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DIFFERENT ARBUSCULAR MYCORRHIZAL FUNGAL SPECIES ARE POTENTIAL DETERMINANTS OF PLANT COMMUNITY STRUCTURE

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Abstract. Almost all natural plant communities contain arbuscular mycorrhizal fungi (AMF). We hypothesized that the species composition of AMF communities could have the potential to determine plant community structure if the growth response to different AMF species or to communities of AMF species varies among plant species. To test the existence of such a differential response we conducted a pot experiment where each of three plant species, *Hieracium pilosella, Bromus erectus,* and *Festuca ovina* were inoculated with each of four AMF species, or with a mixture of these four AMF species, or were uninoculated. The AMF species originated from a calcareous grassland in which the three plant species also coexisted.

We obtained three pieces of evidence suggesting that AMF have the potential to determine plant community structure. First, plant species differed in their dependency on AMF, thus varying in degree of benefit received. Second, specific AMF species and a mixture of these AMF species had significantly different effects on several plant growth variables, and these effects were not the same on each plant species. Third, the amount of variation in the growth response of a plant species to four AMF species and to the mixture of AMF species differed among the plant species. *Hieracium* differed greatly in its growth response to several AMF species while *Bromus* did not exhibit much variation in its response to different AMF species.

The varying mycorrhizal dependency of different plant species has previously been proposed as a mechanism determining plant community structure. However, we found that the mycorrhizal dependency of a plant species can vary greatly because of differential growth responses to specific AMF species compared to the growth of the uninoculated plants. Consequently mycorrhizal dependency, as a measure indicating how much a plant depends on AMF for its growth, is not necessarily a fixed value and therefore cannot be used as a definitive term. In addition, those plant species with highly variable responses to single AMF species or to combinations of AMF species (AMF communities) will be strongly affected by the specific species of AMF that occupy their roots, in contrast to plant species that do not respond differently to different AMF species. We conclude that, through their differential effects on plant growth, AMF species that co-occur as natural AMF communities have the potential to determine plant community structure, and that future studies on plant population and community structure need to consider the strength of their role as a determinant.

Key words: arbuscular mycorrhizal fungal diversity; Bromus erectus; *community structure;* Festuca ovina; Hieracium pilosella; *mutualism; mycorrhizal symbiosis and dependency; plant–fungi interactions; vesicular–arbuscular mycorrhiza (VAM).*

INTRODUCTION

A major aim in plant ecology is to understand the determinants of plant community structure, i.e., the diversity of plant species, their spatial distribution, and their relative abundance. Plant–soil interactions, plant–climate interactions, interactions among plants (Grime 1979, Tilman 1982), and interactions of plants with herbivores (Brown and Gange 1989, Brown 1990) and pathogens (Dobson and Crawley 1994) are thought of as major determinants of plant community structure. The symbioses between arbuscular mycorrhizal fungi (AMF; class Zygomycetes, order Glomales) and plants could also be important because ~80% of all terrestrial plant species form this symbiosis (Smith and Read 1997).

Several studies show that the presence of AMF alters plant community structure by affecting the relative abundance of plant species and plant-species diversity (Grime et al. 1987, Gange et al. 1990, Sanders and Koide 1994). Grime et al. (1987) suggested that the mechanism by which the presence of AMF affects the floristic diversity of plant communities is interplant transport of assimilates from the dominant species in the canopy via a common mycorrhizal hyphal network to subordinate plant species. Another mechanism by which AMF may affect plant community structure is the differential growth response of plant species to col-

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onization by AMF, their so-called ''mycorrhizal dependency'' (Gerdemann 1975, Plenchette et al. 1983, Habte and Manjunath 1991).

Most natural ecosystems comprise several AMF species, forming AMF communities (Walker et al. 1982, Johnson et al. 1991, 1992). Because AMF are thought to lack any host specificity (Law 1988, Fitter 1990), each plant species could potentially be colonized by each AMF species from that community. Which of these AMF species colonizes a plant might be important for its fitness because AMF species are known to vary in their ability to take up phosphorus (Jakobsen et al. 1992) and in their stimulation of plant growth (Haas and Krikun 1985). Furthermore, different plant species may respond differently to specific AMF species. We therefore hypothesize that differential responses of plant species to specific AMF species exist. This would mean that the species composition of AMF communities could potentially affect the way plant species coexist and therefore be a determinant of plant community structure.

