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Interaction between Grass and Trees Mediated by Extraradical Mycelium of Symbiotic Arbuscular Mycorrhizal Fungi

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Abstract

We investigated the effects of the arbuscular mycorrhizal fungi (AMF) *Glomus mosseae* BEG99 and *Gigaspora rosea* BEG9 on plant growth, soil aggregation and ^{32}P transfer between grass and tree seedlings via the extraradical mycelium (ERM). Two microcosm experiments were conducted in rhizoboxes, where the grass *Agrostis capillaris* and seedlings of *Acer pseudoplatanus* (maple), *Alnus glutinosa* (alder) and cutting-derived plants of *Salix purpurea* (willow) were grown separately (1), interacting via roots (2), or interacting via the ERM (3). In Experiment 1, alder biomass was significantly lower in treatment where plants interacted via roots than where grass and trees interacted only via the ERM or grew separately. In spite of having significant enhancement of mycorrhiza development, the grass was a relatively strong competitor to the trees when interacting via roots. In Experiment 2, both AMF species varied in the effect on grass and three tree species interaction and in mycorrhiza development. Trees were infected by ERM hyphae from the quickly-growing grasses, and the ERM linking roots facilitates ^{32}P transfer between the tree and the grass. Apart from this role, the ERM had positive effects on soil aggregation and their presence can represent a significant contribution to erosion control.

Keywords: Arbuscular mycorrhizal fungi, grass and tree seedlings, extraradical mycelium network, transfer of ^{32}P, soil aggregation, plant coexistence

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1. Introduction

Not only do arbuscular mycorrhizal (AM) plants acquire more nutrients, they are also able to share them via an underground network of hyphal connections (extraradical mycelium-ERM) linking individuals within and between species (Hart and Klironomos, 2002), so they are important for interactions between plants. Two mechanisms of interaction have been suggested: 1) the same or different plant species can be linked via the ERM (Newman, 1988; Newman et al., 1994), which facilitates interplant nutrient transport (Grime et al., 1987), thus AMF have the ability to regulate plant species coexistence by the sharing of nutrients (Heijden et al., 2003); 2) an inter-specific competition may result from the different mycorrhizal dependence of plant species (Bergelson and Crawley, 1988). The role of the ERM in nutrient transfer was suggested to be important mainly in the nutrient transfer from decaying (Johansen and Jensen, 1996) and dying (Newman and Eason, 1989) roots to the roots of living plants. It has been shown repeatedly that the hyphal network associated with a living plant is capable to induce colonisation of other plants growing in its vicinity and to provide a significant support for establishing new seedlings (Read et al., 1976; Francis and Read, 1994; Malcová et al., 2001).

According to Heap and Newman (1980b) there are several mechanisms of nutrient (and in particular P) transfer from one mycorrhizal plant to another: 1) Phosphorus could pass in soluble form from the donor roots into the soil solution, move by diffusion or mass flow to the receiver roots and be taken up. 2) Phosphorus could pass into the soil solution as before, be taken up by AMF hyphae attached to the receiver and be translocated by them into the receiver roots; 3) If mycorrhizal hyphae form links between the two root systems, the P could pass into the fungus within the donor root and be translocated into the receiver roots without ever being in the soil solution. The young seedlings being linked to mature plants via ERM of AMF can use the network links for transfer and exploration of nutrient sources from mature and efficiently assimilating plants to their tissue, which decreases the cost of development and maintenance of AM symbiosis. That can give mycorrhizal plants competitive advantage, especially in stressful environments.

Mycorrhizas should be considered as important components of soil and vegetation stability on anthropogenic sites, and their presence can have significant ecological implications on succession and vegetation structure on these artificial substrates. The responsiveness of plant species to AMF infection is very variable (Sanders et al., 1995). Zobel and Moora (1997) demonstrated that the presence of AMF inoculum makes competition more unbalanced (plant weight differences increase) or it has no effect on competition in comparison with community-level experiments, where AM presence results in higher diversity and consequently in more balanced competition in greenhouse

experiments. Vosátka et al. (1999) observed better growth parameters of *A. pseudoplatanus* seedlings growing together with *Festuca rubra* in mycorrhizal than in non-mycorrhizal treatments. They also demonstrated that majority of native AMF isolates from coal mine spoil banks were found in association with native grasses, namely *Calamagrostis epigejos*, and some of them also in association with planted hardwood trees, but only in the case of plantations over 8 years old (unpublished). Grasses seem to be important agents for AM distribution and can facilitate mycorrhization of planted trees in anthropogenic substrates (Enkhtuya et al., 2005).

