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LETTER

Belowground biodiversity effects of plant symbionts support aboveground productivity

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Abstract

Soil microbes play key roles in ecosystems, yet the impact of their diversity on plant communities is still poorly understood. Here we demonstrate that the diversity of belowground plant-associated soil fungi promotes plant productivity and plant coexistence. Using additive partitioning of biodiversity effects developed in plant biodiversity studies, we demonstrate that this positive relationship can be driven by complementarity effects among soil fungi in one soil type and by a selection effect resulting from the fungal species that stimulated plant productivity the most in another soil type. Selection and complementarity effects among fungal species contributed to improving plant productivity up to 82% and 85%, respectively, above the average of the respective fungal species monocultures depending on the soil in which they were grown. These results also indicate that belowground diversity may act as insurance for maintaining plant productivity under differing environmental conditions.

Keywords

Complementarity effect, facilitation, fungal diversity, insurance hypothesis, mycorrhizal fungi, quantitative PCR, selection effect, soil biodiversity.

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INTRODUCTION

Recent work has highlighted the role of biological diversity as a regulator of ecosystem functions (Zavaleta *et al.* 2010). In grassland ecosystems, greater plant species richness is often associated with increased plant productivity (Hector *et al.* 1999; Tilman *et al.* 2001). This positive effect of biodiversity can be explained by the presence of a particular productive species driving a species-rich community (selection effect), as well as niche differentiation and facilitative interactions (complementarity effect) in more species-rich communities (Hooper *et al.* 2005). Both these effects can operate simultaneously and sum to the net effect of biodiversity on ecosystem functioning (Loreau & Hector 2001). The majority of such studies focusing on biodiversity – ecosystem functioning have focused on aboveground organisms while relatively few address the role of biodiversity in the belowground soil microbial community (Balvanera *et al.* 2006). Furthermore, the use of additive partitioning of biodiversity effects has yet to be used to assess the functioning of soil microbial communities.

Soil microbes play critical roles in a number of ecosystem processes and services and several reports emphasise the major role soil microbial diversity plays in sustaining ecosystem functioning (Wardle 2006; van der Heijden *et al.* 2008). Deciphering the importance of soil microbial diversity is a key issue in ecology as many studies have found belowground diversity to be reduced by anthropogenic effects, particularly agricultural practices (Helgason *et al.* 1998; Jansa *et al.* 2002; Van der Wal *et al.* 2006). For instance, a number of studies have shown that richness of arbuscular mycorrhizal (AM) fungi is reduced by agricultural intensification (Oehl *et al.* 2004; Verbruggen *et al.* 2010). These fungi are a group of obligate root endophytic fungi ubiquitous in most terrestrial ecosystems (Smith & Read 2008).

Previous studies have shown that plant productivity and diversity are functions of increasing AM fungal richness (van der Heijden *et al.* 1998; Maherali & Klironomos 2007). This generally is attributed to complementarity among AM fungi that is explained by niche segregation and facilitation. There is evidence that complementarity in their function may occur as different phylogenetic groups of AM fungi possess different life strategies and have different host effects (Maherali & Klironomos 2007). Moreover, a high degree of genetic and functional variability can occur within an AM fungal community (Koch *et al.* 2006). Such multifunctionality of AM fungi could translate to greater host benefits in more AM fungi-rich communities as each could theoretically provide additional services to host plants that contribute to greater plant productivity (Koide 2000).

Conversely, the single most productive AM fungal species can have a similar effect as mixtures of AM fungi (Vogelsang *et al.* 2006). This suggests that the relationships between AM fungal richness and plant productivity may be driven by the selection effect, whereby the likelihood of including the fungal species with the strongest effect on functioning increases with AM fungal richness; also referred to as a 'sampling effect' (see Huston 1997). However, it is impossible to assess either selection or complementarity effects (*sensu* Loreau &

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Hector 2001) of AM fungal richness without knowing which fungi contributed to aboveground effects in mixed communities of AM fungi. To date no study has partitioned out the complementarity and selection effects in AM fungal communities and as a result, it is unclear how these effects influence AM fungal richness – plant productivity relationships. This has generated some debate regarding the mechanisms behind AM fungal richness – plant productivity relationships (e.g. van der Heijden *et al.* 1999; Wardle 1999).

There are a number of additional spatial, temporal and environmental factors that can influence the functioning of AM fungal communities within soils. For example, soil abiotic conditions are known to shift AM fungal community assembly (Lekberg *et al.* 2007) and the mycorrhizal relationship (Johnson *et al.* 1997). This is likely an additional factor resulting in the observed variation of the effects of AM fungal diversity on plant hosts. For instance, soil resource availability may determine whether soil fungi can coexist (Kennedy 2010) and may thus influence whether the effect of AM fungal diversity is driven by a complementarity or selection effect. The relationship between AM fungi and plant communities under differing environmental characteristics is also crucial with regard to the question whether species richness of AM fungi may act as insurance for stability in ecosystem functioning in spatially heterogeneous environments or under altered environmental conditions (Yachi & Loreau 1999).

