the competitive effects of the tight phenotypes (Fig. 2). This was particularly the case for species of cluster group C (Fig. S3c), including the tallest species among subordinates, i.e. the likely least stress-tolerant species (*sensu* Grime 1974). For these species the presence of herbivores shifted cushion effects from competitive (in absence of herbivores) to facilitative, regardless of cushion phenotypes, overall decreasing the competitive effects of the tight phenotype and increasing the positive effects of the loose one. This shows that the contrasting positive and negative effects of the two phenotypes on subordinate species were definitely not induced by differences in grazing intensity across the phenotypes' habitats. Associational defences (protection against herbivores) have been widely recorded in highly grazed communities (Bertness & Callaway 1994; Baraza et al. 2006), in particular within systems including spiny shrubs (Rousset & Lepart 2000; Milchunas & Noy-Meir 2002; Rebollo et al. 2002; Baraza et al. 2006; Smit et al. 2007). Consistently, in our system both spiny *O. cornuta* phenotypes acted as a physical barrier under heavy herbivory, protecting grazing-intolerant subordinate species from grazers. Thus, the presence of herbivores increased indirectly the positive effects of *O. cornuta* cushions. Moreover, grazing-induced changes in cushion traits might have modified the direct cushion effects on subordinate species. Cushion surface increased for the loose phenotype, likely as a compensatory growth response to grazing (McNaughton 1983). This increase in cushion surface might have increased the direct positive effects of the loose phenotypes on subordinate species. In contrast, grazing reduced cushion surface and height for the tight phenotype, decreasing tight phenotypes' interference (direct negative effects) on subordinate species. This decrease in cushion size may be the result of a direct effect of grazing, although tight phenotypes are certainly poorly grazed due to their stiff spines. Additionally, shepherds frequently burn old cushions in grazed areas, and it is likely that they select preferentially tight phenotypes because of their higher lignin content, which may also affect the class size distribution of tight phenotypes.

To summarize, grazing increased the positive effects of both phenotypes because of their shared spiny form, and because changes in cushion traits with grazing enhanced the positive effects of the loose phenotypes and decreased the negative effects of the tight phenotypes. This shows that the contrasting phenotypic effects of *O. cornuta* observed across habitats could not be caused by higher herbivory on loose phenotypes than on tight ones. Indeed, this alternative hypothesis would have been supported only if the difference in facilitative effects of both phenotypes had vanished with the cessation of grazing, consistent with Bertness & Callaway (1994).

To conclude, our results showed that neither drought stress related to exposure nor grazing disturbance appeared to be the main drivers of the observed differences in phenotypic effects on subordinate species across habitats. Differences in effects of *O. cornuta* phenotypes on subordinate species across habitats are thus very likely due to heritable differences in traits between phenotypes. Genomic investigation of the mechanism determining phenotypic variation (candidate gene approaches or transcriptome comparisons) could provide irrefutable evidence of the genetic basis of phenotypic differences within *O. cornuta*. In contrast, reciprocal transplantations of adult phenotypes are unfortunately unrealistic in the field with such a long-lived shrub species to really provide the additional evidence of genetically driven phenotypic effects on subordinate species suggested by our study.

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Supporting Information

Fig. S1. Subalpine xerophytic community of western Mount-Lebanon (Lebanon) dominated by two phenotypes of the spiny cushion shrub *Onobrychis cornuta*: a facilitative Loose-cushion phenotype (mostly downslope) with very few purple-pink flowers and a high abundance of subordinate, and a competitive Tight-cushion phenotype (mostly mid-slope) with many flowers and very few individuals of subordinate species. Photo taken at Ouyoun El Simane - Kfardebian, Mount-Lebanon, Lebanon. Photo courtesy of Patrick AL HAYEK.

Fig. S2. The two phenotypes of *Onobrychis cornuta*. The loose phenotype is on the left part of the photo, with very few flowers and a high abundance of subordinate species (mostly *Bromus tomentellus*) and the tight phenotype on the right part, with many purple-pink flowers and very few individuals of subordinate species. Photo taken at Ouyoun El Simane - Kfardebian, Mount-Lebanon, Lebanon. Photo courtesy of Patrick Al Hayek.