Until recently there was little evidence to support our hypothesis because experiments demonstrating differential effects of AMF species were carried out on single crop plants and used AMF species originating from different soils, which may never have naturally co-occurred with the study plant (Jensen 1982, 1984, Powell et al. 1982, Raju et al. 1990, Ravnskopf and Jakobsen 1995). However, one recent study has been made in an ecological context on the responses of two closely related plant species to different AMF species, all of which originated from the same natural community (Streitwolf-Engel et al. 1997). This study demonstrated that the AMF species strongly determined clonal growth traits of the two plant species, such that they would have the potential to affect population structure. The effects of each AMF species were not the same in the two plant species, and the differential effects were independent of the differential colonization rates of the different AMF species. The results of Streitwolf-Engel et al. (1997) indicate the need to investigate the effects of different AMF species with more co-existing plant species that exhibit a greater diversity in growth form and that occupy different orders of dominance within plant communities, in order to assess the potential of different AMF to determine plant community structure.

To test our hypothesis, three plant species were separately inoculated with four single AMF species, or with a mixture of these four species, or were uninoculated. Both the plant species and the AMF species originated from the same calcareous grassland. Two of the plant species are frequently dominants of calcareous grassland in Europe and the third species is subordinate. Our aims were to investigate whether (1) plant species, which naturally coexist, respond differently to AMF species that co-occur in the same community, (2) plant species respond differently to a community of AMF species, and (3) plant species vary in their response to inoculation with different AMF species and AMF communities.

Methods

Plant and fungal material

Fungal material used in this experiment originated from a field-study site, Nenzlinger Weide; a calcareous grassland in the Jura Mountains, Switzerland (grid reference 255 609 of the Landeskarte der Schweiz sheet 1067 [Bundesamt für Landestopografie, 3084 Wabern, Switzerland]). Nenzlinger Weide is a plant-species-rich calcareous grassland with a diverse community of mycorrhizal fungi (Sanders et al. 1995). Four cultures of morphologically different AMF were isolated from Nenzlinger Weide. These cultures will be called ''AMF species,'' although some of the fungi have not yet been taxonomically described. Cultures of *Glomus geosporum* (Nicol. and Gerd.) Walker (isolate BEG 18), *Glomus* sp. (isolate BEG 19) and *Glomus* sp. (isolate BEG 21) originated from single spores of AMF that were extracted from field soil by a sucrose centrifugation technique (Walker et al. 1982) and were propagated on *Plantago lanceolota*. The fourth culture, *Glomus* sp. (isolate Basle Pi), was obtained from roots of a *Prunella vulgaris* L. plant which was growing at Nenzlinger Weide. The roots of this *Prunella* plant were carefully washed to remove soil and planted into a soil mixture of sand and expanded clay (1:1 volume : volume). This mixture of sand and expanded clay, which contained sporocarps, was used to inoculate plants. So far only sporocarps of one morphological type were observed in this inoculum. *Glomus* sp. (BEG 19) shares morphological characteristics of *Glomus constrictum* Trappe and of *Glomus botryoides* Rothwell and Victor. *Glomus* sp. (BEG 21) is similar in morphology to *Glomus laccatum* Blaskowski. Sporocarps of the fourth *Glomus* sp. (Basle Pi) were similar in morphology to *Glomus microcarpum* Tulasne and Tulasne. All cultures are maintained in the Botanical Institute of the University of Basle (Switzerland) or are available through the *Banque Européenne des Glomales*.[1]

The three plant species used in this experiment are *Bromus erectus* Huds. and *Festuca ovina* L., two perennial grass species that dominate Nenzlinger Weide, and *Hieracium pilosella* L., a subordinate species at Nenzlinger Weide.

AMF inoculation and plant growth conditions

Seeds of each plant species were germinated on quartz sand and seedlings were planted on 8 November 1994 into 560-cm^3 plastic pots containing 400 g of an autoclaved (121°C; 30 min) soil mixture (1:1 quartz sand : soil from Nenzlinger Weide). The seedlings of each plant species were grown with each of the four single AMF species, with all four AMF species to-

[1] URL:http://biont.ukc.ac.uk/beg/asp/default.asp

gether, or without AMF, in a total of 144 pots. Each pot, which received three seedlings, was either inoculated with 2.5 g of soil inoculum of one of the four AMF species, with 2.5 g of mixed soil inoculum comprising of an equal proportion of each of the four AMF species, or with 2.5 g autoclaved (121°C; 30 min) mixed soil inoculum. The treatment that received mixed soil inoculum is termed the ''mixed-AMF species'' treatment and that which received the autoclaved mixed soil inoculum is termed the ''non-AMF'' treatment. After 7 d the two smallest seedlings were removed from each pot. All 144 pots received 2 mL of inoculum washing (20 g inoculum of the mixed inoculum sieved through a 30-μm mesh sieve with 600 mL water) to correct for possible differences in bacterial and fungal communities from the different inocula (Koide and Li 1989).

