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Co-Adaptation of Plants and Communities of Arbuscular Mycorrhizal Fungi to Their Soil Conditions

Hana Pánková · Zuzana Münzbergová · Jana Rydlová · Miroslav Vosátka

Abstract The co-adaptation model formulated on the basis of the reciprocal interaction among plants, arbuscular mycorrhizal fungi (AMF) and soil hypothesizes that plants are adapted to their native AMF. Studies focused on this adaptation bring inconsistent results, however. Previously, we showed that different genotypes of *Aster amellus* exhibit different percentages of root colonization and that the species is adapted to the abiotic-soil environment combined with its native AMF isolate, but not to a specific AMF isolate alone. Here we asked whether plant populations of *A. amellus* are adapted to the whole native AMF community and whether there is co-adaptation between plants growing in their native soil and the native AMF community. In the first experiment, we used plants of one population of *A. amellus* from a marl region and plants of one population from a limestone region and planted them in non-sterile soil from both regions in a full factorial design. In the second experiment, plants from both regions were grown in their native sterilized soil and inoculated with their native AMF community or with the AMF community from the second region. Plants from each region established a specific level of root colonization, and AMF from each region formed a specific abundance of particular morphological structures in *A. amellus* roots. While we did not find any evidence that the plants are adapted to their whole-soil environment in terms of their growth, we did find evidence that adaptation of plants to native soil conditions could occur via adaptation of AMF to their native soil and plants and association of plants with specific type of AMF colonization. This type of adaptation could lead to the absence of adaptation in terms of plant growth.

Keywords AMF community · Arbuscular mycorrhiza · Local adaptation · Negative plant-soil feedback · Reciprocal transplant experiment · Soil pathogens

H. Pánková · Z. Münzbergová · J. Rydlová · M. Vosátka
Institute of Botany, Academy of Sciences of the Czech Republic, CZ-252 43 Průhonice, Czech Republic

Z. Münzbergová (✉)
Department of Botany, Faculty of Science, Charles University, Benátská 2,
CZ-128 43 Praha 2, Czech Republic
e-mail: zuzmun@natur.cuni.cz

Introduction

Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs. This means that they are not able to grow without their host plants, which provide them carbon. On the other hand, AMF improve plant acquisition of nutrients, especially phosphorus (Smith and Read 2008). The mutualistic relationship between AMF and plants usually occurs in soils with limited nutrient availability. It has been suggested that in nutrient rich soils carbon costs of mycorrhizal symbiosis can exceed the benefits to the plant and that mycorrhizal symbiosis may switch to parasitism (e.g. Clapperton and Reid 1992; Johnson et al. 1997; but see Treseder and Cross 2006 for no such pattern). Recently, however, this view has been disputed by studies showing that even plants without any growth response to AMF may acquire phosphorus via the mycorrhizal pathway (Smith and Smith 2012; see also Johnson and Graham 2013 and Smith and Smith 2013 for discussion of the issue).

The close relationship between AMF and plants and the strong effect of the abiotic soil environment on this relationship has led to the formulation of the co-adaptation model, which proposes that due to selection, non-mutualistic associations are suppressed, while mutually beneficial associations are supported (Sanders 1993; Johnson et al. 2010). This co-adaptation model has also been supported by demonstrations of the ability of plants (e.g., Jordan 1992; Kindell et al. 1996; Galloway and Fenster 2000; Raabová et al. 2007) as well as AMF to adapt to their native abiotic soil environment (e.g. Johnson 1993; del Val et al. 1999; Hildebrandt et al. 1999; Ipsilantis and Sylvia 2007). Recently, some studies have tested for plant adaptation to their abiotic soil environment via AMF, but these present inconsistent results. While Johnson et al. (2010), Taheri and Bever (2011), Pánková et al. (2011) and Ji et al. (2013) found clear evidence of adaptation of plants and AMF to each other and to their home whole soil environment or to the abiotic soil environment with native AMF species, Bever (1994) and Wagner et al. (2011) found that plants did not perform better in their native whole-soil environment.

The discrepancy in the results of different studies can be explained by the fact that the authors used very different types of inoculation, including inoculation by a single AMF isolate (Pánková et al. 2011), a mixture of AMF isolates (Schultz et al. 2001), non-sterilized soil collected in the field (Johnson et al. 2010) or soil pre-cultivated in the greenhouse (Taheri and Bever 2011). The results of studies on plant adaptations could also differ depending on plant and fungal genotype (Pánková et al. 2008, 2011; Streitwolf-Engel et al. 1997, 2001) and could also be affected by abiotic soil conditions of the experiment (e.g. Lackie et al. 1988; Douds et al. 1993). The abiotic soil environment, the AMF genotype as well as the host plant can modify the type and proportion of morphological structures formed by the fungi (e.g. Brundrett and Kendrick 1990; Cavagnaro et al. 2001; Yamato and Iwasaki 2002). All these statements suggest that each change in abiotic soil conditions, AMF communities or vegetation, may in turn modify the plant response to AMF.

The standard approach to testing plant adaptations to their local conditions is a reciprocal transplant experiment in the field, which takes into account all naturally co-occurring organisms such as pathogens and herbivores and reflects the exact environmental conditions of the sites (Antonovics and Primack 1982; Kawecki and Ebert 2004; Bischoff et al. 2006; Nuismer and Gandon 2008; Raabová et al. 2011).

Nevertheless, when we test plant adaptation to a specific factor, full factorial greenhouse experiments combining plant populations and the native whole soil environment are more useful because they allow us to keep all treatments under the same environmental conditions (e.g. Joshi et al. 2001; Kawecki and Ebert 2004; Raabová et al. 2007). In these experiments, plants are grown in non-sterilized soil, which allows including all soil microorganisms into the experiments. To be able to separate the effect of AMF from those of other soil microorganisms, it is, however, necessary to use sterilized soil inoculated with AMF (single species or the whole AMF community) and to add filtrates from non-sterilized soil and from the fungal inoculum into all pots to make other microbial conditions similar in all treatments (Koide and Li 1989).

To test for adaptation of plants and AMF to the whole soil environment, we used the obligately mycorrhiza-dependent perennial herb species *Aster amellus*. Raabová et al. (2007) had shown, in a reciprocal transplant experiment in the field, that populations of this species are adapted to the whole soil environment. Results of field sampling, pot experiments in the greenhouse (Pánková et al. 2008, 2011) as well as a field experiment (Pánková et al., unpubl.) show that the level of root colonization by AMF of *A. amellus* differs between different genotypes (defined as populations of origin) with populations from the marl region forming significantly higher levels of root colonization as compared to populations from the limestone region. In a pot experiment with native isolates of the AMF *Glomus mosseae*, we found that plant populations performed better when grown in their native whole soil environment (abiotic environment plus added microorganisms except for AMF) inoculated with their native AMF isolate, but we found no adaptation of plants to a specific AMF isolate independent of the whole soil environment (Pánková et al. 2011). All the above facts indicate that the local adaptations of *A. amellus* to the soil conditions could take place via changes in root colonization by AMF.

In the present study, we asked whether plant populations of *A. amellus* are adapted to the whole native AMF community and whether there is co-adaptation between the plants growing in their native soil and their native AMF community. To answer these questions, we carried out two greenhouse experiments. In the first experiment, we combined plants and non-sterilized soil (whole soil environment) from both regions in a full factorial design. A significant interaction between the plant population and soil would provide indication that the plants are adapted to the whole soil environment at their localities. In the second experiment, plants from both regions grown in their native sterilized soil were inoculated with native non-sterilized soil or with non-sterilized soil from the other region. In this case, we tested the adaptations of plants to AMF when grown in their native abiotic soil environment. A significant interaction between AMF and plant in its native sterilized soil would suggest co-adaptation of the plant in its native abiotic soil environment and AMF to each other.

