Effects of Subordinate Plant Species in Plant and Soil Community Structure and Ecosystem Functioning

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À mes grand-parents et ma famille

L’écologie est une magnifique occasion, peut-être même
l’ultime occasion, de redonner du sens au progrès.

Nicolas Hulot

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Summary

The relevance of biodiversity to human health is an increasing international political issue as it causes concern for ethical and aesthetic reasons, but also has a strong impact on ecosystem properties and ecological goods and services utilised by humanity. However, humans have applied increasing pressure on worldwide biodiversity through pollution, land-use and climate change, over exploitation and the introduction of invasive species which can lead to major alterations of biological communities and a consequent decrease in biodiversity.

When communities are assembled at random from a pool of species, more diverse mixtures have a higher probability to contain species or species-groups with high capacity to drive ecosystem processes. Therefore, it is relevant to ask "how may we classify species in a community in terms of functionality?", and "which species-group is important to maintain the productivity and stability of ecosystems?" In this thesis, I chose to classify species according their frequency and their cumulative relative cover in a plant community (i.e. plant hierarchy) and to differentiate three species-groups: dominant, subordinate and transient, which contribute towards biodiversity.

Dominant species are clearly very important for ecosystem functioning, due to the large amount of biomass they produce, but there is growing evidence that subordinate species, which represent a low amount of plant biomass in grassland ecosystems, are also of considerable importance for ecosystem functioning. Given this recognition, a further objective was to explore the ecosystem level effects of these subordinate plant species, which is currently not well known. This research focused on two aspects: the persistence of subordinate species in the community, and their role in grassland ecosystems. The approach to satisfy these objectives encompassed four years of experimental field studies and glasshouse microcosm experiments.

In order to explain the persistence of subordinate species in semi-natural grasslands, we performed two greenhouse experiments to test the effects of root competition exclusion and the inoculation of arbuscular mycorrhizal fungi (AMF) on the competitiveness of dominant and subordinate species, directly measured by their biomass production. The effects of subordinate species on ecosystem functioning were assessed through long-term field experiment in two sites in the Swiss Jura Mountains, where dominant, subordinate and transient species were preliminarily determined. At both site (Les Amburnex, La Frétaz), a removal experiment was carried out in randomly replicated plots with three different treatments: control without perturbations, removal of all subordinate species and partial removal of dominant biomass. In one site, a summer drought treatment was added using rainout shelters to simulate an extreme climatic event. Biomass production, litter decomposition and soil respiration were monitored during four years in each plot and complemented in some cases by measurements of soil dissolved inorganic nitrogen concentrations, soil microbial communities (bacteria and AMF) and carbon and nitrogen isotopes in plant leaves.

The results of the different experiments conducted in this thesis highlight the importance of cattle activity and AMF on the persistence of subordinate plant species in semi-natural grasslands. Indeed, cattle activities (i.e trampling) provide spatial heterogeneity, through gap creation (areas of root competition reduction), and thus favours the growth of less competitive subordinate species. While AMF acted as a parasite in the greenhouse experiment, the current findings suggest that the competitiveness of dominant species was reduced through the action of the fungus, which indirectly enhanced subordinate species. The interactions between subordinate species and soil organisms were confirmed in the field as bacterial and AMF communities shifted in plots where subordinate species were removed compared to the control treatments. Moreover, the absence of subordinate species had many negative effects on ecosystem functioning by reducing litter decomposition, soil respiration, nitrogen mineralization and community above-ground production. These findings suggest that plant-soil feedbacks explain the importance of subordinate species on ecosystem functioning despite only representing a very low quantity of biomass in plant communities. During the summer drought, subordinate species increased the resistance of the plant community and maintained productivity. Therefore, these outcomes confirm the role of drought-resistant subordinates in the functioning of grassland ecosystems threatened by climate change.

The synthesis of this thesis has important implications for the way grasslands should be managed for biodiversity and confirms that extensive grazing must be maintained as it promotes species coexistence and the persistence of key subordinate species. Moreover, this work demonstrates the important role of subordinate species in ecosystem functioning, showing that not only dominant species determine ecosystem properties. This thesis serves as a contribution to the advances in our understanding of ecosystem functioning and above and below-ground linkages, and provides basis for future research in this domain.

Keywords:

Above- and below-ground interactions, ecosystem functioning, grasslands, hierarchical classification, insurance hypothesis, plant-soil feedbacks, species coexistence, subordinate species.

Résumé

La biodiversité est devenue une préoccupation internationale, tant au niveau politique qu'au niveau du public, puisqu'elle est reconnue comme la source première des services rendus par les écosystèmes. Cependant, les activités anthropiques ont entrainé une forte pression sur la biodiversité mondiale à cause de la pollution, des modifications d'exploitation des terres agricoles, du changement climatique, de la surexploitation des milieux ou de l'introduction d'espèces invasives.

Dans une communauté où les espèces sont assemblées de façon aléatoire, les communautés les plus diversifiées ont davantage de probabilités de contenir des espèces ou groupes d'espèces ayant des effets importants dans le fonctionnement de l'écosystème. De cette façon, il est judicieux de se demander : "Comment peut-on classer les espèces en terme de fonctionnalité ? Et quels groupes d'espèces sont importants pour maintenir la productivité et la stabilité des écosystèmes ?" Dans cette thèse, j'ai choisi de sélectionner les espèces selon leur fréquence d'apparition et leur recouvrement relatif cumulé dans la communauté végétale. Trois grands groupes d'espèces qui participent à la biodiversité ont alors été différenciés : les espèces dominantes, subordonnées et transitoires.

Tandis que les espèces dominantes ont des effets très importants sur le fonctionnement des écosystèmes en raison de la grande quantité de biomasse qu'elles produisent, de récentes études montrent que les subordonnées pourraient avoir un rôle plus important que ce que leur faible abondance suggère. Ainsi, notre objectif était d'explorer les effets de ces espèces subordonnés qui n'ont, jusqu'à présent, jamais été étudiés dans l'écosystème. Cette étude s'est concentrée sur deux aspects différents : les mécanismes de persistance des espèces subordonnées et leur rôle dans les pâturages.

Dans le but d'expliquer la persistance des espèces subordonnées dans les pâturages, nous avons mis en place deux expériences en serre où l'exclusion de compétition racinaire et l'inoculation de mycorrhizes ont été testées sur la compétitivité des espèces dominantes et subordonnées. Le rôle des espèces subordonnées a été mis en évidence à l'aide d'expériences de terrain sur deux sites des montagnes du Jura Suisse où les espèces dominantes, subordonnées et transitoires ont été déterminées. Dans chaque site (Les Amburnex et La Frétaz), une expérience de suppression a été mise en place sur différentes placettes avec trois traitements différents : contrôle sans perturbations, suppression des espèces subordonnées, suppression de biomasse d'espèces dominantes. Sur un des deux sites, nous avons ajouté un traitement de sécheresse estivale à l'aide de toits interceptant les précipitations pour simuler un évènement climatique extrême. Durant les quatre années d'expérience, nous avons suivis la production de biomasse végétale, la décomposition de la litière et la respiration du sol dans chacune des placettes. Des analyses d'isotope du carbone ($\delta^{13}C$) dans les feuilles, de l'azote inorganique dissous dans le sol et des communautés microbiennes (bactéries et mycorrhizes) sont venues compléter les mesures dans certains cas.

Les résultats des différentes expériences de cette thèse ont mis en évidence l'importance de l'activité du bétail et des mycorrhizes dans la persistance des espèces subordonnées au sein des prairies semi-naturelles. En effet, le bétail favorise l'hétérogénéité spatiale (piétinement) et crée des trouées dans la communauté (zone de réduction de la compétition racinaire) permettant aux subordonnées moins compétitives de se développer. Bien que les mycorrhizes agissaient comme des parasites dans notre expérience en serre, nos résultats ont montré que la compétitivité des espèces dominantes était réduite par l'action négative des champignons, permettant ainsi aux subordonnées, moins affectées, de croître davantage. Les interactions entre les espèces subordonnées et les organismes du sol ont été confirmées sur le terrain puisque l'absence des subordonnées dans certaines placettes a modifié les communautés bactériennes et mycorrhiziennes du sol. De plus, la perte des espèces subordonnées a entrainé des effets très négatifs sur le fonctionnement de l'écosystème en réduisant le taux de décomposition de la litière, la respiration du sol, la minéralisation de l'azote et la productivité végétale. Nos résultats suggèrent que les effets induits sur les communautés microbiennes et les processus du sol affectent en retour la communauté végétale, expliquant le rôle important des espèces subordonnées sur le fonctionnement de l'écosystème malgré leur faible abondance. Pendant la sécheresse estivale, les subordonnées ont augmenté la résistance de la communauté végétale et maintenu la productivité. Nos résultats confirment alors que les espèces subordonnées pourraient être plus résistantes à la sécheresse et ainsi promouvoir le fonctionnement des pâturages, aujourd'hui menacés par les changements climatiques.

La synthèse de ce travail entraîne d'importantes implications sur la façon dont les prairies devraient être gérées et confirme que le pâturage extensif par le bétail doit être maintenu puisqu'il permet la coexistence des espèces végétales ainsi que la persistance d'espèces clés que sont les subordonnées. Par ailleurs, nous avons démontré que les espèces subordonnées avaient un rôle majeur à jouer dans le fonctionnement des pâturages montagnards du Jura Suisse et les conclusions de cette thèse participent à la meilleure compréhension du fonctionnement des écosystèmes et des interactions entre les plantes et le sol.

Keywords: coexistence, espèces subordonnées, fonctionnement de l'écosystème, hiérarchie d'espèces, interactions sol-plantes, pâturage, résistance de la communauté.

Chapter 1

General introduction

Biodiversity and its components

Biodiversity or biological diversity is the term given to the variety of life on Earth (like plants, animals, bacteria, protists, etc.) which forms the web of life. The relevance of biodiversity to human health is becoming an international political issue as it causes concern for ethical and aesthetic reasons but has also a strong impact on ecosystem properties and ecological goods and services (EGS) provided to humanity (Hooper et al. 2005, Bunker et al. 2005, Bell et al. 2005), such as biomass production (food and timber), carbon storage (climate regulation), landscape heterogeneity and scenery (leisure), etc. Therefore, biodiversity has a market place through ecosystem services and its replacement in terms of cost has been evaluated to US$ 33 trillion per year (Costanza et al. 1997). Biodiversity is recognized worldwide as a driver of ecosystem functioning and has been largely studied especially in plant communities (Hector et al. 1999; Grime 1997; Van der Putten et al. 2000; Loreau et al. 2001; Hooper et al. 2005; Hector & Bagchi 2007).

Nevertheless, the term "biodiversity" can be described in different ways, depending on its scale of observation (genes, species, communities, habitats, etc.) and need to be clarified when exploring the effects of biodiversity. Recent meta-analyses revealed that research on plant biodiversity loss focuses primarily on the decrease of species richness to explain changes in ecosystem functioning (Balvanera et al. 2006; Cardinale et al. 2006; Hector & Bagchi 2007). However, species richness seems to be not enough to explain the importance of biodiversity and is actually much related to sampling process. Under the sampling effect hypothesis (SEH) (Aarssen 1997; Huston 1997; Tilman et al. 1997), when communities are assembled at random from a pool of species, more diverse mixtures have a higher probability to contain species or species-group with high capacity to drive ecosystem processes. Therefore, it is much more relevant to ask 'How we may classify species in a community in term of functionality? And which species-group is important to maintain the productivity and stability of ecosystems? Many studies tried to classify species in plant communities and to demonstrate the importance of functional groups (Wardle et al. 2003; MacLaren & Turkington 2010), functional traits (Lavorel et al. 2011), relative biomass proportion (Grime 1998) or keystone species (Lyons & Schwartz 2001; Boeken & Shachak 2006) for ecosystem functioning. These different components of biodiversity can have different effects on ecosystem functions (Hooper et al. 2005) but are not necessarily independent.

In his attempt to define functional role of species, Whittaker (1965) recognized that the easiest way to classify components of biodiversity is to order species according their relative abundance or productivity. This classification was taken up by Grime (1998) with the mass ratio hypothesis which suggests that dominant species, accounting for a large proportion of the total community biomass, determine to a large extent ecosystem properties. Plant classification from dominance-diversity profiles, summarized here as an abundance-frequency curve (Fig. 1.1), proposed that within herbaceous vegetation samples, three components may be distinguished: dominant, subordinate and transient which may vary in species richness, functional traits and groups.

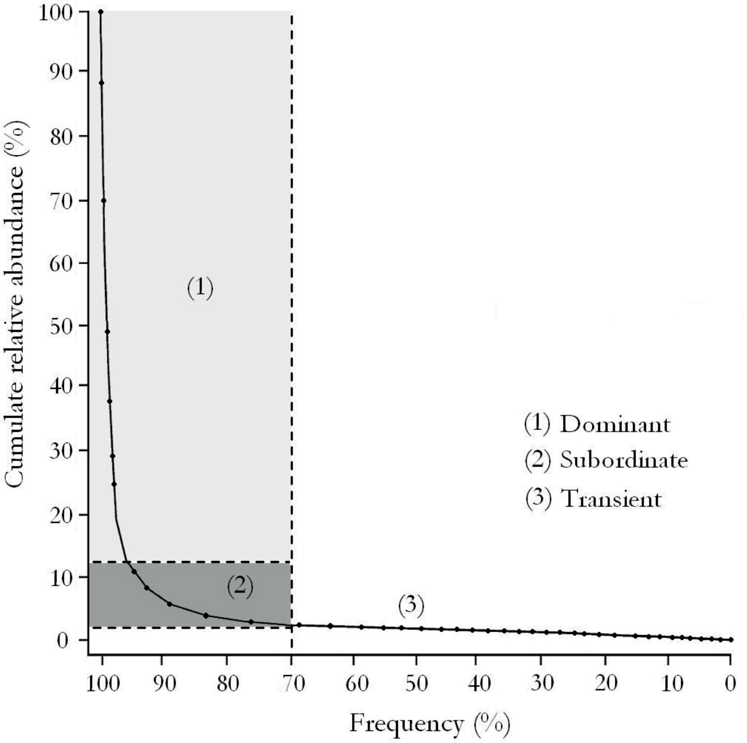


Figure 1.1 – An idealized abundance-frequency curve (adapted from Whittaker 1965; Grime 1998) for a sample of herbaceous vegetation in high diverse grassland ecosystems. Dominant and subordinate species are distinguished from transient species by their frequency in plots higher than 70%. The difference between dominant and subordinates is based upon the cumulate relative abundance (i.e. species ranking) which varied between 2 and 12% (representing 10% of cover) for subordinates and higher than 12% for dominants (arbitrary choice).

According to Grime (1987, 1998), dominant species are few in number, taller and more expansive in morphology and produce higher quantities of biomass in the ecosystem. Therefore, the immediate influence of plant communities on the properties of ecosystems is expected to be primarily determined by traits of dominant species. By contrast, transient species generally do not persist over years, appearing only briefly as seedlings that fail to survive.

As the transients appear to be strongly related to seed bank in the soil, these species may be potential colonizers in case of critical disturbance by exploiting new resources opportunity, better than the other species (founder effects). At an intermediate level, subordinate species are frequent in plant communities, generally more numerous, but smaller in stature and forming a low proportion of the community biomass. The filtering hypothesis (Grime 1998) suggests that subordinate species might exercise controls on the identity, functional diversity and relative abundance of dominants. However, the effects of subordinate species on plant and soil communities and ecosystem functioning has been poorly studied so far.

Species coexistence and plant diversity

Theoretical models suggest that plant species coexistence requires a strict dominance hierarchy where superior competitors (i.e. dominants) can displace inferior competitors (i.e. subordinates) from occupied patches but inferior competitors cannot displace superior competitors (Tilman 1994; Amarasekare 2003). Therefore, subordinate species cannot have access to patches occupied by dominants but because of their better ability for resource recruitment, they can establish in patches that dominants are not able to colonize creating spatial niche differentiation between both species-groups and complementarity for resource use. Species coexistence is also extremely influenced by spatial heterogeneity which is enhanced in plant communities submitted to permanent disturbance. Disturbance and fertility in species-rich communities regulate the relative proportion of dominant, subordinate and transient species, and influence species diversity (Fig. 1.2a). Increasing fertility leads to increasing dominance as niche differentiation is reduced and benefits to dominant species. Increasing disturbance modifies life conditions and promotes transient species (i.e. colonizers). According to the model of Grime (1987), subordinate species seem to increase at intermediate levels of disturbance and fertility.

The amplitude of this curve which determines species diversity may also be modified if a species-group is directly favoured whereas the other is reduced. For example, selective herbivory, from soil herbivores or cattle, can increase or reduce species diversity (Fig. 1.2b) depending on the type of species (dominant or subordinate) which is affected (Klironomos 2002; De Deyn et al. 2003; Wardle et al. 2004, Van der Putten 2005). Moreover, at low level of soil fertility, arbuscular mycorrhizal fungi (AMF) begin to be more important for structuring plant communities (Van der Heijden et al. 1998a,b; Van der Heijden & Horton 2009) and act along the mutualism-parasitism continuum (Johnson et al. 1997; Klironomos 2003). Indeed, AMF can increase or reduce plant growth and modify the abundances of dominants or subordinates (Fig. 1.2c) depending on their positive or negative effects on both

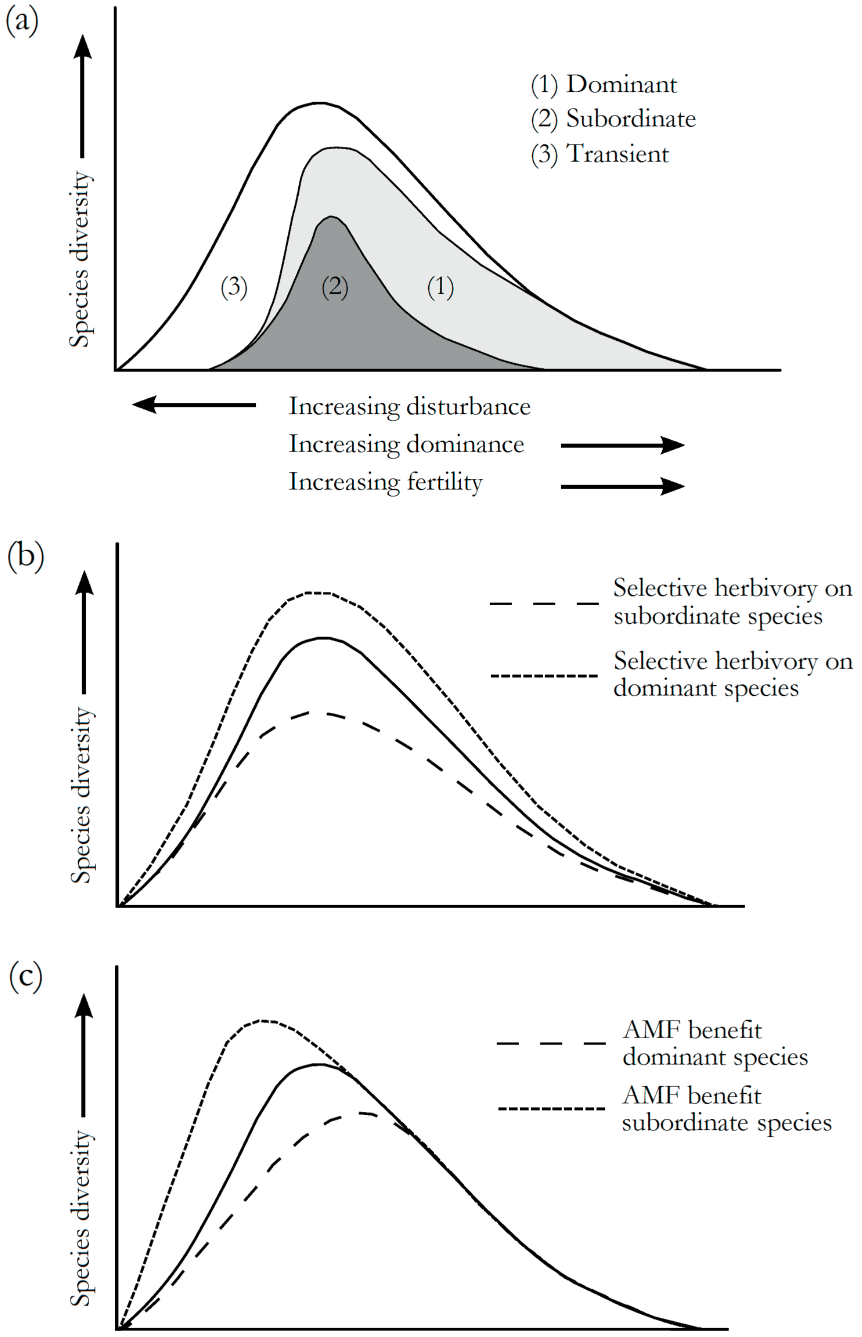


Figure 1.2 - Relationship between disturbance/dominance/fertility and species diversity according to Al-Mufti et al. 1977 and Grime 1987 (taken in Van der Putten 2005 and adapted). (a) Distributions of the three components of plant biodiversity (dominant, subordinate and transient species) in the humped-back model (Grime 1973, 1987). (b) Herbivory increase species diversity when selecting dominant species (De Deyn et al. 2003) and reduce diversity when selecting subordinate species (Klironomos 2002; Wardle et al. 2004). (c) AMF enhance diversity when favoring subordinate species (Grime et al. 1987; Van der Heijden et al. 1998b) and reduce diversity when benefiting dominant species (Hartnett & Wilson 1999).

species-groups which alter competitive relationships (Grime et al. 1987; Hartnett et al. 1993; Hartnett & Wilson 1999; Bever 2003; Scheublin et al. 2007). These responses have been developed into a model whereby the relative response of dominants and subordinates to AMF determines plant community composition and dominance hierarchies (Fig. 1.3; Urcelay and Diaz 2003) but the parasitic effects of AMF has not been included because of the lack of knowledge on this phenomenon.

The persistence of subordinate plant species in grassland ecosystems seems to be intimately associated with grazing disturbance (trampling gaps and selective herbivory) and the presence of soil organisms (AM fungi, root herbivores and pathogens) but these statements have not been intensively studied through experiments. Nevertheless, the persistence of subordinates and the fluctuations in the relative contribution of both species group (dominant vs. subordinate) have important implications for species coexistence and consequently for diversity in term of species richness and functional diversity.

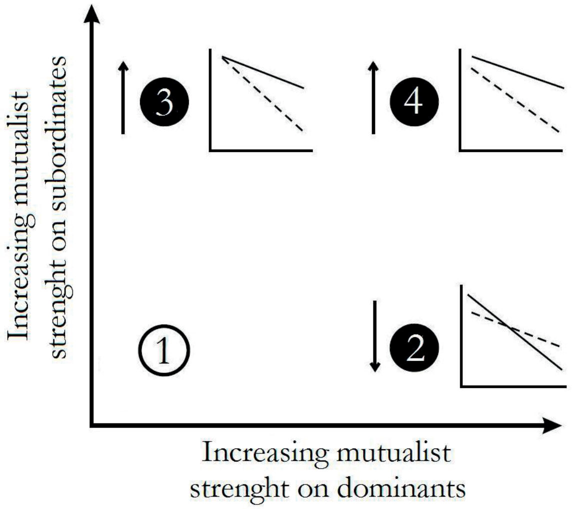


Figure 1.3 - Model of interactions among arbuscular mycorrhizal fungi (AMF) and dominant and subordinate plants under mutualistic conditions (Taken in Urcelay & Diaz 2003). Filled circles show situations where AM fungi have strong effects on plant diversity. The small figures illustrate the effect of the presence (solid lines) or absence (dashed lines) of AMF on the dominance-rank curve (Grime 1998): a steep curve indicates a high dominance whereas a flat curve indicates higher species coexistence. The arrows represent the direction of the effect of AMF on plant diversity (increase/decrease).

Above- and below-ground linkages

Plants represent most of the biomass of terrestrial ecosystems and provide organic matter to feed all heterotrophs in the ecosystems. There has been growing recent interest in better understanding the linkages that may exist between above-ground and below-ground components of terrestrial communities (Wardle 2002; De Deyn & Van der Putten 2005). This issue has been addressed from a biodiversity perspective in a number of recent studies, both in terms of how plant diversity and identity may affect the composition of soil communities (e.g. Koricheva et al. 2000; Stephan et al. 2000; Hedlund et al. 2003; Ledeganck et al. 2003; Wardle et al. 2003; Johnson et al. 2004; Wanner & Xylander 2005; Orwin & Wardle 2005; Bardgett et al. 2006) and how diversity of soil organisms may affect plant communities (Van der Heijden et al. 1998b, De Deyn et al. 2003, Urcelay and Diaz 2003, De Deyn et al. 2004, Bezemer et al. 2005, Bonkowski & Roy 2005, Van Ruijven et al. 2005). The various observed responses reveal the complexity of the involved processes including a high variety of organisms (e.g. bacteria, amoebae, nematodes, earthworms, fungi) interacting in complex food webs. Overall, the number of studies of local above-ground and below-ground biodiversity is still too limited to reveal any general pattern (De Deyn & Van der Putten 2005).

Over the past few years, the exploration of above- and below-ground interactions and their potential consequences for ecosystem properties have progressed as a result of the use of new approaches (De Deyn & Van der Putten 2005). Recent advances have been achieved in the field on environmental microbiology applied to plant and soil studies, as for instance in sampling strategies (Ranjard et al. 2003), total DNA extraction procedures (Luna et al. 2006) and fingerprinting techniques (Blackwood et al. 2003). The generalization of our mechanistic understanding of the results across spatial and temporal scales is however still limited, because most experiments have used individual species in low diversity settings over short time spans. Moreover, above- and below-ground biodiversity links appear to be highly context-dependent (De Deyn & Van der Putten 2005) and operate over a hierarchy of temporal scales, ranging from days to millennia, with differing consequences for ecosystem structure and function (Bardgett et al. 2005). The understanding of the above- and below-ground relationships presents a current major challenge, which requires interdisciplinary ecology and explicit recognition of these relationships as regulatory forces in terrestrial ecosystems (Bardgett et al. 2005).

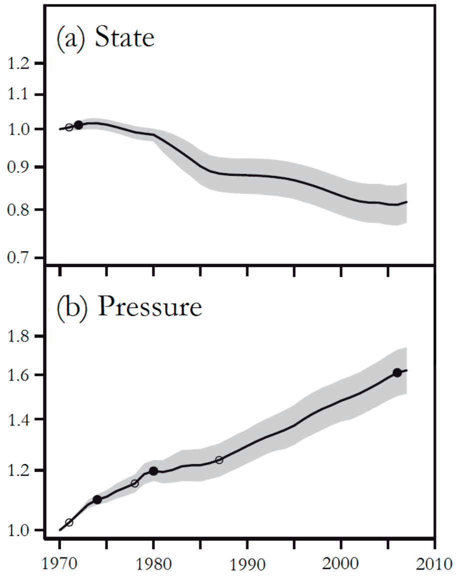


Figure 1.4 - (a) Aggregated indices of the state of biodiversity based on nine indicators of species population trends, habitat extend and condition, and community composition. (b) Pressures on biodiversity based on five indicators of ecological footprint, nitrogen deposition, numbers of aliens species, overexploitation, and climatic impacts. (taken in Butchart et al. 2010).

Biodiversity and ecosystem functioning in a changing world

Human have been modifying aquatic and terrestrial ecosystems and applied therefore an increasing pressure on worldwide biodiversity through pollution, land use and climate change, overexploitation or invasive species which leads to major alterations of biological communities (Millennium Ecosystem Assessment 2005) and consequently decrease of biodiversity (Fig. 1.4). The effects of human-induced climate change are believed to be responsible of a dramatic loss of global natural biodiversity (Haines-Young 2009; Butchart et al. 2010) and greatly impact ecosystem properties and services (Schläpfer & Schmid 1999; De Groot et al. 2002; Balvanera et al. 2006). However, at the ecosystem level, biodiversity is the driver of ecosystem maintaining and stability face to global change. According to the diversity-insurance hypothesis (Yachi & Loreau 1999), an increase of species diversity in an ecosystem corresponds to an increase of the range responses to environmental change or perturbation. Therefore, more diverse communities have higher chance to contain species which are more adapted to perturbations and can compensate for the decline of less resistant species. While the relationship between biodiversity and ecosystem functioning is demonstrated, the trajectory of this relationship may differ. Indeed, many hypotheses emerged from previous experiment (Schläpfer & Schmid 1999; Loreau et al. 2002) to explain the degree of damage due to biodiversity loss (Fig. 1.5). These hypothetical relationships can be classified in different categories depending on the importance of species or species-groups in the system: 1) species are equivalent and each species has potential effect leading to a linear relationship between biodiversity loss and ecosystem functioning; 2) species are redundant and species loss may be compensated by other species more resistant to perturbations; 3) species impacts are context dependent (idiosyncratic) and the impact of species loss depends on environmental conditions (fertility, disturbance, competition); 4) species are singular and the loss of keystone species cause irremediable and dramatic change in ecosystem functioning. Therefore, the effect of plant diversity on ecosystem functioning and stability seems to be context dependent and these different trajectories must be taken into account to understand the impact of biodiversity loss face to global change.

Although warming is a major factor in climate change, recent models predict an increase of water stress in certain regions. Indeed, decreasing summer precipitation and severe drought are expected, especially in Central Europe (Christensen et al. 2007), and play also an important role in the potential impact of climate change (Ciais et al. 2005; Engler et al. 2011). Many experiments have been carried out to determine the effects of plant diversity on plant community insurance to drought but the results are still contradictory with positive (Tilman & Downing 1994; Kahmen et al. 2005; Tilman et al. 2006; Van Ruijven & Berendse 2010) or negative (Pfisterer & Schmid 2002, Van Peer et al. 2004; De Boeck et al. 2008) diversity effects. However, these experiments were generally performed in constructed grasslands and based on the hypothesis that diversity–insurance relationship is linear with random species loss decreasing community insurance.

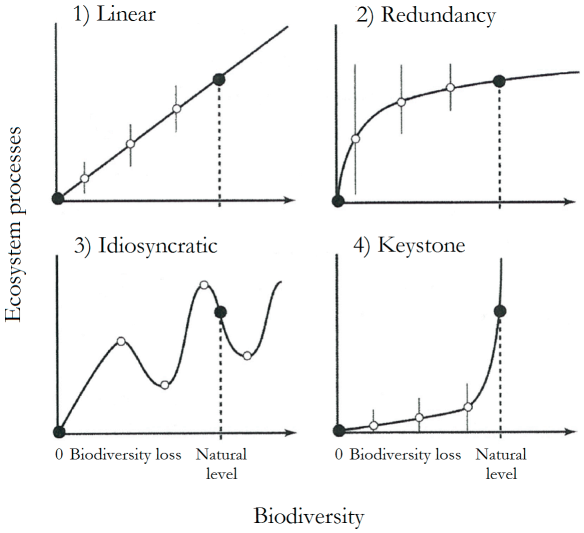


Figure 1.5 - Graphical representations of hypothetical relationships between biodiversity and ecosystem processes. The first point of interest is the point at or near zero biodiversity which means that if there is no diversity there is no ecosystem functioning. The second point is the natural level of biodiversity where there is a highly predictable amount of functioning. (taken in Loreau et al. 2002).

By contrast, Wang et al. (2007) suggested that susceptibility to drought is mainly biomass-dependent with high biomass systems being less resistant to drought compared low biomass systems. In high biomass ecosystems, dominant plants limit light availability of drought-resistant species (high dominance, Fig. 1.2a). In low biomass ecosystems, light limitation is lower and drought-resistant species are more numerous and can compensate for the loss of drought-sensitive species (i.e. dominants). In a constructed old-field ecosystem, Kardol et al. (2010a) showed that the proportion of subordinate species increased in dry compared to wet conditions and suggested that dominant species may respond more strongly to the direct impact of drought whereas subordinate species may respond to altered competition interactions with the dominant species and increase their biomass. Therefore, more than species diversity (i.e. species richness), it is probably the presence of drought-resistant subordinate plants which is important, considering also that loss of these species might induce a drastic decrease of ecosystem processes (i.e. keystone hypothesis, Fig. 1.5). These findings have not been specifically tested and come from indirect observations. Nevertheless, this highlight the hypothesis that subordinate species might increase community insurance to drought and have important implications for ecosystem functioning.

Semi-natural grasslands

Semi-natural grasslands are high diverse ecosystems with more than 35 plant species by square meter but among the most endangered ecosystems in Europe (Etienne 1996), and specifically in Switzerland, threatened by both agricultural intensification and abandonment (Gillet & Gallandat 1996; Buttler et al. 2009). Semi-natural grasslands of Swiss Jura Mountains are generally situated at an altitude ranging from 800 to of 1500 m a.s.l. Climate is predominantly oceanic with a mean of annual rainfall of about 1600 mm at 1200 m a.s.l. (snow cover from November to April) and a mean annual temperature of 7°C. Because of high-elevation, mountain ecosystems are disproportionately exposed to climate change (Beniston et al. 1996; Nogués-Bravo et al. 2007), and these grassland communities are expected to be greatly affected by extreme climatic events (Smith 2011a).

Semi-natural grasslands are continuously disturbed by small and large herbivores (Cahill & Casper 2002; Kohler et al. 2005) which create spatial heterogeneity through the consumption of plants (herbivory), trampling (canopy and root gaps) and the production of fertilizers (dung). Productivity is intermediate in these ecosystems and competition for resources is expected to be intense resulting from the low fertility of soil. Plant communities composition show a typical lognormal rank-abundance curve with a few dominant species accounting for a high proportion of the total community biomass (Grime 1998) and frequent subordinate species which represent a low relative cover. Both species-groups coexist and participate to the diversity of these grazed grassland communities which reflects intermediate level of disturbance, dominance and fertility. Indeed, these high diverse plant communities, threatened by global change (climate and land-use), serve as an ideal model to explore the importance of subordinate plant species on plant and soil communities and ecosystem functioning.