Beside the above-mentioned roles, the network of AMF ERM is also important in binding soil particles and this can have a fundamental function in soil stabilisation and erosion control. The ERM proliferating from colonised roots to the soil appear to be the most important mediator of soil aggregation (Tisdall, 1994; Rillig et al., 1999; Miller and Jastrow, 2000). AMF have been proven to increase formation of soil macro-aggregates and thus prevent water and wind soil erosion (Miller and Lodge, 1997; Tisdall, 1994). Miller and Jastrow (1992) suggested that the ERM can improve the structure of the soil through the formation of water stable soil aggregates by physical entanglement and production of binding agents which increase its resistance to erosion. According to Miller and Jastrow (1992) and Wright et al. (1998) some species of *Gigaspora* are more effective in soil aggregation than isolates of *Glomus* species. In addition, glomalin recently discovered glycoprotein produced in copious amounts by AMF hyphae (Wright et al., 1998), plays a major role in soil aggregate stabilisation (Wright and Upadhyaya, 1998; Rillig et al., 2002).

The aim of our study was to investigate the effect of inoculation with AMF a) on the growth of trees commonly used for re-cultivation of coal mine spoil banks, b) to highlight the role of their interaction with a grass spontaneously colonising some man-made ecosystems, and c) the role of fungal ERM in the transfer of ^{32}P between plants and in soil aggregation.

2. Materials and Methods

In the experiments, the grass *Agrostis capillaris* L. naturally spreading in man made or disturbed ecosystems in the Czech Republic and three hardwood species as the target species for reforestation of mine spoil banks were used: maple (*Acer pseudoplatanus* L.), alder (*Alnus glutinosa* L. Gaerth.) and willow (*Salix purpurea* L.). Even though most plants form one type of mycorrhiza, some plants form both AM and ectomycorrhiza, e.g. species, which we have chosen, alder (Molina et al., 1994) and willow (Lodge, 1989; Dhillion, 1994). Furthermore alder forms also symbiosis with actinonmycete *Frankia* (Baker

and Schwintzer, 1990). However, in this study we concentrated only on AM symbioses of these tree species. Grass seeds were surface sterilised in 10% NaOCl for 10 min and rinsed with deionised water. Seeds of maple and alder were surface sterilised by activated charcoal [7440-44-0] EEC No. 231-153-3 (Sigma), willow was propagated from cuttings. The trees were precultivated in a growth chamber for 6 weeks. During both experiments plants were grown in inert attapulgite clay substrate (Agsorb 18/9 Oil Dri, USA) and greenhouse temperature was kept at 25°C/20°C (day/night). In these experiments there was no additional fertilization.

Experiment 1

Various types of interactions between mycorrhizal and nonmycorrhizal *A. glutinosa* and *A. capillaris* were tested. Plants were grown together in 1560 ml plastic rhizoboxes, divided into two side compartments: 600 ml grass compartment and 960 ml tree compartment. The compartments were separated in three ways: 1) complete separation using hard screen to prevent any interaction of plants, 2) separation by a nylon mesh (aperture diameter 42 μm) allowing interaction of plants by ERM hyphae but not by roots, 3) non-separated compartments with non-restricted root and ERM contacts between plants. Grass seeds were sown to one compartment of each rhizobox and placed in the greenhouse. Two weeks after grass seedling emergence, seedlings were thinned to 12 per pot. Precultivated tree seedlings and grass were inoculated with *G. mosseae* BEG99 isolated from the Brezno spoil bank and cultured for 6 months on maize in sand based substrate. Each rhizobox received 14 ml of inoculum consisting of spores, colonised root fragments and the ERM. Only grass compartments were inoculated in the treatment separated with nylon mesh. Controls were left uninoculated. There were eight rhizoboxes per treatment, and the plants were harvested after 6-months of cultivation. At the harvest, growth of trees (shoot dry weight and height), grass shoot dry weight, AMF development (root colonisation, ERM length and NADH diaphorase activity of ERM), soil aggregation and transfer of ^{32}P from grass to tree seedlings via ERM were evaluated.

