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Trait-mediated effect of arbuscular mycorrhiza on the competitive effect and response of a monopolistic species

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Summary

1. Cessation of agricultural practices often leads to a dramatic decline in species diversity concomitant with the increase in abundance of monopolistic species, which have been hypothesized to be strongly arbuscular mycorrhiza (AM) dependent. AM can affect competitive interactions and influence plant species diversity. Few studies have tested AM effects on the competitive strategy of monopolistic species although it has been shown to be a key parameter to explain their high dominance. In addition, it is not clear whether AM effects on plant interactions can be explained by density-mediated interactions or by trait-mediated interactions.

2. We measured the competitive effect of *Festuca paniculata*, a widespread monopolistic species from subalpine communities, on itself and on two additional target species with and without AM (benomyl treatment) under contrasted fertilised treatments. AM effects on target species traits were quantified.

3. The three target species exhibited contrasted AM dependency and only *F. paniculata* was positively affected by AM presence in fertilised conditions. In the fertilised treatment, AM decreased intra-specific competition and increased inter-specific competition. Changes in competitive responses were explained by the AM effect on P inflow and species lateral spread.

4. Our results highlight AM ability to modify the performance of monopolistic species under different environmental conditions. AM provide an important mechanism by which monopolistic species can maintain a high level of dominance and dramatically decrease species diversity following agricultural abandonment.

Key-words: AM dependency, arbuscular mycorrhiza, competitive effect and response, fertilisation, land use change, monopolistic species, plant functional traits, subalpine grasslands

Introduction

Theoretical studies have suggested that land-use change will be one of the most important drivers of future global diversity change for terrestrial ecosystems (Vitousek *et al.* 1997; Chapin *et al.* 2000). In semi-natural grasslands, increasingly submitted to cessation of traditional agricultural practices (e.g. mowing and grazing), a large decline of species and functional diversity is observed (Tasser & Tappeiner 2002; Quétier, Thebault & Lavorel 2007a). This drop is often a consequence of community invasion by one main species type, i.e. monopolistic species (Willems & vanNieuwstadt 1996; Anthelme *et al.* 2003; Quétier, Thebault & Lavorel 2007a; Liancourt, Viard-Cretat & Michalet 2009). Monopolistic species occur across a wide range of ecological conditions from mediterranean (Fynn, Morris & Kirkman 2005; Violle, Richarte & Navas 2006) to lowland (Hartnett & Wilson 1999), montane (Corcket *et al.* 2003; Liancourt,

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Viard-Cretat & Michalet 2009), and subalpine grasslands (Quétier, Thebault & Lavorel 2007a). These tall plant species are characterized by a slow growth rate and conservative plant trait syndrome (i.e. high nutrient use efficiency, dense tissues, see Gross, Suding & Lavorel 2007 and references therein). They can reach a high level of dominance in undisturbed habitat (over 80% of the community) (Liancourt, Viard-Cretat & Michalet 2009 and references therein) leading to stable community states (Petraitis & Latham 1999; Quétier *et al*. 2007b; Liancourt, Viard-Cretat & Michalet 2009). The success of this species type (also defined as conservative competitor, see Liancourt, Viard-Cretat & Michalet 2009) can be explained by their high competitive response and effect (*sensu* Goldberg & Landa 1991) (Elberse & Berendse 1993; Violle, Richarte & Navas 2006; Gross *et al*. 2009; Liancourt, Viard-Cretat & Michalet 2009), which have been hypothesised to be mediated by mycorrhiza (Petraitis & Latham 1999).

Arbuscular mycorrhiza (AM) are a non-specific association, present in the roots of most plant species (over 80%, Smith & Read 2008). This association ranges along a mutualism-parasitism continuum depending on the life history of plant species and ecological conditions (Johnson, Graham & Smith 1997; Jones & Smith 2004). AM can determine the outcome of plant–plant interactions (Hartnett *et al*. 1993; van der Heijden, Wiemken & Sanders 2003; Scheublin, Van Logtestijn & Van der Heijden 2007) and some species benefit more from AM than others (Smith & Read 2008). Links between species AM dependency (a species is AM dependent when more biomass is produced with AM presence than without AM, in the absence of plant–plant interactions) and the outcome of plant–plant interactions are likely to influence species diversity in natural plant communities (Urcelay & Diaz 2003). AM increase species coexistence when the dominant species is less AM-dependent than subordinate species (Grime *et al*. 1987; van der Heijden *et al*. 1998). In this case, AM could promote species coexistence by increasing intra-specific competition and decreasing inter-specific competition (Moora & Zobel 1996). Conversely, AM can decrease diversity, favouring highly-AM-dependent dominant species, by increasing inter-specific competition and decreasing intra-specific competition (Hartnett *et al*. 1993; Hartnett & Wilson 1999, 2002). Despite recent theoretical and empirical developments (Hartnett & Wilson 2002; Urcelay & Diaz 2003; Collins & Foster 2009), few studies have tested the effect of AM on the competitive strategy of monopolistic species, although it have been shown that these species, e.g. *Brachypodium pinnatum* (Liancourt, Viard-Cretat & Michalet 2009), are strongly AM dependent (van der Heijden *et al*. 1998).

Plant competitive response can be related to variations in competitor biomass, or alternatively is determined by trait-mediated interactions (per gram effect, *sensu* Goldberg *et al*. 2001), i.e. the competitive intensity observed on target species is not correlated with variations in competitor biomass (Okuyama, Benjamin & Bolker 2007). Surprisingly, few studies have investigated the effect of AM on plant functional traits and how this could be related to the competitive response of individual target plants (Smith & Read 2008). By modifying nutrient availability and C economy within the plant, AM may for instance affect belowground and aboveground interactions through the modification of leaf morphology (Grimoldi *et al*. 2005), plant stature (Hartnett & Wilson 2002) or root traits (Eissenstat *et al*. 1993).