Material and Methods

Study System

Aster amellus L. (Asteraceae) is an endangered, perennial, self-incompatible plant species (Münzbergová et al. 2011) that grows in dry grasslands. Its European

distribution extends from northern Italy to Lithuania; the southern distribution limit crosses northern Italy and Macedonia (Merxmüller et al. 1976). Outside Europe, its range extends to the Black Sea and the northern Caucasus (Meusel and Jäger 1992).

Aster amellus occurs as a hexaploid and a diploid in the Czech Republic (Mandáková and Münzbergová 2006; Münzbergová et al. 2011). For this study, we selected one diploid population from a marl region and one diploid population from a limestone region (populations CS1 and CK1 in Pánková et al. 2008). Although it would be better to use more plant populations per region, we used only one to keep the experiment feasible. Based on results of our previous studies, we supposed that using more plant populations per region would probably lead to comparable results (Pánková et al. 2008, 2011).

The plant species is highly genetically variable, and the two populations are clearly genetically separated (Raabová et al. 2007; Mandáková and Münzbergová 2008; Münzbergová et al. 2013). It is thus reasonable to consider the populations as separate genotypes in the subsequent text. The marl and the limestone region are situated about 70 km apart. Vegetation in the marl region belongs to the *Bromion* community (Chýlová and Münzbergová 2008), while the limestone region is characterized by limestone bedrock and oak-hornbeam forests of the association *Quercion pubescenti-petraeae* (thermophile oak wood; Ellenberg 1998). The soil from the marl region has a significantly higher pH, concentration of available calcium, total carbon and carbon in carbonates than the soil from the limestone region (Table 1). The marl soil has significantly lower concentrations of available magnesium and potassium, total nitrogen, organic C and C/N ratio than the soil from the limestone region (Table 1). The two types of soil do not differ in the concentration of available phosphorus (Table 1).

Soil for the experiments was collected at each of the two localities from at least three sampling points spread over the site. After collection, all the soil was partially air-dried, sieved through a 4 mm sieve and sent for γ-irradiation (25 kGrey) to eliminate native AMF. The sterilized soil was used for the second experiment (see below). When the γ-irradiation was finished, we again collected both soils from the same sampling points, partially air-dried them and sieved them through a 4 mm sieve. This second soil was used for setting up the first experiment and to prepare the inoculum for the second experiment (see below). Both experiments were set up within 1 week after collection of this soil.

Seeds of *A. amellus* were collected at each site directly from flowering stalks to prevent contamination with AMF propagules, which may occur when the seeds fall to the soil surface (Pánková et al. 2008). The seeds were collected from at least 20 different mother plants per population.

Table 1 Chemical characteristics of the soils from the marl and limestone regions

Region	pH_{KCl}	pH_{H2O}	Ca	Mg	K	P	N	Ctot	C (CO_3^{2-})	Cox	C/N
			(mg kg^{-1})				(%)				
Marl	**7.3**	**7.9**	**8,300**	**75**	**168**	7.5	**0.2**	**8.2**	**6.2**	**2**	**9.9**
Limestone	**4.5**	**5.4**	**4,700**	**191**	**271**	6.7	**0.4**	**6.6**	**0.02**	**6.5**	**15**

Values significantly different between regions at $P \leq 0.05$ are in bold. The data were previously published in Pánková et al. (2008) – populations CS1 and CK1.

Pot Experiments

In the first experiment, we combined plants and non-sterilized soil from both regions in a full factorial design. Each non-sterilized soil was filled into 500 ml plastic pots. While the pots may seem quite small, their size was selected based on our previous experience with the very slow growth rate of the plant species under study. The pots were not full of roots even at the end of the experiment, and therefore plant performance was probably not limited by the soil volume. Seeds of *A. amellus* were rinsed several times with deionized water and germinated in sterilized sand in the greenhouse (day temperature 25°C, night temperature 10°C, 14-h photoperiod). One 4-week-old plant was transplanted into each pot.

We added 5 ml of microbial filtrate from the other soil (i.e. marl soil obtained filtrate from limestone soil and vice versa) to all pots to make the results comparable with the second experiment (see below). Microbial filtrates were prepared by shaking 30 g of non-sterilized soil from the collected soil at each site with 300 ml of deionized water for 30 min and filtering twice through filter paper with a 15 μm pore size to eliminate mycorrhizal propagules. Added microbes, however, represent probably only a very small fraction of the overall microbial community in the soil, so it should be possible to interpret the results from this experiment as tests for adaptation of plants to the whole soil environment (see discussion for details on this issue).

In the second experiment, we tested co-adaptation of the plant in its native soil and AMF to each other. Soil from both regions was sterilized by γ-irradiation (25 kGrey) to eliminate native AMF. Plant seedlings from both regions were prepared as for the first experiment and planted to their native sterilized soil (plants in their native sterilized soil are hereafter called an abiotic soil-plant complex) inoculated with inoculum containing native AMF community or the AMF community from the other region. The inocula were prepared from the soil collected at each site on the second sampling date. The soil was sieved through a 4 mm sieve; roots were removed, cut to small segments and returned back to the soil. Each inoculated pot received 5 ml of the inoculum. While 1 % inoculum volume may seem rather low, plants in the experiment showed 66–92 % root colonization, suggesting that the amount was quite sufficient. In a previous study, Johnson et al. (2010) used 2 % of non-sterile soil for inoculation of experimental plants. We also aimed to keep the inoculum volume as small as possible not to affect the abiotic soil environment in the experimental pots. The control non-inoculated plants received 5 ml of heat-sterilized inoculum (the inoculum was autoclaved twice at 121°C for 25 min, 24 h apart) plus 5 ml of microbial filtrate from both soils. To obtain the net effect of the AMF community, we added into each pot 5 ml of microbial filtrate from the non-sterilized soil from the other site than the origin of the inoculum (i.e. marl soil obtained the filtrate from the limestone soil and vice versa). Microbial filtrates were prepared as in the previous experiment. The microbial filtrate is supposed to contain the majority of soil microorganisms except for AMF (Koide and Li 1989).

In both experiments, all the soils thus contained microorganisms present in both soils. They either received them via a microbial filtrate, or they were present already in the non-sterilized soil. Both experiments were running simultaneously in the greenhouse (day temperature 25°C, night temperature 10°C, 14-h photoperiod) from March 2006 to August 2007. Every month during the experiments, we measured number of leaves and length of the longest leaf. At the harvest, the aboveground biomass was cut,

the roots were washed of soil and weighed. Approximately one third of the roots from the middle part of the root system was separated, weighed and used for evaluation of mycorrhizal colonization. The remaining part of the root system and aboveground biomass was dried to a constant weight at 70°C for 72 h and weighed. The weight of the root sample used for the determination of AMF colonization was added to the root dry weight after recalculating its wet weight to dry weight using the information on the dry/wet weight ratio based on the rest of the sample. The phosphorus concentration was determined in the aboveground biomass following the method of Olsen and Sommers (1982). The total content of phosphorus in the aboveground biomass was calculated as well.

Roots appointed for determination of mycorrhizal colonization were stained with 0.05 % Trypan blue in lactoglycerol (Koske and Gemma 1989), and colonization was evaluated according to Trouvelot et al. (1986) in 30 root segments under a compound microscope at 200× magnification. This method allows separate quantification of different morphological AM structures. We measured the intensity of colonization in the root system (i.e. both colonized and non-colonized root segments were included into the calculation) and the abundance of arbuscules, vesicles and hyphal coils in the colonized parts of the root system (these parameters are thus independent of the level of root colonization). Each morphological structure (arbuscules, vesicles and hyphal coils) in each microscopic field of view was assigned a number (between 0 and 3) according to its abundance (proportion of the root cortex occupied by the appropriate type of structure). In total, 300 microscopic fields of view were scored for each sample. The abundance of arbuscules, vesicles and coils was then estimated as a parameter using the computer program Mycocalc (Trouvelot et al. 1986).

At the beginning of the experiment, we had 10 replicates in each experimental treatment. Some plants, however, died during the experiment, so at the end of the experiment, we had between 8 and 10 replicates per treatment. All of the control non-inoculated plants in the second experiment died during the first month and were thus not included into the evaluation of the experiment.

