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ORIGINAL PAPER

Extraradical mycelium of arbuscular mycorrhizal fungi radiating from large plants depresses the growth of nearby seedlings in a nutrient deficient substrate

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Abstract The effect of arbuscular mycorrhiza (AM) on the interaction of large plants and seedlings in an early succession situation was investigated in a greenhouse experiment using compartmented rhizoboxes. *Tripleurospermum inodorum*, a highly mycorrhiza-responsive early coloniser of spoil banks, was cultivated either non-mycorrhizal or inoculated with AM fungi in the central compartment of the rhizoboxes. After two months, seedlings of *T. inodorum* or *Sisymbrium loeselii*, a non-host species colonising spoil banks simultaneously with *T. inodorum*, were planted in lateral compartments, which were colonised by the extraradical mycelium (ERM) of the pre-cultivated *T. inodorum* in the inoculated treatments. The experiment comprised the comparison of two AM fungal isolates and two substrates: spoil bank soil and a mixture of this soil with sand. As expected based on the low nutrient levels in the substrates, the pre-cultivated *T. inodorum* plants responded positively to mycorrhiza, the response being more pronounced in phosphorus uptake than in nitrogen uptake and growth. In contrast, the growth of the seedlings, both the host and the non-host species, was inhibited in the mycorrhizal treatments. Based on the phosphorus and nitrogen concentrations in the biomass of the experimental plants, this growth inhibition was attributed to nitrogen depletion in the lateral compartments by the ERM radiating from the central compartment. The results point to an important aspect of mycorrhizal effects on the coexistence of large plants and seedlings in nutrient deficient substrates.

Keywords Degraded site · Mycorrhizal growth response · Nitrogen · Non-host · Nutrient depletion

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Introduction

It is widely recognised that arbuscular mycorrhiza (AM) affects the structure and diversity of plant communities (Hart et al. 2003; van der Heijden 2002; Zobel et al. 1997). Among the questions addressed within this field of research, attention is being paid to the effect of AM on the establishment and growth of seedlings nearby large plants. As has been analysed by van der Heijden and Horton (2009), this effect is highly variable and ranges from support to suppression.

While antagonistic effects of intact networks of AM fungal extraradical mycelium (ERM) on the growth of non-host species are consistently documented (Francis and Read 1995; Ocampo 1986; Sanders and Koide 1994), the situation is less clear for AM host species. Their establishment and growth nearby large plants was improved by AM symbiosis (Carey et al. 2004; Francis and Read 1995; Marler et al. 1999; van der Heijden 2004), but an opposite effect of mycorrhiza has been documented as well (Nakano-Hylander and Olsson 2007). Furthermore, Moora and Zobel (1998) and Kytoviita et al. (2003) demonstrated that mycorrhizal benefits are smaller in seedlings competing with large plants than in seedlings growing alone. This is in line with the density dependency of mycorrhizal effects on

plant growth (Allsopp and Stock 1992; Facelli et al. 1999; Koide 1991).

Mycorrhizal benefits generally decrease with increasing soil fertility, especially with *P* availability (Smith and Read 2008) and the effect of AM symbiosis on plant coexistence also depends on the soil nutrient levels as outlined already by Janos (1980). Van der Heijden and Horton (2009) suggested that experimental conditions, especially the factor soil fertility, are responsible for the wide range of mycorrhizal responses observed in the seedlings. This is also illustrated by the study of Malcová et al. (2001) who reported a range of different responses to inoculation in large plants and seedlings of *Calamagrostis epigejos* co-cultivated in several anthropogenic substrates. Further studies are, however, needed to determine the relative importance of soil nutrient availability for the role of AM symbiosis in plant coexistence generally (Hart et al. 2003), and even more in the large plant–seedling interaction.

The effect of AM hyphal networks in soil on the establishment of seedlings may have different ecological consequences depending on habitat. In established grasslands, AM symbiosis may promote seedling recruitment and thus increase plant diversity (van der Heijden 2004). In ruderal habitats, the ERM network radiating from established plants can favour the establishment and growth of AM host plant species in competition with non-hosts (Püschel et al. 2007b). In early succession ecosystems, comparing plant growth and coexistence with or without AM—coarse-scale effects on plant coexistence cf. Hart et al. (2003)—is relevant, because the soils, which have undergone disturbance, have often a low mycorrhizal inoculation potential (Hart et al. 2003).