In this study, we use a grass-clover model system to test whether AM fungal richness enhances plant productivity in two different soils to reveal the impact that the abiotic soil environment can have on AM fungal richness – plant productivity relationships. Subsequently, we assess whether the AM fungal richness – plant productivity relationships are due to a complementarity or selection effect using additive partitioning (Loreau & Hector 2001). As the identification of each species present and its effect on the ecosystem response in both monocultures and mixtures is a pre-condition for partitioning selection and complementarity effects (Loreau & Hector 2001), we used quantitative PCR to detect the abundance of the AM fungi in monocultures and in mixtures in which four AM fungal taxa were inoculated in all possible combinations. These data enabled us to estimate the relative contribution to aboveground plant productivity of each AM fungal species in mixtures such that additive partitioning of biodiversity effects could be used to assess the role of selection and complementarity effects in increasingly rich mixtures of AM fungi.

METHODS

Preparation of fungal inocula, plants and soils

We used four AM fungi, *Glomus intraradices* (isolate BEG 21, see van der Heijden *et al.* 2006 for description), *G. mosseae* (isolate BEG161, Jansa *et al.* 2002), *G. claroideum* (isolate JJ132, Jansa *et al.* 2002) and *Diversispora celata* (FACE 234, Gamper *et al.* 2009); each belonging to a unique *Glomus* group; Aa, Ab, B and C, respectively (Schüßler *et al.* 2001; Gamper *et al.* 2009). These fungi occur in Swiss agricultural and grassland ecosystems and can differ in function (Jansa *et al.* 2005; Thonar *et al.* 2010). Inocula, including the non-mycorrhizal control inoculum, were propagated on *Plantago lanceolata* L. for 6 months after which the soil was dried and roots were cut into < 5 cm pieces and homogenously mixed. *Glomus claroideum, G. intraradices*, *G. mosseae* and *D. celata* inocula were observed colonising 30.4%, 90.5%, 42.1% and 17.5% of the root length of *P. lanceolata* with 22, 38, 2 and 121 spores per cm^3 of soil, respectively. No AM fungal propagules were observed in the control inoculum.

The field soil used for the experiment was collected from a natural grass-clover field located at Agroscope Reckenholz research station in Zürich, Switzerland (047° 25′ 38.71″ N, 8° 31′ 3.91″ E), sieved through a 0.5 cm mesh and mixed with quartz sand in the ratios of 1 : 4 and 4 : 1 soil to sand (v/v) to create the two soil types, 'high-sand' and 'low-sand' respectively. Soils were sterilised by autoclaving for 99 min at 121 °C. The soil characteristics are provided in Table S1.

Pots, 1 L in volume, were filled with 1.15 kg (dry weight) of one of the two soil types. Pots were inoculated with AM fungi in all possible combinations including a non-mycorrhizal control producing AM fungal richness treatments from 0 to 4 for a total of 16 AM fungal treatments. All pots received 50 mL of inoculum, thus, pots inoculated with 2, 3 or 4 AM fungi received *c.* 25 mL, 16.5 mL and 12.5 mL of inoculum of each fungal species respectively. Soil and inoculum were mixed thoroughly in each pot to create a homogenous substrate and each soil × AM fungal treatment was replicated six times resulting in 192 pots. Pots were randomly distributed in two adjacent greenhouses.

We used *Trifolium pratense* L. var. Milvus and *Lolium multiflorum* Lam. var. Daxus as the host plant community. We chose this grass-legume community for its key role in both agricultural and natural grassland ecosystems where the species of the two involved plant functional groups commonly coexist (e.g. Nyfeler *et al.* 2009). Seeds, originating from agricultural plots located at Agroscope Reckenholz research station, Zürich, Switzerland, were surface sterilised by agitation in 1.25% sodium hypochlorite for 5 min, followed by rinsing in H_2O and placed on 1.5% water agar until germination. Four seedlings of each plant species were planted into each pot. Seedlings not surviving transplant were replaced within 2 weeks post-planting. To standardise the non-mycorrhizal microbial community with a natural soil microbe community native to a natural grass-clover-field, a microbial wash was created by sieving 1 L fresh field soil with 5 L H_2O through a series to < 11 μm of which 10 mL was added to each pot. Numerous root nodules were observed on clover indicating this microbial wash contained active micro-organisms including nodule-inducing rhizobia.

Plants were grown for 25 weeks with 16 h per 25 °C days under natural light maintained above 300 W m^{-2} by 400-W high pressure sodium lights and 8 h per 16 °C nights and received H_2O to maintain soil moisture at 10–20% by weight.

Data collection

After 9 and 16 weeks, shoots were cut 4 cm above the soil surface to simulate mowing. The final harvest occurred at 25 weeks and plants were cut directly at the soil surface. Plant material from each harvest was separated into species, dried at 70 °C and weighed. Data were pooled across harvests for all subsequent analyses, as biomass responses were similar at all harvests (data not shown). At the final harvest, roots were washed and stored at −20 °C until they could be processed for microscopy and DNA extraction. Frozen roots were thawed and cut into small fragments (1–2 cm). For microscopy, a random subsample of 1–2 g of fresh roots was cleared and stained with 5% pen-ink vinegar as described in Vierheilig *et al.* (1998). Stained roots were scored for the presence of colonisation by AM fungi using the intersect method outlined in McGonigle *et al.* (1990) for 100 intersects.

