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Hassan MUHAMED

Pour obtenir le grade de **DOCTEUR**

SPÉCIALITÉ :

Écologie évolutive, fonctionnelle et des communautés

Le rôle des interactions biotiques dans la régénération des chênes au niveau des communautés de forêts dunaires de la région Aquitaine (Sud-Ouest de la France).

Soutenue le 18 Septembre 2012

Philippe Balandier (CR. IRSTEA, Nogent sur Vernisson, France)	Rapporteur
Francisco Pugnaire (DR. CESIC, Almeria, Spain)	Rapporteur
Thomas G Whitham (Professeur en etablissment etranger NAU, USA)	President
Blaise Touzard (MCU. Bordeaux 1)	Examinateur
Emanuele Lingua (A.Pr. University of Padova, Italy)	Examinateur
Richard Michalet (PR. Bordeaux 1)	Directeur de thèse

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Hassan MUHAMED

To obtain the degree of DOCTOR

SPECIALITY:

«Evolutionary, functional and community Ecology"

The role of biotic interactions for oak regeneration in the coastal sand dune forest communities of the Aquitaine region (south-western France)

Thesis defense in 18 Septembre 2012

Philippe Balandier (CR. IRSTEA, Nogent sur Vernisson, France)	Reporter
Francisco Pugnaire (DR. CESIC, Almeria, Spain)	Reporter
Thomas G Whitham (Regent Professor at NAU, USA)	President
Blaise Touzard (MCU. Bordeaux 1)	Examiner
Emanuele Lingua (A. Pr. University of Padova, Italy)	Examiner
Richard Michalet (Pr. Bordeaux 1)	Director of thesis

Laboratoire BIOdiversité, GEnes,
COmmunautés (BIOGECO)
Équipe Écologie des Communautés
Bâtiment B2, Avenue des Facultés
33405 Talence

Dedicate this thesis to
My family
For their continued support

Summary

Although biotic interactions are known to be important determinants of species establishment it is still uncertain what factors determine the net balance between positive and negative interactions thus, under what conditions biotic interactions could enhance or impede species regeneration. This thesis aims to understand the role of biotic interactions of shrubs with oak seedlings for the regeneration of three oak species in the coastal dune forests of Aquitaine (France) in the context of climate change. We analysed how net interactions change with increasing drought stress, from the south to the north of Aquitaine, with canopy opening, from closed forests to gaps, and for three oak species, *Q. robur*, *Q. ilex*, and *Q. suber*.We used two approaches, an observational approach using spatial point pattern data, and an experimental approach by transplanting oak seedlings. The results show that the spatial variation in the nature of biotic interactions was strongly dependant on environmental conditions. The shrub-oak seedling interactions were very sensitive to increasing summer drought and canopy opening; the interaction strength was facilitative in gaps and in the dry northern dunes and switched to competitive in closed forests and in the wet southern dunes. The direction of interactions did not vary significantly with oak species strategies and ecological requirements. In contrast, the functional group of shrubs seems to be determinant with more negative interactions with Ericaceae and more positive interactions with Fagaceae shrubs. Overall, our results largely support the original formulation of stress gradient hypothesis (SGH) that predicts an increase in facilitation with increasing severity and highlights that shading by shrubs is rather recommended for a successful oak regeneration. In this perspective, forest managers should conserve understory shrubs, in particular in gaps, in order to allow a better regeneration of oak seedlings. This thesis highlights the importance of considering biotic interactions in oak regeneration under current harshness climatic conditions with an expectation to have an ambitious role in the mitigation of future climatic change.

Keywords: biotic interaction, facilitation, nurse shrub, oak seedling, spatial association, stress gradient hypothesis

Résumé

Bien que les interactions biotiques soient connues pour être déterminantes dans l’établissement des espèces, il est encore difficile de savoir quels facteurs déterminent la balance entre les interactions positives et négatives. Il est de fait difficile de savoir sous quelles conditions les interactions biotiques peuvent favoriser ou empêcher la régénération des espèces. Cette thèse vise à étudier le rôle des interactions biotiques d'arbustes avec des semis de chêne sur la régénération de trois espèces de chênes dans les forêts de dunes côtières d'Aquitaine dans le contexte du changement climatique. Nous avons en particulier analysé comment la balance des interactions variait en accroissant la sécheresse, du sud au nord de l’Aquitaine, en accroissant la lumière, en forêts fermées et en coupes et pour trois espèces de chênes *Q. robur, Q. ilex,* et *Q. suber*. Ce travail a été effectué en utilisant deux approches, une approche observationnelle en utilisant la méthode des patrons de points répartis dans l’espace, et une approche expérimentale en transplantant les semis des trois espèces de chêne. Les résultats montrent que les variations d’interactions biotiques sont fortement dépendantes de la sévérité environnementale, avec des interactions positives en climat sec et en trouée et des interactions négatives en climat humide et en forêts closes. La facilitation domine largement dans les trouées du nord aquitain et la compétition dans les forêts fermées du sud aquitain.En revanche nous n’avons pas observé de différence de réponses aux interactions pour les trois espèces de chênes. En outre, les interactions négatives sont plutôt observées avec les Ericaceae et les positives avec les Fagaceae. De manière générale les résultats de cette thèse confirment la formulation originale du SGH qui prédit une augmentation de la facilitation en lien avec une augmentation de la sévérité environnementale et souligne le fait que la réduction du stress hydrique atmosphérique par des arbustes est nécessaire à la régénération des semis de chêne en forêts dunnaire. Dans cette perspective, les sylviculteurs devraient conserver les arbustes du sous-étage, en particulier dans les trouées, afin de permettre une meilleure régénération des plants de chêne. Cette thèse met en évidence la nécessaire considération des interactions biotiques dans la régénération du chêne dans les conditions actuelles les plus stressantes et un rôle sans doute crucial de ces interactions dans la réponse des espèces aux changements climatiques futurs prédits pour la région Aquitaine.

Mots-clés: interaction biotique, facilitation, arbuste infirmière, semis de chêne, association spatiale, l'hypothèse de gradient de contrainte

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In Kurdish,

زورسوپاس بو : سه روكاتيا زانكويا دهوكى بتايبه تى **پشكا** په يوه ندیین **ژه رڤه** ,راكريا فه كولتيا جاندنى ودارستانى
ئو بو سه رو كاتيا سكولا دارستانى وماموستايين وى بو **پـــل** پـشتيا وان تا كو ئه ف كاره ب دوماهي هاتي.....
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Contents

Literature cited

General context

1. *Context*

Knowledge of the main mechanisms responsible for structuring the composition of a community is central to community ecology where the main questions are: (i) why certain species are present or absent in a community, and (ii) why does the composition of the community change spatially and temporally in response to changes in the environment? (Lortie *et al*., 2004). Definitive answers to these long-standing questions will have important implications in solving many ecological problems such as restoration of a disturbed community, the control of exotic invasions, and understanding the consequences of climatic change for natural communities. (Lortie *et al*., 2004).

The first attempts to understand community composition started with ecological determinism (deterministic processes theory), i.e., the view that community composition is structured by links with environmental conditions, niche requirements and biotic interactions and consequently, that plant communities follow a predictable track through time (Clements, 1916). However, this view was challenged by ecological stochasticity (the theory of stochastic processes) (Grossman *et al*., 1982), which stated that species coexistence as well as extinction are the product of random processes, and emphasized that the plant communities are not strictly defined since they vary both spatially and temporarily (Jenkins and Buikema, 1998; Hubbell, 2001). The new view among ecologists today is a combination of the two theories, alongside stochastic processes deterministic processes can help to understand community composition (i.e., random processes, local environmental conditions, and species interactions). This new view of plant communities is called ‘the integrated community’ (Lortie *et al*., 2004).

According to the integrated community view, to be present in an extant community, plant species must pass through three main filters (pair of horizontal lines in Fig.1), (i) stochastic and deterministic biogeographically events (i.e., dispersal, distance to new environment); (ii) local environmental conditions (i.e., abiotic factors and management); and (iii) species interactions (i.e., plant-plant interactions) with the likelihood that these filters also interact or behave synergistically (dotted lines in Fig.1) (Bertness and Callaway, 1994).

If a certain species is able to reach a suitable habitat, the probability of its early establishment is governed by its capacity to tolerate local environmental conditions, which include not only the abiotic environment but also the biotic environment generated by the species already *in situ* (Fig.1). One of the most crucial cases of interacting filters is certainly variation in plant-plant interactions in response to variations in climatic stress. Such fluctuations in the net interaction play an important role in regulating the composition of communities and ecosystems. Although biotic interactions are known to occur at local scale, their consequences for community composition may occur at much larger scales (Brooker *et al.*, 2009) and have impacts that may go beyond the composition of plant communities to spread throughout ecosystems, for example through their effects on resource availability and habitat structure (Brooker, 2006). In the context of tree regeneration, both biotic and abiotic drivers have unique and interrelated filtering influence at early regeneration stages. For example, climate conditions or plant-plant interactions can directly affect germination, survival, and growth, and indirectly affect tree regeneration through their relationship with each other. For these reasons, understanding the individual and joint explanatory power of these driver sets during early regeneration stages is critical to estimating their long-term impacts on forest composition dynamics in particular in the context of climate change (Prentice *et al.*, 1991; Castro *et al.*, 2004a; Frelich and Reich, 2010).

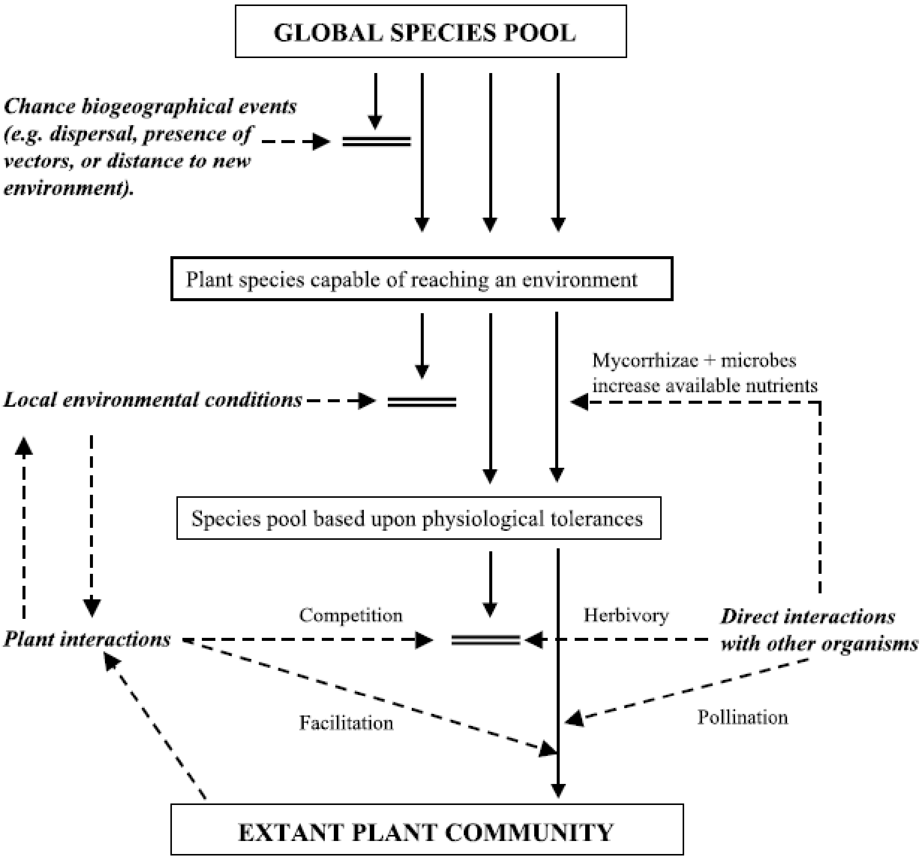


Figure 1. The integrated community view. The composition of plant communities is determined by both random and deterministic processes see text for further details**.** The pair of horizontal lines represents the three main filters that structure a plant community and the corresponding description is in bold italics adjacent to the symbol. Solid arrows show the movement of species through the filters, and dashed lines show where filters can interact or behave synergistically (Lortie *et al*,. 2004).

However, questions have been raised about the role of biotic interactions in mitigating the consequences of climatic change on plant regeneration since different scenarios appeared to contradict the linear role of the original stress-gradient hypothesis of Bertness and Callaway (1994) (Fig. 2). For example, recent studies showed that interactions related to environmental severity gradients can be neutral or negative, such as symmetrical and asymmetrical hump-shaped SGH scenarios (i.e., Maestre and Cortina, 2004; Lortie and Callaway, 2006; Michalet *et al*., 2006; Brooker *et al.*, 2008; le Roux and McGeoch, 2010). Under such a scenario, biotic interactions could either have no effect or have an even more negative influence on the early establishment of a species under increasing climatic severity.

(Maestre and Cortina, 2004; le Roux and McGeoch, 2010). There is thus a need for more experiments on the ongoing effects of climate change on plant-plant interactions and their consequences for community composition and dynamics (Callaway *et al.*, 2002).

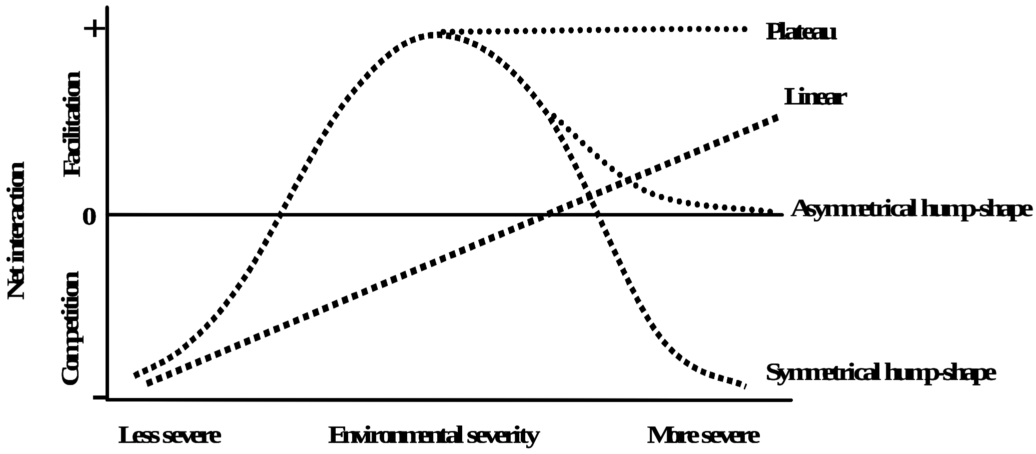


Figure 2. Schematic of possible variations in the relationship between an environmental stress gradient and the strength of species interactions under harsh environmental conditions. (le Roux and McGeoch, 2010).

My thesis addresses two major plant community composition filters, biotic represented by shrubs and seedlings interactions under two overstory canopy conditions and abiotic represented by climatic conditions vary across sites. In fact, many authors have considered shrubs as important regeneration niches for tree species (Callaway, 1995, 1997; Gomez-Aparicio *et al.*, 2005). However, the majority of "nurse shrub" studies only considered the role of canopy shrubs on seedling regeneration in open areas (Castro *et al.*, 2004b; Gomez-Aparicio *et al.*, 2005; Cuesta *et al.*, 2010; Armas *et al.*, 2011). Studies assessing the variation in nurse-seedling associations along a climatic gradient with respect to the overstory canopy and species functional response are notably rare (Gomez-Aparicio *et al.*, 2008). To our knowledge, there is an almost complete lack of studies addressing the characteristics of the nurse phenomenon within the forest communities of the coastal dunes in the Aquitaine Region. My aim with this thesis was therefore to assess the effect of variations in the strength of shrub-seedling associations on regeneration of three oak species with respect to climatic conditions variation between sites and overstory canopy treatments in the context of climate change. This research was conducted in coastal dune forest communities in Aquitaine Region (south-western France) between 2009 and 2012.

2. Thesis outline :

- Chapter 1 is a review of the main topics in the literature related to my PhD research area, i) Natural regeneration ii) Plant-plant interactions and nurse-plant syndrome iii) Climate change and plant-plant interactions ; iv) Spatial point pattern analysis and potential of examining forest regeneration dynamics, and v) Thesis objectives and questions.
- Chapter 2 provides a brief description of the study site and the experimental design.
- Chapter 3, (**article 1, under review in Annals of Forest Sciences)** describes spatial study of the biotic association patterns of the seedlings of three oak species (2 evergreen 1 deciduous) with neighboring shrubs and how associations can vary with respect to a summer aridity gradient, canopy opening and targeted species.
- Chapter 4, (**article 2, in preparation for submission to Canadian Journal of Forest Research**) tests if oak regeneration is spatially associated with specific shrub structures through a broad assessment of the types and frequency of non-random inter- and intraspecific associations of four functional groups of shrubs using spatial and non spatial analysis methods
- Chapter 5, **(article 3, under review in Forest Ecology and Management)** experimentally addresses the same questions as in the chapter 3 to test results obtained and additionally to identify the seedling functional responses to the net shrub-oak seedling balance through a transplanting seedling experiment.

- Finally, Chapter 6, I generally discussed the main results of this thesis and outlines perspectives for the future. The extensive bibliography used in the Ph.D is listed at the end.

Chapter one

Introduction: From natural regeneration to spatial point pattern analysis and potential of examining forest regeneration dynamics, a literature overview

Below, I review the relevant literature about the natural regeneration, spatial variation in plant-plant interactions along ecological gradients in the context of climate change and potential ability of the spatial pattern analysis to describe this variation that are the focus of my thesis in detail.

1- Natural regeneration

In forests, the term regeneration refers to processes involving the production, dispersal, germination of tree seeds and the subsequent establishment of seedlings; vegetative recovery following death of the aboveground portions of mature trees can also be termed regeneration (Price *et al.*, 2001; Dorji, 2004). Whether natural or artificial, the dynamics of forest regeneration processes are highly stochastic phenomena in which a wide range of adaptation mechanisms and a large number of interrelated factors come into play, particularly in the early establishment stages (Ammer, 1996; Dobrowolska, 1998; Pascarella *et al.*, 2000; Paluch, 2005). However, in relation to a single stand, the range of probable determinants is restricted to the mode of seed dispersal beside seed avaliabilty and validity, local neighboring interactions and the spatial heterogeneity of microhabitats that controls the emergence and survival of trees recruits (Caccia and Ballare, 1998; Beckage *et al.*, 2000; Collins and Battaglia, 2002; HilleRisLambers and Clark, 2003). During the tree's life cycle, regeneration is an important way to maintain species composition in forests; it allows species to colonize new regions and contributes to changes in the species composition of the arrival area and consequently the migration of tree species across landscapes. Thus, identifying the processes that promote or limit tree recruitment is a scientific way to ensure the natural regeneration of forests and is useful for restoration and afforestation planning (Marañón *et al*., 2004).

Like for other forest species, oak regeneration is the outcome of a complex balance of successive growth stages from seeds to adults and includes acorn production, animal dispersion, germination and establishment in the dispersed habitat and finally growth to adult trees (Schupp, 1990). Together these processes determine the success or failure of oak regeneration and the final fit of a species to a given set of conditions (Clark *et al.*, 1999; Pausas *et al.*, 2006). Because regeneration is a sequence of demographic stages; the breakdown of any one of them will hamper or halt the overall process even if other conditions are optimal (Schupp, 1990). For instance, the absence of a dispersal vector will limit regeneration even when a massive crop of acorns of sound quality is available.

The acorn regeneration mechanism of a species of oak is mostly similar to that of many oak species with which they share seed-dispersal agents i.e., the European jay *Garrulus glandarius* (Gomez, 2003), acorn predators e.g., weevils (*Curculio elephas*) or rodents and seedling establishment problems i.e., the impact of the first summer drought and of browsing herbivores. Expected mortality is high during the regeneration phase of oak forests where possible causes of failure can occur at any stage, for example, when the number of available acorns (due to under production by parent trees) limits recruitment (Diaz *et al.*, 2004; Bonal *et al.*, 2007). Limitation, on the other hand, may occur when recruitment is limited by the non-dispersal of available acorns, which has been suggested to be an important cause of the lack of oak recruitment in the Mediterranean landscape (Gomez *et al.*, 2003; Den Ouden *et al.*, 2005; Pulido and Diaz, 2005). Both biotic and abiotic limitations i.e., the severity of summer drought, and overgrazing by domestic or wild herbivores can reduce the probability of acorn germination, seedling emergence and abundance of established seedlings (Rey and Alcantara, 2000; Zamora *et al.*, 2001; Gomez-Aparicio *et al.*, 2004) (Fig. 3).

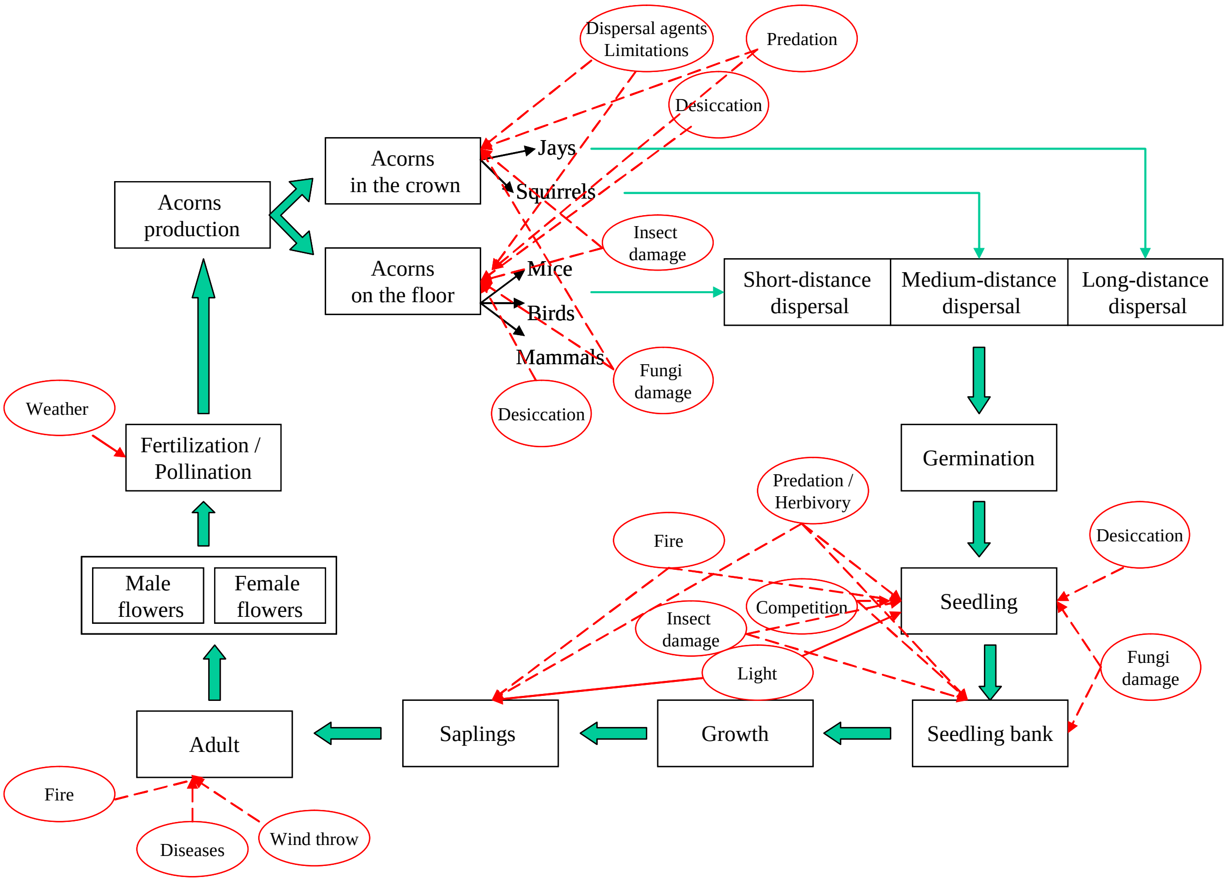


Figure 3. Simplified conceptual model of oak regeneration process. Solid arrows indicate the pathway of successful regeneration while dashed red rows represented the main hazards that may hamper the process at each stage and solid red rows have either positive or negative effect. Adapted from (Tyler *et al.*, 2006) and (Pausas *et al.*, 2009).

Regeneration niche concept

The abundance and distribution of plant species are determined during early regeneration stages, the different mechanisms acting mainly at the seedling stage and effective natural selection take place, as it is most vulnerable to their immediate environment (Kitajima, 1994; Quero *et al.*, 2008). Seeds may be capable of dispersal to many sites and can start germinating under most environmental conditions (Kyereh *et al.*, 1999), but it is the early environmental requirements together with a high mortality rate during the seedling stage that determine whether a species regenerates mostly due to the high natural selection pressures at this stage. This is why seedling regeneration is considered as a bottleneck for successful species establishment (Poorter, 2007). Therefore, identifying the abiotic and biotic factors that correspond to the regeneration niches of different species is crucial to understanding and predicting forest dynamics (Marañón *et al*., 2004).

The so-called ‘‘regeneration niche’’ was defined by Grubb (1977) as the environmental conditions in the earliest stages in the life cycle of a plant (seedling emergence). The classical niche concept assumes that when two species grow as neighbors, negative impacts or a niche-shrinking effect will emerge (i.e., competition, predation and parasitism) (Higashi, 1993), in the other words, no two species can share the same habitat. However, given the strong competition for resources, what processes are behind the large number of tree species that stably coexist in a small area, as is the case with rainforest trees? The classical ecological theory based on Gause’s principle of competitive exclusion is that the stable coexistence between competing species requires them to occupy different niches (Tokeshi, 1999), Inclusion of facilitation into niche theory as incorporating facilitative species can explain better such stable coexistence, where a species can extend and enlarge the restricted environmental conditions that actually occupied by the species after exclusion or minimize the effect of competitors and other enemies (Bruno *et al.*, 2003). Hence, the actual niche of a species can be larger even than the spatial range predicted by the fundamental niche (Higashi, 1993) (Fig. 4). For example, positive biotic interactions between plants can minimize or eliminate the effects of niche-shrinking factors, in the same way as plant densities and distributions can be increased by animals (as acorns dispersal agents) and reduce the effects of predators (Wilson and Agnew, 1992).

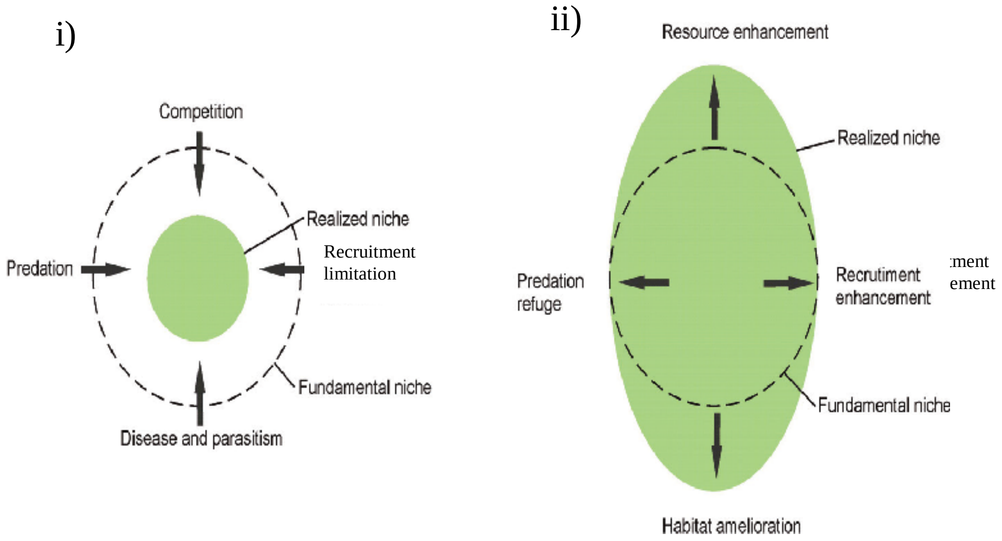


Figure 4. The concept of ecological niche (Hutchinson, 1957), i) the realized niche (in green) is considered to be smaller than the potential fundamental niche because of negative interactions, ii) however, when positive facilitation processes are considered, the realized niche can actually become larger than the expected fundamental niche (according to Bruno *et al*., 2003)

2- Plant-plant biotic interactions and nurse-plant syndrome

Once an acorn has landed and a seedling has emerged, the oak seedling becomes sealed and there is little escape from the local environment and its neighboring plants (Murrell, 2009). Thus, seedling interacts with their close neighbors and responds to a set of local environmental conditions (i.e., the “plant eye view of the community”) (Turkington, 1989; Purves and Law, 2002). In addition to climatic factors, biotic interactions between species are among the most important determining factors of community structure and of the dynamics that affect the growth, survival and fecundity of individuals (Stachowicz, 2001; Eckstein, 2005). Plants growing near each other affect each other in many ways resulting in a wide range of detrimental or beneficial outcomes. Generally, in addition to neutral, two outcomes can expected from plant-plant interactions, positive (facilitation) and negative (competition) effects (Callaway *et al.*, 1996). Facilitation and competition are the results of the net outcome of changes in the environment of a plant influenced by the presence of other plants (Holmgren *et al.*, 1997). When negative effects dominate, the interaction results in competition due to sharing limited resources (e.g., water, nutrients, light, and space) may exclude a species from abiotically desirable areas that are within their spatial dispersal zone (Hochberg and Ives, 1999; Case and Taper, 2000). On the contrary, many sessile organisms can make their habitat more hospitable for their offspring and for other species by buffering environmental stress or increasing resource availability (Bertness and Callaway, 1994; Holmgren *et al.*, 1997). Thus, positive interactions may extend the distribution of some species beyond their fundamental niches (Bruno *et al.*, 2003; Crain and Bertness, 2006). Plant nursing is favorably a mechanism to modify biotic and abiotic conditions (i.e., reduce physical stresses, predation, resource limitation and competition) allowing species to grow in environments that would otherwise be too harsh for their survival (Choler *et al.*, 2001).

One of the most obvious forms of nurse-plant syndrome is shrub-seedling association. The sub-canopy of shrubs can facilitate the regeneration of many seedling species due to the habitat modifying capacity of shrubs both climatically and edaphically (Gomez-Aparicio *et al.*, 2005). However, in natural communities, not all species have the same positive effect, usually only sub-group of shrubs improves their sub-canopy environment while the effects of other species present may vary from competitive to neutral (Callaway, 1995).

Nurse shrubs can play a facilitating role in all critical stages of oak regeneration from acorn dispersal e.g., they can intercept acorns, as well as attracting birds by providing perches and in so doing enhancing the availability of acorns (Toh *et al.*, 1999; Pausas *et al.*, 2006) while, protecting them from predators or from desiccation, which aid germination of acorns that land near or under the shrub canopy (Rousset and Lepart, 1999; Gomez-Aparicio *et al.*, 2008). Shrubs can improve the establishment and growth of oak seedlings by protecting them from herbivores and physical damage, and increase air humidity. Oak seedlings may also gain in from the nutrition provided by leaf litter and fix nitrogen via some nitrogen fixation shrubs consequently enhancing seedlings regeneration (Flores and Jurado, 2003; Gomez-Aparicio *et al.*, 2005; Salas *et al.*, 2006)

Competition-Facilitation net balance and stress gradient hypothesis (SGH)

The high rate of regenerated seedlings close to nurse shrubs does not imply their negative effects have been excluded, but ensure that positive influences overwhelm the negative ones (Padilla and Pugnaire, 2006). The net balance of these positive and negative interactions between plants can strongly influence the structure and composition of communities (Hacker and Gaines, 1997; Michalet *et al.*, 2006). Plant-plant interactions vary spatially and temporally (Kitzberger *et al.*, 2000; Tielborger and Kadmon, 2000; Armas and Pugnaire, 2005; Gomez-Aparicio *et al.*, 2005), according to the morphological, physiological, and life stage traits of the interacting species (Callaway, 1997; Holmgren *et al.*, 1997; Michalet *et al.*, 2006), the abiotic stress experienced by the interacting species (Bertness and Callaway, 1994) as well as the measured response can affect the estimation of the net interactions (Travis *et al.*, 2006; Kawai and Tokeshi, 2007). For example, this type of relationship is mainly postive (i.e., it increases a species' probability of benefiting from interactions with benefactor species) depend on its "beneficiary ability" (Tewksbury and Lloyd, 2001) to exploit environmental conditions improved by other plants, its competitive-response ability to tolerate the negative effects of growing in close association with other plants and all that must occur under sub-optimal environmental conditions for the survival or productivity of the species concerned (Choler *et al.*, 2001; Liancourt *et al.*, 2005; Michalet *et al.*, 2006) (Fig. 5).

However, the factors that regulate the net balance of these types of interactions in different communities are still not well known (Callaway, 1997; Stachowicz, 2001; Brooker, 2006), although abiotic environmental conditions (e.g., stress gradient hypothesis) and certain species traits appear to be the most important and in turn, affect the outcome of plant-plant interactions (Liancourt *et al.*, 2005; Wang *et al.*, 2008). For example in some studies focused on beneficiary responses to biotic plant-plant interactions, stress-tolerant light-demanding species have been shown to be less facilitated than stress-intolerant shade-demanding species (Liancourt et al., 2005; Pages & Michalet, 2006). Also the effect of the functional type of nurses involved in the interactions is obvious in the literature for example, Gomez-Aparicio et al. (2004) showed in a meta-analysis conducted on 11 woody seedlings species planted within 16 different nurse shrubs species that the most important positive effects were found when seedlings were planted within legumes and the most important negative effects within

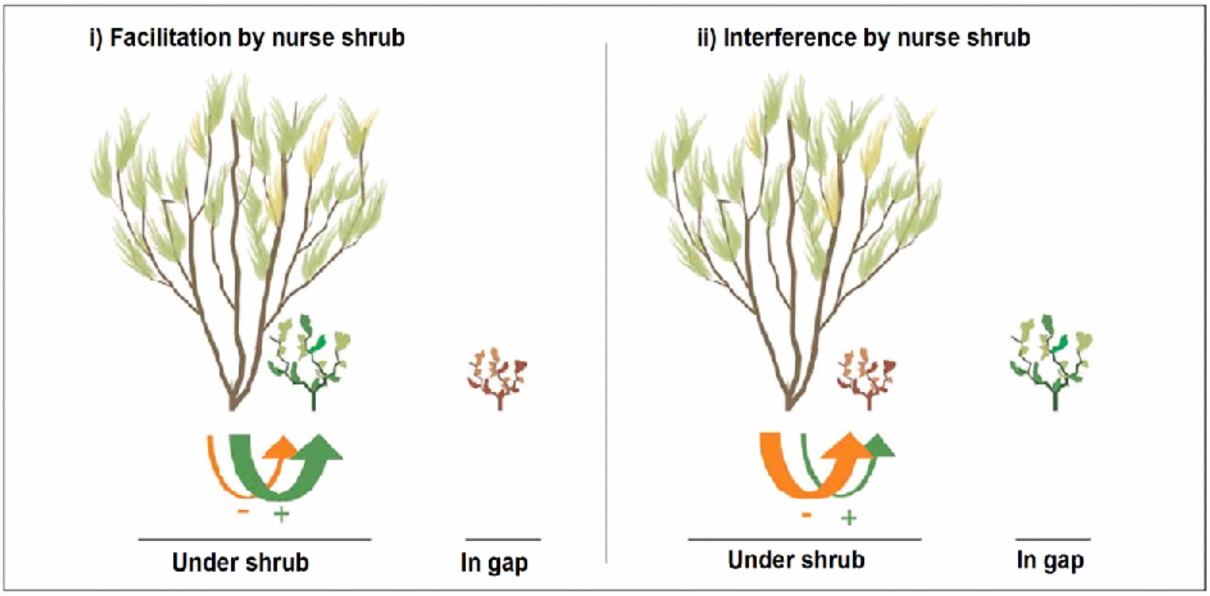


Figure 5. Facilitation and interference under nurse shrub. The net balance outcome from simultaneously acts of positive- negative effects determines the relative importance of the process driving shrub-seedling interaction, (i) when positive effects outweigh negatives ones, seedling can use environmental conditions that have been modified by shrubs consequently increase its performance in compare to seedlings located in gap; (ii) inverse can be found when negative effects outweigh positive ones (Padilla and Pugnaire, 2006).

rockroses (Cistus species, known for their allelopathic compounds), with intermediate positive effects for other shrubs.