Vast spoil banks created by opencast brown-coal mining are typical representatives of early successional sites. In the North-Bohemian coal basin, their original Miocene clay surface is covered by a loess layer in the process of reclamation to form a topsoil horizon more suitable for plant growth in terms of fertility and physical properties. Since this substrate is exposed to environmental factors at its temporary disposal sites, it contains plant seeds and some propagules of AM fungi, while further propagules rapidly colonise the soil already within the first year after the creation of the top soil layer (Püschel et al. 2008). Ruderal annuals (belonging mostly to the families Chenopodiaceae, Brassicaceae, Polygonaceae, and Asteraceae) soon appear in patches, emerging plant individuals of host species form AM symbiosis and maintain an ERM network in the surrounding soil.

In this study, we focused on the role of AM in the one-to-one interaction of a large plant and seedlings of a host plant species (*Tripleurospermum inodorum*) and a non-host (*Sisymbrium loeselii*). Based on previous results (Püschel et al. 2007b), we postulated that the established ERM network will favour the growth and nutrient uptake of the AM host species seedling while it will reduce the growth of the non-host species. In addition, we compared these mycorrhizal effects in two substrates: the loess collected directly at the model locality and this loess diluted with sand. Dilution decreased the nutrient contents in the substrate, which enabled us to compare the interaction at two different nutrient levels. We expected that the mycorrhizal response of both the large plants and the seedlings would be more pronounced in the less fertile, sand-diluted substrate.

Material and methods

Material

The annual dicots *T. inodorum* (L.) and *S. loeselii* L. were selected as two model species of plant communities colonising freshly formed coal mine spoil banks of the Most coal basin (North-Bohemian, Czech Republic). *T. inodorum* (Asteraceae) is a facultatively mycotrophic, highly mycorrhiza-responsive species (cf. Janos 2007; Püschel et al. 2007b), while *S. loeselii* belongs to the typically non-mycotrophic Brassicaceae. The plants were cultivated in two cultivation substrates: (1) loess collected from the freshly formed spoil bank of the Vršany coal-mine with the following characteristics: pH_{KCl} 7.6, Olsen-P (0.5 M $NaHCO_3$ extractable) 9.6 mg kg^{-1}, *N* 0.07%, C_{org} 0.83%; (2) the loess mixed with river sand in the ratio 1:2 (*v*/*v*). In comparison with the field-collected loess, the loess–sand mixture had higher pH_{KCl} of 8.2, lower Olsen-P of 4.3 mg kg^{-1}, *N* 0.01% and C_{org}<0.01%. Both substrates were sterilised by γ-irradiation (25 kGy) prior to the establishment of the experiment. Three inoculation treatments were established in each substrate: (1) non-inoculated (=non-mycorrhizal) control, (2) inoculated with *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe isolate BEG95; (3) inoculated with *Glomus intraradices* Schenck & Smith isolate BEG140. The former isolate originated from a spoil bank, the latter from a pyrite smelter sedimentation pond and both were previously shown to develop well in the spoil bank substrate used in the experiment (Püschel et al. 2007b).

Establishment and growth of the experiment

Altogether, 120 plastic rhizoboxes (12×12×9 cm) were used, each rhizobox was separated into three equal compartments (4×12×9 cm) by a nylon mesh with mesh diameter 42 μm to exclude root competition. These partitions enabled the spread of ERM between the compartments but not that of roots. Sixty rhizoboxes were filled with loess, 60 with the loess–sand mixture. *T. inodorum*

seedlings, germinated and pre-grown in sterilized sand for 3 weeks, were planted into the central compartments, one plant per pot. One third of the plants per substrate were left non-inoculated, the other two thirds were inoculated at planting with *G. intraradices* or *G. mosseae*. The inoculation was performed with 10 ml of inoculum suspension per plant containing colonised roots, spores, and ERM fragments from multispore cultures of the corresponding isolate. Plants of the non-inoculated treatment received 10 ml of heat-sterilized inoculum. All rhizoboxes received bacterial filtrate from the non-sterile spoil bank loess (10 ml per rhizobox) prepared by passing a suspension from the inocula through a filter paper (Whatman no. 1). In order to equalise microbial conditions in the different inoculation treatments, the central compartments of the rhizoboxes were also irrigated with 10 ml of bacterial filtrate prepared from the inocula.

The plants, termed "large *T. inodorum* plants" were cultivated for 2 months in a greenhouse with light supplement (12 h, metalhalide lamps, 400 W). Then, ERM presence was determined in the lateral compartments of the inoculated rhizoboxes, reaching average values per treatment (cultivation substrate × fungal isolate) between 0.8 and 3.2 mg^{-1} soil. *T. inodorum* and *S. loeselii* seedlings, prepared in the same way as described above, were planted into the lateral compartments. The shoots of the large *T. inodorum* plants were gently bound by an elastic band to avoid shading of the seedlings. The two lateral compartments of one rhizobox were planted with seedlings of the same species, one seedling per compartment. The seedlings received no additional inoculation and were thus inoculated only by the ERM spreading from the large *T. inodorum* plant in the central compartment. The experiment was therefore a factorial combination of (1) cultivation substrate (loess or loess–sand mixture), (2) inoculation treatment (non-inoculated, inoculated with *G. intraradices* or with *G. mosseae*), and (3) seedling species (*T. inodorum*, *S. loeselii*). Each combination of the factors comprised ten replicates. Seedlings, which did not survive the transplanting, were replaced by new seedlings 1 week after planting. The experiment was cultivated for further 2 months under the same cultivation conditions.