Here, we investigated the impact of AM on the competitive strategy of the monopolistic species *Festuca paniculata* (Fig. 1). This species dominates low diversity subalpine grasslands where mowing has been abandoned for ca. 30 years and productivity is intermediate (Quétier, Thebault & Lavorel 2007a). Under these conditions, strong competitive responses and effects have been shown to directly explain its high dominance (Gross *et al*. 2009). In a mesocosm experiment, we quantified the competitive effect of *F. paniculata* with or without AM on three target species which co-occur in subalpine grasslands (multiple target species approach) at two levels of fertilisation (to mimic low and intermediate levels of productivity). Target species were: *F. paniculata* used to quantify intra-specific interactions; *Bromus erectus* and *Dactylis glomerata* to measure inter-specific interactions. These two grass species are subordinate in subalpine grasslands where *F. paniculata* is highly dominant and are characterized by contrasting ecological strategies and abundance patterns in the field (Gross, Suding & Lavorel 2007). We predicted that: (i) the monopolistic species *F. paniculata* is more dependent on AM than other subordinate grass species (Reynolds *et al*. 2005); (ii) the competitive effect of *F. paniculata* increases with AM presence; and (iii) inter-specific competition increases whereas intra-specific competition decreases with AM presence.

To understand whether competitive responses of individual target species are mediated by AM effects on their traits, AM effects on target species functional traits were quantified in a greenhouse experiment at low and intermediate levels of fertility. Although AM are thought to benefit plant species in nutrient-stressed environment, AM dependency is species-specific and context dependent (van der Heijden *et al*. 1998; Cahill *et al*. 2008; Smith & Read 2008). Patterns of

Fig. 1. View of a *Festuca paniculata* grassland (foreground) near the Lautaret Pass, France, in July 2005. Photo credit: Nicolas Gross.

abundance observed in the field (Quétier, Thebault & Lavorel 2007a) suggest that AM might be more beneficial for *F. paniculata* at intermediate level of fertility. Therefore, we suggest that AM increase competitive effect of *F. paniculata* with increasing soil fertility.

Materials and methods

STUDY SITE

The experiment was located at the experimental garden of the Station Alpine Joseph Fourier, Lautaret Pass, central French Alps (Villar d'Arêne, 45·04°N, 6·34°E, elevation 2100 m a.s.l). The climate is subalpine with a pronounced continental influence. Mean annual precipitation is 956 mm and mean monthly temperatures range between −7·4 °C in February and 19·5 °C in July. The growing season starts after snowmelt, between mid-April and early May, and finishes at the end of September. A more exhaustive description of the field site and vegetation can be found in Quétier, Thebault & Lavorel (2007a).

TARGET SPECIES

Festuca paniculata (L.) Schinz and Thellung is a C3 tussock grass (Fig. 1). This species is widespread over a range of ecological conditions in subalpine grasslands and becomes highly dominant (over 80% of community biomass) when mowing is abandoned (Quétier, Thebault & Lavorel 2007a) and where water is not limiting (Gross *et al*. 2008). Grasslands dominated by *F. paniculata* have an intermediate level of aboveground productivity (ANPP = 0·0532 kg m^{-2} day^{-1}) compared to other temperate grassland systems, but this corresponds to the highest level of productivity in subalpine grasslands encountered at our field site (Quétier, Thebault & Lavorel 2007a).

To measure the competitive effect of *F. paniculata,* we chose three C3 perennial grasses differing in their abundance across subalpine grasslands. We selected: *F. paniculata* (to test intra-specific interactions), *B. erectus* (L.) and *D. glomerata* (L.) (to test inter-specific interactions). *Dactylis glomerata* dominates fertilised and mown subalpine grasslands with comparable levels of productivity to *F. paniculata* meadows (ANPP = 0·0459 kg m^{-2} day^{-1}) (data source Quétier, Thebault & Lavorel 2007a). This species is characterized by an exploitative competitive strategy (Grime 1977; Gross, Suding & Lavorel 2007). A previous study (Reynolds *et al*. 2005) showed that the growth of this species type is generally negatively affected by AM. *Bromus erectus* dominates dry and unproductive subalpine grasslands (ANPP = 0·0323 kg m^{-2} day^{-1}) and is classified as having a stress-tolerant strategy (Grime 1977). This species has been found to not be affected by AM presence (van der Heijden *et al*. 1998). Mycorrhizal associations and their effects have not been documented for the monopolistic species *F. paniculata* prior to this study. Tillers of the three target species were collected in the field where *F. paniculata* is highly dominant (unmown *F. paniculata* grassland) in October 2004 and propagated in the experimental garden in their natural soil community during the following winter and spring.

THE COMPETITION EXPERIMENT

Matrix construction

To test the competitive effect of this species, *F. paniculata* mono-specific stands (henceforth matrices) were established. Tillers were collected in August 2003 in the field and conserved individually in pots in their natural soil community in the experimental garden. In October 2003, sixteen tillers of similar size were randomly selected and planted in a regular pattern in 33-cm diameter pots (16 L) (See Appendix S1 in Supporting Information). Bare soil pots were also installed at this time in order to provide additional plant isolated individuals of the target species.

Pots were filled with a soil composed of 2/3 of sand, 1/4 of calcinated clay and 1/12 commercial potting compost (Fertiligène®, Scotts, Eully, France). Two soil treatments were established to mimic low and intermediate level of fertility levels in the field. Field N concentration has been quantified by previous studies at the study site (Quétier, Thebault & Lavorel 2007a; Robson *et al*. 2007). *P* concentration was also measured by these previous studies using standardised protocols (Olsen *et al*. 1954; F. Quétier unpublished data*)*. The soil mixture in the unfertilised pots corresponded to the lowest nutrient level measured in subalpine grasslands (Tosca & Labroue 1986) dominated at the study site by *B. erectus* (Matrix mineral N and P concentration: $N = 6{\cdot}1 \pm 0{\cdot}1$ mg kg^{-1} of soil; $P = 12 \pm 0{\cdot}3$ mg kg^{-1} of soil). The other half of the pots were fertilised yearly by adding one 15-g dose of commercial slow-release fertiliser (12% N, 10% P, 12% K, 2% Mg), which mimicked intermediate levels of fertility in *F. paniculata* grasslands (Matrix mineral N and P concentration: $N = 81 \pm 0{\cdot}2$ mg kg^{-1} of soil; $P = 74 \pm 0{\cdot}3$ mg kg^{-1} of soil) (Quétier, Thebault & Lavorel 2007a; Robson *et al*. 2007).