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For DNA extraction, roughly half the remaining root fragments were randomly selected, containing a representative root sample of both host plants and lyophilised. Approximately 20–25 mg of lyophilised roots were used for DNA extraction using the Qiagen DNeasy plant mini kit following manufacturer recommendations for the purification of total DNA from plant tissue (Qiagen Sciences, Germantown, MD, USA). The primer sequences and hydrolysis probes used were specific to each of the four AM fungi and targeted the nuclear large ribosomal subunit (LSU). The qPCR reactions were carried out using the Light Cycler 2.0 (Roche Applied Science, Rotkreuz, Switzerland) and the cycle threshold values were used to determine the number of LSU copies per mg of lyophilised root (see Wagg *et al.* 2011). The primers and probes used did not show any interference from the presence of non-target AM fungal DNA (Fig. S1). Further details on the primers and probes used, as well as the reagents and cycling conditions for the qPCR are described elsewhere (Thonar 2009; Wagg *et al.* 2011).

Contribution of AM fungi to plant productivity

We estimated the contribution of each AM fungus to aboveground plant biomass in the AM fungal mixtures by weighting each of the AM fungal species in the mixture according to its relative abundance (*RY*) and the aboveground biomass the plants produced when mono-inoculated (β). The *RY* of each AM fungal species in the mixture was calculated as the observed abundance of an AM fungus in the mixture (*O*) proportional to its average abundance in monoculture (*M*); equivalent to the relative yield outlined by de Wit (1960), such that $RY = O/M$. The *RY* of each AM fungus was then multiplied by β (the aboveground biomass in the respective mono-inoculated plants). The contribution to aboveground plant biomass (*P*) of each AM fungus in the mixture was then calculated as

$$P_i = \frac{RY_i \cdot \beta_i}{\sum_{i=1-n} RY_i \cdot \beta_i} \times \alpha$$

where RY_i is the relative abundance of AM fungal species i in the mixture, β_i is the aboveground plant biomass when mono-inoculated with this fungus i and α is the aboveground plant biomass produced in the AM fungal mixture. By using this weighting method, we assume that the relative abundance of an AM fungus in the mixture also reflects its influence on plant biomass in the mixture relative to its effect in monoculture regardless of other factors, such as enhanced or reduced abundance of other fungi (e.g. symbiotic efficiency per unit biomass is not dependent on the abundance of other AM fungi).

Biodiversity effects

For each treatment with two or more AM fungi, additive partitioning (Loreau & Hector 2001) was used to determine the biodiversity effects of AM fungi colonising the belowground root system on the aboveground biomass of *T. pratense*, *L. multiflorum* and their combined total. The net biodiversity of AM fungi on plant productivity was defined as the difference between the plant biomass observed in AM fungal mixtures and the average plant biomass observed in AM fungal monocultures of the fungal species making up the mixture. The net biodiversity effect was then partitioned into a selection effect (strong influence of particular AM fungal species in mixture) and a complementarity effect (several AM fungal species contributing more to plant productivity in AM fungal mixtures than expected from their AM fungal monocultures) following Loreau & Hector (2001).

The estimated contribution to plant biomass (*P*) was used as the 'observed yield' for each AM fungal species in the mixture. The plant biomass in the mono-inoculated treatments was used as the 'monoculture yield'. The 'expected yield' of each AM fungi was calculated as plant biomass in the mono-inoculated treatment divided by the number of AM fungi in the mixture, such that the sum of the 'expected yield' of the AM fungi in the mixed inoculum treatment was equal to the average of the biomass produced in mono-inoculated treatments following the null hypothesis of Loreau & Hector (2001). It should be noted that an increased effect of an AM fungal mixture over the best mono-inoculated AM fungus, analogous to 'transgressive overyielding' in plant biodiversity studies, is only one possible outcome of complementarity (see Hector *et al.* 2002).

Data Analyses

The effect of soil × AM fungal treatment on the aboveground biomass of *T. pratense*, *L. multiflorum* as well as their combined total was assessed using ANOVA with soil type, AM fungal treatment, initial AM fungal richness, realised AM fungal richness and the interactions with soil type as sources of variation. The non-mycorrhizal treatment was removed from the data in the analysis to remove any confounding influence it may have in assessing AM fungal diversity. Both initial and realised AM fungal richness were used in the model as not all AM fungi could be detected after harvest by qPCR in 18 of the 180 pots inoculated with multiple AM fungi (see Table S3 for details). The variation among AM fungal treatments and their interaction with soil, if significant, was then partitioned out by contrasts using step-wise addition of contrast terms (the presence/absence of a fungus in all combinations), in order by which they explained the greatest amount of variation with fewest interactions. The abundance of AM fungi, their interactions with each other and soil treatment were then added to the model. Step-wise deletion of terms was used to test for linear and nonlinear relationships after accounting for the presence/absence of an AM fungus. Contrasts within separate ANOVAs for each soil treatment were used to test differences between mycorhizal and non-mycorrhizal treatments as well as whether AM fungal mixtures differed from the corresponding AM fungal monoculture that had the strongest effect on plant productivity to detect any potential facilitative effects.

The abundance of each AM fungal species was assessed using a two-way ANOVA with soil and AM treatments as well as their interaction as sources of variation. To determine whether AM fungi were significantly altered in abundance in AM fungal mixtures, the relative abundance (*RY*) of each AM fungus was assessed for each soil separately by one-way ANOVA with AM fungal treatment as the source of variation. The *RY* was log transformed to improve homoscedasticity in the data that produced positive and negative values such that a difference from 0 (no change in abundance) could conveniently be tested.