The plants were watered three times a week with distilled water to an amount that was equal to 10% soil mass. No additional nutrients were given during the experiment. The pots were kept in the greenhouse and additional lighting was provided with halogen lights to a day length of 15 h.

Measurements and harvesting

After 80 d the plants were harvested. Plants were separated into shoots and roots. Roots were washed to remove soil, mixed, and divided into two subsamples and fresh mass was determined on both. One root subsample and the shoot of each plant were oven dried (90°C) and weighed. The root dry mass of the other root subsample was calculated by multiplying the fresh mass with the fresh mass to dry mass ratio of the oven-dried root subsample. The sum of root dry mass of both subsamples gave total root dry mass. The sum of root dry mass and shoot dry mass gave total plant dry mass. Dried plant material was ground and mixed thoroughly, and phosphorus concentrations of roots and shoots were determined by the molybdate blue ascorbic-acid method (Watanabe and Olsen 1965). For a few samples, especially non-AMF *Hieracium* plants, not enough plant material was available to determine root or shoot phosphorus concentrations.

The root length was determined on one fresh root subsample (Marsh 1971) and the total root length was calculated based on the known fresh mass of both root subsamples. The specific root length was determined by dividing total root length with total plant dry mass. Specific root length can be used to compare different treatments, independently of effects on total plant dry mass. After determining the root length of the fresh subsample the roots were cleared with 10% KOH and stained with trypan blue (Phillips and Hayman 1970). The percentage of root length colonized by AMF was estimated by a modified line intersection method (McGonigle et al. 1990), where a minimum of 50 line intersections per root sample were scored for the presence of hyphae, vesicles, and arbuscules. From these measurements the total percentage of root length colonized by AMF (which equals the amount of root length occupied by hyphae) and the percentage of root length occupied by arbuscules and vesicles was estimated. The total percentage of root length colonized by AMF and the root length occupied by arbuscles and vesicles are referred to as ''fungal variables'' in this study. All other variables are referred to as ''plant variables.''

Experimental design and statistical analysis

The experiment was set up as a complete randomized block design with two factors. One factor, plant species, contained three levels, and the second factor, AMF treatments, contained six levels, making a total of 18 treatment combinations. Each treatment was replicated eight times. Each replicate was assigned to a block, making a total of 8 blocks. The position of both the blocks and the pots within a block were randomized weekly. A significant block effect did not occur for any of the variables, and therefore no correction for block effect was made in the analyses.

Analysis of variance (ANOVA) was performed on percentage of root length occupied by hyphae, percentage of root length occupied by arbuscules, percentage of root length occupied by vesicles, root dry mass, shoot dry mass, root phosphorus concentration, shoot phosphorus concentration, and $\log_e$-transformed specific root length, using the GLM procedure (SAS Institute 1989). Where we tested whether the effects of different AMF species on a given variable differed from each other, only the four single-AMF species treatments formed the AMF treatments factor in the ANOVA. Means comparisons were made among AMF treatments for each plant species treated separately (Tukey's test, Zar 1984). A probability of $P \leq 0.05$ was considered as representing a significant difference in this study. Seven plants that were inoculated with AMF but that did not become colonized were excluded from the analysis.

To analyze several variables at once, a multivariate analysis of variance (MANOVA) was carried out using procedure GLM, option MANOVA (SAS Institute 1989). The power of MANOVA is that the effects of treatments on several variables can be analyzed and summarized all at once, and this provides a description of the overall response of plants to a treatment. MANOVA was executed with the following eight independently measured variables: root phosphorus concentration, shoot phosphorus concentration, root dry mass, shoot dry mass, total root length, and percentage of root length colonized by hyphae, arbuscules, and vesicles. The AMF treatments factor in the MANOVA consisted of four single-AMF species and the mixed-AMF treatment (non-AMF treatment excluded). This was carried out in order to test whether there were differences among treatments with AMF.

As in other studies using multivariate analyses the

distribution of the plant individuals within one AMF treatment differed among some treatments (St. John and Koske 1988, Bever et al. 1996). Consequently, Bartlett's modification of the likelihood ratio test (Morrison 1990) showed that heterogeneity of within-group covariance matrices occurred in our data set. Although this is biologically interesting it could result in a bias of MANOVA. The robustness of multivariate test statistics with unequal covariance matrices is, however, relatively high, so that type I errors are negligible (Olson 1976, Stevens 1979).

