Mycorrhizal fungal identity and diversity relaxes plant-plant competition

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Abstract

There is a great interest in ecology to understand the role of soil microbial diversity for plant productivity and coexistence. Recent research has shown increases in species richness of mutualistic soil fungi, the arbuscular mycorrhizal fungi (AMF), to be related to increases in aboveground productivity of plant communities. However, the impact of AMF richness on plant-plant interactions has not been determined. Moreover, it is unknown whether species-rich AMF communities can act as insurance to maintain productivity in a fluctuating environment (e.g. upon changing soil conditions). We tested the impact of four different AMF taxa and of AMF diversity (no AMF, single AMF taxa and all four together) on competitive interactions between the legume Trifolium pratense and the grass Lolium multiflorum grown under two different soil conditions, showing low and high sand contents. We hypothesized that more diverse mutualistic interactions (e.g. when four AMF taxa are present) can ease competitive effects between plants, increase plant growth and maintain plant productivity across different soil environments. We used quantitative PCR to verify that AMF taxa inoculated at the beginning of the experiment were still present at the end. The presence of AMF reduced the competitive inequality between the two plant species by reducing the growth suppression of the legume by the grass. High AMF richness enhanced the combined biomass production of the two plant species and the yield of the legume, particularly in the more productive soil with low sand content. In the less productive (high sand content) soil, the single most effective AMF had an equally beneficial effect on plant productivity as the mixture of four AMF. Since contributions of single AMF to plant productivity varied between soil conditions, higher AMF richness.
Mycorrhizal fungal identity and diversity relaxes plant-plant competition

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Abstract

There is a great interest in ecology to understand the role of soil microbial diversity for plant productivity and coexistence. Recent research has shown increases in species richness of mutualistic soil fungi, the arbuscular mycorrhizal fungi (AMF), to be related to increases in aboveground productivity of plant communities. However, the impact of AMF richness on plant–plant interactions has not been determined. Moreover, it is unknown whether species-rich AMF communities can act as insurance to maintain productivity in a fluctuating environment (e.g. upon changing soil conditions).

We tested the impact of four different AMF taxa and of AMF diversity (no AMF, single AMF taxa and all four together) on competitive interactions between the legume *Trifolium pratense* and the grass *Lolium multiflorum* grown under two different soil conditions, showing low and high sand contents. We hypothesized that more diverse mutualistic interactions (e.g. when four AMF taxa are present) can ease competitive effects between plants, increase plant growth and maintain plant productivity across different soil environments. We used quantitative PCR to verify that AMF taxa inoculated at the beginning of the experiment were still present at the end.

The presence of AMF reduced the competitive inequality between the two plant species by reducing the growth suppression of the legume by the grass. High AMF richness enhanced the combined biomass production of the two plant species and the yield of the legume, particularly in the more productive soil with low sand content. In the less productive (high sand content) soil, the single most effective AMF had an equally beneficial effect on plant productivity as the mixture of four AMF. Since contributions of single AMF to plant productivity varied between soil conditions, higher AMF richness
would be required to maintain plant productivity in heterogeneous environments. Overall this work shows that AMF diversity promotes plant productivity and that AMF diversity can act as an insurance to sustain plant productivity upon changing environmental conditions.

Key words: AMF richness; plant competition; symbioses; biodiversity effect; insurance effect, overyielding, quantitative PCR
There is currently great interest in understanding the role of species richness and diversity in regulating ecosystem processes (Hooper et al. 2005). The relationship between plant diversity and plant productivity has already received much attention (e.g. Tilman et al. 1996, Hector et al. 1999, Loreau et al. 2002); however, the significance of soil microbial diversity for aboveground plant productivity is still poorly understood (Balvanera et al. 2006). Few studies have investigated whether soil microbial diversity can influence plant community productivity and plant–plant interactions (see Rillig 2004; van der Heijden et al. 2008). Here we focus on arbuscular mycorrhizal fungi (AMF), a group of obligatory root endophytes that form mutualistic associations with the majority of land plants by improving nutrient uptake in plant hosts (Smith and Read 2008).

Recently, Bastolla et al. (2009) illustrated how an increased number of mutualistic interactions can relax competition in species networks and thus increase biodiversity. In a similar manner, greater diversity of AMF taxa may ease belowground competitive interactions between plants as AMF taxa can have specific host preferences (Bever et al. 2001, Vandenkoomhuyse et al. 2003) and differ in functional compatibility between host plants (Ravnskov and Jakobsen 1995). Moreover, the variation in host benefits provided by differing AMF taxa, such as improved pathogen resistance (Newsham et al. 1995b, Maherali and Klironomos 2007) and nutrient uptake abilities (Jansa et al. 2005) may enhance plant species complementarity and thus overall plant productivity (Koide et al. 2000; Rillig 2004).