Shoot dry biomass of plants was assessed after drying in an oven at 80°C for 72 hours. Washed root samples of grass and trees were cleared and stained with 0.05% Trypan blue in lactoglycerol (Koske and Gemma, 1989). The percentage of root length colonised by AMF was evaluated by the modified grid-line intersects method (Giovannetti and Mosse, 1980) under a microscope using an ocular grid at 100× magnification. For estimation of ERM length, a 15-ml core of the substrate was removed from the middle of each rhizobox. A weighed sub-sample was mixed with 200 ml of H_2O in a blender and 0.5 ml of the suspension

was pipetted onto a nitro-cellulose membrane filter (24 mm in diameter and 0.4 μm pore size) and vacuum filtered. The membrane filter was then placed on a microscope slide and stained with 0.05% Trypan blue in lactoglycerol. The total length of the ERM was evaluated under an Olympus BX60 microscope using a grid inside the eyepiece at 100× magnification (Brundrett et al., 1994). For evaluation of NADH diaphorase activity of the ERM, a 50 g sample was wet sieved through two sieves (0.25 and 0.036 mm). The ERM clusters from the finer sieve were collected using sharp tweezers and put into an Eppendorf microtube with 300 μl of the staining solution of the enzyme (Sylvia, 1988). Staining solution for NADH diaphorase activity was prepared by mixing INT (1 mg/ml) and NADH (3 mg/ml) in 0.2M tris buffer pH 7.4. After incubation in the dark at room temperature (25°C) for 12 hours, enzyme activity was estimated. The percent proportion of the ERM length, which contained red precipitate, was measured after mounting mycelium clusters from Eppendorf tubes on the microscope slides at magnification of 200×.

Soil aggregation was measured as percentage of water-stabile soil macro-aggregates larger than 0.5 mm from the total weight of soil sample. Soil samples were taken from compartments with trees, air-dried and used for the wet sieving to determine soil aggregation (Robles, 1999). Weighed soil samples were put on a 0.5 mm sieve and immersed five times into water to approx. 20 cm depth. The soil fraction that remained on the sieve was dried for 5 days at room temperature, weighed and the percentage of stable soil aggregate mass from the total sample mass was determined.

To study ^{32}P transfer between plants via ERM, the rhizoboxes were moved to a growth chamber (14 h photoperiod at 25°C during the day and 19°C at night) and left to acclimatise for two weeks. Then 3.75 ml of acidified (0.02 N HCl) aquaeous solution of $H_3{}^{32}PO_4$ (activity concentration 1.682 MBq ml^{-1}, ICN Biomedical Research Products, UK) was applied into the rhizosphere of *A. capillaris* (donor plant) and then watered with 25 ml of distilled water. Tree seedlings as receiver plants in neighbouring compartments of the rhizoboxes were harvested after three weeks. The content of ^{32}P in their dry shoots was evaluated after wet-digestion and mineralisation (in 96% H_2SO_4 and 30% H_2O_2, under temperatures 250–300°C) using liquid scintillation counting with a spectrometer TRICarb 2900TR (Canberra-Packard Co.). The transfer of ^{32}P was expressed as a relative activity concentration (g^{-1}), i.e. the ratio of the ^{32}P activity concentration in dry matter (dpm g^{-1}) to the total administered activity of ^{32}P (dpm).