Harvest and determination of plant and fungal parameters

The large plants and the seedlings were harvested at the same time. Root samples from each plant were stained with 0.05% Trypan blue in lactoglycerol (Koske and Gemma 1989) and mycorrhizal colonisation was evaluated according to Trouvelot et al. (1986). Four 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; *M*, intensity of mycorrhizal colonisation of the root system; *A*, abundance of arbuscules in the root system, and *V*, abundance of vesicles in the root system. Root colonisation values of the large *T. inodorum* plants were determined in eight to ten replicates per treatment, each based on the evaluation of 30 root segments of 1 cm. Some of the seedlings had a very small root system so that root colonisation was determined in fewer replicates (at least seven per treatment) on a smaller number of root segments (at least five per replicate).

Shoot and root dry weight of the large *T. inodorum* plants was determined after drying at 80°C. *P* concentration in the shoot biomass was analysed by spectrophotometric molybdenum-blue method at a wavelength of 630 nm (Unicam UV4-100) after digestion of the shoot biomass in 65% HNO_3 and 30% H_2O_2. *N* concentration was determined using CHN Analyzer (Carlo Erba NC 2500) with a TCD detector. The element contents were evaluated in three replicates per treatment (cultivation substrate × inoculation × seedling species).

Only shoot dry weight was determined for the seedlings, because the whole root system was used up for the assessment of root colonisation in the inoculated treatments. Seedlings with no green leaves were regarded as dead and not included into the data set on shoot biomass (in averages, 3.3 seedlings of *T. inodorum* and 0.7 seedlings of *S. loeselii* per treatment). The biomass of the two seedlings per rhizobox was combined to calculate mean seedling biomass per rhizobox, which was later used in the statistical analyses as one replicate. When only one seedling per rhizobox was alive, the shoot biomass of this seedling was regarded as one replicate. The seedlings did not produce enough shoot biomass to determine both *P* and *N* concentration. Based on the high mycorrhizal response of the large *T. inodorum* plants in terms of *P* uptake (see "Results" section), it was decided to use the seedling biomass for the determination of *P*. Total contents of *P* in the samples were determined in mineral extracts obtained by dry decomposition (Mader et al. 1998). The ash was dissolved in 1.5% nitric acid. The element contents were determined using inductively coupled plasma-optical emission spectrometer (ICP-OES) with axial plasma configuration (VistaPro, Varian, Australia). Calibration solutions were prepared with concentrations of 10–100 mg l^{-1} *P*. The operating measurement wavelength for ICP-OES was 214.9 nm. Six replicates were analysed per treatments, which were always based on the pooled shoot biomass of two seedlings, preferentially from the two lateral compartments of one rhizobox. The element contents per shoots were calculated based on the average shoot biomass of these two plants.

Response to mycorrhiza (MR) in terms of shoot dry weight, *P* or *N* shoot concentration and *P* or *N* shoot

content was determined for each replicate according to the equation $MR(\%) = (M - NM_{mean})/NM_{mean} \times 100$; where M is the value recorded in the given replicate and NM_{mean} is the mean value of this parameter in the corresponding non-mycorrhizal treatment.

Data analysis

The data for the large *T. inodorum* plants were first analysed by three-way ANOVA with the factors substrate, inoculation, and seedling species. As the factor seedling species and its interactions with the other factors were non-significant for the biomass and element content data, the data sets were analysed by two-way ANOVA (factors substrate and inoculation) and are presented pooled across the two seedling species treatments.

The data for the seedlings were analysed for each seedling species separately by two-way ANOVA with the factors substrate and inoculation. Prior to ANOVA, some data sets were either logarithmically [$y=\ln(x+1)$] or square root ($y=x^2$) transformed in order to meet the requirement of ANOVA on homogeneity of variance (determined by Levene's test).

The data on MR were arcsine-transformed and analysed by two-way ANOVA with the factors substrate and inoculation. Comparisons among multiple means were carried out by Tukey's post hoc test at significance level $P<0.05$. Analyses were performed using the SPSS 15.0 software.