As we used tillers from the field, we assume that AM infection was conserved. Additionally, to ensure successful mycorrhizal infection, each pot was inoculated with a soil solution (100 g soil per litre tap water) taken from the field where *F. paniculata* is highly dominant. Pots were watered daily using an automated system. In total, 40 matrices of *F. paniculata* were planted and 84 pots with only bare soil were prepared for the experiment. Matrices were grown for 2 years at the experimental garden before they achieved sufficient biomass to mimic the biomass of *F. paniculata* under field conditions. Soil fungal community inoculation (using soil from unmown *F. paniculata* grassland) and fertilisation were performed every year as described above. During winter, pots were buried to protect roots from frost.

The experimental design

An experiment using a full-factorial design was conducted over one growing season (from 15 May to 29 August 2005, 107 days). Target species responses to *F. paniculata* competition (with or without neighbours), to fertilisation (with and without fertilisation), to the presence of AM (with and without Benomyl) and their interactions were tested.

Before planting, each target species was cut to 3-cm in length for shoots and 5-cm depth for roots and weighed (total initial Fresh Biomass, FBM_i). Fifteen tillers of each target species were then selected; their shoots and roots were separated, dried at 60 °C for 48 h and weighed. The initial allocation to shoot (AS_i) of selected tillers for each species was calculated, and Dry Mass Content (DMC) was calculated as: DMC, dry weight/fresh weight. The initial aboveground dry biomass (above BM_i) of each tiller used as a target species in the experiment was calculated as: $aboveBM_i = FBM_i \times DMC \times AS_i$.

Target species (one tiller per species) were planted either within the matrices (with neighbours) or singly at the centre of the bare soil pots (without neighbour) (see Fig. S1 in Appendix S1). We assume that during the experiment, interactions among the three target species were negligible due to their small biomass as compared to dense *F. paniculata* matrices (over all treatments, target species represented

less than 10% of the total biomass of the matrices). At the beginning of the experiment, each target species was under the canopy of *F. paniculata* matrices and received similar amounts of light (on average 748 ± 63 μmol m^{-2} s^{-1}).

To test the role of AM on the competitive effect of *F. paniculata*, we used a commercial fungicide (benomyl), which has no phytotoxic action (Pedersen & Sylvia 1997), to eliminate AM from the plant host. Benomyl was added at the beginning of the experiment (May 2005) and then every 3 weeks (Hetrick, Wilson & Hartnett 1989) in benomyl matrices (50 mg of benomyl in 100 mL of distilled water for 1 kg of soil). The use of benomyl can have potential confounding effects (Cahill *et al.* 2008). For example, benomyl can kill not only AM fungi but also soil pathogenic fungi (Pedersen & Sylvia 1997), which may affect plant growth. An alternative treatment often applied consists in the use of sterile soil and selective inocula, however it is not representative of the highly diverse natural fungi community (Hartnett & Wilson 2002). In our study, it appears that the use of benomyl was one of the best solutions (Smith, Hartnett & Rice 2000; Hartnett & Wilson 2002) as we mimic natural field conditions as closely as possible in the competition experiment. Greatest advances will come when both of these approaches will be used and compared in a diversity of studies (see Cahill *et al.* 2008).

Overall, in the pots without neighbours, six replicates per target species and per fertilisation treatment were made without AM (benomyl treatment) (see Appendix S1). Eight replicates per species and per fertilisation treatment were grown with AM (Control treatment). In the *F. paniculata* matrices (with neighbour), ten replicates were performed per treatment (combined benomyl and fertilisation) and per target species. In total, 204 tillers of target species were planted [(3 species cultivated with benomyl and without neighbours × 2 fertilisation levels × 6 replicates) + (3 species cultivated without benomyl and without neighbours × 2 fertilisation levels × 8 replicates) + (3 species cultivated with neighbours × 2 fertilisation levels × 2 benomyl levels × 10 replicates)]. Pots were moved periodically within the experimental garden throughout the course of the experiment, thereby making the spatial design fully random.

At the peak of biomass production (30 July 2005), matrices height and light availability were measured for each species in each pot. Light interception by the matrices was quantified at 2 cm aboveground using a LI-190 Quantum Sensor (LI-COR®, Eurosep Instrument, Cergy-Pontoise, France) under full sun between 11 AM and 2 PM for 20 random points per pot. We found a weak but significant difference in light interception in the fertilised matrices compared to light interception in the field measured in a previous study at peak biomass (data from Quétier, Thebault & Lavorel 2007a) (15% difference, data not shown), indicating that the cover of *F. paniculata* in matrices was slightly lower than in the field. Average light availability during the experiment was 1850·7 μmol photon m^{-2} s^{-1}.

At the end of the experiment, 98% of target individuals survived, and there were no trends in mortality among treatments. Target species were harvested in the pots with (*F. paniculata* matrices) and without neighbours. Aboveground biomass of the matrices was estimated by harvesting a sub-sample of the biomass (1/4 of the matrices). Green and dead biomass was carefully washed, dried for 72 h at 60 °C and weighed. For belowground biomass, two soil cores were taken (25 cm depth, 4·5 cm diameter) within each pot. Roots were extracted and carefully washed with tap water and tweezers. Sub-samples of roots were kept in ethanol (5%) for mycorrhizal counts. Roots were dried and weighed as for aboveground parts. Total belowground biomass of the *F. paniculata* matrices was calculated as the root biomass recovered in the soil cores X (pot soil volume/core soil volume).

THE TRAIT EXPERIMENT

During the winter 2004, a companion experiment was conducted to test AM dependency of the target species and AM effects on plant functional traits. Traits considered were related to the plant ability to acquire and use below- and aboveground resources (Maire *et al.* 2009): lateral spread (LS), allocation to roots (AR), relative growth rate (RGR), specific leaf area (SLA) and nutrient inflow for P and N. For each target species, single tillers were planted in two-liter pots in the experimental greenhouse of the Centre d'Ecologie Fonctionnelle et Evolutive (CEFE, CNRS), in Montpellier (France), in early January 2004 (average mean temperature 15·4 °C; average light availability 780 μmol photon m^{-2} s^{-1}). To ensure the comparability between the two experiments, we used similar soil, experimental treatments and methods to those previously described for the main competition experiment (two levels of fertilisation, with and without benomyl). Ten replicates per treatment and species were planted. The experiment started on 20 January 2004 and was harvested on 1 April. Each pot was randomly placed and moved weekly within the greenhouse. Plants were watered weekly. Initial and final above and belowground biomasses as well as plant height were measured as described previously in the competition experiment. SLA and LS were measured following standardized protocols (Cornelissen *et al.* 2003). The total N and P concentrations were determined on whole plant using a subsample of four replicates per species and treatment. N concentration was determined using an elemental analyzer (Carlo Erba Instruments, model EA 1108, Milan, Italy). Phosphorus assays of shoot and root tissues were carried out after acid digestion using concentrated sulfuric acid and hydrogen peroxide at 100 °C for 35 min and 360 °C for 2 h. Then phosphorus concentration was determined with a colorimetric autoanalyser (alliance Instrument Evolution II, Frépillon, France), using the molybdenum blue method (Grimshaw, Allen & Parkinson 1989). N and P inflows for each target species and treatment was calculated as:

$$\text{Inflow Nut} = [(\text{BM}_f \times \text{Nut}_f) - (\text{BM}_i \times \text{Nut}_i)]/(\text{RBM}_f - \text{RBM}_i),$$

where BM is total plant biomass (above + belowground), Nut is phosphorus or nitrogen concentration (above + belowground) and RBM is the root biomass. *i* and *f* refer to the measurement performed at the beginning (initial) or the end of the experiment (final) respectively. N and P inflows are thus defined as the quantity of nutrients which were assimilated by target species per unit of root biomass produced during the experiment (modified from Staddon, Fitter & Graves 1999).

QUANTIFICATION OF AM COLONISATION

For the two experiments, AM colonisation was assessed in treatments with and without benomyl using a sub-sample of four replicates from each treatment. Roots (without apparent suberin) were cleared and stained following Grace & Stribley (1991). They were mounted on semi-permanent slides in polyvinyl-lactic acid-glycerol (PVGL). AM colonisation was recognized by well stained, rarely septated, and irregular hyphae, vesicles and arbuscules. Root endophyte quantification was made using the magnified intersection method (McGonigle *et al.* 1990) under a compound microscope (Kyowa optical, Model LSCB-VC-2B-L), magnification was ×400. Eighty to one hundred intersections per sample were scored. All three target species were colonized by AM and benomyl significantly and evenly decreased AM colonisation for each species in each experimental treatment (analyses not shown). Mycorrhizal infection rates were 13 ± 2·2% and

0·16 ± 0·17% for: *Bromus erectus*; 7·6 ± 1·4% and 1·97 ± 0·87% for *D. glomerata*; and 3·8 ± 1·1% and 0·07 ± 0·07% *F. paniculata* in AM versus non AM treatments respectively. Mycorrhizal infection in *F. paniculata* matrices was strong and significantly decreased with benomyl application (75 ± 7% and 44 ± 3%). Hereafter the two AM treatments were thus referred in the text as AM+ (without benomyl) and AM- (with benomyl).

CALCULATIONS AND DATA ANALYSIS

Growth of each target species was estimated by calculating the above-ground relative growth rate (RGR) in the two experiments:

$$\text{RGR} = \ln(\text{final above ground biomass}) - \ln(\text{initial above ground biomass})/(\text{Time})$$

The competitive effect of *F. paniculata* matrices was measured on each target species comparing their growth with or without neighbours using the natural-log response ratio (LnRR) (Suding, Goldberg & Hartman 2003) as:

$$\text{LnRR} = \ln\frac{\text{RGR of the species j with Festuca matrix}}{\text{Average RGR of species j without Festuca matrix}}$$

We calculated this index independently in the treatments with and without benomyl to obtain target species responses with and without the presence of AM, and with and without fertilisation, to estimate how fertilisation mediated competition and AM responses. RGR observed in the greenhouse experiment were strongly related with RGR observed without neighbours in the competition experiment ($r^2 = 0{\cdot}95$, $P < 0{\cdot}0001$). This result indicated that experimental conditions between the two experiments were close enough to ensure comparability.

In order to measure the competitive outcome controlling for size of the competitor (*F. paniculata*) the competitive effect per gram of *F. paniculata* was estimated on the three target species as follows:

$$\text{LnRR (per gram)} = \frac{\text{LnRR of the species j in treatment k}}{\text{Total biomass of } \textit{Festuca paniculata} \text{ matrice in treatment k}}$$

Comparing this index with LnRR per plant allowed us to test whether plant–plant interactions are mediated by variation in competitor biomass (density mediated interactions) or by trait-dependent mechanisms (trait-mediated interactions) (Goldberg *et al.* 2001; Okuyama, Benjamin & Bolker 2007). This index was also used to test the relationship between trait variations in response to experimental treatments and the outcomes of competitive interactions.

The response to fertilisation and benomyl addition was analyzed for biomass production of *F. paniculata* and light interception in matrices using a two-way ANOVA. For target species, two sets of analyses were conducted. We used a three-way ANOVA with fixed factors to test the effects of species and experimental treatments (fertilisation and benomyl) on relative growth rate, and traits (AR, SLA, RGR, LS, N and P inflow) in the trait experiment. A similar analysis was conducted in the competition experiment to evaluate competitive responses of target species (LnRR and LnRR per gram). *Post-hoc* analyses were conducted for each species in each fertilisation treatment using Tukey HSD to test for statistical differences in LnRR and LnRR per g between benomyl treatments. For each fertilisation treatment, the competitive response hierarchy was examined by comparing the mean LnRR between species using Tukey HSD. Similarly, we compared trait values between species and treatments using Tukey HSD. Linear regressions between competitive effect per g (LnRR per g) and competitive effect per plant (LnRR) were conducted separately with and without fertilisation. Finally, to investigate how traits were related with the competitive response of the target species, linear regression analyses were conducted between traits measured in the trait experiment and LnRR (per plant and per g). Data were transformed to meet ANOVA's assumptions when necessary. All statistical analyses were performed using the software JMP 5.0.1. (The SAS Institute, Cary, NC, USA).

Results

TARGET SPECIES RESPONSES TO AM AND FERTILISATION IN THE TRAIT EXPERIMENT

The responses of target species grown without neighbours (trait experiment) to fertilisation differed across target species (Table 1). The RGR of *B. erectus* and *D. glomerata* increased significantly more with fertilisation than that of *F. paniculata* (Fig. 2). The three target species also had contrasting responses to benomyl addition and their responses depended on fertilisation (Table 1). None of the species were affected by AM in the unfertilised treatments. In contrast, target species had contrasted responses to AM in fertilised treatments (Fig. 2). Only *F. paniculata* appeared as AM dependent with fertilisation. *Bromus erectus* was not affected by AM presence, and *D. glomerata* was negatively affected by the AM+ treatment.