Statistical Analysis

Results of tests based on biometric data from the course of the experiment were largely similar to the results from the end of the experiment and are thus not presented for simplicity.

In the experiment with non-sterilized soil (whole soil environment), the independent variables in all tests were soil, plant population and their interaction. In the experiment with sterilized soil (abiotic soil environment), the independent variables were abiotic soil-plant complex, AMF community and their interaction. The dependent variables tested at the end of both experiments were aboveground and belowground biomass of the plants, phosphorus concentration and total content of phosphorus in aboveground biomass. All the values except for total phosphorus content were square root transformed to improve normality of the residuals. Further, we analyzed the effects of the independent variables on mycorrhizal parameters (intensity of root colonization, abundance of arbuscules, vesicles and hyphal coils). These values were arcsin transformed before the analyses to improve normality of the residuals. Data on plant biomass, phosphorus concentration and mycorrhizal parameters were tested using factorial ANOVA. Data on total content of phosphorus in aboveground biomass were tested using a generalized linear model with Gamma distribution.

The relationship among aboveground biomass, phosphorus concentration, phosphorus content and mycorrhizal parameters was tested using linear regression separately for each experiment. Because of strong differences in aboveground biomass as well as phosphorus content between treatments within the experiments, these tests were done over all treatments as well as for each treatment separately.

Differences between growth of plants growing in non-sterilized soil from the first experiment and plants growing in inoculated sterilized soil from the second experiment were tested by one way ANOVA separately for each abiotic soil-plant complex. Specifically, we compared plants growing in their native sterilized soil (abiotic soil environment) and inoculated with native AMF with the plants growing in their native non-sterilized soil (whole soil environment). All tests were done using S-Plus (2000). In all cases, we consider the effect as significant at the conventional P-level $P \leq 0.05$.

Results

Plant Size

In the experiment with non-sterilized soil, all parameters related to plant size (aboveground and belowground biomass and root:shoot ratio) were strongly significantly affected by soil but not by plant population (Table 2). Plants grown in the non-sterilized soil from the limestone region had higher aboveground (Fig. 1a) and belowground biomass, higher phosphorus concentration as well as total phosphorus content in aboveground biomass and a lower root:shoot ratio than plants grown in the marl soil (Table 2). Plants originating from the marl region tended to be larger than those from the limestone in both soils, but this tendency was not significant (Table 2, Fig. 2a). Phosphorus concentration in the aboveground biomass was significantly affected by plant population with higher concentration in plants originating from the limestone region, total phosphorus content in the aboveground biomass, however, was not affected by plant population (Table 2). There was also no significant interaction between plant population and soil for any parameter in this experiment (Table 2).

In the experiment with sterilized soil, aboveground (Fig. 1b) and belowground biomass was significantly affected by abiotic soil-plant complex as well as by the interaction of this complex with AMF (Table 2). Plants from the marl abiotic soil-plant complex had lower aboveground (Fig. 1b) and belowground biomass than plants from the limestone complex, and plants from both complexes had higher aboveground (Fig. 1b) as well as belowground biomass when inoculated by non-native AMF (although in the limestone abiotic soil-plant complex the difference between plants inoculated with marl and limestone AMF was not significant). Phosphorus concentration as well as the total content of phosphorus was significantly affected by the abiotic soil-plant complex with more phosphorus in plants from the limestone abiotic soil-plant complex than from the marl abiotic soil-plant complex (Table 2). Total content of phosphorus was significantly affected also by the interaction of the abiotic soil-plant complex with AMF (Table 2). The root:shoot ratio was significantly affected only by interaction of abiotic soil-plant complex and AMF (Table 2). Plants from the marl abiotic soil-plant complex inoculated with marl AMF invested significantly more into aboveground biomass (i.e. the had a lower root:shoot ratio) but had a lower phosphorus

Table 2 Effect of plant population, soil (including AMF) and their interaction on plant growth and mycorrhizal parameters in the experiment in non-sterilized soil and the effect of abiotic soil-plant complex, AMF community and their interaction on plant growth and mycorrhizal parameters in the experiment in sterilized soil

			Aboveg. biomass (g)	Belowg. biomass (g)	Root:shoot	Phosphorus concentration ($mg.kg^{-1}$)	Phosphorus content (mg)	Intensity of root colonization (%)	Abundance of arbuscules (%)	Abundance of vesicles (%)	Abundance of coils (%)
Non-sterilized soil	Soil	Plant									
	Marl	Marl	0.50±0.05b	1.24±0.11b	2.55±0.16a	726.4±53.3c	351.3+29.3a	91±1a	86±5a	68±7a	9±2c
		Limestone	0.40±0.04b	1.14±0.07b	3.07±0.32a	866.5±43.4b	341.1+32.7a	79±2c	71±6b	43±6b	12±1bc
	Limestone	Marl	2.14±0.18a	4.24±0.38a	2.00±0.14b	1251.5±74.1a	2663.6±248.5a	87±2b	58±9c	67±8a	19±4b
		Limestone	1.81±0.25a	3.59±0.74a	1.76±0.33b	1379.5±64.5a	2455.7±324.5a	66±7d	35±11d	52±10b	31±7a
	Plant		2.78n.s.	1.39n.s	0.38n.s.	**5.27***	0.51n.s.	**24.60*****	**7.41****	**5.91***	**3.63+**
	Soil		**126.93*****	**32.50*****	**12.76*****	**69.56*****	**324.88*****	**4.74***	**13.92*****	0.63n.s.	**13.37*****
	Plant× soil		0.29n.s.	0.93n.s.	2.17n.s.	0.15n.s.	0.02n.s.	0.74n.s.	0.01n.s.	0.32n.s.	1.38n.s.
Sterilized soil	Soil-plant	AMF									
	Marl	Marl	0.774±0.07c	1.03±0.52c	1.73±0.83b	843.8±61.2c	632.7±54.2c	92±1a	90±4a	85±5a	3±1c
		Limestone	1.10±0.25b	2.28±0.94b	2.89±1.17a	952.4±38.6b	1000.8±156.7b	82±2ab	48±5b	78±6a	19±5ab
	Limestone	Marl	1.66±0.10a	3.80±1.08a	2.59±0.74a	1419.2±75.2a	2215.3±103.3a	77±3b	81±4a	80±5a	13±3b
		Limestone	1.41±0.13a	2.85±1.05ab	2.29±0.78a	1452.9±115.8a	2055.9±234.9a	66±5c	44±11b	4+8b	23±5a
	Soil-plant		**23.03*****	**32.15*****	0.32n.s.	**45.53*****	**68.44*****	**6.53***	1.05n.s.	**6.60***	**3.72+**
	AMF		0.2n.s.	1.68n.s.	2.47n.s.	1.03n.s.	0.48n.s.	0.23n.s.	**32.02*****	**8.27****	**13.74*****
	Soil-plant× AMF		**4.27***	**14.83*****	**5.87***	0.50n.s.	**8.08****	**8.58****	0.92n.s.	2.30n.s.	0.56n.s.

The table shows mean ± SE for each treatment, *F* values for the tests and their significance levels: *** – $P<0.001$, ** – $P<0.01$, * – $P<0.05$, + – $P=0.06$, n.s. – non-significant effect. d.f. error = 34 for experiment in non-sterilized soil and d.f. error = 32 for experiment in sterilized soil. Different letters denote significant differences between treatment levels within each experiment separately. Significant F values ($P<0.05$) are shown in bold.