There have been a handful of studies that have begun to address the importance of AMF diversity for the productivity of a plant community (van der Heijden et al. 1998a,
van der Heijden et al. 2003, Balvanera et al. 2006, Vogelsang et al. 2006, Jansa et al.
2008). Some studies show AMF diversity can enhance the productivity of single plant
species (Lekberg et al. 2007, Maherali and Klironomos 2007) or a community of
grassland plants (van der Heijden et al. 1998a). Other studies found that a particular AMF
taxon can be as beneficial or even more beneficial to plant growth than the mixture of
several AMF (van der Heijden et al. 1998ab, van der Heijden et al. 2003, Vogelsang et al.
2006, Jansa et al. 2008), suggesting relationships between AMF diversity and plant
productivity to be mainly due to a sampling probability effect (Wardle 1999). However, it
has yet to be demonstrated how AMF diversity may affect plant competition and plant
complementarity effects, i.e. the other major mechanism (Loreau and Hector 2001,
Cardinale et al. 2007) underlying positive plant biodiversity effects on plant productivity.

Studies investigating whether AMF can alter competitive interactions have mainly
concentrated on comparisons of AMF taxa (Scheublin et al. 2007) or the absence versus
presence of AMF inoculum on plant competition (Fitter 1977, Hartnett et al. 1993,
and Foster 2009). It is conceivable that the wide range of AMF–host plant interactions
may reduce the overlap of resource niches among coexisting plants, thus reducing
interspecific competition and increasing complementarity between host plants and
potentially increasing total plant community productivity.

Most ecosystems harbour a diverse AMF community in the soil (Bever et al.
2001). Different AMF taxa can dominate under particular environmental conditions, such
as in different soil types (Oehl et al. 2010). Hence, the functioning of specific AMF taxa
may be depressed in one soil type and enhanced in another, yet the influence of the AMF
community on aboveground productivity in different soil types would be maintained.

This “insurance hypothesis” (Yachi and Loreau 1999) of AMF richness for the maintenance of plant coexistence and productivity in heterogeneous soil environments has yet to be tested.

In this study, the effects of AMF species richness and soil conditions on competitive interactions between a grass (*Lolium multiflorum* Lam.) and a legume (*Trifolium pratense* L.) were tested. We chose a grass-clover plant community as model system as these two species commonly coexist in agricultural and natural grassland ecosystems (Nyfeler et al. 2009), and because grasses and clover respond differently to AMF, with clover usually benefitting from AMF and many grasses being unresponsive (van der Heijden et al. 1998; Scheublin et al. 2007). We hypothesize that (1) co-inoculation of four AMF taxa improves the co-existence between two competing host plant species compared to inoculation of single AMFs and (2) the influence of AMF, inoculated individually and as a community, on competitive interactions between plants differs depending on the soil conditions. To test these hypotheses we grew *T. pratense* and *L. multiflorum* in a replacement series in the absence of AMF (AMF richness = 0), inoculated with one of four AMF taxa individually (low AMF diversity: richness = 1) and the combination of all four AMF taxa (high AMF diversity: richness = 4). This model system was replicated in two soil-sand mixtures to demonstrate the role soil conditions can play in mediating AMF community effects on plant–plant competitive outcomes. The four AMF taxa originated from Swiss grasslands where they commonly co-occur. We used quantitative PCR to confirm the presence of the AMF used as inocula at the end of
the experiment in the plant roots to provide confirmation of the co-colonization of roots by all four AMF within the high diversity treatment.

METHODS

Soil and inoculum preparation

Field soil was collected from a long-term grassland field harboring native *Lolium* and *Trifolium* species located at the Agroscope Reckenholz research station in Zürich, Switzerland (047° 42’ 74’’ N, 008° 51’ 78’’ E). Collected soil was then sieved through a 1 cm mesh in order to remove large stones and root fragments. This soil was mixed with sand by volume in the ratios of 1:4 and 4:1 soil to sand to create two different soil conditions with a “high sand” and “low sand” content, respectively. The two soil-sand mixtures were autoclaved for 99 min at 121 °C. Two samples of approximately 1 kg from each soil type were taken for nutrient analysis after autoclaving.

The high sand soil had a pH of 7.7 with 0.1 % organic C, 2.45 % clay, 8.2 % silt and 89.2 % sand and contained 20.5 mg·kg⁻¹ of water soluble inorganic N (NO₃⁻ and NH₄⁺) determined with a Skalar segment flow analyzer. Plant available P₂O₅ and K₂O, extracted by CO₂-saturated water, was 0.71 mg·kg⁻¹ and 5.0 mg·kg⁻¹ respectively. The ammonium acetate-EDTA (pH 4.65) extracted amounts of Ca, P, K and Mg in mg·kg⁻¹ were 7.02 × 10³, 33.5, 2.85 and 96.6, respectively. The low sand soil had a pH of 7.5 with 0.9 % organic C, 12.2 % clay, 20.8 % silt and 64.4 % sand and contained 50.5 mg·kg⁻¹ of water-soluble inorganic N. Plant available P₂O₅ and K₂O was 0.32 mg·kg⁻¹ and 7.5
mg·kg\(^{-1}\) respectively. The ammonium acid-extracted Ca, P, K and Mg in mg·kg\(^{-1}\) was
4.26 \times 10^3, 17.7, 24.4, and 160.9, respectively.