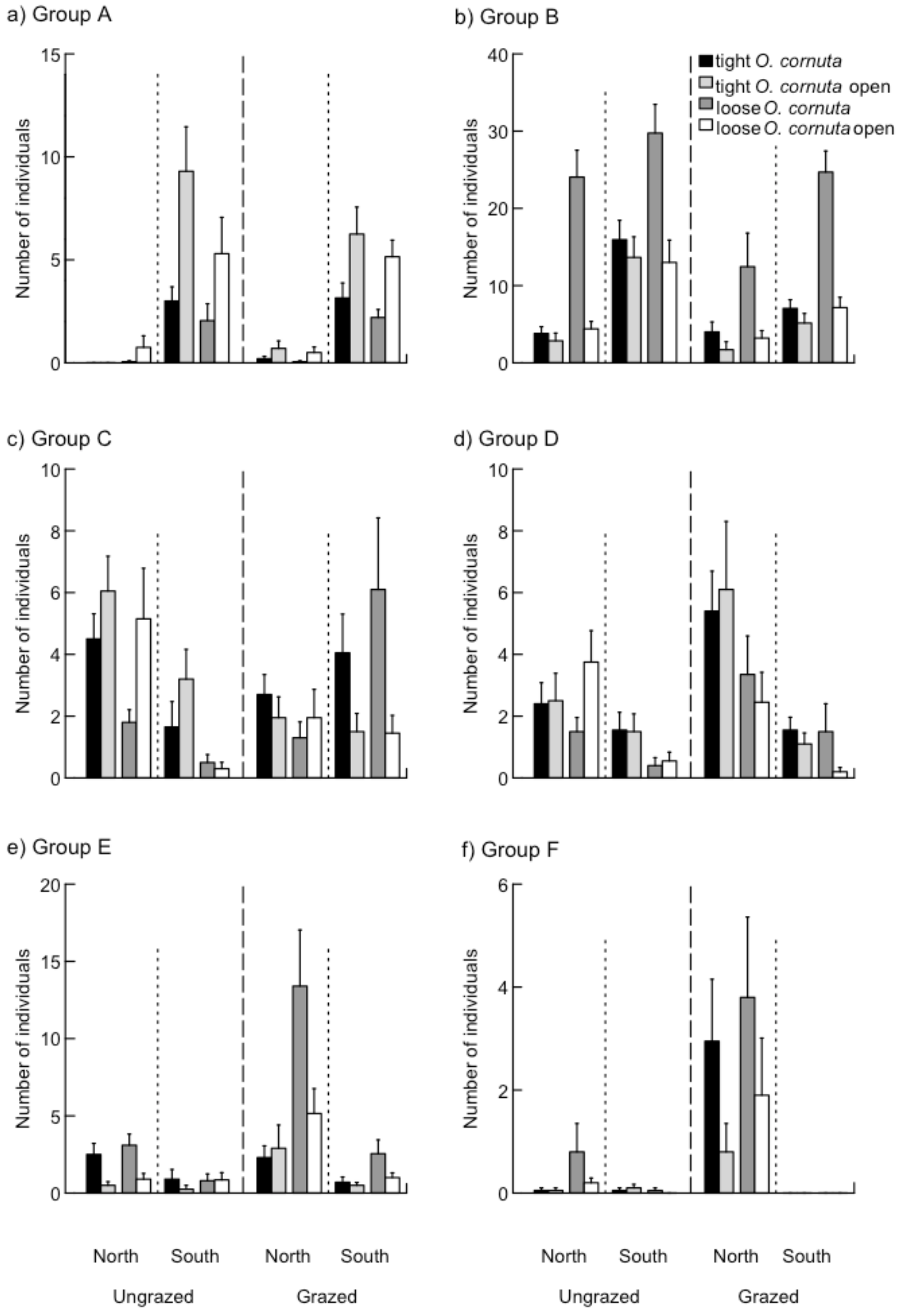

Fig. S3. Means ± SE (n = 20) of cumulated abundances of subordinate plant species for the species cluster groups A, B, C, D, E and F within tight and loose cushions and paired open plots for the two phenotypes of *O. cornuta* under ungrazed and grazed conditions in northern and southern exposures.