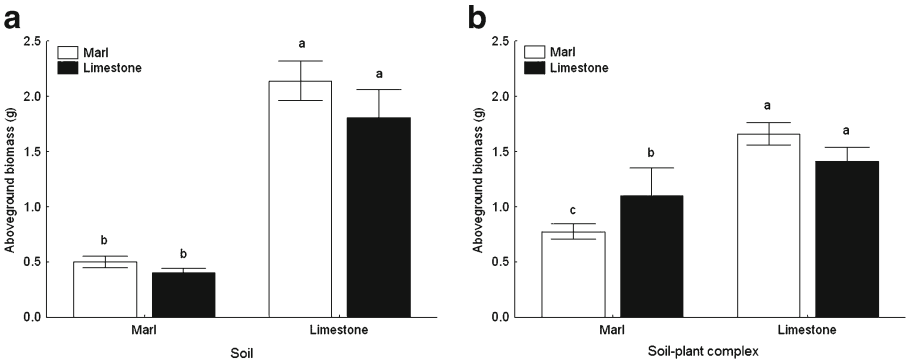

Fig. 1 Aboveground biomass of *Aster amellus*. **a** Experiment in non-sterilized soil. *White boxes* represent plant population from the marl region; *black boxes* represent plant population from the limestone region. **b** Experiment in sterilized soil. *White boxes* represent inoculation with AMF community from the marl region; *black boxes* represent inoculation with AMF community from the limestone region. Soil-plant complex indicates the origin of the soil and the plant population (the plants are always grown in their native soil). All values represent the mean ± SE. Columns sharing the same letter are not significantly different ($P>0.05$)

content than plants inoculated with limestone AMF. In the limestone abiotic soil-plant complex, the difference in the root:shoot ratio as well as phosphorus content between plants inoculated with either AMF was not significant (Table 2).

Mycorrhizal Parameters

In the non-sterilized soil, the intensity of root colonization, abundance of arbuscules, vesicles and coils were significantly affected by plant population (Table 2). A higher intensity of root colonization (Fig. 2a) and a higher abundance of arbuscules (Fig. 2c) and vesicles was observed in plants originating from the marl region in comparison with plants from the limestone region. On the other hand, the abundance of coils was higher in plants from the limestone region than in plants from the marl region (Fig. 2e). The intensity of root colonization, abundance of arbuscules and abundance of coils were further affected by soil (Table 2). Plants grown in the marl soil had a higher intensity of root colonization (Fig. 2a), a higher abundance of arbuscules (Fig. 2c) but a lower abundance of coils (Fig. 2e) than plants grown in the limestone soil. There were no significant interactions between the plant population and soil (Table 2), but the highest intensity of root colonization and abundance of arbuscules and the lowest abundance of coils were observed in the treatment with plants originating from the marl region growing in the marl soil. By contrast, the lowest intensity of mycorrhizal colonization (Fig. 2a) and abundance of arbuscules (Fig. 2c) and the highest abundance of coils (Fig. 2e) were observed in plants from the limestone region growing in the limestone soil (Table 2).

In the sterilized soil, the intensity of root colonization was significantly affected by the abiotic soil-plant complex as well as by the interaction between the abiotic soil-plant complex and the AMF community (Table 2). The highest intensity of root colonization was observed in plants from the marl abiotic soil-plant complex inoculated with the marl AMF community (Fig. 2b). By contrast, the lowest root colonization intensity was observed in the limestone abiotic soil-plant complex inoculated with the limestone AMF community (Fig. 2b). The abundance of arbuscules was significantly

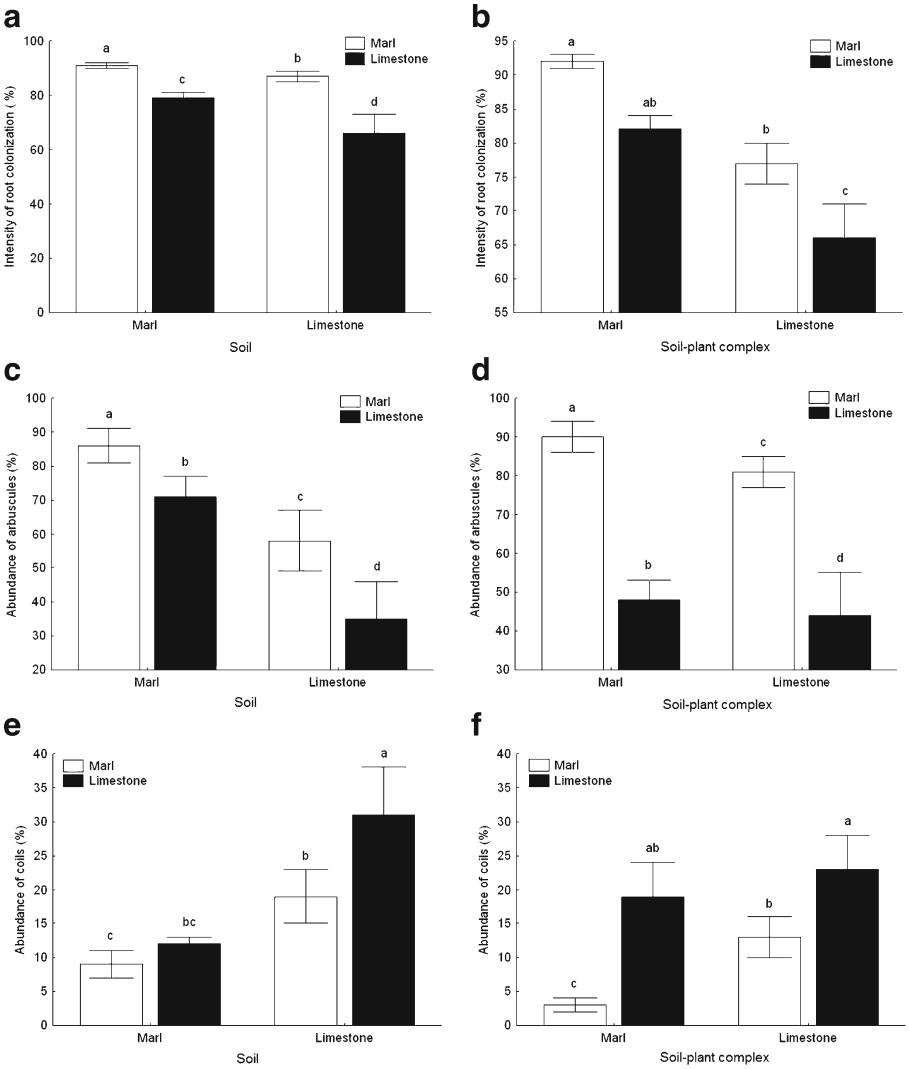

Fig. 2 **a**, **b** Intensity of root colonization, **c**, **d** abundance of arbuscules and **e**, **f** hyphal coils in the roots of *Aster amellus*. **a**, **c**, **e** Experiment in non-sterilized soil. *White boxes* represent plant population from the marl region, *black boxes* represent plant population from the limestone region. **b**, **d**, **f** Experiment in sterilized soil. *White boxes* represent inoculation with AMF community from the marl region, *black boxes* represent inoculation with AMF community from the limestone region. All values represent the mean ± SE. Columns sharing the same letter are not significantly different ($P>0.05$)

affected only by AMF with a higher abundance observed in plants inoculated with the marl AMF community (Table 2, Fig. 2d). The abundance of vesicles as well as coils was affected by the abiotic soil-plant complex and AMF, but the interaction between these two factors was not significant (Table 2). For coils, the effect of abiotic soil-plant complex was only marginally significant. Plants from the marl abiotic soil-plant complex or those inoculated with marl AMF had higher abundance of vesicles than plants from the limestone complex or plants inoculated by the limestone AMF. There was also a marginally significant tendency of these plants to have a lower abundance of

coils than plants from the limestone complex or plants inoculated by the limestone AMF (Fig. 2f).

When comparing the two experiments, aboveground biomass was significantly lower and the root:shoot ratio significantly higher in plants growing in their native non-sterilized soil (whole soil environment) compared to plants growing in their native sterilized soil (abiotic soil environment) inoculated with their native AMF community (Table 3, Fig. 3) when using data from the marl region. No such difference was observed in plants from the limestone region. Belowground biomass, phosphorus concentration and content in aboveground biomass did not differ between the two experiments in plants from any of the two regions (Table 3).