Experiment 2

In this experiment, influence of two different AMF *G. mosseae* BEG99 and

Gigaspora rosea BEG9 on the ERM-mediated interaction of trees *A. pseudoplatanus*, *A. glutinosa* and *S. purpurea* and grass *A. capillaris* was tested. Plants were planted into plastic rhizoboxes with two 80 ml compartments. These two compartments (one for grass and the other for tree seedling) were separated by nylon mesh allowing the growth of ERM but not roots. Every compartment contained one precultivated plant, either grass or tree seedling. Rhizoboxes were filled with autoclaved substrate (the same as in Experiment 1), and there were 5 replicates per treatment. In half of the rhizoboxes, grass compartments were inoculated with 5 ml of AMF inoculum suspension consisting of spores, colonised root fragments and the ERM of one of two AMF species and the other half was left uninoculated as a control treatment. Plants were harvested after 20-weeks cultivation. Tree seedling height, root collar diameter, AMF colonisation of tree seedlings and grass, ERM total length and NADH diaphorase activity of the ERM in the compartments with tree seedlings, ^{32}P transfer from the grass to tree seedlings (3 replicates per each treatment) via ERM and soil aggregation were evaluated using the same methods as in Experiment 1.

Statistical analysis of data

Statistical analysis was done using SOLO 4.0/BMDP Statistical Software. All experimental data were checked for normality. Data showing normal distribution were analysed by ANOVA, whereas data with non-normal distribution were analysed by a non-parametric the Kruscall-Wallis test. The Duncan Multiple Range Test ($P<0.05$) was used to estimate the differences between the treatments.

3. Results

Experiment 1

In this experiment, alder seedlings were taller and had greater aboveground biomass in treatments where interaction between grass and alder seedlings was completely prevented, compared with the other two types of interaction (Fig. 1). In contrast, grass shoot dry weight was best in root-interaction treatments compared with the treatments without contact and ERM-interaction (Fig. 2). In treatments where plants interacted either via roots or only via ERM, AMF inoculation had positive influence on grass shoot dry weight (Fig. 2). In treatments with free root-interaction, grass presence negatively affected growth of alder seedlings (Fig. 1) even though it supported development of AMF (Table 1). In treatments with ERM-interaction, alder seedlings were

successfully colonised by the ERM network spreading from the grass growing in the adjacent compartment. However, inoculation did not have any effect on the growth (height and shoot dry weight) of alder in this treatment (Fig. 1). In contrast the inoculation positively affected both height (Fig. 1a) and shoot dry weight (Fig. 1b) of alder seedlings in root-interaction treatments and in treatments completely separated.

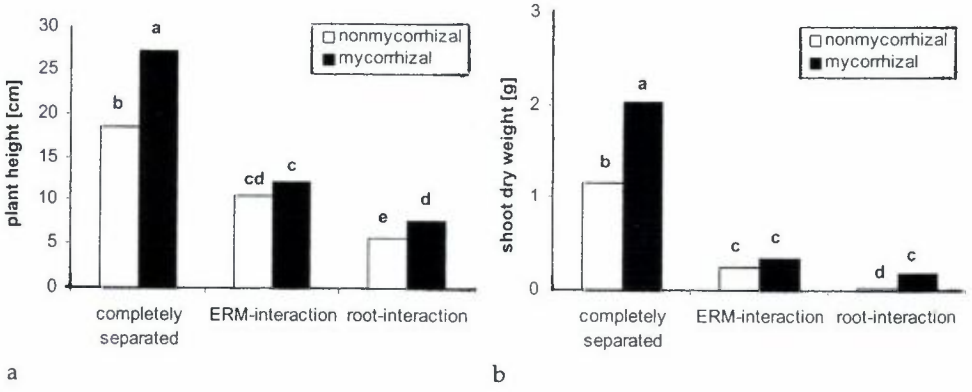

Figure 1. Effect of inoculation with *Glomus mosseae* BEG99 and various types of interaction between grass and *Alnus glutinosa* seedlings on height (a) and shoot dry weight (b) of *Alnus glutinosa*. Presented values are means of eight replicates. Columns marked with the same letter are not significantly different according to Duncan Multiple Range test ($P \leq 0.05$), (Experiment 1).