Since the influential paper of Bertness and Callaway (1994), the stress gradient hypothesis (SGH) has been a major concept in understanding the effects of environmental stress on plant community distribution in a wide range of environments (Brooker, 2006; Lortie and Callaway, 2006). The original formulation of SGH predicted that the strength of positive interactions compared with negative ones would increase monotonically with increasing abiotic and biotic stress (Bertness and Callaway, 1994). This model subsequently extended to gradients in disturbance and generalized across most environmental severity gradients (Brooker and Callaghan, 1998). Today, the stress-gradient hypothesis (SGH) provides a prime reference for understanding how variations in the balance of positive and negative interspecific interactions occur (Kawai and Tokeshi, 2007; Brooker *et al.*, 2008). SGH assumes that under environmentally severe conditions (i.e., climate stress and/or customer pressure) plants benefit from the presence of neighboring individuals because the net positive effects of environmental modification by neighbors override the negative effects due to competition for resources. Conversely, under adequate conditions, SGH predicts a net negative impact of neighboring plants since the effects of competition with nearby individuals will override any positive effects of alleviating environmental stress. Thus, net plant interactions hypothesized gradually to become more positive with gradually increase severe environmental gradient, because of changes in the strength of constituent positive and negative interactions (Fig. 6) (Bertness and Callaway, 1994; Brooker and Callaghan, 1998).

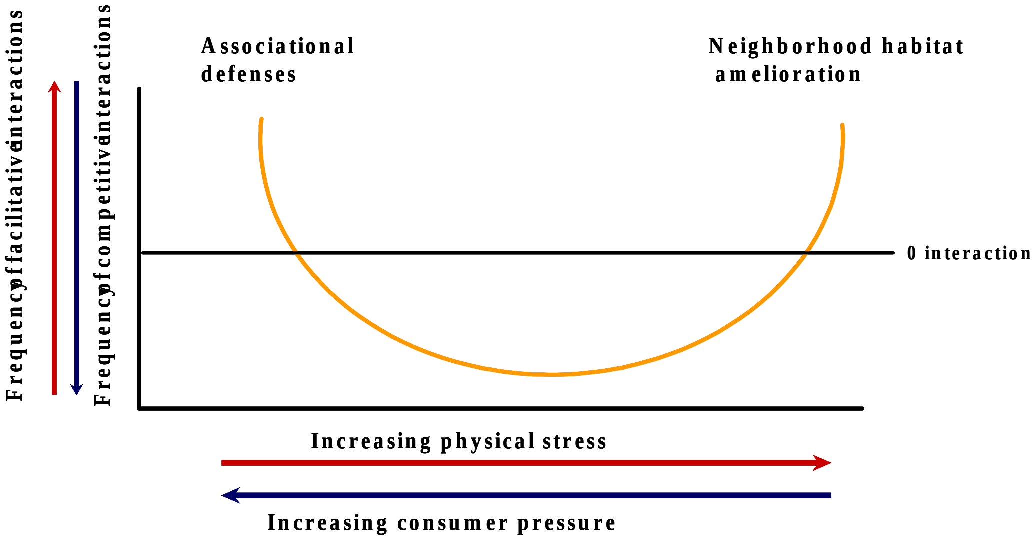


Figure 6. Conceptual model of Bertness and Callaway (1994) expecting under which conditions positive interactions (facilitation) expected be important processes in community structure. Positive interactions are hypothesized to be rare under gentle physical conditions and low consumer pressure. Neighbor amelioration of physical stress and associational defenses hypothesized to lead to positive interactions under harsh physical conditions and intense consumer pressure, respectively.

In Figure (2) Le Roux and McGeoch (2010) listed four distinct scenarios for the severity-interaction relationships (i) linear (SGH), when the relative intensity of facilitation increases over the complete range of environmental severity to reach a maximum before environmental conditions become sufficiently severe to completely exclude the species (Bertness and Callaway, 1994; Lortie and Callaway, 2006; le Roux and McGeoch, 2008; Anthelme *et al.*, 2011); (ii) symmetrical hump-shaped when net facilitation appears at moderate environmental severity and evenly negative at both extremes of the gradient where competition between plants does not decrease with increasing severity (Maestre and Cortina, 2004; Maestre *et al.*, 2010); (iii) asymmetrical hump-shaped, when beyond the moderate environmental conditions at which maximum facilitation occurs, net facilitation shifts to neutral with an increase in the harshness of the conditions, implying that stress conditions cannot be completely ameliorated by neighbors and the costs and benefits of growing close to neighbors are in balance (Michalet *et al.*, 2006; Kawai and Tokeshi, 2007); and (iv) plateau shaped, this model predicts that net facilitation will increase with increasing environmental severity up to an asymptote in parallel with the independent line with constant effect (Tokeshi, 1990; Kawai and Tokeshi, 2007), some authors have supported such a prediction (i.e., Callaway *et al.*, 2002; Graff *et al.*, 2007).

Shortly, in this thesis and considering the study factors and in the context of climate change I am interested to detect which scenario could be applicable under the conditions of coastal dune forest communities of the Aquitaine Region.

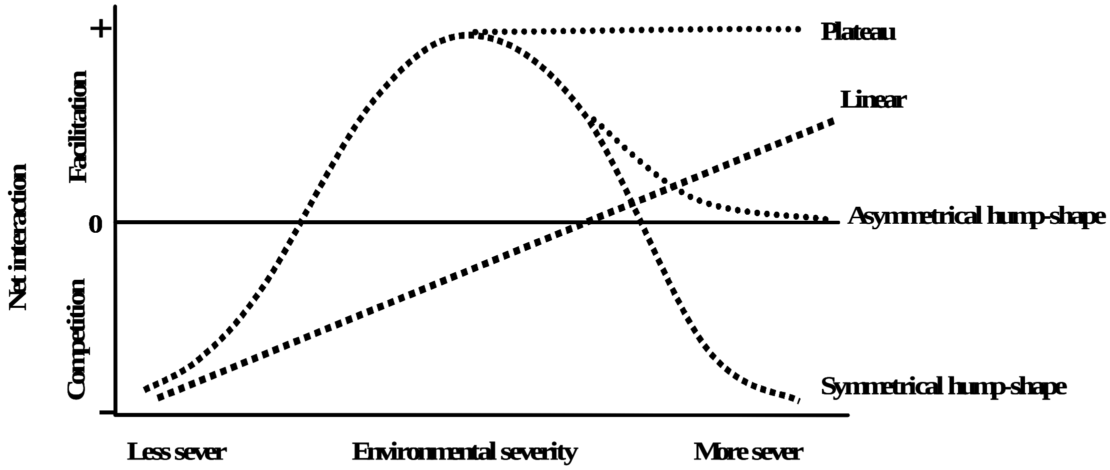


Figure 2. Schematic showing possible variation in the relationship between an environmental stress gradient and the strength of species interaction under severe environmental conditions. (le Roux and McGeoch, 2010).

3- Climate change and plant- plant interactions

According to the Intergovernmental Panel on Climate Change (2007), the earth's ecosystem biodiversity is currently undergoing rapid change. Global modification of biodiversity has been going on for millions of years, and was previously considered to be a natural phenomenon. Complete taxonomic groups have been stochastically wiped out and mass extinction has occurred in relatively short periods of time due to catastrophic accidents, climate change, and the evolutionary advantages of increasing the fitness of one species over another. However, with the rise of human civilization, in the last 100 years, the natural environment has been subject to directed changes, instead of random or system-inherent changes and these changes are starting to have diverse ecological impacts (Pimm, 2001; Parmesan, 2006; IPCC, 2007).

In temperate oceanic climates, like that of south-western France the temperature is expected to increase between +2.5-3.5 C by 2100, summer is likely to become dryer and hotter with more frequent and higher intensity extreme events (IPCC, 2007) (Fig. 8). The main symptoms of this climate change will be rising temperatures with a decrease in spring and summer rainfall in the south of Europe. These will also lead to numerous hazards of extreme weather events especially as prolonged drought like the European heat wave in 2003, which led to a dramatic decrease in primary plant productivity and will likely occur more often in the future (Schar and Jendritzky, 2004; Ciais *et al.*, 2005). These rapid changes in climate have had dramatic ecological consequences, affecting both the abundance and distribution of many species, globally altering the composition, and functioning of communities and ecosystems (Walther *et al.*, 2002; Parmesan, 2006). At species level, three patterns have emerged (i) direct changes in species growth and biomass output; (ii) spatial redistribution of species or changes in species phenology, and (iii) changes in species interactions either with habitat (adaptation to changed climatic conditions) or with organisms (biotic interactions) (Hughes, 2000). For example some species have benefited strongly from climate chang throught lengthening their growing season and increasing their growth and reproductive rates as Antarctic vascular plant species or by contrast, other species have been negatively affected and couldn’t alter its phenology and adapt its physiological tolerances in response to climate change, leading to its extinction (Battisti et al. 2006; Davies et al. 2005; Thomas et al. 2001).

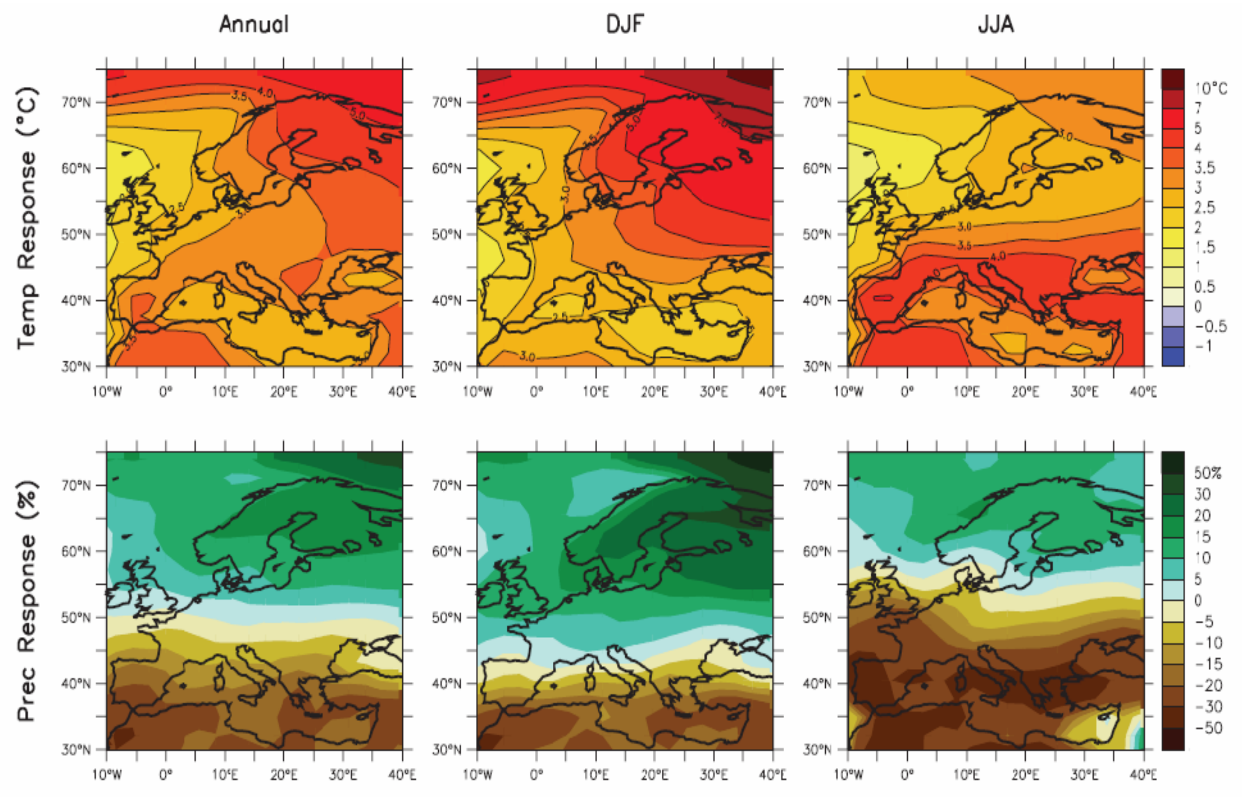


Figure 8. Predected changes in temperature and precipitation over Europe depending on the MMD-A1B* simulations. First row represent the annual mean, DJF (December, January and February) and JJA (June, July and August) temperature change between 1980 to 1999 and 2080 to 2099, averaged over 21 models. Second row: same as first, but for fractional change in precipitation (Christensen and Hewitson, 2007).

MD-A1B* models project a median warming of 3.3°C by the end of the 21st century, which varies seasonally from 3.0°C in JJA to 3.6°C in DJF

Forests are particularly sensitive to climate change. On one hand, the long life span of trees does not enable rapid genetic adaptation to environmental changes and as sessile organisms woody species are less able to shift poleward with changing climatic conditions. Forest management strategies need to be planned to adapt to expected changes in growing conditions since forests that are regenerated today will have to cope with the future climate conditions for approximately 100 years. Over different EU bioclimatic regions, climatic changes will have negative impacts on forests by exacerbating the levels of abiotic stress that affect biotic interactions and influence plant performance and will further hamper natural regeneration (i.e., Callaghan and Jonasson 1995; Guisan *et al.*, 1995). Changes in the performance of one species or functional group will seriously influence current relationships within forest communities i.e., changes in competition or facilitation between co-occurring species that will increase in damaged forest areas and consequently will result in an increase in forest area to be regenerated under less favorable climatic conditions (Klanderud, 2005). Clear evidences appeared that the effect of biotic interactions will be regulated by variation in climate conditions and that the climate change likely to influence the role of facilitation and competition within plant communities (Brooker, 2010). For example, changes in the environmental severity through climate change, will lead to a predictable changes in the dominate type of interaction occurring within community. Thus, under severe environmental conditions relatively stress-sensitive species would be less likely to survive in the absence of stress-tolerant facilitators (Brooker, 2010).

Despite the fact that natural regeneration processes and biotic interactions can be targeted by climate change, they also may represent a direct and immediate opportunity to select tree species or provenances that are believed to be better adapted or adaptable to the changing climatic conditions. Regeneration is a particularly sensitive stage to changes in climate (Spittlehouse and Stewert, 2003) as young seedlings are sensitive to drought and other extreme climatic conditions (Oliet *et al.*, 2002). Consequently, regeneration processes may ensure modification and adaptation itself by allowing natural selection to take place and drive the population to meet the fitness optimum corresponding to the new local environmental conditions (Bürger and Krall, 2004). Whether natural or artificial, regeneration is the establishment phase of genetic composition and diversity and it is expected that surviving individuals after a severe climate event to have some degree of genetic resistance to drought that would be inherited by the next generation. (Gutschick and BassiriRad, 2003; Parmesan, 2006; Millar *et al.*, 2007). Therefore, a suitable suggested step to ensure the adaptive response of established regeneration is to increase the level of genetic diversity within the seedling population (De Dios *et al.*, 2007; Kolström *et al.*, 2011). Similarly, an increasing body of evidence points to the potential of interspecific interactions to mitigate the ecological consequences of climate change on species establishment as strong interspecific interactions occur between some species along temperature and moisture stress gradients (Lortie *et al.*, 2004; Lortie and Callaway, 2006; Dona and Galen, 2007). However, some recent studies showed that interactions related to environmental severity gradients may be neutral or negative, i.e., symmetrical and symmetrical hump-shaped SGH scenarios (e.g. Maestre and Cortina, 2004; Michalet *et al.*, 2006). Under such a scenario, biotic interactions could either have no effect or have an additional negative competitive effect of species and loss of stress-tolerant facilitators consequently reduces survival probability of “beneficiaries” under increasing climatic severity. To grasp the potential for biotic interactions to influence species establishment under increasing severity it is thus important to understand which factors determine the balance between positive and negative interactions among plants.This may help to explain the ability of plants to expand or contract their ranges in response to climate change (Brooker *et al*. 2008; see also e.g. Rodriguez, 2006).

In this context, I try in this thesis using set of spatial point data and transplanting experiments to detect the potential of woody shrubs to enhance the early establishment of sub-canopy oak seedlings with increasing aridity gradient. Hence, provide relevant information can help to predict this role under ongoing climate change to manage oak regeneration niches in the coastal dune forest communities.

4- Spatial point pattern analysis and its potential for examining forest regeneration dynamics

Since the early work of A. S. Watt “Pattern and process in plant community” in 1947 there has been growing interest in studying spatial patterns in plant ecology. Watt’s influence was his view of the community as a mosaic of patches at different stages with correlated effects. Recognition of the spatial patterns of this mosaic is fundamental to understanding the mechanisms that have given rise to the mosaic by trying to identify the spatial patterns that develop during regeneration dynamics of communities. This is a key ecological question that arose from the famous paper of Watt (1947) and still needs to be answered to improve our knowledge of the mechanisms that sustain biodiversity and allow a large number of species to coexist (Turnbull *et al.*, 2007; Lamosova *et al.*, 2010; Wilson, 2011). Coexistence concerns interactions among organisms. Most of the processes that have been hypothesized to explain the coexistence of plant species and community structure display strong temporal and spatial components, such as direct plant-plant interactions i.e., competition or facilitation (Callaway, 1997; Lortie *et al.*, 2004), dispersal limitation (Nathan and Muller-Landau, 2000; Li *et al.*, 2012), habitat preference (Harms *et al.*, 2001; Gunatilleke *et al.*, 2006; Wiegand *et al.*, 2007) and the Janzen-Connell hypothesis (Janzen 1970; Connell 1971). Population interactions, for example, intra and inter-specific interactions between plants, can induce spatially recognizable patterns (Tirado and Pugnaire, 2003; Wiegand and Moloney, 2004; Armas and Pugnaire, 2005; Fajardo and McIntire, 2007). For example, mainly, observed patterns at small spatial scale have been related to either positive or negative plant interactions that have the potential to structure local plant neighborhoods (Phillips and Macmahon, 1981; Valiente-Banuet et al., 2006). For example, regular plant spatial patterns are often referred as a sign of intense competition between plants for limited resources (Stoll and Bergius, 2005; Rayburn and Monaco, 2011). In contrast, more aggregated patterns (especially interspecific) are interpreted as evidence of positive plant interactions (Eccles et al., 1999; Kefi et al., 2007); the independent patterns suggest no direct strong influence of any process in shaping spatial relationships (Szwagrzyk and Czerwczak, 1993). One way to examine the spatial dynamics of individual trees without a long-term demographical study is to perform a point pattern analysis of fully mapped individual locations using, for example, shrub-stem or shrub-crown positions in relation to other forest individuals (Getzin and Wiegand, 2007).

The study of spatial patterns in forests (the spatial organization of individual trees with respect to one another) is a useful way to analyze the structure and regeneration dynamics of forest communities and to determine habitat quality (Pommerening, 2002). It determines the local environment of each tree seedling and its biotic interactions (competition/facilitation processes) thus the tree’s ability to develop and grow, or its probability of dying (Fig. 9).

The need to study forest spatial patterns using point pattern analysis is comprehensible since point pattern data are widely available for forests and such data can be approximated in many situation because dots represent trees better than other plant communities (i.e., diffusely-growing clonal plants in grasslands) (Wiegand and Moloney, 2004; Law *et al.*, 2009). In addition, the long lifespan of trees does not facilitate experimental research. On the other hand, since trees are stationary, ecological coexistence and historical processes that occur in a forest may be detected by analyzing explicit maps of individual tree without the need for costly long-term demographic censuses (Gavrikov and Stoyan 1995; Hubbell *et al.*, 2001). Thus, it was not a coincidence that one of the first applications of point patterns (Ripley’s *k* function) was a forest, when Ripley (1977) analyzed the positions of redwood seedlings and found them more aggregated than the points of a Poisson process. Points in a point-pattern may contain more information than x, y coordinates, termed marks, (i.e., a species identity, a life stage, or whether the individual survived or died), and many biological questions concern the interactions between points with different marks (i.e., facilitation or competition between different species and life stages of individual trees in a forest) (Wiegand and Moloney, 2004). Considering the above, in patterns analysis one can generate hypotheses about the factors that govern forest community dynamics which will be useful for (i) adapting silvicultural regimes that mimic natural processes; (ii) forest inventory planning and design; (iii) improving models of stand dynamics by including inter- and interspecies patterns of association (Tomppo 1986; Salas *et al.*, 2006).

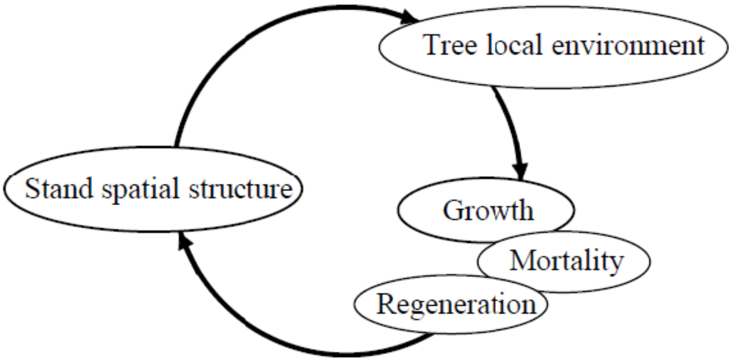


Figure 9. Relationship between stand spatial structure, local environmental conditions and natural processes. (Goreaud *et al.*, 1998)

Inferring processes from patterns

A pattern is a distinctive form that is the reverse of random, meaning that a pattern can be detected and described (Fortin and Dale, 2005). According to Keitt and Urban (2005), the lack of a clear link between a process and spatial pattern is the Rosetta stone of ecology, decoding such a link can shed light on the interactions between and within species in the representative plant community and consequently help gain other insights into ecological functioning. According to Fonteyn and Mahall (1981) and Fortin and Dale (2005) still the attempts to much between observed patterns of ecological phenomenon and causes processes are far from perfect. Many approaches have been used for this purpose including experimentation (e.g., Clark *et al.*, 2004), simulation of processes within a spatial domain (e.g., Pacala *et al.*, 1996) and analyses of spatial patterns (e.g., Fajardo and McIntire, 2007).

Still, ecologists who have used the spatial pattern approach mostly found it difficult to directly infer processes and mechanisms from observed patterns, and concluded that analyses of spatial pattern do not necessarily enable definite conclusions to be drawn about causal relationships (McIntire and Fajardo, 2009). Shipley and Keddy (1987) and Leps (1990) agreed that the mechanism hypothesis should be tested by experimental manipulations and that the descriptive approach can only suggest hypotheses, in other words, the evidence obtained from experimental approach is much more reliable than that based on observed patterns due to the numerous biological phenomena involved. For example, several different processes can generate the same signature pattern, changes in the intensity of a process can produce different patterns, and a well-defined non-random process can create a pattern that is indistinguishable from random. Moreover, the coexistence of species may often be the result of simultaneous processes rather than a single mechanism (Cale *et al.*, 1989; Chesson, 1994; Lavorel and Chesson, 1995). However, inverse that is not necessary always falls where a pattern can also be as mirror that reflects a process since some specific processes can create a distinguishable single pattern, and non-random processes can create highly structured patterns (McIntire and Fajardo 2009). The link between a pattern and a process is currently undergoing reconsideration after rejected by many authors for several decades (McIntire and Fajardo 2009). The main reason being the continuous development of analytical tools for spatial data (e.g., Wiegand and Moloney, 2004; Keitt and Urban, 2005), and testing prior multiple hypotheses which significantly changed the understanding of the ability to bridge between a process and a pattern by reducing the perceived biological limitations. In this context, Jeltsch et al. (1999) proposed three steps to detect underlying processes in nature from a spatial pattern: (i) describing the spatial pattern; (ii) developing prior multiple hypotheses about the underlying processes responsible for the observed pattern; and (iii) evaluating the hypotheses (through experimentation or modeling). Mcintire and Fajardo

(2009) stated in their work “Space as a surrogate” (Fig.10) that the perception of biological limitations behind a process-pattern link is heavily rooted in analytical issues and when using spatial analysis in an inductive description mode (Fig.10 left oval) rather than deductive model (Fig.10 central oval). Therefore, Space as a surrogate” was attempted to move the analysis of spatial pattern from inductive description to deductive inference by merging the three elements that must act together. These elements are accurate ecological knowledge, a prior reasoning and precise application of spatial analytical tools, and then one can start to ask what are the expected underling mechanisms responsible for raised patterns (deductive model), instead, just ask what is the spatial pattern of ecological phenomena (inductive mode). “Space as a surrogate” is an enhanced version of Jeltsch *et al*., (1999) approach by placing process-pattern link via spatial analysis within the context of scientific inference alongside other deductive methodologies (Fig.10)

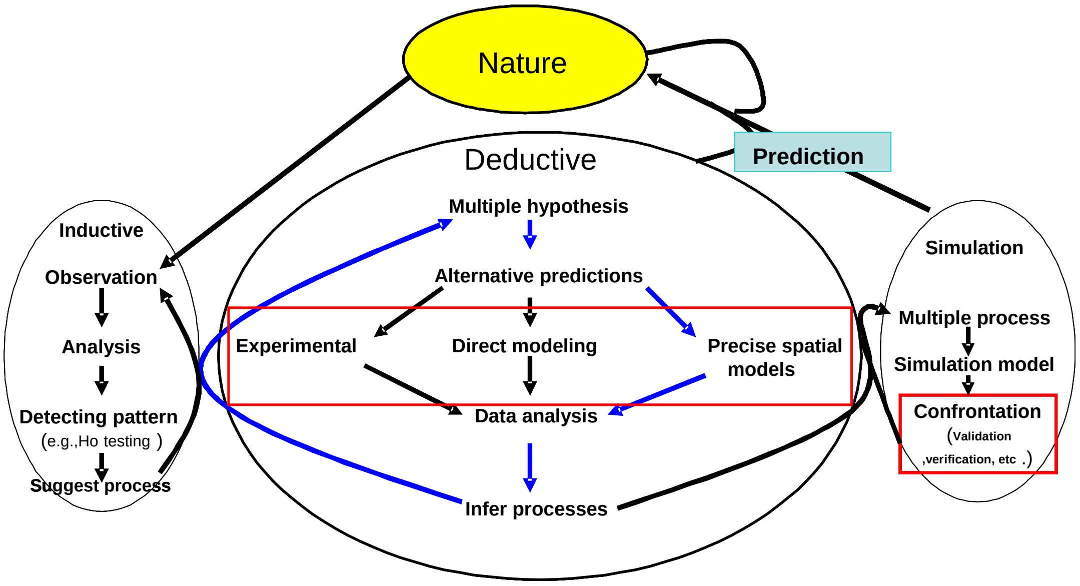


Figure 10. Space as a surrogate conceptual model proposed by McIntire and Fajardo (2009) within the contexts of inductive, deductive, and simulation approaches as an attempt to infer a process underlying detective pattern. The blue arrows represent the frame of this approach depending on the spatial structure of the pattern. Rectangular red boxes indicate where we confront our predictions with data. Following the blue arrows, the approach including three elements : (1) sufficient ecological understanding of spatial structures, both conceptual and empirical, about the system of interest (blue arrows above of central oval); (2) develop precise ecological, multiple a priori hypotheses (or single hypothesis if the model structure is known) that cover all reasonable relevant processes affecting the system under study (upper part of central oval); and (3) evaluate and select the best hypotheses using advanced spatial-analysis techniques, (lower part of central oval).

In (chapters 3 & 4) of this thesis I tried to follow “Space as a surrogate” steps by identifying severity climatic gradient along my study site, set up reasonable a priori hypothesis and using advance static’s tools. This indirect method can improve the knowledge not only of the spatial phenomena themselves but also of their underlying processes, since spatial patterns are the result of interactions between spatial objects (Getzin *et al.*, 2008). In this context, my proposition is (chapter 3 & 4) that spatial point pattern analysis can help me for describe the interaction patterns of regenerating oak species with neighbouring shrubs and provide sounder links to how ecological processes function in coastal dune forest communities, and consequently, help define a more precise ecological hypothesis for my planned transplanting experiment (McIntire and Fajardo, 2009; Schleicher *et al.*, 2011).

Transplantation of oak seedlings under shrubs in gap plot

5- Thesis objectives and questions:

The primary objective of my thesis is to investigate the nature of the association patterns of oak regeneration with dominant shrubs in the context of climate change in the coastal dune forest communities. I tested how associations can vary with respect to a climatic drought gradient, overstory canopy treatments and the functional strategies of the targeted species. This was the aim of two studies described in chapters 3 and 5. The second objective addressed in chapter 4, I tested in this chapter if oak regeneration is associated with a specific shrub structure. Meeting these two objectives should help to understand the mechanisms that may override the early establishment of oak regeneration associated with shrubs thus, how to achieve climate change-adapted silvicultural practices to create regeneration niche for oak species inhabiting these communities.

Using a descriptive and experimental approach, specifically I tried to answer the main following research questions:

In chapters (3 & 5):

1- **How does the magnitude of the net effect of shrubs on the early establishment of oak seedlings shift with an increase in drought between sites and overstory canopy covers?**
2- **Are oak species responses species-specific?**
3- **What mechanisms are involved in the interactions?**

In chapters (4):

4- **Are there differences between the three oak seedlings in their spatial distribution pattern?**
5- **Is oak regeneration spatially associated with a specific shrub structure in coastal dune forest communities?**

Chapter 2

The study site and the experimental design

The experiments were set up in the coastal dunes forest communities located along the Atlantic coast of Aquitaine Region, south-western France. In the following paragraphs, I will briefly describe the main characteristics of my study area followed by the main steps of the used methods and experimental design.

1- Coastal dune forest communities

The Atlantic coastal dune area in France extends about 500 km and is the largest coastal dunes in Europe. In Aquitaine, the biggest dune continues for approximately 240 km from the Gironde estuary in the North (45°32'N, 1°05'W) to the mouth of the Adour river in the South (43° 41 'N, 1°25' W) (Fig. 11 and 12). The coastal dune forest communities have significant heritage value because of their history, original wildlife and biodiversity, and their landscapes, which are highly appreciated by the around 2.5 million tourists who visit the region each year. In the early 19th century, the coastal dunes were planted to protect the sand dunes from erosion and winds. Then, during the 19th and 20th centuries cork and timber production were added to the original aim of physical protection. From 1970 on, tourism developed along the coast and the coastal forest has become a multifunctional forest that has to be managed sustainably to continue to fulfill these functions (Sardin, 2009).

In this area, the forest communities are located at a distance of 1-10 km from the Atlantic ocean along the Aquitaine coast (Fig.11). The main species occupying these communities are maritime pine (*Pinus pinaster*) and three oak species, the decideous Pedunculate oak (*Quercus robur*) and two evergreen species, Holm oak (*Quercus ilex*) and Cork oak (*Quercus suber*). *Quercus robur* is the most abundant hardwood in the coastal dune forest communities, it can be found over the entire coastal dunes latitudinal gradient, but is more common in the central part of Aquitaine coastal dunes, on steep eastern slopes of the fossile dunes where foresters stopped harvesting forests 30 years ago due to the difficulties of access (Sardin, 2009). The distribution of the two evergreen oak species seems to be more restricted by climate heterogeneity conditions which vary between south and north; *Quercus ilex*, which can be found in the Pointe de Grave in the northern part of the Arcachon basin, is the most drought and cold-tolerant species and abundant in the most dry dunes, in the north of Gironde. *Quercus suber* which is less drought and cold-tolerant than *Q. ilex*, is only dominant in the wettest dunes, in the southern Landes (Sardin, 2009). Coastal dune forest communities also include a matrix of shrubs varying in their distribution along the 240 km latitudinal gradient belong to diferent families (Table.1)

Table 1. distributing of main shrubs of the coastal dune forest communities (**Aquitaine region)**

Study plot	Coordinates	Distance to the northermost (km)	Main shrub species
Soulac (So.)	45°32`N, 1°05`W	0	Arbutus unedo, Ruscus aculeatus, Q. ilex
Hourtin (Ho.)	45°09’N, 1°10`W	76	Cytisus scoparius, Q. ilex, Cistus salvifolius,Q. robur , Q. ilex
Biscarrosse1 (Bisc.1)	44°26’N, 1°13`W	102	Q. robur, Ilex aquifolium, Arbutus unedo, Q. robur
Biscarrosse2 (Bisc.2)	44°26’N, 1°12`W	103	Arbutus unedo, Cistus salvifolius, Cytisus scoparius, Ulex europaeus, Q. robur
Born (Bo.)	44°01’N, 1°17`W	222	Cytisus scoparius, Erica cinerea, Ulex europaeus, Q. robur
Seignosse (Se.)	43°41’N, 1°25`W	240	Erica cinerea, Calluna vulgaris, Ruscus aculeatus, Arbutus unedo, Ilex aquifolium, Q. suber

2- Climatic characteristics

The study area is bioclimatically classified as temperate oceanic with temperatures increasing from the north to the south and an average of around 13.5°C (12.8 to 14.10°C, respectively) (Fig.11, i). In the northern part, the climate is moderately to dry in summer. The average rainfall is about 750 to 800 mm per year (Fig.11, ii) and minimum summer rainfull (June-July: less than 40 mm / month on average) with one dry summer month on average (Fig.11, iii) (Sardin, 2009). More towards the center of Aquitaine and surrounding area, the climate tends to moderately dry in summer with rainfall of 800-900 mm per year. In the south, the climate is rainy and wet (1300 mm / year). Generally, frosts are very rare (less than 30 days per year on average) with strong sunlight in summer (2,000 hours per year). The soil is composed of silica sand and has very low organic matter, nitrogen and phosphorus contents (Koijman *et al.*, 1998; Forey *et al*., 2008) and a low water retention capacity. According to Despeyroux (1984), dune soils are generally homogeneous along the Aquitaine coast with possible variations only in the size of the sands.

I chose the coastal dune forest communities in Aquitaine to conduct my PhD study because of the clear south to north drought gradient and the presence of several oak species with a matrix of shrubs. These, were particularly suited to testing my thesis questions relating to the variations in the role of the biotic shrub-oak interactions for oak regeneration dynamics under different environmental conditions. Moreover, providing an excellent space-for-time gradient since there is no soil variation that would allow me to assess changes in oak seedling-understory shrub interactions in the perspective of climate change effect.