Calculation of mycorrhizal dependency

The mycorrhizal dependency of each plant species was determined using an equation that was modified from Plenchette et al. (1983). Mycorrhizal dependency has been defined by Plenchette et al. (1983) as the relationship between the dry mass of plants inoculated with AMF and the dry mass of uninoculated plants. A mycorrhizal dependency >0 means that plants benefit from AMF inoculation. We have used the relationship between the mean dry mass across all AMF treatments compared to the uninoculated plants to describe mycorrhizal dependency, because a plant can respond differently to different AMF species (Eq. 1):

$$\text{mycorrhizal dependency} = 1 - \left(b \frac{n}{\left(\sum_{1}^{n} a_n \right)} \right) \quad (1)$$

where a is the mean plant dry mass of a treatment inoculated with AMF, n is the number of treatments where plants were inoculated with AMF, and b is the mean plant dry mass of the non-AMF treatment.

Calculation of variation in response of each plant species to five treatments with AMF

The total amount of variance among the five treatments with AMF (excluding non-AMF treatment) was determined for each plant species separately using ANOVA with the dependent variable total plant dry mass. The F value was used as a measure of variance because it gives the variance among groups (among treatments with AMF) divided by the variance within groups. The approximate F ratio of a MANOVA can be used for the same purpose. The F ratio was taken instead of the variance among groups (sums of squares) because a correction is made for the size of the response variable, i.e., plants with a higher dry mass are more likely to have a higher sums of squares than small plants, independent of the amount of variance among groups.

RESULTS

Mycorrhizal dependency of plant species

The three plant species differed in their mycorrhizal dependency. The mycorrhizal dependency, based on plant dry mass at the end of the experiment, was 0.23, 0.39, and 0.98 for *Bromus*, *Festuca*, and *Hieracium*, respectively. The mycorrhizal dependency is based on a comparison between plant responses to the non-AMF treatment with the mean of five pooled AMF treatments. *Hieracium* was obligately dependent on AMF. The mean total dry mass of uninoculated *Hieracium* was 0.002 g compared to 0.47 g for the mean dry mass across all other AMF treatments. The mean total dry mass of uninoculated *Bromus* and *Festuca* was 0.57 g and 0.25 g compared to 0.74 g and 0.41 g, respectively, across all other AMF treatments.

TABLE 1. Results of multivariate analysis of variance (MANOVA) on eight dependent variables† of plant and fungal growth, with three plant species, four single AMF species treatments, and the mixed AMF treatment.

Source of variation‡	df	T_0^2§	F	Num. df	Den. df	P
Plant	2	8.27	46.56	16	180	≤0.0001
AMF	4	1.97	5.53	32	358	≤0.0001
Plant × AMF	8	1.24	1.73	64	714	≤0.0006

† MANOVA was executed with the following variables, which were measured at the end of the experiment: root and shoot dry mass, total root length, root and shoot phosphorus concentrations, percentages of root length occupied by vesicles, arbuscules, and hyphae.

‡ Plant = plant species treatments; AMF = AMF treatment. The AMF factor comprises the four individual AMF species treatments and the mixed AMF treatment (the non-AMF treatment was excluded from the analysis).

§ T_0^2 = Hotelling-Lawley trace test statistic. This value can be used to calculate an approximate F ratio with accompanying numerator (num.) and denominator (den.) degrees of freedom (Morrison 1990).

Differential effects of AMF species

Results of multivariate analyses.—When eight plant and fungal variables were analyzed together using MANOVA (executed with the three plant species, four single-AMF species, and mixed-AMF treatment), a significant AMF effect occurred (Table 1). The interaction term between plant species and treatments with AMF was also highly significant (Table 1). This means that responses to AMF species or to the community of AMF species (mixed treatment) differed among plant species. To separate the effects of fungal variables and plant variables in the MANOVA, the same MANOVA was executed with only the five plant variables, and the same results with equal significance levels were obtained (data not shown). This means that the growth responses of the three plant species that were inoculated with AMF varied from each other and that the significant AMF effect seen in the MANOVA was not solely due to differences in fungal growth. When the MANOVA was executed with the four single AMF species (mixed-AMF treatment and non-AMF treatment excluded) a significant main AMF effect ($F_{24, 209} = 8.0$ $P \leq 0.0001$) and a nonsignificant plant species × AMF species interaction term ($F_{48, 416} = 1.30$ $P \leq$

TABLE 2. Results of ANOVA (*P* values) executed for each of three fungal variables and each of five plant variables. The factor ''plant'' consists of three plant species, and the factor ''AMF'' consists of (A) four single AMF species treatments or (B) four single AMF species plus the non-AMF treatment. For (B), results for four plant variables are shown.

