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INVITED VIEWS IN BASIC AND APPLIED ECOLOGY

Mycorrhiza influences plant community structure in succession on spoil banks

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Mycorrhizal symbiosis;
ERM;
Mycorrhizal dependence;
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Summary

The experiment simulated a plant succession stage when perennial grasses (e.g. *Calamagrostis epigejos*) invade communities of annuals with different mycotrophy (e.g. *Atriplex sagittata* and *Tripleurospermum inodorum*) on coalmine spoil banks. Communities of these three model species were planted in 30 l microcosms either in the presence of pre-established mycelium network of three arbuscular mycorrhizal fungi (AMF) species (individually and in a mixture) or without AMF. Different AMF species had significantly different effects on individual plant species' growth, which resulted in changes in plant community structure. While in the no-AMF treatment the non-mycotrophic plant species *A. sagittata* contributed nearly 70% to the total plant biomass, in the presence of the 3 AMF mixture the contribution of this species was only about 10%. Different effects of AMF on tiller formation by *C. epigejos* suggest that some AMF could have greater potential to promote the replacement of annuals by perennial grasses than others. It can be concluded that not merely the presence but also the identity of AMF present on spoil banks can affect the coexistence of plant dominants, the community structure and the progress of plant succession.

Zusammenfassung

Ein Stadium der Pflanzensukzession auf Kohleabbauhalden wurde experimentell simuliert, indem eine Gemeinschaft einjähriger Pflanzen mit verschiedener Mykotrophie (*Atriplex sagittata* und *Tripleurospermum inodorum*) von perennierenden Gräsern (*Calamagrostis epigejos*) invadiert wird. Modellgemeinschaften dieser drei Arten wurden in 30 l-Gefäße gepflanzt, die entweder von intaktem Mycel dreier Arten arbuskulärer Mykorrhizapilze (AM) einzeln oder gemeinsam besiedelt oder AM-frei waren. Der Wuchs der einzelnen Pflanzenarten wurde in signifikant unterschiedlicher Weise von AM beeinflusst, was zu einer Veränderungen in der Pflanzengemeinschaftsstruktur führte. Während die nicht mykotrophe Art

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A. sagittata mit fast 70% zu der Gesamtbiomasse der Modellgemeinschaft beitrug, wenn keine AM Pilze im Boden waren, betrug ihr Beitrag nur etwa 10% in Anwesenheit aller drei AM Arten. Unterschiedliche Effekte der einzelnen AM Arten auf die Stolonbildung von *C. epigejos* legen nahe, dass manche AM Arten die Verdrängung von einjährigen Pflanzen durch Gräser in höherem Maße fördern können als andere. Daraus kann geschlossen werden, dass nicht nur die Anwesenheit von AM Pilzen an sich, sondern auch ihr Artenspektrum, die Koexistenz der dominanten Pflanzen Arten und den Fortgang der Pflanzensukzession beeinflussen können.

Introduction

Opencast coal mining is accompanied by the formation of vast areas of spoil banks that consist of infertile material, the previous overburden of coal seams. Those mostly Miocene clays, mined from a depth of about 200 m, are usually characterised by adverse physical properties such as vulnerability to erosion and low drainage ability. Neither seeds of plants, nor propagules of arbuscular mycorrhizal fungi (AMF) are present in the soil of freshly formed spoil banks. Therefore, within primary vegetation succession, the first invaders belong mostly to plant species with no or low dependence on mycorrhiza and only later in succession mycorrhiza-dependant plants colonise these degraded sites (Janos, 1980; Reeves, 1985). Pioneer plants spontaneously colonise spoil banks already during the first year after deposition, but the process of establishing a complete herbaceous vegetation cover can take up to 15 years (Prach, 1987). Plant communities with dominance of ruderal annuals and biennials can occur between the 7th and 12th year after spoil bank establishment. After approximately 12 years, perennial plants replace communities of these species (Prach, 1987). It is obvious that the presence of mycorrhiza-dependant plants and the presence of AMF are contingent on each other. The predominance of non-mycotrophic plants in the early stages of succession has been attributed to the absence of infective mycorrhizal propagules (Janos, 1980; Reeves, Wagner, Moorman, & Kiel, 1979).

In order to facilitate the formation of vegetation cover in a process of spoil bank reclamation, a layer of loess is often used to form a more fertile topsoil horizon, which also improves physical properties of soil. Since this substrate is exposed to environmental influences in its temporary disposal sites, it can contain plant seeds and even the propagules of AMF. Consequently, when the loess is spread onto a spoil bank, patches of both mycotrophic and non-mycotrophic annuals can soon appear. Perennial grasses, especially *Calamagrostis epigejos*, the dominant plant species even after 30 years of spontaneous succession on spoil banks, often invade these communities (Prach, 1987).