View of the coastal dune forests-Arcachon

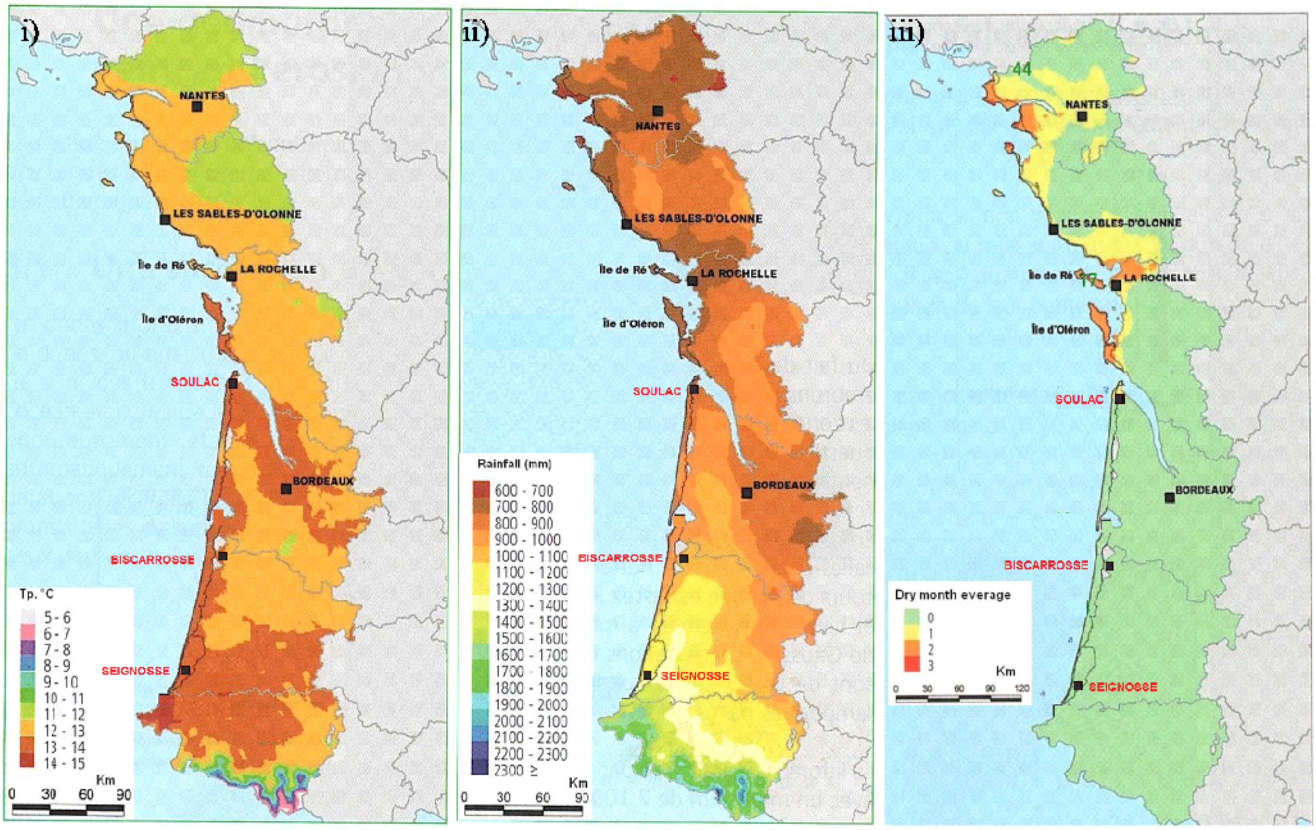


Figure 11. The climatic gradient along the coastal dunes, in red from Seignosse in the south, Biscarrosse in the center and Soulac in the north representing Aquitaine region, i) mean annual temperature, ii) rainfall and iii) average dry months. (Sardin, 2009)

Sampling and data collection

My work was based on two approaches, a descriptive approach using advanced spatial point pattern methods and an experimental approach in which oak seedlings were transplanted at the both extremes of the drought gradient. The two approaches were applied to understand how three oaks regenerations in association with shrubs vary spatially along a drought gradient and in two canopy conditions. This work was conducted between 2009 and 2012.

1-Spatial patterns studies

To detect spatial association patterns between shrubs and the seedlings of the three oak species along the 240 km-drought gradient of coastal dune forests, I chose five latitudinal positions from south to north. At each position (Fig.12), I delimited two uniform and homogeneous permanent 0.315 ha plots, under two canopy conditions, one under a closed forest canopy (tree overstory and midstory shrub layer) and the other one in a recently created gap 3-5 years (with scattered shrubs). Most of the plots were exposed east where the oldest oak forests are still present.

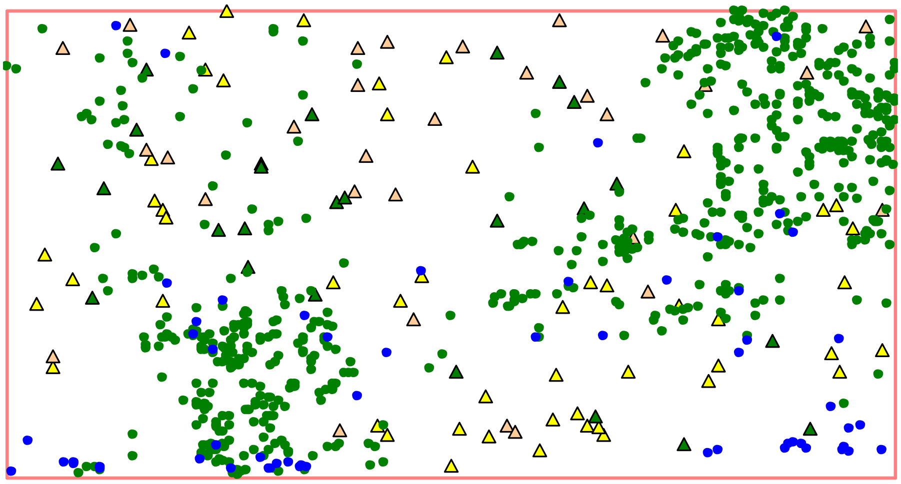
Figure 12. Approximate location of the five sites sampled along the Atlantic coast (Aquitaine, France)

The data I used in my first study (chapter 3) collected by mapping every single woody individual under both canopy conditions including targeted species (*Q. robur*, *Q. ilex*, and *Q. suber* seedlings), neighboring shrubs (Table.1) and adult trees. I divided each plot into 35 $9m^2$ quadrates using rubber tapes (Fig.13.i). Each quadrate was surveyed and individuals were identified and their spatial positions were recorded by cartesian coordinates to the nearest centimetre. I used a metric tape measure to measure the distance from the main stem of the individual to the reference point (x, y) = (0.0), located in the lower right corner of each plot (Fig.13.ii). Additionally, I recorded four coordinate points on shrub crowns to form an approximately ellipse shape to allow crown centres and crown surface areas to be considered rather than stem points when detecting associations between a functional groups of shrubs and targeted oak seedlings (chapter 4) (Fig.14). A total of 1438 shrubs and 4963 oak regenerated seedlings were recorded and mapped, i.e. an average of 451 seedlings per plot (1431.7 ha^{-1}) belonging to three Quercus species (31.61%, 34.21% and 34.17% for *Q. robur*, *Q. ilex* and *Q. suber*, respectively).

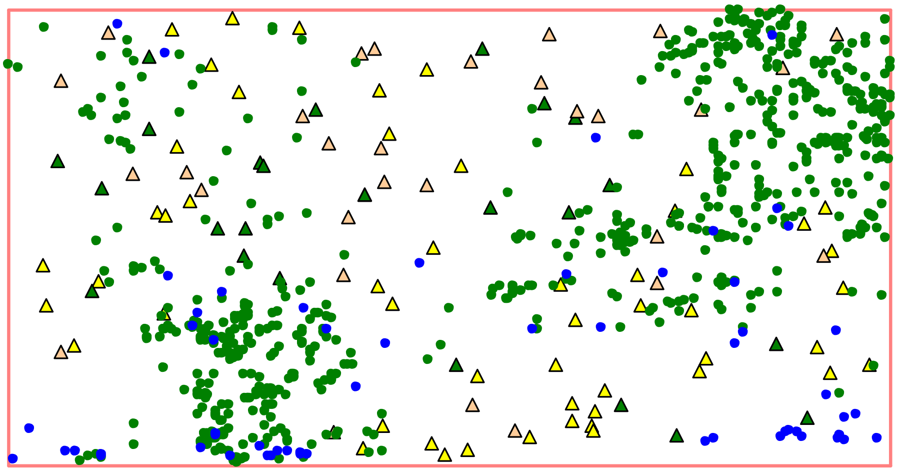
In these studies (chapter 3 & 4), I used three different methods of analysis; two belong to Point Pattern Analysis, pair correlation function and Ripley's *k* function (Fig.15) to test if on average there were more seedlings located around focal shrubs than expected under a certain null method. I also used the Chi-square to test if the presence of seedlings beneath the shrub canopy was independent of the functional group of shrubs (Fig.14).

Figure 13. i) Plot design under forest canopy, ii) Mapping shrubs and oak regeneration in 35 9 m^2 quadrates

Seignosse forest (Stem centroid method)



Seignosse forest (Crown centroid method)



Seignosse forest (Crown area)

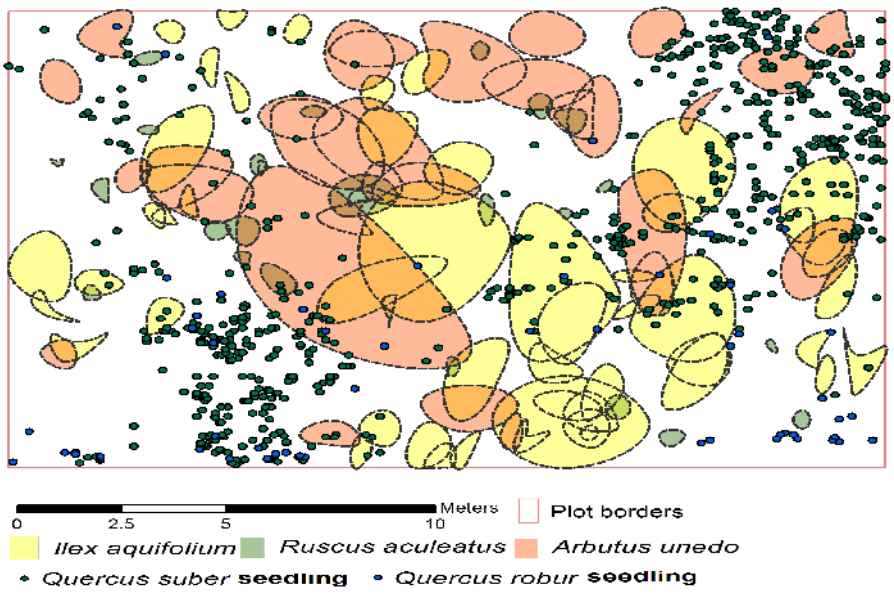


Figure 14. The three mapping methods which have been used to detect shrub-oak seedling spatial association patterns, stem method depending on the x,y coordinates of the stems of shrubs, crown centroid, depending on the centroid points of the crowns of shrubs and canopy area by drawing the crown area of shrubs.

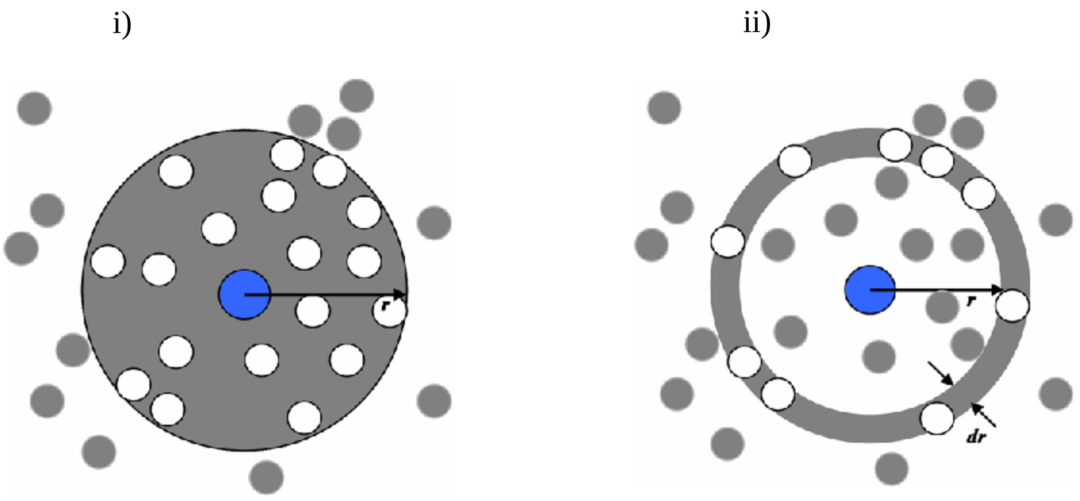


Figure 15. Numerical implementation of i) Ripley's *k*- function and ii) pair correlation function. Points of pattern 2 (seedlings) are represented by white and grey circles, the focal point of pattern 1 in blue represented shrub. For numerical implementation of Ripley's bivariate *k* function we count the number of the points of pattern 2 (white circles) inside the circle around the point of pattern 1 (blue circles) which falls inside the study plot (i.e., the grey area). For bivariate pair correlation function we count the number of the points of pattern 2 inside the ring around the points of pattern 1 which falls inside the study plot. Simply when the intensity λ_2 of the points 2 in circle or ring around the focal points at a certain distance of *r* more or less than the λ_2 over the study plot then we will detect attraction (facilitation) and repulsion (competition) respectively between points of pattern 2 and points of pattern 1. However, *k*-function and the pair correlation statistic respond to slightly different biological questions where *k*-function detect association pattern cumulatively up to a certain distance, whereas the pair correlation can detect association at a given distance *r* (Wiegand and Moloney, 2004).

Mapping of *Q. suber* (left) and *Q. robur* (right) regenerated seedlings

2-Transplant experiment

A transplant experiment was set up firstly to allow me to conduct a straightforward test of the functional strategy of the target species (by considering the same set of species at different levels of environmental conditions) which was not capable via spatial study since these species occur naturally in different climatic conditions. Secondly, it was because only a transplant experiment can allow to identifying directly the association-promoting mechanisms that may govern the early establishment of oak seedlings. I conducted the transplant experiment at the two ends of the gradient, since the central locations (Biscarrosse and Hourtin) appeared as transition zone between the two ends of gradint (chapter 3). In October 2010, I set up a split-split plot design with four factors (i) site representing the dry north and the wet south, (ii) canopy treatment (forest and gap) and (iii) neighbour effect (with and without shrubs) and (iv) species (*Q. robur, Q. ilex,* and *Q. suber* seedlings). In the closed forests and in the gaps, I delimited six blocks each block including 10 naturally occurring shrubs, half of the shrub individuals from each block was removed by severing stems just above-ground. At each of the experimental sites, I applied the experiment treatments by planting 1-2 year old bare-rooted oak seedlings under both canopy conditions in manually dug holes beneath shrubs and in the area where shrubs had been removed (Fig.16 i, ii, iii and iv.). Finally, I planted five seedlings per treatment and all replicated six times (*n* = 120 seedlings). Hence, 720 seedlings planted in all the experiment. The experiment lasted 18 months from October 2010 to March 2012.

The methods used are in more detail (i.e., specific measurements and statistical analyses) in each of the three studies presented in this Ph.D thesis (chapters 3, 4 & 5).

***Quercus ilex* seedlings**

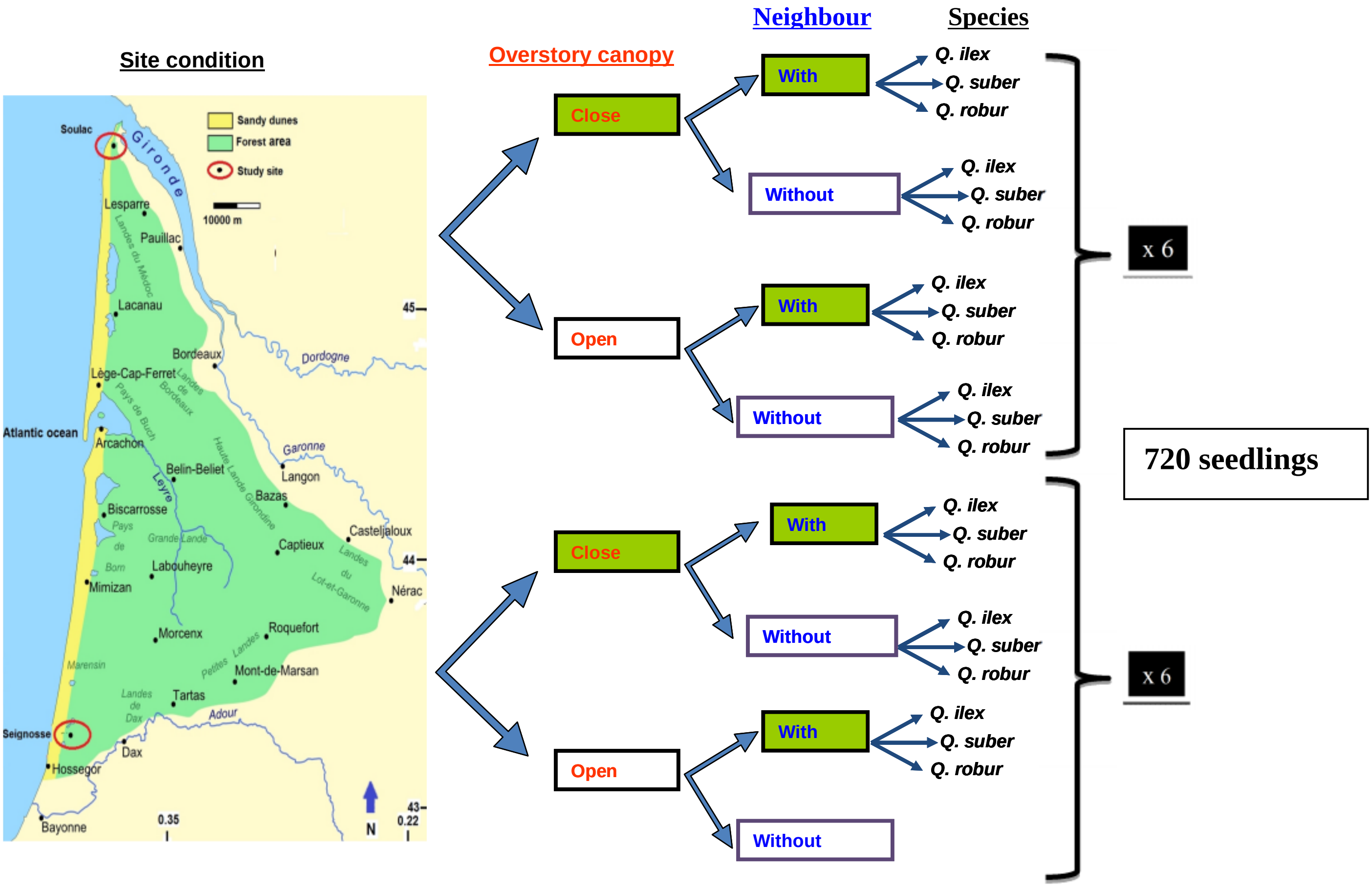


Figure 16. Schematic of the transplantion experimental design. (i) site condition representing the dry north and the wet south, (ii) canopy treatment (forest and canpy opening) and (iii) neighbour effect (with and without shrubs) and (iv) species (*Q. robur*, *Q. ilex*, and *Q. suber* seedlings), each treatment replicated six times (*n* = 240 seedlings of each species). Hence, 720 seedlings planted in all the experiment.

Figure 17. The effect of neighboring shrubs on the early survival of three oak seedlings species, *Q. suber*, *Q. Ilex* and *Q. robur* planted i) under shrubs in a forest plot ii) without shrubs in a forest plot iii) under shrubs in a gap plot iv) without shrubs in a gap plot. HOBO-Pro RH/Temp Data Loggers were set up to calculate VPD values under all treatments.

Chapter 3:

Summer drought and canopy opening increase positive associations between oak seedlings and understory shrubs in coastal sand dune forest communities.

Hassan MUHAMED, Jean-Paul MAALOUF, Richard MICHALET

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Abstract

- **Context:** The knowledge of how shrub-seedling interactions vary with summer drought, canopy opening and tree species is crucial for adapting forest management to climate change.
- **Aims**: The aim of this study is to assess variation in shrub-oak recruitment associations along a south- north drought climatic gradient and between two levels of canopy cover in coastal dune forest communities in a climate change-adapted forest management perspective.
- **Material and Methods:** Mapped data of associational patterns of seedlings of three oak species with inter-specific pooled shrubs were analyzed using a bivariate pair correlation function in 10 (0.315 ha) regeneration plots located in forest and recent gap sites along the climatic gradient. An index of association strength was calculated in each plot and plotted against a summer moisture index.
- **Results:** The association strength increased with increasing summer drought from wet south to dry north and from closed forests to gaps.
- **Conclusion:** Consistent with facilitation theory, our results suggest that climate change may shift associational patterns in coastal dune forest communities towards more positive associations, in particular in canopy gaps. In a perspective of climate change foresters may need to conserve understory shrubs in gaps, in order to promote oak species regeneration.

Keywords: facilitation; spatial association; point pattern analysis; stress gradient hypothesis.

Introduction

The threat of climate change is now recognized as an imminent issue in forestry and incorporating adaptation to climate change into forest management is crucial for the long-term sustainability of forest ecosystem services and diversity (Millar et al. 2007). Several options for climate change adaptation have been explored, such as the control of climatically unfavored species, provenance testing, assisted migration, insect and disease control, or modifying the components of timber harvesting (Steenberg et al. 2011). Another aspect of climate change-adapted forest management which has been rarely addressed is the adaptation of sylvicultural techniques to changes in interactions between tree regeneration and understory species due to climate change (Fotelli et al. 2001; Saccone et al. 2009), although a number of studies have shown that forest understory composition is currently changing with climate change (i.e. Mendoza et al. 2009; Suarez and Kitzberger 2008).

Interactions between understory species and tree regeneration are known to be crucial in forest management but most studies have rather emphasized negative effects of understory species (often called forest weeds) for both productivity and regeneration success (Balandier et al. 2006; Nambiar and Sands 1993). However, positive effects of woody or herbaceous species on tree seedlings or saplings are increasingly shown to occur in forest or woodland communities, in particular in severe environmental conditions (Callaway et al. 1996; Rodriguez-Garcia et al. 2011a). The “nurse plant syndrome” mostly includes two different processes, either environmental stress mitigation (i.e., direct facilitation) or associational defences against grazers and browsers (Callaway 2007). In addition, positive effects may arise from indirect interactions among competitors (Callaway 2007; Saccone et al. 2010). For example, Rodriguez-Garcia et al. (2011b), found that overstory canopy of adult trees indirectly facilitated *P. pinaster* seedlings growth via suppression of the direct negative effects of *Cistus ladanifer* shrubs on *P. pinaster* seedlings. It is widely considered that interactions among species in plant communities including forests are dominantly negative when environmental conditions are favourable and turn to positive when environmental stress increases (i.e. Stress Gradient Hypothesis (SGH), Bertness and Callaway 1994). This switch in interactions along stress gradients has been demonstrated in a number of experiments (see Callaway 2007 for a review) and facilitation is now considered as a useful tool for restoring woodlands in arid and semi-arid environments (Gomez-Aparicio 2009; Padilla and Pugnaire 2006).

Climate change is known to influence associations among species in plant communities (Brooker 2006; Saccone et al. 2009). Plant-plant interactions are strongly regulated by variation in climate conditions and climate change has been shown to also alter the direction and importance of interactions within plant communities (Brooker 2010). For example, Gomez-Aparicio et al. (2004) found in their meta analysis conducted in the Sierra Nevada mountains from Spain that the net facilitative effect of nurse shrubs on seedling survival and growth increased at low altitudes and sunny, drier slopes and decreased at high altitudes and shady, wetter slopes. Additionally, they found that facilitation was higher in the dry years than in the wet ones. Thus, interactions between tree seedlings and understory species are expected to change in forest communities with climate change and these changes deserve to be considered in climate change-adapted forest management strategies.

Thus, interactions between tree seedlings and understory species are expected to change in forest communities with climate change and these changes deserve to be considered in climate change-adapted forest management strategies. Based on the SGH either competition or facilitation should increase if climate change decreases or increases climatic stress, respectively (Brooker 2006). For example, in southern European latitudes where climate change is expected to increase summer drought (Intergovernmental Panel on Climate Change

2007), if the competitive effect of understory species on tree seedlings decreases, then there will be less need to control forest weeds for regeneration success. Furthermore, if facilitation increases, then foresters should rather conserve understory nurses to limit regeneration mortality due to drought.

However, it has been shown that competition may also remain important in water-stressed systems, in particular for tree seedlings competition with strong belowground competitors such as grasses (Fotelli et al. 2001). The role of facilitation in dry environments has been strongly debated but there is increasing evidence in recent studies that both competition and facilitation may occur in a same site depending on the species and the ecological factor involved in the interaction (Gomez-Aparicio 2009; Michalet 2007; Rodriguez-Garcia et al. 2011a; Saccone et al. 2009). Specifically, stress-intolerant species have been shown to be more sensitive to facilitative effects and stress-tolerant species to competitive effects (Liancourt et al. 2005). Furthermore, facilitation generally involves mostly non-resource factors (such as decrease in Vapor Pressure Deficit and extreme temperatures), whereas competition rather involves resource factors (such as water or nutrient availability), as shown by Saccone et al. (2009).

Additionally, species have both negative and positive effects on each other, generally at different times during a year or during ontogeny, which makes important to consider the net effect or the outcome of interactions (Callaway and Walker 1997) over a long time period, in particular in a forest regeneration perspective where regeneration success may only be evaluated after several years. A practical method to assess the long-term role of interactions for regeneration success is through the measurements of spatial associational patterns (Fajardo et al. 2006; Wiegand et al. 2006; Wiegand and Moloney 2004). For example, in a forest community a high frequency of seedlings located close to nurse shrubs does not mean that they never compete, but rather ensures that positive effects overwhelm negative ones on the long term (Padilla and Pugnaire 2006). Spatial patterns have recently received a strong renew of interest in ecology and spatial structure is considered to play an important role for species coexistence (McIntire and Fajardo 2009). This is in particular true for direct plant-plant interactions (Callaway 1997) and dispersal limitation (Nathan and Muller-Landau 2000) and in general for all coexistence processes that display strong temporal and spatial changes. Additionally, in forest communities there is a strong potential to study spatial patterns since there is a high availability of point pattern data (horizontal and vertical data arrangement) as compared to grasslands (Law et al. 2009; Wiegand and Moloney 2004).

The main goal of our study was to assess variation in the direction of tree seedlings-understory shrubs associational patterns with climatic drought and canopy opening in a perspective of climate change-adapted management.

The coastal dune communities of the Aquitaine region (south-west France) are very good systems to address this issue since there is a 240km-long strip of natural forest communities in similar sandy habitats but with varying climatic drought, ranging from wet south toward dry north. Additionally, three oak species of contrasting functional strategies are located in the sandy coastal forest communities along a latitudinal gradient, the temperate deciduous oak *Quercus robur* throughout the gradient and two Mediterranean evergreen species, *Quercus ilex* in the dry north, and *Quercus suber* in the wet south. Thus, in this system we may use space for time to test for the effect of climate change (time effect) through studying spatial variation in ecological processes across sites differing in their current climatic conditions (space effect) since there were few, if any, other confounding factors along the gradient, and in particular no soil variation.

We quantified associational patterns of the three oak seedlings with understory shrubs in forests and recent gaps across the climatic gradient. Consistent with the stress gradient hypothesis (SGH) and with the results of studies on species-specific responses to interactions (i.e., Gomez-Aparicio 2009; Liancourt et al. 2005), we make the following predictions:

1. Understory shrub - oak seedling associational patterns should shift from negative to positive with increasing summer drought along the south - north climatic gradient.
2. The removal of the forest canopy should increase positive associations between understory shrubs and oak seedlings due to increasing drought stress.
3. Association patterns may be species-specific (i.e. vary depending of the oak species) with drought-tolerant species more negatively associated with understory shrubs than drought-intolerant species.

Materials and methods

Sites, study species and forest canopy treatments

The study was conducted in the coastal dunes forests of the Aquitaine region (south-western France) stretching along the Atlantic Ocean from the Gironde estuary in the north (45°32’N, 1°05’W) to the mouth of the Adour River in the south (43° 41 ’N, 1°25’ W). All along the sand strip there are two kinds of dunes occurring in the region: mobile dunes with herbaceous communities close to the beach, and inland fossile dunes with forest communities at 1-10km distance from the beach. The climate is overall temperate oceanic with the rainiest period during winter, but mean annual precipitation decreases with increasing distance from the Pyrenees mountain range, from 1200 mm in the south to 750 mm in the north. Maximum temperatures also slightly decrease from 25.9°C and 17.6°C (summer and spring, respectively) in the south to 23.6°C and 15.4°C in the north (Meteo-France data, 2007). Three oak species dominate the forest communities, *Quercus robur, Quercus suber* and *Quercus ilex. Q. robur* is a common oak species in Europe, in particular in its northern and central parts where climate is temperate with no summer drought, whereas the two evergreen species are mostly found in the Mediterranean climatic area of southern Europe and north-Africa. These two Mediterranean oaks may also occur within the warm temperate or submeditteranean parts of southern Europe where there is no summer drought, but only on dry soils, like is the case in the coastal dunes of the Aquitaine region. Thus, there is a strong difference in drought-tolerance between the deciduous temperate *Q. robur*, and the two evergreen Mediterranenan species (Ozenda 1985). However, there are also differences in drought- and cold-tolerance between *Q. suber* and *Q. ilex* (David et al. 2007), the former inhabiting warmer and wetter Mediterranean climates than the latter (Ozenda 1985, Michalet 1991). In the coastal dunes of the Aquitaine region *Q. suber* is indeed mostly present in the south where the climate is wetter and milder, whereas *Q. ilex* is very abundant in the north where the climate is drier and less mild.

Five latitudinal positions were chosen along the south - north gradient of decreasing rainfall to select our plots in this study (Table 1). At each latitudinal position, for the canopy treatment a forest and a gap plot was selected when available. However, in the north there was only a forest plot in the northernmost site (Soulac) since we did not find a suitable gap. Additionally, only the gap of the second northernmost site (Hourtin) was analysed here since the forest plot did not include the inter-specific associations required in this study. Most plots were located in an east exposure, where remained the oldest oak forests, since foresters stopped logging oak forests only on this exposure at least 30 years ago. All forest sites (F) were mature forests with at least 75% canopy closure by mixed oak tree overstory and all open plots (G) were recent gaps created (3-5 years) by opening overstory canopy through seed tree cutting method. In each plot a uniform and homogeneous permanent 0.315 ha (21 × 15 m) plot was established in the middle part of a moderate to steep slope (between 15 and 25° slope). Because of topographic limitations, the Bisc. 2 G plot was 9 × 35 m arranged.

Table 2. Main characteristics of the study plots

Study plot	Canopy treatment	Coordinates	Distance to the northermost (km)	Aspect	Target oak species	Main shrub species
Soulac (So.)	Forest	45°32`N, 1°05`W	0	E	Q.robur, Q.ilex	Arbutus unedo, Ruscus aculeatus, Q. ilex
Hourtin (Ho.)	Forest	45°11’N, 1°07`W	76	E	Q.robur, Q.ilex	Q. ilex
	Gap	45°09’N, 1°10`W		E	Q.robur, Q.ilex	Cytisus scoparius, Q. ilex, Cistus salvifolius,Q. robur
Biscarrosse1	Forest	44°26’N, 1°13`W	102	E	Q.robur	Ilex aquifolium, Arbutus unedo, Q. robur
(Bisc.1)	Gap	44 °01’N, 1°12`W		E	Q.robur	Arbutus unedo, Q. robur
Biscarrosse2	Forest	44°27’N, 1°13`W	103	W	Q.robur	Cytisus scoparius, Arbutus unedo
(Bisc.2)	Gap	44°26’N, 1°12`W		W	Q.robur	Arbutus unedo, Cistus salvifolius, Cytisus scoparius, Ulex europaeus, Q. robur
Born (Bo.)	Forest	44°02’N, 1°17`W	222	E	Q.robur, Q.suber	Erica cinerea, Ulex europaeus, Q. robur
	Gap	44°01’N, 1°17`W		E	Q.robur, Q.suber	Cytisus scoparius
Seignosse (Se.)	Forest	43°41’N, 1°25`W	240	E	Q.robur, Q.suber	Arbutus unedo, Ilex aquifolium, Ruscus aculeatus
	Gap	43°41’N, 1°24`W		E	Q.robur, Q.suber	Erica cinerea, Calluna vulgaris, Ruscus aculeatus, Q. suber

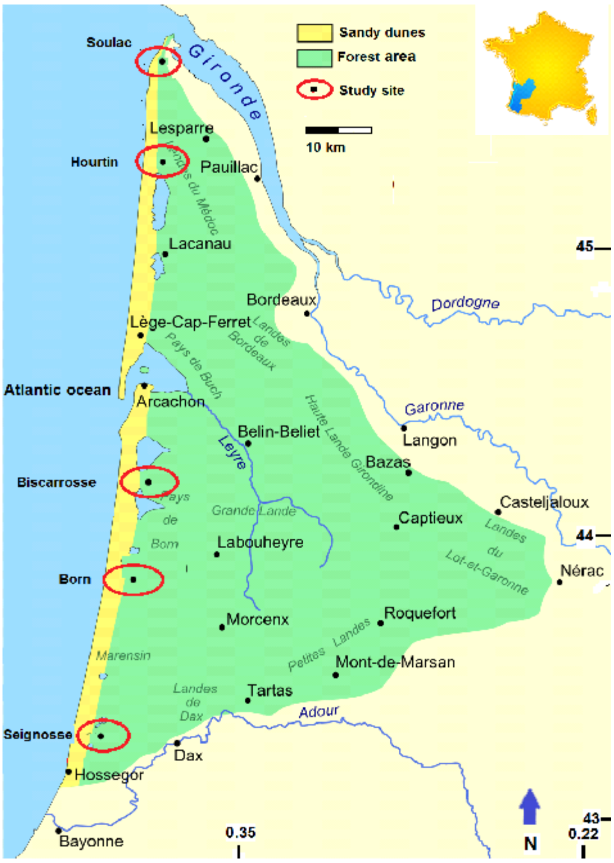

Fig. 1. Location of the five sites sampled along the Atlantic coast (Aquitaine, France).

Data collection and statistical analysis

In September 2009 the locations of oak species and shrub neighbours growing in each of the 10 plots were recorded (Table 1). Each plot was divided into 35 9m^2 quadrats using rubber tapes, and each quadrat was exhaustively surveyed. Individuals were classified in two categories: (i) seedlings (height < 25cm), (ii) shrubs (height > 50cm and DBH < 4cm). Because the minimum number of stems required to run the point pattern analyses (n=30) was not found for sapling (height between 25 and 50cm) and adult tree (DBH ≥ 4cm) categories in all plots, saplings and seedlings of each oak species were pooled into one category (oak seedlings) and the adult trees were excluded from the data set (Meyer et al. 2008). The cartesian coordinates (± 1cm) of all individuals of woody plants of a plot were recorded by using metric tapes along the longer (x-axis, 21m) and shorter (y-axis, 15m) sides of the plot. Additional tapes were placed every 3 m (35 9m^2 quadrats) to measure the coordinates of the centre of the main stem of each tree individual relatively to the reference point (x, y) = (0.0) of the plot, located in its lower right corner. The total number of mapped was 5731 with 4514 oak seedlings and 1217 shrubs individuals with average canopy area 223 cm^2.

Associational spatial patterns of oak seedlings with their shrub neighbours were analysed using the pair correlation function (Wiegand and Moloney 2004). This function is useful for analyse the second-order properties of fully mapped spatial point patterns within increasing distance scales. Compared to the more commonly used Ripley’s K function in point pattern data analysis, the pair correlation function has the advantage of being non-cumulative, where spatial pattern at finer scales does not influence the function at broader scales. Moreover, the pair correlation function tests for interactions between points separated by a distance r, whereas the cumulative K function confounds effects at larger scales with effects at finer scales and refers to interactions within a distance r (Getis and Franklin 1987).