Removal experiments

The dominant protocol to study the effect of plant diversity on ecosystem functioning has involved synthetically assembled communities, in which the experimental design determines species composition (Hooper et al. 2005). By contrast, the composition of naturally assembled communities is determined by environmental filters (Zobel 1997), species recruitment and dispersal, and other assembly processes. Consequently, natural communities and ecosystems can differ from synthetic systems in their reaction to changes in diversity (Thompson et al. 2005). Removal experiments, in which the diversity of naturally assembled communities is manipulated by removing various components, complement the synthetic-assemblage experiments in exploring the relationship between diversity and ecosystem functioning (Diaz et al. 2003). This approach preserves the original community composition, dominance structure and species interactions and allows for natural response to experimental manipulation. Moreover, it removes the confusion between species richness effects and effect of other variables (e.g. soil fertility or disturbance regime correlated with diversity) on the measured response. Results of recent removal experiments (Wardle et al. 1999; Symstad & Tilman 2001; Lyons & Schwartz 2001; Smith & Knapp 2003; Buonopane et al. 2005; Del-Val & Crawley 2005; Wardle & Zackrisson 2005) suggest that they are indeed more useful than the synthetic community approach for understanding the ecosystem effects of local non-random extinctions, changes in abundance of species and complex interactions.

Research objectives and outline of the thesis

The objectives of this thesis are 1) to determine which are the factors influencing the persistence of subordinate species in semi-natural grassland ecosystem (i.e. species coexistence); 2) to study the importance of subordinate species in plant and soil community and for ecosystem functioning through above- and below-ground linkages; and 3) to highlight the role of subordinate species in community insurance to severe drought event which are forecasted for the future. My approach to obtain these objectives encompasses experimental field studies and glasshouse microcosm experiments.

In the first chapter (**Chapter 2**), we present the results of an explorative multi-site study which explore plant community structure in semi-natural grasslands. Using vegetation surveys of 11 sites from Switzerland and France, we determined dominant, subordinate and transient species. We observed their relative importance in the diversity-productivity relationship and then their changes in abundance after abandonment of pasturing. In **Chapter 3**, we explore the importance of root competition in competitive interactions between dominant and subordinate species. We set up a microcosm experiment where five dominant and three subordinate species were grown in pairwise combinations under full competition or under root competition exclusion. In a second experiment (**Chapter 4**), we compare the effects of arbuscular mycorrhizal fungi (*Glomus intraradices*) on the growth and competitiveness of two dominant and two subordinate species by using monocultures and mixtures of two and four species.

We then show the results of a long-term field experiment (**Chapter 5**) in the Swiss Jura Mountains grasslands (Les Amburnex) where we determined dominant and subordinate species and carried out a removal experiment. In 18 plots, we randomly applied three treatments with removal of subordinate species, removal of dominant species in the same biomass than removed for subordinates and control without perturbations. We then monitored biomass production, soil respiration, litter decomposition and soil nitrogen availability during 3-years and determined shift in plant and soil community composition. This removal experiment was repeated in an additional field site (La Frétaz) but with a drought treatment applied during summer (2 months). In this second experiment (**Chapter 6**), we followed biomass production before, during and after the drought period and measured carbon isotope abundance in plant leaves to evaluate the importance of subordinate species in the resistance, recovery and resilience on plant community. Since the chapters correspond to scientific papers, we apologize for redundancies that could occur, especially in introductive and methodological parts.

The last part of the thesis (**Chapter 7**) gives a synthesis of the results obtained in the different experiment and replace them in a larger context. The general discussion focuses on the persistence of subordinate species in grasslands and the importance of these low abundant plant species for ecosystem functioning in a changing world.

Chapter 2

How do subordinate species in semi-natural grasslands relate to productivity and land-use changes?

Mariotte P., Buttler A. & Spiegelberger T.

In preparation

Abstract

Current changes in agricultural practices (intensification or abandonment) represent major perturbations for the vegetation of semi-natural mountain grasslands and are expected to completely modify plant community structure.

Using vegetation surveys of 11 sites in semi-natural grasslands of the Jura and Alps mountains, we determined plant community structure in grazed and abandoned communities. The proportions of dominant, subordinate and transient species were calculated and we observed their changes along a productivity gradient (analysis A) and in response to abandonment of pasturing (analysis B).

The results highlighted a diversity-productivity relationship which follows a polynomial model with lower species diversity in low and high productivity and higher diversity in intermediate levels of productivity. The proportion of subordinate species was also higher in intermediate productivity whereas dominant species did not change along the productivity gradient. Grazed communities with low and high diversity were more affected by cattle abandonment and the decrease of diversity was mainly due to higher loss of subordinate species.

Our explorative study demonstrates the relative importance of subordinate species in the diversity of grazed communities especially at intermediate level of productivity (hump of the model). However, the abundance of subordinates has been shown to decrease in consequence of cattle abandonment through land-use change, thus reducing diversity. These findings make a major contribution to understanding species coexistence and have important implications for the way grasslands should be managed for biodiversity.

Key-words

Diversity-productivity dependency, grazing, land-use changes, plant community structure, species coexistence.

Introduction

Semi-natural mountain grasslands are widespread components of north-temperate landscapes and have an important role in providing grazing for livestock (Dorland et al. 2006) and acting as reservoirs of both carbon (Follett & Reed 2010) and biodiversity (Cremene et al. 2005; Baur et al. 2006). These ecosystems host species-rich communities (up to 40 species by square meter) but are among the most endangered ecosystems in Europe, threatened by both land-use (Silva et al. 2008; Fava et al. 2010) and climate change. Indeed, current changes in agricultural practices are leading to either agricultural intensification or abandonment (Gillet & Gallandat 1996) which both impact these well-established vegetation communities (Buttler et al. 2009). The response of plant communities to land-use change depends on community structure (i.e. species diversity and composition) which drive community stability (Loreau et al. 2001) and ecosystem functioning (Tilman et al 2006). Therefore, it seems essential to understand which structural components of plant community mostly participate to diversity and how they are affected by the perturbations.

In his attempt to define functional role of species, Whittaker (1965) recognized that the easiest way to classify components of biodiversity is to order species according to their relative abundance. Grime (1987, 1998) used a plant classification from dominance-diversity profiles and distinguished three components, which may vary in species richness, functional traits and groups, and called dominant, subordinate and transient species. According to Grime, dominant species are few in number, taller and more expansive in morphology and produce higher quantities of biomass in the ecosystem. Therefore, the immediate influence of plant communities on the properties of ecosystems is expected to be primarily determined by traits of dominant species ("mass ratio theory", Grime 1998). Subordinate species are frequent, generally more numerous, but smaller in stature and forming a low proportion of the total community biomass. By contrast, transient species generally do not persist after years, appearing only briefly as seedlings that fail to survive. As the transients appear to be those of the seed bank in the soil, these species may be potential colonizers in case of critical disturbance by exploiting new conditions, better than other species (founder effects).

Species-rich ecosystems, such as semi-natural grasslands, are driven by species coexistence which requires a strict dominance hierarchy where superior competitors (i.e. dominants) can displace inferior competitors (i.e. subordinates) from occupied patches but inferior competitors cannot displace superior competitors (Tilman 1994; Amarasekare 2003). Subordinate species cannot have access to patches occupied by dominants but because of their better ability for resource recruitment (i.e. filtering hypothesis, Grime 1998) they can establish in patches that dominants are not able to colonize creating spatial niche differentiation between both species-groups and complementarity for resources. Species coexistence, through niche partitioning, is mainly due to light availability and soil fertility which are intimately linked to productivity. Productivity has been demonstrated to be function of species diversity in artificial conditions (Tilman et al. 2001; Hooper et al. 2005; Spehn et al. 2005) but Grace et al. (2007) suggested also, through a meta-analysis, that it is the diversity which is function of productivity in natural grasslands. Productive grasslands (i.e. high soil fertility and/or light availability) are site of competitive exclusion which allows dominance. Low productive grasslands (low soil fertility and/or light availability) are stressful environment for plant species (Al-Mufti et al. 1977). Indeed, high and low productivity reduce species diversity and the diversity-productivity relationship of species-rich communities follows the humped-back model (Grime 1973) where diversity is higher in intermediate levels of productivity, fertility and disturbance (Odum 1963; Connel 1978; Hughes et al. 2007). Grime suggested that subordinate species represent the higher proportion of species within the hump of the model which represents the equilibrium between disturbance and dominance. However, this statement has not been confirmed by field experiments and merits to be verified.

In semi-natural mountain grasslands, extensive grazing by large herbivores is an important driver for maintaining biodiversity and many studies showed that species diversity decrease after abandonment of pasturing (Sebastià et al. 2008; Marion et al. 2010; Parolo et al. 2011). The persistence of subordinate species in these ecosystems seems to be also related to the activity of large herbivores which create gaps and areas of reduced root competition from dominants (Chapter 3) or because subordinates have a better regrowth after grazing compared to dominants (Tahmasebi Kohyani et al. 2009). Moreover, the exclusion of grazing can lead to the dominance of tall and competitive dominant species (Tasser & Tappeiner 2002; Mayer et al. 2009). These findings therefore confirm the hypothesis that intermediate disturbance through cattle activity may benefit to subordinates and that cattle abandonment and land-use changes reduce the abundance of subordinate species.

In this explorative study, we aimed to describe the structure of plant communities in semi-natural mountain grasslands and to understand how this structure and the relative importance of species-groups influence species diversity. As plant community structure is expected to change following productivity, we selected 11 study sites which differed in their above-ground biomass production and determined the abundance of dominant, subordinate and transient species (i.e. species-groups). We then compared the abundance of species-groups in grazed and abandoned plots from 9 experiments in semi-natural grassland ecosystems to test whether the abundance of subordinate species decreases after abandonment.

Materials and methods

Data sources

The study included 11 sites selected in semi-natural grasslands of the Jura and the Alps (Table 2.1) from Switzerland (*n* = 9) and France (*n* = 2). The altitude of sites ranged from 980 to 1755 m a.s.l and community above-ground biomass (i.e. productivity) from 99 to 590 g dwt.m^{-2}. The vegetation data (relative cover in percents) used in the analysis have usually been collected in mid-summer and were sampled using Braun-Blanquet index (site 3), biomass sorting (site 7, 10 & 11), point quadrats (site 1, 4 & 6) or estimated to the nearest percent (site 2, 5, 8 & 9). The sampled surface corresponded to 1 m^2 (except in site 11 with 0.2 m^2). Each experiment ran for a minimum of 4 years and included 5 replicates (i.e. 5 plots) except for site 1, 5 and 6 with 3 replicates.

Code	Site	Region	Altitude (m)	Community aboveground biomass (g dwt.m-2)	Mean species diversity in grazed plots	Analysis	Duration of abandonment (years)	Reference
1	Orvin	Jura, CH	1210	99 ± 7	25.7 ± 1.7	A, B	4	unpublished
2	Chablais	Alps, FR	1470	164 ± 15	23.8 ± 1.6	A, B	5	Spiegelberger et al. 2006
3	La Frétaz	Jura, CH	1200	169 ± 10	33.2 ± 1.5	A	-	Vandenberghe et al. 2008
4	La Métairie d'Evilard	Jura, CH	1210	221 ± 16	32.7 ± 0.7	A, B	3	Kohler et al. 2005
5	Bauges	Alps, FR	1607-1755	366 ± 14	36.8 ± 1.1	A, B	5	Spiegelberger et al. 2006
6	Le Haut des Joux	Jura, CH	1240	431 ± 17	30.3 ± 1.2	A, B	4	unpublished
7	Les Amburnex	Jura, CH	1300	455 ± 29	21.2 ± 1.3	A, B	4	see Chapter 6
8	Chablais	Alps, CH	1409-1517	506 ± 29	24.0 ± 1.4	A, B	5	Spiegelberger et al. 2006
9	Lac de Dix	Alps, CH	1507-1646	546 ± 99	22.0 ± 2.0	A, B	5	Spiegelberger et al. 2006
10	La Frétaz	Jura, CH	1200	532 ± 33	20.6 ± 2.2	A, B	4	see Chapter 5
11	Früebüel	Alps, CH	980	590 ± 77	13.2 ± 0.6	A	-	Gilgen & Buchmann 2009

Table 2.1 - General characterisation of the study sites ranged from the less to the highest productive grassland. In site 5, 8 and 9, the first altitude corresponds to grazed communities and the second to abandoned communities.

Plant community structure (analysis A)

In the 11 sites, vegetation surveys were carried out in natural plant communities. For each site, we determined plant diversity (Table 2.1) calculated as mean of species number recorded in plots (*n* = 3 or 5). We also selected dominant, subordinate and transient species according to their cumulate relative cover and their frequency in plots. A species was classified as dominant if its frequency was greater than 50% (100% frequency means that the species is present in all plots) and its cumulative relative cover greater than 12%. A species was classified as subordinate if its frequency was greater than 50% and its cumulative relative cover between 2 and 12% (adapted from Grime 1998). The other species were classified as transient plants. Then, we determined the contribution of the three species-groups to the diversity along a productivity gradient (i.e.11 sites). The proportions (in percents) of dominant, subordinate and transient species correspond to the number of species by species-group divided by the total number of species in plot.

Abandonment of pasturing (analysis B)

In a second experiment, 9 sites were used to analyse the effect of cattle abandonment (Table 2.1) on the structure of plant community and in relation to changes of diversity. For each site, vegetation surveys were sampled in grazed and abandoned communities either in the same place (site 1, 4, 6, 7 & 10) or with a maximum of 5 km distance between them (site 2, 5, 8 & 9) but in similar conditions of exposition and inclination (see Spiegelberger et al. 2006). The mean number of species per site was determined in abandoned communities (n = 3 or 5) and compared with the diversity of grazed communities in the same location. As previously, dominant, subordinate and transient species were determined in ungrazed communities and changes in the abundance of the three species-groups (i.e. plant community structure) in abandoned plots were observed in relation to the diversity in grazed communities. Proportional changes in species-groups were calculated as the difference in log between the mean number of species among species-groups (dominant, subordinate or transient) in abandoned plots and in grazed plots and corresponds to the (negative, neutral or positive) effects of abandonment.

Statistical analysis

In the first analysis (A), we focused our analysis on detecting relationships between diversity and productivity and then, the relative importance of species-groups in this relationship. Diversity, productivity and abundance of species-groups were summarized into one mean value of each metric by site. In the second analysis (B), we explored the relationship between the species diversity in abandoned and grazed communities and observed the effect of abandonment on species-groups abundance. Polynomial models (degree 2) were constructed with the orthogonal polynomial function (poly {stats}) in R version 2.14.1 (R Development Core Team, 2011). Statistical significance of models (p-value) and multiple R^2 values were obtained using ANOVAs to estimate the goodness-of-fit. Polynomial curve were drawn in graphs only when the model was significant.

Results

Plant community structure (analysis A)

The mean diversity per site ranged from 20.6 ± 2.2 to 36.8 ± 1.1 along the productivity gradient ranging from 99 ± 7 to 590 ± 77 g dwt.m$^{-2.}$ A diversity-productivity relationship clearly appeared when we observed the mean species diversity in function of the mean productivity per site (Fig. 2.1a) and this pattern was modelled by a significant polynomial function ($F_{2,8} = 11.525$, $P < 0.01$, $R^2 = 0.75$). Dominant species represented between 41 and 61% of the species diversity but their contribution to the total diversity did not show a clear pattern along the productivity gradient (Fig. 2.1b). By contrast, the contribution of subordinate species, ranging from 15 to 36% of the species diversity (Fig. 2.1c), differed along the productivity gradient following a significant polynomial function ($F_{2,8} = 10.065$, $P < 0.01$, $R^2 = 0.72$). As for species diversity, the contribution of subordinate species decreased in low and high productive sites whereas it increased at intermediate levels of productivity. Inversely, the contribution of transient species, ranging from 9 to 41% (Fig. 2.1d), was higher in high productive sites and this pattern was fitted by a significant polynomial function ($F_{2,8} = 6.671$, $P < 0.05$, $R^2 = 0.63$).

Abandonment of pasturing (analysis B)

While the productivity did not affect species diversity in abandoned plots (no significant regressions), the effect of cattle abandonment was linked to the diversity in grazed plots (assumed as initial diversity). For the 9 sites used in this experiment, the mean species diversity in abandoned communities was maintained or decreased compared to the mean diversity in grazed communities (Fig. 2.2a). The loss of species, due to abandonment, was more important in lower (site 9) and higher diverse sites (site 5). These effects followed a significant polynomial function ($F_{2,6} = 62.606$, $P < 0.001$) with a high multiple R-squared (0.95). When we analysed the loss of species at species-group level, we observed that the number of transient species (Fig. 2.2d) did not show important changes after abandonment (-12% to +19%) whereas dominant (polynomial model, $F_{2,6} = 12.616$, $P < 0.01$, $R^2 = 0.81$) and subordinate species (polynomial model, $F_{2,6} = 11.501$, $P < 0.01$, $R^2 = 0.79$) showed similar patterns as observed for the species diversity. The number of dominant species (Fig. 2.2b) was maintained in 4 sites, increased in one site (+14%) and decreased in 4 sites (respectively -58 and -45% in lower and higher initial diversity). The number of subordinates (Fig. 2.2c) decreased in 8 of the 9 plots (-6% to -94%) and were more reduced by abandonment than the number of dominants, especially in sites with high diversity (25% less).

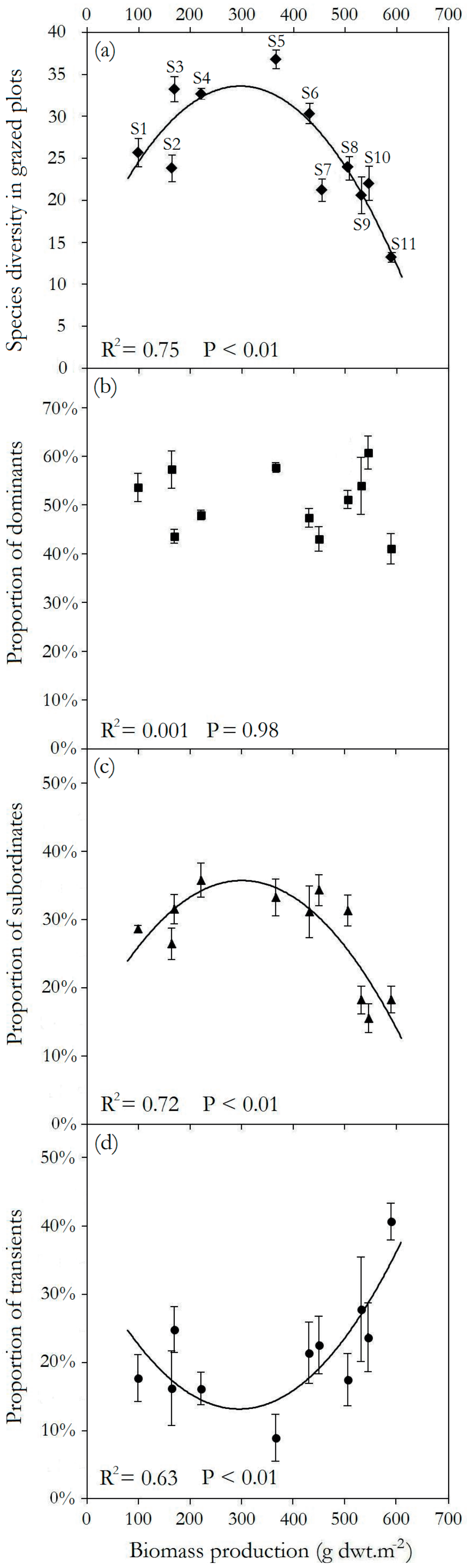
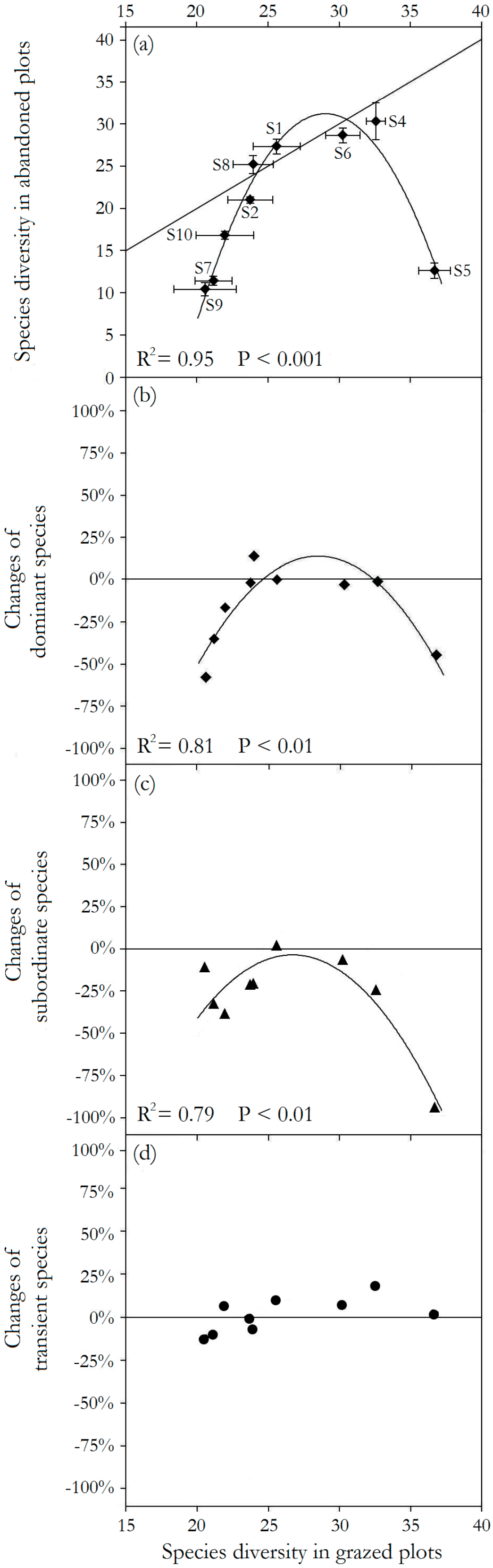


Figure 2.1 - Relationships between (a) the diversity (mean ± 1 SE of species number per site), (b) the proportion of dominant species, (c) the proportion of subordinate species, (d) the proportion of transient species and the productivity (mean biomass production per site) in 11 sites of semi-natural grasslands. Curves represent the significant polynomial models fitting the different relationships with productivity.

Figure 2.3 - Relationship between (a) the diversity (mean ± 1 SE of species number per site) in abandoned plots, (b) change in species number of dominant species, (c) change in species number of subordinates, (d) change in species number of transients- after abandonment and the diversity (mean ± 1 SE of species number per site) in grazed plots for 9 sites of semi-natural grasslands. Polynomial curves represent the significant models fitting the different relationships with the diversity in grazed plots. Linear curves represent neutral effects of abandonment.



Discussion

Plant community structure

In this explorative study, we proposed to determine the plant community structure of semi-natural grasslands along a productivity gradient including 11 sites of the Jura and of the Alps. In the analysis A, we highlighted a diversity-productivity relationship which was represented here as a polynomial model. These results confirm the diversity-productivity dependency which was suggested by different authors (Al-Mufti et al. 1977; Grime 1973; Grace et al. 2007). In semi natural grassland ecosystems, plant communities are continuously disturbed by cattle activity. As all of the 11 sites were selected in extensively grazed grasslands, we assumed that disturbance was similar in each site and that grazing is a constant effect. Therefore, we suggest that the productivity gradient which appears is related to different soil fertility. Indeed, additional data from the experiments showed that the site 2, 7, 9 and 10 showed increasing concentrations of dissolved inorganic nitrogen in soil. In our study, the maximum diversity (30-35 species) appeared at intermediate level of productivity (between 180 and 420 g dwt.m^{-2}) and confirms the humped-back model of Grime (1973; 1987) where diversity is predicted to be higher at intermediate level of productivity and fertility (Odum 1963). Therefore, our findings confirm that intermediate productivity favour species coexistence in grasslands.

Interestingly, the contribution of species-groups differed along the diversity-productivity relationship. Grime (1987) proposed that the proportion of dominant increases in high productive systems, the proportion of transient increases in low productive systems and the proportion of subordinates increases in intermediate productive systems. Indeed, in our analysis, the proportion of subordinates increased in intermediate productive sites and considering that the pattern of change for subordinates was similar to the change of diversity along the productivity gradient, we propose that intermediate productivity favours the presence of subordinate species (i.e coexistence), and thus increases species diversity. By contrast to the hypothesis of Grime (1987), the proportion of transient species increased in low and high productive site of our analysis whereas dominant species did not change along the productivity gradient and corresponded to around 50% of the diversity. In contrary to Grime (1987), we based our analysis on semi-natural grasslands only. In these ecosystems, cattle activities, even at low intensity, tend to prevent potentially competitive species from attaining maximum size in high productive site (grazing and trampling, Grime 1973; Lauda et al.1990; Augustine & McNaughton 1998) but favours also competitive dominants by fertilizing soil patches (dunging) in low productive sites (Gillet et al. 2010). Therefore, we suggest that large herbivores may stabilize the proportion of dominant species along the productivity gradient whereas transient species simply replace subordinates (i.e. founder effects, Grime 1998).

Land-use changes

In the second experiment (analysis B), we showed that plant diversity decreased in abandoned communities compared to grazed communities which confirm previous observations in species-rich pastures (Sebastià et al. 2008; Marion et al. 2010; Parolo et al. 2011). However, the decrease of diversity associated with land abandonment was, interestingly, a function of the initial diversity of the site with high and low diverse sites being more affected by abandonment of pasturing. Plant productivity changed in ungrazed communities but we did not find a clear relation with the decrease of species diversity and/or the changes in plant community structure.

Abandonment of pasturing modified plant community structure and these changes were also function of diversity in grazed plots of the site. Indeed, we showed that, similarly to the diversity loss in abandoned plots (Fig. 2.2a), the number of dominant and subordinate species showed changed shape curve along the species diversity gradient (Fig. 2.2b,c) whereas transient species were not affected (Fig. 2.2d). Moreover, the number of subordinate species decreased more than the number of dominants and indeed low abundant species were always more affected by abandonment of pasturing. Overall, the loss of diversity after land-use change was dependent on initial diversity but the effect on plant community structure was similar along the diversity gradient. Nevertheless, the mechanisms underlying this pattern are difficult to explain as various components of cattle activity may influence plant community, such as grazing, trampling and dunging (Kohler et al. 2005).

Selective grazing is especially high at low grazing pressure (Klapp 1971) as it was the case in the sites of this study. Selective grazing is expected to increase diversity when dominant species are targeted (De Deyn et al. 2003; Van der Putten 2005, see also Fig. 1.2b in Chapter 1) and decrease diversity when targeting subordinates (Klironomos 2002; Wardle et al. 2004). As we observed that diversity decreased after abandonment, we suggest that selective herbivory on dominants occurred in grazed communities. A major response to grazing is the promotion of ground-layer species due to the vertical defoliation (Hadar et al. 1999; Sternberg et al. 2000; Dupré & Diekmann 2001) and small subordinate species are expected to be less affected whereas plants with higher nutrient requirements or growth rate are most likely vulnerable to foliage losses (Lauda et al.1990; Augustine & McNaughton

1998). Tahmasebi Kohyani et al. (2009) showed that subordinate species regrowth better after grazing compared to dominants, due to their physiological plasticity or because they benefit from the reduction of competition from grazed dominants. However, although grazing disturbance significantly influenced competitive relations in favor of subordinate species, increasing nutrient levels counteracted the negative effect of grazing on dominant species (Tahmasebi Kohyani et al. 2009). Gillet et al. (2010) showed also that the fertilizing effect of dung decomposition favours competitive dominants (nutrient demanding tall species) against stress-tolerant or perturbation-resistant subordinates. The absence of selective grazing might indirectly reduce the growth of subordinates whereas the absence of dunging might reduce dominant species. Trampling by cattle activity must also be considered as it provides spatial heterogeneity (gaps) which may benefit to subordinate species (Kohler et al. 2005; Chapter 3). Therefore, we propose that the absence of trampling could be responsible of the higher decrease of subordinates in abandoned plots. Transient species were not affected by abandonment of pasturing but, appearing and disappearing in time, they are generally considered as seed bank in soils and very resistant to critical disturbance.

Conclusion, limits and perspectives

Findings of our explorative study showed that plant diversity of semi-natural grasslands is dependent of productivity and that intermediate productivity (i.e soil fertility) favour species coexistence, allowing the persistence of subordinate species which then represent higher proportion in plant communities. Moreover, the 4-years abandonment of pasturing has been shown to reduce diversity with higher impacts on subordinate species. These findings are important to understand species coexistence and have important implications for the way grasslands should be managed for biodiversity. Moreover, recent studies showed that subordinate species may have a larger influence on ecosystem processes and functioning than their relative abundance would suggest (Lyons et al. 2005; Boeken & Shachak 2006; Polley et al. 2006) and indeed, abandonment of pasturing, through land-use change, might have an important impact on ecosystem functioning in relation to the reduction of subordinate species in plant communities (see Chapter 5 & 6). For example, cattle abandonment reduces the net ecosystem CO_2 exchange (Schmitt et al. 2010) or slow down the nitrogen cycling in mountain grasslands (Farris et al. 2010; Robson et al. 2010), and these effects are always associated with changes in the plant community.

We recognize that many direct or indirect factors may also influence plant community structure after abandonment. For example, we did not measure changes in plant community height and changes in biomass or traits of dominant species. Indeed, while the number of dominant species decreased, our observations suggest that community height and the relative cover of some dominant species (mainly grasses) increased in ungrazed plots, and may also limit subordinate species (less light availability or nutrient resources).

This explorative study serves as an introduction to this thesis by providing some preliminary patterns to better understand the structure of plant community in semi-natural grasslands and how they relate to land-use change. Nevertheless, we acknowledge that a more intensive dataset and complementary analysis would be needed to validate our findings.

Acknowledgments

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Chapter 3

Exclusion of root competition increases competitive abilities of subordinate plant species through root-shoot interactions

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Abstract

Subordinate species may have a large influence on ecosystem processes and functioning but the mechanisms regulating their persistence in plant communities remain unclear. Here we test the hypothesis that exclusion of root competition increases competitive abilities of subordinate plants.

The dominance hierarchy from a mountain wood-pasture ecosystem was used to identify five dominant and three subordinate species. These species were grown in pair-wise combinations under full competition and in the absence of root competition enabling us to calculate indices of competitive effect and response and overall size-asymmetry.

Root competition exclusion led to a decrease in the competitive abilities of dominants whereas subordinates became overall more competitive. Total size-asymmetry also decreased indicating reduced competition between both species-groups. The exclusion of root competition increased both below- and above-ground growth of subordinates, whereas for dominants below-ground growth was unaffected and above-ground growth decreased.

We show that root competition through root-shoot competition interactions is an important factor driving the competitive dominance of species and the structure of grazed grassland communities. Locally, reduction of root competition involved in gap creation might explain persistence of subordinate species within the vegetation community and lead to an aggregated spatial pattern of subordinates involved in species coexistence in grasslands.

Key-words

Competitive effect and response, competitive hierarchy, plant-plant interactions, root competition, size-asymmetry, subordinate species.

Introduction

Semi-natural grassland communities are generally composed of dominant and subordinate species (Grime 1987; Olff & Bakker 1998). Dominant species, which are usually a small fraction of the total number of species, are generally tall and account for a high proportion of the total community biomass. Subordinate species consistently co-occur with particular dominants. They are often small in stature and contribute marginally to the total biomass of the community, although they are the most diverse component of communities (Whittaker 1965). The role of dominant species on ecosystem functioning has received a lot of attention and according to the "mass ratio" theory (Grime 1998), ecosystem properties are determined by dominant species independent of changes in species richness, such as variations in the number of subordinate species (Smith & Knapp 2003). However, more recent studies show that less abundant species may have a larger influence on ecosystem processes and functioning than their relative abundance suggests (Lyons et al. 2005; Boeken & Shachak 2006; Polley et al. 2006). Subordinate species may maximize their resource capture locally within their habitat (Aan et al. 2006; Dassler et al. 2008), leading to the hypotheses that subordinate species affect ecosystem functioning because of more efficient utilization of resources and through facilitating recruitment of certain dominant species during recolonization following a disturbance event (Grime 1998).

Competition is an important biotic interaction that plays a major role in structuring plant communities and regulating relative abundance of species in grassland communities. Intensity of competition refers to the degree to which a plant population is reduced by the presence of neighbours and is directly linked to the competitive abilities of plant species. Competitive ability includes two different components: i) the competitive effect, which is the ability of an individual to use resources better than its neighbours or to actively suppress them, and ii) the competitive response, which is the ability of neighbours to resist suppression by one species (Keddy 2007). Competitive effect and response are not necessarily correlated (Cahill et al. 2005; Fraser & Miletti 2008). Wang et al. (2010) suggested that competitive effect ability is linked to intrinsic plant traits, such as length of leaves or the extent of root systems. This would mean that dominant plants have a higher potential for resource acquisition and are able to suppress the growth of other plants (Goldberg & Landa 1991; Keddy et al. 1998). In contrast, competitive response ability was not consistently correlated to a species trait and may represent a collection of ways of avoiding or tolerating competition. Competitive effect and response abilities of each species are often quantified by relative yield per plant (RYP) through the use of pairwise species combinations (Engel & Weltzin 2008; Fortner &

Weltzin 2007). In a pairwise experiment, the growth of a species in combination with another species is compared with its growth in a monoculture. The best competitor is defined as the species with superior performance, i.e. with greater biomass in the mixture of plant species (interspecific competition) compared to the monoculture (intraspecific competition).