Once mycorrhizal associations with appropriate plant species have established, a network of extraradical mycelium (ERM) spreads through the soil from the colonised roots and creates an effective matrix that can inoculate surrounding plants or emerging seedlings more quickly and efficiently than resting propagules (Sỳkorová, Rydlová, & Vosátka, 2003; Malcová, Albrechtová, & Vosátka, 2001). The ERM network links roots of the same or different plant species (Heap & Newman, 1980; Newman, 1988). Although some studies suggest that both nutrients and assimilates can be transported between co-occurring plants (Whittingham & Read, 1982; Martins, 1993), recent work rather supports the theory that the carbon remains in hyphal structures in the roots of recipient plant and is not integrated into the biomass of that plant (Pfeffer, Douds, Bücking, Schwartz, & Shachar-Hill, 2004). However, the recipient host plant may still benefit from carbon transported from donor plants, because such movement would reduce the carbon demand of the fungus from the recipient plant while allowing it to provide soil nutrients (Newman, 1988; Zabinski, Quinn, & Callaway, 2002).

Although AMF diversity in man-made habitats seems to be low (Vosátka, Rydlová, & Malcová, 1999), AMF communities of low diversity can still contain considerable functional heterogeneity (Munkvold, Kjøller, Vestberg, Rosendahl, & Jakobsen, 2004). AMF species can differ from each other in their ERM formation, the efficiency of phosphorus uptake, mechanisms of phosphate metabolism in the ERM or phosphorus translocation and/or transfer to the plant (Jakobsen, Abbott, & Robson, 1992a, 1992b; Pearson & Jakobsen, 1993; Boddington & Dodd, 1998, 1999). Some AMF species are more beneficial to certain host plants than others (van der Heijden, Klironomos, Ursic, Moutoglis, Streitwolf-Engle, & Boller et al., 1998) and species or even isolates of the same AMF species have different growth effects on the same host plant

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(Munkvold et al., 2004). Moreover, several fungi frequently coexist within the same root (Allen, 1996; van Tuinen, Jacquot, Zhao, Gollotte, & Gianinazzi-Pearson, 1998). Thus, carbon costs and functional benefits to plants linking to the mycelial networks are probably fungal specific and, because of variations in AMF physiology and host specificity, are not shared equally by all plants in a community (Leake et al., 2004).

Because interactions between AMF species and plant species are remarkably rich in their variations and intensity, AMF can play an important role in plant coexistence and can affect plant community structure. Grime, Mackey, Hillier, and Read (1987) showed that floristic diversity was higher in the presence of AMF inoculum. van der Heijden, Klironomos et al. (1998) showed that not merely the presence or absence of inoculums but the diversity and identity of AMF were the determinants of plant diversity and biomass production. They found that different plant species responded differently to AMF, thus alterations in AMF composition caused changes in plant community structure and composition. With increasing AMF diversity, plant diversity and biomass production increased. Changes in community structure induced by AMF that influenced traits of vegetative reproduction of clonal plants was also proved (Streitwolf-Engel, Boller, Wiemken, & Sanders, 1997; Streitwolf-Engel, van der Heijden, Wiemken, & Sanders, 2001).

In this study, a greenhouse experiment with spoil-bank loess substrate, we attempted to simulate the advanced succession stage when vegetation cover on spoil banks is not completely developed but the patches of annuals with different mycorrhizal dependence are already invaded by perennial grasses. At this stage, mycotrophic plants are growing in mycorrhizal symbiosis. Therefore, a system simulating this already developed ERM network had to be created. ERM of 3 AMF species (separately and in a mixture) was pre-developed on a mycotrophic nurse plant in rhizobox systems and then the seedlings of 2 model annuals – the mycotrophic *Tripleurospermum inodorum* and the non-mycotrophic *Atriplex sagittata* – and the mycotrophic grass *Calamagrostis epigejos* were planted into this matrix. We tested the hypothesis whether different AMF species can specifically influence the coexistence of spoil-bank plant dominants in this simplified model community.

Material and methods

The loess (pH 8.0; $C_{org.}$ 0.38%; C/N 1.54; P 24.8 mg/kg) was collected from the freshly formed spoil bank of the Vršany coalmine (North-Bohemian coal basin, near the town of Most, the Czech Republic). For the purposes of the experiment it was mixed with perlite 1:1 (v/v) and sterilised by a dose of 25 kGy of γ-radiation.

Experimental microcosms – round vessels (diameter 50 cm, height 16 cm) containing 30 l of soil – consisted of three concentric compartments – central, intermediate and outer – separated by nylon mesh with mesh diameter of 42 μm. In this design, the mesh blocked the plant roots from growing from one compartment to another and thus prevented direct root interaction between plants in different compartments. However, the ERM could grow through the nylon mesh (Fig. 1).

The experiment encompassed five inoculation treatments: (a) *Glomus mosseae* BEG95, (b) *G. claroideum* BEG96, (c) *G. intraradices* BEG140, (d) mixed inoculums consisting of all these AMF species, (e) non-inoculated control treatment. The AMF species used in the experiment were isolated from man-made ecosystems – spoil bank (a) and pyrite smelter sedimentation pond (b, c). Each treatment included seven replicates.