The intensity of root colonization and abundance of arbuscules did not significantly differ between the two experiments (Table 3). On the other hand, the abundance of vesicles and coils differed significantly between sterilized and non-sterilized soil in the marl abiotic soil-plant complex (Table 3), with higher abundance of vesicles and lower abundance of coils found in the sterilized soil.

Correlation of Plant Growth and Mycorrhizal Parameters

In the experiment with non-sterilized soil, we found many significant correlations between plant growth and mycorrhizal parameters when the tests were done over all treatments. Specifically, aboveground biomass was negatively correlated with the abundance of arbuscules and positively with the abundance of hyphal coils. There was also a positive correlation between belowground biomass and the abundance of hyphal coils and a higher root:shoot ratio was found in plants with a higher intensity of root colonization and abundance of arbuscules (Table 4). There was a negative correlation between the concentration of phosphorus in aboveground biomass and the intensity of root colonization and abundance of arbuscules; however, there was a positive correlation between the concentration of phosphorus and the abundance of hyphal coils. The total content of phosphorus was negatively correlated with the abundance of arbuscules and positively correlated with the abundance of hyphal coils. When the relationship between plant growth and mycorrhizal parameters was tested separately for each treatment, however, all the differences lost their significance ($P>0.05$ in all cases).

Similarly, in the experiment with sterilized soil, there were several significant correlations when the tests were done over all treatments. The only significant relationship between aboveground biomass and mycorrhizal parameters was a positive correlation between aboveground biomass and the intensity of colonization (Table 4). There was also a significant positive relationship between belowground biomass and the root:shoot ratio with intensity of root colonization and abundance of coils (Table 4). The concentration and total content of phosphorus in aboveground biomass was negatively correlated with the intensity of root colonization and abundance of vesicles (non-significant for total content of P) and positively correlated with the abundance of hyphal coils (Table 4). When testing each treatment separately, there was a positive correlation only between aboveground biomass and the intensity of root colonization (d.f. error=6, $R^2=0.46$, $P=0.05$) and a negative correlation between aboveground biomass and the abundance of arbuscules (d.f. error=6, $R^2=0.484$, $P=0.05$) in the limestone abiotic soil-plant complex inoculated with the limestone AMF. All other significant correlations disappeared ($P>0.05$ in all cases).

Table 3 Comparison of the effects of different experimental approaches (non-sterilized soil *vs* inoculated sterilized soil) on plant growth and mycorrhizal parameters

Soil-plant complex	Experiment in soil	Aboveg. biomass (g)	Belowg. biomass (g)	Root:shoot	Phosphorus concentration ($mg.kg^{-1}$)	Phosphorus content (mg)	Intensity of root colonization (%)	Abundance of arbuscules (%)	Abundance of vesicles (%)	Abundance of coils (%)
Marl	Non-sterilized	**0.50+0.05a**	1.24+0.11a	**2.55+0.16a**	726.4+53.3a	351.3+29.3a	91+1a	86+5a	**68+7a**	**9+2a**
	Sterilized	**0.774+0.07b**	1.03+0.52a	**1.73+0.83b**	843.8+61.2a	632.7+54.2a	92+1a	90+4a	**85+5b**	**3+1b**
		0.387**	n.s	**0.279***	n.s	n.s	n.s	n.s	**0.233***	**0.208***
Limestone	Non-sterilized	1.81+0.25a	3.59+0.74a	1.76+0.33a	1379.5+64.5a	2455.7+324.5a	66+7a	35+11a	52+10a	31+7a
	Sterilized	1.41+0.13a	2.85+1.05a	2.29+0.78a	1452.9+115.8a	2055.9+234.9a	66+5a	47+11a	49+8a	23+5a
		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

All tests were done separately for each abiotic soil-plant complex. The table shows mean ± SE for each treatment, *F* values for the tests and their significance levels: *** – $P<0.001$, ** – $P<0.01$, * – $P<0.05$, + – $P=0.06$. d.f. error = 18 for marl region and d.f. error = 15 for limestone region. Different letters denote significant differences between treatment levels within each abiotic soil-plant complex separately. Significant differences ($P\leq0.05$) are highlighted in bold.

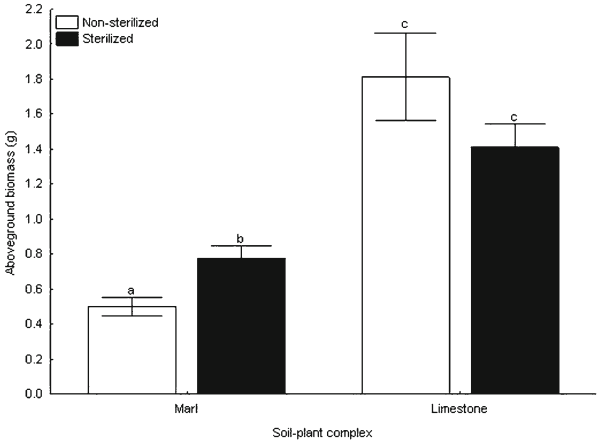

Fig. 3 Comparison of aboveground biomass of *Aster amellus* between the experiment in non-sterilized soil (*white boxes*) and the experiment in sterilized soil (*black boxes*). All values represent the mean±SE. Columns sharing the same letter are not significantly different ($P>0.05$)

Discussion

The aim of the present study was to test the existence of local adaptation in populations of the model species *A. amellus* and the importance of the AMF community for this adaptation. Using data on aboveground biomass as the response variable, results of the two experiments do not indicate either adaptation of *A. amellus* to the whole soil environment or the mutual co-adaptation of the plant in its native abiotic soil environment and AMF. We, however, found that plants from the different regions tend to develop different levels of root colonization and differ also in the abundance of different AM morphological structures. While the differences in the level of root colonization between populations of *A. amellus* from the two regions were already shown in our previous greenhouse studies (Pánková et al. 2008, 2011) as well as in a field study (Pánková et al., unpubl.), in this study we demonstrate that AMF from the two regions form different abundances of particular morphological structures in *A. amellus* roots. While AMF from the marl region form more arbuscules, AMF from the limestone region tend to produce more hyphal coils. The data also provide some indication that the plants of different origin tend to have different abundances of fungal structures in their roots when exposed to the same fungal community. While we do not know whether these differences are due to plants associating with different fungal species from the same fungal community or whether different fungal structures are created by the same fungal species within the roots of different plants, the results suggest that the association between plants and fungi depends on the plant genotype and could contribute to local adaptation of the plants. This pattern, however, occurred mainly in the non-sterilized soil and differed for different fungal structures. In agreement with our study, it was previously demonstrated that the occurrence of morphological AM structures in plant roots could be determined by both the fungal and the plant partner (Cavagnaro et al. 2001; Dickson 2004; Dickson et al. 2007). The observed pattern is thus probably a combined result of these two effects.

Table 4 Correlations between mycorrhizal characteristics and characteristics related to plant performance

		Non-sterilized soil				Sterilized soil			
		Int. root colon.	Abund. arbuscules	Abund. vesicles	Abund. coils	Int. root colon.	Abund. arbuscules	Abund. vesicles	Abund. coils
Aboveground biomass	r	0.03	**0.34**	0.14	**0.44**	**0.38**	0.03	0.19	0.24
	p	0.92	**0.03**	0.4	**<0.001**	**0.002**	0.83	0.273	0.164
	sign	NA	−	NA	+	+	NA	NA	NA
Belowground biomass	r	0.26	0.20	0.10	**0.32**	**0.89**	0.18	0.14	**0.42**
	p	0.1	0.26	0.49	**0.05**	**<0.001**	0.303	0.373	**0.01**
	sign	NA	NA	NA	+	+	NA	NA	+
Root:shoot	r	**0.40**	**0.33**	0.14	0.30	**0.68**	0.28	0.07	**0.38**
	p	**0.014**	**0.04**	0.44	0.06	**<0.001**	0.104	0.688	**0.024**
	sign	+	+	NA	NA	+	NA	NA	+
Phosphorus concentration	r	**0.44**	**0.40**	0.68	**0.53**	**0.40**	0.22	**0.32**	**0.33**
	p	**<0.001**	**0.01**	0.19	**<0.001**	**0.02**	0.21	**0.05**	**0.05**
	sign	−	−	NA	+	−	NA	−	+
Phosphorus content	r	0.07	**0.34**	0.03	**0.50**	**0.48**	0.15	0.28	**0.33**
	p	0.66	**0.04**	0.93	**<0.001**	**<0.001**	0.38	0.1	**0.05**
	sign	NA	−	NA	+	−	NA	NA	+

The tests were done over all treatments separately for the experiment in the sterilized soil and separately for the experiment in the non-sterilized soil. d.f. error = 34 for the experiment in sterilized soil and d.f. error = 36 for the experiment in non-sterilized soil. Significant differences ($P \leq 0.05$) are highlighted in bold.