The four AMF fungi used were: *Glomus mosseae* (isolate BEG161, Jansa et al. 2002), *G. intraradices* (BEG 21, van der Heijden et al. 2006), *G. claroideum* (isolate JJ132, Jansa et al. 2002) and *Diversispora celata* (FACE 234, Gamper et al. 2009). These four AMF belong to the family Glomeraceae, each representing a specific clade (*Glomus* group Aa, Ab, B and C, respectively) and are common in Swiss arable and grassland soils (Schüessler et al. 2001, Schwarzott et al 2001, Gamper et al. 2009, Oehl pers. com.). These fungi were cultured on *Plantago lanceolata* L. in pots of 1 L volume for 5 months. The substrate was sand mixed with approximately 15 % field soil, receiving 20 ml Hoagland’s nutrient solution (Hoagland and Arnon 1950) with ¼ original concentration KH\(_2\)PO\(_4\) every two weeks and watered to maintain 20 % soil moisture by weight. A control inoculum (no AMF) was prepared in the same way as the four AMF inoculants. *Glomus claroideum, G. intraradices, G. mosseae* and *D. celata* inoculants were observed colonizing 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 AMF spores or colonization of roots were observed in the control inoculum.

**Preparation of AMF treatments and plant seedlings**

For the experiment, the cultured AMF material was transferred to 1 L pots containing 1.15 kg (dry weight) of one of the two soil-sand mixtures, high sand or low sand. Pots inoculated with a single AMF received 50 ml of inoculum, containing substrate and root fragments, of one of the four AMF. Treatments co-inoculated with all
four AMF species received 12–14 ml of roots and substrate of each AMF inoculum;
totaling again 50 ml of inoculum. The inocula were mixed throughout the soil substrate
within each pot.

Seeds of *Trifolium pratense* var. Milvus and *Lolium multiflorum* var. Daxus,
originating from seed multiplication plots located at the Agroscope Reckenholz research
station in Zürich, were surface sterilized by agitating them in 1.25 % sodium hypochlorite
diluted household bleach) for 5 minutes followed by a thorough rinse in dH$_2$O. The
seeds were then allowed to germinate on 1.5 % water agar during 2–4 days. Seedlings
were then transplanted evenly spaced into the AMF-inoculated pots. Pots were covered
with cellophane for three days to allow seedlings to establish. Seedlings that did not
survive were replaced up to two weeks after initial planting.

A microbial wash was created by using 1 L of the same un-autoclaved field soil
used to create the two soil treatments and wet sieving it through a series of sieves with the
smallest being 11 µm with 5 L of dH$_2$O. Ten ml of this was added to each pot after
planting in order to standardize the microbial community within each pot with a natural
grass/clover soil microbial community including rhizobia bacteria (evidenced by
numerous root nodules on red clover).

Pots were randomly distributed in two adjacent greenhouses. Plants were allowed
to grow for 25 weeks with 16 h / 25 °C days and 8 h / 16 °C nights. Plants received
natural light and supplemental illumination was provided by 400-W high-pressure
sodium lamps to maintain a light level above 300 W/m$^2$. Pots were watered with dH$_2$O by
weight as required to maintain soil moisture in the range of 10–20 %.
Data collection

Shoots were harvested 9, 16 and 25 weeks after planting in order to reduce aboveground competition for light and to simulate mowing/graazing as is usually done in managed grass-clover meadows/pastures. At the time of harvest at 9 and 16 weeks shoots were cut at approximately 5 cm above the soil surface. During the final harvest at 25 weeks shoots were cut directly at the soil surface and roots were rinsed clean of soil and frozen at –20 °C until they could be processed further. Shoots were dried at 80 °C after each harvest and the biomass recorded to the nearest tenth of a mg. The aboveground biomass of each plant species was pooled across harvests and used in all subsequent analyses.

Frozen roots were thawed, cut into 1–2 cm fragments, and mixed for molecular assessment of AMF (see below) and for determining AMF root colonization. To determine the level of colonization of each single-AMF inoculated treatment and for assessing the viability of the inoculants, a random sample of 1–2 g of fresh root was fixed in 50 % ethanol overnight, cleared with 10 % KOH in an 80 °C water bath for 45 min and then stained with 5 % pen-ink vinegar (Vierheilig et al. 1998) for 10 min in an 80 °C water bath. A random selection of the cleared and stained roots were mounted on glass slides with 50 % glycerine under a cover slip and scored for the presence AMF using the intersect method (McGonigle et al. 1990) for 100 intersects.