Since we aimed at studying spatial associations between two point patterns (shrubs: pattern 1 and oak seedlings: pattern 2) at different spatial scales, we used the bivariate version of the pair correlation function. Mathematically, the pair correlation function is the normalized density of neighbouring pattern 2 points (i.e. the density of pattern 2 points at the plot scale) as a function of distance r from average pattern 1 points; Wiegand and Moloney 2004). It is defined as:

$$g_{12}(r) = \frac{\sum_{i=1}^{n_1} P_2\left[R_i^w(r)\right]}{\sum_{i=1}^{n_1} A\left[R_i^w(r)\right]} \Big/ \lambda_2 \qquad (1)$$

Where n_1 is the number of points of pattern 1; $R_i^w(r)$ is the ring with radius r and width w = 0.5 m centred around the ith point of pattern 1; P_2 is the number of points of pattern 2 within $R_i^w(r)$; A is the area of the ring; λ_2 is the intensity of point pattern 2. When, $g_{12}(r) > 1$, seedlings display attraction to shrubs (i.e. positive association). When $g_{12}(r) < 1$, seedlings exhibit repulsion from shrubs (i.e. negative association). When $g_{12}(r) = 1$, seedlings and shrubs are independent. Within each plot, we computed the bivariate pair correlation function considering radii ranging from 0.25 m to 3 m, with intervals of 0.25 m.

Since shrubs species composition naturally varies across plots we pooled the shrubs in each plot. In order to detect possible interspecific positive or negative associations between oak seedlings and pooled shrubs at increasing distances, we compared our data to the antecedent conditions null model to test whether during the establishment the pattern of oak seedlings is influenced by the pattern of shrubs. For that purpose the locations of the oak seedlings were randomized and shrub locations were fixed (assuming that oak seedlings may occur anywhere in the plot and that the shrub pattern did not change during the development of seedlings) (Wiegand and Moloney 2004). To test the significance of patterns against the null model, approximate (two - sided) 95% confidence envelopes were generated by calculating for each distance r of the 5th lowest and highest values using 99 Monte Carlo simulations of the null model; this yields an approximate 5% error rate. We performed goodness-of-fit tests in order to avoid the risk of inflating type I errors due to the simultaneous testing of the null hypothesis at several spatial scales (Diggle 2003). We used the Programita software for all point pattern analyses (Wiegand and Moloney 2004).

Effects of summer drought and canopy opening on the strength of the oak seedlings-shrub spatial association

In order to test if associational patterns of oak seedlings with pooled understory shrubs were influenced by the climatic drought and canopy opening, we calculated at each site an index of association strength (IA, Fajardo et al. 2006).

$$IA(r) = \frac{g_{12}(r) - 1}{|CE - 1|} \qquad (2)$$

This index $IA(r)$ is a ratio between the observed value of $g_{12}(r)$ and the corresponding y coordinate of the upper (if $g_{12} > 1$) or lower (if $g_{12} < 1$) confidence envelope (CE) at distance r. We calculated this index at three distances (1, 2 and 3 m), assuming that beyond 3 m the patterns are more influenced by environmental heterogeneity than by biotic interactions (Wiegand et al. 2004). In order to characterize the climatic drought stress acting at each of our 10 plots, we calculated a Summer Moisture Index (SMI) derived from the Lang index (1920):

$$SMI = \frac{P_{summer}}{\overline{T}_{\max}} \qquad (3)$$

Where P and T representing the precipitation and the mean of maximum temperatures calculated for the three summer months (June, July and August). This index decreases with increasing summer drought, from south to north in our study. The climatic data used here were the average of the 1998-2008 period obtained from nearest meteorological stations to the study sites (Meteo-France data. 2008).

Since the three oak species were not present naturally at all sites, the three species were pooled. At each of the three studied distances, effects of summer drought and canopy opening treatments on IA values of shrub-oak seedlings associations were analysed using quadratic Analysis of Covariance (ANCOVA) models including one factor (canopy treatment: forest or gap) and one covariate (summer drought represented by the SMI gradient). For each of the three distances taken into consideration, the ANCOVA model may be summarized as follows:

$$Y_{ij} = \mu + \alpha_i + \beta \, SMI_{ij} + \gamma_i SMI + \delta \, SMI_{ij}{}^2 + \eta_i SMI_{ij}{}^2 + \varepsilon_{ij} \qquad (4)$$

Where Y_{ij} is the index of association strength (IA) measured on individual j under canopy condition i, μ is the global mean of IA, α_i is the mean contribution of canopy condition i to the mean of the IA index, β is the mean slope, SMI_{ij} is the summer moisture index undergone by individual j under canopy condition i, γ_i is the contribution of canopy condition i to the slope, δ is the mean quadratic coefficient, η_i is the contribution of canopy condition i to the quadratic term, and ε_{ij} is the residual error. Normality and homoscedasticity of residuals assumptions were met in all models. All analyses were performed using the R software for statistical computing (version 2.10.1, 2010).

Results

Overall, the spatial pattern analysis revealed dominant significant positive associations in the two northern sites (Soulac and Hourtin) and dominant significant negative associations in the southernmost site (Seignosse), whereas most spatial patterns were not significant in between (i.e. in the centre of Aquitaine (Biscarrosse) and in the other southern site (Born)) , Table 2. More precisely in the north, and for both *Q. robur* and *Q. ilex* seedlings the spatial associations were positive at all distances in the gap plot (Ho.G), but only at small distances in the forest plot (So.F), with even independent associations occurring up to 3 meters distance (Table 2; Figs. 1a and 1b). In the southernmost site, where both *Q. robur* and *Q. suber* seedlings were present, spatial associations for both species were significantly negative at all distances in the forest (Se.F) but only at small distances in the gap (Se.G), with even positive associations occurring at 2 meters distance for *Q. robur* (Table 2; Figs. 1g and 1h). Finally, in between, i.e. in the Born and Biscarrosse sites, 6 cases among 8 were not significant and the two significant cases observed in gaps for *Q. robur* were quite complex to understand; at small distances there were positive associations in the Born site and negative associations in the Biscarrosse 1 site but these patterns turn to independent up to 3 meters distance (Table 2; Figs.1d, and f).

Table 2. Signs of the spatial associational patterns between oak seedlings and interspecific shrubs based on bivariate $g_{12}(r)$ function for the 10 plots. +: significant positive association; - : significance negative association; no symbol indicates no significant association. See Table 1 for site abbreviations. F and G are forest and gap plots, respectively. n1 and n2 correspond to the number of shrubs and of target oak species, respectively. *P*-value computed through goodness-of-fit test used for overall significance of patterns over the complete range of *t*

			Distance *t* (m)												
Site	**Target oak species**	**n1/n2**	**0.25**	**0.5**	**0.75**	**1**	**1.25**	**1.5**	**1.75**	**2**	**2.25**	**2.5**	**2.75**	**3**	***P***
So.F	***Q. robur***	155/34			+	+									0.01
	Q. ilex	47/1067			+	+	+	+	+						0.02
Ho.G	***Q. robur***	122/147	+	+	+	+	+	+	+	+	+	+	+	+	0.01
	Q. ilex	85/192	+	+	+	+	+	+	+	+	+	+	+	+	0.01
Bis.1F	***Q. robur***	130/198				+									0.4
Bis.1G	***Q. robur***	53/134	-	-		-	-								0.01
Bis.2F	***Q. robur***	162/41													0.69
Bis.2G	***Q. robur***	134/80	-	-											0.06
Bo.F	***Q. robur***	48/63							-						0.34
	Q. suber	101/66	-			-									0.15
Bo.G	***Q. robur***	83/92	+	+	+								-	-	0.01
	Q. suber	96/58													0.7
Se.F	***Q. robur***	107/58	-	-		-	-	-	-	-	-	-	-	-	0.01
	Q. suber	107/627	-	-	-	-	-	-	-	-	-	-	-	-	0.01
Se.G	***Q. robur***	127/735			-	-	-				+	+	+	+	0.01
	Q. suber	114/922	-	-		-									0.04

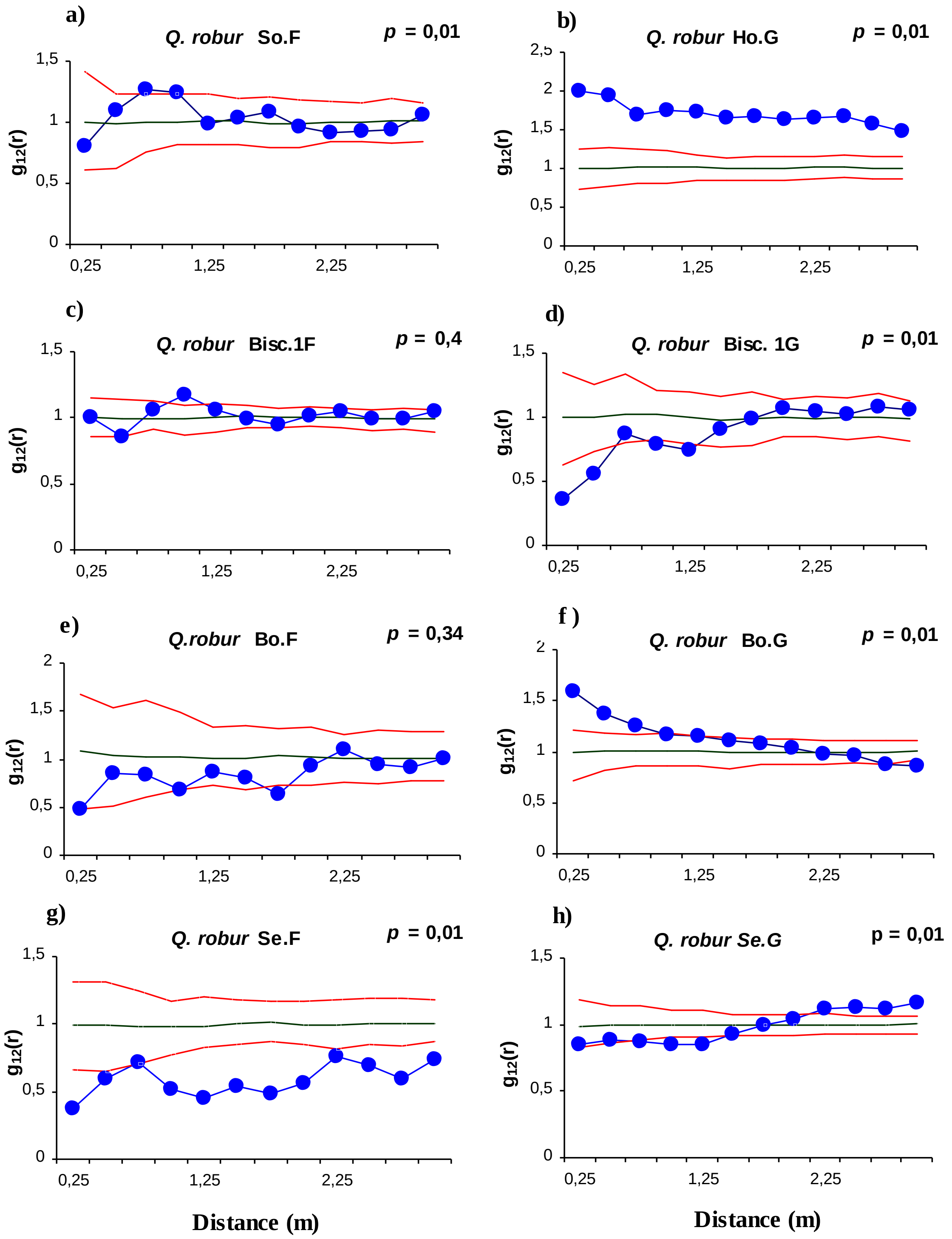

Fig. 2. The interspecific association patterns of shrub-*Q. robur* seedling up to 3 m scale along 240 km climatic gradient . The blue dots line represents the observed pair correlation values $g_{12}(r)$. The red lines represent the confidence envelope constructed by using the 5th-heights and 5th-lowest value of 99 Monte Carlo simulations of the null model. Values above and below the envelope indicate significant positive and negative associations, respectively. See Table 1 for site abreviations.

The index of the association strength was significantly affected by the summer drought of the sites at the three analysed distances (from 1 to 3m) (P = 0.03, 0.02 and 0.03 respectively), with a shift from positive values in the dry north to negative values in the wet south (Table 3; Fig. 2). There was also a significant effect of the canopy treatment, but only at 2m and 3m distance (P = 0.03, and 0.03 respectively) (Table 3; Fig. 2). Furthermore, there was a significant interaction between the summer drought and canopy treatments (P = 0.05, 0.03 and 0.03 respectively) due to the occurrence of significant effects of the canopy treatment only at the driest and wettest ends of the summer drought gradient, but not at intermediate position (Table 3; Fig. 2). Finally, the occurrence of significant quadratic interaction terms at all distances shows that the IA curve switches from convex to concave when passing from the forest to the gap (Table 3; Fig. 2). Considering the summer moisture index interval of this study (see Fig. 2), this means that the IA reached zero asymptotically at the wet end of the gradient in gaps, whereas its decrease towards negative values was accelerated along the drought gradient under forest canopies.

Species effects could not be tested statistically since all species were not present at all sites. However, there were graphically no obvious differences in species responses within sites, i.e. between *Q. robur* and *Q. ilex* at the two northern sites and between *Q. robur* and *Q. suber* at the two southern sites (Fig. 2).

Table 3. Results of ANCOVAs tests for the effects of summer drought, canopy opening and their interaction on the index of association strength at 1, 2 and 3 m distances.

Treatments	Distances					
	1m		2m		3m	
	F	*P*	*F*	*P*	*F*	*P*
Canopy	4.934	0.052	6.690	0.027	6.772	0.026
Summer moisture	6.787	0.026	9.109	0.012	5.875	0.035
Summer moisture²	7.612	0.020	9.947	0.010	6.333	0.030
Canopy x Summer moisture	4.921	0.050	6.716	0.026	6.671	0.027
Canopy x Summer moisture²	4.982	0.049	6.886	0.025	6.708	0.026

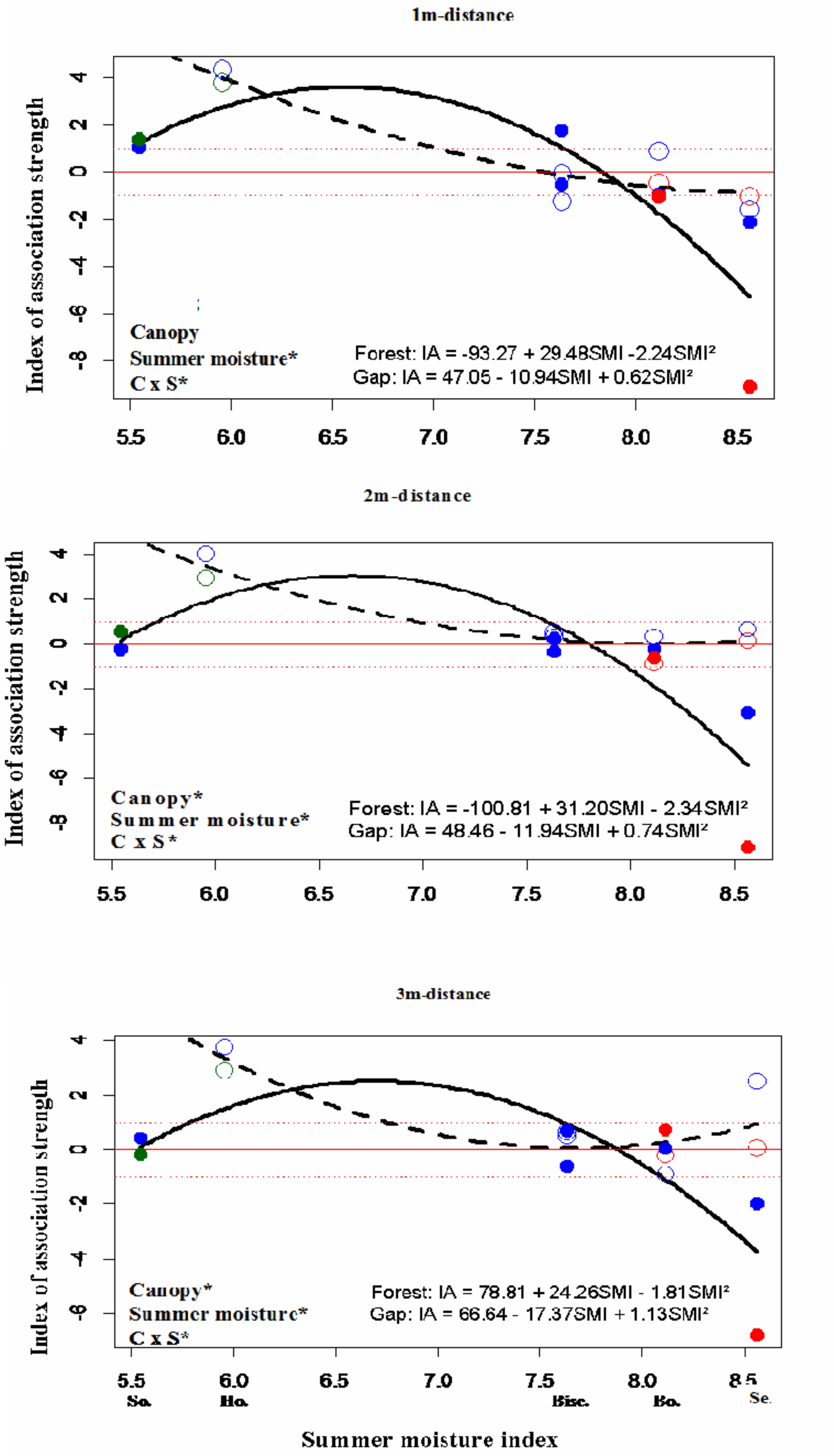

Fig. 2. Regressions of the Index of association strength, calculated at three distances (1, 2 and 3m), against the summer moisture index for the 10 plots and the three target oak species. The full and empty circles represent the forest and gap plots, respectively, whereas the blue, red and green circles represent *Q. robur*, *Q. suber* and *Q. ilex* targets, respectively. The full line represents the regression for the forest plots, whereas the dashed line the regression for the gap plots. The two dashed red lines represent the confidence envelope area around the expected 0 value of $g_{12}(r)$.See Table 1 for site abbreviations.

Discussion

In agreement with our two first hypotheses, both summer drought and canopy removal increased positive associations between oak regeneration and understory shrubs in the coastal sand dune forest communities of the Aquitaine region. Although our design did not allow us to fully assess the species effect in our study, no obvious differences in species responses were recorded within sites (i.e. between *Q. robur* and *Q. ilex* at the two northern sites and between *Q. robur* and *Q. suber* at the two southern sites (Fig. 3)), which shows at least that the response of the deciduous *Q. robur* did not differ from that of the two evergreen species.

Positive associations increase with summer drought and canopy opening

Our results showed that positive spatial associations between oak seedlings and understory shrubs significantly dominated in the dry climate of the north of Aquitaine and spatial negative associations were more frequent in the wet climate of the south of Aquitaine. Additionally, canopy opening increased the positive effect of the shrubs in the dry north but decreased its negative effect in the wet south toward random or positive patterns. These results support the SGH (Bertness and Callaway 1994) arguing that positive effects of woody or herbaceous species on tree seedlings or saplings are more frequent in open forests or woodland communities from severe environments than in closed forests from benign climatic conditions (Callaway et al. 1996; Rodriguez-Garcia et al. 2011a; Saccone et al. 2009). However, it has also been shown that both facilitation and competition may occur through time in a single site (Fotelli et al. 2001) but that the net balance depends on the species and the ecological factor involved in the interaction (Gomez-Aparicio 2009; Michalet 2007; Saccone et al. 2009; Pugnaire et al. 2001). Specifically, Michalet (2007) proposed that facilitation should rather involve non-resource factors (such as decrease in Vapor Pressure Deficit, extreme temperatures and photoinhibition), whereas competition is more likely to occur when the limiting factors are resources (such as water or nutrient availability). For example, Saccone et al. (2009) showed during the 2003 European heat-wave in a single system that the forest canopy facilitated seedlings of *Abies alba* through atmospheric stress mitigation but that adult trees competed for water with the seedlings of *Picea abies*. Although we did not measure environmental conditions in our study, it is very likely that the positive effects of understory shrubs in the dry north of Aquitaine was not due to decrease in soil water stress in these sandy soil conditions but rather to decrease in irradiance, vapor pressure deficit and extreme temperatures. Indeed, in the north, positive associations with shrubs were much stronger in the gaps than in the forests, likely because oak seedlings were protected from atmospheric stress by the tree canopy in the forests but not in the gaps. Additionally, all neighbours in our study were shrubs, known to provide strong aboveground positive effects and low belowground negative effects, as compared to grasses (Castro et al. 2004; Gomez-Aparicio 2009; Michalet 2007). However, in the gaps of the dry north positive associations may also be due to indirect facilitative effects of shrubs, i.e. grass competition release (Callaway 2007; Cuesta et al. 2010).

Although we are aware that only a removal study may allow us to conclude on the real occurrence of shrub-oak seedling interactions in our study, our results are very unlikely to have been influenced by differences in soils between shrubs and open areas since our observations showed that the sandy soils were very similar between both patches. The occurrence of important differences in soils depth between patches within communities is indeed more common in arid and semi-arid rocky areas than in sandy oceanic communities as those of our study, due to the importance of soil erosion processes in the former (Michalet 2006). Thus, the interest to have assessed spatial patterns instead of real plant interactions in our system is certainly to have gained access to the net balance of interactions likely to occur through long-term interactions (Callaway and Walker 1997). Such information is certainly of higher value for managing tree regeneration than short-term removal study.

Spatial patterns were not influenced by target species functional strategies

We were not able with our design to assess the difference in response between the two evergreen oaks since they naturally occur in different climatic conditions. However, our design allowed us to assess differences in responses to the shrubs between *Q. robur* and *Q. ilex* or *Q. suber* at the northern and southern sites, respectively. Since temperate deciduous oaks, and in particular *Q. robur*, are known to be less tolerant to drought stress than evergreen Mediterranean oaks (Ozenda 1985), we hypothesized that the former will be more demanding of environmental amelioration by neighbours than the latters. Indeed, a number of authors have shown that drought-intolerant species are better candidates for facilitation than drought-tolerant ones since the benefit of having neighbours mitigating drought stress is higher for the former than for the latter (Liancourt et al. 2005; Michalet 2007; Saccone et al. 2009). Additionally, since there is a trade off between drought-tolerance and shade-tolerance in particular for tree species (Smith and Huston 1989), the addition of shade by neighbours is inducing a higher cost for a drought-tolerant species than for a drought-intolerant one.

If, however, most of the facilitative effect of understory shrubs is not improving soil water availability but rather a mitigation of atmospheric stress and photoinhibition through decreasing irradiance as stressed above, then our three species may not differ enoughly in their atmospheric and light requirements to produce contrasting patterns of association with shrubs. Indeed, the three species have not contrasting light requirements since both evergreen species are late-successional species in a sunny Mediterranean context, i.e. avoiding atmospheric stress in full light conditions (Cuesta et al. 2010; Penuelas et al. 2001; Perez-

Devesa et al. 2008), whereas *Q. robur* is rather considered as a post-pioneer species, requiring gaps for regeneration in a shady temperate context (Cater and Batic 2006; Rozas 2003).

Conclusions

Our study showed that spatial patterns of association between understory shrubs and oak seedlings are very sensitive to increasing drought and canopy opening independent of the oak species. Climate change models predict a strong decrease in summer rainfall in the Aquitaine region with a shift from a current warm temperate climate toward a submediterranean climate at the end of the century. Our results suggest that with climate change the positive effect of understory shrubs on tree seedlings should increase in the forest gaps of the north of Aquitaine, Thus, in a perspective of climate change-adapted management foresters should rather conserve understory nurses in gaps in order to limit oak seedlings mortality due to drought and extreme irradiance. However, we are aware that measuring seedling performance parameters in relation to presence and absence of the shrubs may further support our observational results of this study. Therefore, experiments including transplantations of the three species all along the gradient are currently being conducted in this system. Such design will allow us to also more straightforwardly test the role of target species functional strategy, since all oak species do not occur in all climatic conditions. Additionally, environmental measurements (light, soil moisture and VPD) should be conducted in order to analyse the relative roles of atmospheric vs. soil water stress in driving changes in biotic interactions between understory shrubs and oak seedlings along complex environmental gradients.

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Chapter 4:
Spatial associations between oak seedlings and shrubs: gaining insight into regeneration dynamics in coastal dune forest of south-western France

Hassan MUHAMED, Emanuele LINGUA , Jean-Paul MAALOUF, Richard MICHALET

Under review in Canadin journal of forest research

Abstract.

- **Context and aims:** The assessment of spatial association patterns of tree seedlings with understory species in forest communities is an important first step for understanding the role of biotic interactions as well as seed dispersal in tree regeneration. In this study, we applied spatial and non-spatial analysis methods to asses the relative importance of different functional groups of shrubs for the early establishment of oak regeneration seedlings in the coastal forest communities of south-western France.
- **Material and Methods:** 11 315 m^2 regeneration plots were selected in forests and gaps located in the costal dune forest communities of the Aquitaine Region. Within each plot, oak seedlings and shrubs canopy were mapped. The spatial pattern of the seedlings of three oak species (*Q. robur*, *Q. suber* and *Q. ilex*) was analysed separately for each species using univariate pair correlation function. Association patterns between the seedlings of the three oaks, pooled in a single group, and 4 functional groups of shrubs (Fagaceae, Ericaceae, Legumes and Spiny shrubs) were analysed using two methods, the pair correlation and Ripley's *k*-functions for shrub canopy centroids data, and the chi-square tests for total shrub canopy areas data.
- **Results:** Oak seedling species were spatially aggregated at short distance scales in most regeneration plots. The functional types of shrubs differed in their associational patterns with oak seedlings. Associations with Fagaceae shrubs were positive but mostly intraspecifically, and there were negative associations with Ericaceae and neutral patterns with the two other groups.
- **Conclusion:** Different oak species likely distributing in aggregation in the early establishment phase. We concluded that the aggregated distribution of seedlings of the three oak species was due to short-distance seed dispersal, which also was likely the primary cause of positive spatial associations between oak seedlings and Fagaceae shrubs, although the

contribution of intraspecific shrubs in facilitation oak seedlings should be assessed in future experimental studies. We recommand to forest managers to focus on the control of Ericaceae, while preserving Fagaceae in order to enhance oak recruitment in this system,

Keywords: Ericaceae, facilitation, nurse shrub, oak seedling, spatial association, tree regeneration.

Introduction

Natural regeneration represents a highly complex process in forest dynamics since many mechanisms are involved (Paluch, 2005). Within a single forest community, this process is mainly the result of seed dispersal strategies, spatial heterogeneity of microhabitats and local neighbouring interactions which may separately or jointly control the establishment of tree regeneration (Collins and Battaglia, 2002; HilleRisLambers and Clark, 2003). During the early phase of the regeneration cycle, tree seedlings are influenced by the presence and abundance of overstory canopy. In particular, shrubs are recognized as important tree regeneration niches, because their canopies could have facilitative effects on the establishment of different tree species (Callaway, 1995; Gomez-Aparicio *et al.*, 2005; Rodriguez-Garcia *et al.*, 2011). However, only some shrub species have a positive effect on tree regeneration through amelioration of climatic and edaphic conditions below their canopies (i.e., interspecific facilitation or the "nurse plant effect", Calloway 1995) while others have effects varying between competitive to neutral (Callaway, 1995, Gomez-Aparicio, 2009). "Fertile islands" formed by shrubs are frequently more favourable to other species recruits than to their own offsprings (Garner and Steinberger, 1989), although intra-specific facilitation has been observed between individuals of different-sized cohorts with large individuals protecting small seedlings of the same species (Fajardo and McIntire, 2011).

In forest ecosystems, the initial horizontal distribution pattern of seedlings and their vertical spatial associations with the overstory canopy are considered a fundamental step to be identified in natural regeneration studies. These two-dimensional spatial patterns of individual trees in a forest govern long-term forest community dynamics (Lookingbill and Zavala, 2000), by influence competition levels, growth rates, and understory development (Duncan, 1991; Chen and Bradshaw, 1999; Goreaud *et al.*, 1999). Fine-scale spatial patterns of woody plant regeneration have been studied to uncover causal mechanisms that facilitate species coexistence (Goreaud and Pelissier, 2003; Wiegand *et al.*, 2007; McIntire and Fajardo, 2009), since coexistence mechanisms operating in the forest should leave a spatial signature that could be detected through maps of individual locations (Wiegand *et al.*, 2007). Spatial point pattern analysis is considered one of the most common approaches used to infer positive and negative plant-plant interactions (Haase, 1996; Dale 1999), and had been used in various ecosystems and for many species. (Wiegand and Moloney, 2004). Mainly, observed patterns at small spatial scale have been related to either positive or negative plant interactions that have the potential to structure local plant neighborhoods (Phillips and Macmahon, 1981; Valiente-Banuet *et al.*, 2006). For example, regular plant spatial patterns are often referred as a sign of intense competition between plants for limited resources (Stoll and Bergius, 2005; Rayburn and Monaco, 2011). In contrast, more aggregated patterns (especially interspecific) are interpreted as evidence of positive plant interactions (Eccles *et al.*, 1999; Kefi *et al.*, 2007); the random patterns suggest no direct strong influence of any process in shaping spatial relationships (Szwagrzyk and Czerwczak, 1993).

In coastal dunes communities, the major limiting factors for plant regeneration are high solar radiation and summer temperatures, and the scarcity of available water and nutrients (Barbour et al. 1985; Ehrenfeld, 1990; Ishikawa *et al.*, 1995; Maun, 1998). Microenvironmental changes provided by nurse plants are important for seedling establishment particularly in seasonally dry ecosystems (Li and Wilson, 1998), since there is a high seedling mortality rate during this critical stage (Pugnaire and Luque, 2001; Franks, 2003). For example, in Mediterranean, sub-Mediterranean, and Atlantic coastal sand dunes where water stress and summer temperatures impose severe constraints on seedling establishment, shrubs frequently improve seedling regeneration (Shumway, 2000; Martinez, 2003; Sternberg *et al.*, 2004; Forey *et al.*, 2009; Cushman *et al.*, 2010). In the Aquitaine region (south-western France), the coastal dune forest communities are inhabited mainly by three oak species within a spatially heterogeneous matrix of shrubs. In a previous study conducted in the same system, we showed that the three species do not differ significantly in their association patterns with shrubs (Muhamed *et al*., 2012, submitted). The main purpose of this study was to evaluate spatial associations between oak seedlings (independent of species) and different shrub groups to better understand oak regeneration dynamics. We analyzed spatial associations using both spatial and non-spatial methods. Additionally, we analyzed the spatial patterns distribution of oak seedlings separately for each species. Our study may help to understand the possible ecological mechanisms occurring during the early establishment of oak species in the coastal dune forests. Furthermore, our results may provide information on how to design appropriate management practices through the identification of the role of the different functional groups of shrub species in the natural establishment of oak seedlings.

Based on previous work, we expected that (i) all three species of oak seedlings would be aggregated at fine scales, and (ii) pooled across species; oak seedlings would display different associational patterns with the four groups of shrubs.

Materials and methods

1-Study site and target species

The study was conducted in the coastal sand dune forests of the Aquitaine Region (south-western France). This region comprise an approximately 240 km-long strip of coastal dunes stretching along the Atlantic ocean from the Gironde river estuary in the north (45°32’N, 1°05’W) to the mouth of the Adour river in the south (43°41’N, 1°25’W). All along the coast, oak forest communities occur in the inland fossil dunes at approximately 1-10 km distance from the ocean. The climate is overall temperate oceanic with the rainiest period occurring during winter. Mean annual precipitation decrease with increasing distance from the Pyrenees mountain range northward (1300 mm in the south to 750 mm in the north). Mean annual temperatures also decrease slightly but significantly along this latitudinal gradient, from14.1°C in the south to12.8°C in the north (Sardin, 2009). The soil is very homogeneous all along the coast and is a young podzosoil with a pure coarse sand texture (Forey *et al.*, 2008).

The study sites were distributed within five positions all along the coast (Table 1). At each position, we selected two plots, one in the forest and one in the gap. However, in the northernmost site (Soulac) we only established one forest plot since we could not find suitable gap for the study. All plots had an eastern exposure, where the oldest oak forests remained because foresters stopped logging oak trees in this exposure since 30 years ago. However, in the central part of the latitudinal gradient, we established two additional plots with a west-facing slope. Within each plot, a uniform and homogeneous permanent 0.315 ha plot was delimited in the middle part of a moderate slope (steepness varying between 15 and 25°).

We chose to study the regeneration of the three dominant oak species in the region, the evergreen sclerophyllous *Quercus ilex* and *Quercus suber* and the deciduous *Quercus robur*. Although the three species have different stress-tolerance strategies, they are all highly or intermediately shade-tolerant since they are rather mid-succesional species as most Fagaceae species (Rameau *et al.*, 1992). *Q. ilex* is certainly the most drought-tolerant of the three species and occurs mostly in the drier part of the gradient, in the north. *Q. suber,* which is more cold-and drought-sensitive than *Q. ilex*, mainly occurs in the wettest and mildest part of the gradient in the south (Michalet, 1991; Davis *et al.*, 1998; Garcia-Mozo *et al.*, 2001). *Q. robur* occurs all along the gradient but is abundant in the central part of the gradient where the two other species are rare (Sardin, 2009).

Table 1. Main characteristics of the study plots

Study plot	Canopy treatment	Coordinates	Aspect	Target oak species	Main shrub species
Soulac (So.)	Forest	45°32`N, 1°05`W	E	*Q. robur, Q. ilex*	*Arbutus unedo, Ruscus aculeatus, Q. ilex*
Hourtin (Ho.)	Forest	45°11’N, 1°03`W	E	*Q. robur, Q. ilex*	*Q. ilex*
	Gap	45°09’N, 1°10`W	E	*Q. robur, Q. ilex*	*Cytisus scoparius, Q. ilex, Q. robur*
Biscarrosse 1	Forest	44°26’N, 1°13`W	E	*Q. robur*	*Ilex aquifolium, Arbutus unedo, Q. robur*
(Bisc.1)	Gap	44 °01’N, 1°12`W	E	*Q. robur*	*Arbutus unedo, Q. robur*
Biscarrosse 2	Forest	44°27’N, 1°13`W	W	*Q. robur*	*Cytisus scoparius, Arbutus unedo*
(Bisc.2)	Gap	44°26’N, 1°12`W	W	*Q. robur*	*Arbutus unedo, Cytisus scoparius, Ulex europaeus, Q. robur*
Born (Bo.)	Forest	44°02’N, 1°17`W	E	*Q. robur, Q. suber*	*Erica cinerea, Ulex europaeus, Q. robur*
	Gap	44°01’N, 1°17`W	E	*Q. robur, Q. suber*	*Cytisus scoparius*
Seignosse (Se.)	Forest	43°41’N, 1°25`W	E	*Q. robur, Q. suber*	*Arbutus unedo, Ilex aquifolium, Ruscus aculeatus*
	Gap	43°41’N, 1°24`W	E	*Q. robur, Q. suber*	*Erica cinerea, Calluna vulgaris, Ruscus aculeatus, Q. suber*

2-Data collection

In September 2009, we mapped the woody vegetation within the 11 0.315 ha rectangular plots by dividing each plot into 35 9m^2 quadrats and exhaustively searching each quadrat. The Cartesian coordinates of each plant were recorded by using a measuring tape to estimate the distance (± 1 cm) of the main stem of each individual to a reference point (x,y = 0.0) located in the lower right corner of the plot (Batllori et al., 2010). We assigned each plant to one of four size categories: (i) seedlings (height < 25cm, (ii) saplings (25<height<50cm), (iii) adult trees (diameter at breast height [DBH] ≥ 4cm, and (iv) shrubs (height > 50cm and DBH < 4cm). For shrubs, we also recorded the four coordinate points defining the crown (approximated to an ellipse). Because of low sample size, we excluded adult oak trees from the analysis and pooled oak seedlings and saplings into one category. Moreover, since shrub species composition and abundance varied widely across plots we pooled shrubs into four functional groups based on taxonomy and morphological traits. (i) Fagaceae (*Q. robur, Q. suber, Q. ilex*); (ii) Spiny shrubs (*Ilex aquifolium, Ruscus aculeatus*); (iii) Fabaceae (*Ulex* *europaeus, Cytisus scoparius,*) and (iv) Ericaceae (*Arbutus unedo, Erica cinerea, Calluna vulgaris*).