A)

Response variable	Source of variation		
	Plant species (df = 2)	AMF (df = 3)	Plant × AMF (df = 6)
Fungal variables			
Colonization by hyphae	0.0001	0.0001	0.60
Colonization by arbuscles	0.0001	0.0001	0.09
Colonization by vesicles	0.07	0.0001	0.05
Plant variables			
Shoot dry mass	0.0002	0.49	0.02
Root dry mass	0.0001	0.67	0.11
Shoot phosphorus concentration	0.16	0.0001	0.74
Root phosphorus concentration†	0.0001	0.0005	0.73
Specific root length	0.0001	0.0001	0.01

B)

Response variable	Source of variation		
	Plant species (df = 2)	AMF (df = 5)	Plant × AMF (df = 10)
Plant variables			
Shoot dry mass	0.0001	0.0001	0.0001
Root dry mass	0.0001	0.0002	0.038
Shoot phosphorus concentration	0.16	0.0001	0.0001
Specific root length	0.0001	0.0001	0.013

Note: Error df = 78 in (A) except as otherwise noted; in (B) error df = 119.
† Error df = 74.

0.092) occurred. The inclusion of the mixed-AMF treatment in the MANOVA resulted in a 33% increase in the *F* ratio of the plant species × AMF species interaction term. This indicates that the three plant species responded differently to the mixed-AMF treatment compared to the single AMF species treatments.

Univariate analyses: fungal variables.—The percentage of root length occupied by hyphae and arbuscules varied significantly among different AMF species, irrespectively of plant species (Table 2a). The percentage of root length colonized by hyphae of *Glomus* sp. (BEG 21) was lower than that of any of the other AMF species in all three plant species (Fig. 1A). *Glomus* sp. (BEG 21) did not produce arbuscules in the roots of any of the plant species (Fig. 1B). None of the plant roots in the non-AMF treatment contained AMF structures (Fig. 1A–C). There was no significant plant species × AMF species-interaction term for the percentage of root length occupied by hyphae or arbuscules when only the four individual AMF species were included in the analyses (Table 2A). This means that each of the AMF species grows differently but that this is irrespective of which plant species they colonize.

The percentage of root length colonized by vesicles varied significantly among different AMF species, irrespectively of plant species (Table 2A). *Glomus* sp. (Basle Pi) was the only fungal species to produce vesicles in the roots of *Festuca* (Fig. 1C). All fungi except *Glomus* sp. (BEG 21) produced vesicles in the roots of *Bromus* and *Hieracium* (Fig. 1C). There was a significant plant species × AMF species-interaction term for the percentage of root length occupied by vesicles (Table 2A), indicating that AMF species produced different numbers of vesicles as a function of the plant species they colonized.

Univariate analyses: plant variables.—AMF species did not significantly affect shoot dry mass (Table 2A). The shoot dry mass of *Hieracium* inoculated with the mixed-AMF treatment differed significantly from those plants inoculated with *Glomus geosporum* (BEG 18) or *Glomus* sp. (BEG 21). The shoot dry mass of *Bromus* and *Festuca* plants inoculated with the mixed-AMF treatment did not differ from any of the other treatments (Fig. 1d). In contrast to the absence of a main AMF species effect, a significant plant species × AMF species interaction term occurred for shoot dry mass (Table 2A). This means that the shoot dry mass differed depending on the combination of plant species and AMF species.

AMF species did not significantly affect root dry mass (Table 2A). The root dry mass of *Hieracium*, *Bromus*, and *Festuca* did not differ among the AMF treatments that received mycorrhizal inoculum (Fig. 1E). The root dry mass of uninoculated *Hieracium* plants differed from inoculated *Hieracium* plants. There was no significant plant species × AMF species interaction term for root dry mass.

AMF species significantly affected shoot phosphorus concentration irrespectively of plant species (Table 2A). Uninoculated *Hieracium* plants had significantly lower shoot phosphorus concentrations than inoculated plants (Fig. 1F). The shoot phosphorus concentration of *Hieracium* plants inoculated with *Glomus* sp. (BEG 21) was significantly lower than *Hieracium* inoculated