The presence of the four different AMF in the high-diversity AMF treatment was determined using quantitative real-time PCR (qPCR) with hydrolysis probes targeting species-specific motifs of the large ribosomal subunit (LSU) of *G. mosseae*, *G. intraradices* and *G. claroideum*, following the protocol developed by Thonar (2009); see
Appendix A for details. For the qPCR quantification of *D. celata*, novel primers and a hydrolysis probe were designed (see Appendix A). The primers for *D. celata* also targeted a fraction of the LSU ribosomal gene copies, similar to the three other AMF taxa.

**Experimental design and statistical analyses**

The experiment was set up as a randomised block design with two blocks (replicates evenly divided between 2 greenhouses), two soil conditions (high sand and low sand) and six AMF treatments (no mycorrhiza, AMF I, AMF II, AMF III, AMF IV and AMF I+II+III+IV). The twelve combinations of two soil conditions × six AMF treatments were factorially combined with five plant-competition treatments. These reflected a replacement series between *T. pratense* and *L. multiflorum*, i.e. individuals of the two species were planted in the following ratios: 8:0, 6:2, 4:4, 2:6, or 0:8. Each treatment combination was replicated six times for a total of 360 pots.

Two pots were found to be contaminated with AMF not initially inoculated into the pots by both light microscopy and real-time PCR. These two pots were removed from the data set. One pot, with 6 *T. pratense* and 2 *L. multiflorum* in high sand soil, initially non-mycorrhizal, was found to be colonized by *G. claroideum*. The second pot of 8 *T. pratense* in low sand soil, which was initially inoculated with *D. celata*, was contaminated with *G. intraradices*.

The aboveground biomass of *T. pratense* and *L. multiflorum* was assessed with a three-way analysis of variance (ANOVA) with soil conditions, planting ratio, AMF treatment and their interactions as main sources of variation. One-way ANOVAs and
Tukey HSD tests were used to further assess the variation in aboveground biomass among AMF treatments within each soil condition × planting ratio. The two greenhouses in which plants were grown was used as a block effect in all ANOVAs.

Competitive interactions between the two plant species were determined by assessing the growth per individual plant within a mixture relative to that in monoculture; which was calculated as the relative yield per individual (RY\textsubscript{ind}) by the equation:

\[ RY_{\text{ind}} = \frac{O_{ij}}{M_{ij}}. \]

Here \( O_{ij} \) is the observed aboveground biomass per individual of plant species \( i \) grown in mixture within a pot of a soil condition × AMF treatment combination \( j \) and \( M_{ij} \) is the mean aboveground biomass per individual within the monoculture of plant species \( i \) present within a pot of the same soil condition × AMF treatment combination \( j \) (de Wit 1960). The relative yield per individual (RY\textsubscript{ind}) portrays the mean change in shoot biomass production of an individual plant as conspecifics are replaced by heterospecifics under the same planting density.

In addition, the relative yield per stand (RY) was also calculated from the observed aboveground biomass per species in mixture divided by the aboveground biomass per species in monoculture. The relative yields per stand of the two plant species in mixtures were added to obtain relative yield totals (RYTs) for each soil condition × AMF treatment combination (de Wit et al. 1966). The RYT provides an overall summary of changes in the total aboveground biomass in mixtures relative to monocultures and is often used to assess overyielding in grass-clover mixtures, where values greater than 1
indicate a greater biomass production in mixtures than the average of the two plant species in monoculture (see Weiner 1980, Kirwan et al. 2007, Marquard et al. 2009).

A three-way ANOVA was used to test the effects of AMF treatment, soil condition and planting ratio, as well as their interactions, on the RY$_{ind}$ of *T. pratense* and *L. multiflorum* and on RYT in mixtures, with the greenhouse in which the plants were grown added as a block effect. The RY$_{ind}$ and RYT were assessed for differences from 1 (RY$_{ind}$ = 1 and RYT = 1, respectively) within each soil condition × AMF treatment combination in order to determine the influence of each AMF on the competitive interactions between the two host species as well as their influence on overyielding in plant mixtures. The effect of each AMF on the RY$_{ind}$ of *T. pratense* and *L. multiflorum* and the RYT was also assessed using contrasts to determine differences in individually inoculated AMF treatments from the non-mycorrhizal control as well as the high-diversity AMF treatment with all AMF co-inoculated.

In order to improve homoscedasticity in the data, Box-Cox transformations were used for the assessment of aboveground biomass and relative yield measures were log transformed prior to analyses. Means were considered to differ significantly at a type-I error level of $\alpha < 0.05$. Statistical analyses were carried out using R 2.10.1 (R Foundation for Statistical Computing 2009).