We interpret our results as being due to different responses of different plant genotypes. Based on our previous studies (Raabová et al. 2007; Mandáková and Münzbergová 2008; Münzbergová et al. 2013), it is clear that the species is highly genetically variable, and the two populations are thus really genetically distinct. In spite of this, we are working with field collected seeds and thus cannot exclude the possibility that the differences in behavior of the plants are due to differences in the maternal environment (e.g. Galloway 1995, 2005; Münzbergová and Plačková 2010). We are, however, not aware of any study which would demonstrate that root colonization by AMF could be due to differences in the maternal environment.

The absence of local adaptation in terms of plant growth, but evidence for it in terms of the level of root colonization and different abundances of particular AM morphological structures, corresponds to the results of previous studies (e.g. Sultan and Bazzaz 1993; Grassein et al. 2010), indicating that differences in some traits (in our case, AMF colonization) may lead to the absence of differences in other traits (in our case, aboveground biomass).

The prevalence of arbuscules in roots of plants colonized by AMF from the nutrient-poor marl environment is supported by findings of some previous studies. Specifically, colonization with prevailing arbuscules is typical of cultured sites (Yamato 2004), deserts (O'Connor et al. 2001) and open sites (Smith and Smith 1997). By contrast, colonization with prevailing hyphal coils is typical of plants growing in forests (Yamato

and Iwasaki 2002). This corresponds with the forested nature of the localities in the limestone region.

It has been shown that both arbuscules and coils are important for phosphorus transfer from AMF to plants (Karandashov et al. 2004; Bucher 2007). Dickson and Kolesik (1999) showed that, although arbuscules had both a smaller volume and surface area than coils, the surface area:volume ratio of arbuscules was much higher. On the other hand, van Aarle et al. (2005) showed that metabolic activity of both colonization types was similar. Finally, Smith et al. (2004) concluded that coils may be less efficient in phosphorus transport than arbuscules due to their higher biomass per cell than in arbuscules and thus their higher carbon demands. However, both arbuscules and coils have the ability to transfer phosphorus to plants. In this study, we found a positive correlation between the concentration and content of phosphorus in plant tissue and the abundance of coils, and a negative correlation for the abundance of arbuscules. This pattern is not in agreement with any previous record concerning the importance of arbuscules and coils for phosphorus uptake. The pattern may be largely driven by the composition of AMF communities from different regions, as they tend to create different amounts of particular mycorrhizal structures. The functional relationships between the abundance of different mycorrhizal structures and phosphorus acquisition in this system, however, still remain to be elucidated.

In the present study, we did not find adaptation of *A. amellus* to the whole soil environment or the co-adaptation of the plant in its native abiotic soil environment and AMF to each other in terms of plant growth. Specifically, we did not find a significant growth response to the interaction between plant population and soil in the experiment with non-sterile soil (whole soil environment); however, we did find a significant growth response to the interaction between AMF and abiotic soil-plant complex in the experiment with sterilized soil (abiotic soil environment). In contrast to our expectations, the plants in both sterilized soils performed better when inoculated with the non-native AMF. This contradicts our previous studies in which *A. amellus* plants performed best when grown in their native soil inoculated with their native AMF (Pánková et al. 2011) as well as with previous studies of other authors (e.g. Johnson et al. 2010; Taheri and Bever 2011). On the other hand, our results are in agreement with some other studies (Bever 1994; Wagner et al. 2011) that found better performance of plants grown in non-native whole soil environment as compared to plants grown in their home soil conditions (including AMF). This could indicate the occurrence of negative plant-soil feedback in our system (see e.g. Bever et al 2010; van der Putten et al 2013).

The mismatch between the findings of the present study and our previous results as well as the results of some other studies could be explained by the fact that previously we inoculated plants with single native AMF isolates, while the whole AMF community was used in the present study. In studies with sterilized soil (abiotic soil environment) inoculated by one AMF species, microbial communities are regenerated using a filtrate from non-sterilized soil as well as from the fungal inoculum (Koide and Li 1989). It is likely that not all microorganisms are really added into the soil via the microbial filtrates. On the other hand, using non-sterilized soil as an inoculum or growing plants directly in non-sterilized soil (whole soil environment) likely means that all soil microorganisms are present directly in the inoculum or in the soil (Bever 1994; Wagner et al. 2011). The possible negative effect of native pathogens could

therefore compensate the positive effect of native AMF and soil and could lead to the absence of differences in plant growth in different treatments (possible negative soil feedback, e.g. Bever et al. 1997; Callaway et al. 2004). In fact, we frequently observed pathogenic Oomycetes in our samples but unfortunately did not quantify their abundance (Pánková, pers. observation). In addition, inoculation with a single AMF isolate may provide very different results as regards plant performance than inoculation with more AMF species or isolates (e.g. van der Heijden et al. 1998; Bennett and Bever 2007; Jansa et al. 2008). The latter is almost certainly the case of using non-sterilized soil as the source of inoculum.

Differences in the amount and/or identity of the (partly added) soil biota could probably also explain the somewhat different behavior of plants and AMF in the two presented experiments. Plants growing in the non-sterilized marl soil were smaller than plants growing in the sterilized marl soil re-inoculated with marl inoculum from non-sterile soil. These two treatments should contain the same soil, AMF and plants; they thus differed only in the mode of inoculation. While in the experiment with non-sterilized soil pathogens, AM fungi as well as other soil organisms were present in the whole volume of the soil (500 ml per pot), re-inoculated sterilized soil obtained soil pathogens, AM fungi as well as other soil organisms with the non-sterilized soil inoculum (5 ml of non-sterilized soil per pot). As both soils in both experiments obtained the same amount of microbial filtrate from the other soil, the compared treatments should differ only in the amount of native soil microorganisms. We thus suppose that the plants in the non-sterilized soil were smaller due to presence of a higher amount of soil pathogens. This corresponds to previous studies indicating that native soil pathogens can have a strong negative effect on plant growth, causing the so-called negative plant-soil feedback (e.g. Bever 1994; Bever et al. 2010; van der Putten et al. 2013).

It can also be speculated that the somewhat different results of our two experiments might be ascribed to the fact that different AM fungal species could develop from the dose of 500 ml *vs* 5 ml of non-sterilized soil in the first and second experiment, respectively. Results of the two experiments also suggest that growing plants directly in non-sterilized soil need not provide the same results as inoculation of plants growing in sterilized soil with a small volume of non-sterilized soil. Any comparison of results based on studies using different experimental approaches should thus be made with caution.

Limitations of the Study

In this study, we attempted to set up experiments that considered the most probable factors affecting plant-soil co-adaptation, namely plant population, soil abiotic as well as biotic components. This attempt, however, represents an important limitation of our study. The comparison of the plants grown in their own soil with their own soil community from the two experiments suggested that using non-sterilized soil and inoculating plants with non-sterilized soil leads to different results. This, unfortunately, means that the two experiments are hardly comparable. From the methodological point of view, however, this is a very important finding, as both approaches (cultivation of plants in non-sterile

soil and inoculation of sterile soil with non-sterile soil) are commonly used and often compared in the discussions of studies.