The inoculum was pre-cultivated on *Zea mays* in large containers filled with a heat-sterilised

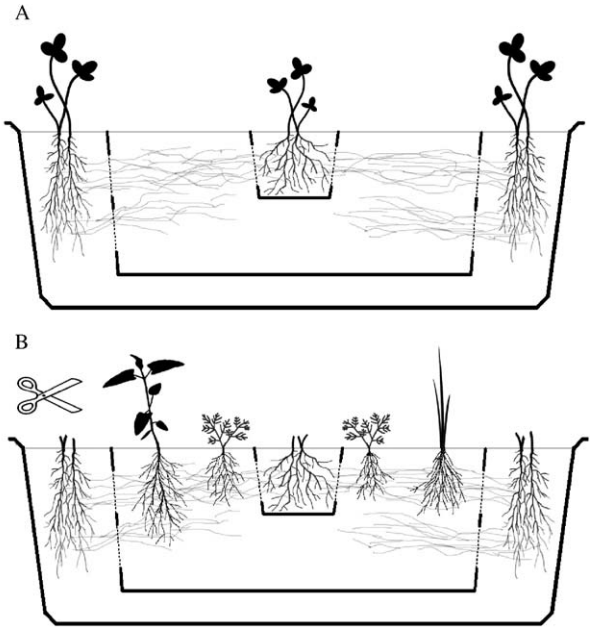

Figure 1. The scheme of two stages of the experiment. Within the first stage (A), the network of ERM grew from the nurse plant *Trifolium pratense* to the intermediate compartment, in which the model plants *Tripleurospermum inodorum, Calamagrostis epigejos* and *Atriplex sagittata* were transplanted at the beginning of the second stage (B).

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sand-zeolite substrate. The 500 ml dose of soil inoculum (containing roots of culture-pot plants, spores of AMF and fragments of mycelium) was added into the outer and central compartments of the experimental vessels of mycorrhizal treatments. The control treatment was supplied with the same quantity of heat-sterilised mixed inoculum plus 250 ml of inoculum-filtrate to get a similar quantity of organic matter and bacterial conditions in all treatments. Furthermore, 250 ml of filtrate from the original soil containing indigenous soil bacteria was added to all treatments.

The experiment was carried out in a climate-controlled greenhouse. The temperature was maintained at 25 °C in the day and 19 °C in the night. Ambient light was supplemented with 600 W metal halide bulbs with a 14 h photoperiod. The position of microcosms was regularly randomised once a week.

At the beginning of the first stage of the experiment, the seeds of a nurse plant, *Trifolium pratense*, were sown into inoculated compartments. There were two requirements about the nurse plant: although it was necessary to prevent shading to model plants by biomass cutting of the nurse plant, its survival throughout the experiment was essential. The death of nurse plant could dramatically change the possible carbon flow between the plants in the community and influence the possible benefits from mycorrhizal symbiosis. Therefore, *T. pratense* was selected as the nurse plant – it is a regenerating species tolerant to the biomass cutting. Besides, *T. pratense* is often incorporated as a member of studied plant communities as well. Its seeds can be dispersed to the sites with spontaneous succession from those areas where it was used as a reclamation plant.

The *Trifolium* plants were maintained at a constant number of 64 plants in the outer compartment and 3 plants in the central compartment of each microcosm throughout the experiment. The roots became colonised already within 2 weeks and ERM grew through the nylon mesh out of the outer/central compartment into the intermediate compartment (Fig. 1).

The second stage of the experiment began after 4 months. The shoot biomass of *Trifolium* was cut, dried at 70 °C to constant weight and weighed. To verify that ERM had developed in the intermediate compartment, the soil samples from the intermediate compartment were taken for ERM length detection according to the same methodology as described below (for *Trifolium* growth parameters and ERM length parameters see Table 1). The seedlings of *A. sagittata*, *T. inodorum*, and *C. epigejos* were pre-germinated in heat-sterilised sand for 2 weeks. Then the plantlets were transplanted into the intermediate compartment – 6 seedlings of each species per compartment. The layout of the plantlets was designed to form six equilateral triangles, each triangle containing 1 plantlet of each species. The position of the species in triangles rotated to guarantee even distribution of each species (Fig. 2). After the first week of growth, 150 ml of modified White's nutrient solution P2N3 (Gryndler, Vejsadová, & Vančura, 1992) was added to the intermediate compartment to provide a start dose of nutrients for model plants. During the model plants' growing period, the regenerating plants of *Trifolium* were regularly cut to prevent shading effect. Cut biomass was dried and weighed (Table 1).

Model plants were harvested after 12 weeks. Shoot and root biomass of each plant in the compartment was separately cut, dried and weighed. Mycorrhizal dependence was calculated for each species of model plants according to the formula by van der Heijden (2003):

Table 1. Biomass production of the nurse plant *T. pratense* and the ERM length in the intermediate compartment in different inoculation treatments.