3-Data Analysis

A previous spatial pattern study in the same system has shown no significant variation in the interactions between the three oak species (*Q. robur, Q. ilex* and *Q. suber)* and pooled interspecific shrub species (Muhamed et al., 2012 submitted). In addition, because the three oak species do not occur across all plots (i.e., *Q. ilex* is only present in northern plots while *Q. suber* only occurs in southern plots) for the associations analysis in this study we pooled the seedlings of the three oak species in one category. Moreover, since shrub functional groups are not constantly replicated in all sites and canopy conditions, the effect of canopy types and sites conditions was also disregarded here. Thus, the main aim of this study is to identify the associational patterns of different functional groups of shrubs with seedlings of pooled-oak species in the coastal dune forests. For this main question, we used both spatial and non-spatial methods to evaluate seedling-shrub associations. In addition, we also analysed the spatial distribution patterns, separately for the seedlings of each of the three oak species, using univariate pair correlation function (Wiegand and Moloney 2004, see 3-1).

3-1-Univariate distribution patterns

To characterize the horizontal distribution of the seedlings of each oak species we used the univariate pair correlation function adopting the null model of complete spatial randomness (CSR) (Wiegand and Moloney, 2004). The univariate pair correlation function $g_{11}(r)$ can be defined as the expected density of points 1 (oak seedlings) in a ring of radius *r* and width *dw* centered in an arbitrary point, divided by the intensity (λ) (λ= n1/area of plot) of points 1. Values of $g_{11}(r)$ greater than, equal to or lower than 1 indicate spatial aggregation, randomness, and segregation distribution, respectively at a given distance (Wiegand and Moloney, 2004).

3-2- Bivariate association patterns

To detect the association patterns between oak seedlings and the four functional groups of shrubs, we first used two widely common spatial point analysis functions, Ripley's k and pair correlation $g_{12}(r)$, used with the x,y coordinates of shrub crown centroids as input. Second, non-spatial analysis was used for testing the independency between the abundance of oak seedlings individuals and estimated canopy surfaces of the different functional groups of shrubs (chi-square independency tests). The Chi-square independency null model is non-spatial in the sense that it does not depend on the x and y-coordinates of the plant. In other words, the presence of oak seedling depends only on shrub canopy area; see Schleicher et al. (2011).This second method may help to overcome the spatial point pattern limitation where shrub canopies are reduced to point locations (Wiegand and Moloney, 2004).

3-2-1- Ripley's *k* and pair correlation functions (Spatial method)

To assess the influence of each functional group of shrubs on oak seedlings regeneration we relocated shrub stems x, y coordinates to the crown centroids expecting that interactions are more likely mediated by local light conditions beside the crown asymmetry among our studied shrubs (Umeki, 1995). Spatial associations between crown centroid points and oak seedling stem locations were analysed using bivariate Ripley's k and pair correlation functions. These analyses allow distinguishing the various types of intra or inter-specific spatial associations from those that may arise purely by chance. The bivariate k-function is a ccumulative function defined as $k_{12}(r)$ = E [# (points of pattern 2 *up to* a distance $\leq r$ from an arbitrary point of pattern 1)] / λ_2, where E [ ] is the expectation operator; pattern 2 and pattern

1 represents oak seedlings and shrubs respectively; λ_2 is the intensity of pattern 2. Positive and negative values of $k_{12}(r)$ indicate attraction and repulsion between the two patterns up to a distance *r*, respectively. The pair-correlation function is a non-cumulative function, $g_{12}(r)$ is the analogue of Ripley's $k_{12}(r)$ when replacing the circles of radius *r* by rings with radius *r*. $g_{12}(r)$ = E [# (points of pattern 2 *at* a distance *r* from an arbitrary point of pattern 1)] / λ_2, $g_{12}(r) > 1$ and $g_{12}(r) < 1$ indicate attraction and repulsion between the two patterns at a distance *r*, respectively (Wiegand and Moloney 2004). The pair correlation function and Ripley's *k*-function are closely related; since both are based on the distribution of distances of all pairs of points of the patterns. However, the two functions respond to slightly different biological questions: while Ripley's *k*-function detects association patterns cumulatively up to a certain distance *r*, the pair correlation detects association at a given distance *r*. Therefore, Wiegand and Moloney (2004) argue to not only use *k*-function but also the complementary pair correlation function. For both functions, we extracted statistics at three fine scales (r = 1, 2 and 3 m) in order to capture information at scales where internal processes such as biotic interactions are more likely to act. The general effects of functional shrubs were tested using values derived from Ripley's *k*-function at the three distances scale in one-Way Analysis of Variance (ANOVA) models followed by Tukey's multicomparison tests.

For both functions, we compared the observed bivariate point patterns with the antecedent conditions null model (see Wiegand and Moloney, 2004). To detect the significance of patterns against the null models for both univariate and bivariate analyses, approximate (two-sided) 95% simulation envelopes of the null models were created by calculating for each distance *r* the 5th lowest and highest values of the summary statistic from 99 Monte Carlo simulations of the null model; this yields an approximate 5% error rate. However, because of simultaneous inferences (i.e., we tested *r* at several spatial scales simultaneously), type I error may occur if the value of the chosen statistic is close to a simulation envelope (i.e., the null model may be rejected even if it is true when values of the evaluated function are close to values of the simulation envelopes). We therefore combined the common simulation envelope method with a goodness-of-fit test (GoF) recently advocated by Loosmore and Ford (2006). In all analyses, we used a distance lag of 0.25 m with 0.5 m *dr* (ring width) calculated up to 3 and 7m for association and distribution patterns consequently. Point pattern analyses were performed using the software Programita (Wiegand and Moloney 2004, improved version of 2010).

3-2-2 Chi-Square Test (Non-spatial Method)

A limitation of point pattern analysis is that plants are reduced to point locations. The points approximation is applicable when plant size is small in comparison to the investigated spatial scales. Otherwise it may obscure the real spatial relationships at smaller scales that in which ecologists are interested when studying interactions among plants (i.e., Prentice and Werger, 1985; Wiegand *et al.*, 2006). In order to overcome this limitation we compared observed-to-expected oak seedling occurrence under each shrub functional group using chi-square (χ^2) independence tests considering shrub canopy surfaces rather than centroid point coordinates. Expected frequencies were derived by multiplying the percent canopy cover of a given functional group of shrubs by the total frequency of oak seedlings (Callaway *et al.*, 1996; Brett and Klinka, 1998). A Yates continuity correction was applied to 2 × 2 χ^2 tests to provide a better match with the χ^2 distribution**.** The null hypothesis was that presence of seedlings beneath the shrub canopies is independent of the functional group of shrubs (Schleicher *et al.*, 2011).

The shrub canopy polygons were drawn in a computer-aided design (CAD) system (AutoCAD, Autodesk Inc., Sausalito, CA) by fitting bidimensional splines to the four coordinates endpoints recorded in the field. We exported the polygons to Arc GIS 10.1 (ESRI

Inc., Redlands, CA), and the *x, y* coordinates of polygon centroids were extracted (i.e., Fig. 1a, b and c). Chi-square analysis, ANOVA and Tukey's tests were performed using the R software for statistical computing (version 2.10.1, 2010).

Results

1- Univariate distribution patterns of the seedlings of each of the three oak species

A total of 4963 oak seedling individuals belonging to three oak species were recorded and mapped, with an average of 451 seedlings per plot (1431.7 seedlings.ha^{-1}) (31.61%, 34.21% and 34.17% for *Q. robur*, *Q. ilex* and *Q. suber*, respectively). By applying univariate pair correlation analysis, the significant GoF test values ($P<0.01$) generally revealed similar aggregated patterns of regeneration for the three oak species (Fig 1, Appendix: Table 1) which confirms the visual inspection of the mapped patterns (Fig. 2a, b and c). An exception was recorded in Born. G. plot ($P = 0.33$) where *Q. suber* did not show significant non-random patterns (Fig. 1). Generally, the highest values of $g_{11}(r)$ occurred at short distances (0.25m to 1m) and faded away with increasing distance (Fig. 1).

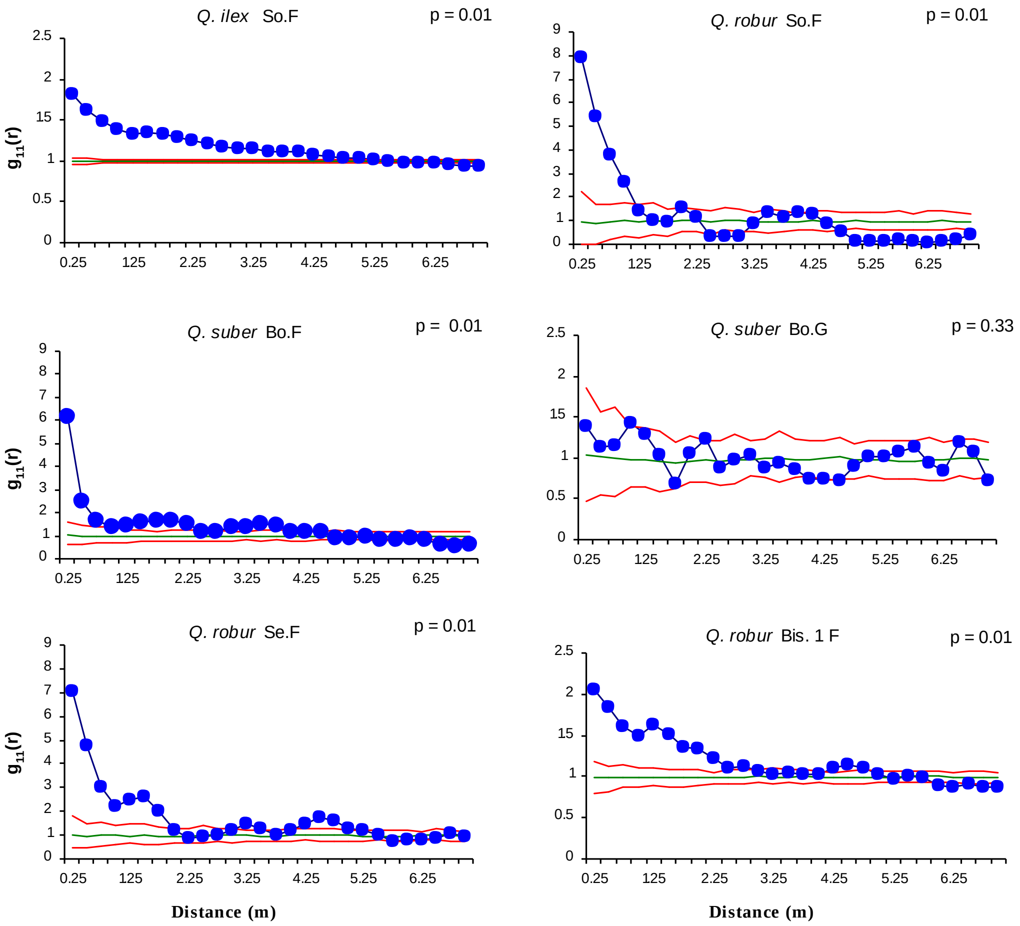

Fig.1. Examples of univariate spatial distribution for the seedlings of the three oak species using pair correlation function. Dotted lines indicate ring statistics g(r); red lines indicate the upper and lower limits of the 99% confidence envelope of the univariate CSR (complete spatial randomness) null model. Points above, under and within envelopes indicate aggregation, regular and random spatial distribution, respectively, green straight line is the estimated empirical function value. See Table 1 for site abbreviations.

2- Bivariate shrub-oak association patterns

2-1- Ripley's *k* and pair correlation functions

One-Way ANOVA analysis for the values derived from $k_{12}(r)$ up to 1, 2 and 3 m distance scales showed the seedlings of pooled-oak species significantly in a positive association (attraction) with Fagaceae and in a negative association (repulsion) with Ericaceae up to both 2 ($P = 0.039$) and 3 m ($P = 0.018$) distances from the shrub canopy centroids. There were no significant differences of both Fabaceae and Spiny shrubs over the three distance scales (Table 2 and Fig. 3).

The GoF tests of the bivariate inter and intra-specific pair correlation function detected significant associations for 33 shrub-oak pairs out of potential 43 tests (17 cases of positive and 16 cases of negative associations (Table 3, Appendix: Table 2)). Seedlings of oak species displayed significant negative spatial association with Ericaceae shrubs in 7 (58.33 %) out of 12 cases. Results for seven spatial association tests between Spiny shrubs and oak seedling exhibited three cases of both positive (42.86%) and negative (42.86%) ($P = 0.01$) associations and one case of independence ($P = 0.09$). Out of 8 cases, Fabaceae shrubs showed 3 (37.5%) positive and independent interactions with 2 (25%) negative interactions (Table 3). Among the 16 Fagaceae shrub-oak seedling cases, there were nine (56.25%) positive, four negative (25%) and three independent (18.75) interactions. It is interesting to notice that the majority (i.e., seven) of these nine positive association cases were intraspecific and only two were interspecific (Table 3, Appendix: Table 2).

In short, bivariate analysis under the null model of antecedent condition (facilitation /competition between shrubs and seedlings) exhibited a higher density of oak seedlings neighbouring Fagaceae shrubs than the rest, as positive cases occur more frequently with intraspecific Fagaceae (56.25%). Negative interactions mainly occurred with Ericaceae shrubs (58.33%), while 37.5 % of Fabaceae interactions showed basically independence (Fig.3, Table 3 and Appendix: Table 2).

2-2- Chi-square

Chi-square analyses showed that oak seedlings significantly disassociated with the Ericaceae canopies in 50% of the plots whereas both positive and independent associations were found in 25% of each plots. Out of 16 cases Fagaceae shrubs showed attraction in 7 cases (43.75%) 6 cases of them were due to intraspecific interaction, repulsion was found with the same frequency 7 (43.75%), but with more interspecific interaction (4), whereas there were only 2 independent patterns (12.5%) (Table 3; Fig.4). From (8) and (7) association cases of Fabaceae and Spiny respectively we did not find a strong tendency toward positive or negative association where the three association patterns (positive, negative and independent) were relatively equally occurred (Table 3; Fig.4). In summary, Chi square analysis results were almost similar to those emerging from pair correlation and Ripley's *k* analysis.

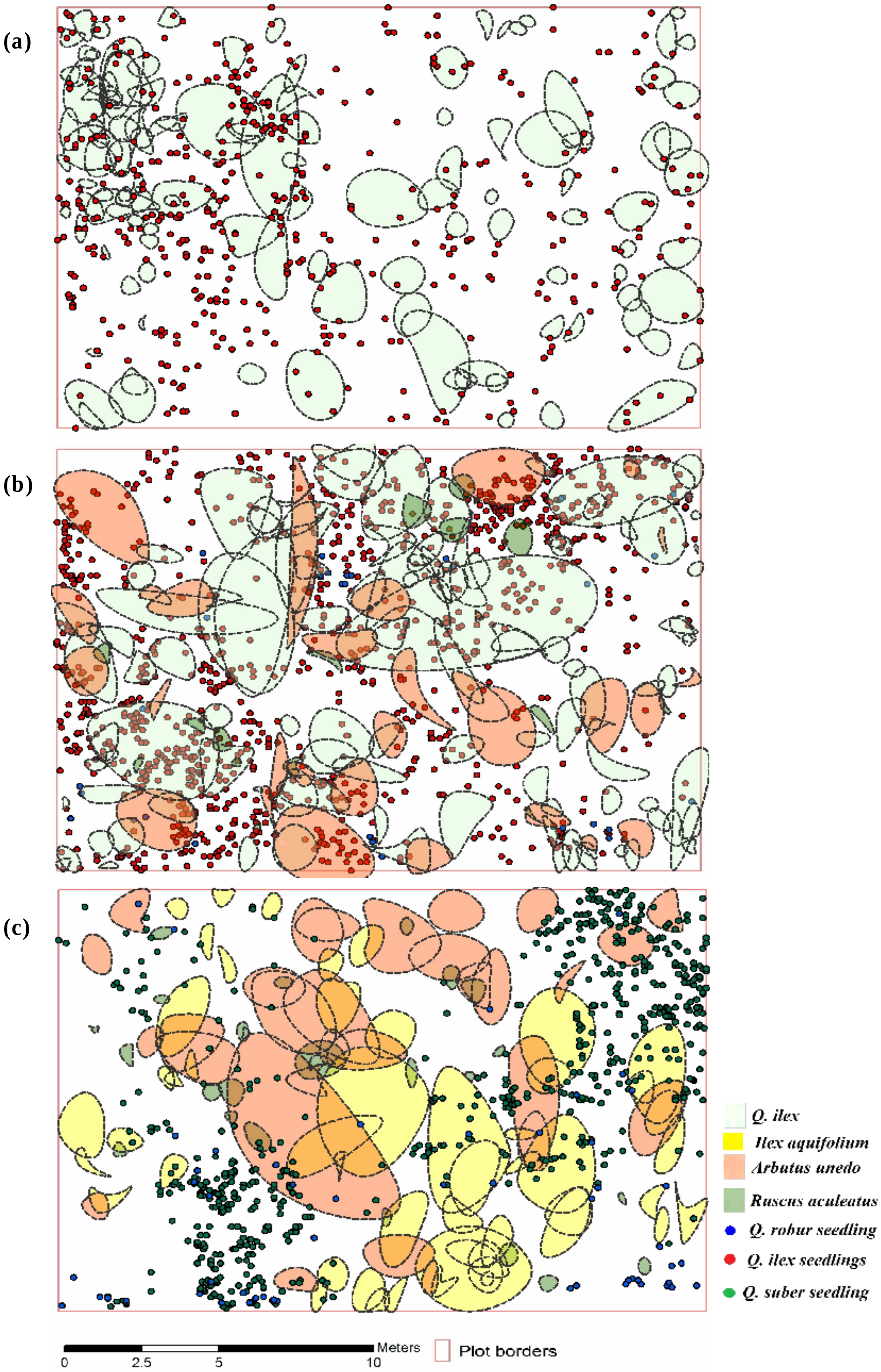

Fig. **2**. Examples of crown projections of shrubs canopy and distribution of oak regeneration in 315 m^2 plots**,** showing the aggregation pattern of oak seedlings and generally **(a)** facilitation (attraction) in Hourtin forest **(b),** independence association in Soulac forest and **(c**) competition (repulsion) in Seignosse forest..

Table 2. Summary statistic of one – way ANOVA showing the effect of different functional groups of shrubs on oak regeneration at three distance scales. Significance values: $P < 0.05$.

	1m				**2m**			**3m**		
S.O.V	**df**	**MS**	***F***	***P***	**MS**	***F***	***P***	**MS**	***F***	***P***
Species	3	5.5134	2.237	0.1006	11.3489	3.084	0.0393*	14.933	3.792	0.0184*
Residuals	36	2.4644			3.6799			3.938		

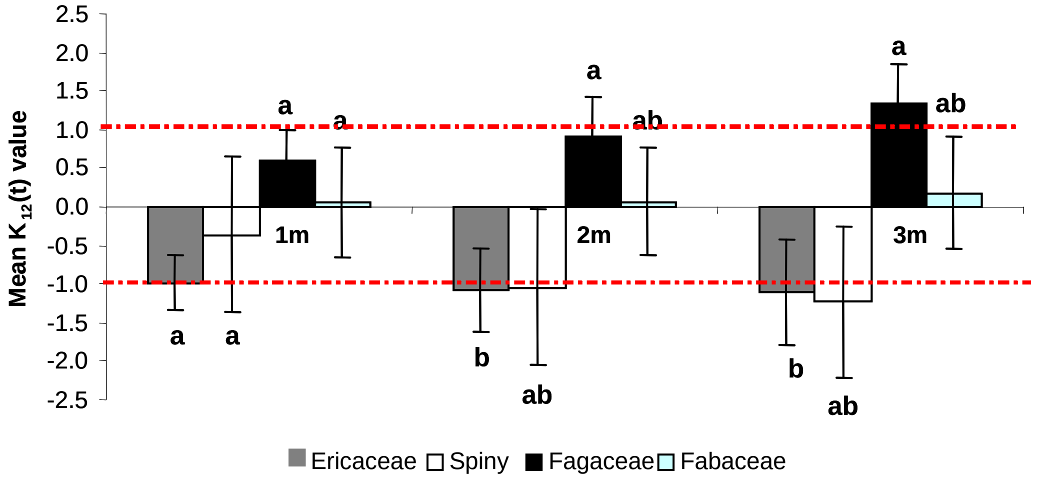

Fig. 3. Spatial association types and intensity between different functional shrubs characteristics with overall oak seedlings up to three scale distances. Different letters indicate significant differences among the functional groups of shrub at each scale distance ($P < 0.05$ from Tukey's HSD tests). Dashed red lines show the confidence envelope area around the expected k_{12} (r) value (0).

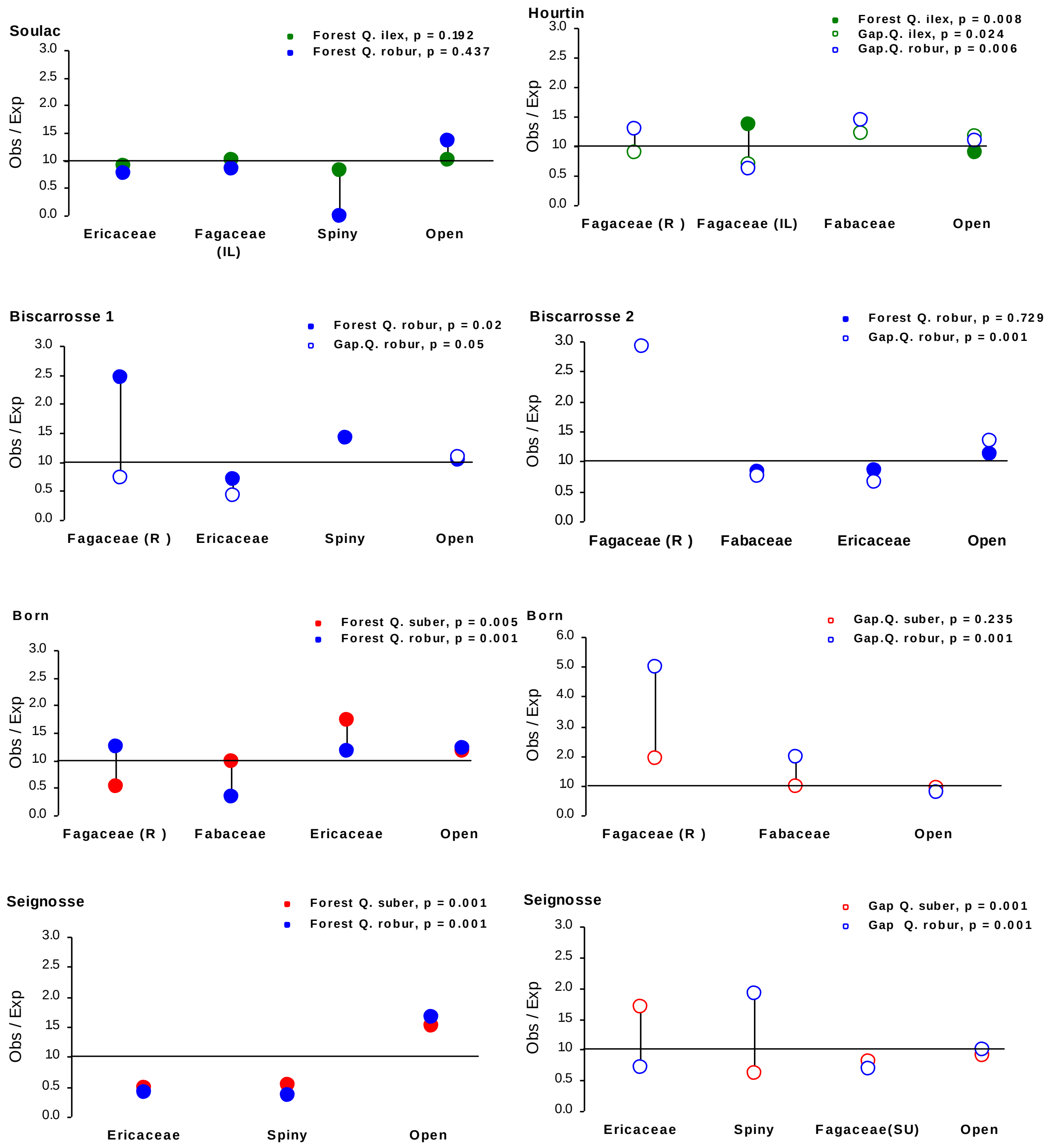

Fig. 4. Observed-to-expected frequency distribution of the three oak seedlings with different functional groups of shrubs species and in different canopy and site conditions based on chi-square test. Symbols IL; R and SU represented *Q. ilex*, *Q. robur*, and *Q. suber* shrubs respectively. See Table 1 for site abbreviations.

Table 3. Association percentage and Frequency patterns of the seedlings of pooled-oak species with the four functional groups of shrubs in the 11 315 m^2 plots extracted from the results of pair correlation function and chi-square. The values in bold correspond to the highest percentage and frequency of association pattern recorded among the four functional shrubs. Fagaceae shrubs sub-divided in to intra- and interspecific association.

Analysis Method	Functional group	Positive	Negative	Independent	Total
Pair correlation function	**Fagaceae**	**56.25 (9)**	25(4)	18.75 (3)	16
	Intra-specific	**43.75 (7)**	12.5 (2)	6.25 (1)	10
	Inter-specific	12.5 (2)	12.5 (2)	12.5 (2)	6
	Spiny	42.86 (3)	42.86 (3)	14.29 (1)	7
	Fabaceae	37.5 (3)	25(2)	**37.5(3)**	8
	Ericaceae	16.67 (2)	**58.33 (7)**	25(3)	12
Chi square	**Fagaceae**	**43.75 (7)**	43.75 (7)	12.5 (2)	16
	Intra-specific	**37.5 (6)**	18.75 (3)	6.25 (1)	10
	Inter-specific	6.25 (1)	25(4)	6.25 (1)	6
	Spiny	28.57 (2)	42.86 (3)	28.57 (2)	7
	Fabaceae	37.5 (3)	25 (2)	**37.5 (3)**	8
	Ericaceae	25(3)	**50 (6)**	25(3)	12

Discussion

Consistent with our expectations, we found that (1) seedlings of all three oak species tended to be spatially aggregated at fine scales (sub-meter scale) in most plots, and (2) independent of species, oak seedlings had contrasting association patterns in relation to the four functional shrub groups. Association patterns were predominantly positive for Fagaceae, negative for Ericaceae, and more diverse for Fabaceae and Spiny shrubs.

1- Aggregated distribution of oak seedlings

It is generally accepted that both random and regular distributions of tree regeneration tend to be an exception rather than a rule (Pardos *et al.*, 2008). Tree seedlings are often aggregated at fine scales (e.g., < 1m, Salas et al., 2006). We found in the 10 (among 11) significant cases of aggregated univariate patterns that strong $g_{11}(r)$ values occurred mostly at short distances (0.25-1 m). Our findings are in line with results reported for seedlings of different oak species in natural stands including *Q. pubescens* Mill (Kunstler *et al.*, 2004), *Q.*

ilex and *Q. pubescens* (McDonald *et al.*, 2003), *Q. faginea*, *Q. suber* (Maltez-Mouro *et al.*, 2007) and *Q. robur* (Andersson, 1991). Aggregated patterns may be caused by limited seed dispersal (Houle, 1997; Kunstler *et al.*, 2004), habitat heterogeneity (Harms *et al.*, 2001) and temporary local release from a regeneration limitation or a combination of these factors (Skarpe, 1991). Hence, for *Quercus*, a genus characterized by large acorns typically distributed by gravity, short-distance seed dispersal could be a plausible reason for the observed aggregated patterns.However; biotic interactions could also in part explain such pattern of aggregation within the influence zone of particular shrubs. We did not find obvious differences in aggregation patterns among the three oak species. Indeed, the acorns of the three species have approximately the same size, and all three are dispersed primarily by gravity and secondarily by similar animal dispersal agents such as the Eurasian jay *Garrulus glandarius* (Gomez, 2003). However, the effect of rodents or birds was not documented in our study, but since most of these agents would lead to overdispersion patterns, our results suggest that they played a minor role in creating the patterns of oak seedlings in the studies system.

2- Positive intraspecific shrub-oak association patterns

The results of both methods used to quantify shrub-oak seedlings association patterns showed that Fagaceae shrubs were overall positively associated with pooled-species oak seedlings and that most of these positive associations were intraspecific. Most studies conducted in arid environmental conditions have considered that positive associations of tree seedlings with shrubs are evidence of facilitation (Tirado and Pugnaire 2003; see review by Brooker et al. 2008). However, under temperate oceanic climate conditions this pattern needs to be interpreted with some caution since several other processes may drive suuch positive associations, including environmental heterogeneity (Michalet et al., 2006; Perry et al., 2009) and seed dispersal (Schurr et al., 2005. Although we did not specifically document within-site environmental variation in our study, we assume that environmental heterogeneity was not the cause of these bivariate association patterns considering the relatively small size of our plots and the homogeneity in topography, exposure and soil texture conditions of our sites. Rather, this positive association may have been caused by acorn dispersal and/or facilitation, e.g., improved conditions expected beneath host shrubs (Cuesta *et al.*, 2010; Schleicher *et al.*, 2011). As a rule, facilitation dominates when niche overlap is limited (interspecific) while competition for shared resources otherwise dominates (intraspecific) (Keddy, 2001). The stronger intraspecific than interspecific competition stressed by classic competition theory (Begon, 1996; Gavilan *et al.*, 2002; Dullinger *et al.*, 2007) has not always been supported by empirical work (Franks, 2003; Fajardo and McIntire, 2011). Intraspecific facilitation may occur particularly among individuals of the same species but from different life stages, with large individuals protecting small seedlings causing nurse effects to recruitment (Callaway, 1995). This may partially explain our positive association pattern (naturally occurring oak seedings around and beneath intraspecific Fagaceae shrubs). However, because this positive pattern was observed in different sites in addition to the strong aggregation univariate patterns in all sites regardless of environmental stress due to climatic conditions, limited acorn dispersal in the vicinity of adult trees is likely the dominant driver of these intraspecific positive associations between oak seedlings and Fagaceae shrubs. Although we are aware that shrubs do not produce acorns both the shrubs and the oak seedlings may have recruit in the close neighborhood of the adult trees and that both processes (seed dispersal mode and facilitation) could potentially explain this pattern. For example, when subsequent aggregated acorn dispersal could be later enhanced by their intraspecific shrubs. It is a considerable challenge to distinguish the attraction pattern (positive association) created due to acorn dispersal from the effect of direct plant interactions, given that separating such processes is difficult to achieve in general with an observational approach (Wiegand *et al.*, 2007; Schleicher *et al.*, 2011). Removal experiments as well as seed dispersion studies in our system may allow us to disentangle the respective roles of intraspecific facilitation and limited seed dispersal.

3- Negative and independent interspecific association

The clear negative association between Ericaceae and oak seedlings was presumably due to the allelopathic leachates characteristic of this family (Ballester, 1982), particularly in the case of *Calluna* spp, *Erica* spp, and *Arbutus* spp. Our results are in line with Gonzalez-Martinez and Bravo, (2001) which found a negative correlation between scotch pine regeneration and a dense cover of Ericaceae shrubs (*Daboecia cantabrica*, *Erica cinerea*, *Erica vagans* and *Calluna vulgaris)* due to the inhibiting role of their aboveground parts and roots. Similler results were found by Walker *et al.*, (1999) and Eppard *et al.*, (2005) where the litter and organic layer substrates of Ericaceae severely limited the regeneration of seedlings of *Q. rubra*, *Tsuga canadensis* and *Pinus rigida* .

The lower number of positive association cases of both Fabaceae and Spiny shrubs in this study were somewhat unexpected because many studies have shown that both functional groups of shrubs have overall positive effects on other species, because of their direct enhancement of soil nutrients or indirect protection effect against herbivores, respectively (e.g., Callaway, 1995; Pugnaire *et al*., 1996; Pugnaire and Luque, 2001; Rebollo *et al*., 2002; Smit *et al*., 2005). For Spiny shrubs, this result may be due to the low overall herbivore pressure in this coastal dune system, while the low nutrient requirement of oak species may explain the lack of a positive association with Fabaceae shrubs. However, a lower replication cases than for other functional groups may also explain this neutral pattern observed in our study.

Conclusion:

Our study provides further evidence for spatial aggregation of three oak regeneration species in the coastal dune forests. This result, observed with the univariate analyses, as well as the significant positive intraspecific association pattern found between the seedlings of pooled-oak species and Fagaceae shrubs with the bivariate analyses, suggests the importance of seed dispersal for explaining spatial patterns of oak recruitment. Further studies, and in particular removal experiments, are needed to really assess the possible contribution of intraspecific facilitation in this pattern. We found in general that interactions between oak seedlings and shrubs were strongly dependant on the functional groups of shrubs also because of the importance of negative associations observed between oak seedlings and Ericaceae species. Our study allows us to recommend to the forest managers to focus on the control of Ericeae shrubs to enhance the regeneration of oak forests, while preserving the Fagaceae shrubs, at least as regeneration insurance, in expectation of oncoming experimental studies that will assess the importance of intraspecific facilitation in this system.

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Appendix: Tables

Table 1. Spatial structures of the three oak species in the 11 sites using the univariate pair correlation function. Symbols C and R indicate significant clumped and regular distribution at distance *r*, respectively. No symbol indicates random distribution. Symbols F and G are forest and gap respectively. Significance is using a 99% confidence envelops with 0.25 m step. *P*-value computed through goodness-of-fit test used for overall significance of patterns over the complete range of *t.* See Table 1 for site abbreviations.