Results

Root colonisation of the large *T. inodorum* plants reached high F of 90–100% (average value per treatment). The M and A values were significantly higher in plants inoculated with *G. intraradices* than in those with *G. mosseae* (Table 1). The M values of *G. mosseae* tended to be higher in the pure loess while the M values of *G. intraradices* tended to be higher in the loess–sand mixture, which was reflected by the significant interaction of the factors substrate and inoculation. Vesicles were formed by *G. intraradices* only, and they were significantly more abundant in the roots of plants cultivated in the loess–sand mixture than in the pure loess.

The *T. inodorum* seedlings had root colonisation F between 72% and 79% (average value per treatment). Similarly as in the large *T. inodorum* plants, M values were higher in the *G. intraradices*-inoculated plants, whereas A values did not significantly differ between the inoculation treatments (Table 2). Vesicles were formed by *G. intraradices* only, and their abundance did not significantly differ between the two substrates.

Table 1 Intensity of colonisation (M), abundance of arbuscules (A) and vesicles (V) in the roots of large *T. inodorum* plants inoculated with *G. mosseae* (GM) or *G. intraradices* (GI) and co-cultivated in rhizoboxes with seedlings of *T. inodorum* (TI) or *S. loeselii* (SL)

Substrate	Inoculation	Seedling	*M* (%)	*A* (%)	*V* (%)
Loess	GM	TI	69 (14) b	5 (3) b	0
	GM	SL	58 (23) b	3 (1) b	0
	GI	TI	89 (5) a	50 (27) a	6 (3) b
	GI	SL	94 (1) a	42 (21) a	9 (4) ab
Loess+sand	GM	TI	51 (18) b	3 (2) b	0
	GM	SL	57 (11) b	7 (4) b	0
	GI	TI	93 (3) a	44 (16) a	12 (2) a
	GI	SL	94 (4) a	62 (19) a	13 (5) a
				F value/significance	
	Substrate (A)		0.5 *n.s.*	2.1 *n.s.*	12.3 **
	Inoculation (B)		217.8 ***	338.0 ***	*n.d.*
	Seedling (C)		1.0 *n.s.*	1.2 *n.s.*	1.0 *n.s.*
	A×B		6.1 *	0.2 *n.s.*	*n.d.*
	A×C		0.1 *n.s.*	9.2 **	0.4 *n.s.*
	B×C		3.5 *n.s.*	0.4 *n.s.*	*n.d.*
	A×B×C		2.2 *n.s.*	0.5 *n.s.*	*n.d.*

Data are means of eight to ten replicates (SD). Values within each column marked by the same letter are not significantly different ($P<0.05$, Tukey's multiple range test)

Effects of factors according to ANOVA: *n.s.* non-significant effect, *n.d.* not determined

*$P<0.05$, **$P<0.01$, ***$P<0.001$

Table 2 Intensity of colonisation (*M*), abundance of arbuscules (*A*) and vesicles (*V*) in the roots of *T. inodorum* and *S. loeselii* seedlings inoculated with *G. mosseae* (GM) or *G. intraradices* (GI)

Substrate	Inoculation	*T. inodorum*			*S. loeselii*	
		M (%)	*A* (%)	*V* (%)	*M* (%)	*V* (%)
Loess	GM	41 (14) ab	10 (7) a	0	0	0
	GI	58 (22) a	8 (14) a	9 (6) a	3 (3) b	0.2 (0.2) b
Loess+sand	GM	35 (19) b	4 (4) a	0	0	0
	GI	57 (25) a	7 (12) a	18 (18) a	13 (9) a	2.0 (1.3) a
				F value/significance		
Substrate (A)		0.3 *n.s.*	1.7 *n.s.*	1.5 *n.s.*	40.6 **	42.5 **
Inoculation (B)		11.7 *	0.0 *n.s.*	*n.d.*	*n.d.*	*n.d.*
A×B		0.2 *n.s.*	0.7 *n.s.*	*n.d.*	*n.d.*	*n.d.*

Data are means of seven to 19 replicates (SD). Values within each column marked by the same letter are not significantly different ($P<0.05$, Tukey's multiple range test)

Effects of factors according to ANOVA: *n.s.* non-significant effect, *n.d.* not determined

*$P<0.01$, **$P<0.001$

S. loeselii seedlings were colonised only by *G. intraradices*, while no intraradical AM fungal structures were found in the *G. mosseae*-inoculated treatments. The *F* of *G. intraradices* colonisation was significantly higher in the loess–sand mixture (25%) than in the pure loess (8%), similarly as the *M* and *V* values (Table 2). No arbuscules were found in *S. loeselii* roots.