Inoculation	Total dry shoot biomass of *T. pratense* (g)				Total ERM length (mm/g soil)	
	End of the 1st stage		End of the experiment		End of the 1st stage	
G. mosseae BEG95	48 ± 1.9	b	70 ± 1.7	b	1468 ± 290	a
G. claroideum BEG96	57 ± 2.3	a	82 ± 2.4	a	745 ± 113	b
G. intraradices BEG140	51 ± 1.7	b	70 ± 1.6	b	507 ± 104	b
Mixed inoculum	52 ± 1.4	ab	75 ± 1.9	b	726 ± 120	b
NM	8 ± 1.2	c	13 ± 2.0	c	–	–
	$F = 130.2$ df effect = 4		$F = 206.8$ df effect = 4		$F = 5.75$ df effect = 3	

NM – non-inoculated. Data are means of seven replicates ± SE. Values within columns marked by the same letter are not significantly different ($P < 0.05$; Duncan multiple range test).

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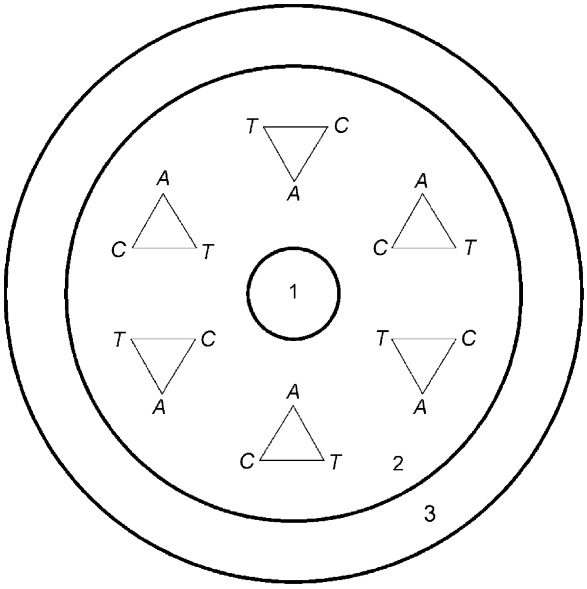

Figure 2. The scheme of the microcosms. The microcosms consisted of three concentric compartments. The nurse plant *Triforium pratense* was planted in the central (1) and outer (3) compartments, and model plants were planted in the intermediate compartment (2) in a triangle-layout. The position of model plant species in triangles were rotated to guarantee even distribution of each species. A – *Atriplex sagittata*, C – *Calamagrostis epigejos*, T – *Tripleurospermum inodorum*.

$$\text{If biomass of } \sum_{1}^{n} a_n > bn,$$

then mycorrhizal dependence

$$= \left(1 - \left(\frac{bn}{(\sum_{1}^{n} a_n)}\right)\right) \times 100\%$$

$$\text{If biomass of } \sum_{1}^{n} a_n < bn,$$

then mycorrhizal dependence

$$= \left(-1 + \left(\frac{(\sum_{1}^{n} a_n)}{bn}\right)\right) \times 100\%,$$

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. Values of mycorrhizal dependence range from −100% to +100%.

In the case of *C. epigejos*, the number of tillers was measured as an additional growth parameter. In addition to the biomass of single model plants, the total shoot biomass and root biomass of the 18 model plants in the microcosm community were calculated.

Root samples from each plant were stained with 0.05% Trypan blue in lactoglycerol (Koske & Gemma, 1989) and mycorrhizal colonisation was evaluated according to Trouvelot, Kough, and Gianinazzi-Pearson (1986) on 30 root segments 1.5 cm in length. Three parameters of mycorrhizal colonisation were calculated using the programme "Mycocalc" (http://www.dijon.inra.fr/mychintec/Mycocalc-prg/download.html): *F* – frequency of mycorrhiza in the root system (reflects the frequency of occurrence of mycorrhizal structures in the root system), *M* – intensity of mycorrhizal colonisation in the root system (reflects how intensively are colonised those parts of the roots where mycorrhiza is present), *A* – arbuscule abundance in the root system (quantifies the extent of arbuscule occurrence in parts of the roots, where those structures are present). One soil core (15 ml each) was extracted from the central part of three triangles in the intermediate compartment of each microcosm and the total ERM length was assessed using a modified membrane filtration technique (Jakobsen et al., 1992a). First the soil sample was homogenised by hand, then 3 g of soil were put into a household blender with 500 ml of deionised water and blended for 30 s. About 1 ml of the supernatant was pipetted onto a nitro-cellulose membrane filter (24 mm diameter, 0.40 μm pore size) and vacuum-filtered. The extracted ERM was stained with 0.1% Trypan blue in lactoglycerol. The total length of the ERM was assessed under a compound microscope at 100× magnification and expressed in milimetres of hyphae/g of dry soil. The ERM extraction was also performed in the non-inoculated control treatment and obtained data – mean value 36 mm/g (the end of the 1st stage) and 44 mm/g (the end of the experiment) – were subtracted from the inoculated treatment values.