Site	Quercus spp.	Type and scale of distribution pattern *r* (m)																													
		No.	0.25			1				2				3				4				5				6				7	*P*
SoF	***Q. ilex***	1066	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C			R	R	R	R	R	0.01
	Q. robur	34	C	C	C	C					R	R	R							R	R	R									0.01
H F	***Q .ilex***	440	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C		R	R		R					C	C	C	C	0.01
HG	***Q. ilex***	192	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C		C	C	C	0.01
	Q. robur	147	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	0.01
Bisc1F	***Q. robur***	196	C	C	C	C	C	C	C	C	C	C	C						C	C	C					R	R	R	R	R	0.01
Bisc1G	***Q. robur***	134	C	C	C	C	C	C	C	C							R						R	R						C	0.01
Bisc2F	***Q. robur***	41	C	C	C	C						C		C											R	R				R	0.01
Bisc2G	***Q. robur***	73	C	C	C	C	C		C	C								R	R	R	R			R	R	R	R				0.01
	Q. suber	85	C	C	C	C	C	C	C	C	C			C	C	C	C										R	R	R	R	0.01
BoF	***Q. robur***	63	C	C		C	C	C	C			C	C	C	C				R		C										0.05
	Q. suber	59																	R								C				0.33
BoG	***Q. robur***	102			C	C	C	C	C	C	C								R						R	R				R	0.01
	Q. suber	630	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C		R	R	R	R	R	R	R	R	R	R	0.01
SeF	***Q. robur***	58	C	C	C	C	C	C	C						C	C			C	C	C	C			R						0.01
	Q. suber	922	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C		R	R	R	R	R	R	R	0.01
SeG	***Q. robur***	721	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	0.01

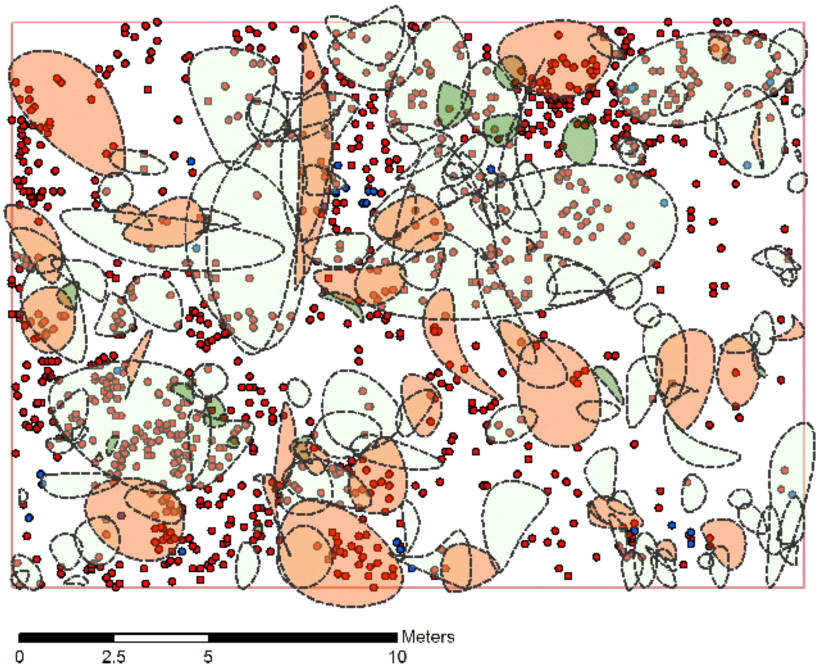
Table 2. Patterns of intra and inter-specific associations of the three oak species seedlings with canopy centroids of four functional groups of shrub in the 11 sites.

Site	**Functional shrub**	**Oak recruits.**	**Type and scale of association *r* (m)**													
			n1/n2	**0.25**	**0.5**	**0.75**	**1**	**1.25**	**1.5**	**1.75**	**2**	**2.25**	**2.5**	**2.75**	**3**	***P***
So.F	**Spiny**	***Q. ilex***	16/1066		+	+	+	+	+	+	+	+	+	+	+	**0.01**
		Q. robur	16/34						-							**0.09**
	Ericaceae	***Q. ilex***	31/1066		-			-	-	-	-	-		-	-	**0.03**
		Q. robur	31/34									-	-	-	-	**0.05**
	Fagaceae (*Q. ilex*)	***Q. ilex***	108/1065	-	-	-	-	-	-	-	-	-	-	-	-	**0.01**
	Fagaceae (*Q. ilex*)	***Q. robur***	108/34		+	+	+	+					+	+		**0.01**
Ho.F	**Fagaceae** (*Q. ilex*)	***Q. ilex***	124/440	+	+	+	+	+	+	+	+	+	+	+	+	**0.01**
Ho.G	**Fabaceae**	***Q. ilex***	50/192		+	+	+	+	+		+	+		+	+	**0.01**
		Q. robur	50/147	+	+	+	+		+		+	+		+	+	**0.01**
	Fagaceae (*Q. robur)*	***Q. ilex***	35/192				+						+	+	+	**0.22**
		Q. robur	35/147	+	+	+	+	+	+	+	+	+	+	+	+	**0.01**
	Fagaceae (*Q. ilex*)	***Q. ilex***	72/192						-	-	-	-	-	-	-	**0.01**
		Q. robur	72/147				-	-					-	-	-	**0.02**
Bisc1 F.	**Ericaceae**	***Q. robur***	51/196	-	-	-									+	**0.01**
	Spiny	***Q. robur***	79/196	+	+	+	+	+				+				**0.01**
	Fagaceae (*Q. robur)*	***Q. robur***	18/196			+	+	+			+	+	+			**0.01**
Bisc.1G	**Ericaceae**	***Q. robur***	53/134		-		-	-	-	-			-		+	**0.02**
	Fagaceae (*Q. robur)*	***Q. robur***	55/134	+							+		+	+		**0.2**
Bisc.2F	**Fabaceae**	***Q. robur***	107/41													**0.41**
	Ericacaea	***Q. robur***	55/41				-				-	-		-		**0.26**
Bisc.2G	**Fabaceae**	***Q. robur***	81/73	-	-	-	-									**0.03**
	Ericacaea	***Q. robur***	53/73			+										**0.41**
	Fagaceae (*Q. robur)*	***Q. robur***	19/73		+	+	+	+	+			+				**0.01**
Bo.F	**Fabaceae**	***Q. suber***	32/85			-										**0.45**
		Q. robur	32/63	-				-	-		-					**0.03**
	Ericaceae	***Q. suber***	16/85							+	+	+	+			**0.05**
		Q. robur	16/63										-			**0.68**
	Fagaceae (*Q. robur)*	***Q. suber***	53/85	-	-	-		-	-	-						**0.01**
		Q. robur	53/63				+	+	+	+	+	+			+	**0.02**
Bo.G	**Fabaceae**	***Q. suber***	83/59													**28**
		Q. robur	83/102	+	+	+	+	+	+	+			+			**0.01**
	Fagacaea (*Q. robur)*	***Q. suber***	13/59													**0.21**
		Q. robur	13/102	+	+	+	+	+	+							**0.01**
Se.F	**Ericaceae**	***Q. suber***	31/630	-	-			-	-	-	-	-	-	-	-	**0.01**
		Q. robur	31/58	-	-	-	-	-	-	-	-	-	-	-	-	**0.01**
	Spiny	***Q. suber***	76/630	-	-	-	-	-	-	-	-	-	-	-	-	**0.01**
		Q. robur	76/55	-		-	-	-	-	-	-	-	-			**0.01**
Se.G	**Ericaceae**	***Q. suber***	88/922						+	+	+	+	+	+		**0.05**
		Q. robur	88/721	-	-	-	-	-	-	-	-	-	-	-	-	**0.01**
	Spiny	***Q. suber***	26/922	-	-	-	-	-	-	-	-	-	-	-	-	**0.01**
		Q. robur	26/721	+		+	+		+				+	+		**0.01**
	Fagaceae (*Q. suber*)	***Q. suber***	13/922									+	+	+	+	**0.01**
		Q. robur	13/721								+	+	+	+		**0.05**

Symbols F and G are forest and gap respectively. n1,. n2 corresponds to the numbers of shrubs in a functional group and numbers of oak seedlings of the three oak species (*Q. ilex* and *Q. robur* and *Q. suber*) respectively. +: significant positive association; - : significance negative association; no symbol indicates independence association based on antecedent null method of g_{12} (r) function. Significant is evaluated using 99% Monte Carlo confidence envelops (99 simulations) with 0.25 m spatial scale intervals. *P*-value computed through goodness-of-fit test used for overall significance of patterns over the complete range of *t.* See Table 1 for site abbreviations.

Appendix: Figures

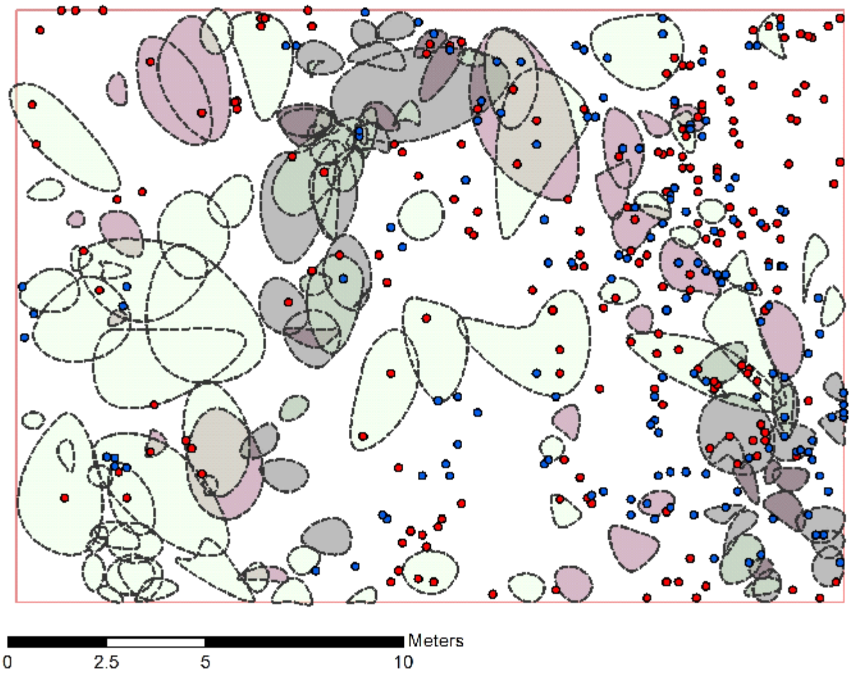
The following figures showing the crown projections of three oak seedlings species with four functional groups of shrubs (Spiny, Ericaceae, Fagaceae and Fabaceae) in 315 m^{2}plots .The figures correspond with the distribution and association pattern results showed in fig.2,3 and the both tables 1 and 2 in the Appendix.



Soulac Forest

▭ ***Quercus ilex*** 🟧 ***Arbitus unedo*** 🟩 ***Ruscus aculeatus***

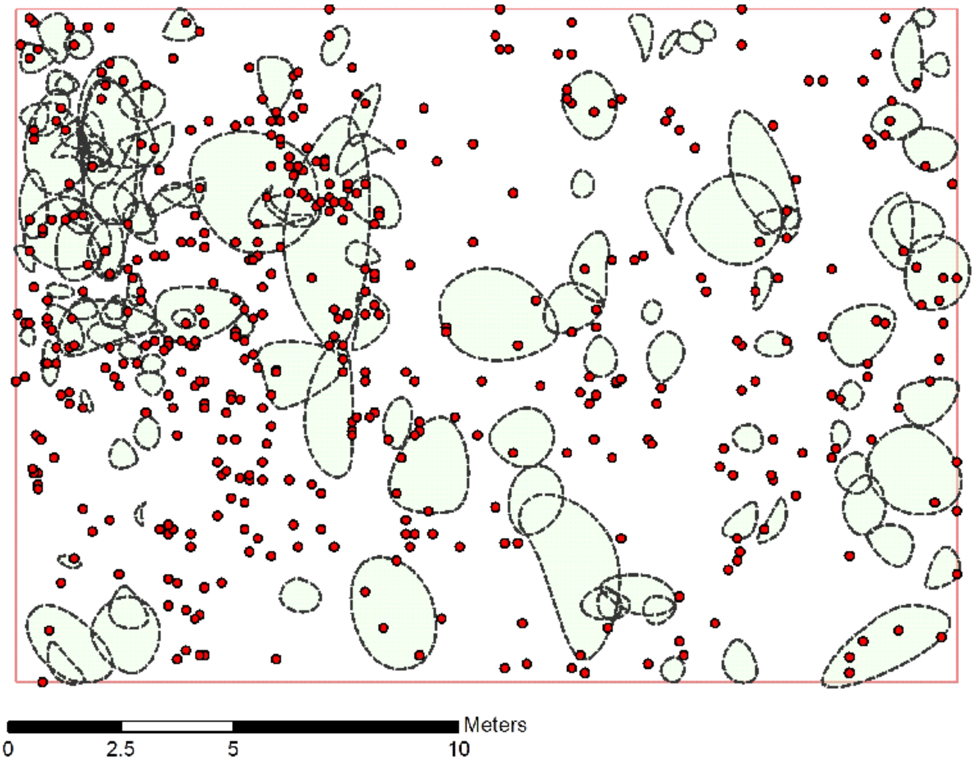
● ***Quercus robur* regeneration** ● ***Quercus robur* regeneration**



Hourtin Gap

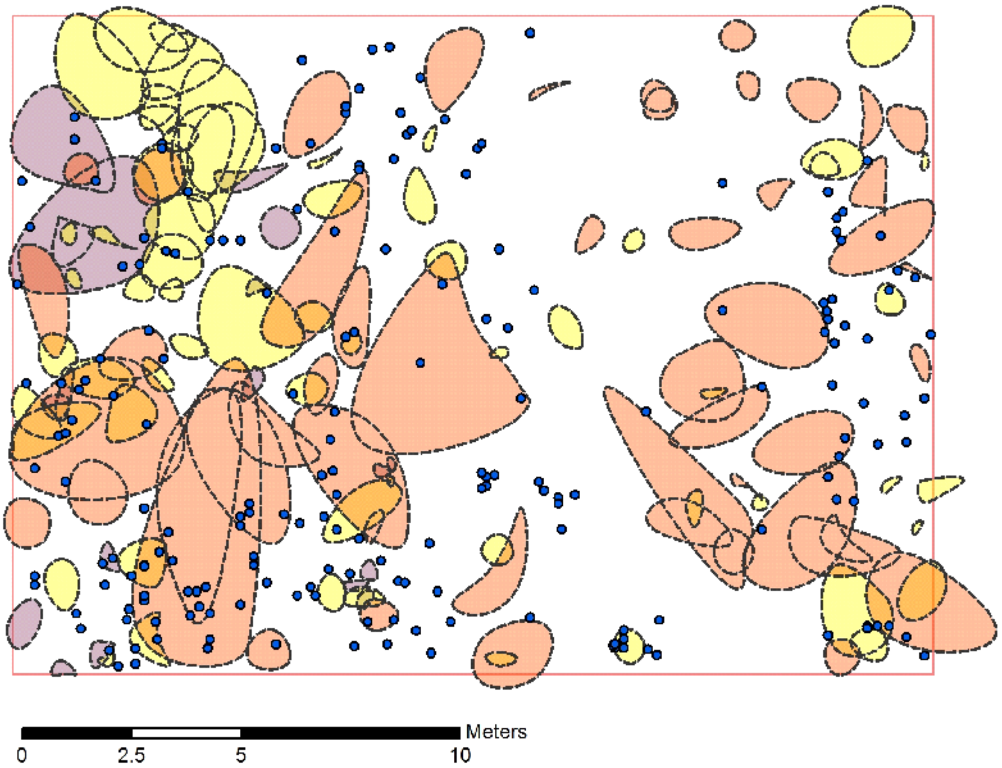
▭ ***Quercus ilex*** ▬ ***Cytisus scoparious*** ▭ ***Quercus robur***

● ***Quercus ilex* regeneration** ● ***Quercus robur* regeneration**



Hourtin Forest

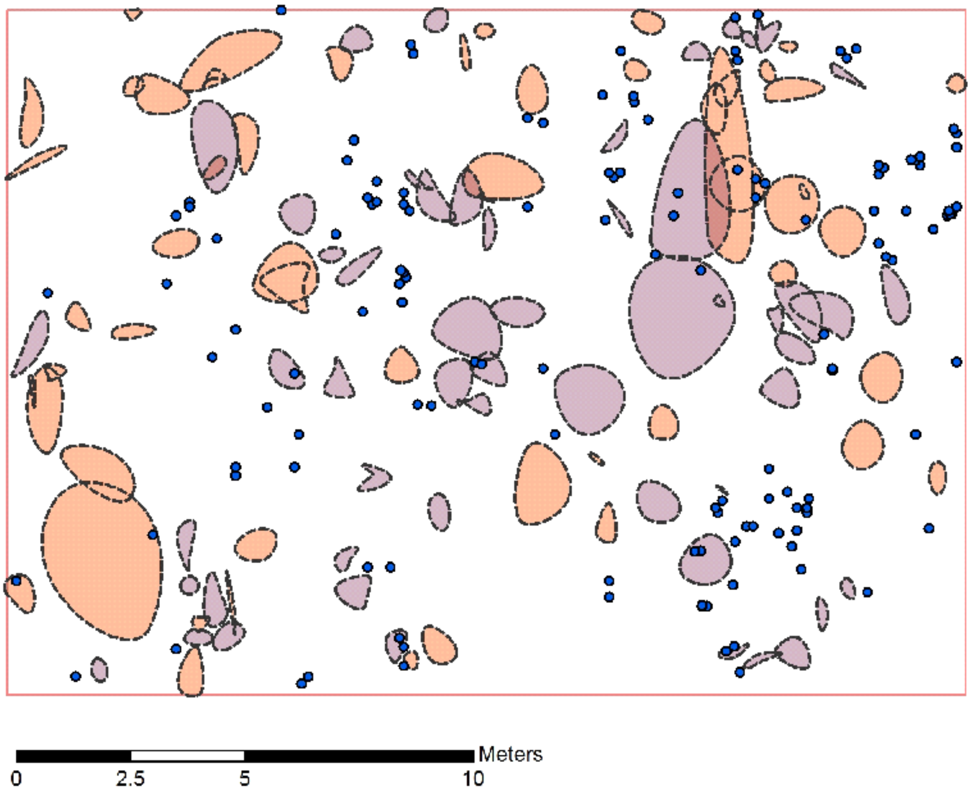
🟩 *Quercus ilex* ● *Quercus ilex* regeneration



Biscarrosse Forest 1

🟨 *Ilex aquifoluim* 🟧 *Arbitus unedo* 🟪 *Quercus robur*

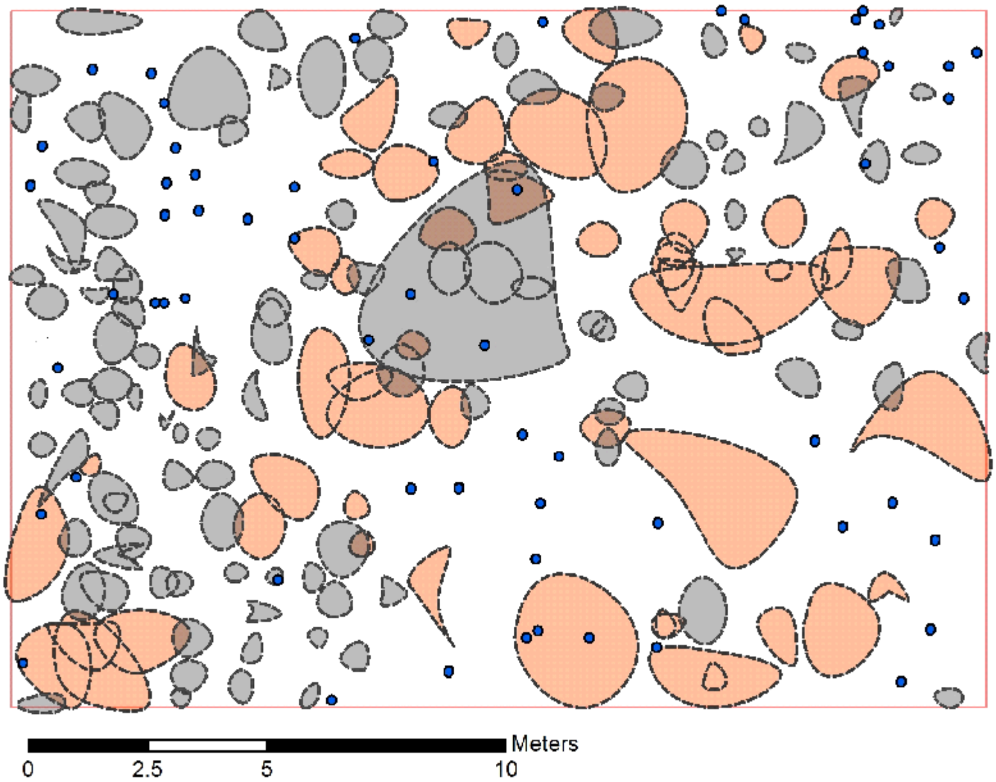
● *Quercus robur* regeneration



Biscarrosse Gap 1

🟧 ***Arbitus unedo*** 🟪 ***Quercus robur***

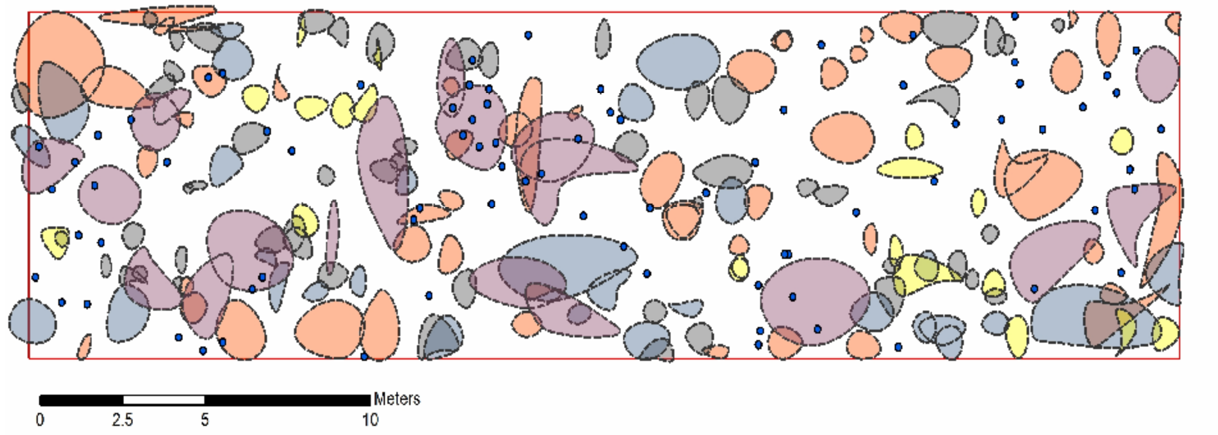
● ***Quercus robur* regeneration**



Biscarrosse Forest 2

🟧 ***Arbitus unedo*** 🟪 ***Cytisus scoparious***

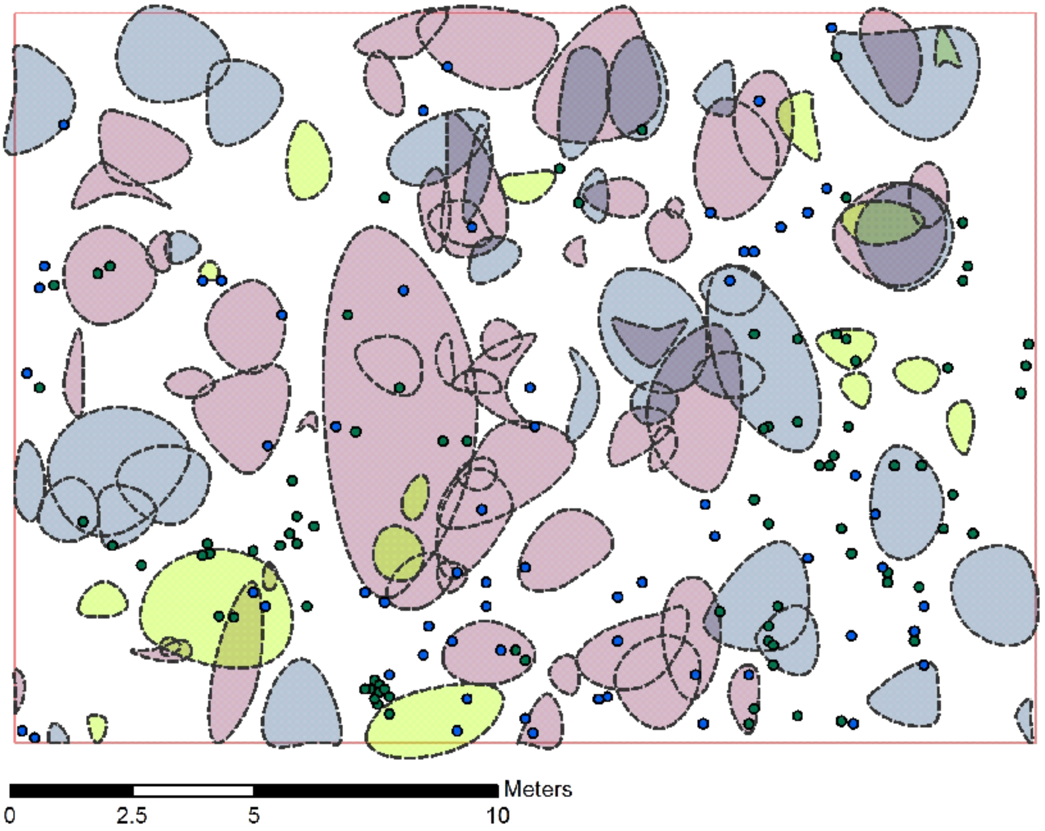
● ***Quercus robur* regeneration**



Biscarrosse gap 2

🟧 ***Arbitus unedo*** 🟪 ***Quercus robur*** 🟨 ***Cistus salvifolius***

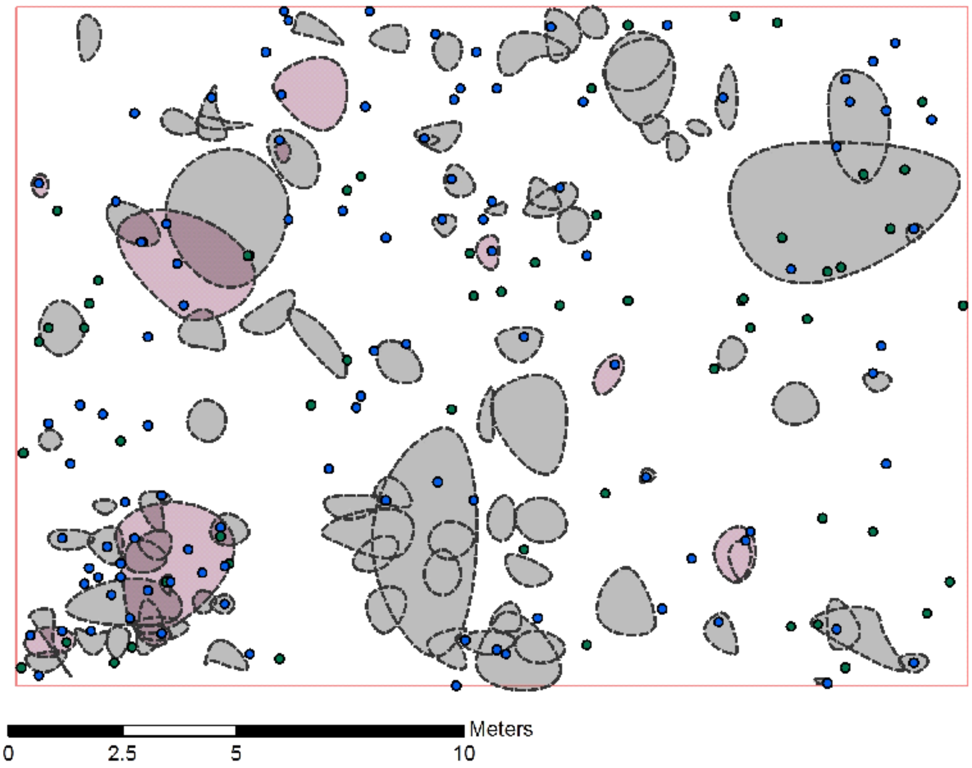
▬ ***Cytisus scoparious*** ● ***Quercus robur* regeneration**



Born Forest

🟦 ***Ulex europaeous*** 🟩 ***Erica cinarea*** 🟪 ***Quercus robur***

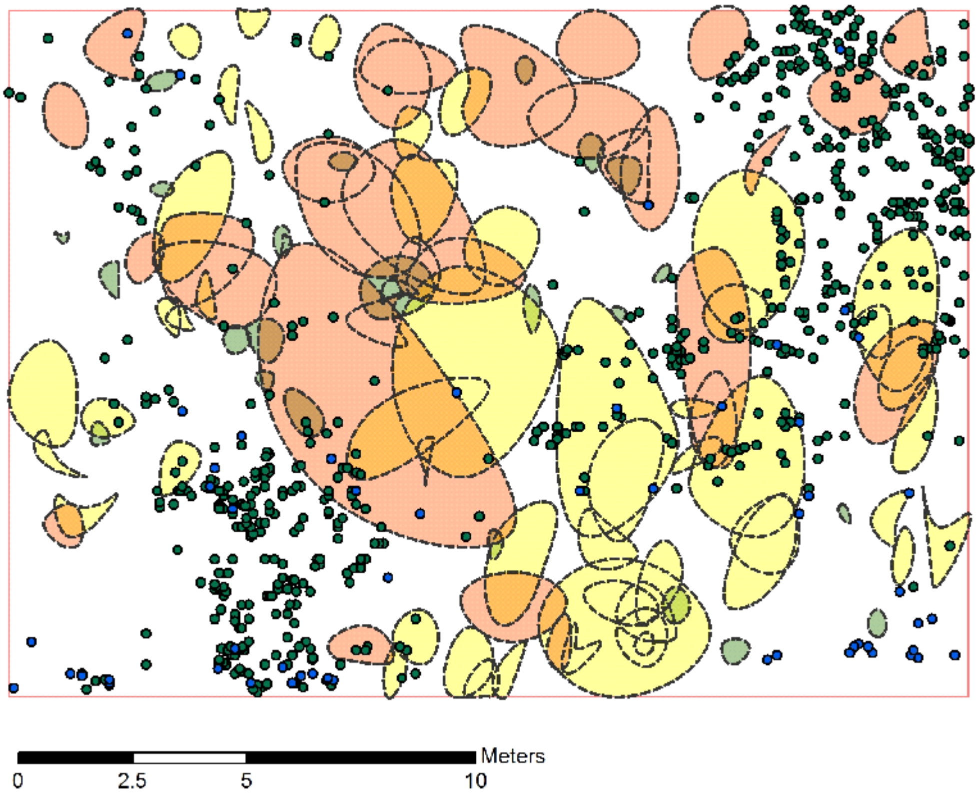
● ***Quercus suber* regeneration** ● ***Quercus robur* regeneration**



Born Gap

▬ ***Cytisus scoparious*** ▭ ***Quercus robur***

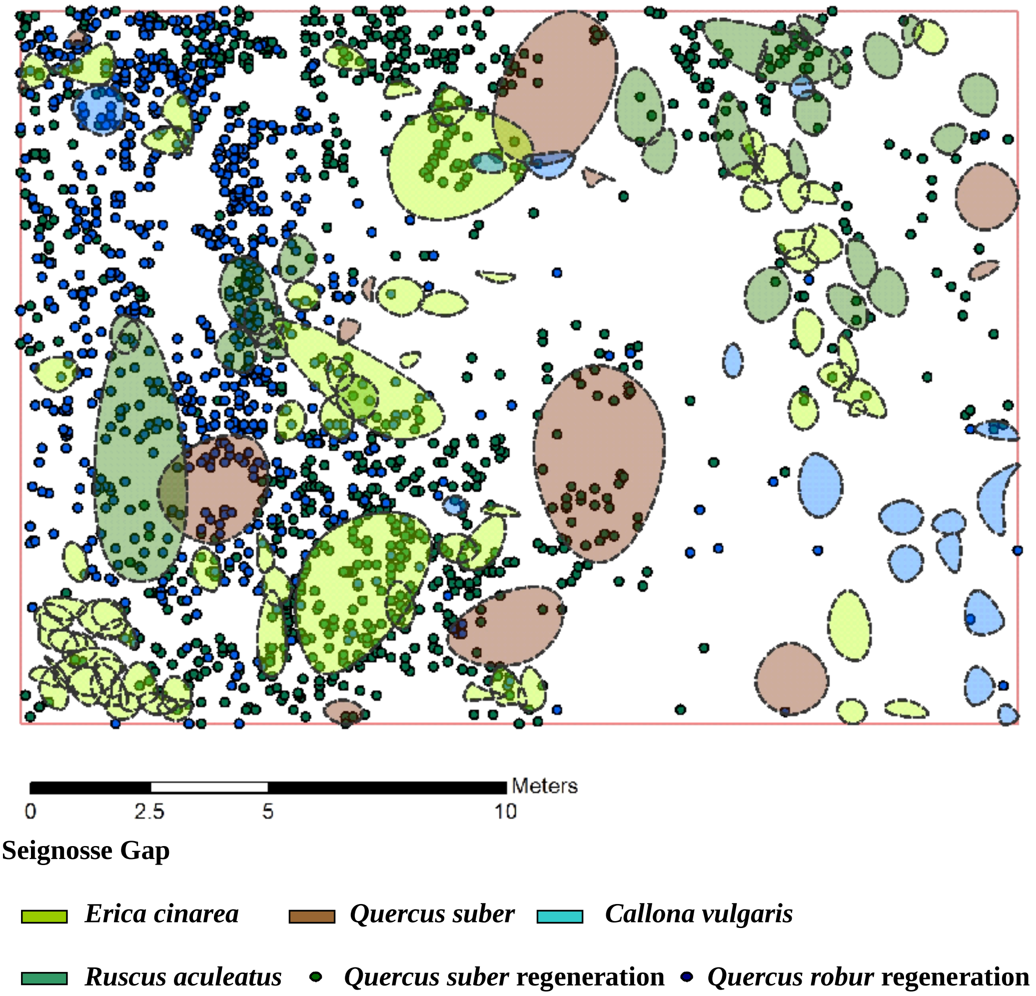
● ***Quercus suber*** **regeneration** ● ***Quercus robur*** **regeneration**



Seignosse Forest

▭ ***Arbitus unedo*** ▭ ***Ilex aquifolium*** ▬ ***Ruscus aculeatus***

● ***Quercus suber*** **regeneration** ● ***Quercus suber*** **regeneration**


Meters
0
2.5
5
10
Seignosse Gap
Erica cinarea
Quercus suber
Callona vulgaris
Ruscus aculeatus
Quercus suber regeneration
Quercus robur regeneration

Chapter 5:

The role of biotic interactions for the early establishment of oak seedlings in coastal dune forest communities

Hassan Muhamed, Yoann Le Bagousse-Pinguet, Blaise Touzard, Richard Michalet

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Abstract

Although we are aware that forest management should adapt to current changing climate, the task is still challenging due to the difficulty for predicting local species and communities responses to climate change. We aim to focus on one aspect of climate change-adapted forest management which has rarely been addressed, the adaptation of sylvicultural techniques to changes in interactions between tree recruitment and understory species due to climate change. We used a space for time design taking advantage of the coastal dune community system of the south west of France including a 240 km-long natural gradient of increasing water stress on similar sandy soil conditions. We transplanted at both extremes of the gradient seedlings of three oak species of contrasted functional strategies both in forest and gap plots and with and without understory shrubs. We also measured Vapor Pressure Deficit in all treatment conditions. We found strong canopy and climatic conditions effects on interactions between understory shrubs and oak transplants. Competition was dominant in the forest plots of the wettest site and facilitation in the gap plots of the driest site. Oak survival without shrubs (but not with shrubs) was strongly related to VPD values, which suggests that the positive effect of shrubs in the most stressful conditions was due to decreased atmospheric stress below their canopy. In contrast, we found that understory shrubs/oak seedlings interactions were weakly affected by oak species functional strategies. Our results provide strong evidence that future oak regeneration management should take into account changes in interactions with understory shrubs due to climate change. In particular, we recommends to conserve understory shrubs in the most stressful sites in order to maintain a sufficient oak regeneration for the long term dynamics of coastal oak forest communities under changing climate.

Keywords: atmospheric stress, coastal dune forest, competition, facilitation, nurse shrub, oak seedling.

1. Introduction

Although we are aware that climate change will have considerable effects on community composition, diversity and services (Parmesan, 2006; Lenoir et al., 2008; Lindner et al., 2010), adapting natural ecosystems conservation and management to climate change is not an easy task (Millar et al., 2007; Hobbs et al., 2009; Milad et al., 2011). The difficulty mainly lies in the uncertainty of predictions at local scales, due to the complexity of ecological drivers to include in the models (Cheaib et al., 2012), as well as the idiosyncrasy of species and local community responses (Liancourt et al., 2005; Maalouf et al. 2012). Thus, the inherent complexity of local-scale processes makes it difficult to extrapolate from small-scale studies to ecosystem, landscape, and regional scales (Harrison and Cornell 2008).