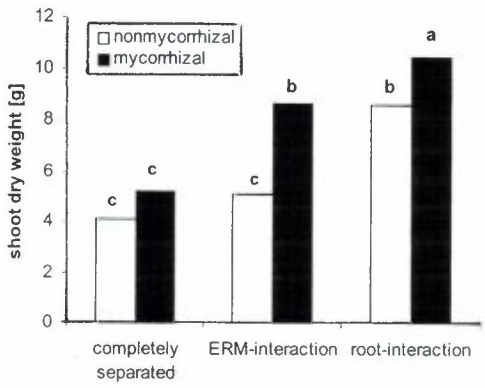

Figure 2. Effect of inoculation with *Glomus mosseae* BEG99 under various types of interaction between grass and *Alnus glutinosa* seedlings on grass (*Agrostis capillaris* L.) shoot dry weight. Presented values are means of eight replicates. Columns marked with the same letter are not significantly different according to Duncan Multiple Range test ($P \leq 0.05$), (Experiment 1).

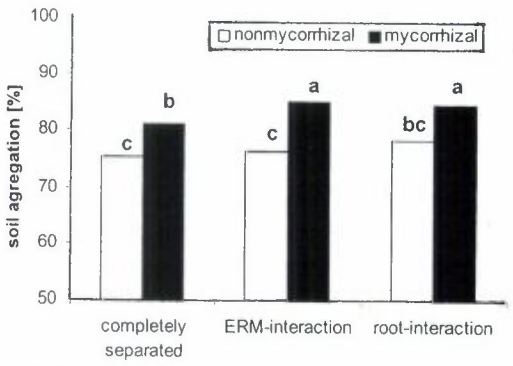

Fig. 3.

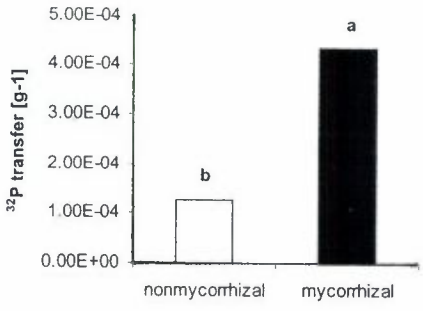

Fig. 4.

Figure 3. Effects of inoculation and various types of interaction between grass and *Alnus glutinosa* seedlings on percentage of water stable macroaggregates. Presented values are means of eight replicates. Columns marked with the same letter are not significantly different according to Duncan Multiple Range test ($P \leq 0.05$), (Experiment 1).

Figure 4. Effects of inoculation on transfer of ^{32}P from grass to *Alnus glutinosa* seedlings via ERM in ERM-contact treatment. Presented values are means of five replicates. Columns marked with the same letter are not significantly different according to Kruscall-Wallis test ($P \leq 0.05$), (Experiment 1).

In root-interaction treatments root colonisation was significantly higher than in completely separated or in treatments with ERM-contact (Table 1). Similar results were found for grass seedlings, where average colonisation in root-interaction treatments was 82%, in treatment with ERM-interaction 63%, and in completely separated treatment 62%. The ERM total length in tree compartments was significantly higher in root-interaction and ERM-interaction treatments in comparison with the completely separated treatment (Table 1). NADH diaphorase activity of the ERM was significantly higher in the root-interaction treatment as compared to the completely separated treatment (Table 1). Inoculation with AMF significantly increased the percentage of water-stable soil macro-aggregates in comparison with non-inoculated controls for all treatments (Fig. 3). In the ERM-interaction treatment, inoculated plants showed a greater transfer of isotope ^{32}P from the donor grass to the receiver trees as compared to plants in the non-inoculated treatment (Fig. 4).

Experiment 2

As in the first experiment, the roots of alder, maple and willow seedlings were successfully colonised by the ERM network spreading from compartments

with grass. Inoculation with both *G. mosseae* BEG99 and *Gi. rosea* BEG9 significantly stimulated growth of alder and maple (Table 2). *Gi. rosea*, but not *G. mosseae*, significantly increased height of willow, while inoculation with *G. mosseae* did not significantly affect this growth parameter (Table 2). There were no significant differences among inoculation treatments for root collar diameters of willow (Table 2).

Table 1. Effects of various types of interactions (root-interaction, ERM-interaction and separation) between *Agrostis capillaris* and *Alnus glutinosa* seedlings on mycorrhizal parameters of AMF isolate *Glomus mosseae* BEG99 associated with *Alnus glutinosa* seedlings. Means in columns followed by the same letters are not significantly different according to Duncan's Multiple Range test at the level $P<0.05$. Data are means of eight replicates (Experiment 1).