Table S1. Results of three-way ANOVAs of the effects of Grazing (G), Exposure (E), Phenotype (P) and their interactions on cushion traits (surface, height, penetration, number of stems and number of flowers) and cushion environmental conditions (convexity and stone cover). (*): $P < 0.1$; *: $P < 0.05$, **: $P < 0.01$; ***: $P < 0.001$

		Traits									
		Surface		Height		Penetration		Number of stems		Number of flowers	
	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Grazing	1	0.10	0.990	12.19	<0.001 ***	0.78	0.377	0.00	0.964	87.89	<0.001 ***
Exposure	1	0.00	0.750	19.39	<0.001 ***	1.02	0.313	0.58	0.447	3.10	0.080 (*)
Phenotype	1	16.61	<0.001 ***	40.52	<0.001 ***	230.40	<0.001 ***	86.73	<0.001 ***	49.85	<0.001 ***
G x E	1	6.26	0.013 *	3.76	0.054 (*)	2.30	0.131	0.58	0.447	13.10	<0.001 ***
E x P	1	1.01	0.318	0.57	0.543	6.40	0.012 *	3.87	0.051 (*)	7.99	0.005 **
G x P	1	10.24	0.002 **	8.93	0.003 *	0.78	0.377	3.77	0.054 (*)	0.03	0.875
G x E x P	1	0.62	0.432	0.15	0.699	0.40	0.528	0.08	0.772	3.03	0.084 (*)
Error	152										

		Environmental conditions			
		Convexity		Stone cover	
	df	*F*	*P*	*F*	*P*
Grazing	1	9.55	0.002 **	12.47	<0.001 ***
Exposure	1	20.40	<0.001 ***	3.72	0.056
Phenotype	1	146.97	<0.001 ***	10.17	0.002
G x E	1	6.84	0.010 **	1.94	0.165
E x P	1	0.23	0.635	0.55	0.458
G x P	1	16.33	<0.001 ***	1.45	0.231
G x E x P	1	0.51	0.477	0.11	0.746
Error	152				

Table S2. Results of three-way ANOVAs of the effects of Grazing (G), Exposure (E), Phenotype (P) and their interactions on $\mathrm{RII}_{\mathrm{Richness}}$ and $\mathrm{RII}_{\mathrm{Abundance}}$ of subordinate plant species. (*): $P < 0.1$; *: $P < 0.05$; ***: $P < 0.001$

		RII species richness		RII abundance	
	df	*F*	*P*	*F*	*P*
Grazing	1	4.05	0.046 *	0.11	0.744
Exposure	1	3.47	0.064 (*)	6.78	0.010 *
Phenotype	1	2.45	0.120	52.42	<0.001 ***
G x E	1	0.09	0.767	0.78	0.377
E x P	1	4.10	0.045 *	2.52	0.114
G x P	1	0.03	0.867	1.79	0.183
G x E x P	1	1.76	0.186	0.12	0.726
Error	152				

Table S3. Results of mixed model ANOVAs on the effects of Grazing (G), Exposure (E), Phenotype (P), Neighbour (N) and their interactions the relevés scores of CA axes 1 and 2, respectively. (*): $P < 0.1$; *: $P < 0.05$, **: $P < 0.01$; ***: $P < 0.001$

		Axis 1		Axis 2	
	df	*F*	*P*	*F*	*P*
Grazing	1	17.46	<0.001 ***	1.25	0.266
Exposure	1	436.55	<0.001 ***	2.28	0.134
Phenotype	1	13.35	<0.001 ***	27.59	<0.001 ***
G x E	1	1.22	0.270	8.00	0.005 **
G x P	1	3.89	0.050 (*)	0.34	0.563
E x P	1	7.72	0.006 **	0.95	0.332
G x E x P	1	5.36	0.022 *	0.53	0.469
Error	152				
Neighbour	1	0.15	0.695	106.82	<0.001 ***
G x N	1	6.49	0.012 *	0.35	0.556
E x N	1	18.02	<0.001 ***	4.26	0.041 *
P x N	1	2.27	0.134	2.84	0.094 (*)
G x E x N	1	0.58	0.447	0.04	0.850
G x P x N	1	0.04	0.850	1.18	0.280
E x P x N	1	0.01	0.939	0.05	0.821
G x E x P x N	1	0.07	0.787	1.46	0.229
Error	152				