**RESULTS**

*Aboveground biomass*
Overall the total shoot biomass of *T. pratense* was strongly influenced by planting ratio and AMF treatment, but not by soil conditions (Table 1). The total biomass of *T. pratense* was greater in mixtures where it was more abundant (Fig. 1A and B). Inoculation with AMF increased *T. pratense* biomass up to 15 times compared to the non-mycorrhizal treatment depending on soil and planting ratio (Fig. 1A and B), resulting in a significant three-way interaction effect (Table 1). In all planting ratios within the high sand soil, the greatest *T. pratense* biomass occurred in the inoculation treatments with *D. celata* or with all four AMF; with *D. celata* frequently being the greater of the two, followed by the inoculation treatments with *G. intraradices*, *G. claroideum* or *G. mosseae*, which were generally similar in effect (Fig. 1A). Within the low sand soil, the high-diversity AMF, *D. celata* and *G. claroideum* treatments had large beneficial effects on *T. pratense* biomass and were commonly similar in effect; however, in mixtures with *L. multiflorum*, inoculation with all four AMF consistently yielded the greatest *T. pratense* biomass (Fig. 1B).

The shoot biomass of *L. multiflorum* was most strongly influenced by soil conditions, followed by AMF treatment and planting ratio (Table 1; Fig. 1C and D). Shoot biomass of *L. multiflorum* was greatest in the low sand soil as well as in mixtures in which it occurred in high proportion (Fig. 1D). However, unlike *T. pratense*, the *L. multiflorum* biomass did not vary consistently among AMF treatments and was generally greatest in the non-mycorrhizal treatment (Fig. 1C and D). In no case was the shoot biomass of *L. multiflorum* significantly improved by the presence of AMF (Fig. 1C and D). Whether AMF inoculation resulted in a significant depression in *L. multiflorum* biomass depended on soil conditions and planting ratio (Fig. 1C and D).
Relative yields

The relative yield per individual (RY\textsubscript{ind}) of \textit{T. pratense} was strongly influenced by soil conditions, AMF treatments and planting ratio (Table 2). In mixtures with \textit{L. multiflorum}, the RY\textsubscript{ind} of \textit{T. pratense} was depressed below its RY\textsubscript{ind} in monoculture by 80\% in the high sand soil (Fig. 2A) and 90\% in low sand soil (Fig. 2B) in the absence of AMF. The presence of AMF significantly enhanced the RY\textsubscript{ind} of \textit{T. pratense} in both soil conditions compared to the non-mycorrhizal treatment (all p < 0.0001, Fig. 2A and B), demonstrating that AMF reduced competitive pressure by \textit{L. multiflorum}. In the high sand soil, both the high-diversity and \textit{D. celata} AMF treatments were similar in effect (p > 0.50) and resulted in a RY\textsubscript{ind} that did not differ from 1 (p = 0.2, Fig. 2A), indicating that competitive effects of \textit{L. multiflorum} depressing aboveground growth of \textit{T. pratense} were completely alleviated in these two treatments. However, all other AMF treatments differed strongly from the high-diversity AMF treatment (all p < 0.0001). Intriguingly, the same effect was not seen in the low sand soil, where although all AMF treatments improved the RY\textsubscript{ind} of \textit{T. pratense}, all were significantly lower than 1 (all p < 0.0001, Fig. 2B). Furthermore, all \textit{T. pratense} plants inoculated with single AMF had significantly lower RY\textsubscript{ind} than the plants inoculated with the high-diversity treatment with all four AMF (all p < 0.01, Fig. 2B; Table 2).

The RY\textsubscript{ind} of \textit{L. multiflorum} was also found to be heavily influenced by soil conditions, AMF treatment and planting ratio (Table 2). In all cases, the RY\textsubscript{ind} of \textit{L. multiflorum} was significantly greater than 1 in both soils (all p < 0.0001) and the \textit{L. multiflorum} individuals also obtained greater biomass when grown in mixture with \textit{T. pratense}. 
pratense (Fig. 2C and D). Within the high sand soil, the RY$_{\text{ind}}$ of L. multiflorum (Fig. 2C) showed an inverse ranking of AMF treatments in biomass production compared to that of T. pratense (Fig. 2A), demonstrating an AMF-mediated T. pratense competitive effect on L. multiflorum. Moreover, L. multiflorum RY$_{\text{ind}}$ was significantly greater in the non-mycorrhizal treatment than all other AMF treatments (all p < 0.05, Fig. 2C). The RY$_{\text{ind}}$ of L. multiflorum in the low sand soil was only significantly depressed below the non-mycorrhizal treatment in the presence of D. celata (p < 0.01). The effect of the high-diversity AMF treatment on the RY$_{\text{ind}}$ of L. multiflorum also differed from that of G. intraradices (p < 0.0001, Fig. 2D).

The relative yield total (RYT) varied between soils as well as among AMF treatments and planting ratios (Table 2). Overyielding (RYT values > 1) occurred more frequently in the high sand soil resulting in an overall greater RYT than in the low sand soil (Fig 2E and F). Regardless of soil conditions, inoculation with G. mosseae and the non-mycorrhizal resulted in similar RYT values; both of which did not result in overyielding and differed significantly from inoculation with G. intraradices and the high-diversity AMF treatment (all p ≤ 0.01, Fig. 2E).