All data were checked for normality. Statistical analysis was carried out using: (i) one-way ANOVA – growth parameters of plants and mycelium with inoculation as a factor; (ii) two-way ANOVA – parameters of mycorrhizal colonisation with plant species and inoculation as factors. Significant differences were tested by Duncan multiple range test (STATISTICA 5.1 '98 Edition). Because plants grown in the same microcosm were not independent, we used means of all the plants of the same species grown in the same microcosm as replicates in the analysis.

Results

Plant growth

The total shoot biomass produced by the whole community of 3 plant species was, in general, higher in inoculated treatments compared to the

control. Inoculation with *G. claroideum* and with the mixed inoculum appeared to be more effective than inoculation with *G. mosseae*. The community produced the most extensive root system in the presence of the mixed inoculum. Conversely, the inoculation with *G. mosseae* resulted in a less developed root system, which did not differ from the control (Fig. 3).

Different AMF had a different effect on the contribution of each plant species to total plant biomass produced by the community (Fig. 4).

A. sagittata apparently suffered in inoculated treatments (mycorrhizal dependence −52%) and this was reflected in all measured parameters. The highest *A. sagittata* shoot and root biomass production and total shoot biomass contribution reaching nearly 70% occurred in the non-inoculated treatment (Figs. 4 and 5A). Among mycorrhizal treatments, more biomass was formed in treatments with *G. mosseae* and *G. claroideum*, while *G. intraradices* and the mixed inoculum caused a significant reduction of shoot biomass. These two mycorrhizal treatments also had the most negative effect on the root biomass of *A. sagittata* (Fig. 5A).

Mycorrhizal dependence of *T. inodorum* was 79%. This species produced the most shoot and root biomass and had highest total biomass contribution in the treatment inoculated with the mixed inoculum (Figs. 4 and 5B). All these parameters were lowest in the non-inoculated treatment.

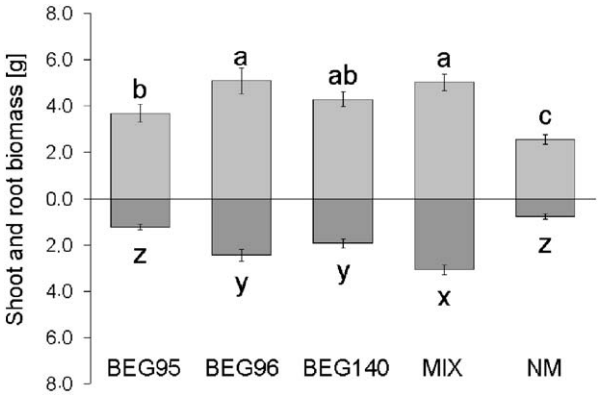

Figure 3. The effect of mycorrhizal inoculation with *Glomus mosseae* BEG95, *G. claroideum* BEG96, *G. intraradices* BEG140 and the mixture of these three isolates (MIX) on total shoot and root biomass production of the plant community in microcosms. NM (no mycorrhiza): control treatment. Data are means of seven replicates ± SE. Columns marked by the same letter are not significantly different ($P<0.05$; Duncan multiple range test).

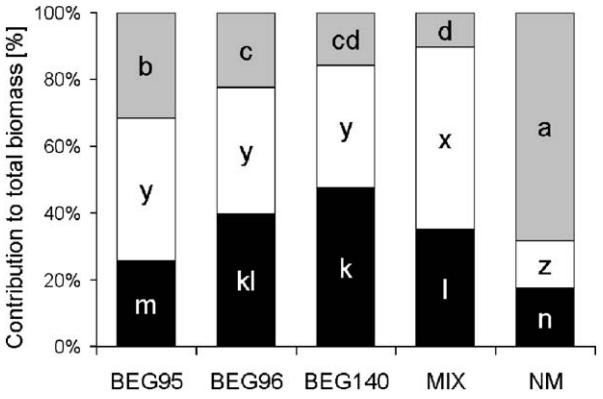

Figure 4. The effect of mycorrhizal inoculation with *Glomus mosseae* BEG95, *G. claroideum* BEG96, *G. intraradices* BEG140 and the mixture of these three isolates (MIX) on contribution of each model plant species to total community biomass production. *Atriplex sagittata* (grey columns), *Tripleurospermum inodorum* (white columns), *Calamagrostis epigejos* (black columns). NM (no mycorrhiza): control treatment. Data are means of seven replicates. Columns marked by the same letter are not significantly different ($P<0.05$; Duncan multiple range test).