In plant communities, species may experience above-ground competition for light and space, and below-ground competition for mainly water and nutrients. Competition is generally linked with asymmetry (Freckleton & Watkinson 2001) which is a natural outcome of local neighbour interactions (Weiner 1990; Bauer et al. 2004). Asymmetric competition measures the degree to which one species is suppressed by the presence of the other. Competition for light is expected to be asymmetric and creates a positive feedback where larger individuals (often dominants) are able to outcompete smaller individuals (often subordinates), capturing a important fraction of the available light (Schwinning & Weiner 1998). Root competition is intense in many plant communities (Cahill 1999; Keddy 2001) but is expected to be symmetric and cannot magnify small initial differences between species. The lack of feedback mechanisms through which symmetric root competition can eventually lead to competitive exclusion seems to explain why root competition can have little direct impact on plant community structure (Lamb & Cahill 2008). However root competition can indirectly influence plant community structure by reducing evenness through root-shoot competition interactions especially when root competition is more intense than shoot competition (Lamb et al. 2009). Root-shoot competition interactions occur when root competition reduces shoot growth and thus affects competition for above-ground limiting resources such as light. Jumponnen et al. (2005) found that dominants were mainly limited by the supply of nutrients whereas the greatest limitation faced by subordinate plant species was light availability. The importance of root competition on subordinates might be underestimated as the growth of subordinates could be limited through root-shoot competition interactions, especially in highly diverse and productive plant communities where the importance of root competition is supposed to increase (Belcher et al. 1995; Eisenhauer et al. 2009).

Investigations of root traits were recently recognized as an important tool to provide deeper insight into the competitive abilities of plants (Frank et al. 2010) and their responses to varying environmental factors such as grazing (May et al. 2009) or gap creation (Hook et al. 1994). Gap creation in plant communities is an important factor in determining the species composition and plant competitive hierarchies (Suding & Goldberg 2001). Gaps are sites of reduced below-ground competition (Casper & Jackson 1997; Cahill & Casper 2002) and Vandvik (2004) showed that locally infrequent species become relatively more dependent on gaps for local population persistence during succession. In grazed grassland, short-term reduction of root competition related to gap creation could therefore facilitate locally less abundant species such as subordinate species and favour their persistence.

In the Swiss Jura wood-pastures, plant communities result from a traditional management with regular grazing and trampling disturbance with well-established vegetation communities (Buttler et al. 2009). These communities serve as an ideal model to explore changes in competition intensity on plant interactions since they are very diverse (up to 40 vascular species per square meter) and show a typical lognormal rank-abundance curve with a few dominant species accounting for a high proportion of the total community biomass (Grime 1998). In these (intermediate) productive communities, the reduction of root and shoot competition, caused by gap creation due to the activity of large grazers, might be important for the persistence of subordinates. In this study, we created mesocosms in which we investigated the effect of root competition exclusion on the competitive abilities of dominant and subordinate species determined in the field according to their relative abundance. We predicted that exclusion of root competition will facilitate subordinates and change the dominance hierarchy through a decrease of overall size-asymmetric competition between dominant and subordinate species.

Methods

Field site

The field research site was an extensively-grazed and species-rich (30 - 40 species per square meter) pasture situated in a well-drained limestone karst valley of the Swiss Jura Mountains (Les Amburnex, western Switzerland, 6°13’50” E, 46°32’50”N) with a vertic cambisol (after the World Reference Base for Soil Resources - IUSS Working Group WRB, 2006) deeper than 1 m and an intermediate productivity with an average standing biomass (± 1 SE) of 258 ± 14 g dwt/m^2. The site is situated at an altitude of 1300 m a.s.l. and has a suboceanic climate. The pasture is part of the wood-pasture system typical for this area, surrounded by small and large patches of Picea-dominated forests, and is grazed by cattle following a rotational system during the vegetation growing season lasting from June to September.

Dominants and subordinates field selection

Within a 25 by 25 m cattle-excluded area, 30 plots of 1.2 by 1.2 m were established at a regular distance of 1.6 m from each other. In July 2008, absolute plant cover was determined within all plots using an adapted Braun-Blanquet index (1: cover less than 5%, 2a: 5-10%, 2b: 10-25%, 3: 25-50%, 4: 50-75%, 5: 75-100%). A total of 41 species were identified. Frequency was calculated from the occurrence of the species in the 30 plots. A species was classified as dominant if its frequency was greater than 75% and its cumulative relative cover greater than 25%. A species was classified as subordinate if its frequency was greater than 75% and its cumulative relative cover between 2 and 12% (adapted from Grime 1998). This method resulted in the determination of seven dominant and seven subordinate species.

Greenhouse pairwise experiment

Eight species including five dominant (1) *Trifolium repens L.* (N fixer), (2) *Festuca nigrescens* Lam., (3) *Taraxacum officinale* agg., (4) *Plantago media* L., (5) *Agrostis capillaris* L. and three subordinate species (6) *Trifolium pratense* L. (N fixer), (7) *Prunella vulgaris* L., (8) *Achillea millefolium* L. in the field were selected for the greenhouse experiment (undertaken at PRIMPLANTS SA, Lonay, Switzerland). The uneven number of dominant and subordinate species was due to the availability of seeds which were gathered in the Swiss plateau (supplier: UFA SAMEN by FENACO SA, Yverdon-les-bains, Switzerland). In March 2009, three seeds of each of the eight species were sown within cubes (36 cm^3) of mixed soil (25% compost, 75% black peat). One seedling was kept after germination. After one month, species were assembled in all possible inter- and intra-specific combinations (28 inter-specific pairs and 8 intra-specific pairs) in pots (4 L) filled with a mix of compost (20%) and sand (80%) which contained low level of nutrients to recreate field conditions. Two individuals of both species were placed centrally within the pot (Fig. 3.1).The 36 pairs were replicated and a competition treatment including full competition (FC) and root competition exclusion (RCE) was applied. Root competition was suppressed by placing a PVC barrier from the surface to the bottom of each pot resulting in four equal below-ground compartments separating in equity the individuals in pots. Each compartment contained a sufficient volume of soil to not limit plant growth (1 L). The 72 pairs (i.e. pots) were randomly placed at a distance of 25 cm within blocks (3.5 m x 3.5 m). Finally, we created four replicates for each species in each combination and competition treatment (i.e. four blocks).

All 288 pots were automatically watered two times a day (morning and evening) during the experiment. Seven weeks after assembling the species, plants reached their maximum above-ground biomass (i.e. after flowering), and the biomass of each species was harvested, dried in an oven at 60°C for 72h and weighed. Roots were observed to use nearly the entire below-ground space of the pot and they were collected by washing, and then dried and weighed as described for above-ground biomass. The average of the two (inter-specific combinations) or four (intra-specific combinations) individuals per species in each pot was calculated to obtain the overall measure of root and shoot biomass per species. In full competition pots, roots of both species could be relatively easily dissociated as the roots of the different species differed in morphology and colour. Nervertheless, in some cases, we were unable to retrieve the root systems (dead or rotten) resulting in a loss of data and unequal replication.

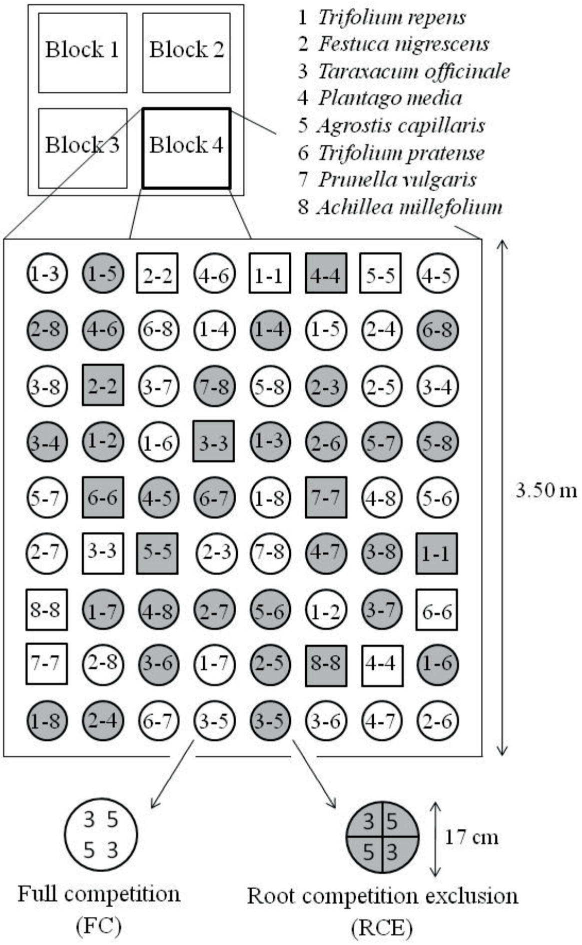

Figure 3.1 - Experimental layout and plant competition scheme. Pots were arranged in four blocks. Two individuals per species were placed in each pot resulting in a total of 4 seedlings per pot. In each block, all 36 plant combinations including intra- (square) and inter-specific (circle) pairs of dominant (1 to 5) and/ or subordinate species (6 to 8) were reproduced two times. Half of the pots had full competition between species (open symbol) and the other half had root competition exclusion (solid symbol) using PVC barrier from the surface to the bottom of pots. The arrangement of 72 pots was randomized at the block level.

Competitive effect and competitive response

We firstly determined plant hierarchy in full competition (FC) and under root competition exclusion (RCE) to test the hypothesis that species classified as dominants (or subordinates) react similarly to competition treatments (i.e. at the species level). Then, we grouped species into dominants and subordinates (i.e. species-group level) to explore the effect of root competition exclusion on competitive abilities of both species-groups to test the hypothesis that subordinate species benefit from root competition exclusion with dominants.

We determined the competitive dominance of a species under FC and RCE by calculating the relative yield per plant (RYP) from total biomass (shoot and root biomass, Appendix 3.1) for each species,

$$RYP_{ij} = Y_{ij} / Y_{ii}$$

where RYP_{ij} is the relative yield per plant of species *i* (i.e. target) grown with species *j* (i.e. neighbour), Y_{ij} is the yield of plant *i* when grown with species *j*, and Y_{ii} is the yield of plant *i* when grown in monoculture (Keddy 2001; Weigelt & Jolliffe 2003).

Relative yield per plant was calculated in each competition treatment for each species, resulting in 2 matrices (FC matrix and RCE matrix) comparing the 8 species (8 columns *i*) in interaction with each other, (8 rows *j*) per block, yielding a total of 512 RYP scores (Appendix 3.2). In each block and each competition treatment, competitive effect (CE) of the species *i* is defined as the mean RYP of the target species *i* grown with each of the eight neighbour species (mean of the *i* column). Competitive response (CR) of the species *j* is defined as the mean RYP of the species *j* grown as neighbour with each of the eight target species (mean of the *j* row). Competitive hierarchy was determined at species level under each competition treatment by ranking CE and CR of each species. CE and CR were summed to obtain the final ranking. Species with a greater CE and lower CR are the best competitor, whereas species with lower CE and higher CR are less competitive.

Since the final ranking of competitive hierarchy in FC was still similar with the a-priori field classification as dominant (higher CE, lower CR) or subordinate (lower CE, higher CR) species, we grouped species in dominants (5 species with the highest ranking in FC) and subordinates (3 species with the lowest ranking in FC) for further analyses.

Root competition exclusion effect

We distinguished the effect of root competition exclusion following different species interactions: among subordinates (intra-specific: $n = 12$, inter-specific: $n = 24$), among dominants (intra-specific: $n = 20$, inter-specific: $n = 80$) and on interacting dominant and subordinate species ($n = 60$ for dominants vs subordinates and $n = 60$ for subordinates vs dominants) to explore more detail the root competition processes. Root exclusion effect (REE) was determined for above- and below-ground plant biomass,

$$REE_{ij} = \ln (Y_{ij}RCE/Y_{ij}FC)$$

where $Y_{ij}RCE$ represents plant biomass of a species *i* growing in absence of root competition with species *j* and $Y_{ij}FC$ the biomass of the same species *i* growing in full competition with species *j*. We averaged REE_{ij} of species following different species interactions of subordinates and/or dominants (see above). Negative values of REE indicate a negative growth response of a species to root exclusion and inversely, positive values indicate better growth in the absence of root competition with neighbours.

Total asymmetry of competition

Overall asymmetry was only calculated for plant pairs that included a dominant and a subordinate species ($n = 60$). A distinction was made between situations in which dominant species obtained an advantage against subordinates or inversely when subordinate was the stronger competitor. Asymmetry was measured from the RYP matrix as the ratio of the stronger competitor effect to the effect of the lesser competitor (Wilson 1988; Keddy 2007):

$$A = \ln (RYP_{ij}/RYP_{ji}) \text{ with } RYP_{ij} > RYP_{ji}$$

High asymmetry (generally close to 1) indicates an important impact of one species on the second whereas low asymmetry (close to 0) indicates that each species has similar impact on the other. Calculation with RYP controls for the initial size differences due to intrinsic traits of species because RYP considers biomass of plant in mixture compared to monoculture.

Statistical analysis

All of the analyses were carried out with R version 2.11.1 (R Development Core Team, 2010). The differences in CE and CR between species were analysed separately for FC and RCE (after log-transforming) using one-way ANOVA with block as a random factor (Appendix 3.2). Competitive effect and response were analysed at species-groups level using linear mixed-effects models specifying neighbour species nested into target species nested into block as random factors and root competition (FC/RCE) and species type (dominant/subordinate) as fixed factors. Root competition was tested at target species level using target nested into block as error term ($n = 32$). Species type was nested at the lowest level using neighbour:target:block as error term ($n = 256$). Data were square-root transformed to fulfill the assumptions of normality and homogeneity of variances. Root exclusion effect (REE) and asymmetry (A) were analysed at species-group level using linear mixed-effects models specifying neighbour species nested into target species nested into block as random factors.

	CE	Rank	CR	Rank	Overall competitive hierarchy
A Full competition					
Trifolium repens	1.265 ± 0.22^{ab}	2	1.140 ± 0.09^{bc}	5	3
Festuca nigrescens	1.093 ± 0.10^{ab}	4	1.134 ± 0.09^{bc}	4	4
Taraxacum officinale	1.456 ± 0.07^{a}	1	0.421 ± 0.05^{a}	1	1
Plantago media	0.919 ± 0.10^{b}	7	1.446 ± 0.20^{bc}	7	7
Agrostis capillaris	1.218 ± 0.08^{ab}	3	0.941 ± 0.06^{b}	2	2
Trifolium pratense	0.921 ± 0.13^{b}	6	1.151 ± 0.10^{bc}	6	6
Prunella vulgaris	0.786 ± 0.10^{b}	8	1.424 ± 0.13^{c}	8	8
Achillea millefolium	1.038 ± 0.32^{ab}	5	1.038 ± 0.09^{bc}	3	5
B Root competition exclusion					
Trifolium repens	1.011 ± 0.09^{bc}	5	1.218 ± 0.13^{b}	5	5
Festuca nigrescens	0.818 ± 0.05^{b}	8	1.290 ± 0.09^{b}	7	8
Taraxacum officinale	1.310 ± 0.07^{ac}	2	0.655 ± 0.06^{a}	1	1
Plantago media	0.870 ± 0.04^{b}	7	1.270 ± 0.12^{b}	6	7
Agrostis capillaris	1.007 ± 0.07^{bc}	6	1.002 ± 0.06^{ab}	3	4
Trifolium pratense	1.515 ± 0.19^{a}	1	1.062 ± 0.06^{ab}	4	2
Prunella vulgaris	1.012 ± 0.08^{bc}	4	1.327 ± 0.13^{b}	8	6
Achillea millefolium	1.173 ± 0.07^{abc}	3	0.977 ± 0.08^{ab}	2	3

Table 3.1 - Means competitive effect (CE) and competitive response (CR) for each species (± 1 SE) in A Full competition (FC) and in B with root competition exclusion (RCE). Species with a greater CE and lower CR are the best competitor. Overall competitive hierarchy was determined by ranking the numerical sum of target and neighbour score rank with priority to CE in case of equality. Within columns, means with the same letter do not differ significantly (P > 0.05). The order of the species in the list corresponds to the field abundance hierarchy with five dominants at the top and three subordinates at the bottom.

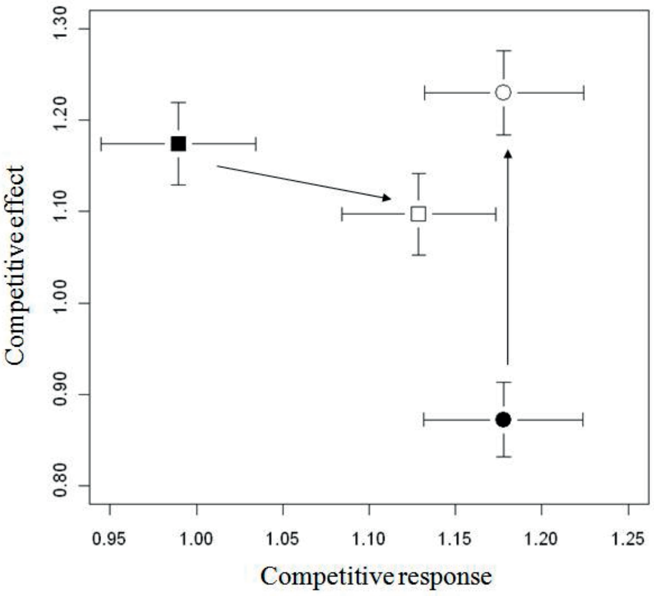

Figure 3.2 - Relationships between mean competitive effect and competitive response (± 1 SE) of dominant (square) and subordinate (circle) plant species with FC (solid symbol) or with RCE (open symbol).

Results

Competitive effect and response among dominant and subordinate species

Under full competition, the overall competitive hierarchy was similar to the one observed in the field (Table 3.1) with dominant species ranked higher (except P. media) and subordinates ranked lower: (1) *T. officinale*, (2) *A. capillaris*, (3) *T. repens*, (4) *F. nigrescens*, (5) *A. millefolium*, (6) *T. pratense*, (7) *P. media*, (8) *P. vulgaris*. Under root competition exclusion, all subordinates increased their competitive ranking whereas dominants maintained or decreased their ranking: (1) *T. officinale*, (2) *T. pratense*, (3) *A. millefolium*, (4) *A. capillaris*, (5) *T. repens*, (6) *P. vulgaris*, (7) *P. media*, (8) *F. nigrescens*.

At the species-groups level, when dominant species were grown in full competition with neighbours, they were good competitors resulting in high competitive effect and low competitive response. By contrast, subordinates had a low competitive effect and high competitive response and were thus poor competitors (Fig. 3.2). Root competition exclusion had a significant opposite effect on the competitive effect ($F_{1,221} = 15.33$, $P < 0.001$) and competitive response ($F_{1,221} = 4.67$, $P = 0.03$) of dominant and subordinate species (Appendix 3.3). Dominant species did not show change of their competitive effect but an increase of their competitive response. Exclusion of root competition increased the competitive effect of subordinateswhile their competitive response remained unchanged .

Root competition exclusion effect

Root competition exclusion mostly affected interacting dominant and subordinate species. The below-ground biomass of subordinate species was favoured by the absence of root competition of dominant species, whereas below-ground biomass of dominant species was unaffected by the absence of root competition from subordinates (Fig. 3.3). In absence of root competition, subordinates produced more biomass above-ground while dominants produced less biomass. In addition, root competition exclusion between subordinates in intra-specific combinations (not shown in graphs) led to a significant decrease in their root biomass (ANOVA, $F_{1,21} = 4.82$, $P = 0.04$), whereas no effects were found above- and below-ground between subordinates (inter-specific) and between dominant species (intra- and inter-specific).

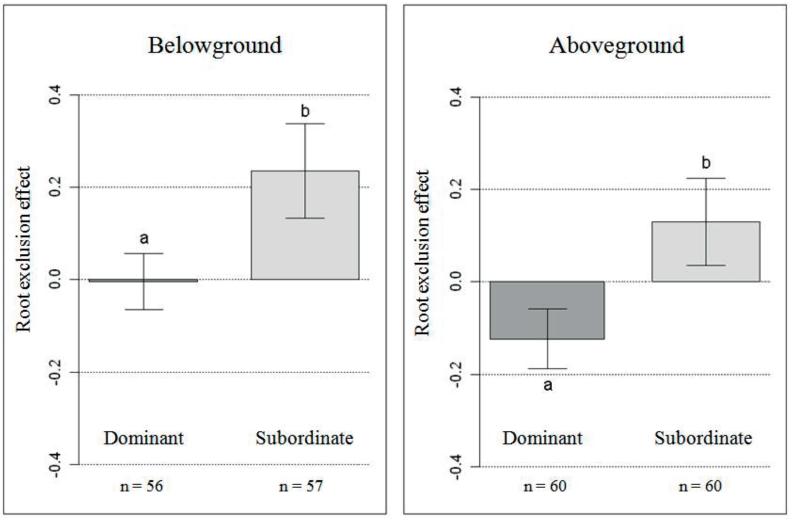

Figure 3.3 - Mean root exclusion effect (± 1 SE) on below- and above-ground biomass of interacting dominants and subordinates. Positive values correspond to a better growth with root competition exclusion and negative values correspond to a decrease of growth performance when roots are isolated from their neighbours. Bars with different letters are significantly different ($P < 0.05$). Unequal replication of root biomass in some cases led to lower number of observations.

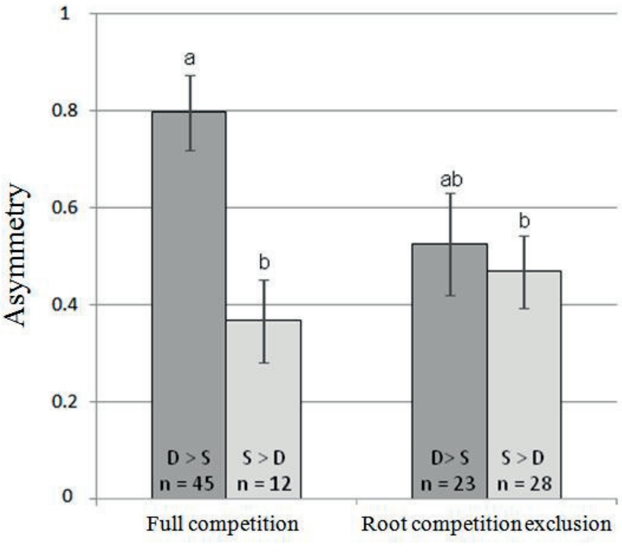

Figure 3.4 - Means (± 1 SE) of asymmetric competition for each combination of dominant and subordinate species under full competition and under root competition exclusion. Dark bars indicate cases where dominants were more competitive than subordinates (based on higher RYP) and light bars indicate cases where subordinates had better performance and n is the number of cases in each category. Bars sharing a letter are not significantly different ($P > 0.05$). D: dominant, S: subordinate species. Unequal replication of root biomass in some cases led to lower number of observations.

Degree of overall asymmetry

Under full competition, dominant species were mostly more competitive than subordinates ($n = 45$ out of 57) indicating a high asymmetry between both species-groups (Fig. 3.4). In only a few cases ($n = 12$), subordinates were better competitors than dominants but with significantly lower asymmetry. These results were mostly due to the effect of *P. media* (8 cases out of 12) which did not respond as a dominant species in this greenhouse experiment. When competition from roots was excluded between dominant and subordinate species, we observed a decrease in the number of cases where dominants were the best competitors ($n = 23$). Asymmetry also decreased compared to full competition. Consequently, the number of cases where subordinates were more competitive increased ($n = 28$) but the asymmetry in these interactions remained low

Discussion

Competitive abilities of dominant and subordinate species

In our experiment, the overall competitive hierarchy of dominant and subordinate species remained very similar to the species ranking determined in the field from relative abundances. Moreover, there appeared to be no association between competitive ability and the ability of plants to fix nitrogen as *Trifolium repens* was ranked higher and *Trifolium pratense* was ranked lower. Among eight species in the greenhouse experiment, only *Plantago media* (a dominant species) appeared lower in the ranking compared to the field. In the field, competitive interactions can differ from those in pairwise experiments, and depending on the growing conditions, intransitive interactions (i.e. non-hierarchical) may occur to slow competitive exclusion (Laird & Schamp 2006) and maintain subordinate species in communities. Indeed, *P. media* was the ‘loser’ in pairwise interactions with the three subordinate species in this experiment whereas it probably outcompetes more species in the field (including around 40 species) leading to its position as a dominant. Nevertheless, in this competition experiment, we assumed that plant communities are mainly governed by hierarchical competitive interactions (i.e. transitivity, Grace et al. 1993) and we based our results on the prediction that globally, dominant species are the best competitors whereas subordinate species are less competitive.

As expected, dominant and subordinate species differed significantly in their competitive abilities. Wang et al. (2010) concluded that competitive effect ability is related to a general characteristic or trait of a species, e.g. plant height or lateral spread. As such, bigger plants more easily suppress the growth of other plants since they have a higher potential for resource acquisition such as light (Goldberg & Landa 1991; Keddy et al. 1998). It has been shown (Liancourt et al. 2009) that under controlled conditions dominant plants have better ability to exclude other species by closing the canopy and competing for light. Our results point to this linkage between plant traits and competitive effect since dominants had a high competitive effect, while subordinates had a lower competitive effect. Competitive response is not only related to specific traits of the target species but also to the traits of neighbours and resource levels (Pennings et al. 2005; Wang et al. 2010). Indeed, dominant species had a higher competitive effect and performed better at resisting suppression by neighbours, whereas subordinate species were suppressed by other plants. The differences in competitive abilities between these groups of plants are confirmed by very high overall asymmetry of competition (A = 0.8) between dominants and subordinates.

Root competition exclusion and competitive abilities

Competitive effect and response of dominants and subordinates were shifted when competition from roots was excluded. The competitive effect of subordinate species increased but it did not change for dominants, whereas the competitive response of dominants increased while it did not change for subordinates. Jastrow & Miller (1993) showed that neighbouring plants can have a great influence on the root architecture of target species. Indeed, root exclusion of neighbours probably led to a change in root morphology of subordinates allowing for better growth and explaining their increase of competitive effect. The increase in competitive response of dominant species once root competition is excluded is most likely linked to the improvement of subordinate's competitive performance, and supports findings from North American prairie systems (Wang et al. 2010).

When root competition was excluded, the competitive abilities of dominants and subordinates became almost equivalent. With root exclusion, the decrease of competitive asymmetry between species when dominants were more competitive, together with the increase in the number of cases where subordinates were more competitive than dominants, confirms a reduction of competition between dominants and subordinates. As a consequence, the competitive superiority of dominants is attenuated and is beneficial for subordinates. However, when root-competition exclusion increased the competitiveness of subordinates, asymmetry between subordinates and dominants remained low indicating that even under these favourable conditions, subordinates remain weaker competitors than dominant species.

Root competition processes

In full competition, dominants had greater effects on subordinates, both above- and below-ground, which confirms the general finding in literature (Goldberg & Landa 1991; Keddy et al. 1998). Dominant species produced equal biomass below-ground in full competition and with root competition exclusion, showing that root competition from subordinates had no effect on the root growth of dominants. This result confirmed also that reduction of soil volume (by PVC barriers) did not affect growth of dominant species. However, when negative effects from the roots of dominants are removed, subordinates obtain an advantage in soil use through their roots, growing better below-ground. This increased root growth by subordinates is reflected above-ground by an increase of shoot biomass, probably because subordinates may have a larger access to nitrogen and phosphorus. Subordinates become more competitive for light resource capture due to root-shoot interactions and their effects on dominant species increases leading to a decrease of dominant shoot biomass. Frank et al. (2010) showed that the capacity of a species to produce shoot biomass is linked with large and well-proliferated root systems which emphasize the importance of roots in establishing above-ground dominance.

Root competition from dominant species is an important mechanism maintaining competitive hierarchy between dominant and subordinate species and consequently influences the plant community diversity and composition. In dominant and subordinate species interactions, root competition gives an advantage to dominants below-ground, which limits the growth of subordinate roots and subsequently their shoots. Competition between subordinates and dominants shows high degree of asymmetry and smaller subordinates are not able to compete strongly with dominants for light resource capture, explaining their low position in the species ranking. Inversely, the growth performance of subordinates increases if root competition is suppressed, for example due to the creation of gaps which enable more extensive root systems and greater nutrient acquisition, and so they compete more effectively with dominants. This study showed that the exclusion of root competition increases root-shoot competition interactions of subordinates and reduces the overall asymmetry between dominants and subordinates. Our results support Lamb et al. (2009) who demonstrated that root competition indirectly structures plant communities by modifying shoot biomass and success of plants in above-ground competition. Root competition therefore seems to reinforce shoot competition asymmetry between plants. In addition, root competition exclusion had negative effects on the root biomass of subordinates in monoculture and suggests that subordinate species facilitate themselves below-ground in intra-specific interactions.

Semi-natural grassland communities are continuously disturbed by both large and small herbivores (Cahill & Casper 2002; Kohler et al. 2005) that create canopy and root gaps. Gaps create spatial heterogeneity which is recognized as a driving factor of species co-existence in pastures (Vandvik 2004; Dufour et al. 2006). Subordinates might therefore depend more than previously thought on these frequent above and below-ground disturbance events and subsequent changes in root-shoot competition interactions. Reduction of root competition due to gap formation can promote coexistence of dominant and subordinate species and these findings have important consequences on competitive coexistence in spatially varying environments. Indeed, more studies about the effect of root competition and their inclusion in meta-analyses are necessary to complete the important gaps in the theory on the persistence of inferior competitors and/or populations with low density, which has received insufficient attention in spatial models (Chesson 2000; Amarasekare 2003). Examinations of the patterns of subordinates in the field are also needed to further increase our understanding of the persistence of subordinates in grassland communities.

In summary, this study suggests that root-shoot competition interactions may structure plant communities by maintaining the competitive hierarchy between dominants and subordinates through increasing overall asymmetry of competition. Inversely, when root competition is removed, root-shoot competition interactions can favour the persistence of subordinates locally in grassland communities by a decrease of overall asymmetry between dominant and subordinate species.

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Chapter 4

Arbuscular mycorrhizal fungi reduce the differences in competitiveness between dominant and subordinate plant species

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Abstract

In grassland communities, plants can be classified as dominants or subordinates according to their relative abundances but the factors controlling such distributions remain unclear. Here we test whether the presence of the arbuscular mycorrhizal (AM) fungus *Glomus intraradices* affects the productivity and competitiveness of two dominant and two subordinate species.

Plants were grown in controlled conditions in pots in the presence or absence of the fungus, in monoculture and in mixture of both species-groups with two and four species. The AM fungus developed extensive vesicles and had an overall negative effect on the growth of species indicating it was acting towards the parasitic end of the mutualism-parasitism continuum.

Subordinate and dominant species when colonised by the fungus decreased their biomass in monoculture. When dominants and subordinates were grown together, the negative effects of the AM fungus were most pronounced for the dominant species, particularly when grown in a mixture of four species, whereas subordinates were unaffected. In the absence of *G. intraradices*, dominant species were clearly more competitive than subordinate species. The presence of the AM fungus significantly shifted the competitive effect of plants and reduced the differences in competitiveness between dominant and subordinate species.

Our results indicate that the effects of *Glomus intraradices* varied with species composition highlighting the importance of plant community type to mediate the effects of AM fungi. Subordinate species benefited from colonisation by the AM fungus in mixtures containing dominants, and our findings predict that the dominance hierarchy may flatten in plant communities in situations where the cost to the plant of the symbiosis outweighs the benefit.

Key words

Competitive effect, *Glomus intraradices*, grasslands, mutualism-parasitism continuum, plant diversity.

Introduction

Semi-natural grasslands are widespread components of north-temperate landscapes and have important roles in providing grazing for livestock and acting as reservoirs of both carbon (Follett & Reed 2010) and biodiversity (Cremene et al. 2005; Baur et al. 2006). In particular, grasslands developed on calcareous substrates tend to contain a large diversity of plants including several rare, threatened and iconic species. For example, in the Swiss Jura wood-pastures, plant communities result from a traditional management with regular grazing and trampling disturbance and have a long succession history with well-established vegetation communities where up to 40 species may inhabit one square meter (Gigon & Leutert 1996; Buttler et al. 2009). Given these crucial and contrasting ecosystem services, it is important that species-rich grasslands are conserved. This aim requires understanding of the processes that govern the composition and stability of species-rich plant communities.

Observations of plant species abundances in semi-natural grasslands reveals distinct frequency distributions, with some species found frequently and in high abundance (dominants) and some found frequently as well, but in low abundance (subordinates; Grime et al. 1987; Olff & Bakker 1998). Some species may also be considered transients because they rarely persist (Whittaker 1965). Dominant species are generally few in number, tall and account for a large proportion of the total community biomass. In contrast, subordinate species consistently co-occur with particular dominants, are often small in stature and contribute marginally to the total biomass of the community, although they are the most diverse component of communities. The role of dominant species in ecosystem functioning has received considerable research attention and according to the “mass ratio” theory (Grime 1998), ecosystem properties are determined by dominant species independent of changes in species richness that involve variations in the number of subordinate species. However, more recent studies show that less abundant species may have a larger influence on ecosystem properties and functioning than their relative abundance suggests (Lyons et al. 2005; Boeken & Shachak 2006). Thus, it becomes important to consider the factors that determine the abundance of not only dominants but also subordinates.