Another weakness of the study is that we attempted to separate the effect of AMF from the effect of other soil organisms, but such separation is unfortunately not entirely possible. We used a standard approach of adding soil filtrate containing all the other microorganisms into all the soils to eliminate their effects. As shown from the comparison of the two experiments, the recovery of soil microbial communities, however, did not fully work. Again, we suggest that this is an important methodological message, as such an approach is common in the literature.

In this study, we used only one population for each region. Indeed, it would be much better to use multiple populations per region as was done in our previous studies (Pánková et al. 2008, 2011). To keep the experiment feasible, we decided not to do this. Based on our previous results demonstrating large differences in response to AMF between regions and small differences between populations within regions, we, however, assume that using multiple populations per region would not change the overall conclusions.

The experiment with sterilized soil could also include the treatment with plants grown in the foreign soil. While we decided not to do this due to the overall complexity of the experiment, we assume that this would not increase the overall understanding of the system due to the limitations mentioned above.

An important finding in our study is that the plants from different origins differ in the level of root colonization. Higher root colonization may not, however, always mean higher acquisition of nutrients because acquisition of nutrients largely depends of the amount of extraradical mycelia (e.g. Gavito and Olsson 2003; Janoušková et al. 2011). The amount of extraradical mycelia was unfortunately not quantified in our study.

Finally, we found different mycorrhizal structures in the roots of plants of different origin growing in different soils. While the data provide indication that the differences are partly driven by the plant, we cannot tell if this was due to different plant genotypes associating with different fungal species or if the same fungal genotype formed different mycorrhizal structures under different circumstances. Molecular identification of the fungi associated with plant roots in each of the treatment combinations would thus be needed to answer this question (see Ji et al. 2013 for such identification).

Conclusions

In this study, we found evidence that adaptation of plants to soil conditions can take place not only via the specific level of AM colonization but also via differences in the abundance of particular morphological fungal structures.

The native AMF community from the nutrient-poor marl soil showed higher infectivity and developed more arbuscules, which have a high surface area to volume ratio and a fast biomass turn-over. In addition, plants originating in the marl region seem to induce higher development of arbuscules and higher overall root colonization. On the other hand, AMF from the limestone soil, which contains more nutrients, invest energy into more long-term coils. They show lower infectivity, however. Also the

plants from the limestone region seem to induce higher development of coils but lower overall root colonization.

This adaptation of AMF to their native soil and plants and association of plants with a specific type of AMF colonization could lead to absence of adaptation of the plant species which could be detected in terms of plant growth. The positive effects of native AMF could also be overwhelmed by negative effects of native soil pathogens.

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References

Antonovics J, Primack RB (1982) Experimental ecological genetics in *Plantago*. 6. The demography of seedling transplants of *P. lanceolata*. *J Ecol* 70:55–75

Bennett AE, Bever JD (2007) Mycorrhizal species differentially alter plant growth and response to herbivory. *Ecology* 88:210–218

Bever JD (1994) Feedback between plants and their soil communities in an old field community. *Ecology* 75: 1965–1977

Bever JD, Dickie IA, Facelli E, Facelli JM, Klironomos J, Moora M, Rillig MC, Stock WD, Tibbett M, Zobel M (2010) Rooting theories of plant community ecology in microbial interactions. *Trends Ecol Evol* 25: 468–478

Bever JD, Westover KM, Antonovics J (1997) Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *J Ecol* 85:561–573

Bischoff A, Vonlanthen B, Steinger T, Müller-Schärer H (2006) Seed provenance matters – effects on germination of four plant species used for ecological restoration. *Basic Appl Ecol* 7:347–359

Brundrett M, Kendrick B (1990) The roots and mycorrhizas of herbaceous woodland plants. I. Quantitative aspects of morphology. *New Phytol* 114:457–468

Bucher M (2007) Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. *New Phytol* 173:11–26

Callaway RM, Thelen GC, Rodriguez A, Holben WE (2004) Soil biota and exotic plant invasion. *Nature* 427: 731–733.

Cavagnaro TR, Gao LL, Smith FA, Gao LL, Smith FA, Smith SE (2001) Morphology of arbuscular mycorrhizas is influenced by fungal identity. *New Phytol* 151:469–475

Chýlová T, Münzbergová Z (2008) Past land use co-determines the present distribution of dry grassland plant species. *Preslia* 80:183–198

Clapperton MJ, Reid DM (1992) A relationship between plant growth and increasing VA mycorrhizal inoculum density. *New Phytol* 120:227–234

Del Val C, Barea JM, Azcón-Aguilar C (1999) Assessing the tolerance to heavy metals of arbuscular mycorrhizal fungi isolated from sewage sludge-contaminated soils. *Appl Soil Ecol* 11:261–269

Dickson S (2004) The *Arum-Paris* continuum of mycorrhizal symbioses. *New Phytol* 163:187–200

Dickson S, Kolesik P (1999) Visualisation of mycorrhizal fungal structures and quantification of their surface area and volume using laser scanning confocal microscopy. *Mycorrhiza* 9:205–213

Dickson S, Smith FA, Smith SE (2007) Structural differences in arbuscular mycorrhizal symbioses: More than 100 years after Gallaud, where next? *Mycorrhiza* 17:375–93

Douds DD, Janke RR, Peters SE (1993) VAM fungus spore populations and colonization of roots of maize and soybean under conventional and low-input sustainable agriculture. *Agric Ecosyst Environm* 43:325–335

Ellenberg H (1998) *Vegetation ecology of Central Europe*. Cambridge University Press, Cambridge

Galloway LF (1995) Response to natural environmental heterogeneity: maternal effects and selection on life-history characters and plasticities in *Mimulus guttatus*. *Evolution* 49:1095–1107

Galloway LF (2005) Maternal effects provide phenotypic adaptation to local environmental conditions. *New Phytol* 166:93–99

Galloway LF, Fenster CB (2000) Population differentiation in an annual legume, local adaptation. *Evolution* 54:1173–1181

Gavito ME, Olsson PA (2003) Allocation of plant carbon to foraging and storage in arbuscular mycorrhizal fungi. *FEMS Microbiol Ecol* 45:181–187

Grassein F, Till-Bottraud I, Lavorel S (2010) Plant resource-use strategies: the importance of phenotypic plasticity in response to a productivity gradient for two subalpine species. *Ann Bot (Oxford)* 106:637–645

Hildebrandt U, Kaldorf M, Bothe H (1999) The zinc violet and its colonization by arbuscular mycorrhizal fungi. *J Pl Physiol* 154:709–717

Ipsilantis I, Sylvia MD (2007) Interactions of assemblages of mycorrhizal fungi with two Florida wetland plants. *Appl Soil Ecol* 35:261–271

Janoušková M, Rydlová J, Pueschel D, Száková J, Vosátka M (2011) Extraradical mycelium of arbuscular mycorrhizal fungi radiating from large plants depresses the growth of nearby seedlings in a nutrient deficient substrate. *Mycorrhiza* 21:641–650

Jansa J, Smith FA, Smith SE (2008) Are there benefits of simultaneous root colonization by different arbuscular mycorrhizal fungi? *New Phytol* 177:779–789

Ji BM, Gehring CA, Wilson GWT, Miller RM, Flores-Renteria L, Johnson NC (2013) Patterns of diversity and adaptation in Glomeromycota from three prairie grasslands. *Molec Ecol* 22:2573–2587

Johnson NC (1993) Can fertilization of soil select less mutualistic mycorrhizae? *Ecol Appl* 3:749–757

Johnson NC, Graham JH (2013) The continuum concept remains a useful framework for studying mycorrhizal functioning. *Pl & Soil* 363:411–419

Johnson NC, Graham JH, Smith FA (1997) Functioning of mycorrhizal associations along the mutualism–parasitism continuum. *New Phytol* 135:575–586

Johnson NC, Wilson GWT, Bowker MA, Wilson JA, Miller RM (2010) Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *Proc Natl Acad Sci USA* 107:2093–209