**Arbuscular mycorrhizal colonization**

All AMF were found to colonize roots of host plants when inoculated individually. Irrespective of soil type and planting ratio, G. intraradices colonized the greatest percentage of roots (79.5 %, SE = 1.5), followed by G. claroideum (35.1%, SE = 1.7), D. celata (22.0 % SE = 1.4) and G. mosseae (17.7 %, SE = 1.8). All four AMF were detected by qPCR in 43 of the 60 replicates where all four AMF were co-inoculated. In 7
cases *G. mosseae* and in 8 cases *G. claroideum* were not detected, while in two cases both *G. claroideum* and *G. mosseae* were not detected. *Glomus intraradices* was the most abundant within roots, when all four AMF were co-inoculated, with an average of $16.2 \times 10^5$ (SE = $1.84 \times 10^5$) LSU copies per mg of dried root, followed by *D. celata* ($6.09 \times 10^4$, SE = $0.50 \times 10^4$), *G. claroideum* ($5.66 \times 10^4$, SE = $1.07 \times 10^4$) and *G. mosseae* ($2.87 \times 10^3$, SE = $0.72 \times 10^3$). The percent colonization and the number of LSU copies of each of the AMF taxa differed among planting ratios and soils depending on AMF taxa (see Appendix B). In general, all AMF taxa were abundant within the *T. pratense* monoculture and the high sand soil, with the exception of *G. claroideum*, which was more present within roots in the low sand soil and showed preference for the *L. multiflorum* monoculture in the high sand soil (Appendix B).

**DISCUSSION**

Our results demonstrate that AMF identity and diversity has a large impact on competitive interactions between the grass *L. multiflorum* and the legume *T. pratense*, favoring the legume. Moreover, in support of our hypothesis, the high-diversity AMF treatment with all four AMF in all but one case improved the biomass production of individual *T. pratense* plants more than did the individual AMF taxa, irrespective of soil conditions, enabling it to better coexist with *L. multiflorum* in mixtures (see Fig. 2). The effect of AMF species identity and AMF diversity on plant productivity varied between the two soil conditions. For example, in the less productive high sand soil, the best single AMF species and the diverse AMF mixture had an equally beneficial effect on the competitive ability of the legume. Conversely, in the more productive low
sand soil the diverse AMF species community was more beneficial than the best individual AMF in supporting legume competitive ability. The differences between soil conditions in the relative importance of AMF species identity versus diversity provides support for the insurance effect of biodiversity (Yachi and Loreau 1999), demonstrating the role of AMF richness to be an important mediator of plant productivity across heterogeneous soil conditions.

Effects of AMF on plant productivity between soils

Earlier work has shown that AMF and different AMF taxa alter competitive interactions and coexistence between plants (Fitter 1977, Hartnet et al. 1993, Hetrick et al. 1994, Zobel and Moora 1995, Scheublin et al. 2007, Schroeder-Moreno and Janos 2008, Collins and Foster 2009). However, effects of AMF diversity on competitive interactions were not yet investigated. This study now shows that AMF species richness indeed influences plant competition and that, especially in heterogeneous environments, the effects of a diverse AMF community may result in greater effects on the competitive interactions between plants than most of the individual AMF of which the AMF community is comprised.

Furthermore, our results show that AMF can enhance overall plant productivity by easing competitive interactions between plants resulting in overyielding. Within the more productive low sand soil the greater $R_{\text{ind}}$ of $T. \text{pratense}$ in mixtures in the high-diversity treatment reveals a more diverse AMF community is of greater importance for plant co-existence than any individual AMF. This demonstrates complementarity within a diverse belowground community of mutualists to be a mechanism behind plant
complementarity aboveground. However, the effect of the diverse AMF community on aboveground plant productivity within the high sand soil was similar to that of a single AMF taxa, *D. celata*, suggesting a sampling / selection effect within the high AMF diversity treatment may be behind the functioning of the AMF community (see Wardle 1999 / Loreau and Hector 2001). This reveals AMF identity can be of greater importance than diversity *per se* depending on abiotic soil conditions.

Importantly, earlier studies investigating effects of AMF richness on plant coexistence and community structure (e.g. van der Heijden et al. 1998a, Klironomos 2000, Vogelsang et al. 2006) did not test whether the AMF taxa co-inoculated at the start of the experiment were still present at the end. Using quantitative PCR we could verify that in the majority of pots all four AMF inoculated at the start where still present at the end of the experiment. Moreover, the AMF taxa identified as being most effective (*D. celata*), was present in all co-inoculated pots at the end of the experiment. This provides for the first time empirical evidence in support of conclusions that effects of a more diverse AMF community can be driven by the dominance of the single most effective AMF.