Table S4. Results of four-way ANOVAs of the effects of Grazing (G), Exposure (E), Phenotype (P), Neighbour (N) and their interactions on species cumulated abundances for each species cluster group. (*): $P < 0.1$; *: $P < 0.05$, **: $P < 0.01$; ***: $P < 0.001$

		Group A		Group B		Group C	
	df	*F*	*P*	*F*	*P*	*F*	*P*
Grazing	1	0.36	0.551	15.67	<0.001 ***	0.25	0.621
Exposure	1	82.43	<0.001 ***	31.84	<0.001 ***	0.24	0.128
Phenotype	1	3.03	0.084 (*)	36.91	<0.001 ***	2.64	0.107
G x E	1	0.89	0.347	1.88	0.173	15.42	<0.001 ***
G x P	1	0.22	0.642	0.25	0.615	3.61	0.059 (*)
E x P	1	3.92	0.050 *	0.01	0.921	0.46	0.498
G x E x P	1	1.16	0.283	2.98	0.086 (*)	0.79	0.377
Error	152						
Neighbour	1	27.45	<0.001 ***	88.23	<0.001 ***	0.08	0.774
G x N	1	0.97	0.325	1.32	0.252	13.82	<0.001 ***
E x N	1	17.95	<0.001 ***	0.71	0.400	8.54	0.004 **
P x N	1	0.60	0.440	54.94	<0.001 ***	0.03	0.859
G x E x N	1	1.30	0.256	1.58	0.211	0.95	0.332
G x P x N	1	0.43	0.515	1.97	0.163	0.04	0.837
E x P x N	1	1.37	0.244	0.35	0.555	3.74	0.055 (*)
G x E x P x N	1	1.23	0.269	2.96	0.087 (*)	0.00	0.989
Error	152						

		Group D		Group E		Group F	
	df	*F*	*P*	*F*	*P*	*F*	*P*
Grazing	1	2.93	0.089 (*)	10.05	0.002 **	7.27	0.008 **
Exposure	1	18.97	<0.001 ***	15.47	<0.001 ***	11.73	<0.001 ***
Phenotype	1	3.67	0.057 (*)	8.50	0.004 **	0.83	0.364
G x E	1	2.40	0.123	6.29	0.013 *	8.00	0.005 **
G x P	1	1.25	0.266	5.79	0.017 *	0.15	0.704
E x P	1	0.28	0.601	3.80	0.053 (*)	0.95	0.331
G x E x P	1	2.70	0.103	3.17	0.077 (*)	0.10	0.754
Error	152						
Neighbour	1	0.03	0.857	23.14	<0.001 ***	9.22	0.003 **
G x N	1	2.54	0.113	2.43	0.121	5.08	0.026 *
E x N	1	1.89	0.171	10.36	0.002 **	9.22	0.003 **
P x N	1	0.00	0.971	10.80	0.001 **	0.09	0.769
G x E x N	1	0.06	0.800	0.61	0.437	5.08	0.026 *
G x P x N	1	3.02	0.084 (*)	13.14	<0.001 ***	0.39	0.536
E x P x N	1	0.19	0.665	8.10	0.005 **	0.03	0.871
G x E x P x N	1	0.96	0.330	5.00	0.027 *	0.24	0.625
Error	152						