The large *T. inodorum* plants produced less biomass and had lower *P* and *N* contents in shoots when cultivated in the loess–sand mixture than in the pure loess (Table 3). The dilution of the loess substrate with sand also slightly decreased the *P* concentration in shoots, but had no effect on *N* shoot concentration. Inoculation generally improved the nutrient uptake and enhanced shoot growth of the large *T. inodorum* plants, while it had no effect on root growth. The effects of the two isolates on shoot biomass were similar, while *P* and *N* uptake was improved more effectively by *G. intraradices* than by *G. mosseae.* MR was most pronounced in *P* uptake (Fig. 1), the MR in shoot growth and *N* uptake was lower. MR was also significantly higher in the loess–sand mixture than in the loess, except for shoot *N* concentration.

Table 3 Growth, *P*, and *N* uptake of large *T. inodorum* plants when non-inoculated (NI), inoculated with *G. mosseae* (GM) or *G. intraradices* (GI)

Substrate	Inoculation	Dry weight (g)		Shoot concentration (mg g^{-1})		Shoot content (mg)	
		Shoots	Roots	*P*	*N*	*P*	*N*
Loess	NI	0.55 (0.12) b	0.39 (0.12) ab	1.3 (0.4) c	7.9 (0.9) b	0.7 (0.2) d	4.2 (0.2) c
	GM	0.74 (0.20) a	0.49 (0.16) a	2.3 (0.4) b	9.4 (1.0) b	1.6 (0.5) c	6.5 (1.6) b
	GI	0.85 (0.21) a	0.47 (0.20) a	4.4 (0.6) a	12.9 (2.0) a	3.9 (0.8) a	11.2 (2.4) a
Loess+sand	NI	0.28 (0.10) c	0.28 (0.12) b	0.9 (0.2) c	8.3 (1.5) b	0.2 (0.1) e	2.1 (0.5) d
	GM	0.56 (0.15) b	0.28 (0.11) b	2.2 (0.1) b	9.1 (1.4) b	1.3 (0.2) c	5.4 (0.9) bc
	GI	0.56 (0.14) b	0.31 (0.10) b	4.2 (0.7) a	12.3 (1.7) a	2.3 (0.3) b	6.7 (0.9) b
					F value/significance		
Substrate (A)		72.4 **	39.4 **	6.1 *	0.1 *n.s.*	43.6 **	52.8 **
Inoculation (B)		39.5 **	1.4 *n.s.*	188.8 **	30.4 **	223.1 **	102.8 **
A×B		1.8 *n.s.*	0.9 *n.s.*	2.8 *n.s.*	0.3 *n.s.*	3.9 *	5.0 *

Because data are pooled across the two seedling treatments, the presented values are means of 20 replicates (dry weights) or six replicates (shoot concentrations and contents), with SD in parentheses. Values within each column marked by the same letter are not significantly different ($P<0.05$, Tukey's multiple range test)

Effects of factors according to ANOVA: *n.s.* non-significant effect

*$P<0.05$, **$P<0.001$

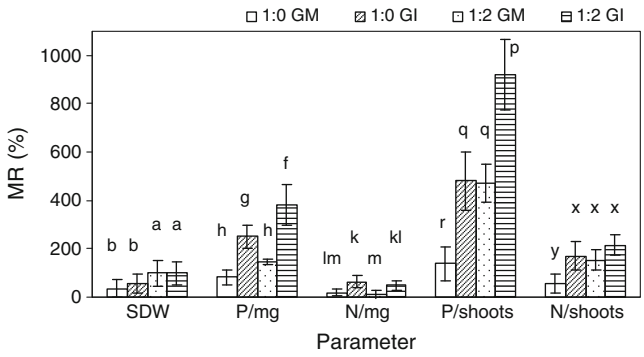

Fig. 1 Response to mycorrhiza (MR) of large *T. inodorum* plants when grown in the pure loess (1:0) or in the loess–sand mixture (1:2), inoculated with *G. mosseae* (GM) or with *G. intraradices* (GI). MR is presented for the following parameters: shoot dry weight (SDW), *P* concentration in shoots (*P*/mg), *N* concentration in shoots (*N*/mg), *P* content in shoots (*P*/shoots) and *N* content in shoots (*N*/shoots). Because data are pooled across the two seedling treatments, the columns are means of 20 replicates (SDW) or six replicates (the other parameters) with indicated SD. Values within each parameter marked by the same letter are not significantly different ($P<0.05$, Tukey's multiple range test)

In contrast to the large *T. inodorum* plants, the growth of the seedlings, both *T. inodorum* and *S. loeselii*, was reduced by the presence of the AM fungi (Table 4). In the inoculated treatments, the seedlings produced about ten times lower shoot biomass than in the corresponding treatment without inoculation. The *P* concentration in the shoot biomass, in contrast, was either higher in the inoculated than in the non-inoculated seedlings (five treatments) or was not significantly affected by inoculation (three treatments, Table 4). The *P* shoot concentration was also generally higher in the loess–sand mixture than in the pure loess. In both seedling species, it was significantly affected by the interaction of the factors inoculation and substrate (Table 4). In *T. inodorum*, the effect of each isolate depended on substrate, while in *S. loeselii*, the significant interaction was based on a more pronounced effect of both isolates in the loess–sand mixture than in the pure loess (Fig. 2). The total *P* content in shoots was decreased by inoculation in both seedlings, similarly as the shoot biomass (Table 4, Fig. 2).