*Competitive interactions between host plants*

Our findings show *L. multiflorum* to have a strong interspecific competitive effect on *T. pratense* under both soil conditions tested, but particularly in the absence of AMF and in the more productive low sand soil. In the absence of AMF, the competitive suppression of growth in *T. pratense* by *L. multiflorum* was considerable. This corresponds to previous studies that observed *Lolium* spp. as a strong competitor (Stone
et al. 1998, Hodge et al. 1999, Cralle et al. 2003). The depression in *L. multiflorum* productivity in AMF-inoculated treatments compared to the non-mycorrhizal treatment would suggest AMF supply soil resources toward *T. pratense* away from *L. multiflorum*. This result is similar to that of Fitter (1977), who found growth of *L. perenne* to be greatly reduced by AMF when competing belowground with *Holcus lanatus* resulting from AMF-mediated nutrient uptake. This effect of indirect competition has also been observed in other AMF-related plant competition studies where the species that was better able to utilize AMF associations to increase its own nutrient uptake caused a growth depression in the neighboring competing plant (Zobel and Moora 1995; Moora and Zobel 1996; Marler et al. 1999; Zabinski et al. 2002). This study also confirms previous studies that show strong positive responses by *Trifolium* species to AMF inoculation (Crush 1995, Li et al. 1997, Takacs et al. 2006, Wang et al. 2007, Sudová 2009) and that *T. pratense* depends heavily on AMF to acquire soil resources (e.g. up 53–65 % of soil P in the study by Feng et al. 2003).

The improved *L. multiflorum* growth in mixtures relative to monocultures is likely a response to reduced intraspecific competition as well as the fact that *T. pratense* can fix nitrogen, which subsequently may have increased *L. multiflorum* growth, in line with previous observations in grass–legume mixtures (Turkington and Klein 1991; Elgersma and Hassink 1997; Elgersma et al. 2000; Lucero et al. 1999) and a series of plant biodiversity experiments (Temperton et al. 2007, Wacker et al. 2009). However, due to inherent problems with the classic replacement series (see Connolly 1986; Snaydon 1991; Gibson et al. 1999; Jolliffe 2000 for a discussion) these two effects are inseparable in our study. In either case, the increased biomass production of individual plants of *L.*
multiflorum and the alleviation of the L. multiflorum competitive suppression of T. pratense biomass production in the presence of AMF resulted in overyielding in mixtures reflecting the relaxed plant-plant competition, where AMF benefits to T. pratense outweighed the negative effects on L. multiflorum.