All three biodiversity effects (net, selection and complementarity) were assessed by ANOVA using soil type, AM fungal treatment and AM fungal richness (both initial and realised) as well as the interaction of soil type with AM fungal treatment and richness as sources of variation. Separate regression models for each biodiversity effect in each soil type were then used to determine whether the magnitude of effects corresponds with increasing AM fungal richness. The

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greenhouse in which plants were grown was added as a block effect in all ANOVA models. All statistics were calculated using R 2.11.1 (The R Foundation for Statistical Computing 2010).

RESULTS

Responses in aboveground plant productivity

Overall, AM fungal richness enhanced aboveground biomass of grass-clover mixtures (Fig. 1a) resulting from a positive effect of AM fungal richness on *T. pratense* biomass (Fig. 1b) and a marginal negative effect on *L. multiflorum* biomass (Fig. 1c). The realised AM fungal richness explained a greater proportion of variation in all three measures of aboveground biomass than the initial AM fungal richness, which did not explain any further variation in biomass after the realised richness was entered into the model. Thus, initial AM fungal richness is not present in the model (Table 1). Overall, the presence of AM fungi altered the relative abundance of *T. pratense* biomass from 3% and 11% in the non-mycorrhizal treatment up to 27% and 66% in the most beneficial AM fungal treatment in the low- and high-sand soils, respectively.

Soil and AM fungal treatment strongly influenced all three biomass measures (Table 1). Overall, *T. pratense* produced greater biomass in the high-sand soil and *L. multiflorum* produced greater biomass in the low-sand soil (Fig. 1). The presence of AM fungi significantly enhanced *T. pratense* biomass in both soils (high-sand: $F_{1,79} = 268$, $P < 0.001$, low-sand: $F_{1,79} = 85.4$, $P < 0.001$, Fig. 2). The presence and abundance of *D. celata* significantly influenced *T. pratense* biomass resulting in a 9–10 fold increase above non-mycorrhizal treatments in monoculture in both soils (Table 1a; Fig. 2). Moreover, the effect of *D. celata* presence on *T. pratense* biomass depended on soil and AM fungal treatment (Table 1a; Fig. 2). Combinations of AM fungi, particularly those involving *G. claroideum* and *G. intraradices*, resulted in greater effects on *T. pratense* biomass than the most effective AM fungi in mono-inoculated plants (Fig. 2). *Lolium multiflorum* biomass was reduced in the presence of AM fungi by *c.* 20–40% and 15–20% in the high- and low-sand soils respectively (Fig. 2) compared with the non-mycorrhizal treatment (high-sand: $F_{1,79} = 28.5$, $P < 0.001$, low-sand: $F_{1,79} = 13.9$, $P < 0.001$). The presence of *G. claroideum* in AM fungal treatments contributed to the largest depressions in *L. multiflorum* growth followed by *G. intraradices* (Table 1b, Fig. 2). In addition, the abundance of *G. mosseae* significantly influenced *L. multiflorum* biomass (Table 1b). The effects of the various AM fungal combinations as well as the relationships in abundance are also evident in the analysis of the combined biomass of the two plant species (Table 1c).

AM fungal abundance in roots

All four AM fungi colonised roots when mono-inoculated, demonstrating their viability. Of the four AM fungi used, *G. intraradices* was the most infective, although not the most beneficial, in monoculture, colonising 79.5% (SE = 1.54) of root length, pooled for both soils, followed by *G. claroideum*, *D. celata* and *G. mosseae* which colonised 35.1% (SE = 1.74)%, 22.0% (SE = 1.37) and 17.7% (SE = 1.82) of root length, respectively. No AM fungal colonisation was observed in any of the roots of control plants. The root-length colonisation by AM fungi correlated well with the number of LSU copies detected (Spearman's rho = 0.74, $P < 0.001$, pooled for all mono-inoculated treatments). The abundance of each AM fungal species differed between soils and among AM fungal treatments (Table S2). Generally, *G. intraradices* was most affected by other AM fungi while the abundances of *G. mosseae*, *G. claroideum* and *D. celata* were most affected by the soil in which they were inoculated (Table S2). Overall, the abundance of each AM fungus was frequently less in AM fungal mixtures than their respective mono-inoculated treatments (Table S3).

The abundance of *G. claroideum* and *G. mosseae* was significantly less than its abundance in monoculture in all AM fungal mixtures in both soils (Fig. S2). Intriguingly, *D. celata* did not deviate significantly from its abundance in monoculture in the majority of AM fungal mixtures in the high-sand soil (Fig. S2a), but was significantly reduced in abundance in all AM fungal treatments, including complete absence in the presence of *G. mossseae*, in the low-sand soil (Fig. S2b). *Glomus intraradices* was also absent in roots of all replicates when co-inoculated with *G. mosseae*, but in all other AM fungal mixtures did not differ significantly from its inoculation in monoculture in the low-sand soil (Fig. S2b).