C. epigejos was found to be only slightly less mycorrhiza-dependant (71%). The best growth response was observed in treatments inoculated with *G. intraradices*, *G. claroideum* and also with the mixed inoculum (Fig. 5C). However, contribution to the total shoot biomass was significantly lower in the mixed inoculum treatment compared to the *G. intraradices* treatment (Fig. 4). Dry root biomass was, conversely, higher in the mixed inoculum treatment. Although the shoot and root biomass of *C. epigejos* produced in the non-inoculated treatment was not significantly different from the treatment inoculated with *G. mosseae*, this non-inoculated treatment was the lowest of all for total biomass contribution. The values of the number of produced tillers corresponded with the results of shoot and root biomass: *G. intraradices* (highest number of produced tillers − 9.2) was not different from *G. claroideum* and the mixed inoculum treatment (9.0 and 8.5, respectively). Significantly less tillers were produced in association with *G. mosseae* (5.2), similar to non-inoculated treatment (3.3).

The growth response of the nurse plant corresponded with the other results. The vitality of *T. pratense* gained by inoculation, strongly contrasted with the reduced growth in the non-inoculated treatment. Among the mycorrhizal treatments, inoculation with *G. claroideum* had the highest growth effect (Table 1).

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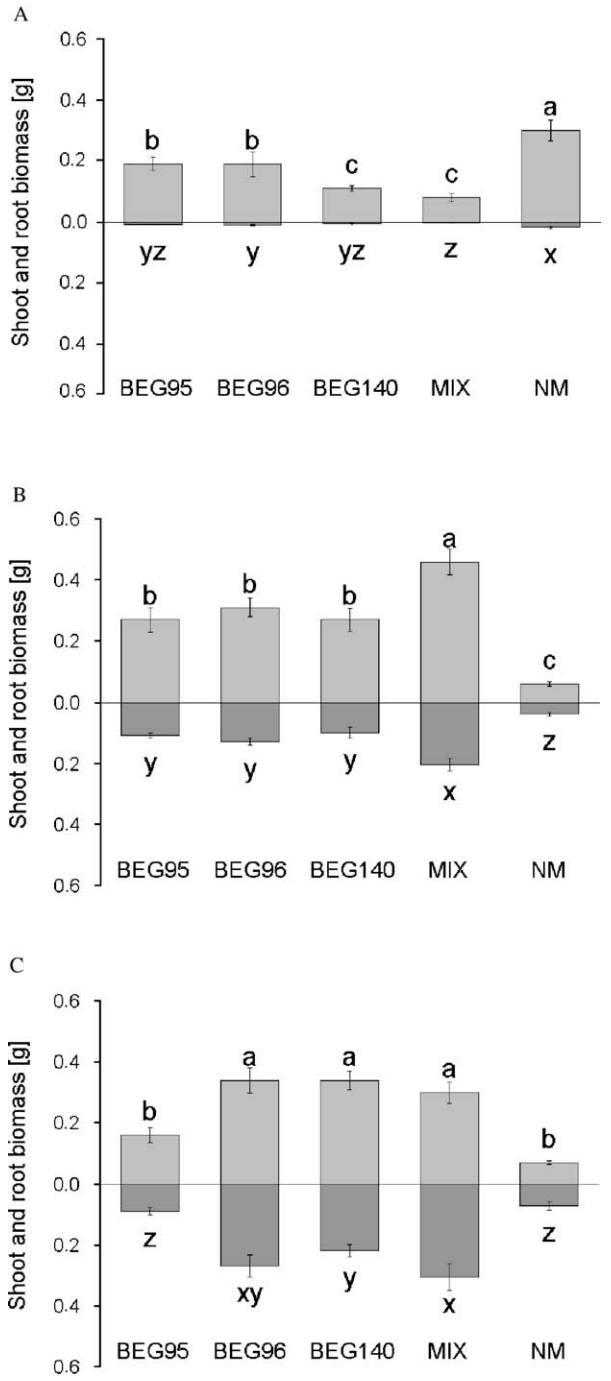

Figure 5. The effect of mycorrhizal inoculation with *Glomus mosseae* BEG95, *G. claroideum* BEG96, *G. intraradices* BEG140 and the mixture of these three isolates (MIX) on total shoot and root biomass production of *Atriplex sagittata* (A), *Tripleurospermum inodorum* (B) and *Calamagrostis epigejos* (C). NM (no mycorrhiza): control treatment. Data are means of seven replicates $\pm$ SE. Columns marked by the same letter are not significantly different ($P<0.05$; Duncan multiple range test).

Development of arbuscular mycorrhizal symbiosis

The significant interaction between plant species and inoculation treatments in all monitored parameters (Table 2) revealed that different AMF isolates differed in their development in the roots of different model plants. No traces of mycorrhizal colonisation were found in the roots of control plants.