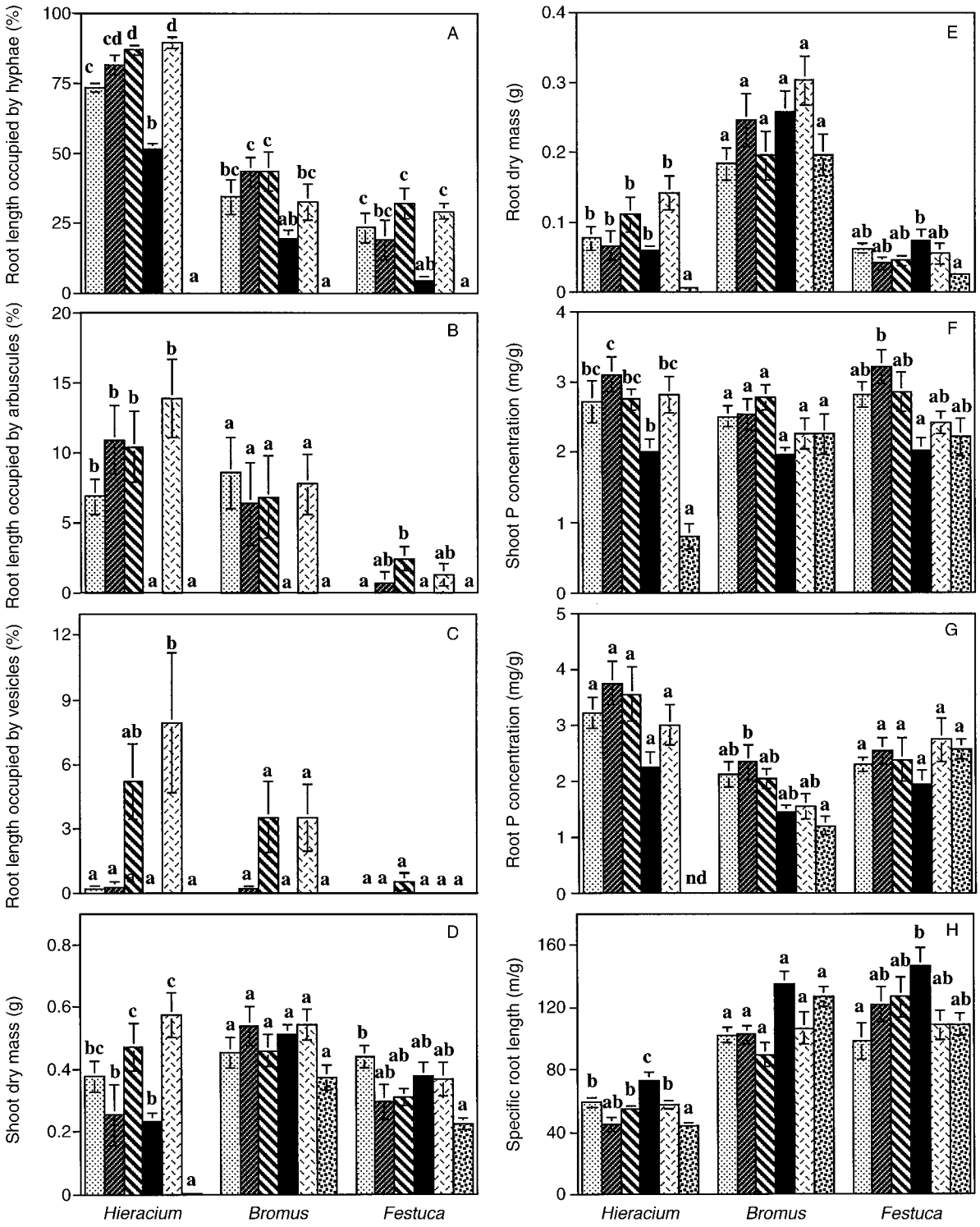

FIG. 1. Mean values of fungal and plant growth variables for *Hieracium*, *Bromus*, and *Festuca* plants inoculated with one of the four arbuscular mycorrhizal fungal (AMF) species, the mixed AMF species treatment, or the non-AMF treatment. (A) Percentage of root length occupied by hyphae, (B) percentage of root length occupied by arbuscules, (C) percentage of root length occupied by vesicles, (D) shoot dry mass, (E) root dry mass, (F) shoot phosphorus concentration, (G) root phosphorus concentration, and (H) specific root length. Data were obtained at the final harvest (80 d). ⬚ = *Glomus* sp. (BEG 19), ▨ = *Glomus geosporum* (BEG 18), ▩ = *Glomus* sp. (Basle Pi), ■ = *Glomus* sp. (BEG 21), ⬚ = mixed AMF, ⬚ = non-AMF. Bars represent ±1 SE; ND = not determined. Mean comparisons are treated separately for each plant species. Different lowercase letters above bars indicate a significant difference ($P \leq 0.05$) among AMF treatments belonging to one plant species, according to Tukey's test. Letters above bars of different plant species cannot, therefore, be compared.

with *Glomus geosporum* (BEG 18). The shoot phosphorus concentration of *Bromus* did not differ among treatments (Fig. 1F). No significant plant species × AMF species interaction term occurred for the shoot phosphorus concentration (Table 2A).

AMF species significantly affected root phosphorus concentration irrespectively of plant species (Table 2A). Uninoculated *Bromus* plants had significantly lower phosphorus concentrations than *Bromus* plants inoculated with *Glomus geosporum* (BEG 18) (Fig. 1G). None of the treatments with *Hieracium* and *Festuca* differed significantly from each other in root phosphorus concentration (Fig. 1G). No significant plant species × AMF species interaction term occurred for the root phosphorus concentration (Table 2A).