Discussion

Early studies hypothesised that large plants may facilitate the establishment of seedlings by providing them with nutrients or carbon via mycelial links of AM fungal ERM (Grime et al. 1987; Ocampo 1986). Later studies, however, did not support this hypothesis and concluded that a common mycelial network does not alter the principally competitive nature of the interaction of large plants and seedlings (Eissenstat and Newman 1990; Kytoviita et al. 2003; Nakano-Hylander and Olsson 2007).

In our study, the presence of the ERM network in the compartmented systems depressed the growth of seedlings, both the host and the non-host species to a similar extent. This is in contrast with studies, where the presence of an ERM network in soil differentially affected host and non-host species (Francis and Read 1995; Landis et al. 2005;

Table 4 Growth, *P* concentrations and contents in shoots of *Tripleurospermum inodorum* and *Sisymbrium loeselii* seedlings when grown in non-inoculated rhizoboxes (NI) or in rhizoboxes inoculated with *G. mosseae* (GM) or *G. intraradices* (GI)

Substrate	Inoculation	*T. inodorum*			*S. loeselii*		
		Shoot dry weight (mg)	*P* shoot conc. ($mg.g^{-1}$)	*P* shoot content (μg)	Shoot dry weight (mg)	*P* shoot conc. ($mg.g^{-1}$)	*P* shoot content (μg)
Loess	NI	74 (21) a	0.9 (0.4) c	68 (30) bc	118 (67) a	1.1 (0.4) cd	127 (58) a
	GM	16 (4) b	0.7 (0.6) c	9 (6) d	21 (15) b	2.2 (0.5) bc	53 (46) abc
	GI	7 (4) bc	2.2 (0.4) b	15 (11) d	11 (6) b	2.4 (0.9) b	29 (17) c
Loess+sand	NI	182 (135) a	3.3 (0.5) b	643 (450) a	160 (39) a	0.8 (0.4) d	116 (42) ab
	GM	17 (12) b	7.0 (1.7) a	158 (115) b	11 (5) b	4.0 (0.4) a	51 (24) bc
	GI	7 (3) c	3.1 (1.2) b	18 (6) cd	11 (7) b	3.9 (0.7) a	38 (22) c
				F value/significance			
Substrate (A)		2.2 *n.s.*	102.0 ***	47.8 ***	0.4 *n.s.*	23.3 ***	0.0 *n.s.*
Inoculation (B)		84.9 ***	3.9 *	36.2 ***	117.0 ***	49.1 ***	15.0 ***
A×B		2.2 *n.s.*	22.1 ***	8.1 **	4.3 *	10.5 **	0.2 *n.s.*

Data are means of eight to ten replicates (shoot dry weight) or six replicates (*P* shoot concentration and content), with SD in parentheses. Values within each column marked by the same letter are not significantly different ($P<0.05$, Tukey's multiple range test)