In *A. sagittata*, mycorrhizal hyphae and vesicles were observed. However, no arbuscules were developed in the roots. Apart from very low values for *G. mosseae*, other mycorrhizal treatments had a similar level of frequency and intensity of colonisation (Table 2). The roots of *T. inodorum* were highly colonised in all mycorrhizal treatments. While the frequency of mycorrhiza did not differ between treatments, significant differences were found in the intensity of mycorrhizal colonisation and abundance of arbuscules (highest in treatments of *G. intraradices*, see Table 2). Inoculation of *C. epigejos* with *G. intraradices* resulted in the highest values of all three mycorrhiza parameters. Although the two other AMF treatments had a high frequency of mycorrhiza and arbuscular abundance, the intensity of colonisation was significantly lower. The mixed inoculum was, for *C. epigejos*, the least successful treatment with respect to the intensity of colonisation (Table 2).

In general, in all three mycorrhizal parameters – frequency/intensity of colonisation and arbuscule abundance – and in all mycorrhizal treatments, the values of *T. inodorum* were higher than the values of *C. epigejos*. The lowest values were found in the non-mycotrophic *A. sagittata*.

At the end of the experiment, the highest values of ERM length were found in the treatment inoculated with *G. claroideum* (approximately 1200 mm/g of soil). In other mycorrhizal treatments, ERM length was significantly lower (the lowest in *G. intraradices*, 770 mm/g of soil).

Discussion

In general, the presence of an AMF network had a positive effect on total biomass production in the model plant community. Furthermore, specific effects of certain AMF on individual model plant species influenced the community structure. The contribution of each plant to total biomass production differed in response to different AMF isolates because each model plant preferred specific mycorrhizal conditions. The ability of AMF to

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Table 2. Mycorrhizal colonisation of *Atriplex sagittata*, *Calamagrostis epigejos* and *Tripleurospermum inodorum*.

Plant	Inoculation	*F* (%)		*M* (%)		*A* (%)	
A. sagittata	*G. mosseae* BEG95	0.2±0.12	b	< 0.1	b	0	
	G. claroideum BEG96	8.5±3.41	a	2.3±1.09	ab	0	
	G. intraradices BEG140	13.5±1.98	a	4.4±0.88	a	0	
	Mixed inoculum	16.0±3.20	a	5.1±1.47	a	0	
C. epigejos	*G. mosseae* BEG95	95.7±1.12	ab	50.9±1.82	b	26.3±1.41	ab
	G. claroideum BEG96	93.3±2.22	ab	49.6±4.77	b	30.3±4.47	a
	G. intraradices BEG140	97.3±1.09	a	63.0±4.92	a	36.4±6.20	a
	Mixed inoculum	91.1±1.51	b	38.2±2.37	c	16.2±2.06	b
T. inodorum	*G. mosseae* BEG95	98.5±0.49	ns	65.9±2.45	c	40.4±1.96	b
	G. claroideum BEG96	97.8±0.94		74.1±3.13	b	45.4±4.70	ab
	G. intraradices BEG140	99.8±0.16		83.6±1.94	a	56.4±4.27	a
	Mixed inoculum	98.8±0.46		78.9±2.52	ab	39.5±3.73	b
F value/significance							
Inoculation (A)	df effect = 3	5.5	**	10.7	***	7.8	***
Plant (B)	df effect = 2	3294.7	***	760.9	***	200.4	***
A × B	df effect = 6	6.5	***	6.2	***	2.4	*

F – frequency of mycorrhiza in the root system, M – intensity of mycorrhizal colonization in the root system, A – arbuscule abundance in the root system. Data are means of seven replicates±SE. Effects of factors according to two-way ANOVA, ns – non-significant effect. *$P<0.05$, **$P<0.01$, ***$P<0.001$. Values within columns marked by the same letter are not significantly different ($P<0.05$; Duncan multiple range test).

regulate plant species coexistence was repeatedly demonstrated by van der Heijden, Klironomos et al. (1998), van der Heijden, Boller, Wiemken, and Sanders (1998), Hartnett and Wilson (2002), Grime et al. (1987). The potential of AMF to influence plant coexistence and total biomass contribution was also reported by van der Heijden, Wiemken, and Sanders (2003).

The mycorrhizal dependence calculated according to van der Heijden (2003) indicates the relative values obtained for the model plants used in the given experimental conditions. The results of *T. inodorum* and *A. sagittata* met the hypothesis of *T. inodorum* being highly mycotrophic/highly dependant (79%), *A. sagittata* was found non-mycotrophic/not dependant (−52%). Relatively high values of *C. epigejos* (71%) were, nevertheless, quite surprising. Rydlová and Vosátka (2001) found a much less dependant response of *C. epigejos* in their pot experiment using soil from spoil banks of four different ages. The positive growth response of *C. epigejos* was observed only in the soil from the youngest spoil bank.