In semi-natural, extensively managed grasslands, the roots of almost all plants are heavily colonised by arbuscular mycorrhizal (AM) fungi (Read et al. 1976). AM fungi often form large mycelial networks throughout soil and greatly facilitate acquisition and uptake of scarce or immobile mineral nutrients, particularly phosphorus (Johnson et al. 2001). In forming mycelial networks, individual mycorrhizal fungi can be supported by several host plants, and so the hyphae can facilitate seedling establishment (Van der Heijden et al. 2004) and affect plant competition. Whilst the AM symbiosis is typically considered a mutualistic one, increasing evidence points to a more diverse range of interactions. In a meta-analysis, around 45% of studies found positive effects of AM fungi on plant growth, 30% showed no effect, and 25% showed negative effects (Van der Heijden & Horton 2009). This analysis supports the idea that AM fungi act along a continuum between mutualism and parasitism (Johnson et al. 1997; Klironomos 2003) and species competitiveness could be increased as well as decreased in the presence of AM fungi. Here the net outcome of the symbiosis is likely dependent on the environment, or may develop in different directions throughout the lifetime of a plant.

The contrasting functional characteristics of individual AM fungi therefore suggest that they may have important roles in shaping plant community composition and stability in grasslands. The effect of AM fungi on plant communities are also related to nutrient availability in soils. It has been shown that low phosphorus status stimulates colonisation of roots by AM fungi, which feeds back to affect plant diversity and productivity (Collins & Foster 2009). Moreover, some studies have demonstrated that subordinate and dominant plant species can show distinct responses to AM fungi with subordinates considered most responsive to colonisation (Van der Heijden et al. 1998a; Yao et al. 2007; Karanika et al. 2008). AM fungi are completely dependent on host plants for carbon, and their biomass and activity means they are substantial sinks for plant assimilate (Johnson et al. 2002) which could therefore completely change the dominance hierarchy (Gross et al. 2010) following its degree of profit for plant species. These responses have been developed into a model whereby the relative response of dominants and subordinates to AM fungi determines plant community composition and dominance hierarchies (Urcelay & Diaz 2003). When mycorrhizal dependence of subordinates is strong, dominance rankings flatten in presence of AM fungi, but are steeper in their absence, so that overall plant species diversity increases. In contrast, when mycorrhizal dependence of dominants is strong and subordinates is weak, dominance rankings steepen in response to AM fungi and flatten in their absence so that overall plant species diversity decreases. To date there have been few studies designed that explicitly test the hypothesis set out by Urcelay & Diaz (2003) but most of the evidence is indirect from other experiments or observations which related the relative influence of mycorrhizal symbiosis (Klironomos et al. 2011).

Plant communities of wooded pastures in the Swiss Jura Mountains result from traditional cattle and forest management and vegetation communities are well-established on unimproved soils with intermediate fertility. These grasslands communities serve as an ideal model to explore the effect of AM fungi on plant interactions since they are very diverse and show a typical lognormal rank-abundance curve (Grime 1998) with a few dominant species accounting for a high proportion of the total community biomass. In this study, we chose four plant species, which are native to species-rich calcareous grasslands. According to their relative abundance in the field, two of these species can be considered to be dominants and two as subordinates. Previous experiments in the field (unpublished) showed that these subordinate species were significantly more colonised by AM fungi (about 20% more) compared to dominants during seedlings establishment. AM fungi could therefore be more beneficial to less competitive subordinates and explain their persistence in plant communities. In this paper, we test the competitive effect of two dominant and two subordinate species in different species composition when grown under control conditions and in the presence or absence of the ubiquitous AM fungus *Glomus intraradices*. We hypothesize that inoculation with *G. intraradices* will promote growth of subordinate plant species and reduce the differences in competitiveness with dominant species which will not or less draw benefit from the fungus.

Materials and methods

Experimental design

Dominant and subordinate species were selected from measurements of community composition in semi-natural calcalreous grassland in the Swiss Jura Mountains (Les Amburnex, western Switzerland, 6°13'50" E, 46°32'50"N). The field research site is an extensively-grazed pasture with a vertic cambisol which contains relatively low level of nutrients (0.18% total phosphorus; 0.67% total nitrogen and 5.8% total organic carbon, pH = 6.4). A species was classified as dominant if its frequency was greater than 75% and its cumulative relative cover greater than 25%. A species was classified as subordinate if its frequency was greater than 75% and its cumulative relative cover between 2 and 12% (adapted from Grime 1998). Based on their field abundance and results of their competitive abilities when growing together in a previous greenhouse experiment (Chapter 3), we assigned *Prunella vulgaris* and *Achillea millefolium* as subordinate species and *Agrostis capillaris* and *Taraxacum officinale* as dominant species. Seeds of the four species were gathered in the Swiss plateau (provided by FENACO SA., Yverdon-les-bains, Switzerland) and stored at 4°C prior to the start of the experiment, sterilized in 5% bleach for 10 minutes to eliminate living organisms, then rinsed 5 times with distilled water. On 18th March 2010, around 700 seeds of each species were germinated on sterile water agar gel (1.6%) in a phytotron over 6 days in the dark at 22°C.

On 25th March 2010, seedlings were transplanted into 1.5 L sterile plastic pots containing 800 ml of a gamma sterilized sand (Berns et al. 2008) mixture (90 % sand and 10% compost). The mixture recreated conditions of the field in term of soil fertility (0.21% total phosphorus, 0.57% total nitrogen and 4.1% total organic carbon). Four individuals were transplanted into each pot within their agar gel cube (1 cm^3) with an inter-plant spacing of 3 cm. The agar gel was covered with sand to minimize stress during transplantation. Non-surviving plants during the first week were replaced. All pots contained 4 individuals, either from the same species (monoculture), from two different species including one dominant and one subordinate (two-species mixture) or from four different species including two different dominants and two different subordinates (four-species mixture).

An inoculation treatment was established by adding on the top soil, 40 ml of *Glomus intraradices* fungal inoculums which embedded the agar gel cube, with corresponding controls comprising 40 ml of autoclaved inoculum (120°C for 30 min). We chose the ubiquitous *G. intraradices* because it has a broad host range and is the most frequently detected phylotype in all grassland systems (Opik et al. 2006) and the most abundant in Swiss meadows (Mathimaran et al. 2005; Sykorova et al. 2007). The fungus inoculum (mix of root fragments, sand and spores), native from a calcareous grassland, was provided by Prof. Van der Heijden, Agroscope ART Zurich, Switzerland (further details about this inoculum in Streitwolf-Engel et al. 1997; Van der Heijden et al. 1998b). A split-block-design was adopted (Fig. 4.1) with three factors: 1) inoculation treatment (with *G. intraradices* or sterile autoclaved inoculum) varying at the block level, 2) mixture type (monoculture, two-species mixture or four-species mixture) varying at the plot level, and 3) plant identity (subordinates: *P. vulgaris* and *A. millefolium*, dominants: *T. officinale* and *A. capillaris*) which varied at the pot level. The interaction of mixture type and species identity yielded to nine different mixture compositions to which the inoculation treatment was applied, resulting of 18 combinations of the three factors. The combination of factors was replicated 6 times, yielding a total of 108 pots arranged in 6 blocks. The pots were maintained in a greenhouse and during the first month, each pot was watered every day with 200 ml of water directly in the receiver to ensure plant survival. They were then watered only when the substrate was dry which corresponds to a 10% humidity. After 105 days, shoot biomass was harvested for each species by cutting at ground level. Roots were collected by washing and sieving each individual. Subsamples of selected individuals were used for determination of AM fungal colonisation. Shoot and root biomass were dried in an oven at 60°C for 72 h and weighted. The average of the two (two-species mixture) or four (monoculture) individuals per species in each pot was calculated to obtain the overall measure of root and shoot biomass per species per pot.

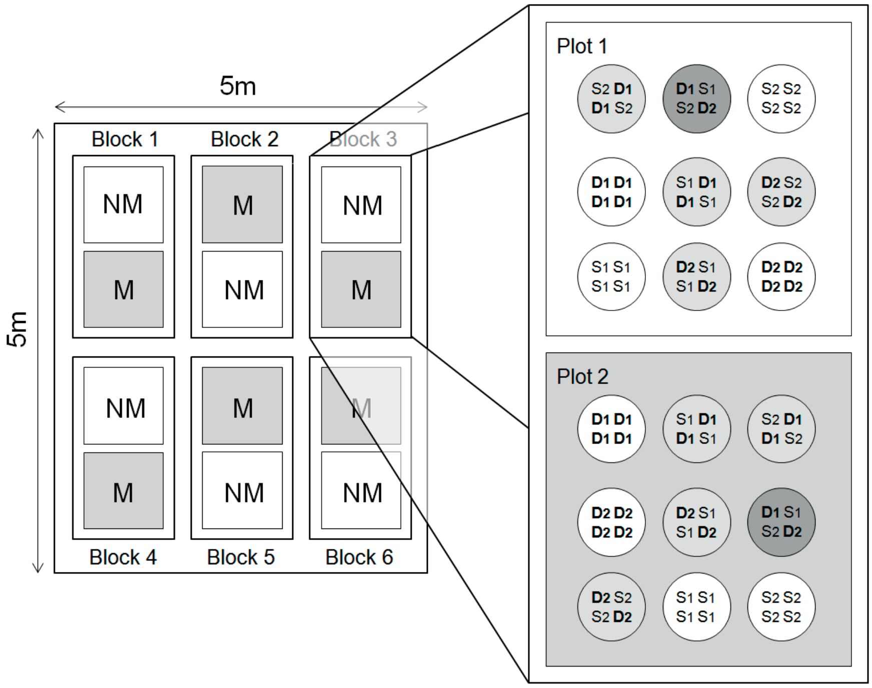

Figure 4.1 -Schematic diagram of the experimental design. Circles represent pots and contain plant combinations of dominant species: *Taraxacum officinale* (D1) and *Agrostis capillaris* (D2), and subordinate species: *Prunella vulgaris* (S1) and *Achillea millefolium* (S2). Each pot contained four individuals of the same species in monoculture (white), two individuals per species (one dominant and one subordinate) in two-species mixture (grey) and one individual per species (two dominants and two subordinates) in mixture of four species (dark grey). Combinations are reproduced either with (M) or without (NM) AM fungal inoculation which varied at the plot level in 6 blocks.

Calculation of AM fungal colonization

Confirmation of the presence of mycorrhizas was undertaken microscopically by staining roots from each plant species in each community composition (4 replicates). The primary objective was to determine whether the inoculum had successfully colonized roots rather than to test for effects of mixture type and plant identity. A minimum of 50 intersections per root sample were viewed and scored for the presence of vesicles, hyphae and arbuscules. The absence of colonization was also confirmed in uninoculated plants.

Relative effect of arbuscular mycorrhizal fungi

We did not observe any differences in root/shoot ratios following inoculation with *G. intraradices*, or by changing the richness and mixtures of species. We therefore focused on total plant biomass as the response variable in our analyses. For each species when grown in monoculture, two-species mixtures and four-species mixtures, the effects of the AM fungus were measured as:

$$\text{AM fungal effect}_{ij} = \ln (Y_{ij}M/Y_{ij}NM)$$

with $Y_{ij}M$ representing total plant biomass (root and shoot) of species *i* growing with species *j* in presence of AM fungal inoculum and $Y_{ij}NM$ the biomass of the same species *i* growing with species *j* without AM fungal inoculum. Positive values of AM fungal effect indicate a better growth in presence of AM fungi and inversely, negative values indicate a negative growth response to inoculation by AM fungi.

Competitiveness of dominant and subordinate species

We calculated the relative yield per plant (RYP) of each species from total biomass (root and shoot) in the mycorrhizal (M) and non-mycorrhizal (NM) conditions in two and four-species mixtures,

$$RYP_{ij} = Y_{ij} / Y_{ii}$$

where RYP_{ij} is the relative yield per plant of species *i* (i.e. target) grown with species *j* (i.e. neighbour), Y_{ij} is the yield of plant *i* when grown with species *j*, and Y_{ii} is the yield of plant *i* when grown in monoculture (Engel and Weltzin 2008). When $RYP_{ij} > 1$, *j* is less competitive than species *i* and conversely, when $RYP_{ij} < 1$, *j* is a better competitor than *i*. When $RYP_{ij} = 1$, species *i* and *j* are equally competitive.

Competitive effect (CE) of dominant species is defined as the mean RYP of *T. officinale* and *A. capillaris* grown with each subordinate species and competitive effect of subordinate species is defined as the mean RYP of *P. vulgaris* and *A. millefolium* grown with each dominant species (Goldberg & Landa 1991). Species with a greater CE are better competitors and inversely, species with a lower CE are less competitive. To test whether competitiveness changed when plants were colonised by *G. intraradices*, the CE of dominant and subordinate species was calculated for both mycorrhizal and non-mycorrhizal plants in two and four-species mixtures.

Statistical analysis

Statistical analyses were undertaken in R version 2.11.1 (R Development Core Team, 2010). Total plant biomass and competitive effect were analysed, after square-root transforming, using linear mixed-effects models with plot nested into block as random factors. For total plant biomass, datasets from dominant and subordinate plant species were analysed separately and competitive effect was analysed separately for two and four-species mixture.

Results

Colonisation of roots by the AM fungus

The root systems of the plant species inoculated with *G. intraradices* were heavily colonized but AM fungal colonisation did not significantly differ between species. Plants were mostly colonised by vesicles (mean of 46% for *T. officinale*, 56% for *A. capillaris*, 80% for *P. vulgaris* and 63% for *A. millefolium*) and hyphae (30% for *T. officinale*, 33% for *A. capillaris*, 11% for *P. vulgaris* and 24% for *A. millefolium*), but showed little development of arbuscules (less than 2% for each species). Plants not inoculated with *G. intraradices* remained uncolonized.

Plant productivity of species-groups

In the absence of AM fungi, *Agrostis capillaris* and *Taraxacum officinale* produced more biomass than the subordinates when in mixture, confirming their classification as dominants. The presence of *G. intraradices* significantly reduced overall biomass of dominant ($F_{1,79} = 85.146$, $P < 0.001$) and subordinate ($F_{1,79} = 6.541$, $P < 0.01$) plant species compared to uninoculated controls by 54% and 25% respectively. The mixture type (i.e. monocultures and two and four-species mixtures) had only a significant effect on biomass of subordinate species ($F_{1,79} = 3.115$, $P < 0.05$) and no interaction was found between mixture type and AM fungal inoculation for both species-group. The effects of the AM fungus were very consistent among species-groups when species were grown in monoculture (Fig. 4.2), significantly reducing biomass of dominants and subordinates by between 42-44% compared to the non-mycorrhizal controls. In mixture, we observed differences between dominant and subordinate species in response to AM fungal inoculation since biomass of dominants was significantly reduced by 53% and 65%, respectively in two and four-species mixture whereas subordinates were not affected by the AM fungus when the plants were grown in a mixture with dominants.

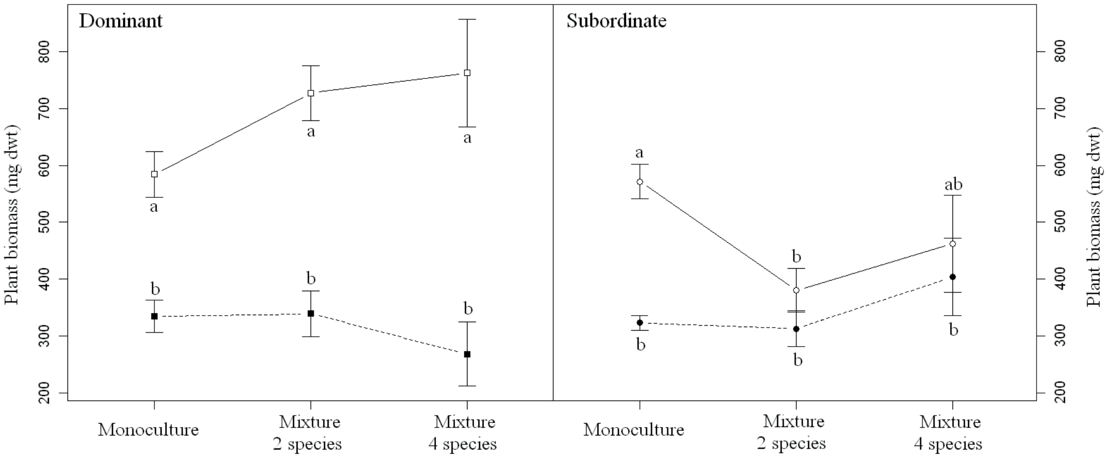

Figure 4.2 - Total plant biomass (± 1 SE) of dominant and subordinate plant species growing with (solid symbol) and without (open symbol) *G. intraradices* in monoculture and in two and four-species mixtures. Points sharing a letter are not significantly different. (P>0.05)

Relative AM fungal effect at species level

The addition of *G. intraradices* had a negative effect on both dominant and subordinate species when they were grown in monoculture (Fig. 4.3) but the effects differed when the species were grown in mixtures. The relative AM fungal effect was always negative for the dominant species (*T. officinale* and *A. capillaris*) regardless of whether they were competing with their conspecifics or with one or two subordinates. The magnitude of the negative effect of *G. intraradices* on *A. capillaris* biomass increased in the four-species mixture compared to the two-species mixture. In contrast, the effect of *G. intraradices* remained constant across monocultures and mixtures for *T. officinale*. The patterns of relative AM fungal effect were the opposite for subordinate species. Here, the magnitude of negative effects tended to decrease in two and four-species mixture. For example, *P. vulgaris* was unaffected by *G. intraradices* in mixture of two and four species, whereas *A. millefolium* remained marginally affected in two-species mixture but was unaffected in mixtures of four species.

Competitiveness of species-groups

In the absence of *G. intraradices* inoculation, the index of competitive effect was high for dominant species (CE > 1) and low for subordinate species (CE < 1) independent of mixture type of interacting species-groups (Fig. 4.4). In contrast, the inoculation of *G. intraradices* significantly modified competitive effect of species-groups (Table 4.1) with a decrease for dominants and an increase for subordinates in two and four-species mixture. In two-species mixture comprising interacting species-groups (Fig. 4.4a), both dominants and subordinates had a competitive effect close to 1 indicating that these two species-groups had the same competitiveness in presence of the AM fungus. In the four-species mixture comprising interacting species-groups (Fig. 4.4b), the competitive effect of dominant species decreased (CE < 1) whereas competitiveness of subordinates increased (CE > 1), indicating that subordinate species were more competitive than dominants.

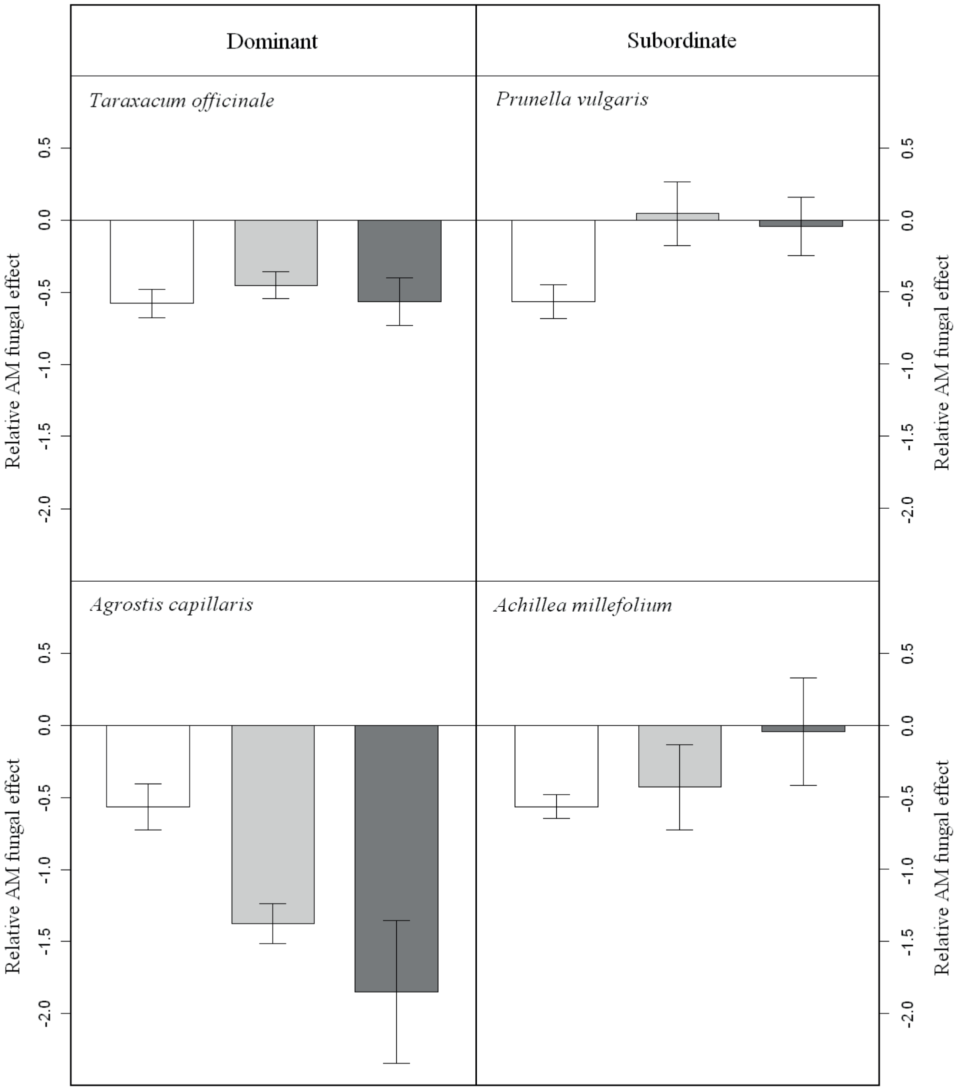

Figure 4.3 - Relative effect of the arbuscular mycorrhizal fungus *Glomus intraradices* on the biomass of dominant (*Taraxacum officinale* and *Agrostis capillaris*) and subordinate (*Prunella vulgaris* and *Achillea millefolium*) species in monoculture (white), two-species mixture (grey) and four-species mixture (dark grey).

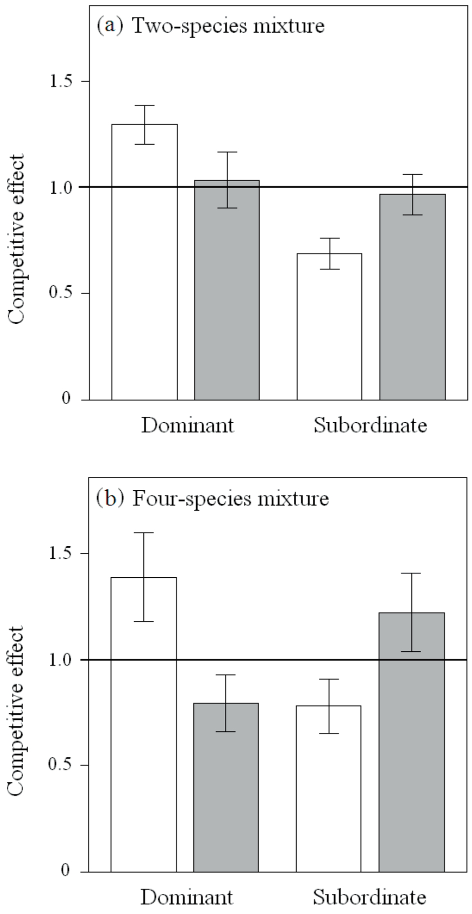

Figure 4.4 - Index of competitive effect (CE ± 1 SE) of interacting dominant and subordinate species when grown in the presence (grey bars) and absence (white bars) of the arbuscular mycorrhizal fungus *Glomus intraradices* in two-species mixture (a) and in four-species mixture (b). Horizontal lines indicate indicates a CE value of 1 whereby species have equal competitiveness. Values above the line indicate a strongly competitive group while values below the line indicate a weakly competitive group.

Two-species mixture	Df	F-value	P-value
Competitive effect			
AMF inoculation	1	0.0028	0.959
Residuals	5		
Type (Dominant/Subordinate)	1	11.958	**0.001**
Residuals	82		
Type x AMF inoculation	1	9.000	**0.004**
Residuals	82		

Four-species mixture	Df	F-value	P-value
Competitive effect			
AMF inoculation	1	0.157	0.709
Residuals	5		
Type (Dominant/Subordinate)	1	0.261	0.613
Residuals	34		
Type x AMF inoculation	1	9.660	**0.004**
Residuals	34		

Table 4.1 - Linear mixed-effects model analysis of competitive effect depending of AM fungal inoculation and species type (dominant/subordinate) in two and four-species mixtures.

Discussion

Mutualism-parasitism continuum

AM fungi affect plant growth and nutrition and thus have been shown to alter competitive relationships (Grime et al. 1987; Hartnett et al. 1993; Hartnett & Wilson 1999; Bever 2003; Scheublin et al. 2007). Whilst this was also the case in our study, the results demonstrated that plants colonised by *G. intraradices* performed worse (i.e. produced less biomass) than they did in the non-mycorrhizal conditions. Such negative effects of mycorrhizal fungi on plant productivity are found only in 25% of published experiments (Van der Heijden & Horton 2009). Thus, it appears that the plant:mycorrhizal system was likely operating within the parasitic end of the mutualism-parasitism continuum highlighted by Johnson et al. (1997). This is also reflected by the large proportion of vesicles observed in the roots of all species (46-80%). Vesicles are thought to be storage structures for carbon (Smith & Read 2008) and so the ratio between carbon allocation and mineral nutrient uptake might not be favourable from the perspective of the host (Johnson 2010). Under the conditions of this experiment (low phosphorus and high light availability), we expected that the fungus act as a mutualistic symbiont (Johnson et al. 1997) but this was not and we suggest that some other mechanisms may affect AM fungi activity in the mutualism-parasitism continuum. Indeed, we manipulated only *G. intraradices* in this experiment and the absence of a diverse soil microbial community (i.e. soil competitors) might also influence the direction of the symbiosis.

Species-groups and AM fungal dependency

Subordinate plant species were expected to be more dependent on AM fungi for growth than dominants (Van der Heijden et al. 1998a; Karanika et al. 2008) and dominants were expected to grow equally well with or without AM fungi (Yao et al. 2007) but our results contradicted these expectations. *G. intraradices* had no effect on subordinate species and strongly detrimental effects on dominant species in the two and four-species mixtures whereas we observed a negative effect for all species in monoculture. AM fungi influenced the growth of dominant and subordinate plants differently due to differential responses to the parasitic effects of *G. intraradices* in mixtures. *A. capillaris* was the only grass in this experiment and was the most negatively affected by AM fungal inoculation. Even if they do not respond all in the same way, grasses are known to be positively affected by AM fungi inoculation in mutualistic conditions (Wilson & Hartnett 1998; Hoeksema et al. 2010) but, inversely, in parasitic conditions, grasses could be more negatively affected by AM fungi.

Nevertheless, the other dominant species, the forb *T. officinale*, was also negatively affected by inoculation of *G. intraradices*. The greatest differences in response to *G. intraradices* among species were observed in four-species mixtures: *P. vulgaris* and *A. millefolium* (subordinates) were unaffected by the AM fungus whereas *A. capillaris* and *T. officinale* (dominants) were negatively affected. Since subordinate species were negatively affected by AM fungus in monoculture, these findings suggest that the neutrally-mutualistic symbiosis between subordinates and *G. intraradices* may only occur in polyculture due to greater intensity of competition between each plant species. Indeed, no competitive hierarchy can be established in monoculture where only one plant species (subordinate or dominant) is available for the fungus. When plants grow in a mixture of subordinate and dominant species and a competitive hierarchy can be recognised, *G. intraradices* appeared to be selective among species-groups in its intensity of parasitism, affecting more negatively the dominant species. We suggest that AM fungi might select plant species with high biomass that potentially can supply larger amounts of mineral nutrients. Subordinate plant species, which produced low biomass when non-mycorrhizal, benefited more when AM fungi were present, while dominant species, which produced high biomass in non-mycorrhizal treatment, were not enhanced, or even reduced (Grime et al. 1987, Van der Heijden et al. 1998b). Our findings confirm therefore the key role of neighbourhood identity and community composition that interacts with AM fungi to affect competitive outcomes of plants (Harnett & Wilson 1993; Collins & Foster, 2009).

Competitive interactions

Urcelay & Diaz (2003) hypothesised that interactions between subordinate and dominant plants are regulated by their increasingly positive growth responses to mycorrhizal fungi which ultimately shapes plant community composition and diversity. Based on this model, we hypothesised that AM fungi would flatten the differences in competitiveness among species because subordinates would have an advantage over dominants. Whilst our findings supported our hypothesis, in terms of reduction of competitiveness of dominant species, the mechanism was the opposite of what we predicted in that it was driven by differential responses of plant species to the parasitic effects of *G. intraradices* and an interaction with species mixture of the communities, rather than the positive effects of the fungus.

The competitive effect of dominant species decreased in presence of *G. intraradices* whereas it increased for subordinate species independently of mixture type. In addition, the effect of the AM fungus on competitive effect was more intense in mixture of four species than in two-species mixture. Actually, in two-species mixture, species-groups became equally competitive (CE = 1) following a reduction of the differences in competitiveness between dominant and subordinates. In four-species mixture, we observed an inversion of the competitive balance between species-groups with subordinates (CE > 1) becoming more competitive than dominant species (CE < 1). In this experiment, dominant species were clearly more competitive than subordinates in absence of *G. intraradices*. In presence of the fungus and depending on species composition, the competitive effect of dominant species decreased, leading to a reduced negative effect on subordinate species which enable subordinates to be more competitive. Our findings highlight the role of myccorhizas to allow species coexistence by favouring less competitive species but also the importance of plant community type to mediate the effects of AM fungi.

Our study therefore gives an opportunity to further our understanding of how interactions between subordinate and dominant plants are regulated when AM fungi are functioning at the parasitism end of the mutualism-parasitism continuum. Under these circumstances, when grown in mixtures of two and four species, dominant plants tended to be more negatively affected by *G. intraradices* than subordinate plants (Figs. 4.2 and 4.3; i.e. a significant negative mycorrhizal effect) leading to the reduction of competitiveness between dominants and subordinates. Our findings therefore provides insight into the parasitic effects of AM fungi, which remain poorly studied, and gives new perspectives for the model of Urcelay & Diaz (2003). According to our findings, we would predict dominant species to be less competitive and flatten the dominance hierarchy in communities, especially with increasing species richness. Clearly, this prediction is restricted to one fungal species and needs to be supported by experiments with a greater number of fungi and plant species than used in the present study but nonetheless our data provide the basis for undertaking such work.

Our results also highlight the potential importance of one species of AM fungi in shaping plant communities (Scheublin et al. 2007) although interactions among different AM fungi and plant species might generate really different outcomes in natural system (Klironomos 2003; Van der Heijden et al., 2006; Wagg et al. 2011). It is likely that parasitic to strongly mutualistic responses to individual mycorrhizal fungi occur simultaneously in nature, and so understanding the conditions that lead to these contrasting responses and quantifying the net effect of mycorrhizal communities on ecosystems is important. Given the contrasting effects of plant species (Vandenkoornhuyse et al. 2003; Johnson et al. 2004; Hausmann & Hawkes 2009) on AM fungal community composition and functioning, below-ground interactions may be a key process driving competition among neighbours especially between dominant and subordinate species.

In conclusion, the present study showed that, in monoculture, the four plant species grew lower in presence of AM fungi whereas contrasting effects were observed among species in mixture. In mixtures of two or four species, the two dominant plant species, which produced the largest biomass in absence of the fungus, tended to grow less when colonised whereas the two subordinates grew identically with or without AM fungi. These results point up the importance of plant composition to regulate the effects of AM fungi with potentially more negative effect on high competitive species. At the species-groups level, it appeared that the presence of AM fungi might confer an advantage to subordinate plant species despite the fungus used was considered to be functioning at the parasitic end of the mutualism-parasitism continuum. In natural plant communities, subordinate plants may therefore not only gain an advantage from associating with functionally mutualistic species of AM fungi (Urcelay & Diaz 2003), but may also be able to persist when the symbiosis is considered parasitic on dominant species, which may occur due to changes in resource availability and AM fungal community structure. Thus, regardless of the overall benefit of colonisation by AM fungi, asymmetry in the response of dominant and subordinate plants to mycorrhizal colonisation is likely to be a key process that regulates plant community structure in grassland.

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Chapter 5

Subordinate plant species impact on soil microbial communities and ecosystem functioning in grasslands: findings from a removal experiment

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In review

Abstract

Subordinate plant species may have a larger influence on ecosystem processes and functioning than their relative abundance suggests but their importance in plant communities remain poorly studied. The aim of our study is to test how subordinate species play a role on the functioning of grazed species-rich grasslands.

The dominance hierarchy from a mountain wood-pasture ecosystem was used to identify dominant and subordinate species (competitive hierarchical classification). A removal experiment was carried out on 18 permanent plots from 2009 till 2011 including three treatments: removal of all subordinate species, partial biomass removal of dominant species and control without any biomass removal. For each treatment, we determined soil microbial community (bacteria and mycorrhizal fungi) by T-RFLP and measured litter decomposition using minicontainers system, soil respiration, plant available nitrogen (DIN) and above-ground biomass production.