One of the most crucial local drivers of species responses to climate change is certainly interactions among species (Brooker, 2006; Saccone et al., 2010). Although biotic interactions are known to occur at local scale, their consequences for community composition and diversity may scale up at much larger scales (Brooker et al., 2009). However, although there is an increasing interest of modelers in including biotic interactions in their scenarii (Boulangeat et al., 2012; Cheaib et al., 2012; Meineri et al., 2012), their high unpredictability and the lack of studies focusing on variation in biotic interactions along climatic gradients certainly impede our ability to progress in this direction (Brooker, 2006). Thus, there is an important challenge for ecologists and managers in trying to both scale up local processes such as biotic interactions for predicting species and communities responses to climate change and to locally adapt management procedures to changes in community composition, diversity and processes due to climate change.

In this study, we aim to focus on one aspect of climate change-adapted forest management that has rarely been addressed, the adaptation of sylvicultural techniques to changes in interactions between tree recruitment and understory species due to climate change

(Fotelli et al., 2001; Saccone et al., 2009). Since tree species are particularely vulnerable to biotic and abiotic conditions during early life-stages (Kitajima and Fenner, 2000), interactions between understory species and tree recruitments are known to be crucial for tree regeneration and thus forest management (Nambiar and Sands, 1993; Groot, 1999; Balandier et al., 2006; Wagner et al., 2006). However, because climate change known to modify interactions among species in plant communities (Brooker, 2006; Saccone et al., 2009), interactions between tree seedlings and understory species expected to change in forest communities with climate warming and/or drying.

We aimed to assess changes in the effects of understory species on tree recruitment in a climate change-adapted forest management perspective in the coastal dune forests of south-western France. In the coastal dune communities of the Aquitaine region, there is a 240km-long homogeneous sand strip colonized by oak forest communities. From the southern end of the sand strip, close to the Pyrenees mountain range, to its northern end, there is a steep gradient of decreasing rainfall, providing an excellent space-for-time gradient to assess changes in oak seedlings-understory shrub interactions in a climate-change adapted forest management perspective. Additionally, three oak species of contrasted functional strategies occur along the gradient, a temperate deciduous oak, *Quercus robur*, all along the gradient and two Meditteranean evergreen species, *Quercus ilex*, in the north, and *Quercus suber*, in the south. These contrasted forest compositions provide an excellent natural context to analyse also the role of functional strategies in tree species responses to the effect of neighbors in contrasted climatic conditions. Muhamed et al. (2012, submitted) analysed the associational patterns of the seedlings of those three oak species with understory shrubs at five positions along the climatic gradient and both in closed forests and recent gaps. They found a switch from dominant negative associations in the wet southern end of the gradient to dominant positive associations in the dry northern end of the gradient and from closed forests to gaps. This result is highly consistent with facilitation theory predicting a switch from competition to facilitation with increasing stress (SGH hypothesis, Bertness and Callaway 1994). Additionally, this result has important consequences for tree regeneration in a climate change-adapted management perspective. If climatic drought strongly increases with climate change, as predicted for this region by modellers (Intergovernmental Panel on Climate Change, 2007), there should be a decrease in the competitive effect of understory species on tree seedlings in the wettest part of the gradient and thus less need to control forest weeds for regeneration success. Additionally, if facilitation increases at the driest end of the gradient, then foresters should rather conserve understory nurses to limit recruitment's mortality due to drought.

However, spatial associational patterns are not evidences of true interactions and the objective of this study was experimentally to test the hypothesis that interactions between oak seedlings turn from negative to positive with increasing drought stress along the climatic gradient and with increasing light levels from forests to gaps. Additionally, we also measured Vapor Pressure Deficit (VPD) in all treatment conditions in order to search for the driving mechanisms of oak seedlings-understory shrub interactions. Finally, Muhamed et al. (2012, submitted) could not adequately test the role of species strategies in their survey of associational patterns since the three oak species do not naturally occur all along the climatic gradient. Thus, in this study we transplanted the three species at the two ends of the gradient to test also the role of functional tree strategies in their response to neighbours in a changing climate. We aim to answer to two main questions (i) Does the net effect of understory shrubs on early oak survival shift from negative to positive with increasing drought stress from the wet southern site to the dry northern one and from closed forests to gaps? (ii) Are oak species responses species-specific?

2. Material and Methods

2.1. Study sites and target species

The field experiment was conducted in the coastal forest sand dune communities of the Aquitaine Region (south- western France). In Aquitaine,coastal dunes occur within an approximately 240 km-long sand strip stretching from the Gironde estuary in the north to the mouth of the Adour river in the south at the vicinity of the Pyrenees mountain range. The oak forest communities are located in inland fossile dunes, at approximately 1-10km distances from the ocean. The soil is homogeneous all along the coast and is a young podzosoil with a pure coarse sand texture (Forey et al., 2008). The climate is overall temperate oceanic with the rainiest period during winter. However, there is a south-north gradient of decreasing mean annual rainfall with increasing distance from the Pyrenees mountain range, with a mean annual rainfall of 1300mm in the south and 750mm in the north. Mean annual temperatures also decrease slightly but significantly along this latitudinal gradient, from14.1°C in the south to12.8°C in the north (Sardin, 2009). Our experiment was conducted in two experimental sites located at both ends of this gradient because of their contrasted climatic conditions, a wet southern site in Seignosse (43°41'N, 1°25'W) and a dry northern site in Soulac (45°32'N, 1°05'W). During the main year of the experiment (2011) annual precipitations, summer precipitations, annual temperatures and maximum summer temperatures were 1040mm, 152mm, 14.2°C and 24.1°C in the northern site, and 1473mm, 215mm, 15.75°C and 25.9 °C in the southern site, respectively (Meteo-France data, 2011).

Three oak species dominate the forest communities, one deciduous species, *Quercus robur*, and two evergreen *Quercus suber* and *Quercus ilex*. *Q. robur* is a common oak species in Europe, in particular in its northern and central parts where climate is temperate with no summer drought, whereas the two evergreen species are mostly found in the Mediterranean climatic area of southern Europe and north-Africa. These two Mediterranean oaks may also occur within the warm temperate or submediterranean parts of southern Europe where there is no summer drought, but only on dry soils, like is the case in the coastal dunes of the Aquitaine region. Thus, there is a strong difference in drought-tolerance between in one hand the deciduous temperate *Q. robur*, and the two evergreen Mediterranenan species (Ozenda 1985). However, there are also differences in drought- and cold-tolerance between *Q. suber* and *Q. ilex* (David et al., 2007), the former inhabiting warmer and wetter Mediterranean climates than the latter (Ozenda 1985, Michalet 1991). In the coastal dunes of the Aquitaine region *Q. suber* is indeed mostly present in the south where the climate is wetter and milder, whereas *Q. ilex* is very abundant in the north where the climate is drier and less mild. However, these differences in geographical distribution may also be influenced by anthropogenic factors since both species are traditionally planted for ornamental reasons and *Q. suber* for cork production.

2.2. Experimental design

In early October 2010, we set up a split-split plot design with four factors: (i) Site (Seignosse in the south and Soulac in the north, see above), (ii) Canopy (forest and gap), (iii) Neighbour (with and without shrubs) and (iv) Species (*Q. robur, Q. ilex*, and *Q. suber* seedlings). Within each site, we selected always in an east exposure, 6 forest plots and 6 gap plots for the canopy treatment, with a minimum of 500m distance between plots. The forest plots were mature oak forests with at least 75 % cover of adult oak trees and the gap plots were recent gaps (3-5 years), with no or very few remaining adult tree individuals in the overstory. For the neighbour treatment, which was embedded in the canopy treatment, we randomly selected, within each of the 12 plots from each site, 10 shrub individuals with approximately 10m distance between them. The shrub species were the same in the gap and forest plots of each site but different across sites, with *Q. ilex* and *Q. robur* in Soulac (north) and *Q. suber* and *Ilex aquifolium* in Seignosse (south). The height and two crown perpendicular diameters of all shrub individuals were measured with a tape; height of the shrubs varied between 120 and 250cm and their crown diameters between 140 and 240cm, with no significant differences between sites and between gap and forest plots. The aboveground canopy of half of the shrub individuals from each plot was removed by severing stems at ground level and all regrowing stems were cut again at each seasonal survey when necessary.

For the species treatment, which was embedded in the neighbour treatment, we used 1-2 year-old bare-rooted oak seedlings of the three species that were collected in natural stands and transplanted in all experimental conditions. *Q. ilex* seedlings were collected at the northern site (Soulac), *Q. suber* seedlings at the southern site (Seignosse) and *Q. robur* seedlings at intermediate latitude (44.4°N, 1.16°W). Seedlings were carefully lifted out from the sandy soils, stored in moist sandy soil in plastic boxes, and then planted at each site within two days. Target seedlings were always planted in 20 x 20 x 20cm manually dug holes located at the northern side of the shrub. One seedling of each of the three species was planted per shrub or shrub removed subplot, with at least 20cm distance between them. Thus, there were 5 seedlings per species, neighbour, canopy, and subsite and there were 6 subsites (n = 240 seedlings of each species). Hence, a total of 720 seedlings were planted in the whole design. In order to decrease the influence of transplantation shock, we replaced all dead seedlings (8.3% in Soulac and 11% in Seignosse), one month after planting, early November 2010.

2.3. Measurements

Survival and growth of each seedling was recorded at 5 times during the experiment, early October 2010 immediately after planting, March 2011 after the first winter, June 2011 after the first spring, October 2011 after the summer and March 2012 after the second winter. Survival was calculated at each date per plot as a percentage of the five individuals transplanted per combination of the site*canopy*neighbour*species treatments. Survival was calculated at each date in a cumulative and non-cumulative way, in order to assess the outcome for the transplanted seedlings and to compare results among seasons, respectively. However, only final survival rates calculated following the former method are presented here. For growth we measured seedling height, stem diameter at collar height and number of green leaves, and calculated a relative growth increment during the whole experiment for each growth measurement ((final measurement/Initial measurement) /initial measurement). However, because of a too high mortality occurring in some treatments, we did not analyse growth data.

We used the Relative Interaction Intensity (RII) index to assess the effect of the shrubs on the oak seedlings (Armas et al., 2004). The RII index compares the performance of targets with and without neighbors as follows:

$$RII_{shrub} = \left(S_{+N} - S_{-N}\right) / \left(S_{+N} + S_{-N}\right) \qquad (1)$$

Where S is the mean survival value of seedlings in the presence of shrubs ($+N$) and absence of shrubs ($-N$).

RII values are symmetrical around zero, varying between -1 and 1, with negative values for competition and positive values for facilitation. RII equal 0 when the net balance of interactions is neutral.

To assess the level of atmospheric stress occurring at each site*canopy*neighbour treatment combination condition, Vapor Pressure Deficit (VPD) was quantified by measuring air temperature and relative humidity at a height of 50cm above ground level, using HOBO-Pro RH/Temp Data Loggers (Onset Computer Corp., Pocasset, MA, USA). We used three HOBOs per site*canopy*neighbour treatment combination. Temperature and relative humidity were registered each hour and the mean value of the maximum VPD per day was calculated for the April-August 2011 period. VPD was calculated as:

$$VPD\ (Pascal) = ((100 - RH)/100) * SVP \qquad (2)$$

Where $SVP\ (Pascal) = 610.7 * 10^{7.5T/(237.3+T)}$ (3)

SVP and RH represent saturated vapour pressure and relative humidity.

2.4. Statistical analysis

Differences in oak survival rates due to the site, canopy, neighbour and species treatments were analysed with a split-split-plot ANOVA model, with site and canopy as main effects, neighbour as subplot effect and species as sub-subplot effect. Differences in RII values due to the site, canopy and species treatments were analysed with a split-plot ANOVA model, with site and canopy as main effects and species as subplot effect. Differences in VPD values due to the site, canopy and neighbour treatments were analysed with a split-plot ANOVA model, with site and canopy as main effects and neighbour as subplot effect. The effects of VPD (covariate) and of shrubs (factor) on final oak survival rates were analysed using an analysis of covariance (ANCOVA model). Finally, we use quantile regressions to estimate whether the association strength represented by the relative interaction index between shrubs and oak seedling species differs at different values of VPD. This procedure makes no distributional assumption about the error term in the model, and it is robust to outliers. Indeed, quantile regression offers considerable model robustness. Data were checked for normality and homogeneity of variance and all variables were log or arcsine-square root transformed before analyses to meet assumptions of parametric tests when necessary. Duncan multiple range tests were used to determine the significant differences between group’s means when significant effects ANOVA occurred at $P < 0.05$. One-sample t-tests were also performed to test significant deviations of RII values from zero. All statistical analyses were carried out in SAS 9.2 (SAS Institute Inc., USA).

3- Results

The three oak species had strong and highly significant differences in survival at the end of the experiment (Table 1, Fig. 1), with the highest survival for *Q. robur* (45.8 ± 4.2%), the lowest for *Q. suber* (16.6 ± 3.2%) and an intermediate survival for *Q. ilex* (25 ± 3.6%) (Duncan tests: a, c and b, respectively). When expressed in a non-cumulative way the strongest decline in survival occurred for the three species during the dry summer period (data not shown). There was also a highly significant canopy effect with a 45 % higher overall survival in the forest plots than in the gaps (Table 1, Fig.1). The neighbour effect was weakly significant but there was a highly significant canopy*neighbour interaction, because shrubs increased survival 4 times in the gaps but decreased it by 1.3 times in the forests (Table 1, Fig.1). There was also a weakly significant higher survival in the wet southern site of Seignosse than in the dry northern site of Soulac (33± 2.9% and 25 ± 3.6 %, respectively). However, there were significant site*neighbour and site*species interactions, since shrubs ameliorated survival in Soulac but not in Seignosse and only *Q. robur* and *Q. suber* (but not *Q. ilex*) had a higher survival in Seignosse than in Soulac. Finally, there were only weakly significant site*canopy and neighbour * species interactions, due to a higher survival in forest plots than in gaps in Seignosse only and to a slightly overall positive effect of shrubs for the survival of *Q. robur* and *Q. ilex* but not of *Q. suber*, respectively (Table 1, Fig.1).

There was a highly significant canopy effect on the RII index, because the shrubs strongly facilitated oak survival in the gap plots (RII = 0.48 ± 0.1) but reduced it in the forest plots (RII = - 0.18 ± 0.1). There was also a significant site effect since the effect of shrubs was overall positive (RII = 0.34 ± 0.1) in the dry northern dunes from Soulac but null (RII = - 0.04 ± 0.1) in the wet southern dunes from Seignosse (Table 2, Fig. 2). Although there were no significant interactions between the species and either the site or canopy treatments, results of sample *T*-tests show that only *Q. robur* and *Q. ilex* were significantly facilitated in the gap plots of Soulac and only *Q. robur* and *Q. suber* suffered from competition in the forest plots of Seignosse (Figure 2)**.**

VPD was highly significantly affected by the canopy conditions, increasing from forest plots (1.10 ± 0.04) to gap plots (1.53 ± 0.05) (Table 3). VPD values were also higher between shrubs (1.40 ± 0.08) than beneath shrubs (1.23 ± 0.07) and in the dry northern dunes from Soulac (1.39 ± 0.07) than in the wet southern dunes from Seignosse (1.24 ± 0.08) (neighbour and site effects, respectively, Table 3). There were no significant interactions among any of the three factors (Table 3). Interestingly, variation in VPD values strongly explained differences in survival among oak seedlings from removed shrubs plots, but not among seedlings from with shrubs plots ($F_{\mathrm{VPD\ x\ Neighbors}} = 6.4$, $df = 1$, $P = 0.02$, Fig. 3). This suggests that shrubs facilitated oak seedlings from highly stressed conditions by reducing VPD values. Finally, there was a significant (0.01) postive correlation between the relative interaction index and Vapor Pressure Deficit (VPD) (Fig.4).

Table 1: Results of the four-way ANOVA for the effects of site, canopy, neighbors, species and their interactions on survival rates of oak transplants. Significant effects at *P < 0.05* indicated in bold.

Source of variation	df	Anova SS	Mean Square	F Value	Pr > F
Block	5	0.774	0.155	1.82	0.11
Site	1	0.385	0.385	4.53	**0.03**
Canopy	1	2.462	2.462	28.94	**<.0001**
Neighbour	1	0.409	0.409	4.8	**0.03**
Species	2	4.465	2.233	26.24	**<.0001**
Site x Canopy	1	0.343	0.343	4.03	**0.04**
Site x Neighbour	1	0.741	0.741	8.71	**0.003**
Site x Species	2	1.130	0.565	6.64	**0.001**
Canopy x Neighbour	1	2.720	2.720	31.97	**<.0001**
Canopy x Species	2	0.232	0.116	1.36	0.25
Neighbour x Species	2	0.477	0.239	2.81	**0.04**
Site x Canopy x Neighbour	1	0.131	0.131	1.54	0.21
Site x Neighbour x Species	2	0.168	0.084	0.98	0.37
Site x Canopy x Species	2	0.416	0.208	2.45	0.09
Canopy x Neighbour x Species	2	0.230	0.115	1.35	0.26
Site x Canopy x Neighbour x Species	2	0.411	0.205	2.41	0.07

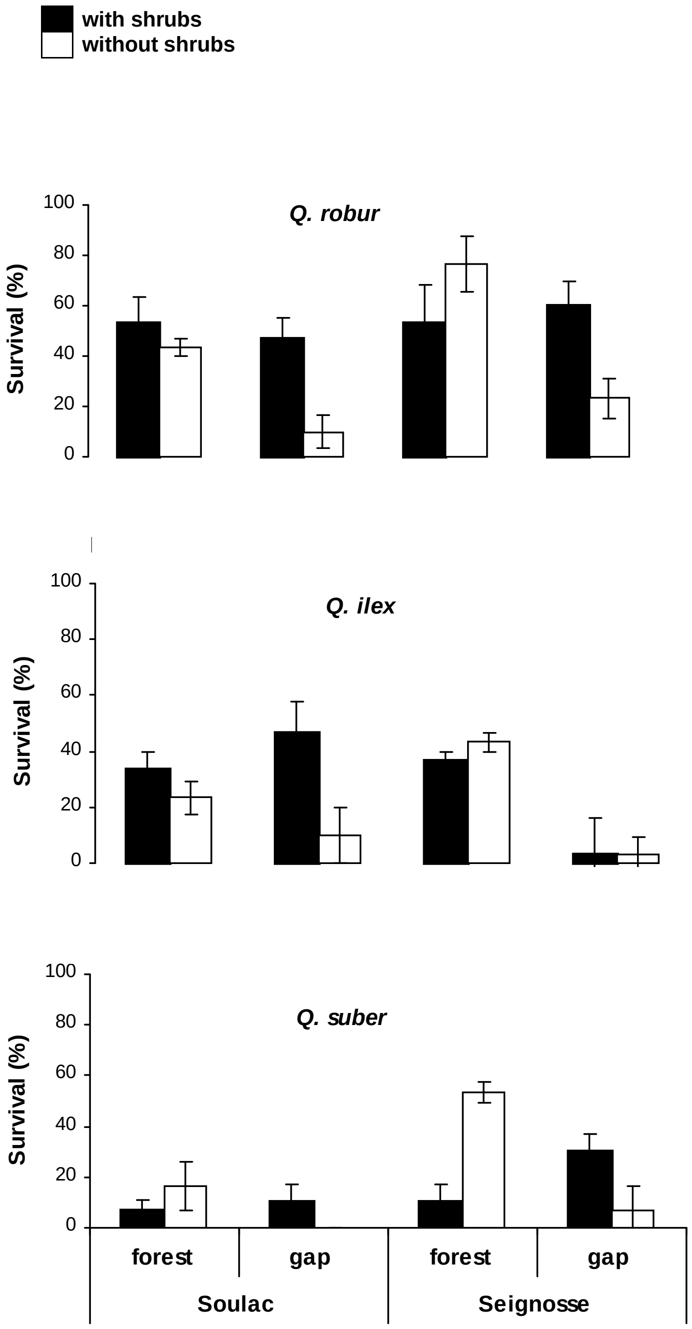

Fig. 1: Mean (N = 6) survival rates of the seedlings of the three oak species in the forest and gap plots of the dry northern site of Soulac and of the wet southern site of Seignosse. Shaded bars represent seedlings transplanted beneath shrubs and empty bars seedlings transplanted in removed-shrubs plots

Table 2: Results of the three-way ANOVA for the effects of site, canopy, neighbors and their interactions on the Relative Interaction Intensity (RII) index for the survival of oak transplants. Significant effects at *P < 0.05* indicated in bold.

Source of variation	df	Anova SS	Mean Square	F Value	Pr > F
Block	5	0.791	0.158	0.54	0.74
Site	1	2.577	2.577	8.74	**0.004**
Canopy	1	7.869	7.869	26.69	**<.0001**
Species	2	1.208	0.604	2.05	0.13
Site x Canopy	1	0.010	0.010	0.03	0.85
Site x Species	2	0.880	0.440	1.49	0.23
Canopy x Species	2	0.681	0.340	1.15	0.32
Species x Site x Canopy	2	0.829	0.415	1.41	0.25

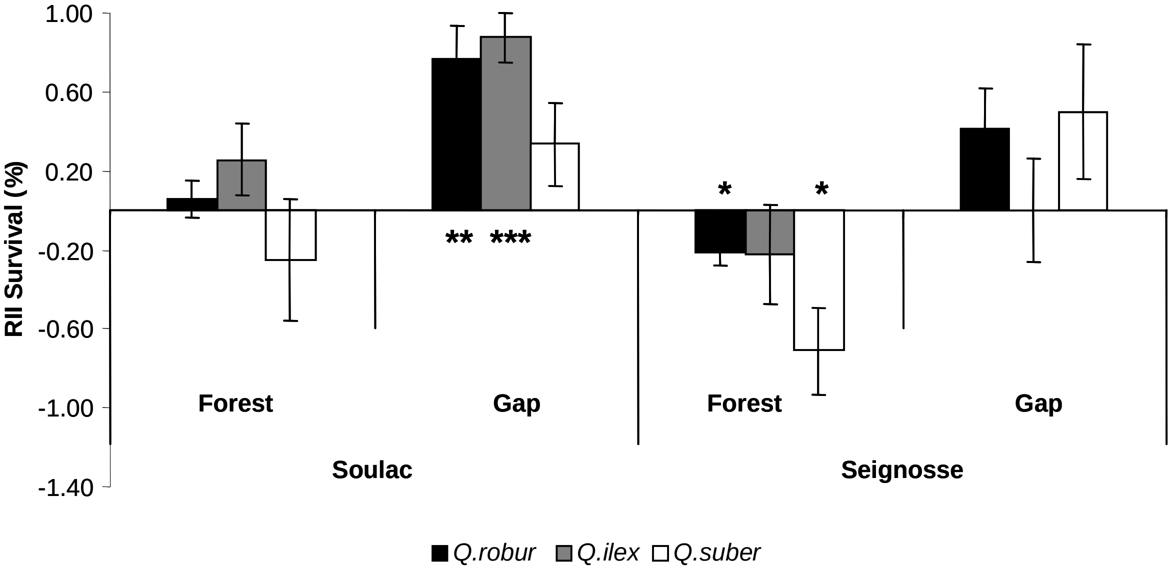

Fig. 2: Mean (N = 6) Relative Interaction Intensity (RII) index calculated for the survival of the three oak species transplanted in the forest and gap plots of the dry northern site of Soulac and of the wet southern site of Seignosse. Asterisks indicate significant deviations of RII values from 0 (one-sample t test): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 3: Results of the three-way ANOVA for the effects of site, canopy, neighbors and their interactions on mean values of VPD measured from April to August 2011. Significant effects at *P < 0.05* indicated in bold.

Source of variation	df	Anova SS	Mean Square	F Value	Pr > F
Block	2	0.002	0.001	0.11	0.89
Site	1	0.145	0.145	12.02	**0.003**
Canopy	1	1.092	1.092	89.97	**<.0001**
Site x Canopy	1	0.001	0.001	0.15	0.70
Neighbour	1	0.179	0.179	14.78	**0.001**
Site x Neighboor	1	0.007	0.007	0.65	0.43
Canpy x Neighbour	1	0.012	0.012	1.02	0.33
Site x Canopy x Neighbour	1	0.015	0.015	1.27	0.27

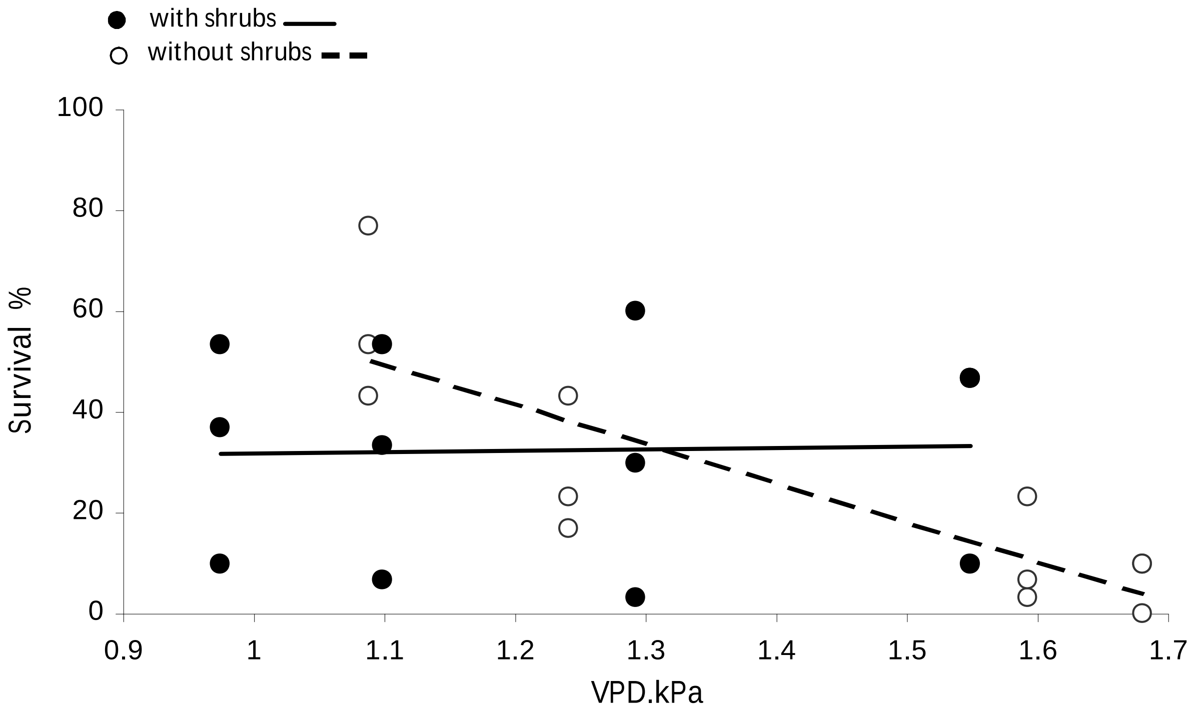

Fig. 3: Oak seedlings survival (%) as a function of the average Vapor Pressure Deficit (VPD) measured beneath shrubs (solid circle) and in the removed-shrubs plots (open circle). The solid line represents the non-significant regression line of the with-shrubs plots and the dashed line the regression line of the removed-shrubs plots.

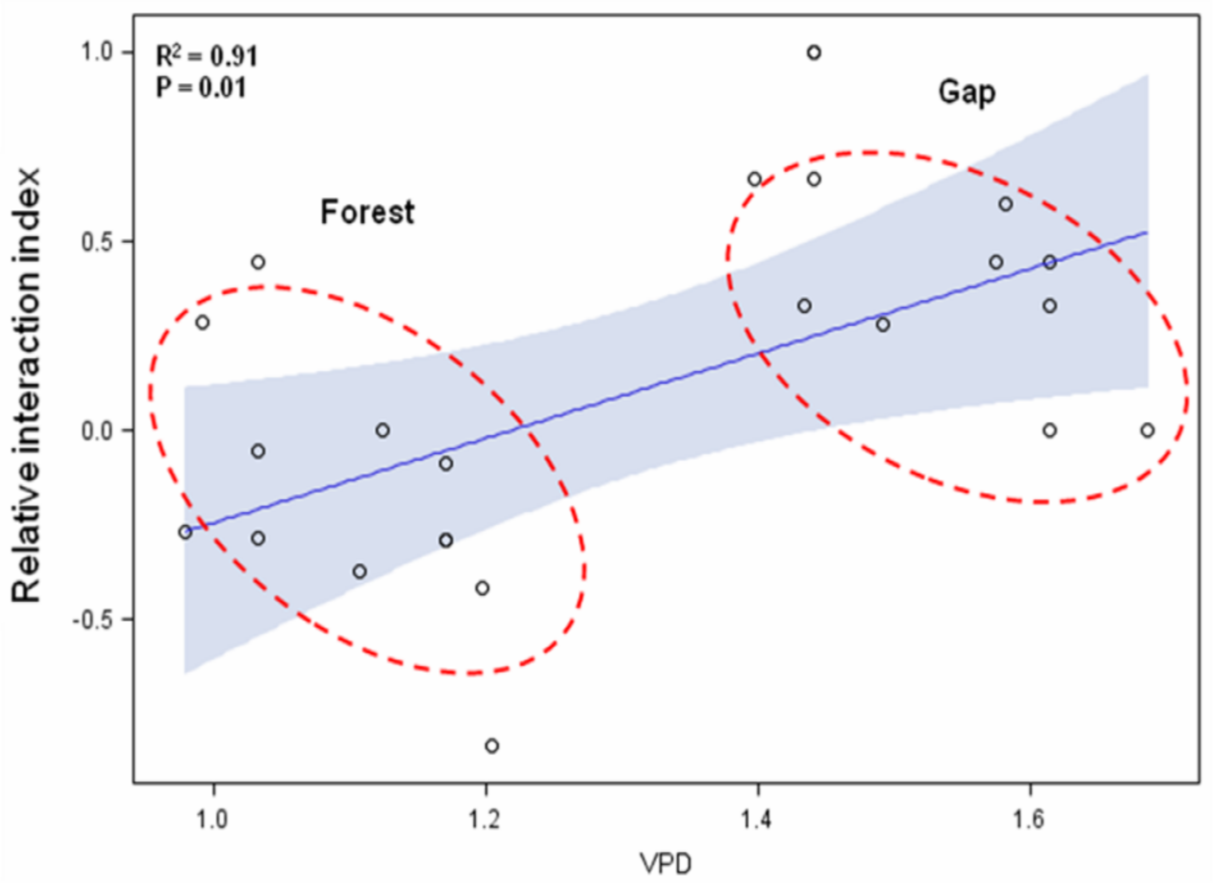

Fig. 4: Illustrates plot of the RII (Relative interaction index) versus VPD (vapor pressure deficit, [kPa]). Open circles are the 24 observations with 22 degrees of freedom, blue line represents the adjusted model and band plot is the confidence interval of the predicted mean. The net effect of shrubs on oak seedlings increase (RII) with increasing atmospheric stress (VPD) from forest to gap plots.

4- Discussion

Our results provide evidences of significant effects of understory shrubs on oak seedling survival in the coastal sand dune forests of the south-western of France. These effects strongly vary among sites and canopy conditions, with a shift from negative to positive interactions with increasing drought along the climatic gradient and from closed forests to gaps. In addition, our VPD measurements and their postive correlation with RII showed that the main process involved in the positive effect of the shrubs in dry conditions was certainly a decrease in atmospheric water stress below their canopies. In contrast, differences in species responses to the effects of understory shrubs were weak and not clearly related to species ecological requirements and geographical distributions. Interestingly, our results were very consistent with the spatial study of associational patterns of oak seedlings of Muhamed et al. (2012, submitted). Thus, although short, our experiment provides robust results highlighting the dominant interactions occurring in this system and allowing us to draw realistic recommendations for managing oak regeneration in a climate change-adapted forest management perspective.

4.1. Variation in oak seedlings responses to understory shrubs with site and canopy conditions

The most significant result of our study was the shift in interactions occurring between understory shrubs and oak seedlings due to the combined effects of canopy and climatic conditions (site effect). The effect of the shrubs on oak seedlings survival strongly shifted from a negative net balance in the forest plots of the wet southern site to a positive net balance in the gap plots of the dry northern site, with no significant interactions in intermediate conditions (i.e., gap plots of the south and forest plots of the north). This result strongly supports the Stress Gradient Hypothesis (Bertness and Callaway, 1994; Callaway, 1997;

Holmgren et al., 1997) proposing that the net effect of neighbors should turn from negative to positive with increasing environmental stress. This is also consistent with the results of a number of experiments conducted along spatial or temporal water stress gradients (Callaway et al., 1996; Greenlee and Callaway, 1996; Gomez-Aparicio et al., 2004; Sthultz et al., 2007; Cuesta et al., 2010).

However, other studies have found conversely an increase in competition with increasing water stress (Davis et al., 1998; Tielborger and Kadmon, 2000; Maestre and Cortina, 2004; Sthultz et al., 2007), which induced an important debate on variation in plant interactions along water stress gradient (Maestre et al., 2005; Lortie and Callaway, 2006). It has been suggested that these discrepancies may be due to different stress factors involved along complex aridity gradients, with an increase in water resource stress more likely inducing an increase in competition and an increase in atmospheric water stress more likely inducing an increase in facilitation (Michalet, 2007; Gomez-Aparicio, 2009; Maestre et al., 2009). This hypothesis was supported by Saccone et al. (2009) who found, for different tree species depending on their functional strategies, either an increase in facilitation with increasing Vapor Pressure Deficit (VPD) or an increase in competition with increasing soil water stress. Our measurements of VPD strongly suggest that the positive effects of understory shrubs on oak seedlings in the gap plots of the dry northern site was due to reduced atmospheric stress below their canopy. Indeed, we found a significant negative correlation between VPD values and oak survival for seedlings planted without shrubs but not for seedlings planted below shrubs. Furthermore we found that the net effects of shrubs positively increase in relation to increasing the atmospheric moisture stress (VPD) from the forest toward the gap. Thus, our study provides additional evidence that facilitation should increase with increasing water stress when the stress for the target species is rather atmospheric than resource-based (Valiente-banuet and Ezcurra, 1991; Gomez-Aparicio et al., 2005; Saccone et al., 2009; Cuesta et al., 2010).

4.2. Species-specificity of oak seedlings responses to treatments

We observed strong and significant differences in species survival, but a weak difference is species responses to understory shrubs. The deciduous oak, *Q. robur*, had a much higher survival than the two evergreen Mediterranean oaks. Additionally, *Q. robur* and *Q. suber* had a higher survival in the wetter and milder climate of the south, whereas there were no differences among sites for *Q. ilex*. The stronger overall survival of *Q. robur* may be explained by its deciduous habit and pioneer strategy and thus a likeable lower sensitivity to transplantation shock (Rameau et al; 1993; Rozas, 2003; Cater and Batic, 2006). The lower sensitivity of *Q. ilex* to site conditions, and in particular its higher survival in the stressed northern site as compared to the two other species in Seignosse, is consistent with its higher tolerance to physical stress and natural dominance in the north. Indeed, ecophysiological comparisons of the two Mediterranean species have shown that *Q. ilex* is more drought-tolerant than *Q. suber* (David et al., 2007). Additionally, the two species have contrasted climatic distributions within the Mediterranean region, the former inhabiting colder and drier climates, both in Europe and north-Africa, than the latter (Ozenda, 1985; Michalet, 1991). This is consistent with the high mortality rate of *Q. suber* in the dry northern dunes of Soulac.