Biodiversity effects

Analysis of the biodiversity effects of AM fungi on aboveground plant productivity revealed soil and AM fungal combination to be important factors influencing the complementarity and selection effects, particularly in the case of *T. pratense* (Table S4). For example, combinations

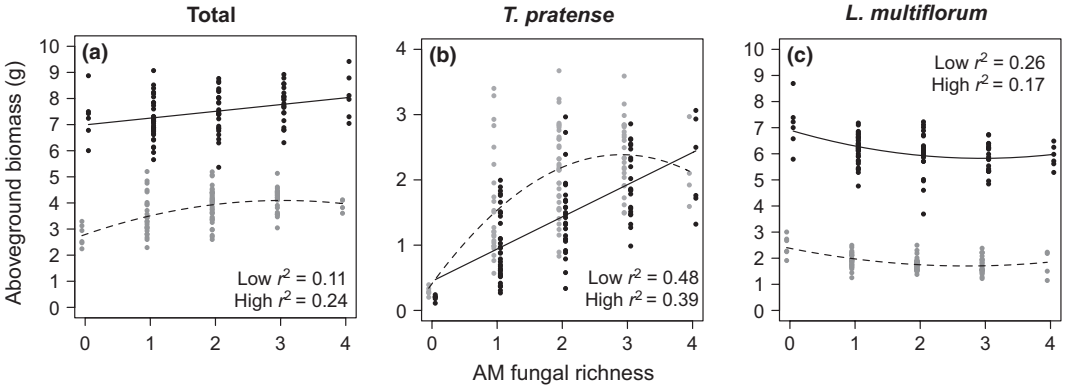

Figure 1 Scatter plots with trend lines showing the relationship of the aboveground biomass of (a) Total aboveground biomass, (b) *T. pratense* and (c) *L. multiflorum* with realised AM fungal richness in both low-sand (black dots, solid line) and high-sand (grey dots, dashed line) soils. All models were significant (all $P < 0.001$).

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Table 1 Summary of ANOVA results for the biomass of (a) *T. pratense*, (b) *L. multiflorum* and (c) their combined total. The significant proportion of the variance explained by the different AM fungal treatments (using only inoculated treatments) and the interaction with soil type is partitioned out by the addition of contrast terms explaining the greatest amount of variation with the fewest terms (indented terms). Transformations were used to improve homoscedasticity in the data (*T. pratense* square-root transformation and *L. multiflorum* transformed to the power of 0.25). Capital letters in contrasts represent the presence or abundance of an AM fungus (M = *G. mosseae*, I = *G. intraradices*, C = *G. claroideum* and D = *D. celata*)

Source of variation	d.f.	MS	*F*
(a) *T. pratense* biomass			
Block	1	0.32	12.2***
Soil	1	2.89	109***
Realised richness	1	3.92	148***
Arbuscular mycorrhizal (AM) fungal treatments	14		
D presence	1	1.62	61.0***
M presence	1	1.19	44.9***
I presence	1	0.16	5.89*
I × D presence	1	0.56	21.0***
C × D presence	1	0.34	12.7***
I × C × D presence	1	0.18	6.61*
AM residual	8	0.05	1.83
D abundance	1	0.24	9.18**
Soil × AM	14		
Soil × D presence	1	0.54	20.3***
Soil × C presence	1	0.68	25.5***
Soil × M × C presence	1	0.25	9.53**
Soil × AM residual	11	0.04	1.34
Residuals	146	0.03	
(b) *L. multiflorum* biomass			
Block	1	6.84×10^{-5}	0.04
Soil	1	7.57	4.62×10^{3}***
Realised richness	1	1.82×10^{-2}	11.1**
AM fungal treatments	14		
C presence	1	2.52×10^{-2}	15.4***
I presence	1	0.81×10^{-2}	4.69*
AM residual	12	0.21×10^{-2}	1.26
M abundance	1	0.91×10^{-2}	5.58*
Soil × AM	14	0.23×10^{-2}	1.38
Residuals	146	0.16×10^{-2}	
(c) Total biomass			
Block	1	1.31×10^{-2}	10.8**
Soil	1	2.88	2.38×10^{3}***
Realised richness	1	5.64×10^{-2}	46.7***
AM fungal treatments	14		
D presence	1	11.3×10^{-2}	93.8***
M presence	1	2.67×10^{-2}	22.1***
C presence	1	0.78×10^{-2}	6.45*
I × D presence	1	1.35×10^{-2}	11.2**
M × I presence	1	1.17×10^{-2}	9.70**
C × D presence	1	0.76×10^{-2}	6.29*
AM residual	8	0.06×10^{-2}	0.50
D abundance	1	0.93×10^{-2}	7.72**
M abundance	1	1.17×10^{-2}	9.69**
Soil × AM	14		
Soil × D presence	1	3.25×10^{-2}	26.9***
Soil × AM residual	13	0.16×10^{-2}	1.29
Soil × M abundance	1	0.64×10^{-2}	5.29*
Soil × M nonlinear abundance	2	0.58×10^{-2}	4.78**
Residuals	142	0.12×10^{-2}	

d.f., degrees of freedom; MS, mean squares. Significance levels of the *F*-test are indicated as: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

with *G. claroideum* and *G. intraradices* resulted in complementarity, as evidenced by their mixtures having a greater effect than the average of their mono-inoculated treatments (Fig. 2). Combinations involving *D. celata* always resulted in a strong selection effect in the high-sand soil (Fig. 2).