Below-ground, the removal of subordinate plant species shifted significantly bacterial communities and marginally mycorrhizal fungi communities leading to a decrease of litter decomposition, soil respiration and dissolved inorganic nitrogen. Above-ground, the absence of subordinate plant species did not modify plant community but reduced above-ground biomass production compared to control plots.

This removal experiment revealed that the absence of subordinate species modified microbial soil communities and ecosystem processes through plant-soil feedbacks. Our findings confirm the importance of non-random species loss on ecosystem functioning and show that not only dominants species but also subordinate species are important drivers of ecosystem processes.

Key-words: arbuscular myccorhizal fungi, bacteria, biomass production, competitive hierarchical groups, dissolved inorganic nitrogen, grasslands, key-species, litter decomposition, soil respiration, T-RFLP.

Introduction

Humans have strongly modified aquatic and terrestrial ecosystems around the world, through pollution, land-use and climate change, overexploitation or invasive species, leading to major alterations of biological communities and reductions in biodiversity (Millennium Ecosystem Assessment 2005). Transformations of land-use, coupled with the effects of human-induced climate change, are believed to be responsible of a dramatic loss of global biodiversity (Haines-Young 2009; Butchart et al. 2010) with consequent impacts on ecosystem properties and services (Schläpfer & Schmid 1999; De Groot et al. 2002). To date, most research on plant biodiversity loss has focused on the influence of changes in species richness on ecosystem functioning (Balvanera et al. 2006; Cardinale et al. 2006). Furthermore, many other studies demonstrated the importance of species type such as functional groups (Wardle et al. 2003; MacLaren & Turkington 2010), functional traits (Lavorel et al. 2011), relative biomass proportion (Grime 1998) or keystone species (Lyons & Schwartz 2001; Boeken & Shachak 2006) to explore the role of biodiversity on functioning of ecosystems.

According to the "mass ratio" theory of Grime (1998), ecosystem properties are mainly determined by competitive dominant species, which are generally few in number, tall and account for a large proportion of the total community biomass. However, more recent studies show that less abundant, or subordinate, species may have a larger influence on ecosystem processes than their relative abundance suggests (Lyons et al. 2005; Polley et al. 2006), which, according to the "complementary resource" and "filtering" hypotheses (Grime 1998), is related to their more efficient utilization of resources and ability to facilitate certain dominant species. Subordinate species are also known to be well adapted to disturbance (Pierce et al. 2007) due to their physiological plasticity (Tahmasebi Kohyani et al. 2008) and have been shown to increase the resistance of plant communities to environmental change (Grime 1998, Chapin et al. 2000; Chapter 6), maintaining ecosystem function by substituting for dominants (Walker et al. 1999). There is also emerging evidence that subordinate species may have higher impact than dominants on bacterial and fungal communities (Peltzer et al. 2009) and consequently on plant litter decomposition (Wardle and Zackrisson 2005). Moreover, subordinates seem to benefit from a preferential association with arbuscular myccorhizal fungi (Van der Heijden et al. 1998) and increase their biomass (Hart et al. 2003) or their competitiveness (Grime et al. 1987; Chapter 4) in presence of AMF relative to that of the dominants. However, despite this knowledge, our understanding of the influence on ecosystem functioning of subordinate plant species remains unclear, especially when considering ecosystem multifunctionality and interacting above- and below-ground processes.

In many studies, the importance of diversity on ecosystem properties is evaluated by experiments involving a random assemblage of plant species, using often only dominant species and without considering the role of less abundant species (Bardgett & Wardle 2010). Therefore, the aim of this study was to explore the ecosystem-level effects of subordinate species on plant and soil naturally established communities and ecosystem functioning. The role of subordinate species, defined as a plant group, was explored on (1) plant community composition, expecting subordinate species to regulate competition between dominant and transient species and maintain plant diversity, (2) bacterial and AM fungi communities, expecting to shift in absence of subordinates, and on (3) litter decomposition, plant available nitrogen, and above-ground plant productivity which are expected to change following (1) and (2). Our experiment was carried out in the Swiss Jura mountains where semi-natural grasslands have well-established vegetation (Buttler et al. 2009) resulting from a traditional management with regular grazing and trampling disturbance. Plant communities are very diverse (up to 40 vascular species per square meter) and include a few dominant species that account for a high proportion of the total community biomass, and many subordinate species that represent low biomass, but contribute significantly to plant diversity. To validate our hypothesis, we used a plant removal experiment which is not yet often used to determine the effects of non-random losses in natural communities (Wardle et al. 1999; Diaz et al. 2003). During 3 years, plots from which subordinates were suppressed were compared to plots in which the same biomass from dominants was removed and to control plots without any removal.

Materials and methods

Site description

The field experiment was carried out in an unfertilised, extensively-grazed and species-rich pasture (mean of 35 species m-2) situated in a well-drained limestone karst valley of the Swiss Jura Mountains (Les Amburnex, western Switzerland, 6°13’50” E, 46°32’50”N). The pasture is part of the wood-pasture system typical for this area, surrounded by small and large patches of Picea-dominated forests, and is grazed by cattle following a rotational system during the vegetation growing season lasting from June to September. The site is situated at an altitude of 1300 m a.s.l. and has a suboceanic climate with a mean annual rainfall of about 1600 mm and a mean annual temperature of 7°C. The soil is a vertic cambisol (after the World Reference Base for Soil Resources - IUSS Working Group WRB, 2006) deeper than 1 m and the ground is covered by snow from November to April with temperature below 0°C on more than 60 days per year. Considering the climatic conditions in these areas, two seasons may be distinguished: the winter season from November to April and the summer season from May to October.

Defining dominant and subordinate species

Within a 25 by 25 m cattle-excluded square, 49 plots of 1.2 by 1.2 m were established at a regular distance of 1.6 m from each other. In July 2008, absolute plant cover was determined within all plots using a modified Braun-Blanquet index (1: cover less than 5%, 2a: 5-10%, 2b: 10-25%, 3: 25-50%, 4: 50-75%, 5: 75-100%). A total of 41 species were identified and classified in competitive hierarchical groups. A species was classified as dominant if its frequency was greater than 70% (100% frequency means that the species is present in all plots) and its cumulative relative cover greater than 12%. A species was classified as subordinate if its frequency was greater than 70% and its cumulative relative cover between 2 and 12% (adapted from Grime 1998). The other species were classified as transient plants and generally do not persist over years. This method resulted in the determination of seven dominant species: (1) *Trifolium repens* L., (2) *Festuca nigrescens* Lam., (3) *Taraxacum officinale* agg., (4) *Ranunculus acris ssp. friesianus*, (5) *Plantago media* L., (6) *Leontodon* sp. (*Leontodon autumnalis* L. and *Leontodon hispidus* L.sl.), (7) *Agrostis capillaris* L. and seven subordinate species: (8) *Trifolium pratense* L., (9) *Prunella vulgaris* L., (10) *Alchemilla monticola* Opiz, (11) *Cardamine pratensis* L., (12) *Achillea millefolium* L., (13) *Plantago major* L.s.str., (14) *Cerastium fontanum* ssp. vulgare.

Removal treatment experiment

In this experiment, we used a removal approach in order to evaluate the ecosystem level effects of subordinate species. This approach has been used in previous studies of plant functional groups (Wardle et al. 1999; Symstad & Tilman 2001; Buonopane et al. 2005) and early-successional plants communities (Polley et al. 2006) in grasslands, and has the advantage of testing for impacts of the loss of plant species in real ecosystems (Diaz et al. 2003). From the 49 plots, we retained the 18 most homogenous ones according to above-ground biomass and species cover as determined in 2008. Removal treatments were randomly applied to the plots: (C) control without removal; (S) removal of subordinate species; and (D) random removal of dominant biomass, with the removal of biomass in S and D being equal. Treatment D was designed to enable us to distinguish effects on ecosystem processes of biomass removal alone and suppression of subordinate species.

In order to estimate the total biomass of the removed subordinate and dominant plants, we established an allometric model for each of the 7 dominant and 7 subordinate species. As removal of individuals of *F. nigrescens* was not feasible because of its growth form (tussocks), this species was not considered for removal. The above-ground and below-ground biomass of 30 individuals of each species was sampled in 2008 in spare plots of the experiment. In the laboratory, three traits were measured on the fresh above-ground biomass of all individuals: number of leaves, length of the largest leaf and length of the stem. Above-ground and below-ground biomass was then dried in an oven at 60°C for 72 hours and weighed. All possible correlations between the three plant traits and total dry weight of each species were tested using the coefficient of correlation R^2. In this way, we were able to select for each species the most suitable above-ground plant trait for establishing an allometric model for total biomass estimation. Each model followed the same equation:

$$b = \gamma x^{\beta}$$

where b is the total biomass of the individual plant and x the value of the plant trait (Appendix 5.1). No correlation was found for *A. capillaris*, and hence mean biomass of the 30 individuals was used to estimate total biomass of this species in the field. Plant removal was carried out between 1st and 15th August in 2009, and again during the first week of August in 2010 and 2011. First, each plot was divided into 144 cells of 10 by 10 cm and all subordinate plants were pulled out by hand in each cell, in a way that as much root material as possible was removed without disturbing the soil. Then, each S plot was randomly paired with a D plot, meaning that once the total biomass of the removed subordinate species was calculated using the allometric models, the same amount of total dominant biomass was removed in the paired D plot. An individual was selected randomly amond dominant species, also taken randomly among the 144 cells, pulled out within the plot and its traits were measured in order to determine the total biomass using its corresponding allometric model. We repeated the removal of dominant individuals until the same biomass as removed in the paired S plots was reached.

In treatment S (and consequently in treatment D), the total estimated subordinate biomass (above- and below-ground) was (mean ± 1 SE) 61 ± 8 $g.m^{-2}$ in 2009, 33 ± 8 in 2010 and 6 ± 1 in 2011. In total, 247 ± 45 individuals of subordinates per square meter were removed in 2009, 177 ± 33 in 2010 and 25 ± 6 in 2011. With respect to dominants, 31 ± 12 individuals of dominants per m^{-2} were removed in 2009, 19 ± 8 in 2010, and 8 ± 3 in 2011. The subordinate individuals found in the S plots during the second and third year of the experiment were mainly due to regrowth.

Plant community composition and above-ground productivity

Species cover was determined in July 2010 in the central subplot of 50 cm by 50 cm using a grid divided in 100 cells of 5 cm by 5 cm in which the presence of all plant species was recorded. Relative species cover was calculated from grid data but subordinate species, which were absent in S plots, were left out in order to compare the shift in species composition between treatments. Above-ground community biomass production was measured by harvesting standing crop above 3 cm in the central subplot for each treatment ($n = 18$), at peak biomass at the end of August 2009, 2010 and 2011. The biomass was dried at 60°C for 72 hours and weighed. The yearly removed plant biomass in D and S plots was added to the standing crop to allow clear comparison with the C plots.

Microbial community analysis by TRFLP

Soil sampling. Soil sampling was done in September 2010 and five soil cores of 10 cm depth were taken randomly in each plot using an auger (2 cm diameter), pooled in a sterile plastic bag (Whirlpack, USA), homogenized manually and stored at -80°C in the laboratory until analysis.

DNA extraction. Total DNA was extracted from soil samples using a modified version of the Maxwell 16 SEV Soil DNA kit (Promega, Madison, USA). About 4 g of each soil sample were layed in a 15 ml sterile Falcon-like tube containing already 1 ml of sterile 0.1 mm-diameter silica – zirconia beads (Biospec, USA) with 4 ml of a Guanidium- Sarcosine extraction buffer (Guanidine thiocyanate 5 M, EDTA 75 mM, Na-Lauroylsarcosine (sarkosyl) 1%, PVP K30 1%, Na Phosphate buffer pH = 8.0 150 mM, DTT 1.2 mM). The tubes were fixed onto a shaker holder (Vortex Genie II) and shaken for 10 min at room temperature, followed by incubation at 65°C in a water bath. The shaking step was resumed once again and the tubes were centrifuged (5000 x g, 5 min, room temperature). Then 0.5 ml of the dark brownish supernatant was sampled and added to a new 15 ml sterile Falcon-like tube, along with 1 ml of phenol:chloroforme:isoamylalcool (25:24:1) stabilized and saturated with TE buffer (pH > 7). The content was mixed manually for 1 min and allowed to stand on ice for 10 min. The tubes were centrifuged (5000 x g, 5 min, 4°C) and 0.2 ml of the supernatant were added to the first well of the Maxwell 16 SEV Soil DNA kit. The extraction robot was run according to the manufacturer instructions and the resulting DNA samples were resuspended in 300 µl of 10 mM Tris-HCl (pH > 7). DNA samples were quantified with a spectrophotometer (Nanodrop, ThermoScientific, USA). Then dilutions in sterile H_2O were carried out to provide DNA concentrations of 2 ng/µl for bacteria and 10 ng/µl for AMF.

PCR amplification for bacteria. Typical PCR amplification reactions were set using 2.5 µl of soil extracted template DNA [2 ng/µl], 25.5 µl of H_2O miliQ, 5µl of $MgCl_2$ (25 mM), 10 µl of Buffer (5X), 4µl of dNTPs (2.5 mM), 1.25 µl of each primer (8f FAM-labeled and 518r) and 0.5 µl of Taq polymerase (5 U/µl, Promega). Thermal cycling was done in a T3 thermocycler (Biometra) with the following conditions: 4 min. initial denaturation at 94°C; 20 cycles of 30 s denaturation at 94°C, 45 s annealing at 50°C, 1.5 min elongation at 72°C and a 10 min final elongation at 72°C.

PCR amplification for AM fungi. A nested-PCR amplification procedure was set, according to Krüger et al. (2009). The first amplification used primers Af and Ar. PCR reactions were set using 2.5 µl of soil extracted template DNA [10 ng/µl], 12.75 µl of H_20 miliQ, 1 µl of $MgCl_2$ (25 mM), 5 µl of buffer (5X), 2 µl of dNTPs (2.5 mM), 0.75 µl of each primer and 0.25 µl of Taq polymerase (5 U/µl, Promega). Thermal cycling was done with the following conditions: 3 min. initial denaturation at 94°C; 40 cycles of 45 s denaturation at 94°C, 1 min annealing at 57°C, 2 min elongation at 72°C and a 10 min final elongation at 72°C. The second reaction mixture included 5 µl of the first PCR products, 25.5 µl of H_20 miliQ, 2.5µl of $MgCl_2$ (25 mM), 10 µl of buffer (5X), 4µl of dNTPs (2.5 mM), 1.25 µl of each primer (Cf FAM-labeled and Br) and 0.5 µl of Taq (5 U/µl, Promega). Similar cycling conditions were used for this nested PCR amplification, except that the annealing temperature was 60°C and only 26 cycles were carried out.

Restriction enzyme digestion. Labeled PCR products were purified with the MSB Spin PCRapace kit (Invitek, Germany) according to the manufacturer instruction. Bacterial and AM Fungi PCR products were digested with HaeIII and SauIII respectively. In both cases, 200 ng of purified PCR products were digested 0.5 µl of enzyme and 1 µl of buffer (10 µl final volume) for 3 hours at 37°C.

Electrophoresis analysis. The digested products were analyzed on a ABI 3130xl capillary sequencer according to Rossi et al. 2009.

Litter decomposition

Litter decomposition rate was measured by using mini-containers as described by Eisenbeis (1999). In November 2009, two PVC bars were inserted vertically in the soil down to 20 cm in all plots. Each bar measured 25 cm and held six mini-containers (diameter 1.6 cm) with a mesh size of 200 µm and filled with 500 mg of plant biomass of the field site (collected in september 2009) excluding subordinate species (bulk chemical content: 2.20% N, 55.58% C and 0.23% P). Before filling of mini-containers, litter was dried at 60°C for 72 hours and mixed to obtain a standardized material. Half of the PVC bars were collected after 6 month (April 2010; i.e. end of winter season) and the other half after 12 months (October 2010). Rates of decomposition were measured from remaining litter that was dried and weighed. For the three removal treatments, we determined the rate of decomposition during the summer season (May to October 2010) by measuring the difference between litter loss during the 12 months and the 6 first months. As depth did not significantly influence decomposition rates, an average value (6 mini-containers ranging from the surface to 20 cm depth) was used for litter decomposition. We repeated the same protocol for the measurment of litter decomposition in the winter season 2010-2011 and in the summer season 2011.

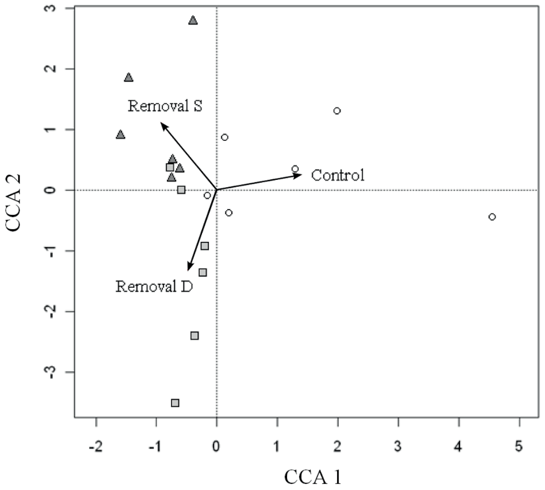
Soil respiration

Soil respiration was measured using a LI-8100A automated soil CO_2 flux system equipped with a LI-8100-102 chamber (LI-COR Biosciences, Lincoln, Nebraska, USA) to obtain instantaneous soil CO_2 efflux measurements (see Liang et al., 2004). In each plot, we measured soil respiration in two PVC collars (10 cm diameter, 5 cm depth) inserted 2 cm into the soil to minimize disturbance to the surface soil and ensure repeated sampling of the same soil area. Collars were kept free of plants and the soil covered with a mesh to maintain humidity. Respiration measurements were made four times in each plot during the summer 2010 (21st July, 9th and 22nd August, 10th September) and 2011 (22nd July, 12th and 24th August, 12th September) and determined from a time period of 2 min with CO_2 efflux recorded each 2 s.

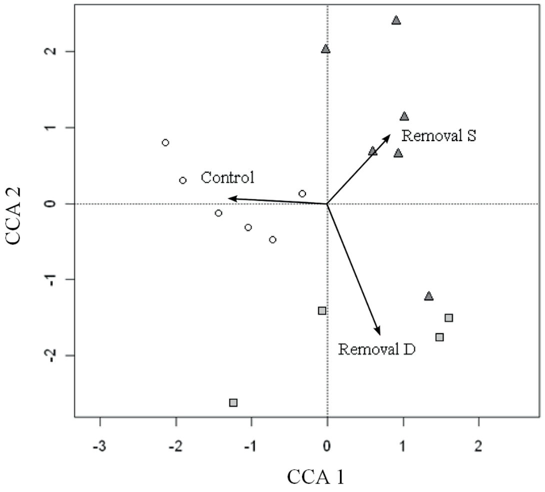
Soil nitrogen

Soil was collected in each plot using an auger (2 cm diameter, 10 cm depth) at the end of the 3-year removal experiment (September 2011) to determine available nitrogen in all plots (C, D and S). Five subsamples per plot were taken, pooled and immediately processed. Plant available mineral nitrogen (DIN) was measured by adding 50 ml 0.5 M K_2SO_4 to 15 g fresh mass of root-free soil, which were then shaken at 150 rpm at room temperature for 3h prior to being filtered through Whatman paper (0.45 µm). Nitrate (NO_3^--N) and ammonium (NH_4^+-N) in the extracts were measured by autoanalyzer procedures (AutoAnalyzer 3, SEAL Analytical, USA).

(a) Vegetation community



(b) AM fungi community profile



(c) Bacterial community profile

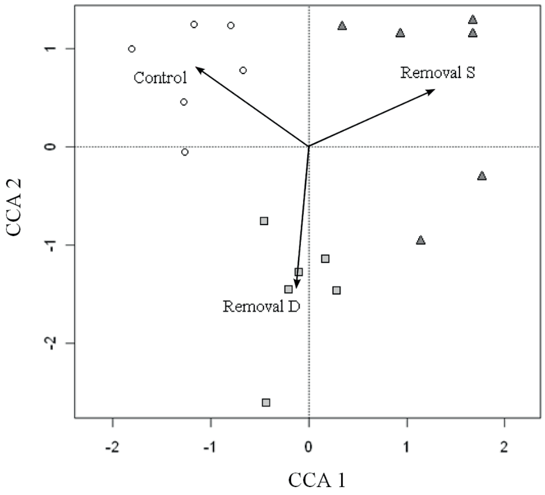


Figure 5.1 - Canonical correspondence analysis (CCA) biplot of the vegetation community (a), AM fungal (b), and bacterial (c) community profile constrained by treatments. Symbols represent control plots (circle), plots with removal of all subordinates (square) and plots with partial removal of dominant plant biomass (triangle) of the removal field experiment. In (a) Axes 1 and 2 hold, respectively, 8.2% ($F_{1,15} = 1.42$, $P = 0.32$) and 4.4% ($F_{1,15} = 0.94$, $P = 0.55$) of the explained variance. In (b) Axes 1 and 2 hold, respectively, 15.9% ($F_{1,13} = 2.57$, $P = 0.03$) and 0.04% ($F_{1,13} = 0.61$, $P = 0.80$) of the explained variance. In (c) Axes 1 and 2 hold, respectively, 12.2 % ($F_{1,15} = 2.26$, $P = 0.003$) and 6.7% ($F_{1,15} = 1.25$, $P = 0.17$) of the explained variance.

Statistical analysis

All analyses were carried out with R version 2.14.1 (R Development Core Team, 2011). A set of canonical correspondence analyses (CCA) constraining the plant communities, bacterial and AM fungal community profiles (T-RFLP) by removal treatments was performed. Permutation tests (Monte Carlo) were used to assess the significance of these multivariate regression models. Community above-ground biomass, soil ammonium, nitrate, and DIN concentrations were analysed, after log-transforming, using ANOVAs followed by Tukey post hoc. Litter decomposition rate was analysed for each period using ANOVAs specifying depth nested into plot as random error. Soil respiration was analysed using linear mixed-effects models specifying time nested into collars nested into plot as random factors.

Results

Plant community composition responses

Plant community composition did not show significant shifts following removal treatments after 2 years ($F_{2,15} = 1.180$, $P = 0.188$, 999 Monte Carlo permutations) according the results of CCA constraining plant cover by removal treatments (Fig. 5.1a). The analysis of the relative importance of the three species-groups (dominant, subordinate and transient) showed a trend with dominant species abundance increasing and transient species decreasing in S plots (not shown).

Soil microbial community responses

After 2 years, Arbuscular myccorhizal fungal communities, measured by T-RFLP profiles of soil samples, differed marginally between treatments ($F_{2,13} = 1.593$, $P = 0.062$, 999 Monte Carlo permutations; Fig. 5.1b) with a dissociation on axis 1 ($F_{1,13} = 2.57$, $P = 0.03$) between plant removal treatments (D and S) and the control (C). The removal treatments significantly influenced bacterial communities ($F_{2,15} = 1.757$, $P < 0.01$, 999 Monte Carlo permutations) with three distinct communities being discriminated along axis 1 ($F_{1,15} = 2.26$, $P = 0.003$) distinguishing the control treatment (C), the biomass removal of dominants treatment (D) and the subordinates removal treatment (S) (Fig. 5.1c).

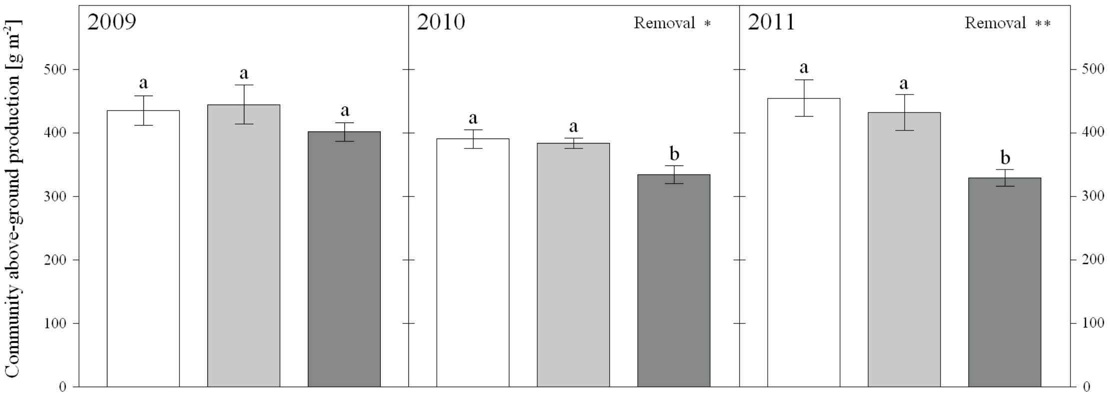


Figure 5.2 - Mean (± 1 SE) of above-ground biomass production in August 2009, 2010 and 2011 following removal treatment with three levels: (C) control without removal (white bars), (D) random removal of dominant biomass (grey bars), (S) removal of subordinate species (dark grey bars). Bars sharing a letter are not significantly different ($P > 0.05$).

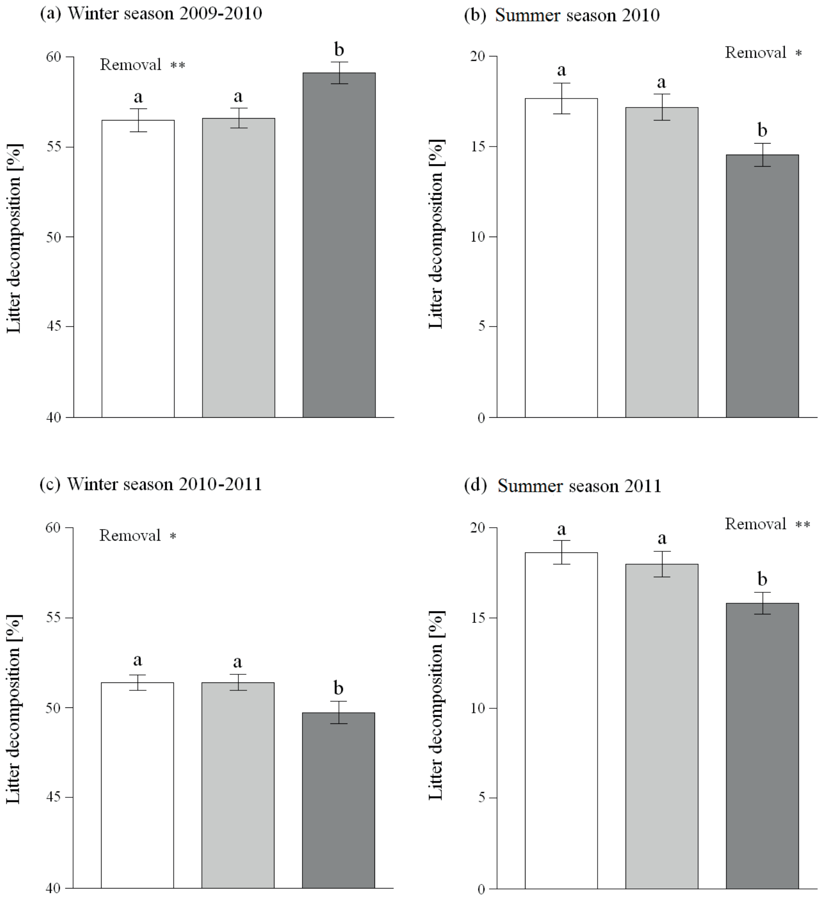


Figure 5.3 - Litter decomposition in soil following treatments: (C) control without removal (white bars), (D) random removal of dominant biomass (grey bars), (S) removal of subordinate species (dark grey bars). during (a) the winter season 2009-2010, (b) the summer season 2010, (c) the winter season 2010-2011 and (d) the summer season 2011. Bars with different letters are significantly different ($P < 0.05$).

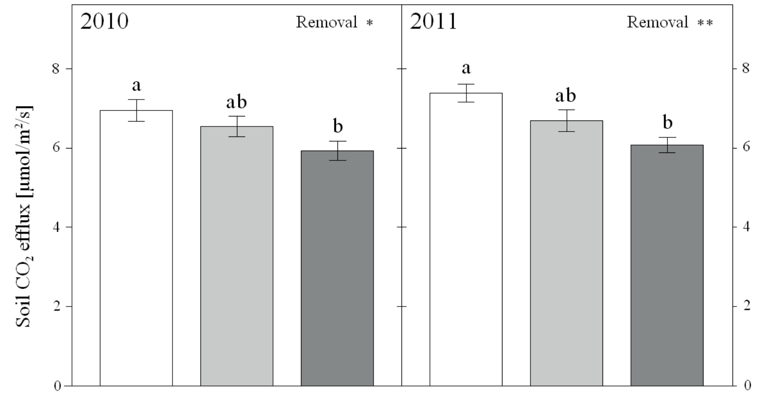


Figure 5.4 - Mean (± 1 SE) of soil respiration following treatments: (C) control without removal (white bars), (D) random removal of dominant biomass (grey bars), (S) removal of subordinate species (dark grey bars). during the summer 2010 and 2011. Values of CO_2 efflux correspond to the means of four measurement dates in experimental plots. Bars with different letters are significantly different ($P < 0.05$).

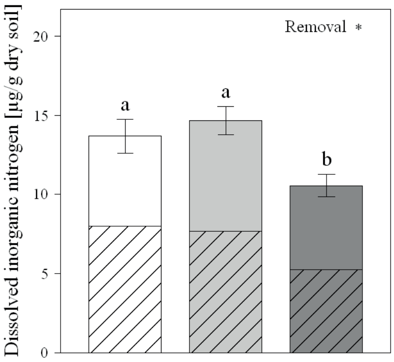


Figure 5.5 - Means (± 1 SE) of dissolved inorganic nitrogen in soil at the end of the 3-years experiment including NO_3^--N (line shaded) and NH_4^+-N (open bars) following treatments: : (C) control without removal (white bars), (D) random removal of dominant biomass (grey bars), (S) removal of subordinate species (dark grey bars). Bars with different letters are significantly different ($P < 0.05$).

Ecosystem processes responses

During the first year of the experiment (2009), we did not observe changes in aboveground biomass between the treatments (C, D and S) but significant effects occurred in 2010 ($F_{2,15} = 5.684$, $P < 0.05$) and increased in 2011 ($F_{2,14} = 10.725$, $P < 0.01$). The differences were due to the subordinate removal treatment which led to a decrease of community aboveground biomass (Fig. 5.2) whereas the plant community produced about the same amount of biomass in C plots and in D plots.

The rate of litter decomposition was higher in winter (November to April) than in summer (May to October) across all treatments. During the winter season 2009-2010, which follow the first campaign of removal, litter decomposition was significantly higher in S plots compared to C and D plots (Fig. 5.3a). From the summer season 2010, litter decomposition was always significantly lower in S plots (Fig. 5.3b,c,d) compared to the other treatments (C and D).

Soil CO2 efflux was significantly different between treatments (C, D and S) after 2 years ($F_{2,15} = 3.830$, $P < 0.05$) and 3 years of experiment ($F_{2,14} = 6.638$, $P < 0.01$). We observed the same pattern in both years (2010 and 2011) with a significant decrease of soil respiration in treatment S where subordinates were removed compared to the control (Fig. 5.4). The soil CO2 efflux in treatment D did not significantly differ from that of C or S treatments.

The dominant extractable inorganic nitrogen form was NO_3^--N with soil concentrations ranging from 8.0 µg g^{-1} dry soil in control plots to 5.3 µg g^{-1} in S plots. Soil concentrations did not differ between treatments (C, D and S) for NO_3^--N ($F_{2,14} = 2.904$, $P = 0.08$) and NH_4^+-N ($F_{2,14} = 2.233$, $P = 0.14$) but there was a clear trend of lower values in the S treatment relative to C and D (Fig. 5.5). Moreover, the removal of subordinates (S) led to a significant decrease in soil DIN (i.e. NH_4^+-N + NO_3^--N) relative to the control and to D treatment ($F_{2,14} = 6.351$, $P = 0.01$).

Discussion

In this 4-years experiment, we aimed to evaluate the role of subordinate species on plant and soil community composition and their consequent impacts on ecosystem processes. While plant community did not change after 2 years of subordinate species removal, soil community composition (bacteria and AMF) changed. In addition, the rate of litter decomposition, soil respiration and soil dissolved inorganic nitrogen significantly decreased in absence of subordinates and led to a reduction of community above-ground biomass through plant-soil feedbacks. We therefore discussed separately each effect of the subordinates removal treatment to understand how these low abundant species influence ecosystem functioning of grasslands.

Shifts in plant and soil community composition

After two years, plant community composition did not change following the removal of subordinate species (Fig. 5.1a). As reported in previous studies, species removal generally has an effect on remaining plant community (Aarssen & Epp 1990) but with a time-lag in the response of plant groups or plant species to changes in plant composition (Wardle et al. 1999; Symstad & Tilman 2001; Buonopane et al. 2005). Indeed, the timescale of observations in this study is probably too short to perceive shifts in plant community composition. However, it seems that the absence of subordinates led to an increase of dominant species and a decrease of transient species which also participate to biodiversity (Grime 1998). Therefore, we suggest that subordinate species, which act as intermediate competitors between dominant and transients, may reduce dominance and promote species diversity (Grime 1987).