All traits were strongly affected by experimental treatments (Table 1, Fig. 3). AM presence generally decreased species LS without fertilisation. Lateral spread of *F. paniculata* increased in presence of AM with fertilisation, whereas LS of *D. glomerata* decreased and remained constant for *B. erectus* (Fig. 3a,b,c). AR generally increased with AM presence for *B. erectus* and *D. glomerata* but no change was observed for *F. paniculata* (Fig. 3d,e,f). Contrasted results were observed for each species when considering SLA (Fig. 3g,h,i). SLA decreased for *B. erectus* in the AM+ treatment, whereas it increased for *D. glomerata*. No effect was observed for *F. paniculata*. Finally, N and P inflows were generally lower in the AM+ treatment for *B. erectus* and *D. glomerata* (Fig. 3j,k,m,n). In fertilised treatment, both P and N inflows increased substantially in the AM+ treatment for *F. paniculata* (Fig. 3l,o).

BIOMASS PRODUCTION OF *FESTUCA PANICULATA* MATRICES

Total biomass production of *F. paniculata* matrices was significantly affected by the experimental treatments (Fertilisation: $F_{1,37} = 152$; $P < 0{\cdot}0001$, benomyl: $F_{1,37} = 3{\cdot}66$; $P > 0{\cdot}05$; Fertilisation x benomyl: $F_{1,37} = 10{\cdot}85$; $P < 0{\cdot}001$; Fig. 4). Overall, fertilisation increased biomass production of the matrices ($P < 0{\cdot}0001$) but the fertilisation effect was modified by benomyl ($P < 0{\cdot}001$). Presence of AM had no

Table 1. Effect of experimental treatments observed in the trait experiment on relative growth rate (RGR) and traits: lateral spread (LS), allocation to root (AR), specific leaf area (SLA), and P, N inflows

Factors		RGR	AR	LS	SLA	Inflow P	Inflow N
	d.f.	*F* ratio	*F* ratio	*F* ratio	*F* ratio	*F* ratio	*F* ratio
Sp.	2	53.4***	230.59***	16.73***	230.59***	109.74***	300.86***
Fert.	1	49.76***	169.01***	16.1***	169.01***	865.93***	1252.81***
Sp. × Fert.	2	33.04***	9.94***	3.49*	9.44***	54.14***	222.17***
Benomyl	1	5.31*	22 97***	1.18 NS	22.97***	> 0.0001 NS	15.19***
Sp. × Benomyl	2	5.8**	7.63**	10.27***	7.63***	30.3***	18.12***
Fert. × Benomyl	1	3.75*	4.27*	17.5***	4.27*	14.14***	27.18***
Sp. × Fert. × Benomyl	2	4.45*	1.26 NS	3.98*	1.26 NS	34.68***	47.34***
Error		99	99	101	102	36	36

d.f., degree of freedom; Sp., species; Fert., fertilisation; we indicated results from full factorial ANOVA: NS, $P > 0{\cdot}05$; *$P < 0{\cdot}05$; **$P < 0{\cdot}01$; ***$P < 0.001$.

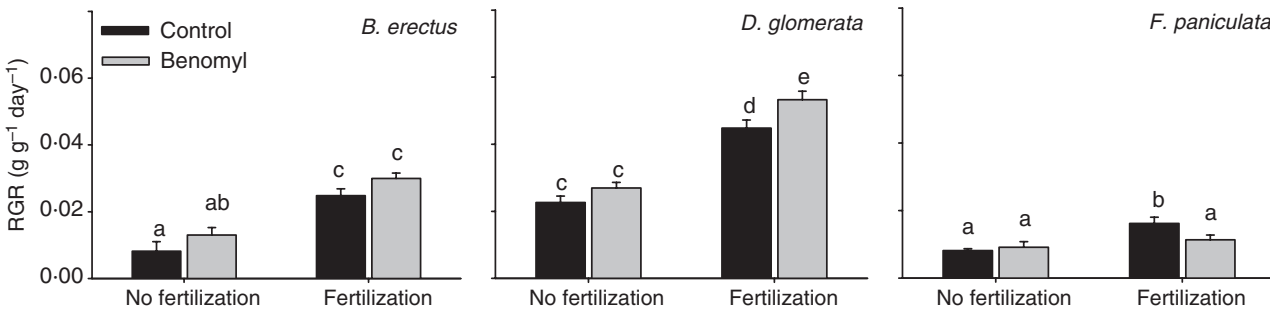

Fig. 2. Mycorhizal dependency of individual target species measured in the trait experiment, for *B. erectus, D. glomerata* and *F. paniculata* with and without fertilisation. Mycorrhizal dependency is assessed as the growth rate increment in the presence of AM. Black bars correspond to the control treatment and grey bars to benomyl application. We compared traits value between species and treatments for each trait (Tukey HSD, small letter). When letters are different it indicates a significant difference among species and treatments.

effect on biomass production without fertilisation (despite a negative trend), whereas biomass was substantially enhanced in the AM+ treatments with Fertilisation. Light interception within *F. paniculata* matrices responded in a similar way than biomass to experimental treatment (data not shown). Light interception peaked in the AM+ and fertilised treatment. There was no effect of AM treatments on light interception without fertilisation.

COMPETITIVE EFFECTS OF *FESTUCA PANICULATA*

The target species responded differently to competition from *F. paniculata* (LnRR) (Table 2, Fig. 5). Without fertilisation, *B. erectus* experienced less competition in the AM+ than in the AM- treatment (Fig. 5a), whereas both *D. glomerata* (Fig. 5b) and *F. paniculata* (Fig. 5c) suffered more from competition in the AM+ treatment. With fertilisation, *F. paniculata* was less affected by competition in AM+ than in AM− (Fig. 5c), indicating that intra-specific competition decreased with AM at higher levels of nutrients. In contrast, *D. glomerata* and *B. erectus* experienced more competition in AM+ than in AM−, indicating that inter-specific competition increased with AM with increasing nutrient availability.