Interaction	Myc. colon. (%)	ERM (cm/g dry soil)	NADH-diaphorase activity (%)
Root-interaction	63 b	77 b	26 b
ERM-interaction	72 b	126 a	29 ab
Separation	88 a	134 a	32 a

Table 2. Effects of inoculation with different AMF species (*Glomus mosseae* BEG99 and *Gigaspora rosea* BEG9) on height and shoot collar diameter of *Acer pseudoplatanus*, *Alnus glutinosa* and *Salix purpurea* and on ^{32}P transfer by ERM from grass *Agrostis capillaris* to tree seedlings. Means in columns followed by the same letters are not significantly different according to Duncan's Multiple Range test (height and shoot collar diameter) and to Kruscall-Wallis test (transfer of ^{32}P) at the level $P<0.05$. Data are means of five (height and shoot collar diameter) or three (^{32}P transfer) replicates (Experiment 2).

Height of trees (cm)			Root collar diameter of trees (mm)			^{32}P transfer by ERM (relative activity conc. g^{-1} shoot dry weight) ($.10^{-5}$)		
Alnus	*Acer*	*Salix*	*Alnus*	*Acer*	*Salix*	*Alnus*	*Acer*	*Salix*
Non-inoculated								
4.5 b	7.4 n	11.5 z	1.0 b	2.0 n	2.3 y	0.40 b	0.19 n	1.08 z
Gi. rosea								
10.7 a	12.7 m	23.5 y	2.0 a	3.5 m	2.2 y	3.16 a	0.12 n	8.26 y
G. mosseae								
9.8 a	13.7 m	15.5 yz	2.8 a	3.4 m	3.0 y	1.56 b	1.90 m	1.66 z

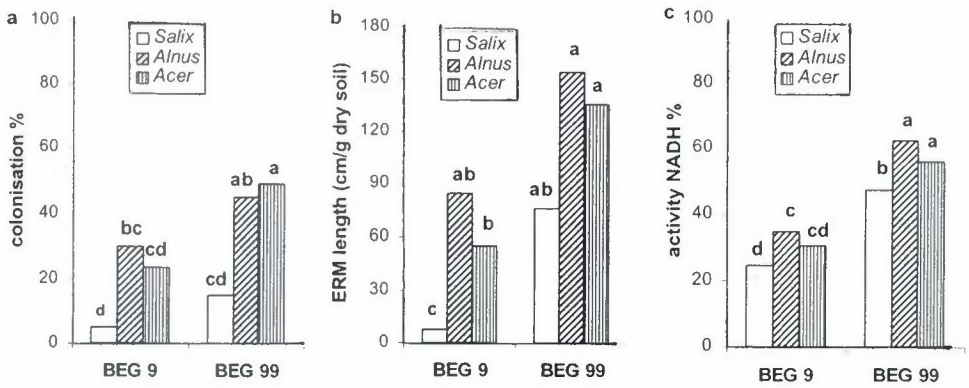

Figure 5. Mycorrhizal colonisation (a), ERM length (b) and NADH-diaphorase activity (c) of *Glomus mosseae* BEG99 and *Gigaspora rosea* BEG9 in association with *Acer pseudoplatanus*, *Alnus glutinosa* and *Salix purpurea*. Presented values are means of five replicates. Columns marked with the same letter are not significantly different according to Duncan Multiple Range test ($P \leq 0.05$), (Experiment 2).

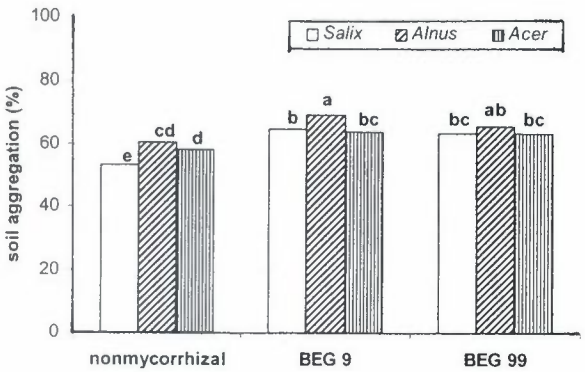

Figure 6. Effects of *Gigaspora rosea* BEG9 and *Glomus mosseae* BEG99 in association *Acer pseudoplatanus*, *Alnus glutinosa* and *Salix purpurea* on percentage of water stable macroaggregates. Presented values are means of five replicates. Columns marked with the same letter are not significantly different according to Duncan Multiple Range test ($P \leq 0.05$), (Experiment 2).