Jordan N (1992) Path analysis of local adaptation in two ecotypes of the annual plant *Diodia teres* Walt. (Rubiaceae). *Amer Naturalist* 140:149–165

Joshi J, Schmid B, Caldeira MC, Dimitrakopoulos PG, Good J, Harris R, Hector A, Huss-Danell K, Jumpponen A, Minns A, Mulder CPH, Pereira JS, Prinz A, Scherer-Lorenzen M, Siamantziouras A-SD, Terry AC, Troumbis AY, Lawton JH (2001) Local adaptation enhances performance of common plant species. *Ecol Lett* 4:536–544

Karandashov V, Nagy R, Wegmüller S, Amrhein N, Bucher M (2004) Evolutionary conservation of phosphate transport in the arbuscular mycorrhizal symbiosis. *Proc Natl Acad Sci USA* 101:6285–6290

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. *Ecol Lett* 7:1225–1241

Kindell CE, Winn AA, Miller TE (1996) The effects of surrounding vegetation and transplant age on the detection of local adaptation in the perennial grass *Aristida stricta*. *J Ecol* 84:745–754

Koide RT, Li MG. (1989) Appropriate controls for vesicular arbuscular mycorrhiza research. *New Phytol* 111: 35–44

Koske RE, Gemma JN (1989) A modified procedure for staining roots to detect VA-mycorrhizas. *Mycol Res* 92:486–505

Lackie SM, Bowley SR, Peterson RL (1988) Comparison of colonization among half-sib families of *Medicago sativa* L. by *Glomus versiforme* (Daniels and Trappe) Berch. *New Phytol* 108:477–482

Mandáková T, Münzbergová Z (2006) Distribution and ecology of cytotypes of the *Aster amellus* aggregates in the Czech Republic. *Ann Bot (Oxford)* 98:845–856

Mandáková T, Münzbergová Z (2008) Morphometric and genetic differentiation of diploid and hexaploid populations of *Aster amellus* agg. in a contact zone. *Pl Syst Evol* 274:155–170

Merxmüller H, Schreiber A, Yeo PF (1976) *Aster* L. In Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA (eds) *Flora Europaea 4*. Cambridge University Press, London-New York-Melbourne, pp 112–116

Meusel H, Jäger E (1992) *Vergleichende chorologie der Zentraleuropäischen flora*. Gustav Fischer Verlag, Jena

Münzbergová Z, Plačková I (2010) Seed mass and population characteristics interact to determine performance of *Scorzonera hispanica* under common garden conditions. *Flora* 205:552–559

Münzbergová Z, Raabová J, Castro S, Pánková H (2011) Biological flora of Central Europe: *Aster amellus* L. (Asteraceae). *Perspect Pl Ecol Evol Syst* 13:151–162

Münzbergová Z, Surinová M, Castro S (2013) Absence of gene flow between diploids and hexaploids of *Aster amellus* at multiple spatial scales. *Heredity* 110:123–130

Nuismer SL, Gandon S (2008) Moving beyond common garden and transplant designs: insight into the causes of local adaptation in species interactions. *Amer Naturalist* 171:658–668

O'Connor PJ, Smith SE, Smith FA (2001) Arbuscular mycorrhizal associations in the southern Simpson Desert. *Austral J Bot* 49:493–499

Olsen SR, Sommers LE (1982) *Phosphorus*. In: Page AL et al (eds) *Methods of soil analysis, Part 2: Chemical and microbiological properties.* Agronomy Monograph no. 9, Ed. 2, ASA and SSSA, Madison, pp 403–430

Pánková H, Münzbergová Z, Rydlová J, Vosátka M (2008) Differences in AM fungal root colonization between populations of perennial Aster species have genetic reasons *Oecologia* 157:211–220

Pánková H, Münzbergová Z, Rydlová J, Vosátka M (2011) The response of *Aster amellus* (Asteraceae) to mycorrhiza depends on the origins of both the soil and the fungi. *Amer J Bot* 98:850–858

Raabová J, Münzbergová Z, Fischer M (2007) Ecological rather than geographic or genetic distance affects local adaptation of the rare perennial herb, *Aster amellus. Biol Conservation* 139:348–357

Raabová J, Münzbergová Z, Fischer M (2011) The role of spatial scale and soil for local adaptation in *Inula hirta. Basic Appl Ecol* 12:152–160

S-Plus (2000) *Professional edition for Windows, release 2*. MathSoft, Inc., Massachusetts

Sanders IR (1993) Temporal infectivity and specificity of vesicular-arbuscular mycorrhizas in co-existing grassland species. *Oecologia* 93:349–355

Schultz PA, Miller RM, Jastrow JD, Rivetta CV, Bever JD (2001) Evidence of a mycorrhizal mechanism for the adaptation of *Andropogon gerardii* (Poaceae) to high- and low-nutrient prairies. *Amer J Bot* 88:1650–1656

Smith FA, Smith SE (1997) Tansley Review No. 96. Structural diversity in (vesicular)-arbuscular mycorrhizal symbioses. *New Phytol* 137:373–388

Smith FA, Smith SE (2013) How useful is the mutualism-parasitism continuum of arbuscular mycorrhizal functioning? *Pl & Soil* 363:7–18

Smith SE, Read D (2008) *Mycorrhizal symbiosis*. Ed. 3. Academic Press, London

Smith SE, Smith FA (2012) Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. *Mycologia* 104:1–13

Smith SE, Smith FA, Jakobsen I (2004) Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. *New Phytol* 162:511–524

Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1997) Clonal growth traits of two Prunella species are determined by co-occurring arbuscular mycorrhizal fungi from a calcareous grassland. *J Ecol* 85:181–191

Streitwolf-Engel R, Van Der Heijden MGA, Wiemken A, Sanders IR (2001) The ecological significance of AMF effects on clonal reproduction in plants. *Ecology* 82:2846–2859

Sultan SE, Bazzaz FA (1993) Phenotypic plasticity in *Polygonum persicaria*. 3. The evolution of ecological breadth for nutrient environment. *Evolution* 47:1050–1071

Taheri WI, Bever JD (2011) Adaptation of *Liquidambar styraciflua* to coal tailings is mediated by arbuscular mycorrhizal fungi. *Appl Soil Ecol* 48:251–255

Treseder KK, Cross A (2006) Global distributions of arbuscular mycorrhizal fungi. *Ecosystems* 9:305–316

Trouvelot A, Kough JL, Gianinazzi-Pearson V (1986) *Mesure du taux de mycorhization VA d'un système radiculaire ayant une signification fonctionnelle*. In Gianinazzi-Pearson V, Gianinazzi S (eds) *Physiological and genetical aspects of mycorrhiza*. INRA Presse, Paris, pp 217–221

van Aarle IM, Cavagnaro TR, Smith SE, Smith FA, Dickson S (2005) Metabolic activity of *Glomus intraradices* in Arum- and Paris-type arbuscular mycorrhizal colonization. *New Phytol* 166:611–618

van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396:69–72

van der Putten WH, Bardgett RD, Bever JD, Bezemer TM, Casper BB, Fukami T, Kardol P, Klironomos JN, Kulmatiski A, Schweitzer JA, Suding KN, van de Voorde TFJ, Wardle DA (2013) Plant-soil feedbacks: the past, the present and future challenges. *J Ecol* 101:265–276

Wagner V, Antunes PM, Ristow M, Lechner U, Hensen I (2011) Prevailing negative soil biota effect and no evidence for local adaptation in a widespread Eurasian grass. *Plos One* 6: e17580. doi:10.1371/journal.pone.0017580

Yamato M (2004) Morphological types of arbuscular mycorrhizal fungi in roots of weeds on vacant land. *Mycorrhiza* 14:127–131

Yamato M, Iwasaki M (2002) Morphological types of arbuscular mycorrhizal fungi in roots of understory plants in Japanese deciduous broadleaved forests. *Mycorrhiza* 12:291–296

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