The competitive response hierarchy was modified by fertilisation and AM treatments (Sp. × Benomyl × Fert., $P < 0{\cdot}0001$, Table 2). Without fertilisation and in AM+, *D. glomerata* and *F. paniculata* experienced more competition than *B. erectus* (best competitive response) (Fig. 5). Without fertilisation and in AM−, *B. erectus* had the worst competitive response, *F. paniculata* was intermediate and *D. glomerata* was the least affected by competition. With fertilisation and in AM+, *B. erectus* experienced the most competition whereas *F. paniculata* and *D. glomerata* had the same competitive response. Finally, with fertilisation and in AM− *F. paniculata* had the worst competitive response, whereas *B. erectus* was intermediate and *D. glomerata* had the best competitive response.

PER GRAM EFFECTS OF *FESTUCA PANICULATA* MATRICES

Per gram competitive effects of *F. paniculata* matrices varied markedly across target species and mirrored LnRR. The per gram competitive effect on *B. erectus* was not affected by AM treatments (Fig. 5d). The AM+ treatment strongly increased the per gram effect of *F. paniculata* on *D. glomerata* with and without fertilisation (Fig. 5e). The per gram effect of

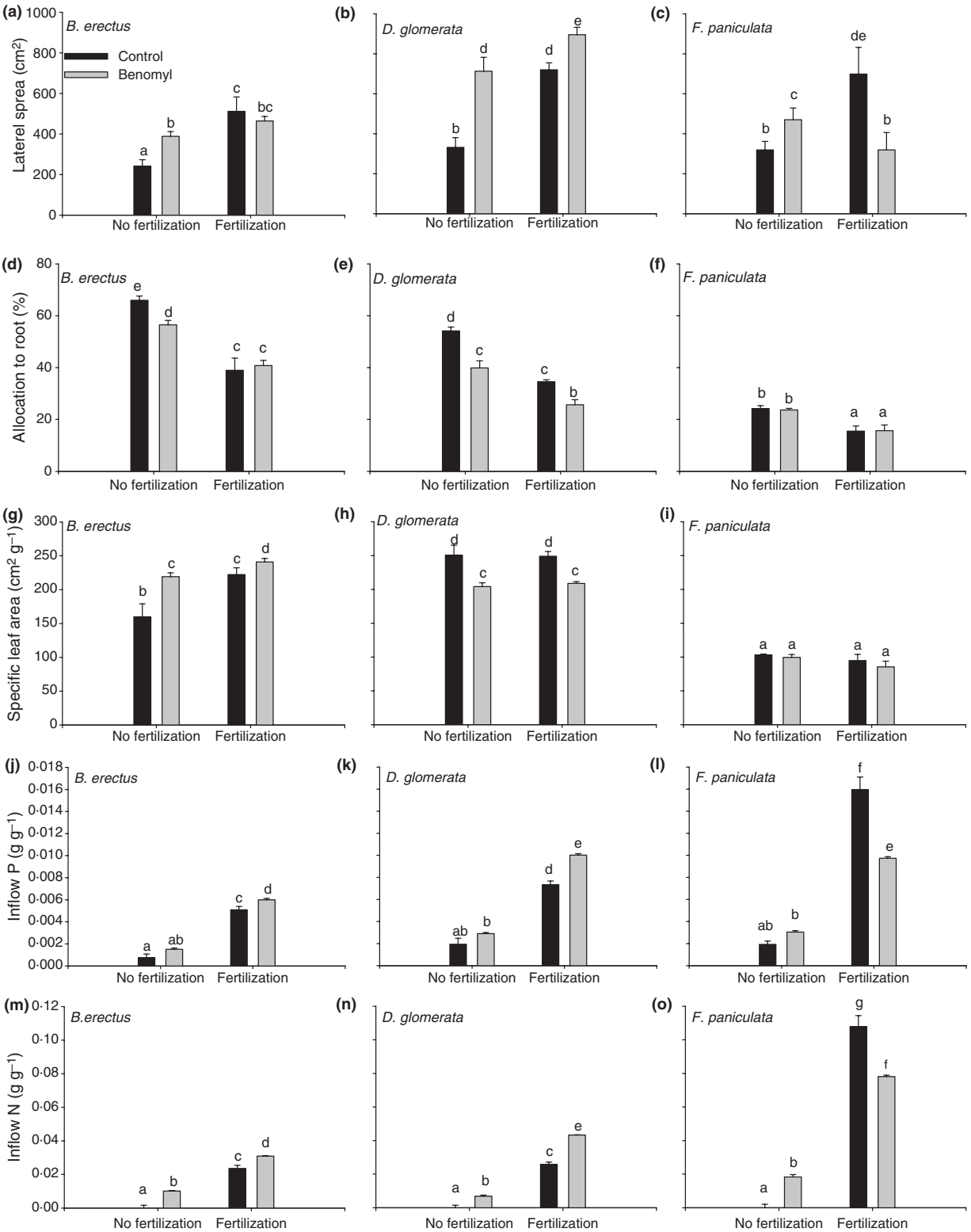

Fig. 3. Trait variations in response to fertilisation and benomyl treatments in the trait experiment: lateral spread (a, b, c); root allocation (d, e, f); specific leaf area (g, h, i); Inflow P (j, k, l); Inflow N (m, n, o). Black bars correspond to the control treatment and grey bars to benomyl application. We compared traits value between species and treatments for each trait (Tukey HSD, small letter).

F. paniculata on itself was higher in the AM + without fertilisation, but was lower in the AM + treatment with fertilisation (Fig. 5f). When considering all species, there was no significant relationship between variation of the intensity of competitive interaction (LnRR) and the total biomass of the matrices (r^2 = 0·06; P > 0·05). In addition, there was a

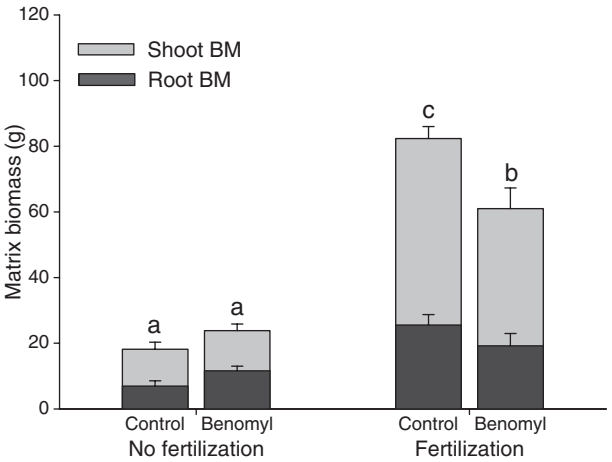

Fig. 4. Growth response to fertilisation and Benomyl treatments for *F. paniculata* matrices. We tested the effect of benomyl application within each fertilised treatment using Tukey HSD test (small letter).