In the development of AMF (mycorrhizal root colonisation and total ERM length) significant differences were found between AMF species in maple and willow, where *G. mosseae* developed significantly better than *Gi. rosea* (Fig. 5a, b). NADH diaphorase activity of the ERM associated with all tree species was significantly higher for *G. mosseae* comparing to *Gi. rosea* (Fig. 5c). In spite of the great variability of data, inoculated trees contained more ^{32}P than non-inoculated ones (Table 2), trees inoculated with *Gi. rosea* 3× and with *G.*

mosseae 12× more ^{32}P, respectively (non-inoculated $0.56.10^{-5}$, *Gi. rosea* $1.69.10^{-5}$ and *G. mosseae* $7.18.10^{-5}$ dpm per gram shoot dry weight). However, when considering particular tree species, *Gi. rosea* was significantly more efficient in transporting ^{32}P between grass and alder and willow as compared to *G. mosseae* (Table 2). The opposite result was found for maple, where *G. mosseae* was more efficient than *Gi. rosea* (Table 2). Both *G. mosseae* and *Gi. rosea* significantly increased soil aggregation in comparison with uninoculated treatments (Fig. 6). *Gi. rosea* was similarly as efficient as *G. mosseae* considering soil aggregation (Fig. 6), even though it had significantly lower ERM total length.

4. Discussion

In both experiments, ERM radiating from the grass roots successfully inoculated tree seedlings in adjacent compartments of the rhizoboxes. The ERM hyphae spreading from nurse plants reached these compartments within a few weeks as shown by Jakobsen et al. (1992). In the first experiment, in treatments with free root-interaction, grass presence significantly supported development of AMF on receiver tree seedlings. Thus, roots and ERM of fast growing grasses provided the source of mycorrhizal colonisation for slow-growing trees. Even though grasses supported mycorrhizal development in tree roots, they negatively affected growth of alder seedlings. In the treatment with free root-interaction, mycorrhizal alders had higher shoot dry weights as compared to nonmycorrhizal plants, while in the treatment where plants were linked via ERM alders did not differ from nonmycorrhizal plants in spite of high mycorrhizal colonisation.

These results are in agreement with findings of Kytoviita et al. (2003), who concluded that a common mycorrhizal network may imply some mutual aid for the connected plants, but competitive interactions within the extraradical mycorrhizal network can suppress any benefits. For grass growth, opposite results were found: inoculated grasses in the root- and ERM-interaction treatments had significantly better shoot dry weight as compared to the completely separated treatment.

It is known that the presence of AMF can enhance growth and resource acquisition of plants (Call and Davies, 1988; Smith and Read, 1997). The existence of ERM-links could have important effects on belowground interaction of plants how they interact and attenuate environment stress effects on nurslings (Valiente-Banuet and Ezcurra, 1991; Carrillo-García et al., 1999). The ERM-links influenced the ability of seedlings to establish as suggested by Eissenstat and Newman (1990) and Ocampo (1986), who did not observe that mycorrhizas diminish competition between large plants and small unshaded seedlings. AMF influence the development and stability of the plant-soil system as colonists of

both root and soil (Carrillo-García et al., 1999). According to them an early integration of mycotrophic seedlings into the community through a pre-established common AM mycelium may increase their survival rate, whereas nonmycotrophic plants would benefit from the improved growth conditions provided by the resource islands formed by nurse plants (Carrillo-García et al., 1999). This was described as nurse plant effect, when highly mycotrophic plants support mycorrhiza formation of less mycotrofic species (Ocampo, 1986). The ERM-links also affect the balance between established plants when growing in low P soil by favouring the species dependent on mycorrhiza (Ocampo, 1986). In consequence the ERM-links can significantly influence plant diversity in plant communities (Grime et al., 1987).