AMF species significantly affected the specific root length irrespective of plant species (Table 2A). The specific root length of *Hieracium* plants inoculated with *Glomus* sp. (BEG 21) was significantly higher than those plants inoculated with the other AMF (Fig. 1H). The specific root length of *Festuca* plants inoculated with *Glomus geosporum* (BEG 18) was significantly lower than those plants inoculated with *Glomus* sp. (BEG 21). The specific root length did not differ among the AMF treatments of *Bromus* (Fig. 1H). A significant plant species × AMF species interaction occurred for specific root length (Table 2A), meaning that the specific root length differed depending on the combination of plant species and AMF species. This result also demonstrates that AMF effects on specific root length were independent of any AMF effects on plant mass. This is because the specific root length is a relative measure, and hence we were able to compare the root length of different treatments independent of plant mass.

There was a significant main AMF effect and a significant plant species × AMF treatment interaction term on the shoot and the root dry mass, the specific root length, and the shoot phosphorus concentration when the mixed-AMF and the non-AMF treatment were included in the analysis (Table 2B). The analysis of variance with the non-AMF treatment and mixed-AMF treatment was not executed for the root phosphorus concentration due to missing values in the non-AMF treatment. Inclusion of the mixed-AMF and the non-AMF treatment caused an increase in the total amount of variation for almost all plant variables (Table 2) because of the high variance in the response of the different plant species to the non-AMF treatment (Fig. 1).

Variation in growth response of each plant species to treatments with AMF

ANOVA of total plant dry mass with all AMF treatments except the non-AMF treatment showed that the variation in response to treatments with AMF is different among the plant species. The *F* ratio, which indicates the amount of variance among the treatments, was the highest for *Hieracium* and was 2.93 and 2.72 times lower for *Bromus* and *Festuca,* respectively (Table 3). The same effect was also obtained with MANOVA (data not shown). This indicates that the amount of variation in the growth response of a plant species to different AMF species or to the mixture of AMF species was much higher in *Hieracium* than in *Bromus* or *Festuca.*

TABLE 3. Results of ANOVA on total plant dry mass with five different AMF treatments (the non-AMF treatment excluded). The analysis was executed separately for each plant species.

Plant species	df	*n*†	SS	*F*	*P*
Hieracium	4	40	1.022	4.184	0.007
Bromus	4	39	0.259	1.427	0.246
Festuca	4	34	0.125	1.537	0.218

† Sample size.

DISCUSSION

Mycorrhizal dependency and its ecological significance

It has been demonstrated that the structure of plant communities can be strongly altered by the presence or absence of arbuscular mycorrhizal fungi (AMF) (Grime et al. 1987, Gange et al. 1990, Sanders and Koide 1994). The varying mycorrhizal dependency of plant species is one of the main factors that has been proposed to explain such effects of AMF on plant communities. Our results confirm that plant species differ in their mycorrhizal dependency and that some species, e.g., *Hieracium,* may even be obligately dependent on AMF for their growth. It has been suggested that differences in mycorrhizal dependency of plant species are especially important in plant succession—primary successional communities lacking AMF comprise nonmycorrhizal plant species or those that have a low mycorrhizal dependency, and plants that are highly dependent on mycorrhiza can only become established when AMF immigrate to the community (Janos 1980). We suggest that this comparative-presence/absence-of-AMF approach may, however, be ecologically less relevant for explaining the current role of AMF in determining the structure of the majority of plant populations or communities—firstly because almost all communities have succeeded beyond a primary community and contain a community of AMF species, and secondly because we have shown that plants grow differently depending on which AMF species is present.

Measurements of mycorrhizal dependency based on their response to one AMF (as proposed by Plenchette et al. 1983) could be highly variable but could also be very similar, depending on the plant species response to the non-AMF treatment. This renders values of mycorrhizal dependency difficult to interpret and in some cases meaningless. For example, a species such as *Hieracium,* which was so strongly AMF dependent but which also varied greatly in biomass among AMF species treatments, did not grow well without AMF. Be-

cause of the extremely low biomass of the non-AMF treatment, estimations of mycorrhizal dependency based on any of the single-AMF species treatments vary little (mycorrhizal dependency varied between 0.99 and 0.97). In contrast, *Festuca* did not vary as much in its growth response to different AMF as *Hieracium*, but because the plants in the non-AMF treatment were also able to grow, the estimations of mycorrhizal dependency based on single-AMF species treatments are highly variable (mycorrhizal dependency varied between 0.50 and 0.25). For this reason, we suggest that ecological interpretations of how species may coexist, based solely on their mycorrhizal dependencies, should be avoided or should include a measurement of their response to different AMF species from communities in which they naturally occur.