Table 2. Effect of experimental treatment observed in the competition experiment on target species competitive response calculated with LnRR per plant and LnRR per g

Factors	LnRR		LnRR per gram	
	d.f.	*F* ratio	d.f.	*F* ratio
Sp.	2	27.51***	2	13.92***
Fert.	1	10.10**	1	237.32***
Sp. × Fert.	2	3.66*	2	2.47 NS
Benomyl	1	10.04*	1	30.47***
Sp. × Benomyl	2	20.74***	2	25.59***
Fert. × Benomyl	1	1.66 NS	1	51.01***
Sp. × Fert. × Benomyl	2	18.86***	2	21.19***
Error	74		74	

d.f., degree of freedom; Sp., species; Fert., fertilisation; we indicated results from full factorial ANOVA: NS, $P > 0{\cdot}05$; *$P < 0{\cdot}05$; **$P < 0{\cdot}01$; ***$P < 0.001$.

positive relationship between the competitive effect per plant (LnRR) and the per gram competitive effect (LnRR per g) without fertilisation ($r^2 = 0{\cdot}88$; d.f. 1,5; $P = 0{\cdot}0048$) and with fertilisation ($r^2 = 0{\cdot}81$; d.f. 1,5; $P = 0{\cdot}01$) (Fig. S2). These results indicate that the competitive effect was independent from the biomass variation of *F. paniculata* matrices across treatments.

Target species responses estimated with LnRR per g were correlated with variation of *P* inflow ($r^2 = 0{\cdot}60$; d.f. 1,7; $P = 0{\cdot}002$) and lateral spread ($r^2 = 0{\cdot}68$; d.f. 1,7; $P = 0{\cdot}001$) observed in the trait experiment in response to experimental treatments (Fig. S3). Although other traits (e.g., Allocation to roots, N inflow, SLA) were strongly affected by the benomyl treatment, none of them were significantly related with LnRR or LnRR per g (Fig. S3).

Discussion

The three target species used in our experiment exhibited contrasted responses to benomyl (Fig. 2). These responses were consistent with previous studies, conducted with selective inocula, which found similar variation in AM dependency for grass species (West 1996; van der Heijden *et al.* 1998; Reynolds *et al.* 2005). As a result, we interpreted benomyl responses observed in our study as a direct effect of AM presence. The strong differences in functional traits and strategies among grass species (Grime 1977; Diaz *et al.* 2004; see Gross, Suding & Lavorel 2007 for the selected target species) can explain the variation in AM dependencies observed within this life form (Hartnett *et al.* 1993; West 1996; van der Heijden *et al.* 1998; Reynolds *et al.* 2005; Grimoldi *et al.* 2005).

MYCORRHIZA AND COMPETITION IN CONTRASTING FERTILIZED CONDITIONS

Consistent with recent theoretical and empirical studies (Hartnett & Wilson 1999; Urcelay & Diaz 2003), our results showed that strong links exist between AM dependency and the outcomes of plant–plant interactions, and that these can be modulated by soil fertility (Collins & Foster 2009). In the fertilised treatment, *F. paniculata* was strongly AM dependent compared to other species, confirming our first prediction (Figs 2 and 4). Accordingly, AM increased the competitive effect of *F. paniculata* on target species with different ecological strategies (*sensu* Grime 1977), whereas they decreased intra-specific competition for *F. paniculata*. These results differ from those of Moora & Zobel (1996) who found that AM can increase intra-specific competition and thus increase species coexistence in species-rich communities (Grime *et al.* 1987; van der Heijden *et al.* 1998). However, in communities characterized by low species diversity, AM have been reported to reduce intra-specific but increase inter-specific competition (Hartnett *et al.* 1993). Our results suggest that under intermediate level of fertility, the high AM dependency of *F. paniculata* can enhance subordinate species exclusion and promote its own competitive success.

Conversely, in unfertilised treatments AM did not favour *F. paniculata* (Fig. 2). Intra-specific competition was higher with AM (Fig. 5c). Only *B. erectus* experienced less competition with AM (Fig. 5a), consistent with the fact that this species dominates unproductive mountain grasslands (Liancourt, Corcket & Michalet 2005; Quétier, Thebault & Lavorel 2007a). AM are known to favour high diversity in grasslands dominated by *B. erectus* (Grime *et al.* 1987; van der Heijden *et al.* 1998), although mechanisms that underpin AM effects on species coexistence are still not well established (Scheublin, Van Logtestijn & Van der Heijden 2007). For instance, our results reveal that AM can have a strong impact on the competitive response hierarchy (Fig. 5). In our experiment, AM reduced the competitive ability of the monopolistic species (*F. paniculata*) and the competitive species (*D. glomerata)* in low fertility conditions. AM may thus promote the coexistence of non AM-dependent and stress-tolerant species such as *B. erectus* (sensu Grime 1977) in low fertility grasslands by decreasing competitive abilities of other species types (Urcelay & Diaz 2003).