Effects of factors according to ANOVA: *n.s.* non-significant effect

*$P<0.05$, **$P<0.01$, ***$P<0.001$

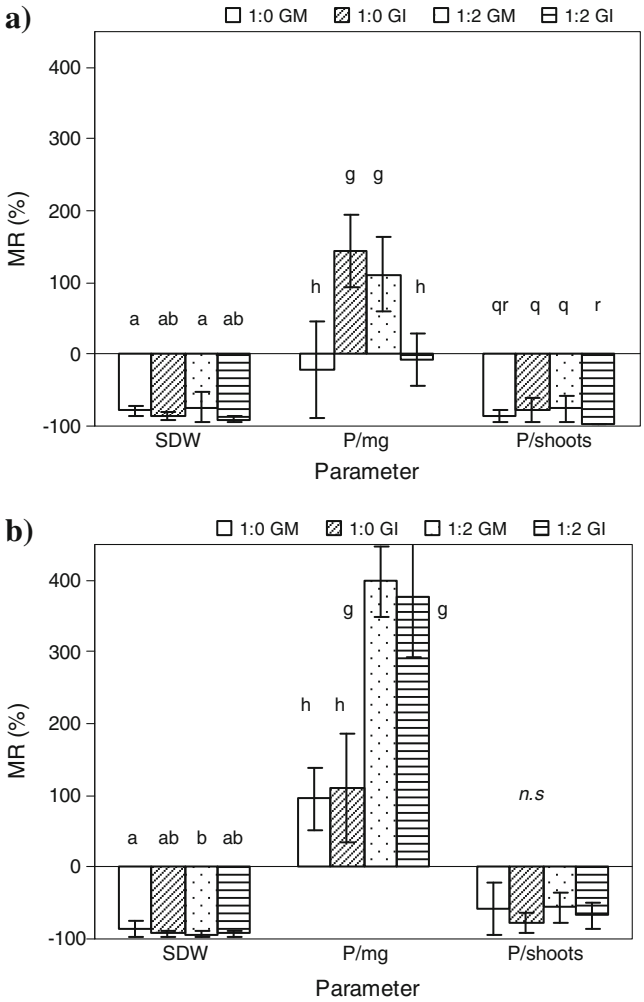

Fig. 2 Response to mycorrhiza (MR) of *T. inodorum* (**a**) and *S. loeselii* (**b**) seedlings when grown in the pure loess (1:0) or in the loess–sand mixture (1:2), inoculated with *G. mosseae* (GM) or with *G. intraradices* (GI). For the abbreviation of the parameters, see Fig. 1. The columns are means of eight to ten replicates (SDW) or six replicates (the other two parameters) with indicated SD. Values within each parameter marked by the same letter are not significantly different ($P<0.05$, Tukey's multiple range test)

Ocampo 1986), and also with our hypothesis based on previous results (Püschel et al. 2007b). The results do not indicate that the seedlings of the host species would have gained any benefit from sharing the mycelial network with the large plant. They rather point to nutrient depletion in the lateral compartment by the ERM radiating from the large plant as to the dominant factor determining the seedling growth. ERM can effectively deplete soil for both *P* (Li et al. 1991) and inorganic *N* (Johansen et al. 1992), and the positive response to mycorrhiza observed in the large *T. inodorum* plants is suggestive for intensive nutrient uptake by ERM also from the lateral compartments. Our results are also consistent with the observations that AM symbiosis increases biomass differences between large plants and seedlings (Moora and Zobel 1998) and variation in plant size within a community (Allsopp and Stock 1992), which is attributed to pre-emption of the fungal-delivered resources by the larger plants. It has been hypothesised that resource flow in a common mycorrhizal network is regulated by the ability of the interconnected plants to supply carbohydrates to the fungus (Pietikainen and Kytoviita 2007). This mechanism would also explain why the highly mycorrhiza-responsive *T. inodorum* seedlings reacted similarly to the presence of ERM as the non-mycotrophic *S. loeselii* seedlings. Though they became interconnected into the mycelial network, they did not obtain significant amounts of nutrients from the fungus, because most of the nutrients taken up by the ERM were directed to the dominant carbon source, i.e., to the large plant.

Such a pronounced growth depression by mycorrhiza in host species seedlings as in our study has been rarely observed before even in similar conditions. In the study of Püschel et al. (2007b), the ERM of pre-cultivated large plants extracted similar or even higher amounts of nutrients per volume of soil from the compartments, which were later planted with seedlings. Despite this, inoculation improved the growth of host species seedlings. This can be probably attributed to the higher fertility of the soil used (about three to six times more available *P* and four to 25 times more total *N* than in our cultivation substrates). Though a negative relationship between soil fertility and mycorrhizal growth response is widely accepted (Smith and Read 2008), mycorrhiza is not effective below a certain nutrient availability (Titus and del Moral 1998). The nutrient levels in the lateral compartments of our experimental systems probably decreased below this critical level during the pre-cultivation of the large plants in the mycorrhizal treatments.

This is also in accordance with the results of Malcová et al. (2001), who followed the growth of seedlings, inoculated by an ERM network, in different substrates. They observed positive growth response only in a substrate with intermediate *P* levels, while in extremely nutrient-deficient sand, AM consistently depressed the growth of the seedlings. The smaller degree of growth inhibition in the study of Malcová et al. (2001) than in our study can be explained by the fact that they cut the shoots of the large plants at planting the seedlings, which relatively increases the mycorrhizal benefit to seedlings in the large plant–seedling interaction as has been consistently shown in later studies (Jakobsen 2004; Pietikainen and Kytoviita 2007).

The mycorrhizal response of the large plant was most pronounced in *P* shoot content, which increased two to ten times by inoculation. However, *P* depletion was not the reason for the depressed growth of the seedlings: seedlings of both species had higher shoot *P* concentration in the inoculated than in the non-inoculated treatments, which discards *P* as the limiting factor for seedling growth in the inoculated treatments. The seedlings in the mycorrhizal

treatments had also higher *P* concentration in the sand-diluted soil than in the non-diluted soil. This leads to the conclusion that the growth depression should be ascribed to another factor than *P* availability in soil, which was more effective in the diluted soil than in the non-diluted soil. This factor was most probably *N* availability, especially as both cultivation substrates were *N* deficient and the diluted soil had lower *N* levels than the non-diluted soil.