Effects of different AMF species

We hypothesized that the species composition of AMF communities might determine plant community structure if plant species respond differently to different AMF species. Our results support this hypothesis—by significant plant species × AMF species interactions on single variables, by differential responses of the three plant species to different AMF species, and by the variation in the response of plant species to different AMF.

The differential response of plant species to AMF in our study is especially interesting because two of the three plant species, *Bromus* and *Festuca*, are frequently co-dominants of calcareous grasslands. This indicates that coexistence among dominants may also potentially be affected depending on which AMF are colonizing the roots. The existence of specific microhabitats for AMF species (Johnson 1993) indicates that the coexistence of plants may depend on which AMF species is present at that site. A previous study showed that two subordinate species from the same calcareous grassland, *Prunella vulgaris* and *Prunella grandiflora*, also responded differently to specific AMF species (Streitwolf-Engel et al. 1997).

In most natural communities several AMF species co-occur. A complex number of interactions may result because two different plants may be colonized by the same or by different AMF species or be multiply colonized by several AMF species (Rosendahl et al. 1990, Clapp et al. 1995). Our observation that the plant species responded differently to the mixed-AMF treatment, and that these differential responses were even stronger than to those of the single-AMF species, indicates that a community of several AMF species may cause differences among the growth responses of plant species. Therefore, we conclude that the species composition and diversity of the AMF community is a potential determinant of plant community structure.

Variation in responses among plant species

In this study we demonstrated that plant species vary in the degree they respond differently to single-AMF species or to a community of AMF species. *Hieracium* exhibits a large variation in its response to different AMF species, while *Bromus* exhibits a low amount of variance to different AMF. The ability of *Hieracium* to coexist with other plant species could therefore be highly dependent on which AMF species it forms a symbiosis with. In contrast, *Bromus* would not directly be affected by which AMF species it is colonized by because its variation in response to different AMF is low. For example, our results concerning AMF effects on plant dry mass (Fig. 1D) suggest that if *Hieracium* would grow with *Bromus* when *Glomus* sp. (BEG 21) or *Glomus geosporum* (BEG 18) were present, growth of *Hieracium* would be greatly reduced, possibly to the point of competitive exclusion because of the low biomass that *Hieracium* can achieve. However the ability of *Hieracium* to coexist with *Bromus* in a community that is dominated belowground by another AMF species, e.g., *Glomus* sp. (Basle Pi) or the mixed-AMF community, would be much greater because the biomass of *Hieracium* when colonized with *Glomus* sp. (Basle Pi) is much higher. We therefore propose that the degree in which a plant species varies in its response to different AMF is a potentially important factor in how AMF could determine the coexistence of plant species.

A significant positive correlation between the biomass and the amount of root colonization by AMF occurred in *Hieracium* ($R^2 = 0.36$), while this relationship did not exist in *Bromus* and *Festuca* ($R^2 = 0.001$ and 0.002 for *Bromus* and *Festuca*, respectively) (all correlations obtained are executed with only those plants that received mycorrhizal inoculum). This indicates that *Hieracium* forms a much closer symbiosis with AMF than *Bromus* or *Festuca* and it can, therefore, give a functional explanation as to why *Hieracium* showed the largest differential response to the different treatments with AMF. It is often thought that AMF increase plant biomass through an increased uptake of phosphorus for the plant (Smith and Read [1997] and references therein), and the amount of phosphorus taken up should be related to the amount of root colonization by AMF (Sanders et al. 1977, Graham et al. 1982). However, in this study we found that this relationship is plant-species dependent. For *Hieracium* and *Bromus* a significant positive correlation between the percentage of root length colonized by AMF and the plant phosphorus concentration and plant phosphorus content was observed, while no significant correlation was found for *Festuca* (data not shown). This implies that phosphorus uptake cannot be used as the only mechanism to explain AMF effects on plants.

Conclusions

The species composition and diversity of AMF communities has a potential to determine plant population and plant community structure. The fact that plant species vary in the degree to which they respond differ-

ently to AMF species has important implications for the growth of individual plant species, and this affects a plant's ability to coexist with other plant species in a community. Furthermore, because of the high variability in the growth response of plant species to AMF species we see the classification of plants based solely on their mycorrhizal dependency as incomplete. We suggest that a measurement of the variation in the response of a plant species to different AMF species will be useful in comparative plant ecology and in defining functional groups of plants within communities. Our results indicate that future studies of plant population or community structure need to consider the composition of AMF communities.

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