CHAPTER SIX:
SYNTHESIS

This Ph.D. was conducted in the purpose of trying to understand the extended consequences of an intraspecific variation within foundation species across heterogeneous environments in mountain ranges. The main scope was to examine the relative contribution of genetics and plasticity to the phenotypic differentiation of two foundation plant species of subalpine and oromediterranean communities (*F. gautieri* in the French Pyrenees, and *O. cornuta* in the Mount-Lebanon range, respectively), and to their differential effects and consequences for the subordinate species. Two relevant points appeared recurrently in my experiments. The first is that morphological differences observed in the field between tight and loose phenotypes had both genetic and environmental (plasticity) determinisms, with however a higher contribution of phenotypic plasticity compared to genetic control. The second is that differences in morphologies induced heritable differences in competitive effects, which were not consistent to the general increase in competition with the decreasing stress dominantly found in the literature on subalpine systems and initially proposed by Grime (1974) and Bertness & Callaway (1994). Consequently, the maintenance of a higher diversity in stable environmental condition (absence of stress, i.e. loose cushions habitat) rather than in stressed conditions (i.e. tight cushion habitat) is only due to genetic effects that overcome the effects of the environment, thus limiting competition.

In the following, I first present evidence of the contribution of both genetics and plasticity to the phenotypic variation and its induced differential effects for *F. gautieri* (results from chapters 3 and 4) and *O. cornuta* (chapter 5), then I discuss the consequences of such variation on the subordinate species. Finally, I propose future studies to complete this work.

1- Determinism of the observed phenotypic variation: the contribution of genetic variation and phenotypic plasticity

1.1- The implication of genetics in the phenotypic variation

Differences in morphological traits between tight and loose *F. gautieiri* cushions observed in the field were maintained throughout the shadehouse experiment (where replicates of both *F. gautieri* phenotypes were grown for two years under uniform environmental conditions), as all measured traits (maximum leaf length, cushion penetration, leaf thickness, cushion surface, and cushion leaf density) remained significantly different at the end of the experiment. These results were confirmed with Reciprocal Transplant Experiments (RTEs hereafter) conducted in field conditions, thus supporting the genetic basis of trait differences. Additionally, patterns of adaptation were observed in the RTEs as each phenotype showed a home-site advantage in terms of cushion surface and leaf density (higher performance of tight cushions in the convex garden, and higher performance of loose cushions in the concave garden). This is consistent with the distribution patterns of tight and loose cushions in the field, and agrees with Le Bagousse-Pinguet *et al*. (2014b) who found contrasting survival responses between the tight and loose phenotypes along a soil moisture gradient in the same system, showing a more adapted tight phenotype to relatively high-stress conditions (stress-tolerant target) and more adapted loose phenotype to low-stress conditions (stress-intolerant target). For *O. cornuta*, such experiments were not feasible due to the very slow growth rate of this long-lived leguminous shrub, and the necessity to have adult plants to assess their effects on other species (Liancourt *et al*. 2009; Le Bagousse-Pinguet *et al*. 2013); however, a support to this likely genetic basis of the phenotypic variation within this species was possible by showing the non-implication of environmental stress and/or disturbance in the phenotypic variation between tight and loose cushions across natural habitats.

1.2- The contribution of plasticity to the phenotypic variation

Plasticity effects, as evidenced in both the shadehouse and the RTEs, in part induced changes in the morphological traits of *F. gautieri* cushions from the concave to the convex habitats in the field. In the shadehouse, watering the cushions increased their surface, leaf length, penetration and leaf density, and decreased leaf thickness. Similar patterns were found in the RTEs with the decrease of stress from the convex to the concave garden, except for leaf density that decreased for the tight phenotype and was not affected for the loose phenotype. This discrepancy between the shadehouse and the RTEs for leaf density might be due to the fact that the conditions in the shadehouse were not exactly representative of the field conditions, suggesting that factors other than water stress might be involved in modifying this trait. For *O. cornuta*, plasticity also affected morphological traits. Southern-exposed cushions were taller than northern-exposed ones and penetration increased for the loose cushions from northern to southern exposure (chapter 5).

Is shape variation plastic or genetic?