In our experiment, inoculation had a strong negative effect on the non-mycotrophic *A. sagittata* in all mycorrhizal treatments. This plant prospered only in conditions without AMF. Similar negative effects of AMF on non-mycotrophic plants have often been reported (Sanders & Koide, 1994). Francis and Read (1995) observed reduced yield and high mortality of several non-mycotrophic plant species grown in the presence of AMF mycelium. AMF hyphae colonized their roots and vesicles were also present in the roots of some plant species. In another experiment (Francis & Read, 1994), the mere presence of ERM of AMF reduced the growth of non-mycotrophic plants, because the examination revealed no hyphal penetration of roots. Our results showed some interesting differences in the extent of negative effects on *A. sagittata* caused by different species of AMF. While *G. intraradices* and mainly the mixed inoculum had negative effects on *A. sagittata*, the presence of *G. mosseae* and *G. claroideum* did not reduce the growth of *A. sagittata* to such a high rate. This conclusion contrasts with the theory of van der Heijden (2003) describing a relationship between mycorrhizal dependence and mycorrhizal species sensitivity (defined as the variation in the growth response of plant species to different AMF species). According to this theory, the higher mycorrhizal dependence of plants is, the higher is also the mycorrhizal species sensitivity. Because *A. sagittata* is a non-mycotrophic plant, it should have low mycorrhizal species sensitivity and the response to different AMF isolates should not differ. Our results, however, are not necessarily in contradiction with the above findings. The treatment inoculated with *G. mosseae* had a less positive effect on *C. epigejos* compared to other isolates. Therefore, the competitive abilities of *C. epigejos* could have been decreased and *A. sagittata*, consequently, could

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profit from these conditions and this benefit was reflected in the increased biomass of *A. sagittata*. This explanation cannot, however, be applied to the treatment inoculated with *G. claroideum*, because no decrease of performance was observed in the other two plant species in the community.

The positive effect of AMF on mycotrophic plants is well known (Grime et al., 1987; Sanders & Koide, 1994; Smith & Read, 1997; van der Heijden, 2003) and was confirmed also in our experiment. The plants in each mycorrhizal treatment prospered much more than those in the non-inoculated treatment. The extent of this positive effect was, however, not the same for each AMF isolate and for each mycotrophic plant species. Highly mycotrophic *T. inodorum* responded best to the mix of all three AMF isolates, while the inoculation with any of these single isolates had a less positive effect. As a highly mycotrophic plant, it should have also high mycorrhizal species sensitivity (van der Heijden, 2003). The question arises, why was not at least one of the single isolates (contained also in the mixed inoculum) preferred by *T. inodorum* in some of single isolate treatments? The simple answer could be that *T. inodorum* needed the combination of more AMF isolates for best growth, because each fungal isolate brought a different benefit to the host plant. More AMF species mean more functions fulfilled and more opportunities for a beneficial relationship to develop (Hart & Klironomos, 2003).

C. epigejos was less specific in preferences of symbiotic AMF; it had the same growth response to inoculation with *G. claroideum* and *G. intraradices*. The same growth response was found in the mixed-inoculum treatment as well, which is not surprising because it contained both of the preferred AMF isolates. The number of tillers of *C. epigejos* followed the results of shoot biomass. This parameter can, obviously, be very important as a factor affecting plant community structure. The results of an experiment focused on clonal growth response of *Prunella vulgaris* (Streitwolf-Engel et al., 1997) to inoculation with different AMF indicated that different AMF species can influence clonal growth traits that would influence the vegetative fitness of a given plant, the number of ramets and their spatial distribution in a population. The potential of the diversity and composition of AMF populations to determine clonal growth and propagation of clonal plants was confirmed also by another experiment of Streitwolf-Engel et al. (2001).

A very strong growth response of the nurse plant to mycorrhizal inoculation suggests its possible utilisation in spoil bank reclamation. *T. pratense* belongs to plant species used within reclaiming processes on spoil banks nowadays; the massive increase of vitality gained by the inoculation could, however, make this process even more effective.

Neither the level of root colonisation nor the length of developed ERM observed in our experiment revealed a correlation with the growth response of model plants. The extent of root colonisation need not, however, be directly proportional to benefits gained by the host plant. According to Graham, Linderman, and Menge (1982), AMF may differ in their ability to enhance phosphorus uptake and host plant growth even when the extent of root colonisation is similar. Such variation could be due either to functional differences at the level of the host–fungus interface, or to differences in characteristics of the external hyphae such as length and distribution, or phosphorus uptake and translocation (Jakobsen, Abbott, & Robson, 1991).

The change in length of developed ERM in different mycorrhizal treatments between the first sampling (the end of the 1st stage) and the second sampling (the end of experiment) suggests, that while some AMF isolates benefited from the increase of plant community diversity, other isolates prospered most in symbiosis with *T. pratense* as a single host plant species.

In summary, our experiment simulated the specific stage of succession, when mycotrophic and non-mycotrophic plants on spoil banks are present at the site together and the mycelium of AMF spreads through the soil from colonised roots. The obtained data revealed the strong effect of the presence and identity of AMF on the growth of individual plant species, which resulted in changes of plant community structure. This effect was not only plant specific, but also AMF specific, because individual AMF species had different effects on particular plant species. It can be concluded that the presence and composition of AMF is one of the key factors that determine the progress in plant community structure within the succession on spoil banks.

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