The selection effect in AM fungal mixtures on all three measures of aboveground plant productivity differed between the two soils (Table S4). In the high-sand soil, the selection effect accounted for up to 82% of the net biodiversity effect on *T. pratense*. The selection effect had an overall significant positive effect on *T. pratense* and total plant biomass while an overall negative selection effect on *L. multiflorum* biomass (Fig. 3). The grand mean of the selection effect in the low-sand soil did not differ from 0 (Fig. 3). The complementarity effect on *T. pratense* accounted for up to 85% of the net biodiversity effect in the low-sand soil and increased with AM fungal richness (Fig. 3) demonstrating increases in AM fungal richness correspond to increases in a complementarity effect. Overall, the effect of AM fungal richness resulted in a positive net effect on the biomass of *T. pratense* that outweighed the overall negative effects on *L. multiflorum* resulting in a significant positive effect on the total aboveground biomass (Fig. 3).

DISCUSSION

It is known that selection and complementarity effects can enhance the performance of species-rich plant communities (Hooper *et al.* 2005). However, until now, the use additive partitioning (*sensu* Loreau & Hector 2001) to determine the role of selection and complementarity effects of soil microbial diversity on aboveground plant communities has not been tested. We observed a positive AM fungal richness-plant productivity relationships, similar to previous studies (van der Heijden *et al.* 1998; Vogelsang *et al.* 2006; Maherali & Klironomos 2007), which were attributed to either a complementarity or selection effect depending on soil conditions. These biodiversity effects resulted from a range of interactions among AM fungi from facilitation to antagonism with consequences for aboveground plant productivity, particularly for *T. pratense*, the more symbiont-dependent plant species. Importantly, although the net biodiversity effect of AM fungi on plant biomass was similar in both soils, different mechanisms (either complementarity or selection effects) were responsible for the AM fungal diversity – plant productivity relationships. This illustrates how the overall effect of AM fungi on a plant community can be maintained under differing environmental conditions by a more AM fungal species-rich community despite the altered functioning of individual fungi.

Biodiversity effects of AM fungi on plant productivity

The overall effect of AM fungal richness on aboveground plant productivity primarily resulted from its strong positive effects on the legume *T. pratense*. In the less productive high-sand soil, this effect was driven by the ability of *D. celata*, the most growth promoting AM fungus, to remain abundant within roots while the abundances of other co-inoculated AM fungi were reduced, indicating its ability to outcompete other AM fungi under these soil conditions. It is possible that the higher spore number in the *D. celata* inoculum favoured its strong influence under these soil conditions. Nevertheless, this resulted in a significant and positive selection effect on *T. pratense* and on total aboveground plant biomass.

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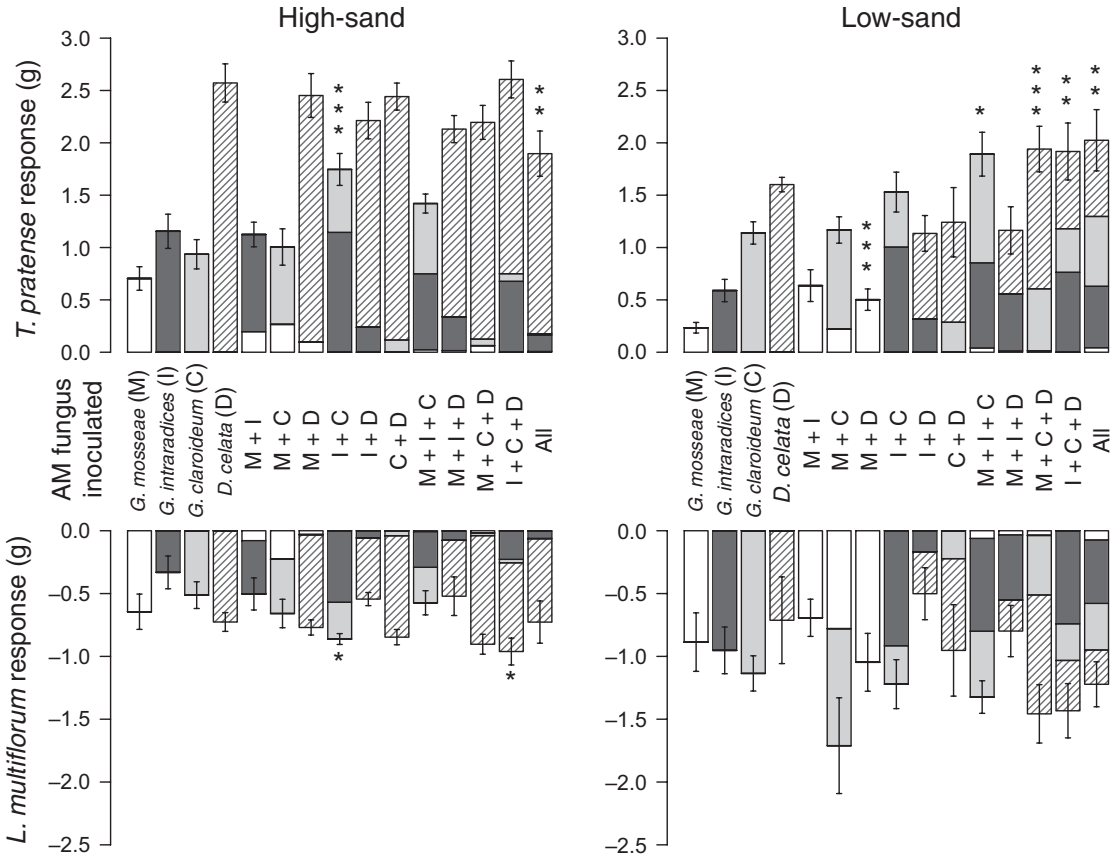