Overall, results of *F. gautieri* showed that for traits variation, plastic and genetic effects acted in the same direction with the strongest trait differences observed between tight phenotypes in relatively high-stress conditions (dried cushions in the shadehouse, and convex garden in the RTEs) and loose phenotypes in low-stress conditions (watered cushions in the shadehouse, and concave garden in the RTEs). This indicates an advantage for each phenotype within its natural habitat, and thus an adaptation to its habitat conditions. However, both in the shadehouse and the RTEs we found that, overall, plasticity had a higher impact than genetics in modifying cushion traits. This is consistent with Bresson *et al*. (2010) who showed for *Fagus sylvatica* and *Quercus petraea* in the French Pyrenees that both genetics and plasticity contributed to the phenotypic variation in leaf morphological and physiological traits, with a higher contribution of plasticity. Also, Vitasse *et al*. (2009) showed the contribution of both heritable and environmental effects to the phenological traits of *Quercus petraea* and *Fraxinus excelsior* in the Pyrenees; however, for *Fagus sylvatica*, they showed opposite heritable and environmental effects, which contradicts our findings. Other authors also showed the implication of both genetics and plasticity in controlling morphological (Michalet *et al*. 2011) or physiological traits (Nielsen & Jorgensen 2003).

2- Consequences of the phenotypic variation within foundation species on the subordinate species

Previous studies have shown that architectural variation in a foundation species can trigger variation in competitive/facilitative effects on subordinate species (Callaway *et al*. 1991; Pugnaire *et al*. 1996; Rudgers & Maron 2003; Michalet *et al*. 2011), and that the genetic basis of morphological, phenological, or chemical composition variation may in turn affect community composition (Whitham *et al*. 2003, 2006; Johnson & Agrawal 2005; Pakeman *et al*. 2006; Bailey *et al*. 2011; Genung *et al*. 2011; Michalet *et al*. 2011). Accordingly, in my study, I show that differences in competitive ability between phenotypes of foundation species, due to genetic and plastic differences in architectural variation, explained differences in diversity or abundance of subordinate species.

2.1- Differences in competitive effects between phenotypes: consequences for subordinate species

2.1.1- Differences in effects due to genetics

Tight cushions were shown to be genetically stronger effect-competitors than loose cushions both in the shadehouse under similar conditions (chapter 3) and in the two gardens (chapter

4), which is probably due to their genetically higher interference acquired with time through the development of more competitive traits (more compact cushions with thicker and stiffer leaves). In accordance with the SGH (Bertness & Callaway 1994), in my system competition increased in the field from the relatively high-stress habitat (tight cushions habitat, stressful conditions) to the low-stress habitat (loose cushions habitat, benign conditions) of *F. gautieri* cushions. In the third chapter of this thesis (article accepted in *Oecologia*), my colleagues and I predicted that:

> "*[…] with increasing water availability from the tight phenotype's habitat to the loose one, the competitive effect of both phenotypes should decrease since both showed an increase in cushion penetration with watering […]*" (Al Hayek *et al*. 2014).

However, this prediction did not hold in the RTEs, since competitive effects increased for both phenotypes from the water-stressed convex garden to the wet concave one, even though cushion penetration increased. This is most probably due to the absence of physical stress in the open areas from the concave garden, which allows the other species of the community to perform equally or even better outside than within neighbouring cushions (see Cavieres *et al*. 2014). Thus the increase in competition from the tight to the loose cushions habitats is certainly not due to genetic variation between phenotypes, but rather to direct environmental effects (i.e. an environmental severity effect, Michalet *et al*. 2014b) with more benign conditions favourable to competition in the latter habitat (Bertness & Callaway 1994; Brooker & Callaghan 1998; Callaway *et al*. 2002; Brooker *et al*. 2005). To summarise, genetic effects enhanced competition with increasing stress, consistent to MacArthur & Wilson (1967), Tilman (1982) and Taylor *et al*. (1990).

2.1.2- Differences in effects due to plasticity

Using a transplantation competition experiment, I showed that the competitive effects of *F. gautieri* cushions decreased from the concave (low stress) to the convex (high stress) garden for both target survival and growth regardless cushion phenotype. This proves the plasticity of cushion effects as they are partially modified by (micro)-environmental changes. Moreover, loose cushions showed a higher plasticity in effects than tight cushions with a shift from competition to facilitation for the former with the increase in stress, but no such changes for the latter. The higher plasticity in the effects of loose than tight cushions goes along with the higher growth rate and lower stress-tolerance of the former as shown by trait measurements, the results of Le Bagousse-Pinguet *et al*. (2014b) and their contrasting field distributions. This plasticity-induced increase in facilitation (or decrease in competition) with increasing stress is in line with Grimes's (1974) model and the SGH (Bertness & Callaway 1994). Thus, for *F. gautieri* both genetic and plastic effects induce variation in competitive effects across phenotypes, but act in opposite directions with the former enhancing competition and the latter decreasing it with the increase in stress.