Synthesis

Our results demonstrate that AMF taxa differ in their ability to influence interspecific plant competition and that a diverse community of AMF can ease plant competition to a greater extent than do the individual AMF taxa. This reveals that a diverse AMF community is a key driver of the productivity of grass–clover ecosystems by relaxing interspecific plant competition and contributing to overyielding (Hector et al. 2002). However, whether or not a diversity of AMF improved productivity more than the best single AMF was dependent on soil conditions. The differing relative effects of AMF diversity in the two soil conditions points to the importance of AMF diversity as an insurance in heterogeneous soil environments, which to date has received surprisingly little attention. Moreover, the use of qPCR proved to be a useful tool in detecting and quantifying co-colonizing AMF and their combined roles in ecosystem functioning for future soil biodiversity studies. What remains clear is that there is still much to be uncovered regarding the role of AMF diversity within fluctuating and heterogeneous environments as is typical in many natural ecosystems.
We thank Agroscope Reckenholz Tänikon research station for providing financial support for the project. We also thank Caroline Scherrer for the preparation of inocula, Beat Boller for the provision of seeds and Hannes Gamper for providing AMF isolates. The helpful comments of two anonymous reviewers are greatly appreciated.
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TABLE 1. Results of the ANOVA testing for the effects of soil conditions (Soil), planting ratio (Ratio) and AMF treatment (AMF) on the overall aboveground biomass of *L. multiflorum* and *T. pratense*. Prior to analyses Box-Cox transformations were used to improve the homoscedasticity of the residuals. The greenhouse in which plants were grown is represented by the ‘Block’ effect.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>F</th>
<th>p</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>1</td>
<td>1.14</td>
<td>0.29</td>
<td>2.44</td>
<td>0.12</td>
</tr>
<tr>
<td>Soil</td>
<td>1</td>
<td>0.93</td>
<td>0.34</td>
<td>5.29 × 10^3</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Planting Ratio</td>
<td>3</td>
<td>1.78 × 10^3</td>
<td>&lt; 0.0001</td>
<td>11.2</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>AMF</td>
<td>5</td>
<td>327</td>
<td>&lt; 0.0001</td>
<td>14.5</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Soil x Ratio</td>
<td>3</td>
<td>136</td>
<td>&lt; 0.0001</td>
<td>3.45</td>
<td>0.02</td>
</tr>
<tr>
<td>Soil x AMF</td>
<td>5</td>
<td>13.5</td>
<td>&lt; 0.0001</td>
<td>3.31</td>
<td>0.007</td>
</tr>
<tr>
<td>Ratio x AMF</td>
<td>15</td>
<td>8.03</td>
<td>&lt; 0.0001</td>
<td>3.50</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Soil x Ratio x AMF</td>
<td>15</td>
<td>2.49</td>
<td>0.002</td>
<td>0.96</td>
<td>0.49</td>
</tr>
<tr>
<td>Error</td>
<td>237</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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</table>
TABLE 2. Results of the ANOVA testing for the effects of soil conditions (Soil), planting ratio (Ratio) and AMF treatment (AMF) on the relative yield per individual (RY$_{ind}$) of *L. multiflorum*, *T. pratense* and the relative yield total (RYT) in mixtures. Data were log-transformed prior to analyses to improve homoscedasticity in the data of all three measures. The greenhouse in which plants were grown is represented by the ‘Block’ effect.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>T. pratense RY$_{ind}$</th>
<th>p</th>
<th>L. multiflorum RY$_{ind}$</th>
<th>p</th>
<th>RYT</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>1</td>
<td>2.61</td>
<td>0.11</td>
<td>5.16</td>
<td>0.02</td>
<td>5.08</td>
<td>0.03</td>
</tr>
<tr>
<td>Soil</td>
<td>1</td>
<td>609 &lt; 0.0001</td>
<td></td>
<td>84.2 &lt; 0.0001</td>
<td></td>
<td>10.1</td>
<td>0.002</td>
</tr>
<tr>
<td>Planting Ratio</td>
<td>2</td>
<td>15.8 &lt; 0.0001</td>
<td></td>
<td>451 &lt; 0.0001</td>
<td></td>
<td>4.61</td>
<td>0.01</td>
</tr>
<tr>
<td>AMF</td>
<td>5</td>
<td>171 &lt; 0.0001</td>
<td></td>
<td>16.7 &lt; 0.0001</td>
<td></td>
<td>8.80</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Soil x Ratio</td>
<td>2</td>
<td>7.98 0.0005</td>
<td></td>
<td>6.27 0.002</td>
<td></td>
<td>0.95</td>
<td>0.39</td>
</tr>
<tr>
<td>Soil x AMF</td>
<td>5</td>
<td>4.16 0.001</td>
<td></td>
<td>4.89 0.0003</td>
<td></td>
<td>1.63</td>
<td>0.15</td>
</tr>
<tr>
<td>Ratio x AMF</td>
<td>10</td>
<td>1.58 0.12</td>
<td></td>
<td>2.55 0.007</td>
<td></td>
<td>0.98</td>
<td>0.46</td>
</tr>
<tr>
<td>Soil x Ratio x AMF</td>
<td>10</td>
<td>1.09 0.37</td>
<td></td>
<td>1.00 0.44</td>
<td></td>
<td>0.89</td>
<td>0.55</td>
</tr>
<tr>
<td>Error</td>
<td>177</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 1. Mean and standard error (SE) of the aboveground biomass of *T. pratense* (grey bars) and *L. multiflorum* (black bars) are shown for each planting ratio (*T. pratense : L. multiflorum*) and AMF treatment combination in both high sand (A and C) and low sand (B and D) soils. AMF treatments are denoted as: N = non-mycorrhizal, M = *G. mosseae*, I = *G. intraradices*, C = *G. claroideum*, D = *D. celata*, A = inoculation with all 4 AMF taxa. Significant differences (Tukey HSD p > 0.05) between AMF treatments within each planting ratio and soil conditions are indicated by different letters. N.S. = not significant.

Fig. 2. The relative yield per individual (RY\textsubscript{ind}) of *T. pratense* (A and B) and *L. multiflorum* (C and D) as well as the relative yield total (RYT, E and F) in both high sand (A, C and E) and low sand (B, D and F) soils. All *T. pratense* RY\textsubscript{ind} values differed from 1, with the exception of *D. celata* and All AMF treatments in the high sand soil (A). In all other cases, regardless of soil conditions, the RY\textsubscript{ind} of *T. pratense* differed from 1. In all cases the RY\textsubscript{ind} of *L. multiflorum* differed from 1. The RYT differed from 1 in all cases in the high sand soil (E) except for the non-mycorrhizal and *G. mosseae* treatments. Inoculation with *G. intraradices* and all four AMF resulted in RYT values significantly greater than 1 within the low sand soil (F).
FIGURE 1.

A

FIGURE 1.

B

C

D

L. multiflorum : T. pratense

L. multiflorum : T. pratense
**Figure 2.**

A

B

C

D

E

F

---

Relative yield per individual T. pratense

Relative yield per individual L. multiflorum

Relative Yield Total (RYT)

T. pratense : L. multiflorum

Non-Mycorrhizal

G. mosseae

G. intraradices

G. claroideum

D. celata

All AMF

---

T. pratense : L. multiflorum