Plant biomass removal (treatment S and D) rather than subordinate species removal (treatment S only) seems to have an effect on arbuscular mycorrhizal fungal (AMF) community composition in our pastured grassland. AMF are obligate symbionts because they completely depend on host plants for carbon (Johnson et al. 2002) and indeed, plant removal is expected to reduce AMF biomass in soil and then, to shift AM fungal community composition. While the plant removal corresponded to a low quantity of biomass, the observed effects on AM fungi communities could be also attributed to the indirect physical disturbance caused by the removal itself (Diaz et al. 2003) which may reduce mycorrhizal colonization (Smith & Read 2008). Considering that AM fungi are generalists and may colonize different plant species, mycorrhizal communities are very resilient to changes in vegetation composition (Wardle et al. 1999; Johnson et al. 2004; Urcelay et al. 2009) and, on a short term, the effect of subordinate species removal might have rather small influence. We suggest that the direct (i.e. biomass) and indirect (i.e. disturbance) effects of the removal (D and S) rapidly affect AM fungi communities whereas the species effect of subordinates (in S only) might be visible only at long term.

The removal treatments (D and S) modified the bacterial community (Fig. 5.1c) relative to the control (C) but considering the gradient along the significant axis 1, the removal of subordinates (or species-group identity effect) had higher impact than the partial removal of dominants (or biomass effect). Two mechanisms have been put forward to explain the effects of plant species on soil food webs: the quantity and quality of litter (e.g. abundance of lignin) that plants return to the soil (Wardle et al. 2006) and root exudates of plant assimilated carbon which stimulate the growth and activity of heterotrophic microbes (Pollierer et al. 2007). Plant traits reflect a species strategy for a rapid acquisition or conservation of resources (Grime et al. 1997; Diaz et al. 2004) and are important drivers of litter quality. Dominant species are generally taller with large leaves (high SLA) allowing better light capture whereas subordinate species are smaller in stature with little leaves (low SLA) (Grime 1998). Therefore, it can be suggested that dominant species are adapted to rapid acquisition of resources resulting in high-quality litter whereas subordinate species have higher capacity for resource conservation leading to a lower litter quality. Thus, the removal of subordinate species in our experiment and consequently modified litter quality inputs might explain the changes in the bacterial community composition. In addition, root exudation is an important mechanism in grazed grasslands (Guitian & Bardgett 2000; Hamilton et al. 2008) and may have also participated to the effects of subordinates on soil communities. We acknowledge that complementary experiments testing the direct effects of subordinate plant litter and their root exudates on soil communities are needed in order to validate the proposed mechanisms. Nevertheless, our findings demonstrate that species which represent only a small proportion of the total plant biomass may affect soil microbial communities as well as do dominants species and therefore highly participate to ecosystem functioning.

The role of subordinates on soil processes

The removal of subordinate plant species significantly affected a range of soil properties including litter decomposition, soil respiration and nitrogen dynamics. The increased litter decomposition rate in the S treatment during the first 6 months (winter season) is likely due to the removal of about 250 subordinate individuals (compared to 30 dominant individuals) through the first campaign of removal. This will have left a significant amount of dead roots in the soil, potentially increasing soil microbial activity and rates of litter decomposition in the short term. After 6 months, the rate of litter decomposition decreased significantly as a result of the removal of subordinates compared to control plots (C and D). Bacteria and fungi are primary decomposers because of their unique capability to directly break down complex organic compounds (Chapin et al. 2002) and therefore, changes in bacterial and fungal communities are expected to modify soil processes. Since bacterial communities were different in plots where we removed subordinate species relative to C and D plots, we may hypothesize that it influenced litter decomposition. Decomposition of dead organic matter through the action of chemical reduction by soil organisms produces CO2 and inorganic nutrients (Chapin et al. 2002). In accordance with the decreased litter decomposition, the removal of subordinates reduced soil respiration during the second and third year of the experiment. Moreover, the quantity of plant available nitrogen (DIN), which results from nitrogen mineralization (i.e. proteins and amino-acids), decreased significantly in absence of subordinate plant species and in return, these effects have important implications for plant nutrition and productivity. These findings provide new evidence that non-random species loss may have more effects than species diversity and confirm the importance of key species on soil functioning.

Plant-soil feedbacks and ecosystem functioning

Above-ground plant community biomass decreased significantly due to the absence of subordinates. As plant community composition did not change due to removal of subordinates, we suggest that the effects observed on above-ground productivity were mainly due to negative plant-soil feedbacks. The absence of subordinate plant species modified soil microbial communities which led to a decrease of litter decomposition and consequent reduction in soil DIN. Nitrogen is a limiting factor for plants in low productive grasslands and we suggest that the reduction of inorganic nitrogen availability in soil, potentially coupled with changes in mycorrhizal communities, led to a reduction in plant community biomass where subordinates were removed.

Plants can exert positive or negative feedbacks depending on the soil community that they promote and these feedbacks may directly affect the dynamics of coexisting plant species (Bardgett & Wardle 2010). Three mechanisms have been proposed to explain such feedbacks, including the promotion of pathogens (Bever 2003), mutualists (Smith & Read 2008; Grime et al. 1987) or decomposers (Wardle 2002). Subordinate species might produce many plant-soil feedbacks, positive and/or negative, following which group of soil organisms they facilitate. Considering our results on soil processes, we believe that the subordinate species in our system promote decomposers which enhance nutrient mineralization and thus above-ground biomass production of the plant community. Moreover, depending on soil fertility AM Fungi may promote subordinate species by reducing the difference in competitiveness between dominant and subordinate species (Grime et al. 1987; Van der Heijden et al. 1998; Hart et al. 2003; Chapter 4). The absence of subordinates in our experiment seems to favour dominant species and reduce transient species and so subordinate species might have also the capacity to limit growth of dominant species through mycorrhizal fungi (mutualists).

Our results support theoretical evidence that plant-soil feedbacks maintain plant species coexistence and diversity if they reduce competition between dominant and subordinate species (Bonanomi et al. 2005; Van der Putten 2005; Kardol et al. 2006; Petermann et al. 2008) or involved positive effects on decomposition and mineralization process. Indeed, when plant-soil feedbacks favour subordinates and disadvantage dominants, the dominance hierarchy may flatten and increase species diversity. Moreover, positive plant-soil feedbacks on nitrogen mineralization led by subordinate species may benefit a range of species and increase coexistence. We recognize that many soil organisms interact in soils and can potentially appear or disappeared in plots where subordinates were removed leading to direct or indirect effects on ecosystem processes. Therefore, complementary experiments are needed to explore more in details mechanisms by which subordinate species impact on soil organisms and soil trophic cascades and consequences for plant-soil feedbacks and ecosystem functioning.

Conclusions

In natural grasslands, land use and climate change are expected to be responsible of an important loss of natural biodiversity and more than only species diversity (random loss), the diversity of plant groups (functional type, functional groups, competitive hierarchical groups) which represent non-random plant species will impact ecosystem functioning. The above-ground and below-ground interactions and feedbacks are recognized to significantly regulate terrestrial ecosystems explaining why non-random species losses may have a great impact on ecosystem functioning especially in the context of climate change. For instance, many issues remain unresolved about the real importance of species-groups in driving ecosystem processes in grasslands especially when considering ecosystem multifunctionality and interacting above- and below-ground. Nevertheless, our results on the effects of subordinate species on plant-soil feedbacks provide novel findings from which specific hypotheses can be generated and subsequently tested.

Acknowledgments

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Chapter 6

Subordinate plant species enhance community insurance to drought in grassland ecosystems

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In review

Abstract

Water limitation constrains net primary production, and summer drought events play a major role in the impact of climate change on grasslands communities. According to the insurance hypothesis, more diverse plant communities have higher probability to be more resistant to drought. While many experiments have been carried out to determine the effects of plant diversity on plant community insurance, the results are still contradictory.

Here, we conducted drought experiment where we tested if independently of diversity, the presence of drought-resistant subordinates species increases plant community insurance. In species-rich grassland of the Swiss Jura Mountains, we combined a removal experiment of subordinate plant species with a summer drought during two months.

Our results showed that subordinate species seem to be more resistant to drought and increased community resistance by enhancing their above-ground biomass. These outcomes are also linked with a decreasing of competitiveness of dominant species whose biomass decreased during drought. Recovery and resilience were not affected by the summer drought showing that semi-natural grassland communities may respond very rapidly to drought in terms of biomass production.

Interestingly, the absence of subordinate species reduced community above-ground biomass, independently of the drought, highlighting the role of subordinates for ecosystem functioning. Our study thus improved the understanding community insurance to drought and confirms the importance of the entire plant community including not only dominant but also subordinate species.

Key-words:

Drought, ecosystem stability, extreme climatic event, insurance hypothesis, carbon isotopes ratios, plant hierarchy,

Introduction

Climate is one of the major factors which affect plant species distribution around the world and climate changes are expected to shift distribution or modify abundance of many plant species (Parmesan 2006; Lenoir et al. 2008). Recent climate change models predict an increase of water stress in certain regions especially in Central Europe where decreasing summer precipitation and severe drought are expected (Christensen et al. 2007). In Switzerland, the mean decrease of summer precipitation might reach 20% in 2070 compared to 1990 with a maximum decrease of 40% (Frei et al. 2006). Water limitation constrains net primary production, even in temperate or boreal ecosystems, and changes in precipitation, not only warming, play an important role in the potential impact of global change on terrestrial communities (Ciais et al. 2005; Engler et al. 2011). High-elevation ecosystems are disproportionately exposed to climate change (Beniston et al. 1996; Nogués-Bravo et al. 2007) and mountain grassland communities are expected to be greatly affected by extreme drought events (i.e. ECE, extreme climatic event, Smith 2011a) with significant consequences for ecosystem functioning (Gilgen et al. 2010). However, the response of plant community to stress depends on species diversity and composition which both drive community stability (Loreau et al. 2001) and understanding which component of plant community promotes ecosystem maintaining during summer drought event is essential (Smith 2011b).

According to the diversity-insurance hypothesis (Yachi & Loreau 1999), an increase of species diversity in an ecosystem corresponds to an increase of the range responses to environmental change or perturbation. Therefore, more diverse communities have higher chance to contain species which are more adapted to perturbations and can compensate for the decline of less resistant species. Insurance of plant community against a drought event includes three different phases for which species diversity can have an effect: the resistance during the perturbation, the recovery after the perturbation, and the resilience (Pimm 1984; Tilman & Downing 1994; Van Ruijven & Berendse 2010). Resistance is the ability of a plant community to withstand perturbation by maintaining biomass or reproductive outputs during the event (e.g. summer drought). Recovery represents the ability of a plant community to compensate for the loss due to the perturbation. Resilience represents the ability of a system to return to its original state following perturbation and combines resistance and recovery to provide a measure of community stability.

Many drought experiments have been carried out to determine the effects of plant diversity on plant community insurance but the results have been contradictory. While some studies showed decreasing resistance against drought with increasing species diversity in constructed communities (Pfisterer & Schmid 2002, Van Peer et al. 2004; De Boeck et al. 2008), other experiments observed increased resistance in natural grasslands (Tilman & Downing 1994; Kahmen et al. 2005). Based on a recent mesocosm experiment, Van Ruijven & Berendse (2010) found that recovery after the drought was enhanced by an increase of diversity, mainly due to one single species, whereas no effects were found on resistance and resilience. The diversity-insurance hypothesis remains difficult to validate because most of the studies were conducted in constructed grassland ecosystems. Moreover, Wang et al. (2007) suggested that susceptibility to drought is mainly biomass-dependent with high biomass systems being less resistant to drought compared low biomass systems. In high biomass plots, tall plants limit light availability of drought-resistant species. By contrast, in low biomass plots, light limitation is lower and drought-resistant species can compensate for the loss of drought-sensitive species. Kardol et al. (2010a) showed that the proportion of subordinate species increases in dry compared to wet conditions and suggested that dominant species may respond more strongly to the direct impact of drought whereas subordinate species may respond to altered competition interactions with the dominant species. Competitive interactions might act as a key factor in community response to drought by favouring drought-resistant subordinate species which compensate for the loss of drought-sensitive dominant species when competition decreases between both species-groups. Therefore, an increase of species diversity, associated with the presence of more resistant species (i.e. insurance hypothesis) might be particularly important for the insurance to drought in low productive grasslands where competitive interactions are likely to decrease between resistant (i.e. subordinates) and sensitive species (i.e. dominants). Actually, more than species diversity (i.e. species richness), it's probably the presence of key species such as drought-resistant plants which seems to be important for community stability in grasslands.

One of the main factors determining the plant drought-resistance under water limitation is water-use efficiency (WUE). An improved WUE is recognized as a plant strategy to enhance biomass production during drought and corresponds to the ability of species to maximize the utilization of water. The analysis of the natural abundance of the stable isotope ^{13}C has been proposed as an indirect measure of WUE due to the dependency of the ^{13}C discrimination from the stomatal and mesophyllic conductance (Farquhar et al. 1989). Therefore, the measurement of δ^{13}C values in plant leaves provides insight about the drought resistance of plants and might help to identify the most resistant species (Condon et al. 2004).

Semi-natural grasslands of the Swiss Jura Mountains are in general low productive but very diverse with up to 40 vascular species per square meter (Buttler et al. 2009). These communities are expected to be rather resistant to perturbations such as severe summer drought which are forecasted for the future (Christensen et al. 2007). Observations of plant species abundances in these grasslands reveal distinct frequency distributions, with some species found frequently and in high abundances (dominants) and some found frequently as well, but in low abundances (subordinates; Grime et al. 1987; Olff & Bakker 1998). Dominant species account generally for a high proportion of the total community biomass and, according to the "mass ratio" theory (Grime 1998), ecosystem properties are determined by dominant species independent of changes in species richness, such as variations in the number of subordinate species. Although dominant species are clearly very important for ecosystem processes, there is growing evidence that subordinates which represent a low amount of plant biomass in grassland ecosystems are likewise important for ecosystem functioning and maintenance especially after drought events (Kardol et al. 2010a, Wang et al. 2007). Both coexisting species-groups participate to the diversity of these well-established grassland communities that are submitted to regular grazing and trampling disturbance (Buttler et al. 2009). The persistence of subordinate species seems to be linked with the activity of large grazers which create gaps and areas of reduced root competition from dominants (Chapter 3) or because subordinates have a better regrowth after grazing compared to dominants (Tahmasebi Kohyani et al. 2008).

In this study, we examine how subordinate species influence community insurance to drought in semi-natural grasslands of the Swiss Jura Mountains which are expected to be highly affected by extreme drought events. We firstly manipulated plant communities using a removal experiment of subordinate species and then controlled water availability using rainout shelters to simulate a summer drought event. Our specific objectives were to show the role of subordinate species in each phase of plant community insurance to drought (i.e. resistance, recovery and resilience) and in ecosystem functioning using carbon isotope natural abundance ($\delta^{13}C$) and community above-ground biomass. Here, we hypothesize that subordinates might be more drought-resistant than dominants and therefore, decreasing contributions of subordinates may influence ecosystem functioning and further reduce insurance of plant communities in the context of climate change.

Materials and methods

Site description

The field experiment was carried out in an extensively-grazed and species-rich (mean of 35 species per square meter) pasture situated in a calcareous slope (30°) of the Swiss Jura Mountains (Agroscope Changins-Wädenswil Research Station, La Frétaz, western Switzerland, 6°34'30" E, 46°50'50"N). The soil was a Cambisol eutric (after the World Reference Base for Soil Resources - IUSS Working Group WRB, 2006) and not deeper than 40 cm. The site is situated at an altitude of 1200 m a.s.l. and has a suboceanic climate with 1393 mm of annual precipitation (mean data from MeteoSwiss station Bullet/La Frétaz, 1999-2008). The pasture is grazed by cattle following a rotational system during the growing season lasting from May to September.

Defining dominant, subordinate and transient species

Within a 25 by 25 m cattle-excluded area, 49 plots of 1.2 by 1.2 m were established at a regular distance of 1.6 m from each other. In July 2008, absolute plant cover was determined within all plots using a modified Braun-Blanquet index (1: cover less than 5%, 2a: 5-10%, 2b: 10-25%, 3: 25-50%, 4: 50-75%, 5: 75-100%). A total of 37 species were identified. A species was classified as dominant if its frequency was greater than 70% and its cumulative relative cover greater than 25%. A species was classified as subordinate if its frequency was greater than 70% and its cumulative relative cover between 2 and 12% (adapted from Grime 1998). The other species, which do not persist following years or show great fluctuations, were classified as transient species. This method resulted in the determination of eight dominant: 1) *Trifolium repens* L., (2) *Cynosurus cristatus* L., (3) *Festuca nigrescens* Lam., (4) *Agrostis capillaris* L., (5) *Carum carvi* L., (6) *Ranunculus acris ssp. friesianus*, (7) *Taraxacum officinale* agg., (8) *Alchemilla monticola* Opiz and five subordinate species: (9) *Trifolium pratense* L., (10) *Achillea millefolium* L., (11) *Leontodon* sp. (*Leontodon autumnalis* L. and *Leontodon hispidus* L.sl.), (12) *Cerastium fontanum* ssp. *vulgare*., (13) *Veronica chamaedrys* L.

Removal experiment

From the 49 plots, we retained the 30 most homogenous ones according to above-ground biomass and species cover as determined in 2008 (Braun-Blanquet index). We applied the following three treatments each with 10 replicates: control (C) without removal, (S) removal of subordinate species, (D) random removal of dominant biomass. The biomass removal in S and D was equal. Treatment D allowed to distinguish the effects of biomass removal alone and suppression of subordinate species on ecosystem processes. As removal of individuals of *Cynosurus cristatus* and *Festuca nigrescens* was not feasible, this species was not considered for the removal treatment D.

In order to estimate the total biomass of the removed subordinate and dominant plants, we established allometric models for each of the 5 dominant and 5 subordinate species used in the removal treatment. The above- and below-ground part of 30 individuals of each species was randomly sampled in 2008 within the exclosure of the experiment. In the laboratory, above- and below-ground biomass were washed, measured and dried in an oven at 60°C for 72 hours and weighed. Three traits were measured on the fresh above-ground biomass of all individuals: number of leaves, length of the largest leaf and length of the stem. The most suitable above-ground plant trait for estimating total biomass from allometric models was found by the best correlations (R^2) between the three plant traits and total dry weight of each species. Each model followed the same equation:

$$b = \gamma x^{\beta}$$

where b is the total biomass of the individual plant and x the value of plant trait (see Appendix 6.1). No correlation was found for Agrostis capillaris and therefore mean biomass of the 30 individuals was used to estimate total biomass of this species in the field.

Plant removal was carried out at the end of June in 2009, 2010 and 2011. Firstly, each plot was divided in 144 cells of 10 by 10 cm and all subordinate plants were pulled out by hand in each cell in a way that a maximum of roots were removed without disturbing the soil structure. Then, each S plot was randomly paired with a D plot, meaning that the same amount of biomass of subordinate species which was removed from the S plot was also removed from the paired D plot as dominants, based on allometric models. In S plots (and consequently in D plots), the total estimated subordinate biomass (above- and below-ground) was (mean ± 1 SE) of 17 ± 3 $g.m^{-2}$ in 2009, 3 ± 1 in 2010 and 2 ± 1 in 2011 which correspond to 1.4% of the community above-ground biomass in 2009, 0.5% in 2010 and 0.3% in 2011.

Summer drought simulation

In combination with the removal treatment, we installed rainout shelter from 9 July to 6 September 2010 to simulate a pronounced summer drought. For true control of shelter effects, we established roofs for both control (n = 15) and drought plots (n = 15), with water collected from the roof added back to the control plot. Interaction of removal and drought treatment resulted in five replicates of each combination of treatments. Rainout shelters (adapted from Yahdjian & Sala 2002) were made of wood frames (1.80 m x 1.80 m) covered with transparent foil that permitted 90% penetration of photosynthetically active radiation (Cello Flex 4SF, Puteaux SA, Clayes-sous-bois, France). The mean height of the roofs was 1.70 m to leave enough space for plant growth. Rainout shelters had a 30° inclination following soil surface topography and on the lowest side, a gutter was installed to collect the intercepted precipitation in a storage tank (60 l). On the four sides, a foil strip was maintained in order to minimize border effect. Nevertheless, to ensure sufficient air circulation, we left two open layers (15 cm), above the soil and under the roof. Control plots were watered after each rainfall event with an amount of water corresponding to local precipitation according to the MeteoSwiss station close to the study site (Bullet/La Frétaz). Microclimatic variables were continuously monitored in the centre of three drought and control plots from the beginning of July till the end of September 2010. Soil moisture (EC-5) and temperature (ECT) sensor were placed at 10 cm depth and air temperature (EHT) sensor with radiation shield were placed at 50 cm above the soil surface. Data were recorded every 10 min using data loggers (Em50, Decagon Devices Inc., Pullman, WA, USA) fixed to the rainout shelter.

Resistance, recovery and resilience

Above-ground biomass was harvested twice a year, at the beginning of July and September 2009, 2010 and 2011, in order to simulate the presence of cattle which rotate two times in this pasture. Cutting height of the vegetation was approximately 3 cm above the soil in the central square (50 cm by 50 cm) of each plot (n = 30). Plant biomass was separated by species in 2010 (July and September), dried at 60°C for 72 hours and weighted. Relative species abundance was calculated from biomass of each species, but subordinate species, which were absent in S plots, was left out of vegetation community analysis to better compare the shift in species composition between the three treatments.

We calculated resistance of plant community to drought stress as the ratio of regrowth biomass after drought (September 2010) to pre-drought biomass (July 2010). It corresponds to the proportion of biomass which regrew during the drought. Resistance was also calculated for each species-group of dominant, subordinate and transient species. We determined also recovery as the ratio of biomass which regrew after the drought (July 2011) to the pre-drought biomass (July 2010). It corresponds to the proportion of biomass which directly regrew after the drought compared to the pre-drought biomass. Resilience was calculated as the ratio of total biomass after the drought (2011) to total biomass before the drought (2009). It corresponds to the proportion of biomass which is produced the year after the drought compared to the year before the drought. Resistance, recovery and resilience were observed in drought plots and compared with watered plots for each level of removal to distinguish between the effects on community stability associated to the removal treatment and the effects induced by the drought treatment.

Carbon isotopes and nitrogen concentration measurements

Carbon isotope ratios ($\delta^{13}C$ values) were determined as an integral measure for water use efficiency (Farquhar et al. 1989; critical review by Seibt et al. 2008). Two dominant species (*Taraxacum officinale* and *Alchemilla monticola*) and two subordinate species (*Achillea millefolium* and *Veronica chamaedrys*) were sampled at the end of the drought treatment (September 2010). We chose these four species as all of them regrew in each plot after the drought event. Leave biomass were ground to a fine powder and we determined carbon concentrations and isotope ratios ($\delta^{13}C$) with an elemental analyzer (Euro EA 3000, HEKAtech, Germany) coupled to an isotope ratio mass spectrometer (Delta V Advantage, Thermo, Germany).

Statistical analysis

All of the analyses were carried out with R version 2.13.1 (R Development Core Team, 2011). Canonical correspondence analysis (CCA) constraining the regrowing plant community (September 2010) by the removal and drought treatments was performed (after Hellinger transformation). Permutation tests (Monte Carlo) were used to assess the significance of these multivariate regression models. Community and species-groups resistance, recovery and resilience were analysed using ANOVAs, specifying removal and drought (and their interactions) as fixed factors, followed by Tukey post hoc tests. In plots without plant removal (C), carbon isotopes measurements were analysed with ANOVAs specifying drought and species (and their interactions) as fixed factors. As drought treatment had an overall significant effect on $\delta^{13}C$, we analysed separately carbon isotopes values for watered plots and drought plots with ANOVAs, specifying species as fixed factor, followed by Tukey post hoc tests. When we compared C and S plots, carbon isotopes measurements were analysed with ANOVAs specifying drought, removal and species as fixed factors.

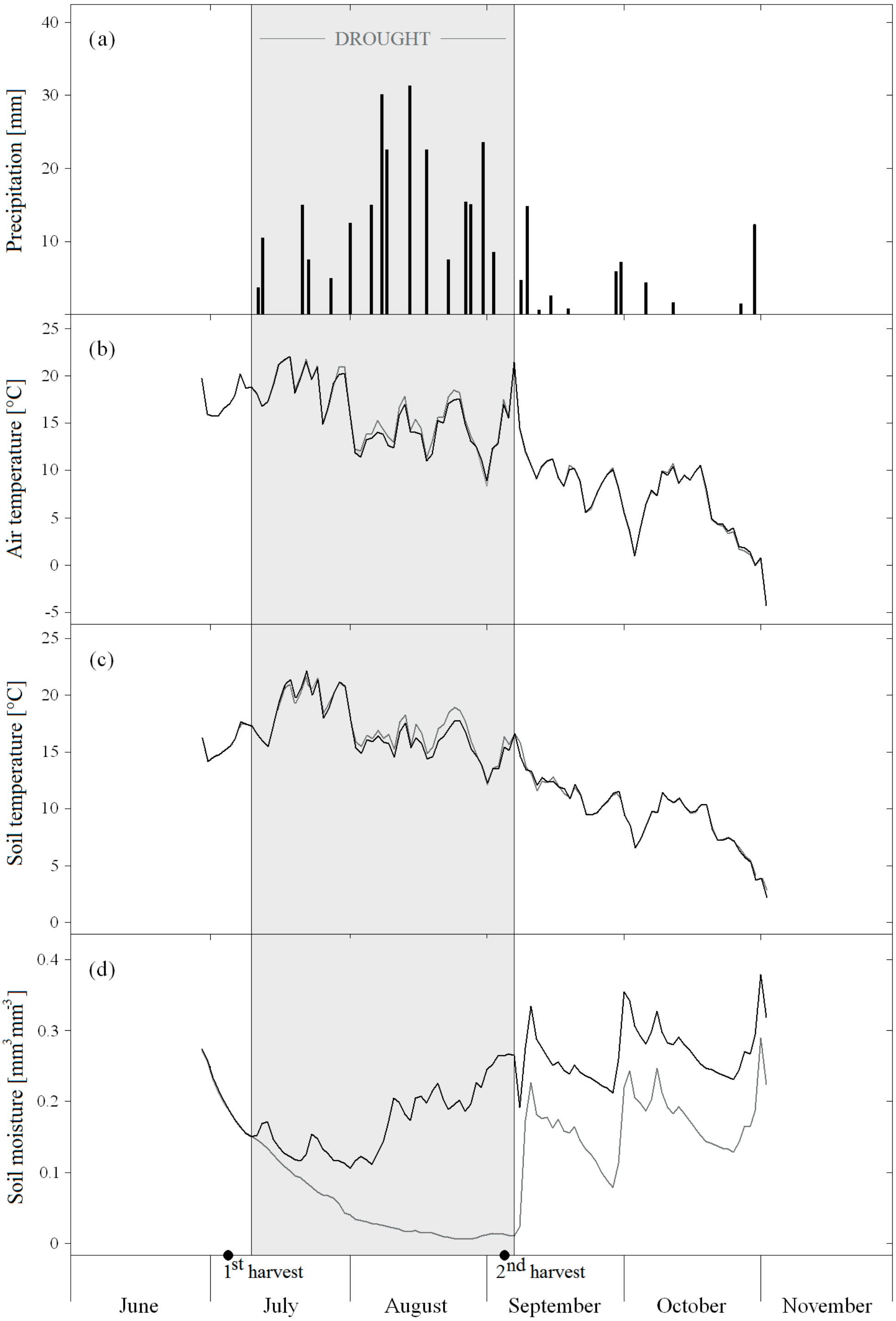

Figure 6.1 - Micrometeorology at La Frétaz from July to October 2010 for drought plots (grey line) and control plots (black line). (a) Daily precipitation sum (data from MeteoSwiss station Bullet/La Frétaz) and water addition during the drought in watered plots, (b) mean daily air temperature at 50 cm, (c) mean daily soil temperature at 10 cm depth, (d) mean daily soil moisture at 10 cm depth. Grey shadow indicates the period of drought by rainout shelters and black points corresponds to the two harvests of community above-ground biomass.

Results

Climatic conditions

The experimental drought by rainout shelters reduced the amount of precipitation by around 268 mm during 2 months in summer 2010 (Fig. 6.1), corresponding to 19% of mean annual precipitation at this site. During the drought treatment, the average air temperature increased by around 0.4°C and the average soil temperature by around 0.2°C under the shelters in drought plots compared to control plots. However this increase was not significant neither for air ($F_{1,116} = 0.137$, $P = 0.71$) nor for soil temperature ($F_{1,116} = 0.152$, $P = 0.70$). As expected the rainout shelters decreased soil moisture at 10 cm depth by an average of 67% during the drought period. The maximum decrease in soil moisture was 97% at the end of August compared to control plots. After the drought, soil moisture remained lower in the drought plots by around 38% from September to October.

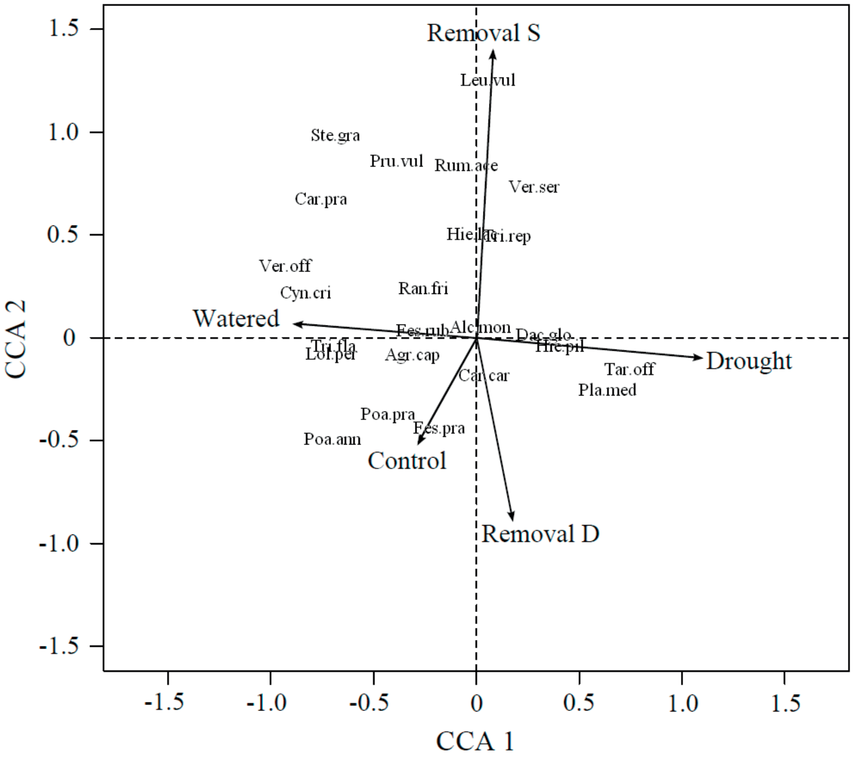

Figure 6.2 - Biplot of canonical correspondence analysis of the vegetation community (subordinates excluded) after drought (September 2010) constrained by the removal (Control, Removal D, Removal S) and drought (Watered, Drought) treatments. Axes 1 and 2 hold, respectively, 11.5% ($F_{1,26} = 3.72$, $P < 0.001$) and 5.3% ($F_{1,26} = 1.72$, $P = 0.04$) of the explained variance.

Community resistance, recovery and resilience

Community above-ground biomass between plots ($n = 30$) did not differ following the removal treatment at the beginning of the experiment (2009), excluding an effect of pre-drought biomass variation. We did not detect significant differences in community above-ground biomass between years (2009, 2010, 2011) in intact control plots (watered and without plant removal, $n = 5$) showing that natural annual variation was negligible during this 3-years experiment.

Plant community composition shifted significantly following treatments ($F_{3,26} = 2.093$, $P = 0.001$, 999 Monte Carlo permutations), as shown by results of CCA constraining plant abundance by treatments (Fig. 6.2). Axis 1 (11.5% explained variance, $P < 0.001$) corresponds to the drought treatment and discriminated watered and drought plots. Axis 2 (5.3% explained variance, $P < 0.05$) corresponds to the removal treatment and discriminated control plots (C and D) and plots where subordinate species were removed (S).

Plant community resistance (Fig. 6.3a) was significantly affected by the drought ($F_{1,24} = 110.061$, $P < 0.001$) and the removal treatment ($F_{2,24} = 3.583$, $P < 0.05$) with a significant interaction between the treatments ($F_{2,24} = 4.348$, $P < 0.05$). In watered plots, plant community resistance, an indicator of grazing resistance, showed about 60% of regrowth and no significant influence of the removal treatment. In drought plots, plant community resistance represented around 20% of regrowth in plots C and D whereas it decreased significantly in S plots with only 2.5% of the biomass regrowing after drought.

The same pattern of response of the whole plant community to drought and removal were found for the resistance of dominant and transient species (Fig. 6.3b). However the effects of drought were higher on dominant species than on transient species with only 5% of regrowth in C plots, 3% in D plots and 0.09% in S plots. In contrast, subordinate species responded differently in watered and drought plots ($F_{1,16} = 6.766$, $P < 0.05$) and increased largely their resistance in watered plots (around 500% of regrowth) and after drought (1500-2000% of regrowth). The proportion of species-group changed also after drought compared to watered plots (Fig. 6.3b), with a decrease of dominant species (25% instead of 60%) and an increase of transient (65% instead of 40%) and subordinate species (12% instead of 2%).

Plant community recovery (Fig. 6.3a) was not affected by the drought and community above-ground biomass recovered completely (around 100% of regrowth compared to the pre--drought biomass) in watered and drought plots for each removal treatment. Plant community resilience was not significantly influenced by the drought treatment (Fig. 6.3a) as resilience was similar in watered and drought plots. However, resilience was significantly affected by the removal treatment ($F_{2,24} = 9.351$, $P < 0.001$) and community above-ground biomass did not return to its original state in plot where subordinates were removed (60% of regrowth compared to 80% in plots C and D).