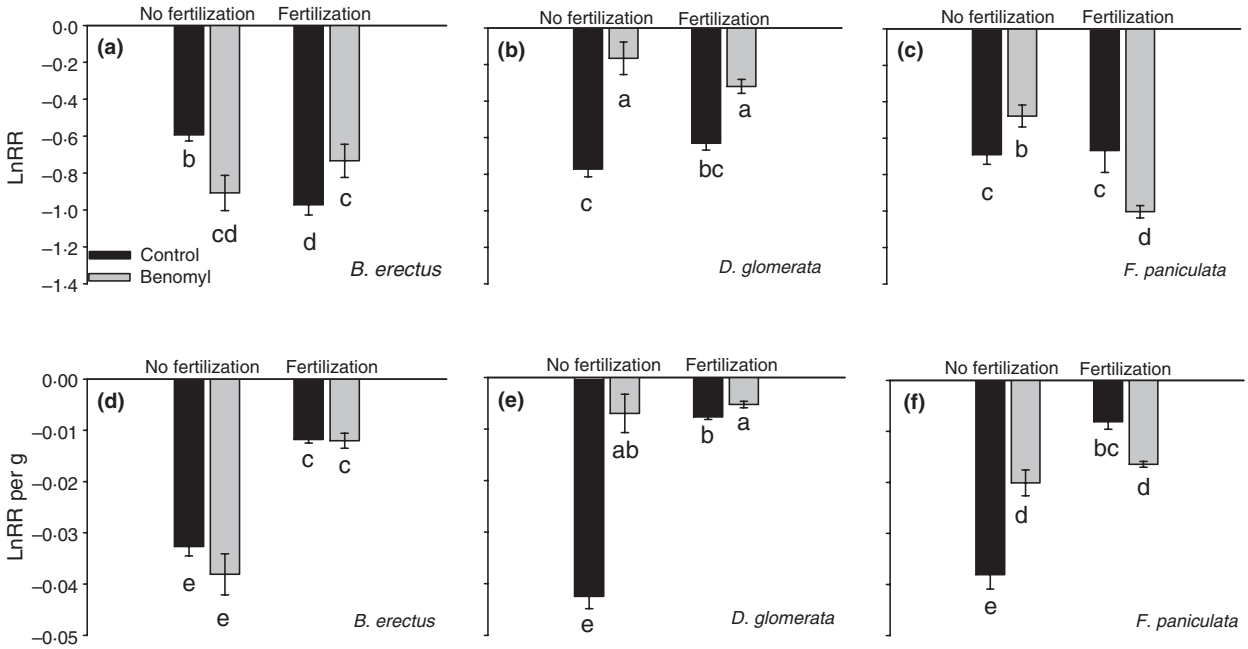

Fig. 5. Competitive effect (LnRR) of *F. paniculata* matrix with and without fertilisation and benomyl application (a, b, c) and competitive effect per gram (LnRR per g) (d, e, f) measured with competitive response of *B. erectus* (a, d), *D. glomerata* (b, e) and *F. paniculata* (c, f). We tested difference between control and benomyl treatments using Tukey HSD test.

The role of soil fertility for AM effect on plant interactions has been recently highlighted by Collins & Foster (2009). Following their theoretical model, AM is likely to favour monopolistic species only for high soil N : P. In our case, the fertilisation treatments should have modified soil N : P. Our soil conditions may have exhibited low N : P ratio in the unfertilized treatment. Differences in soil N : P ratio are thus likely to explain why AM decreased or increased intra-specific competition whether in fertilized or unfertilized conditions respectively, leading to high competitive success of *F. paniculata* only in fertilised treatments. However, our experiment was not explicitly designed to test the effect of the N : P ratio on AM-mediated interactions and further investigations are needed to address this question.

TRAIT-MEDIATED INTERACTIONS AND COMPETITIVE EFFECT OF *FESTUCA PANICULATA*

The intensity of competitive interactions observed in our experiment were not related to variation in competitor biomass (density mediated interaction) (Goldberg *et al.* 2001) but rather to variation in the per gram effect (lnRR per g) under both fertilised and unfertilised conditions (Fig. S2). Trait-mediated interactions (Okuyama, Benjamin & Bolker 2007) are thus possible determinants of the observed competitive outcomes. For instance, we found that observed change in lateral spread in response to fertilisation and AM treatments were able to explain the variation of per gram interactions (Fig. S3). High lateral spread, favoured by presence of AM in the case of *F. paniculata,* may have increased species aboveground space utilisation (Vojtech *et al.* 2009) and, as such, is likely to increase species competitive responses (Navas & Moreau-Richard 2005). Similarly, we found that species with high P inflow exhibited higher competitive responses in the competition experiment (Fig. S3). Particularly, *F. paniculata* exhibited a substantially higher P inflow compared with other species in presence of AM under fertilised conditions. This result is likely to explain its high competitive response in fertilised conditions (Fig. 5). Without AM, P inflow was not significantly different for *D. glomerata* and *F. paniculata* (Fig. 3k,l). However, *D. glomerata* exhibited a higher root allocation under these conditions, which supported its highest competitive response (see Raynaud & Leadley 2004 for sink strength strategies in belowground interactions). Although trait-mediated interactions are likely to explain variation in competitive intensity for inter-specific interactions, it is rather hard to explain the decrease of intra-specific interaction observed for *F. paniculata* in presence of AM (Fig. 3f). Additional mechanisms may have been involved in order to decrease intra-specific interactions. For instance, carbon and nutrient transfer between plants through external mycelial networks have been suggested in several studies (Callaway *et al.* 2001; Grime *et al.* 1987; Simard *et al.* 1997; but see Robinson & Fitter 1999). In our case, transfer of mineral compounds between individuals of *F. paniculata* may favour the observed decrease of intra-specific competition.

Conclusions

Our study illustrates the key regulatory role of AM in the strategy of a monopolistic species, as AM increased

inter-specific competition regardless of target species strategy, and decreased its own intra-specific competition. AM effect on biotic interactions was strongly related to their effect on plant functional traits. By increasing the lateral spread and the P inflow of *F. paniculata* AM can considerably increase the competitive success of this species. As dominance of *F. paniculata* in the field is strongly related with its competitive response and effect (Gross *et al*. 2009), this mechanism may substantially contribute to the observed drop in species diversity associated with abandonment of mowing (Quétier, Thebault & Lavorel 2007a) and the maintenance of a stable community state (Petraitis & Latham 1999; Liancourt, Viard-Cretat & Michalet 2009) dominated by *F. paniculata* (Quétier *et al*. 2007b).

A better understanding of mechanisms that drive AM effects on plant growth, plant–plant interactions, and community structure will help us to better explain the general context-dependency observed in results from empirical studies (Hoeksema *et al*. 2010). For this, recent theoretical models, which aim to link AM effects on plant interactions and the maintenance of species-rich plant communities, need to be tested under a range of environmental conditions (Hartnett & Wilson 1999; Urcelay & Diaz 2003) by explicitly manipulating soil fertility and N:P ratios (Collins & Foster 2009). Such experiments would also take into account fungal diversity given that AM effects have been shown to be linked with AM identity (van der Heijden *et al*. 1998; Hoeksema 2005). In this context, the complex trait-dependent mechanisms by which AM can affect plant growth and competitive outcomes should continue to be explored.

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Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1. Experimental design and links between per gram effect and traits.

Fig. S1. Experimental design.

Fig. S2. Relationship between competitive effect per plant (LnRR) and the competitive effect per gram (LnRR per g).

Fig. S3. Relationship between LnRR per g and traits.

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