Figure 2 The mean and standard error for the difference between mycorrhizal and non-mycorrhizal biomass of *T. pratense* (above) and *L. multiflorum* (below) in both soils (high-sand non-mycorrhizal *T. pratense* = 0.26 g, SE = 0.03 g and *L. multiflorum* = 2.47 g, SE = 0.16 g; low-sand non-mycorrhizal *T. pratense* = 0.19 g, SE = 0.02 g and *L. multiflorum* = 7.11 g, SE = 0.39 g). The estimated contribution of each AM fungus to the effect of AM fungal inoculation on aboveground plant biomass is represented by the proportional shading of each bar, reflecting their relative abundance in the mixture as well as their influence on growth in relation to their monocultures. The presence of an AM fungus is indicated on the *x*-axis (C = *G. claroideum*, I = *G. intraradices*, M = *G. mosseae*, D = *D. celata*). Differences (either greater or less) in plant biomass between a mixed-inocula treatments and the corresponding most effective mono-inoculated AM fungus is indicated by $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

The fact that in the high-sand soil, the AM fungus causing the selection effect also dominated the AM fungal mixture in terms of relative abundance is not surprising; yet not necessary for the selection effect to occur (Loreau & Hector 2001), as theoretically, a subdominant species could cause a positive selection effect. However, the parallel between the selection effect and the high abundance of the most beneficial AM fungus in mixtures is what Huston (1997) and Wardle (1999) predicted under the term 'sampling effect' hypothesis: that improved productivity with increasing species richness is due to the greater probability of adding the most productive species that drives the functioning of mixtures. Complementarity among AM fungi was observed in both soils depending on AM fungal combination, such as the dual inoculation of *G. intraradices* and *G. claroideum*. Overall, in the high-sand soil, a selection effect occurred more frequently, overriding the complementarity effect.

By contrast, in the more productive low-sand soil, more AM fungi-rich mixtures resulted in a greater aboveground plant response in *T. pratense* than any of the single species. Interestingly, the dual inoculation of *G. mosseae* with *D. celata* in the low-sand soil resulted in poorer *T. pratense* biomass production than the average of the two AM fungi mono-inoculated, demonstrating a negative selection effect and indicating an antagonistic interaction between the two fungal species. However, when more than these two species were present in a mixture, such a strong negative selection effect did not occur and the complementarity effect of AM fungal richness had a greater influence. This indicates that *G. mosseae* was less effective at excluding the most productive species when challenged with a greater number of interspecific interactions and as a result, the complementarity effect increased with AM fungal richness driving the overall biodiversity effect.

Mechanisms of AM fungal coexistence and their biodiversity effects

We could verify the coexistence of AM fungi within the root system via qPCR, supporting a number of previous studies (e.g. Abbott & Robson 1984; Vandenkoornhuyse *et al.* 2002; Janoušková *et al.* 2009). The ability for AM fungi to coexist is a key factor as to whether biodiversity effects are driven by complementarity or selection effects, as the ability to coexist via niche segregation is a major component behind the two partitioned effects (Loreau & Hector 2001). In our experimental design, abiotic soil properties determined whether the functioning of AM fungal communities were driven by a single AM fungus (as observed for the high-sand soil) or whether multiple AM fungi coexisted (as observed in the low-sand soil) to influence the aboveground plant community. The substrate in the low-sand soil was perhaps more complex, thus allowing for better coexistence of a more