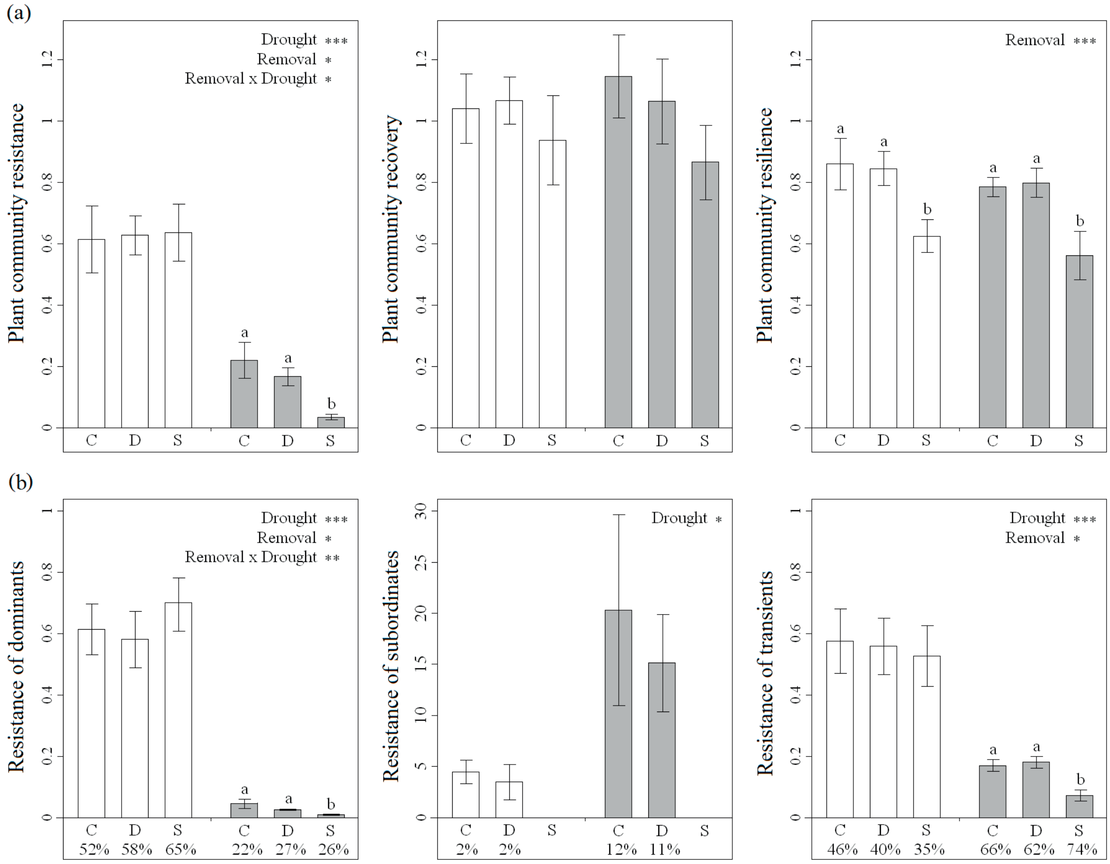

Figure 6.3 - (a) Plant community resistance, recovery and resilience in watered plots (white bars) compared to drought plots (grey bars) following the removal treatment. (b) Resistance of dominant, subordinate and transient species in drought plots (grey bars) compared to watered plots (white bars) following the removal treatment. Percentages correspond to the contribution of each species-group to the community biomass. Significant results of ANOVAs are indicated in each graph (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) and bars with different letters are significantly different ($P < 0.05$).

Carbon isotope composition

Carbon isotope ratios ($\delta^{13}C$) of plant leaves collected in C plots ranged between -31.5‰ and -27.9‰ (Fig. 6.4a). The $\delta^{13}C$ values were significantly affected by the drought treatment ($F_{1,31}$ = 73.799, $P < 0.001$) with an increasing values in plant leaves growing in drought plots. In addition, we found significant effect of species identity on $\delta^{13}C$ ($F_{3,31}$ = 76.071, $P < 0.001$) with lower values in subordinate species leaves (*Veronica chamaedrys* and *Achillea millefolium*) and higher values in dominant species leaves (*Taraxacum officinale* and *Alchemilla monticola*). The interaction between species identity and drought was not significant for the $\delta^{13}C$ values ($F_{1,36}$ = 0.729, $P = 0.40$) indicating that the drought effect was similar for dominant and subordinate species.

The $\delta^{13}C$ values of plant leaves collected in plots without removal (C) and with removal of subordinate species (S) were still significantly affected by the drought and species identity but also by the removal treatment (Fig. 5.4b). The significant interaction between removal and drought treatments ($F_{1,32}$ = 10.146, $P < 0.01$) revealed that, in watered plots, $\delta^{13}C$ of plant leaves increased in S plots compared to C plots whereas no effects of the removal were found in drought plot.

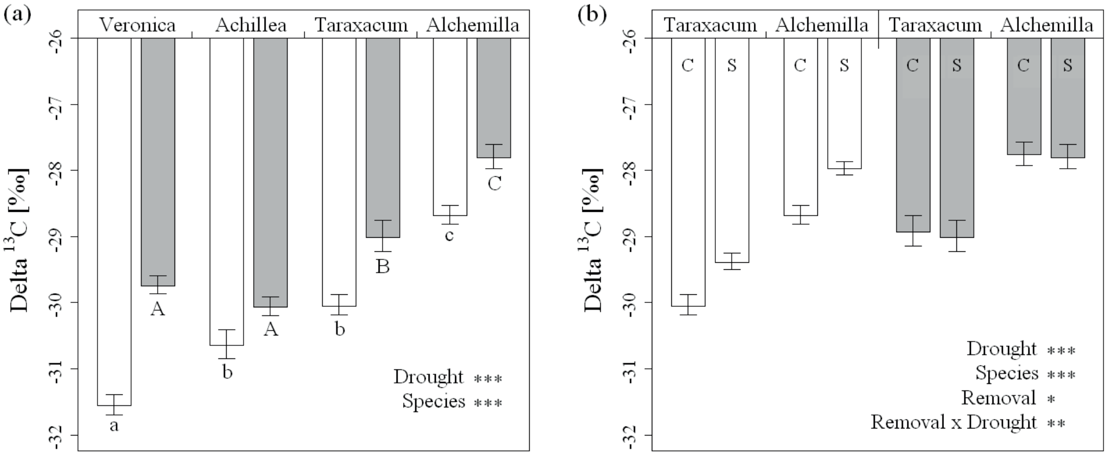

Figure 6.4 - (a) Carbon isotope ratios ($\delta^{13}C$) after drought in plant leaves of two subordinate species (Veronica chamaedrys, Achillea millefolium) and two dominant species (Taraxacum officinale, Alchemilla monticola) growing in watered plots (white bars) or drought plots (grey bars) without plant removal (C). (b) Carbon isotope ratios ($\delta^{13}C$) after drought in plant leaves of two dominant species (Taraxacum officinale, Alchemilla monticola) growing in watered (white bars) or drought (grey bars) conditions in plots without plant removal (C) and in plots where subordinate species were removed (S). Significant results of ANOVAs are indicated in each graph (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$) and bars with different letters are significantly different ($P < 0.05$).

Discussion

In this field experiment, we explored the effects of subordinate plant species removal, summer drought and their interactive effects on plant communities in grasslands. The two months long experimental drought significantly affected plant community composition and the removal of subordinates decreased the resistance of plant community to drought (Fig. 6.2, Fig. 6.3). Plant community varied following removal treatments with different plant composition in communities without subordinates compared to the intact communities. Some studies reported that species removal has generally an effect on the remaining plant community but with a time-lag in the response of plant groups or plant species to changes in plant composition (Wardle et al. 1999; Symstad & Tilman 2001). Other experiments showed changes in plant composition after a reduction of precipitation (Bloor et al. 2010; Craine et al. 2011; Weihßuhn et al. 2011) but severe droughts seem to have generally few effect on plant compositional change compared to plant-plant interactions and senescence (Jentsch et al. 2011). These experiments did not show clear patterns about which species-groups (e.g. functional group or traits) were altered through the drought. In our study, by contrast, we found that overall the proportion of dominants decreased whereas subordinate and transient species increased (Fig. 6.3b).

Our results indicate that the changes in plant community composition through the removal of subordinates were related to the community resistance to drought. As expected drought significantly decreased plant community resistance compared to watered plots (around 20% vs. 60%). However, the decline in resistance was about ten times higher in plots where subordinates had been removed (S) as compared with control plots (C and D) (Fig. 6.3a). Actually, this outcome can be explained by the decrease of dominants and transients resistance in the communities without subordinates which shows that the presence of subordinates might facilitate the regrowth of neighbours during water resources limitation. In constructed communities, Jentsch et al. (2011) observed that competitive and facilitative effects changed strongly in response to drought and therefore, plant-plant interactions might be a key factor in community resistance to drought. Most interestingly, when we consider the natural community (C plots), we observed that subordinates regrew four times more in drought plots than in watered plots after the drought (Fig. 6.3b), which indicates that subordinate species increase the resistance of plant community during drought by increasing their biomass. These findings may be explained either by the reduced competitiveness of dominants, whose biomass decreased during drought, and/or by a better physiological adaptation of subordinates to drought (e.g. a better WUE or a deeper rooting system).

The ^{13}C discrimination has been used as an integral measure for the water-use efficiency, because it is sensitive to the ratio of the CO_2 in the chloroplast to the CO_2 in ambient air and the ratio between photosynthesis and the stomatal as well as the mesophyll conductance (Farquhar et al. 1989; Seibt et al. 2008). Globally, subordinates species had lower water-use efficiency than dominant species and we suggest that water availability is less limiting for subordinate species. The significant increase of $\delta^{13}C$ values in plant biomass under drought (Fig. 6.4) indicated a better WUE as supported by other drought experiments in temperate grasslands where leaf gas exchange had been measured (Gilgen et al. 2010; Otieno et al. 2012). Our results showed that the effects were similar for the two dominant (*Taraxacum* and *Alchemilla*) and the two subordinate species (*Veronica* and *Achillea*). Therefore, dominant and subordinate species did not differ in their physiological adaptation to drought, which confirms that the increase of subordinate resistance to drought was linked to the reduction of competition between both species-groups (Kardol et al. 2010a, Wang et al. 2007). Mariotte et al. (Chapter 3) showed that a reduced root competition by dominants increased the competitive abilities of subordinates which grow better below-ground and subsequently above-ground. In addition, Kahmen et al. (2005) observed an increase of below-ground biomass after drought which was significantly influenced by plant community composition. We suggest that the reduced competitiveness of dominants is beneficial for subordinates, especially below-ground, and enhance their biomass production during drought. Below-ground compartment remains poorly studied and different element in soil may also interact to explain the effects of subordinate species on drought insurance, such as microbial activity, nematodes (Kardol et al. 2010b) or fungal communities (Hawkes et al. 2010).

Interestingly, the removal of subordinate species decreased the ^{13}C discrimination of dominants (*Taraxacum* and *Alchemilla*) under watered conditions (Fig. 6.4b). We hypothesize that the absence of subordinates induces a stress for the neighbouring community similarly to a water resources limitation. The "filtering" hypothesis (Grime 1998) suggests that the important role of subordinate species on ecosystem functioning is related to a facilitation of the recruitment of certain dominant species by creating favorable niches. Indeed, subordinate might increase water availability for the neighboring plants through, for example, interactions with mycorrhiza, which seem to be more beneficial for subordinates (Grime 1987; Van der Heijden et al. 1998; Chapter 4).

While community resistance was affected by drought and the absence of subordinate species, community recovery was not (Fig. 6.3). The removal of subordinates had no significant effect on plant community recovery after drought. Nevertheless, we observed a trend with lower recovery where subordinates were removed and we hypothesize that subordinate species would have been more influential after repeated drought events. Only few studies explored the effect of recurrent summer drought in grasslands (Gilgen & Buchmann 2009; Jentsch et al. 2011) and future research should go in this direction to better understand how plant community may recover following continuous climate change and to identify which species or species-group are most important for the resilience of grasslands. In the Swiss Jura Mountains, plant communities seem to be very resilient to a single drought event since our results showed that one year after the perturbation, plant community produced the same amount of biomass in drought and watered plots. However, independent of the drought event, resilience was significantly reduced where we removed subordinate species showing that community above-ground biomass was negatively affected by the continuous three years removal of subordinate. Therefore, our results highlight the importance of subordinate species for grasslands stability as suggested in previous studies (Lyons et al. 2005; Polley et al. 2006) but for a generalization, more evidence from long term experiments is needed.

Although the diversity-resistance hypothesis remains difficult to validate, we demonstrated that in species-rich grassland communities, subordinate species, which highly participate to plant diversity, act as a key component of community resistance to drought. These findings confirm the importance of ecosystem-level impacts of these low abundant plants which were not explored in experiments with random assembled communities (Bardgett & Wardle 2010). We suggest that the negative diversity effect on drought resistance observed by different authors in constructed grasslands (Pfisterer & Schmid 2002, Van Peer et al. 2004; De Boeck et al. 2008; Van Ruijven & Berendse 2010), might be due to the random assemblage of plant species (generally dominants) which neglects the competitive hierarchy and the presence of subordinates in plant communities. Inversely, in nature, more diverse communities have higher chance to include more subordinate (i.e. drought resistant) explaining the positive diversity effects on the drought resistance observed in intact grasslands (Tilman & Downing 1994; Kahmen et al. 2005).

In summary, our results provide further evidence for the diversity-stability hypothesis (Kahmen et al. 2005; Tilman et al. 2006) underlining the role of subordinate species during drought events and, more generally, for the functioning of grasslands. Independently of functional groups and functional traits which have been intensively studied, our findings confirm the importance of plant hierarchy including not only dominant but also subordinate and transient species. This experiment focused only on above-ground community but form a part of a larger study investigating the role of subordinate species in ecosystem functioning through above- and below-ground interactions. We acknowledge that the mechanisms by which subordinate species maintain above-ground biomass production and increase community resistance must be worth considering, especially in the below-ground, through their potential interactions with microbes.

Acknowledgments

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Chapter 7

Synthesis: general discussion and perspectives

Introduction

The objective of my thesis was to explore the ecosystem level effects of subordinate plant species which have not been much studied so far, by comparing them to dominant species which are recognized to determine ecosystem properties ("Mass ratio theory", Grime 1998). My research focused on two aspects: the persistence of subordinate species in the community and their role in grassland ecosystems. During four years, I carried out field (removal treatment, drought simulation) and glasshouse experiments (competition, AMF inoculation) to demonstrate that subordinate species need to receive more consideration in ecological research. Indeed, although subordinate plants are discreet in plant communities, they highly participate to species diversity and act to promote ecosystem functioning.

The aim of this chapter is to give a general overview of the thesis and to summarize and synthetize the key results of the various chapters in order to highlight the importance of subordinate species and their key role. By using findings of my different experiments and some additional results which are not presented in foregoing chapters, I will show how subordinate species may persist in grassland ecosystems and how, despite their low abundance, these species impact ecosystem functioning through above- and below-ground interactions, and how they are important for ecosystem resistance in the context of climate change.

Plant community structure and species coexistence

Plant community structure of semi-natural grasslands (11 sites) has been shown to change along a diversity-productivity gradient (Chapter 2) with higher diversity in intermediate productivity. Indeed, intermediate levels of productivity and fertility favoured species coexistence and thus, the proportion of subordinates increased to reach 35% of the species diversity. These preliminary results confirmed the hypothesis of Grime (1973, 1987) and showed that subordinate species are an important component of high diverse communities. Moreover, the analysis of 9 sites where pasturing was abandoned (land-use change) showed that the absence of cattle reduced plant diversity mainly in sites where diversity in grazed plots was either low or high. Plant community changed after abandonment of pasturing and the number of both dominant and subordinate species decreased with higher effects on subordinate species number. Findings of this explorative study suggest that large herbivores participate to the persistence of subordinates and may play an important role in species coexistence of semi-natural grasslands (Kohler et al. 2005). We suggest that continuous disturbance, linked with cattle activities (grazing, trampling, dunging), might create favourable niche for subordinates by modifying nutrient availability or plant-plant interactions (facilitation, competition).

Competition and facilitation are the two major factors of plant-plant interactions which influence species coexistence. Both above-ground competition for light and space, and below-ground competition for mainly water and nutrients are important drivers of plant establishment and growth. While shoot competition has been largely studied, the importance of root competition remains unclear. Many studies suggested that root competition has little direct impact on plant communities (Belcher et al. 1995; Peltzer et al. 1998; Cahill 1999; Cahill & Casper 2000). This is probably due to the lack of feedback mechanisms through which symmetric root competition can lead to competitive exclusion (Lamb & Cahill 2008). While root competition seems to have no direct impact, root-shoot competition interactions can indirectly influence plant community. This occurs when root competition reduces shoot growth and thus affects competition for above-ground limiting resources such as light (Lamb et al. 2009). In our experiment on root competition exclusion between dominant and subordinate species (Chapter 3), we observed that root-shoot competition interactions increase the competitiveness of dominant species. Indeed, overall asymmetry between both species-groups increased through the effect of root competition from dominants. When root competition was excluded, root-shoot competition interactions were also removed and subordinate species increased their competitiveness. Reduction of root competition through disturbance might therefore be responsible for subordinate's persistence.

As we showed in Chapter 2, abandonment of pasturing was responsible for a higher decrease of the number of subordinate species compared to dominants. The absence of trampling, which creates gaps in the sward and thus reduces root competition, might be one of the mechanisms explaining the decrease of subordinates in abandoned plots. By contrast, in grazed plots, the activity of large herbivores might enhance the persistence of subordinates. In my thesis, I did not study selective grazing but it might be also an important factor for species coexistence. In a meta-analysis based on 197 studies, Diaz et al. (2006) showed that grazing favoured short plants over tall plants. Considering that subordinate species are generally smaller than dominants, we suggest that selective grazing might also promote subordinates. Moreover, De Deyn et al. (2003) demonstrated in a microcosm experiment that the invertebrate root herbivores were selectively feeding on roots of dominant plants and thus, soil fauna can also reduce root competition between dominant and subordinate species to promote coexistence.

Soil community and species coexistence

Soil organisms are recognized to directly impact plant community through the actions of root-associated biota such as mycorrhizal fungi, which are associated with roots of 80% of all terrestrial plants (Read et al. 1976; Smith & Read 2008). Arbuscular mycorrhizal fungi (AMF) have been largely studied in grasslands and many experiments demonstrated their capacity to facilitate seedling establishment (Van der Heijden et al. 2004), to enhance acquisition and uptake of scarce or immobile mineral nutrients, particularly phosphorus (Johnson et al. 2001), and to provide resistance to pathogens and drought (Gange & West 1994; Smith & Read 2008). In a complementary experiment (not shown), we determined AMF community profiles (by T-RFLP, same methods than in Chapter 5) in soil samples collected in 15 plots before the removal treatment at Les Amburnex. The results showed that two AMF communities were discriminated by the relative cover of dominants or subordinates (Fig. 7.1., $F_{2,12} = 2.505$, $P < 0.01$, 999 Monte Carlo permutations). These findings confirm the interactions between AMF and plant community and suggest two mechanisms which are difficult to disentangle: AMF influence plants and/or plants influence AMF. Plant species may structure AMF communities when host specific associations exist between some AMF species and some dominant or subordinate plant species (Johnson et al. 2004; Scheublin et al. 2007). By contrast, AMF have a significant role in structuring plant community structure (Van der Heijden et al. 1998a,b) and enhancing species diversity (Stampe & Daehler 2003; Van der Heijden et al. 2006). Indeed, AMF communities which promote subordinates and/or reduce the abundance of dominant can increase species coexistence and consequently species diversity.

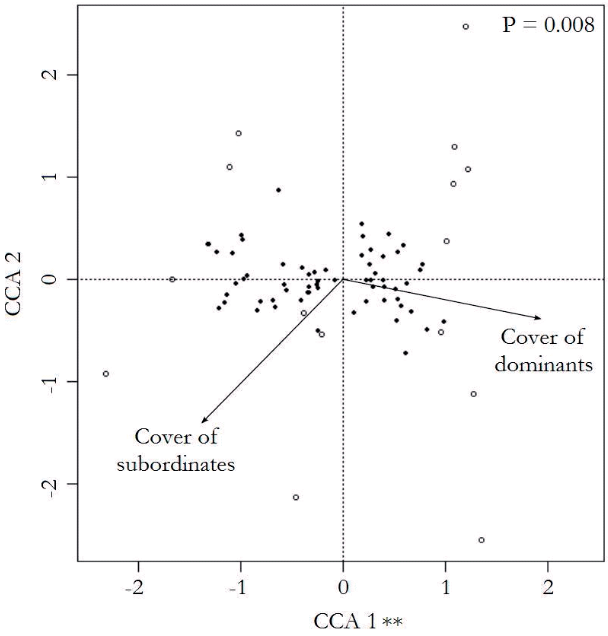

Figure 7.1 - Biplot of canonical correspondence analysis of the AM fungal community profiles determined in soils samples (pool of 5 sub-samples) constrained by the relative cover of dominant and subordinate species in 15 plots at the beginning of the experiment (Les Amburnex). Symbols represent plots (open circle) and T-RFLP peaks (filled circle). Axes 1 and 2 hold, respectively, 25.5% ($F_{1,12} = 4.33$, $P = 0.002$) and 3.9% ($F_{1,15} = 0.68$, $P = 0.79$) of the explained variance.

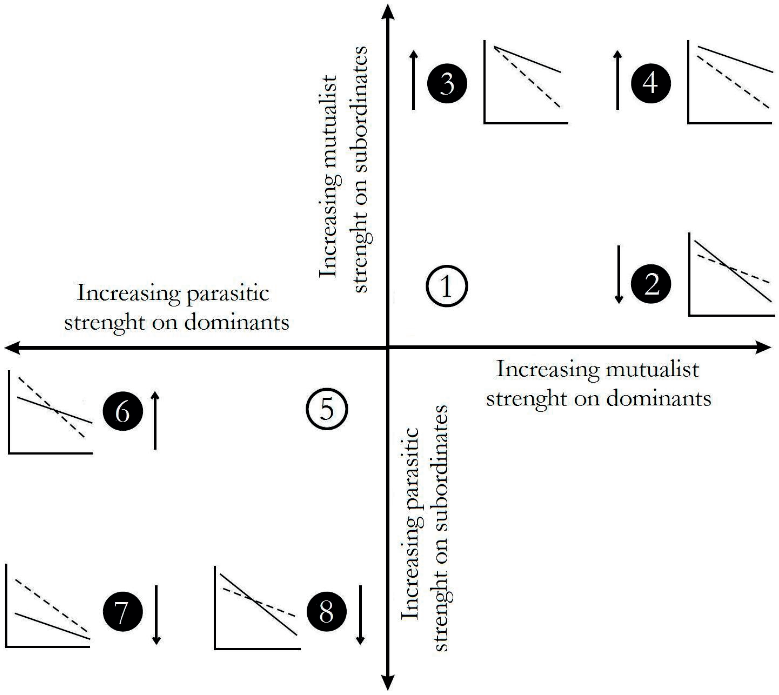

Figure 7.2 - Model of interactions among arbuscular mycorrhizal fungi (AMF) and dominant and subordinate species along the mutualism-parasitism continuum (adapted from Urcelay & Diaz 2003). Filled circles show situations where AM fungi have strong effects on plant diversity. The small figures illustrate the effect of the presence (solid lines) or absence (dashed lines) of AMF on the dominance-rank curve (Grime 1998): a steep curve indicate a high dominance whereas a flat curve indicate species coexistence. The arrows represent the direction of the effect of AMF on plant diversity (increase/decrease).

Previous experiments suggested that subordinate species were most responsive to colonisation (Van der Heijden et al. 1998a; Yao et al. 2007; Karanika et al. 2008) and that AMF may cause a reduction in dominance in favor of subordinate plant species (Grime 1987; Van der Heijden et al .1998b). AMF act along a continuum between mutualism and parasitism (Johnson et al. 1997; Klironomos 2003) and following its degree of profit for plant species, AMF could therefore completely change the dominance hierarchy (Urcelay & Diaz 2003, Fig. 1.3; Gross et al. 2010). Our competition experiment (Chapter 4), where dominant and subordinate grew in presence or absence of AMF (*Glomus intraradices*), was based on the model of Urcelay & Diaz (2003) and we hypothesized that AMF species may benefit subordinate species and reduce the differences in competitiveness with dominants.

Contrary to our expectations, the fungus acted towards the parasitic end of the mutualism-parasitism continuum and had an overall negative effect on the growth of all species. However, this study provided interesting results about the parasitic effect of the fungus in the dominance hierarchy. Indeed, we showed that subordinate species were less affected than dominants and increased their competitiveness, especially in mixture of all species. In natural plant communities, subordinate plants may therefore not only gain an advantage from associating with mutualistic species of AMF (Grime 1987; Urcelay & Diaz 2003), but may also be able to persist when the symbiosis is considered parasitic on dominant species. Therefore, we would predict dominant species to be less competitive, leading to their dominance reduction which can potentially increase species richness. Parasitism and mutualism seem to have opposite impact on dominance hierarchy and species diversity, and in figure 7.2, I propose to extend the model of Urcelay & Diaz (2003) by using our findings in the experiment presented in Chapter 4 (situation 6 in Fig. 7.2) and my hypothesis concerning the negative effects of AMF on dominance (situation 7 and 8).

Insurance to drought

Pasturing by large herbivores is an important factor determining the persistence of subordinate species in semi-natural grasslands (Chapter 2 & 3). Therefore, land-use changes are expected to modify plant community structure and to reduce the abundance of subordinate species. However, the loss of these low abundant species might be dramatic for ecosystem functioning especially under climate change. Indeed, we demonstrated that subordinate species can compensate for the loss of drought-sensitive dominants during summer drought events, and consequently increase the resistance of plant community and maintain productivity (Chapter 6). High diverse plant communities contain a higher proportion of subordinate plant species and we suggest that these communities would be more resistant to drought, which is in agreement with the diversity-stability hypothesis (Kahmen et al. 2005; Tilman et al. 2006). Independently of functional groups and functional traits which have been intensively studied (Wardle et al. 2003; MacLaren & Turkington 2010; Lavorel et al. 2011), our findings confirm the importance of plant hierarchy including not only dominant but also subordinate species. Moreover, our results provide also further evidence that the susceptibility to drought is biomass dependent (Wang et al. 2007) with high biomass systems, which include low abundance of subordinate species (Chapter 2), being less resistant to drought.

While the outcomes presented in Chapter 6 highlight the importance of subordinate species in plant community, additional results from the same drought experiment suggest that the effects of subordinates are also obvious in soil. The removal of subordinate species led to a decrease of litter decomposition in the topsoil (0-10 cm) during and after drought. During the summer season 2010 (May to October), which included 2 months of severe drought, the rate of litter decomposition decreased significantly due to water stress (Fig. 7.3.a) but the effects were higher in plots where subordinate species were removed compared to the other treatments (C and D). The effects of drought on the rate of litter decomposition persisted during the winter season 2010-2011 (Fig. 7.3b) showing that soil processes were less resilient than biomass production (Chapter 6). Nevertheless, the rate of litter decomposition was always lower in plots were subordinates were removed, in both drought and watered plots. These results give further evidence that subordinate species drive also soil processes and probably affect soil microbial communities (i.e. AMF and bacteria, Chapter 5) during and after drought events. Complementary analysis on soil organisms (bacteria, fungi, AMF biomass by PLFA; collembolans and earthworms by species determination) are underway in order to confirm this hypothesis.

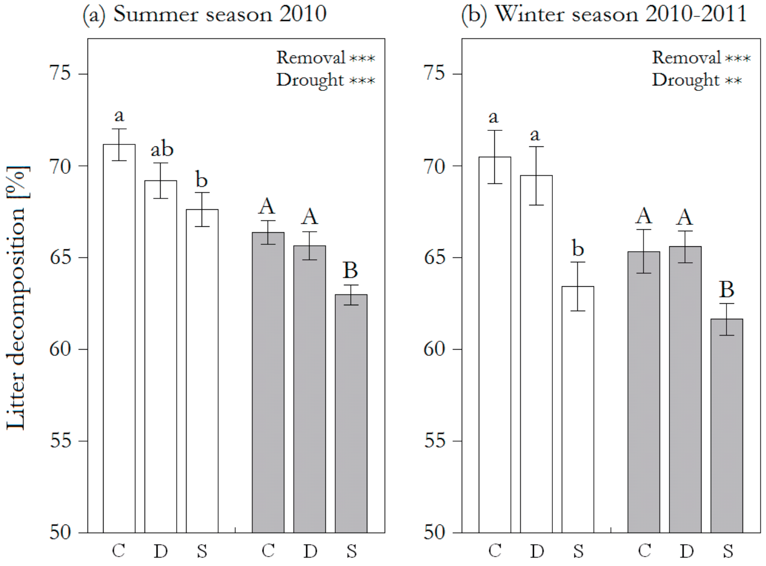

Figure 7.3 - Rate of litter decomposition (± 1 SE) in soil (from 0 to 10 cm depth) following treatments: C (control), D (partial removal of dominant biomass) and S (removal of subordinate species) in watered (white bars) and drought plots (grey bars) during (a) the summer season 2010 and (b) the winter season 2010-2011. Bars with different letters are significantly different ($P < 0.05$). (Measured by minicontainer system, same methods than in Chapter 5).

Following the same protocol as for the measurement of carbon isotope ratios (Chapter 6), we analysed nitrogen isotope ratios ($\delta^{15}N$) in plant leaves of the two dominant species *Taraxacum officinale* and *Alchemilla monticola*, collected at the end of the drought (Fig. 7.4). The results showed that in natural communities (control), $\delta^{15}N$ values in plant leaves of both species was not affected by the drought whereas in absence of subordinates (S plots), $\delta^{15}N$ decreased, with higher impact in watered than in drought plots. Foliar $\delta^{15}N$ values in non-N_2-fixing plants are recognized as valuable tools to estimate net nitrogen mineralization in soil (Garten 1993; Kahmen et al. 2008). Therefore, we suggest that the removal of subordinate species reduced nitrogen mineralization which is reflected above-ground in plant leaves of both dominant species. These findings confirm the effects of subordinate species on nitrogen dynamics which were also highlighted in Chapter 5, with the decrease of dissolved inorganic nitrogen in soil where subordinate were removed. Interestingly, the foliar $\delta^{15}N$ values of *T. officinale* and *A. monticola* measured in plots without subordinate species were lower in watered plots compared to drought plots (i.e. significant removal x drought interaction). We have no clear explanations for these findings but the analysis of soil microbial biomass could probably help to understand the underlying mechanisms involved there. Nevertheless, the overall outcome of this innovative experiment, which combines plant removal and drought treatment, is that subordinate species have important implications for the resistance, recovery and resilience to drought through interacting above- and below-ground compartments.

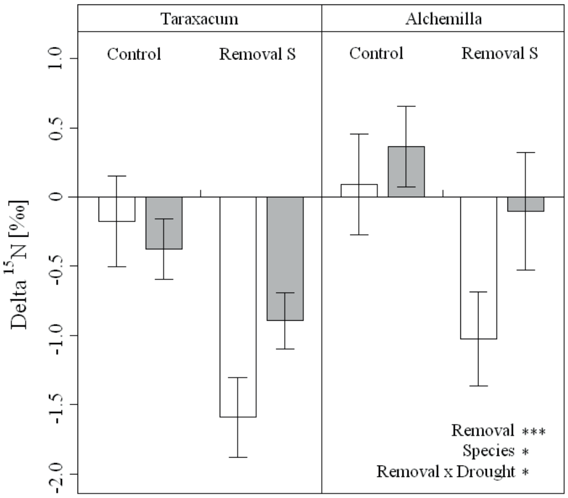

Figure 7.4 -Nitrogen isotope ratios ($\delta^{15}N$) after drought in plant leaves of two dominant species (*Taraxacum officinale*, *Alchemilla monticola*) growing in watered (white bars) or drought (grey bars) conditions in control plots (C) and in plots where subordinate species were removed (S). Significant results of ANOVAs are indicated in each graph (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Plant-soil feedbacks and ecosystem functioning

While subordinate plants enhance the resistance of plant community and maintain productivity in drought conditions (Chapter 6), they have also direct impact on ecosystem functioning (Chapter 5). Our findings showed that the removal of subordinate species significantly modified AMF and bacterial community structure in semi-natural grasslands and confirm therefore the importance of plant communities for driving soil communities (Koricheva et al. 2000; Stephan et al. 2000; Ledeganck et al. 2003; Johnson et al. 2004; Wardle 2002; Wardle et al. 2004; De Deyn & Van der Putten 2005; Bardgett et al. 2006; Bardgett & Wardle 2010). In addition, the absence of subordinates had important impacts on ecosystem processes with a reduction of litter decomposition rate, soil respiration, plant available nitrogen in soil and above-ground biomass production. Considering that the removal of subordinate species represented only 15% of the total community above-ground biomass (first removal), the observed effects on ecosystem functioning after 3 years were astonishingly important.