The *N*/*P* ratio of the shoot biomass of non-mycorrhizal large *T. inodorum* plants was 6.1 in the non-diluted and 9.1 in the diluted soil, which generally indicates conditions of *N* limitation (Koerselman and Meuleman 1996). However, the *N*/*P* ratio decreased even further to up to 2.1 in mycorrhizal plants, because the mycorrhizal response of the large plants was higher in *P* uptake than in *N* uptake. This is in line with the conclusions of Johnson (2010) that plants benefit little from AM symbiosis in terms of *N* uptake. She explained this by the high *N* demand of the fungal tissues and consequent preferential accumulation of *N* in fungal tissues when *N* is so rare that it limits growth of both the plant and the fungus (Johnson 2010). For our experimental systems, we can assume that *N* was sufficiently available to "support" the development of the fungus and a positive mycorrhizal growth response of the large plants based on improved *P* uptake. This, however, may have decreased the *N* availability in the lateral compartments, the *N* being not only transferred to the large plant but also utilized for building up fungal structures.

The dilution of the original loess with sand rendered differences in the growth and nutrient uptake of the large plants, which are consistent with the lower *P* and *N* availability in the sand-diluted loess in comparison with the non-diluted loess. The lower nutrient levels in the sand-diluted substrate did not principally alter the response to inoculation, but made it more pronounced. This is in agreement with the known fact that positive mycorrhizal response is more pronounced at lower soil nutrient levels (Marschner and Dell 1994; Smith and Read 2008). In contrast, the mycorrhizal growth response of the seedlings did not principally differ between the two substrates. This indicates, provided our assumption on *N* depletion is right, that *N* was depleted to similar (very low) availability in both substrates regardless initial concentration. The original spoil bank substrate itself was thereafter so *N*-deficient that seedling establishment was virtually inhibited in the mycorrhizosphere of large plants. In further studies, it would be interesting to study the role of mycorrhiza in this soil–plant system after *N* fertilization, in conditions of *P* limitation.

The two AM fungal species, which were compared in the experiment, differed in their intraradical development and these differences were broadly in agreement with the results of Püschel et al. (2007b). Interestingly, only *G. intraradices*, but not *G. mosseae* colonised the roots of non-host species in both studies, forming intraradical hyphae and vesicles. Regvar et al. (2003) found only the AM fungal species *G. intraradices* in the roots of field-collected *Thlaspi* spp., which indicates that this species is particularly active in colonising the roots of non-host species. Püschel et al. (2007a) reported root colonisation by *G. mosseae* in two non-host species (*Atriplex sagittata* and *S. loeselii*), but the fungus was present in the roots only temporarily (in one of the two performed harvests, depending on plant species). The presence of intraradical structures of *G. intraradices* and absence of that of *G. mosseae* in our study could therefore be also a result of differential dynamics of root colonisation in both fungal species.

The two AM fungal species consistently differed in the *P* and *N* supply to the large *T. inodorum* plants, *G. intraradices* increasing the shoot concentrations of both nutrients more than *G. mosseae*. We should, however, be careful in extrapolating this finding as an intrinsic difference between the two isolates, because it may be specific to the nutrient levels in the substrates or even based on a difference in the initial fungal development in the given conditions (Janos 2007). Consistently with the differences in large plants and the assumption of *N* depletion by ERM, the seedlings tended to be smaller in the treatments inoculated with *G. intraradices*. This difference was, however, negligible in comparison with the overall effect of inoculation, which is not surprising in view of the similar response of the seedlings to inoculation in the two substrates differing in *N* availability, as discussed above.

In conclusion, we demonstrate that AM symbiosis may have opposite effects on the growth of an earlier established (large) plant and a later established seedling. Based on the determined *P* and *N* concentrations in the shoot biomass of the experimental plants, we assume that the growth of the seedlings was depressed by nitrogen depletion by the ERM radiating from the large plant in the mycorrhizal treatments. This illustrates that relationship between soil fertility and mycorrhizal response is complex in systems comprising plants of different age. Based on experiments with single plants, it is generally assumed that plants establishing on degraded sites with nutrient deficient soils profit from mycorrhiza (e.g., Azcon and Barea 1997; Oliveira et al. 2005). Our results, however, indicate that the situation may apply only to the first colonisers. The establishment of later emerging seedlings may be inhibited by depletion zones created by the mycorrhizosphere of already established plants. This effect will be certainly less pronounced in natural conditions than in the artificial conditions of our experiment with limited soil volumes. However, we believe that the results of this experiment point to an important aspect of mycorrhizal effects on the coexistence of large plants and seedlings in nutrient deficient substrates.

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