On the other hand, for *O. cornuta,* plasticity was not the driver of the differences in effects between tight (facilitative) and loose (competitive) phenotypes, even though it contributed to modifying the effects. In the field, the increase in stress from North (wet) to South (dry) exposures exacerbated the differences in effects, as tight competitive cushions become more competitive and loose facilitative cushions become more facilitative. Thus, as the effects of both phenotypes are not similarly affected by the increase in stress [i.e. an increase in competitive effects with increasing stress, consistent with Maestre & Cortina (2004)], plasticity could not be the cause of the difference in effects across phenotypes' habitats within the same exposure. In parallel, the cessation of grazing did not cancel the difference in effects between phenotypes, in contrast to what was predicted if grazing was the driver of the difference in effects between phenotypes (see chapter 5); instead, the cessation of grazing increased the positive effects of both phenotypes, increasing facilitation for the loose cushions and decreasing competition for the tight ones. Thus, grazing contributed to the effects of tight and loose cushions, but was not the driver of the difference. This brings additional evidence that plasticity could not be the cause of the differences in effects between phenotypes.

To summarise, plasticity contributed to generating the difference in effects between the two phenotypes of *F. gautieri* but not of *O. cornuta*. This discrepancy arises likely from the difference in the way cushion traits of each species are affected by the increase in stress. For *F. gautieri,* the increase in stress from the concave to the convex garden enhanced competitive traits for both cushion phenotypes, as both tight and loose cushions were tighter (less penetrating cushions with shorter, thicker and higher density of leaves). However, for *O. cornuta* the increase in stress from North to South exposures had divergent effect on cushions thus enhancing facilitative traits for the loose cushions (higher penetration) and competitive traits for the tight ones (increase in cushion height). Moreover, for *O. cornuta,* the increase in stress from northern to southern exposures increased the pool of sensitive subordinate species, with 4 species (positively affected by loose cushions) in the North vs. 21 species (14 negatively affected by both phenotypes and 7 positively affected by loose cushions) in the South.

2.1.3- Consequences for the subordinate species

Several studies have reported greater abundance of other plant species inside cushions than outside (Cavieres *et al*. 2002; Arroyo *et al*. 2003), and many suggested that the 'nursing effect' should be more important in stressful conditions (Bertness & Callaway 1994; Holmgren *et al*. 1997; Brooker & Callaghan 1998), and that diversity should increase in semi-stressful environments as competition decreases (Grime 1973) and facilitation increases (Hacker & Gaines 1997; Michalet *et al*. 2006). The consistent prediction to these models and findings of the literature would have been that for both *F. gautieri* and *O. cornuta*, tight cushions occurring in semi-stressful habitats would harbour more subordinate species than loose cushions occurring in low-stress habitats. This was not the case neither in my study systems nor in my experiments, as subordinate species richness and abundance were higher within loose than tight cushions. In fact, nursing effects may depend on the nurse's identity and morphological characteristics (Callaway 2007).

I previously showed that tight *F. gautieri* cushions are genetically stronger effect-competitors than loose cushions due to their more competitive traits (specifically, less penetrating cushions with thick leaves), and that plasticity effects reduce competition with increasing stress. Therefore, the decrease in species richness and abundance from the loose cushions (in low-stress habitats) to the tight ones (in semi-stressful habitats) is certainly not due to plasticity effects but rather to the genetically higher competitive effects of tight cushions, mainly expressed through interference, and thus impeding the recruitment of other species. This shows that diversity is more dependent on genetic than plasticity effects, and brings additional evidence that a genetic variation within a species may have consequences at the community level.