As we suggested in Chapter 5, the effects of subordinate species seem to be associated with plant-soil feedbacks which may directly affect coexisting plant species and ecosystem functioning. Bardgett & Wardle (2010) proposed three mechanisms of plant-soil feedbacks (Fig. 7.5; promotion of pathogens, mutualists and decomposers) and our results would confirm these hypothesis. Indeed, we observed that the removal of subordinates tend to increase the abundance of dominant species. Considering the low amount of subordinates which were removed and the small space occupied by these species which generally growunder the canopy of dominants or in gaps, we think that dominants do not only benefit from the empty space left by subordinates but are also enhanced by the removal of negative plant soil feedbacks that subordinates may exert. Subordinate species, when present, might reduce abundance of dominant by promoting soil organisms which attack dominant species (Fig. 7.5a). For example, the AM fungus *Glomus intraradices*, which reduced the biomass of dominant species and increase the competitiveness of subordinates in mixture (Chapter 4), could be considered as a pathogenic organism. By contrast while AMF might be pathogens, these soil organisms might also be considered as mutualists and directly benefit subordinates (Fig. 7.5b; Grime et al. 1987; Van der Heijden et al. 1998b; Urcelay and Diaz 2003). Actually, the capacity of subordinate to control soil pathogens and mutualists directly or indirectly may influence plant dominance and thus species diversity. The findings of our removal experiment (Chapter 5 & 6, Fig.7.3, Fig. 7.4), suggest that subordinate species may accelerate soil processes by promoting decomposers (probably through litter quality), which increase the rate of litter decomposition, soil respiration and nitrogen mineralization (Fig.7.5c). An increase of plant available nitrogen in soil directly enhances productivity. Indeed, in both removal experiments, we observed that above-ground plant community biomass was significantly higher where subordinates were present. While nutrient mineralization enhances productivity and, at the same time, reduces diversity (i.e. diversity-productivity relationship, see also Fig.1.3.a) in high fertile sites, it enhances both productivity and diversity in low fertile sites (Mikola et al. 2002; Van der Putten 2005). Therefore, positive plant-soil feedbacks on decomposers through the action of subordinate species can also promote species coexistence in low productive mountain grasslands.

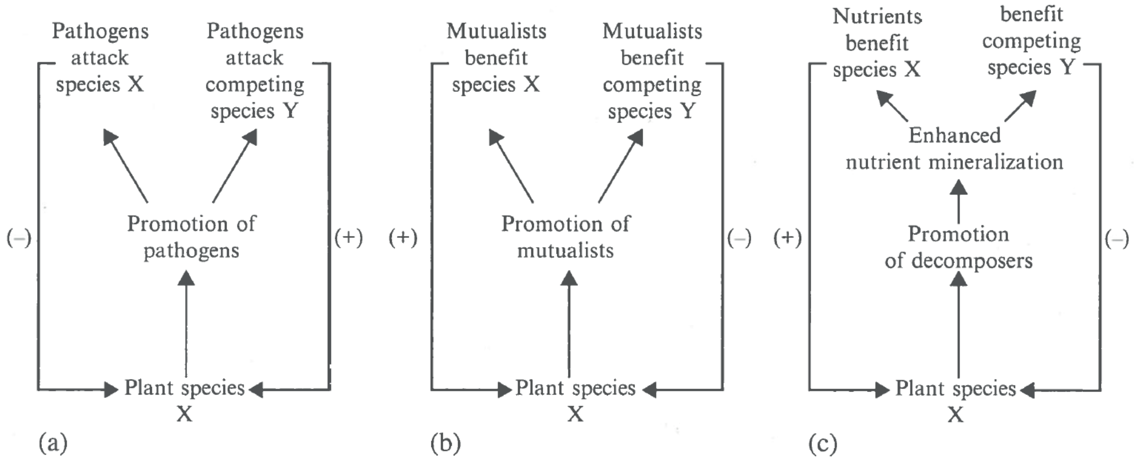

Figure 7.5 - Mechanisms by which a hypothetical plant species X can exert either positive (+) or negative (-) feedbacks with the soil community it promotes, depending on whether the mechanisms mainly affects species X directly or exerts a stronger effect on hypothetical competing species Y. (a) Pathogens, (b) mutualists, (c) decomposers. (Taken in Bardgett & Wardle 2010).

Above- and below-ground interactions and plant-soil feedbacks are recognized to significantly regulate terrestrial ecosystems explaining why subordinate species, which represent a small proportion of community biomass and cover, can have relatively strong effects on ecosystem functioning compared to dominants. The findings of my thesis demonstrate the important role of subordinate species in ecosystem functioning and confirm that not only dominant species determine ecosystem properties. Moreover, the different results obtained through the field and greenhouse experiments (Chapter 3, 4, 5 & 6) highlight the importance of soil in the persistence of subordinates and in their effects in the ecosystem. While subordinate species persist in plant community through root-associated organisms and the reduction of root competition (gaps), they impact mainly soil organisms and processes. In the past, many studies pointed out the importance of above-ground mechanisms (shoot competition, biomass production and shoot traits) and, at the same time, the importance of dominant species. In my opinion, these statements are not independent and the increasing knowledge and research on soil biota and root traits shows the importance of subordinate and transient species. This thesis serves as a contribution to the advances in this domain and provides basis for future research on ecosystem functioning and above- and below-ground linkages.

Management implications in a changing world

Semi-natural grassland ecosystems host species-rich communities but are among the most endangered ecosystems in Europe, threatened by both land-use and climate change. Abandonment of pasturing, through land-use change, might modify plant community structure and be responsible of a drastic loss of subordinate species, thus reducing biodiversity (Chapter 2). We showed that, by their interactions with soil microbial communities and plant-soil feedbacks, subordinate species enhance ecosystem processes (decomposition, mineralization, productivity; Chapter 5 & 6). Indeed, the absence or the low abundance of subordinate species can have negative impact on the functioning of these ecosystems. Our findings have therefore important implications for the way grasslands are managed for biodiversity and we confirm that extensive grazing must be maintained as it promotes species diversity and the persistence of key subordinate species. As we showed, the persistence of subordinate species is also crucial for the resistance of plant and soil community to extreme climatic events such as drought. Therefore, these recommendations for grassland management are ever more important when we considering that mountain grasslands are disproportionately exposed to climate change (Beniston et al. 1996; Nogués-Bravo et al. 2007), especially in Switzerland, where the mean decrease of summer precipitation might reach a maximum of 40% in 2070 compared to 1990 (Frei et al. 2006).

Perspectives

As in many studies, the duration of the field experiments was relatively short considering the processes involved in natural communities and ecosystems. Despite we showed in our experiment that subordinate species affected ecosystem functioning through plant-soil feedbacks, we cannot predict how these plant-soil feedbacks may evolve in time (seasonality/succession) and space (local/global), and under perturbations caused by land-use and climate changes. Further research must underpine the effects (negative, positive or neutral) of subordinate species in different ecosystems over time. The concept of Grime (1998) to classify species, which I took up in my thesis, may be applied to a range of ecosystems, and can be the starting point to explore the effects of plant community structure on ecosystem functioning at larger scale (e.g. biomes) and in relation to the diversity-productivity/stress/disturbance relationships.

In addition, our research on the influence of subordinate species on ecosystem functioning focused mainly on a species-group and the underlying mechanisms involved to explain these effects remain unclear, especially for nutrient storage and availability. For instance, little is known about how subordinate plant species vary in their impact on soil properties and ecosystem functioning (e.g. through different root traits, functions, decomposability, etc.) or whether the effects of subordinate species are mainly due to a single subordinate species or to an association of complementary species with different functional traits. Plant species differ in their functional traits, such as nutrient use traits and their complementarity and facilitation, can strongly affect the quantity of carbon input (Van der Heijden et al. 1998b ; Van Groenigen et al. 2006 ; De Deyn et al. 2008) and ecosystem services (Lavorel et al. 2011). Recent research in a UK grassland showed that two subordinate species, which were also subordinates in both of our sites, can yield significant benefits for soil C and N storage (*Trifolium pretense*, De Deyn et al. 2011) or promote plant C and N sequestration (*Achillea millefolium*, De Deyn et al. 2009). Exploring the effects of species identity seems to be necessary to understand the relative influence of each subordinate species in ecosystem functioning. Moreover, as highlighted in this thesis, the soil compartment (above- and below-ground interactions, plant-soil feedbacks and soil processes) was extremely important in relation to the persistence and the effects of subordinate species, and thus, we confirm that investigations on plant root traits and root-associated organisms are crucial.

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Appendix

Appendix 3.1 - Means (± 1 SE) of root and shoot biomass (in grams per individual) for each plant species (in columns) growing with each species (in rows) under full competition above and under root competition exclusion underneath (grey shaded). Combinations of interacting dominant and subordinates are bordered with thick line.

Shoot	T. repens	F. nigrescens	T. officinale	P. media	A. capillaris	T. pratense	P. vulgaris	A. millefolium
T. repens	2.91 ± 0.64	1.84 ± 0.27	3.70 ± 0.36	2.33 ± 0.21	3.33 ± 0.46	2.29 ± 0.09	2.04 ± 0.31	3.63 ± 0.85
	3.47 ± 0.76	0.96 ± 0.20	2.72 ± 0.54	1.79 ± 0.35	3.11 ± 0.38	3.03 ± 0.42	3.37 ± 0.42	2.69 ± 0.27
F. nigrescens	3.47 ± 0.63	1.42 ± 0.19	3.58 ± 0.23	3.55 ± 0.18	3.66 ± 0.62	2.36 ± 0.40	2.70 ± 0.35	1.46 ± 0.37
	3.33 ± 1.49	1.78 ± 0.11	4.07 ± 0.40	2.33 ± 0.33	2.89 ± 0.77	3.02 ± 0.78	2.87 ± 0.10	2.92 ± 0.25
T.officinale	1.04 ± 0.30	0.55 ± 0.08	2.59 ± 0.29	0.57 ± 0.07	1.06 ± 0.22	0.81 ± 0.15	0.81 ± 0.15	1.01 ± 0.14
	2.27 ± 0.69	0.92 ± 0.17	2.28 ± 0.26	1.50 ± 0.23	1.48 ± 0.22	0.89 ± 0.43	1.72 ± 0.24	1.47 ± 0.34
P. media	7.67 ± 1.83	1.52 ± 0.17	3.97 ± 0.57	2.73 ± 0.46	3.90 ± 0.46	3.13 ± 0.56	2.31 ± 0.66	2.83 ± 0.48
	3.59 ± 1.13	1.60 ± 0.22	3.12 ± 0.44	2.24 ± 0.10	4.01 ± 0.44	2.57 ± 0.66	3.25 ± 0.49	3.53 ± 0.58
A. capillaris	2.80 ± 0.77	0.86 ± 0.17	3.28 ± 0.36	1.78 ± 0.26	2.66 ± 0.09	2.60 ± 0.50	2.90 ± 0.25	2.29 ± 0.16
	3.36 ± 0.71	1.09 ± 0.22	2.76 ± 0.41	2.26 ± 0.07	2.87 ± 0.27	2.07 ± 0.74	2.13 ± 0.39	2.46 ± 0.48
T. pratense	2.20 ± 0.59	1.82 ± 0.39	4.08 ± 0.49	2.46 ± 0.40	3.79 ± 0.51	2.98 ± 0.23	2.00 ± 0.21	2.66 ± 0.66
	4.17 ± 0.95	1.41 ± 0.21	2.51 ± 0.34	1.83 ± 0.16	2.63 ± 0.58	2.08 ± 0.61	3.77 ± 1.06	3.13 ± 0.25
P. vulgaris	3.62 ± 0.48	2.01 ± 0.12	3.84 ± 0.26	2.78 ± 0.43	4.17 ± 0.55	4.92 ± 1.83	3.08 ± 0.46	2.99 ± 0.38
	3.88 ± 0.79	2.00 ± 0.06	3.18 ± 0.29	1.96 ± 0.32	3.65 ± 0.29	4.17 ± 1.45	2.75 ± 0.29	2.83 ± 0.18
A. millefolium	1.81 ± 0.56	1.61 ± 0.40	4.23 ± 0.20	1.95 ± 0.26	3.41 ± 0.11	1.89 ± 0.43	2.94 ± 0.38	2.41 ± 0.20
	3.09 ± 0.62	1.55 ± 0.26	2.86 ± 0.29	1.59 ± 0.16	2.13 ± 0.26	2.59 ± 0.54	1.89 ± 0.12	2.29 ± 0.12

Root	T. repens	F. nigrescens	T. officinale	P. media	A. capillaris	T. pratense	P. vulgaris	A. millefolium
T. repens	1.38 ± 0.10	2.23 ± 0.24	4.42 ± 0.53	2.68 ± 0.86	1.86 ± 0.30	1.06 ± 0.19	0.92 ± 0.09	1.25 ± 0.24
	1.82 ± 0.13	1.79 ± 0.69	2.55 ± 0.67	0.98 ± 0.21	2.86 ± 0.29	1.93 ± 0.46	1.11 ± 0.27	2.49 ± 0.43
F. nigrescens	2.12 ± 0.16	2.06 ± 0.29	5.78 ± 1.32	0.90 ± 0.24	2.63 ± 0.28	1.49 ± 0.37	1.40 ± 0.24	1.65 ± 0.36
	1.96 ± 0.46	1.87 ± 0.10	4.04 ± 0.98	1.02 ± 0.11	1.74 ± 0.13	3.03 ± 0.92	1.45 ± 0.14	1.97 ± 0.16
T.officinale	2.27 ± 0.42	1.14 ± 0.26	3.23 ± 0.16	0.61 ± 0.11	1.56 ± 0.26	1.41 ± 0.33	0.62 ± 0.08	0.91 ± 0.11
	2.41 ± 0.49	1.30 ± 0.14	2.16 ± 0.30	0.89 ± 0.17	1.70 ± 0.29	1.21 ± 0.40	1.10 ± 0.18	1.86 ± 0.23
P. media	2.51 ± 0.62	1.85 ± 0.24	3.87 ± 0.75	0.91 ± 0.12	2.85 ± 0.31	3.05 ± 0.57	0.57 ± 0.13	3.69 ± 0.49
	3.05 ± 0.41	2.08 ± 0.22	3.20 ± 0.47	0.95 ± 0.01	2.09 ± 0.18	2.17 ± 0.41	1.54 ± 0.19	2.20 ± 0.56
A. capillaris	2.01 ± 0.44	0.82 ± 0.29	2.63 ± 0.50	0.66 ± 0.13	1.85 ± 0.06	2.31 ± 0.54	0.99 ± 0.33	1.58 ± 0.39
	1.29 ± 0.31	1.65 ± 0.29	3.22 ± 0.82	1.26 ± 0.18	1.83 ± 0.03	1.73 ± 0.47	1.39 ± 0.28	1.77 ± 0.42
T. pratense	1.44 ± 0.22	4.02 ± 0.53	4.41 ± 1.18	0.62 ± 0.02	2.40 ± 0.46	2.48 ± 0.40	0.89 ± 0.10	1.96 ± 0.54
	2.39 ± 0.17	1.96 ± 0.16	3.34 ± 0.23	1.06 ± 0.14	1.97 ± 0.29	1.28 ± 0.24	1.14 ± 0.18	2.25 ± 0.10
P. vulgaris	2.42 ± 0.33	2.09 ± 0.15	4.61 ± 0.70	0.87 ± 0.07	2.23 ± 0.10	3.15 ± 0.27	1.21 ± 0.16	2.69 ± 0.34
	2.52 ± 0.18	2.32 ± 0.32	3.73 ± 0.83	1.04 ± 0.28	2.79 ± 0.08	2.29 ± 0.37	1.12 ± 0.12	2.27 ± 0.39
A. millefolium	2.28 ± 0.18	2.13 ± 0.09	3.31 ± 0.25	0.69 ± 0.07	2.87 ± 0.24	2.86 ± 0.15	0.95 ± 0.25	2.54 ± 0.33
	2.64 ± 0.38	2.32 ± 0.32	5.00 ± 0.36	0.74 ± 0.17	1.66 ± 0.18	2.10 ± 0.17	1.30 ± 0.43	1.94 ± 0.16

Appendix 3.2 - Matrix of relative yield per plant for the 8 target species (columns) in interactions with the 8 neighbour species (rows), repeated for blocks (4) in full competition and in root competition exclusion. Means of columns correspond to the competitive effect (CE) of species and means or rows correspond to the competitive response (CR) of species.

Full competition

	BLOCK	*T. repens*	*F.nigrescens*	*T. officinale*	*P.media*	*A.capillaris*	*T. pratense*	*P. vulgaris*	*A. millefolium*
T. repens	1	1	0.79	2.19	1.78	1.24	0.56	0.68	0.75
	2	1	1.68	1.19	1.01	0.82	0.66	0.55	NA
	3	1	1.43	1.40	NA	1.56	0.53	0.89	NA
	4	1	1.03	1.00	1.56	1.02	0.71	0.72	1.16
F.nigrescens	1	0.64	1	1.64	1.91	0.91	0.46	1.28	NA
	2	2.04	1	1.97	1.34	1.53	0.44	0.94	0.65
	3	1.51	1	NA	0.93	1.70	1.09	0.93	0.94
	4	1.53	1	1.19	0.95	1.39	0.99	0.77	0.56
T. officinale	1	0.81	0.45	1	0.42	0.37	0.40	0.58	0.36
	2	NA	0.44	1	0.29	0.65	0.30	0.30	0.44
	3	0.49	0.54	1	0.31	0.81	0.27	0.30	0.40
	4	0.92	0.51	1	0.30	0.47	0.69	0.23	0.36
P.media	1	1.37	0.53	1.20	1	1.55	0.97	0.49	1.07
	2	3.77	1.62	1.07	1	1.19	1.55	0.70	1.77
	3	1.23	0.89	1.78	1	1.68	0.80	1.11	1.73
	4	4.45	1.20	1.33	1	1.60	1.18	0.41	0.84
A.capillaris	1	0.96	0.57	1.01	0.94	1	0.70	0.93	0.65
	2	0.59	0.79	1.27	0.79	1	0.84	0.90	0.88
	3	1.34	0.15	0.78	0.41	1	0.82	0.89	0.67
	4	1.53	0.53	0.98	0.62	1	1.36	0.90	0.94
T. pratense	1	0.64	1.37	2.28	0.90	1.07	1	0.99	0.28
	2	1.87	2.38	1.62	0.96	1.45	1	0.44	1.32
	3	0.49	1.64	1.12	0.63	1.19	1	0.84	1.44
	4	0.85	1.65	1.00	0.90	1.74	1	0.62	0.93
P. vulgaris	1	0.96	0.94	1.46	1.69	1.39	0.69	1	1.03
	2	2.35	1.78	1.33	0.81	1.68	1.19	1	0.80
	3	1.10	1.07	1.75	0.83	1.30	1.52	1	1.39
	4	1.82	1.22	1.30	0.88	1.29	2.94	1	1.43
A. millefolium	1	0.94	NA	1.64	1.13	1.69	0.60	0.95	1
	2	NA	1.59	1.02	0.48	1.28	0.84	0.93	1
	3	NA	0.78	1.37	0.73	1.38	0.85	1.01	1
	4	1.09	1.45	1.24	0.68	1.26	1.31	0.77	1

Root competition exclusion

	BLOCK	*T. repens*	*F.nigrescens*	*T. officinale*	*P.media*	*A.capillaris*	*T. pratense*	*P. vulgaris*	*A. millefolium*
T. repens	1	1	NA	0.67	1.04	NA	1.73	1.93	1.58
	2	1	NA	1.01	0.74	1.40	0.90	0.85	1.29
	3	1	1.05	1.41	0.83	1.71	1.16	1.15	0.95
	4	NA	0.55	1.78	0.88	0.87	3.33	0.99	1.08
F.nigrescens	1	NA	1	2.31	1.29	NA	1.84	1.55	1.09
	2	NA	1	1.27	0.83	1.30	1.32	0.98	1.22
	3	1.63	1	1.38	1.21	1.38	1.74	1.04	1.39
	4	NA	1	2.90	0.89	0.67	3.07	1.06	0.98
T. officinale	1	1.58	0.50	1	0.85	NA	1.42	0.81	0.81
	2	0.31	0.49	1	0.60	0.84	0.19	0.50	0.91
	3	0.95	NA	1	0.60	0.52	0.80	0.77	0.56
	4	NA	0.67	1	0.96	0.57	0.32	0.90	0.86
P.media	1	1.86	0.97	1.85	1	NA	3.28	2.15	1.42
	2	0.55	0.93	1.29	1	1.12	1.00	0.70	1.38
	3	0.98	1.36	1.43	1	1.45	0.90	1.32	1.53
	4	NA	0.77	1.14	1	1.31	1.41	1.15	1.13
A.capillaris	1	0.96	0.70	1.67	1.08	NA	1.73	0.97	1.07
	2	1.09	0.93	1.41	1.08	1	1.36	0.84	0.84
	3	0.85	0.94	1.27	0.99	1	0.26	0.88	1.17
	4	NA	0.40	0.97	1.28	1	1.96	0.98	0.95
T. pratense	1	1.20	0.94	1.31	0.72	NA	1	0.94	NA
	2	1.30	0.76	1.06	0.80	1.17	1	1.75	1.44
	3	NA	0.85	1.47	0.91	0.69	1	1.26	1.50
	4	NA	1.16	1.54	1.23	0.94	1	0.99	1.12
P. vulgaris	1	1.33	1.06	1.40	0.63	NA	2.32	1	1.25
	2	0.74	1.08	1.33	1.36	1.34	2.33	1	1.07
	3	1.19	1.49	1.94	0.78	1.43	0.95	1	1.18
	4	NA	1.12	1.55	0.95	1.38	2.79	1	1.30
A. millefolium	1	1.55	1.12	1.93	0.65	NA	NA	0.95	1
	2	0.76	1.28	1.67	0.78	0.82	1.35	0.92	1
	3	0.79	0.66	1.61	0.51	1.13	0.72	0.63	1
	4	NA	1.15	2.01	0.99	0.75	2.59	0.81	1

Appendix 3.3 - Linear mixed-effects model analysis of competitive effect and competitive response depending of root competition and species type (dominant/subordinate) using neighbour species nested into target species nested into block.

	Df	F-value	P-value
Competitive effect			
Type (Dominant/Subordinate)	1	1.265	0.271
Residuals	27		
Root competition	1	0.869	0.352
Type x Root competition	1	15.325	**<0.001**
Residuals	221		
Competitive response			
Type (Dominant/Subordinate)	1	2.582	0.119
Residual	27		
Root competition	1	8.784	**0.003**
Type x Root competition	1	4.670	**0.032**
Residuals	221		

Appendix 5.1 - Allometric models for the total estimation of subordinate and dominant species at Les Amburnex. The models were first applied to the removed subordinate species to know the amount of biomass removed in each S plots, and then applied to the dominant species in order to remove the equivalent biomass in D plots (see explanations in the text).

	Allometric equation (b =)	R^2	Trait (x)
Subordinate species			
Trifolium pratense	$0.023\ x^{1.218}$	0.75	Leaf number
Prunella vulgaris	$0.019\ x^{1.353}$	0.78	Leaf lenght
Alchemilla monticola	$0.105\ x^{2.451}$	0.81	Leaf lenght
Cardamine pratensis	$0.039\ x^{1.737}$	0.73	Leaf lenght
Achillea millefolium	$0.006\ x^{1.250}$	0.73	Leaf lenght
Plantago major	$0.179\ x^{1.014}$	0.73	Leaf number
Cerastium fontanum	$0.001\ x^{1.409}$	0.73	Stem lenght
Dominant species			
Trifolium repens	$0.004\ x^{1.380}$	0.67	Stem lenght
Taraxacum officinale	$0.016\ x^{1.722}$	0.56	Leaf lenght
Ranunculus acris friesianus	$0.050\ x^{1.624}$	0.52	Leaf lenght
Plantago media	$0.027\ x^{1.677}$	0.73	Leaf number
Leontodon sp.	$9.10^{-5}\ x^{3.394}$	0.73	Leaf lenght
Agrostis capillaris	0.128 g per individual	-	-

Appendix 6.1 - Allometric models for the total estimation of subordinate and dominant species at La Frétaz. The models were first applied to the removed subordinate species to know the amount of biomass removed in each S plots, and then applied to the dominant species in order to remove the equivalent biomass in D plots (see explanations in the text).

	Allometric equation (b =)	R^2	Trait (x)
Subordinate species			
Trifolium pratense	$0.025\ x^{1.303}$	0.81	Leaf number
Achillea millefolium	$0.006\ x^{1.026}$	0.65	Leaf lenght
Leontodon sp.	$0.004\ x^{2.260}$	0.79	Leaf number
Cerastium fontanum	$8.10^{-4}\ x^{1.409}$	0.73	Stem lenght
Veronica chamaedrys	$0.026\ x^{2.204}$	0.84	Leaf lenght
Dominant species			
Trifolium repens	$0.016\ x^{0.952}$	0.69	Stem lenght
Agrostis capillaris	0.092 g per individual	-	-
Carum carvi	$0.041\ x^{1.219}$	0.65	Leaf number
Ranunculus acris friesianus	$0.026\ x^{2.364}$	0.83	Leaf lenght
Taraxacum officinale	$0.009\ x^{1.628}$	0.63	Leaf lenght
Alchemilla monticola	$0.084\ x^{2.399}$	0.73	Leaf lenght

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Languages: English, French, German (school level)

Papers in peer-reviewed journals

1. **Mariotte, P.**, Meugnier, C., Johnson, D., Thébault, A., Spiegelberger, T., Buttler, A. (2012) Arbuscular mycorrhizal fungi reduce the differences in competitiveness between dominant and subordinate plant species. *Plant and Soil*. Accepted

2. **Mariotte, P.**, Buttler, A., Johnson, D., Thébault, A. & Vandenberghe, C. (2012) Exclusion of root competition increases competitive abilities of subordinate plant species through root-shoot interactions. *Journal of Vegetation Science*. In press.

3. Spiegelberger, T., Gillet, F., Amiaud, B., Thébault, A., **Mariotte, P.**, Buttler, A. (2012) How do plant community ecologists consider the complementarity of observational, experimental and theoretical approaches? *Plant Ecology and Evolution*, 145, 4-12.

4. Hocquet, D., Plésiat, P., Dehecq, B., **Mariotte, P.**, Talon, D., Bertrand, X. (2010) Nationwide investigation of extended-spectrum β-Lactamases, metallo-β-lactamases, and extended-spectrum oxacillinases produced by ceftazidime-resistant *Pseudomonas aeruginosa* strains in France, *Antimicrobial Agents and Chemotherapy*, 54, 8, 3512-3515.

Papers in non peer-reviewed journals

1. **Mariotte, P.** & Buttler, A. (2011) Chaque espèce compte (German title: Auch schwache Pflanzen sind stark), Swiss Research Magazine *Horizons*, N°91, December 2011, p.21.

Submitted

1. **Mariotte, P.**, Vandenberghe, C., Hagedorn, F., Buttler, A. Subordinate species enhance community insurance to drought in semi-natural grassland ecosystems.

2. **Mariotte, P.**, Vandenberghe, C., Meugnier, C., Rossi, P., Bardgett, R.D., Buttler, A. Subordinate plant species impact on soil microbial communities and ecosystem functioning in grasslands: findings from a removal experiment.

In preparation

1. Kardol, P., De Deyn, G., Hawkes, C., Laliberté, E., **Mariotte, P.** (2013) A hierarchical framework for investigating plant-soil feedbacks in space and time. (Special Issue of Ecological Society of America, Annual meeting 2012). *Journal of Ecology*.

2. **Mariotte, P.**, Buttler, A., Spiegelberger, T. Plant community structure in semi-natural grasslands threatened by land-use changes.

3. **Mariotte, P.**, Vandenberghe, C., Hagedorn, F., Ostle, N., Bardgett, R., Buttler, A. Subordinate plant species maintain soil functioning during summer drought.

Reviewer for peer-reviewed journals

1. *Journal of Applied Ecology*
2. *Plant and Soil*
3. *Basic and Applied Ecology*

Oral communications at conferences

1. Kardol, P., De Deyn, G., Hawkes, C., Laliberté, E., **Mariotte, P.** (2012) Plant-soil feedbacks in a temporally and spatially variable environment. Ecological Society of America (ESA), 97th Annual meeting, 5-10th August, Portland, Oregon, USA (accepted for presentation).

2. **Mariotte, P.**, Vandenberghe, C., Hagedorn, F., Buttler, A. (2012) Subordinate plant species enhance community insurance to drougth in semi-natural grasslands. Ecological Society of America (ESA), 97th Annual meeting, 5-10th August, Portland, Oregon, USA (accepted for presentation).

3. **Mariotte, P.**, Vandenberghe, C., Meugnier, C., Rossi, P., Bardgett, R.D., Buttler, A. (2012) Rôle des espèces subordonnées dans le fonctionnement de l'écosystème de prairie semi-naturelle du Jura Suisse. *ECOVEG* 8, *8th Conference of Plant Community Ecology,* 28th-30th March, Nancy, France.

4. **Mariotte, P.**, Vandenberghe, C., Buttler, A. (2011) Subordinate plant species promote resistance of plant community during summer drought. *54th Symposium of the International Association for Vegetation Science* (IAVS) 20–25th June, Lyon, France.

5. **Mariotte, P.**, Meugnier, C., Vandenberghe, C., Mitchell, E., Buttler, A. (2010) Espèces subordonnées et champignons mycorhiziens: symbiose préférentielle? *ECOLOGIE 2010*, 2–4th September, Montpellier, France.

6. **Mariotte, P.**, Vandenberghe, C., Buttler, A. (2010) Ranking of plant species: from dominant to subordinate, what's effect of root competition? *7th European Conference on Ecological Restoration,* 23–27th August, Avignon, France.

7. **Mariotte, P.**, Meugnier, C., Vandenberghe, C., Mitchell, E., Buttler, A. (2010) Subordinate species and arbuscular mycorrhizal fungi: Preferential symbiosis interactions? *7th European Conference on Ecological Restoration,* 23–27th August, Avignon, France.

8. **Mariotte, P.**, Vandenberghe, C., Buttler, A. (2010) Hiérarchie d'espèces végétales: de dominante à subordonnée, quel rôle de la compétition racinaire? *ECOVEG 6*, *6th Conference of Plant Community Ecology,* 31th March–2nd April, Rouen, France.

9. Vanbriel, M., Vangeel, R., **Mariotte, P.**, Vandenberghe, C., Buttler, A. (2010) De rol van niet dominante planten in begraasde soortenrijke graslanden in het Zwitserse Juragebergte. *Starters in het natuuronderzoek,* 18th March 2010. Brussels, Belgium.

10. **Mariotte, P.**, Vandenberghe, C., Buttler, A. (2009) Rôle des plantes subordonnées dans la structure des communautés végétales et sur le fonctionnement de l'écosystème pâturage. *ECOVEG 5*, *5th Conference of Plant Community Ecology,* 8–10th April, Gembloux, Belgium.

Posters presentation at conferences

1. **Mariotte P.**, Vandenberghe, C., Buttler, A. (2011) Rôle des espèces végétales subordonnées dans la résistance des communautés face au stress hydrique. *ECOVEG 7*, *7th Conference of Plant Community Ecology*, 30th March–1st April, Lausanne, Switzerland. Award for the best poster.

2. Meugnier C., **Mariotte P.**, Mitchell E., Buttler A., (2010) Subordinate species and arbuscular mycorrhizal fungi: Preferential symbiosis interactions? *Networks of Power and Influence. A one day Symposium on Symbiotic Associations between Plants and Mycorrhizal Fungi.* 22th September, Agroscope ART, Switzerland.

3. **Mariotte, P.**, Vandenberghe, C., Buttler, A. (2009) What makes a plant dominant or subordinates in a community? Common garden test of pairwise interactions and above and belowground competition. *Green Days of Environmental Engineering Institute (IEE)*. 3–4th September, Arolla, Switzerland.

Course and meeting

1. Annual Meeting of Soil and Ecosystem Ecology group (R. Bardgett's group) and Center for Ecology and Hydrology (N. Ostle's group) (2011), Lancaster Environment Centre, 29th November, Lancaster, UK.

2. PhD Course on Functioning of Boreal Forest Ecosystems (2011) Swedish University of Agricultural Science, 4–10th June, Umea, Sweden.

3. Adapting Conservation to a Changing Climate (2011) Natural England, British Ecological Society Meeting, 11–12th January, Charles Darwin House, London, United Kingdom

4. Workshop on Grassland Management and Biodiversity (2010), University of Bern, Ecology and Evolution, Conservation Biology, 16-18th June, Bern, Switzerland.

5. First International School of Alpine Ecology and Global Change (2009) Research Group on Ecology of the Alpine Region of Innsbruck University, 4–8th October, University Center Obergurgl, Austria.

Organization of conference

1. Member of organizing committee
   7th Conference of Plant Community Ecology (ECOVEG 7)
   30th March – 1st April 2011, Ecole Polytechnique Fédérale de Lausanne
   CH-1015 Lausanne (Switzerland)
   ecoveg7@epfl.ch
   http://ecos.epfl.ch/ecoveg7/

Student assistantship

1. Janina Milkereit (University of Aberdeen, United Kingdom) – Master project 2011. The importance of arbuscular mycorrhizal fungi for subordinate plants and its implications for community composition.

2. Katrijn Sabbe (KHKempen, Belgium) - Master project 2010-2011. Role of subordinate plant species on plant community after summer drought.

3. Claire Meugnier (BGS, Unine, Switzerland) - Master project 2009-2010. Arbuscular mycorrhiral fungi and subordinate plant species: Preferential symbiotic interactions?

4. Michaël Vanbriel (KHKempen, Belgium) - Master project 2009-2010. The spatial distribution of subordinate species - A field experiment in the Swiss Jura Mountains.

5. Ruben Vangeel (KHKempen, Belgium) - Master project 2009-2010. Hiérarchie compétitive d'espèces végétales - Définir des plantes dominantes et subordonnées.

6. Stéphanie Pilet (SIE, EPFL, Switzerland) - Master project 2008-2009. Etude des interactions de compétition entre plantes subordonnées et dominantes.