In experiments conducted in compartment systems Malcová et al. (2001) observed that AM colonisation initiated from the established ERM network radiating from the nurse plant supported the establishment of colonisation and fitness and growth of early developmental stages of *Calamagrostis epigejos* in the substrates from disturbed ecosystems. The disturbance of ERM links between nurse plants and seedlings delayed AM colonisation of seedlings, however, only negligible effect of ERM disturbance on the growth of seedlings was found. McGee (1985) is described the importance of mycelial network for the survival of plants and establishment of infection in seedlings of *Centaurium erythraea*. Seedlings of *C. erythraea* died in the absence of inoculum of AMF in the soil low in nutrients. Our results also confirm this by positive effects of inoculation on alder and maple seedlings in the Experiment 2 for completely separated and root-interaction treatments in the Experiment 1. On the other hand, the lack of alder growth response to AMF inoculation in the ERM-contact treatments in the first experiment, and of willow in the second experiment is the same as in the study of Lumini et al. (1994). They found no positive growth response of *Alnus cordata* seedlings to inoculation with *G. mosseae* or *G. fasciculatum*, either after 5 months pre-cultivation or after 12 months growth on mine spoils. Lack of growth response of willow is in contrast with the results of Heijden (2000), who showed highly significant positive effects of AMF inoculation on growth of *Salix repens* even at very low colonisation levels. Average colonisation was 7 and 9% for 27 weeks with *G. mosseae* and *Acaulospora laevis*, respectively, but in our second experiment it was 5 and 15% with *Gi. rosea* and *G. mosseae*, respectively.

Similarly to findings of Malcová et al. (1999) and Miller and Allen (1992) the amount of transferred ^{32}P detected by us represented only a small part of that initially applied. We also detected low amount of ^{32}P in the non-inoculated receiver plants, which was in agreement with the mechanisms of P transfer suggested by Heap and Newman (1980b). We concluded that it was not possible to completely ignore diffusion of P in the medium, and hyphae of saprophytic fungi also could have taken part in the transfer. We suggest that

the AMF-mediated below-ground competition between grass and trees is based rather on the inter-specific nutrient transfer as proposed by Grime et al. (1987) than on the different mycorrhizal dependence as proposed by Bergelson and Crowley (1988). Such nutrient transfer could be relevant in a harsh environment with lower availability of nutrients.

In both experiments, inoculation with AMF increased soil aggregation, probably due to binding particles by the ERM network. Similar increase in stabilisation of soil macroaggregates by the ERM hyphae has been shown in several studies (Tisdall, 1991; Tisdall, 1994; Rilling et al., 2002; Enkhtuya et al., 2003). In our second experiment *Gi. rosea* affected aggregation more than *G. mosseae*. This is in agreement with the report of Miller and Jastrow (1992) who showed that the length of ERM of *Gigaspora gigantea* were more positively associated with macroaggregation of soil in the reconstructed prairie grassland than an isolate of *Glomus* species. AMF can differ in a variety of physiological and ecological traits, for example in hyphal production (Giovanetti and Gianinazzi-Pearson, 1994), production of glomalin per hyphal length (Wright et al., 1996), and promotion of aggregate stability (Schreiner and Bethlenfalvay, 1995). Wright et al. (1998) suggested that some species of *Gigaspora* produce more glomalin in the soil than species of *Glomus* and thus are more efficient in soil aggregation. It is also conceivable that different host plants colonised by different subsets of the AMF community, could give rise to species-specific changes in aggregate stability (Rilling et al., 2002). This non-nutritional effect of AMF has potential practical application in stabilisation of substrates in the adverse ecosystems against wind and water erosion.

The results support the hypothesis about the key role of the AMF in the coexistence of grass and trees. Their ERM links are important in the belowground interaction of plants and may change the output of the interaction. We suggest that the nutrient (P) transport can be realised via ERM, and the biological significance of such nutrient transfer between species could play an important role in harsh environments where the nutrient availability is a limiting factor for plant competition ability and survival. In conclusion, the interaction between grass and studied tree species mediated by ERM represents an important mechanism of plant coexistence affected by the symbiotic relationship with mycorrhizal fungi.

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