2.2- Feedbacks of subordinate species on their 'nurse'

For both foundation species, *F. gautieri* and *O. cornuta*, loose cushions with higher cover of subordinate species had significantly less flowers (or inflorescences) than tight cushions with low cover of subordinate species, thus suggesting a potential reproductive cost for loose cushions. Most researches, specifically in the purpose of ecological restoration, focused on the influence of nurse plant species on their dependent species, but not on the feedbacks of the 'nursed' species on their nurse. However, understorey plants can have negative and/or positive effects on their nurse (Pugnaire *et al*. 1996; Barnes & Archer 1999; Holzapfel & Mahall 1999; Michalet *et al*. 2011; Cranston *et al*. 2012; Schöb et *al*. 2014). For example, Pugnaire *et al*. (1996) showed that *Retama sphaerocarpa* had positive effects on its understory species including *Marrubium vulgare*, and that these species had positive feedbacks on their nurse, enhancing its biomass and branches nitrogen contents. In contrast, Barnes & Archer (1999) showed that *Prosopis glandulosa* var. *glandulosa* enhanced the establishment of understory species, but once established, understory species (specifically woody species) had negative feedbacks on their nurse's growth and reproduction. Moreover, experiments in savannah in South Texas showed that nurse plants grew better when understory species were removed (see Ren *et al*. 2008). Consistently, our experimental removal of subordinate species within *F. gautieri* loose cushions (cf. chapter 3) showed a significant increase in inflorescences number, thus supporting the hypothesis that subordinate species had negative feedback effects on their nurse. Positive feedback effects of subordinates on *F. gautieri* loose cushions were not detected, but they may exist for other traits than flowering output, specifically for traits affecting cushion's fitness permitting the persistence of loose cushion phenotype.

3- Perspectives for future studies

Each of my experiments stretched for only one or two years, but it would be interesting to perform longer-term experiments that might reveal farther thorough results, specifically for species with K-strategies which might need time to express their 'real' effect. Still, I have succeeded to demonstrate within a period of one to two years that both genetic and plasticity play a role in generating morphological differences within foundation species, and that community diversity depends primarily on the genetic effects of the foundation species rather than local changes in environmental conditions. In an evolutionary context, this latter point is of considerable importance, as most studies of plant-plant interactions stressed the role of the environment in conditioning the intensity or importance of interactions, underestimating the role of heritable variation between phenotypes which could have noticeable consequences at community and ecosystem levels (Dawkins 1982; Whitham *et al*. 2003, 2006, 2008; Crutsinger *et al*. 2006; Pakeman *et al*. 2006; Adams *et al*. 2011).

A direct investigation of the genetic component of the phenotypic variation

Even though transplant experiments are time-consuming, they still have the advantage of being simple, more or less easy to perform, requiring little technology and relatively inexpensive. Though, nowadays the advances of molecular biology allow a more direct study of genetic variation. Thus, it would be interesting to further investigate the molecular mechanisms underlying the phenotypic variation within the foundation species, using technics such as exome sequencing or transcriptome comparisons, to double-check the genetic basis of this variation, specifically for long-lived species such as *O. cornuta* for which field transplant experiments are unrealistic.

Is there a benefit for harbouring subordinate species?

Studies assessing feedback effects of beneficiary species on their benefactor (i.e. effects of subordinate species on their nurse, Valiente-Banuet *et* al. 1991; Pugnaire *et al*. 1996; Holzapfel & Mahall 1999; Armas & Pugnaire 2005; Michalet *et al*. 2011; Cranston *et al*. 2012; Schöb *et al*. 2013) have showed that the feedback effects can range widely from negative to positive. Bronstein (2009) stress the importance of evaluating the full nature of reciprocal effects of the subordinate species on their nurse for a deeper understanding of the evolutionary consequences of facilitation. In chapter 3, my colleagues and I found that subordinate species had negative feedbacks on their nurse, specifically on loose cushions with many subordinates (since tight cushions harbour very few if any subordinates). However, reciprocal effects of subordinate species were evaluated only for the number of flowers of the nurse, but other traits related to the nurse’s fitness (e.g. pollen quality and quantity) could show an opposite result (i.e. a positive feedback of subordinate species on the nurse). Thus, it would be valuable to test for the occurrence of positive community feedbacks.

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Beginning