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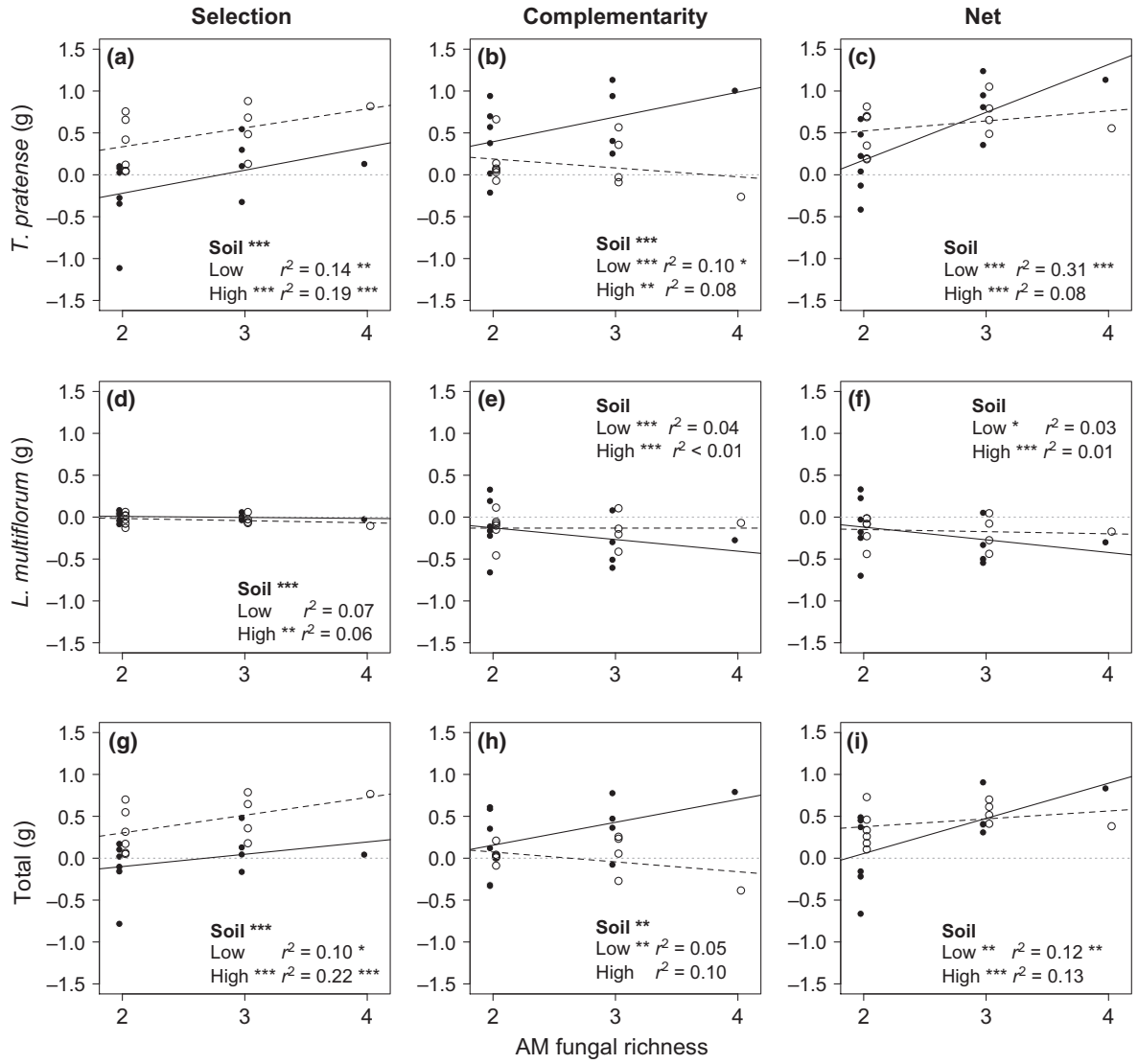

Figure 3 Selection, complementarity and net biodiversity effects of AM fungal richness on productivity of *T. pratense* (a–c), *L. multiflorum* (d–f) and their combined total (g–i) in both high-sand (open circles, dashed line) and low-sand (filled circles, solid line) soils. Initial AM fungal richness is shown on the *x*-axis. Significant differences in the overall effect between soils (Soil) and whether the effect in each soil differs from 0 is indicated by $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Significance levels adjacent to r^2 values indicate whether slopes differ significantly from 0.

functionally dissimilar community. The importance of such a link between resource complexity and the role of richness has been demonstrated by Jousset *et al.* (2011), where a more complex resource environment allowed for greater coexistence of functionally dissimilar *Pseudomonas fluorescens* genotypes, thus enhancing community functioning.

Whether a complementarity or selection effect is behind the AM fungal richness – plant productivity relationship can be due to numerous factors beyond abiotic characteristics, such as functional strategies of AM fungi. For example, similar to Jansa *et al.* (2008) and Janoušková *et al.* (2009), our results show that *G. intraradices* and *G. claroideum* are able to coexist and can facilitate greater benefits to aboveground plant growth than either of the species inoculated individually; perhaps occurring via differences in foraging strategies (Jansa *et al.* 2005; Thonar *et al.* 2010). In addition, spatial separation among AM fungi, such as via host preference in a plant polyculture, may maintain the functioning of a less competitive AM fungi (Bever *et al.* 2009) and thus enhance the complementarity effect of AM fungal communities. This potentially could explain the similarity between AM fungal monocultures and mixtures in their effect on plant productivity in some studies using a plant monoculture (e.g. Jansa *et al.* 2008), but perhaps not others using a plant polyculture (e.g. Vogelsang *et al.* 2006).

Temporal variation among AM fungi has been observed (e.g. Fitter & Merryweather 1998; Oehl *et al.* 2004) and thus their segregation in functioning through time is an additional mechanism by which AM fungi can differentially influence host responses. This could lead to a complementarity effect, however, may not be detected by sampling at a single time point, such as the case in our study. Detecting such an ebb and flow of colonisation by different AM fungi through time that coincides with observable host effects will be an important feature for furthering the assessment of AM fungal biodiversity effects.

In general, it is difficult, if not impossible due to the seemingly limitless possibilities, to determine all the differences in functional and

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resource-based niches that each AM fungal species within a more AM fungal-rich community could occupy to influence whether a complementarity or selection effect drives the AM fungal richness – plant productivity relationship. However, as AM fungal taxa functional and life-history characteristics can be linked to phylogeny (Maherali & Klironomos 2007), the use of phylogenetic over- and under-dispersed AM fungal communities may give rise to understanding the different combinations of AM fungi within a community that best supports the aboveground plant community.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 Soil characteristics and nutrient concentrations.
Table S2 ANOVA results for the number of LSU copies of each AM fungus.
Table S3 Means and standard errors for the number of LSU copies detected.
Table S4 ANOVA results for biodiversity effects.
Figure S1 Results for the test of interference by non-target DNA on the ability to detect target DNA.
Figure S2 Relative abundance of each AM fungal species in mixtures.

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