Although the three target oak species have contrasted ecological requirements and geographical distributions, they had surprisingly very weak differences in responses to the effects of both the understory shrubs and canopy conditions. We only observed a weakly significant species*neighbor interaction in the four way ANOVA model on survival data, due to a slightly overall positive effect of shrubs for the survival of *Q. robur* and *Q. ilex* but not of *Q. suber*. However, there was no significant species effect in the three-way ANOVA model on RII values. This weak species-specificity of oak seedlings in responses to the effects of neighbors is quite surprising since most biotic interactions studies have highlighted that species functional strategies highly contribute to explain variation in species interactions along environmental gradients (Gomez-Aparicio et al., 2004 and 2009; Liancourt et al., 2005; Saccone et al., 2009 and 2010; Forey et al., 2010). Drought-tolerant species have been shown to be very sensitive to competition, whereas shade-tolerant species are in opposition very sensitive to facilitation (Michalet et al., 2006). In our study differences in species responses were weak and rather unclear since, for example for the two Mediterranean species, the least stress-tolerant oak, *Q. suber*, showed a tendency to be less facilitated than the most stress-tolerant one, *Q. ilex*. This low species-specificity observed in our experiment may be due to either low difference in shade tolerance among the three target oak species and/or to overwhelming effects of site and canopy conditions or a too small number of seedlings.

4.3. Implications for oak regeneration in a climate change-adapted management perspective

Our experiment provides clear evidence that (i) macro- and microclimatic conditions due to site and canopy conditions, respectively, strongly determine the direction and strength of interactions of oak seedlings with understory shrubs, (ii) oak seedling functional strategies had conversely no influence on understory shrubs-seedlings interactions strength. This result is highly consistent with the spatial patterns of associations of natural recruits of the same species with understory shrubs surveyed by Muhamed et al. (2012, submitted) in the same system but including a more important number of forest and gap plots. They found a clear shift from negative associations of oak seedlings with understory shrubs in the forest plots of southern wet sites to positive associations in the gap plots of northern dry sites, with neutral associations in intermediate geographical and canopy per site conditions. Additionally, they also found no obvious effects of oak species functional strategies on associational patterns. This suggests that, although short our experiment provides robust evidences of the dominant interactions occurring between understory shrubs and natural oak recruits in this system. In other words, the results of our experiment were not just representative of the particular climatic conditions that stochastically occurred the years of our study.

This certainly allows us to propose realistic recommendations for managing natural oak regeneration in a climate change-adapted forest management perspective. Current knowledge issued from climatic models predicts an increase in temperature and summer aridity for the Aquitaine region, as is the case for other parts of southern west Europe. Following the results of our experiment and the survey of associational patterns of Muhamed et al. (2012, submitted) on a spatial gradient of increasing water stress, and consistent with facilitation theory (Bertness and Callaway, 1994; Brooker, 2006; McIntire and Fajardo, 2009), we may predict that increasing water stress with climate change should induce a decrease in the negative influence of understory shrubs in the most benign physical conditions (closed forest plots from the south) and an increase in their positive effects in the most severe environmental conditions (gaps from the north). Thus, it is reasonable to consider that forest managers may in the future reduce forest weed control in the south of Aquitaine due to decrease competition with oak recruits and rather conserve understory shrubs and/or tree shelter in the north to maintain a sufficient oak recruit’s survival. In contrast, although longer experiments with a higher replication effort may reveal more clear results on the effects of oak species functional strategy, our study does not provide evidence that foresters should adapt their management of understory shrubs to particular oak species in this system.

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Appendix :

Fig.1: Relative Interaction Intensity (RII) index calculated for the survival of the three oak species transplanted in the forest and gap plots of the dry northern site of Soulac and of the wet southern site of Seignosse. Asterisks indicate significant deviations of RII values from 0 (one-sample t test): * *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001

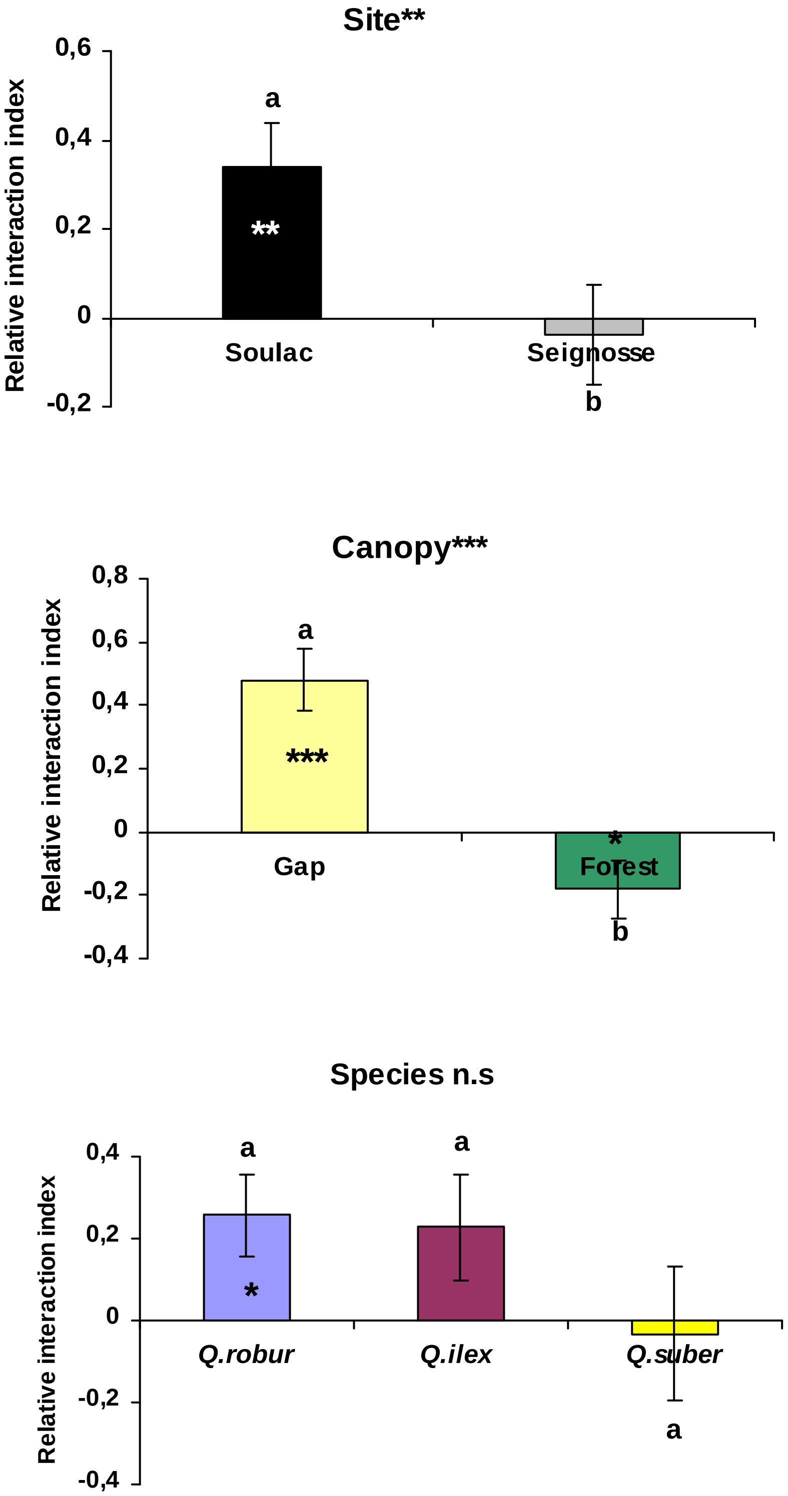


Fig.2. Vapour pressure dificite under different climatic conditions, site (wet south in Seignosse and dry north in Soulac), canopy (closed forest and gap) and neighbouring effect (under shrubs and without shrubs). * *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001

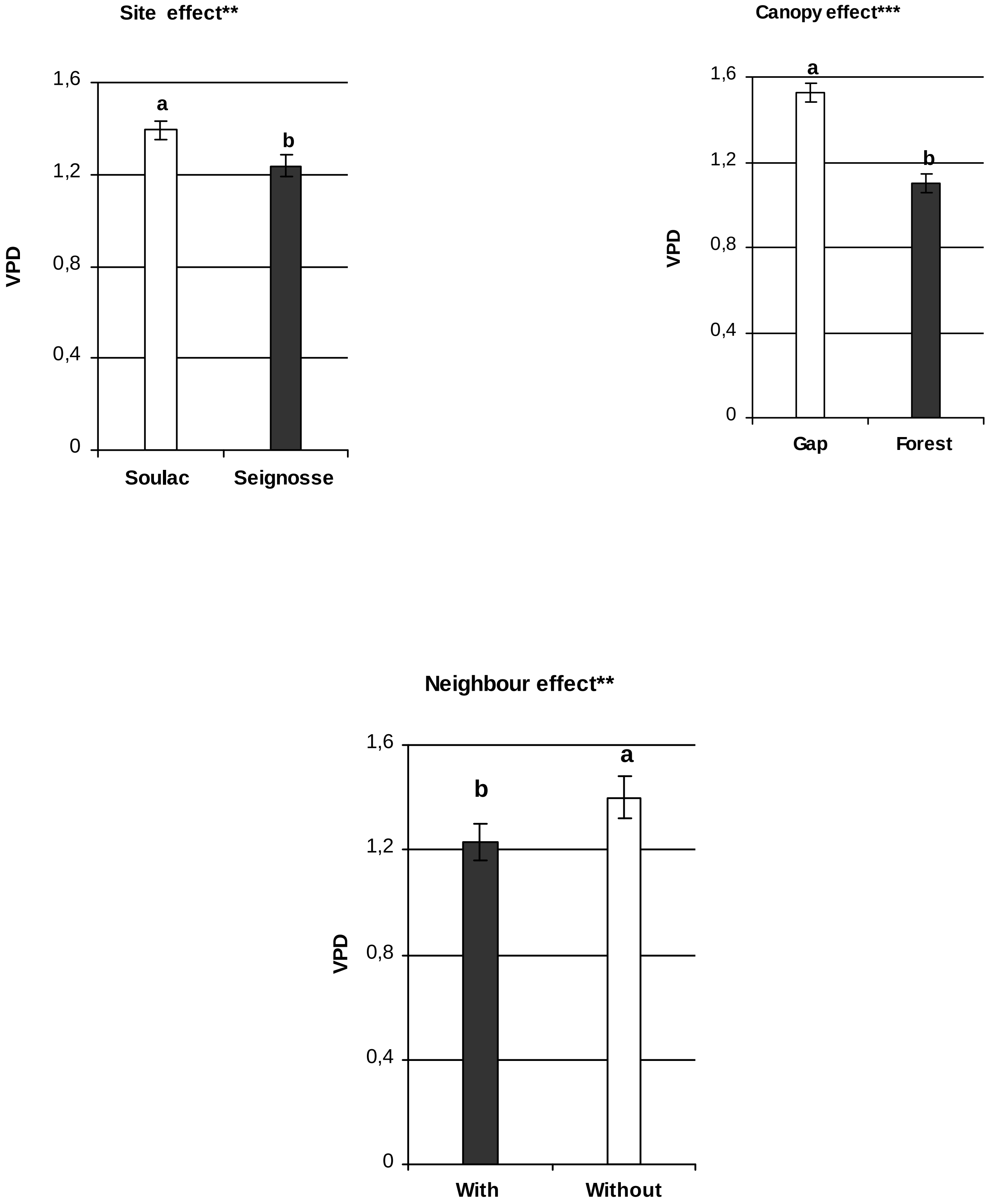
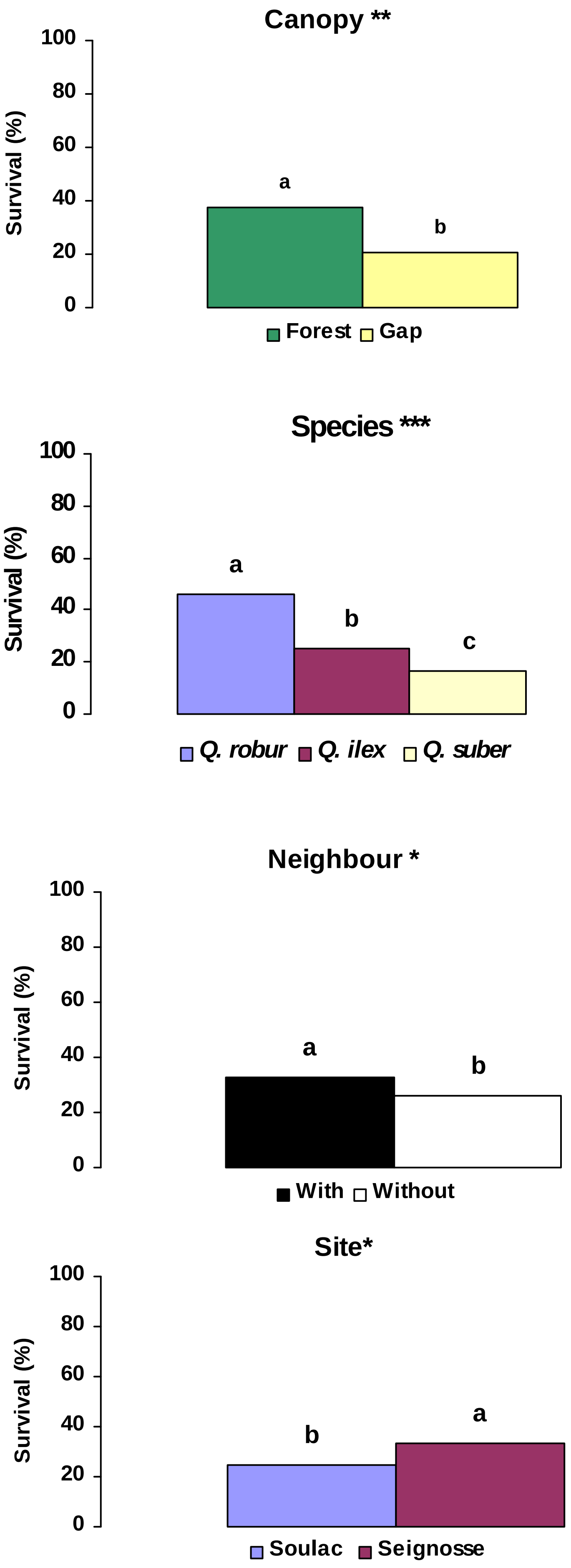
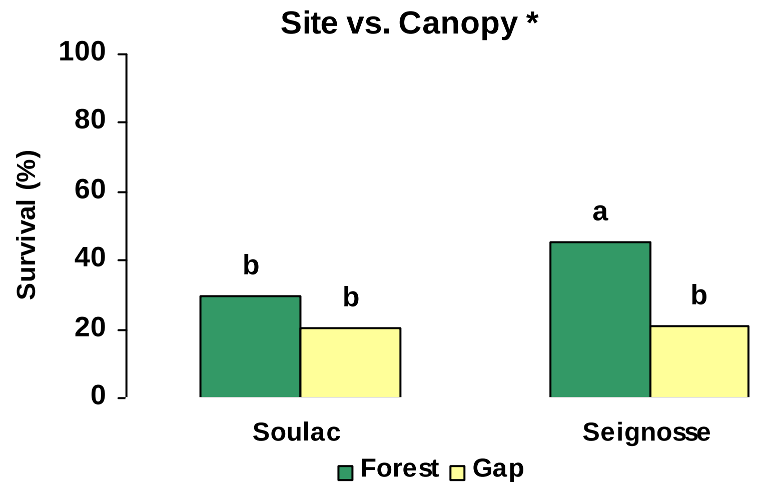
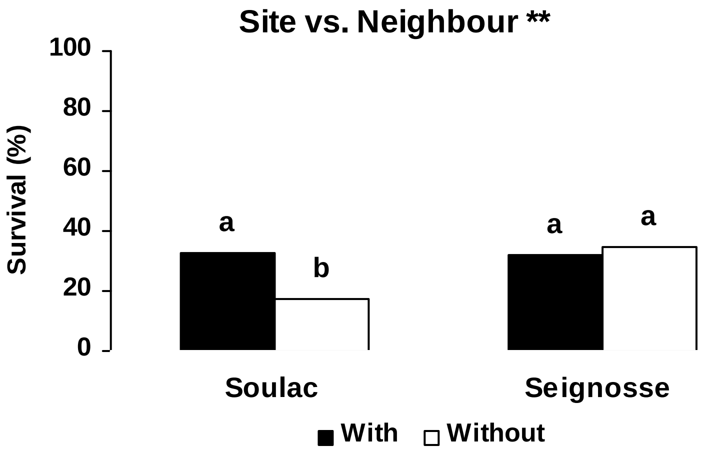
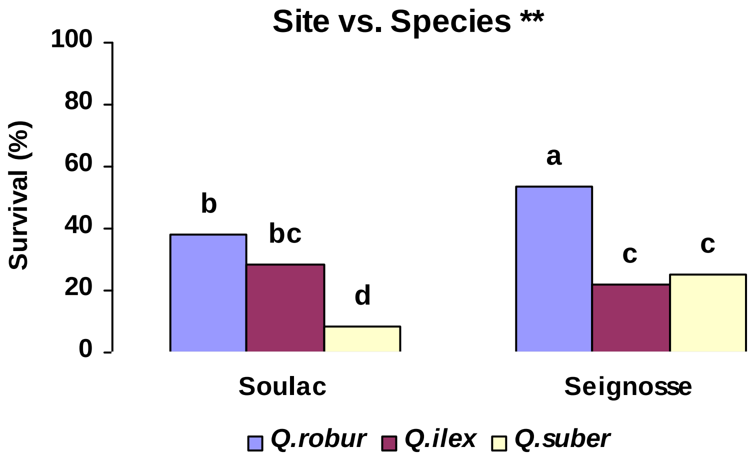


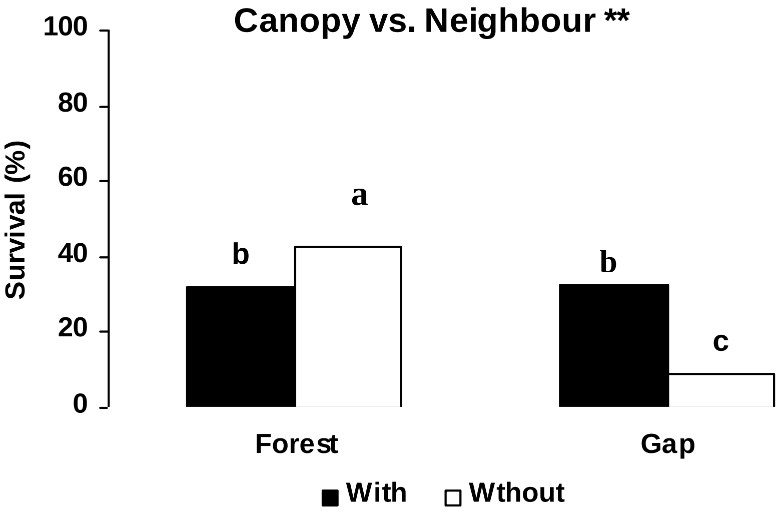
Fig. 3: Mean of survival rates of the seedlings of the three oak species in the forest and gap plots of the dry northern site of Soulac and of the wet southern site of Seignosse. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

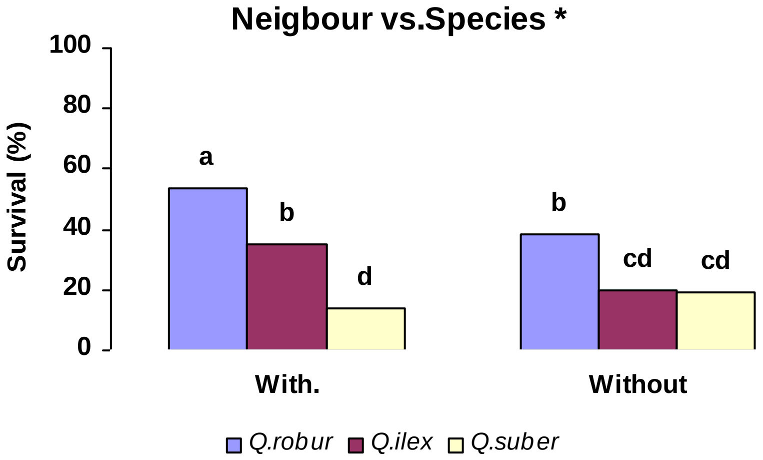


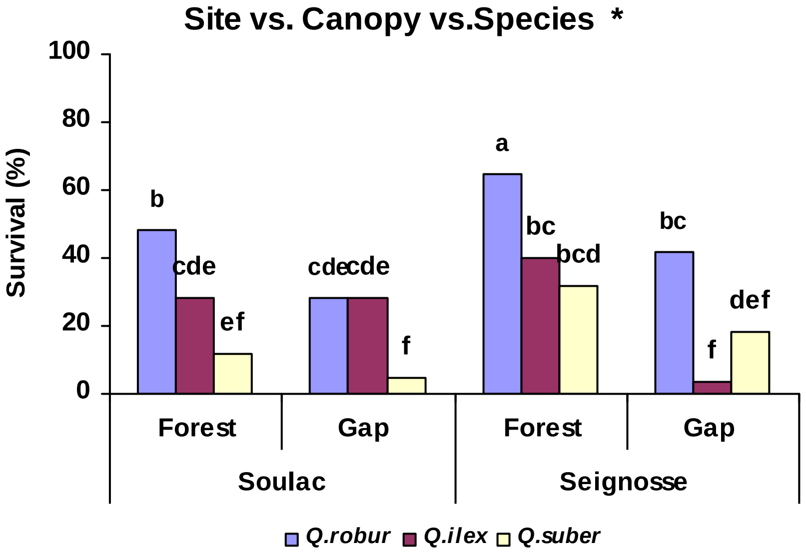

Site vs. Canopy *
Survival (%)
100
80
60
40
20
0
b
b
a
b
Soulac
Seignosse
Forest
Gap


Site vs. Neighbour **
Survival (%)
100
80
60
40
20
0
a
b
a
a
Soulac
Seignosse
With
Without


Site vs. Species **
Survival (%)
100
80
60
40
20
0
b
bc
d
a
c
c
Soulac
Seignosse
Q.robur
Q.ilex
Q.suber


Canopy vs. Neighbour **


Neigbour vs.Species *


Site vs. Canopy vs.Species *

Chapter 6:

General Discussion

A positive shrub-seedling interaction (facilitation) is a successful regeneration mechanism for woody seedlings under stressful climate conditions (Flores and Jurado, 2003; Castro *et al.*, 2004; Padilla and Pugnaire, 2006). However, biotic interaction patterns may change as response to overstory canopy conditions (Rodriguez-Garcia *et al.*, 2011), species' particular strategy (Liancourt *et al.*, 2005) and resource gradients (Callaway 2007). In the Aquitaine coastal dune forests, our understanding about the effect of these factors on the biotic role of shrubs for woody regeneration was still limited. This thesis came to fill this gap of knowledge through assessing biotic interactions role of shrubs with oak seedlings on the early oak regeneration and how this role change along a drought gradient, opening overstory canopy and in respect to the functional strategy of three oak species.

Each of the three studies that make up this thesis (chapters 3, 4 & 5) is an endeavour to gain more insights into the processes that influence the natural regeneration of oak species in these forest communities. Such information could be crucial for improving management in the context of climate change by identifying oaks regeneration niche requirements under current and future environmental conditions. Two major results appeared recurrently across this thesis (i) site-canopy specificity is a main driver determining the associations strength between shrubs and oak seedlings (ii) species functional strategy had conversely no obvious influence on the net associations balance. Accordingly, we found that SGH was the most appropriate model describing the shape of the severity-interaction relationships of the coastal dune forest communities in the Aquitaine region.

In the following sections, I discuss the main results in more detail.

1. Shrub-oak associations along a gradient of climatic severity (chapters 3 & 5)

Generally, the results obtained in the transplanting experiment (chapter 5) were very similar to those obtained in the spatial pattern study (chapter 3); both approaches detected significantly increasing positive shrub-oak interactions and associations with increasing drought from the wet south in Seignosse to the dry north in Soulac and from forest conditions to gap conditions. This finding supports the SGH for defining the spatial variation in the balance of positive and negative shrub-oak interactions between sites and overstory canopy treatments, and underscores the fact that facilitation by nurse shrubs is recommended for oak seedling establishment particularly under stressful conditions like those found in the gaps of the dry north in Soulac.

The positive role of biotic interaction increase with increasing drought between sites and canopy treatments

Results obtained from chapters (3) and (5) showed strong influences of the site and canopy treatments on the net effects of shrubs for oak regeneration. The interactions balance of shrub-oak changed from dominate competition under closed forest canopy in the wet south in Seignosse to dominate facilitation under gap conditions in the dry north in Soulac. These changes in the interactions balance is likely a result of the variation in the macro- and microclimatic conditions between sites and canopy treatments. This fact has been supported by the index of association strength (IA) (chapter 3, Fig.3) and the relative interaction intensity index (RII) (chapter 5, Fig.2), with both increasing significantly with increasing summer aridity index and VPD values from the south to the north and from forests to gaps. Generally, these findings are consistent with the original SGH, which attributes part of the variation in the strength of positive-negative interactions to the spatial variation in environmental severity (Bertness and Callaway, 1994; Brooker and Callaghan, 1998). In our study conditions this variation could be explained as follows: under severe conditions like those found in the dry north in Soulac, under a canopy gap, the ability of the oak seedlings to acquire available key resources was likely to be under the control of stress factors (such as increase in air temperature, irradiation and seedlings evapotranspiration particularly during summer). Therefore, any release for such limiting conditions by shrubs likely enhanced the early establishment of the seedlings (Bertness and Callaway, 1994; Brooker and Callaghan, 1998; Brooker *et al.*, 2008) as long as the positive effects of environmental modification by shrubs greatly exceed the costs of competing with shrubs (i.e., the improvement in plant moisture relations exceeds the cost entailed by a reduction in the level of available light). Conversely, under benign growing conditions like those prevailing in the wet south of Seignosse, under a forest canopy (such as adequate soil water and air moisture), both shrubs and seedlings may grow rapidly and the depletion of a key resource allow competitive effects to predominate (Buckley, 1984; Bertness and Callaway, 1994; Callaway, 1995). In other words, the net effect of the shrub is negative since the effects of competition with shrubs for resources are greater than the positive effects of environmental amelioration. In short, the net shrub-seedling interactions are positively increase with a relative increase in moisture severity due to changes in the strength of constituent positive and negative interactions between sites and canopy treatments (Callaway, 2007; Armas *et al.*, 2011)

Species-specificity: oak species strongly affected by site treatment but weakly responded to understory canopy shrubs.

Differences in survival between oak species (chapter 5) were more related to site conditions than to shrub effects. The highest survival rate of *Q. robur* under shrubs and in the wet south site is in agreement with its deciduous habit and likeable low stress-tolerance strategy (Rameau *et al*; 1993; Rozas, 2003; Cater and Batic, 2006). The strong site effect for *Q. suber* survival was obvious with the highest mortality rate in the dry north in Soulac (dry and relatively cold). This result is consistent with its relatively less drought-tolerant and cold-tolerant strategy as compared to *Q. ilex* which survived more in the north (García-Mozo *et al*., 2001 and Davis *et al.*, 1998). This result is also consistent with differences in geographical distribution in the coastal dunes communities, with the former in the south and the latter in the north.

Species-specificity: oak species particular strategy does not matter in net interaction balance

In contrast, the most stress-tolerant species, *Q. ilex*, was in the north more positively affected by shrubs than *Q. suber,* which is not consistent with the results of other experiments (e.g., Liancourt *et al*. 2005). Indeed, *Q. ilex* survival was enhanced under shrubs in particular in the gaps of the dry north site, indicating that *Q. ilex* was less water stressed and photo-inhibited under the nurse shrub than in the open (Baquedano and Castillo, 2006). Thus, although species functional strategy is considered as one of the important factors that can affect the net balance of plant-plant interactions (Liancourt *et al.*, 2005; Wang *et al.*, 2008), we did not detect important and consistent differences among oak species in their responses to the net biotic effects of the shrubs (chapter 5, Fig.2). This suggests that the dominant environmental conditions at both ends of the gradient did not differ enough in their influence among the three species. Thus, the net amelioration provided by shrubs in the gap of the dry north or the net competition in the forest of the wet south almost evenly distributed among the three species thus did not allow the oak species to produce an observable difference in the balance of shrub-oak interactions. For example most of the amelioration effects of the shrubs were involving non-resources factors (i.e., buffering soil temperature with modify atmospheric stress by reducing irradiance intensity). Consequently, and considering that targeted species could not be expected to vary sufficiently in their atmospheric and light requirements, the three species responded in a similar positive tendency to the net effect of shrubs in the dry site in gap (chapter 5, Fig.2) (Gomez-Aparicio *et al.*, 2008). This pattern is understandable since the three species concerned here do not have notably contrasted light and successional position requirements, i.e., the two evergreen species avoiding atmospheric stress in full light conditions (Espelta *et al.*, 1995; Penuelas *et al.*, 2001; Perez-Devesa *et al.*, 2008; Cuesta *et al.*, 2010), and *Q. robur* requiring gaps for regeneration in a shady temperate environment (Rozas, 2003; Cater and Batic, 2006). The second reason may be that the relatively strong canopy and site effects on both IA (chapter 3) and RII indexes (chapter 5) likely suggest that site-canopy specificity overrode any effects of species specificity on the net balance of shrub-oak interactions.

Reduction of atmospheric moisture stress by shrubs facilitates early establishment of oak seedlings

Interestingly, the significant positive correlation between IA index and increasing summer drought across the gap plots (chapter 3, Fig.3), and the significant negative correlation between the survival of seedlings planted without shrubs and increasing VPD value (chapter 5, Fig.3) reinforced the hypothesis that shading by shrubs plays a key role in the regeneration of oak seedlings under stress climatic conditions. Therefore, the likelihood of oak seedlings becoming established under a shrub canopy in gap plots (32.78 ± 4.92 %) was approximately four times higher than that of seedlings planted without shrubs (8.89 ± 2.92 %) (chapter 5). The shrub canopy certainly helps maintaining low soil temperatures and mitigating atmospheric stress, thereby buffering seedlings from midday temperature extremes and photoinhibition by reducing irradiance. Shading by shrubs has often been used to explain the generally better performance of seedlings situated under or close to a canopy than in the open (Callaway, 1992; Maun, 1998; Shumway, 2000; Gomez-Aparicio *et al.*, 2004; Quero *et al.*, 2006).

2. The functional groups of shrubs differently effects on the seedlings of pooled-oak species (chapter 4)

Species-specificity not only concerns beneficiaries but also benefactors since they may differ in their effects according to their ecological and architectural characteristics (Callaway 1995; Gomez-Aparicio *et al.*, 2004). We tried to consider that in chapter (4) by conducting a wide assessment of types and frequency of inter and intra-specific associations of four functional groups of shrubs with oak seedlings. Generally, we found strong dissociation of Ericaceae with the seedlings of pooled-oak species; we regarded this pattern consistent with the allelopathic characteristics of this family and in line with other previous studies (Walker *et al.*, 1999; Ballester, 1982; Gonzalez-Martinez and Bravo, 2001; Eppard *et al.*, 2005). The relatively more important frequency of positive associations found with intra-specific Fagaceae than with other functional groups of shrubs does not imply that it is overriding the overall inter-specific (Ericaceae, Fagaceae, Fabaceae and Spiny) in enhancing oak seedlings. Indeed, 10 positive cases of inter-specific associations were recorded in comparison to 7 cases for intra-specific Fagaceae shrubs (chapter 4, Table 3). This shows that intra-specific Fagaceae may have a positive role beside other positive cases of the rest of functional shrubs particularly if we consider acorn dispersed under Fagaceae shrubs could be later enhanced by their intraspecific shrubs. However, the lack of data about the patterns of seedling survival and performance under and out the canopy of shrubs particularly for Fagaceae beside uneven frequency in number of the replicates of the functional shrubs over our study area may not allow us to strongly confirm our results. Nonetheless, one can be more confident about the negative effect of Ericaceae and just hypothesized for future experiments that Fagaceae shrubs probably play a role in facilitating the early establishment of oak species in Aquitauine coastal dunes forests.

3. Considerations for management under climate change

Certain shrubs can offer the benefits of proximity (e.g., microclimate amelioration, increased soil fertility, association defence) at minimum costs in terms of competitive effects (Gomez-Aparicio *et al.*, 2009). For example, when positive shrub-seedling interactions dominate, seedlings exploit efficiently the environmental conditions that have been modified by shrubs (Tewksbury and Lloyd, 2001). In the perspective of biotic interactions, shrub-seedling interactions have been suggested to facilitate the establishment of oak seedlings under severe conditions (Callaway 1992; Gomez-Aparicio *et al.*, 2005 and 2008; Cuesta *et al.*, 2010). In the light of the Intergovernmental Panel on Climate Change report (2007), this role will become much more necessary in the future since the temperature in oceanic climate regions expected to increase 3°C by 2100, and summer is likely to become dryer and hotter. These expected changes in climate are likely to increase the degree of the current abiotic stress and may further impede natural oak recruitment, particularly in the dry northern dunes where summer drought is considered one of the main limiting factors that hamper tree regeneration at seedling stage (Sardin, 2009). In this thesis our results implies that the removal of shrubs is not appropriate for oak natural regeneration particularly in gap areas.

Conserving shrubs vegetation covers in dry northern dune forests

Increasing both IA and RII between shrubs and understory oak seedlings with increasing summer drought and VPD values in gap plots, suggests that positive association can play a role in alleviating the ecological consequences of climate change in the dry north site (chapters 3 & 5) (Lortie *et al.*, 2004; Dona and Galen, 2007; Maestre *et al.*, 2010).

Considering that and the fact that removing shrubs in gap plots in our study markedly increased mortality, we suggest that the aim of management of oak regeneration niches should be to maintain a shrub vegetation cover to enhance the natural regeneration of oak under current and increasing climate severity. Moreover, naturally occurring shrubs could be used also as a regeneration tool to promote the early establishment of artificial regeneration either direct-seeding acorns or seedlings planting. In this context, we are not able to recommend particular management of understory shrubs for specific species since our results showed similar response of oak species in their response to understory shrubs (chapters 3 & 5). However, one can recommend controlling Ericaceae species to avoid any inhibiting role on early oak regeneration (chapter 3).

Reduce forest weed control in the wet southern dune forests

Increasing facilitation in the dry northern dunes (chapters 3 & 5) mostly will be concomitant to decreasing competition strength in the wet southern dunes due to increasing drought stress with climatic change. Traditionally, foresters in this site consider shrubs and other vascular species as a competing sources limiting woody natural regeneration. However, with increasing severity due to climate change this will lead decreasing in competition strength consequently the need to control understory weeds (including shrubs) will be less. Therefore, we recommand to decrease this activity particularly with Fagaceae shrubs, because such increase in severity with ongoing climate change may limit the uptake of resources by seedling, allowing habitat amelioration provided later by shrubs to become on of the major determinant of oak natural regeneration in this site.

4. Future outlook

According to the three studies that were the cornerstone of this thesis, we found some limitations precluding clearly considering the species functional strategies of both targeted seedlings and nurse shrubs effects on the oak regeneration. These limitations are certainly due to (i) no sufficient replications of involved species over the study sites (chapters 3 & 4); (ii) short term duration of the transplanting experiment (chapter 5). Therefore, we believe a long-term experimental work including transplanting the three oak species under canopy of different shrubs species at both ends of gradients with examining multiple traits of targeted seedlings will provide valuable information to select the best nurse species capable to improve oak regeneration and the success of restoration projects with increasing climatic severity.

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