Native arbuscular mycorrhizal fungal communities differentially influence the seedling performance of rare and common *Pulsatilla* species

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Summary

1. An experiment was carried out to determine whether the community composition of root-colonizing arbuscular mycorrhizal fungi (AMF) influences the growth and nutrient status of two congenic *Pulsatilla* species, one rare and one common in Estonia. We hypothesized that: (i) establishment and growth of plants is influenced by the composition of native AMF communities; (ii) growth of congenic plant species with different abundances differs due to their response to specific AMF communities; and (iii) distribution of a plant species may depend on the composition of local root symbiotic AMF communities.

2. Rare *Pulsatilla patens* and common *Pulsatilla pratensis* were grown in pots, under homogeneous soil nutrient and microbial community conditions, containing either one from two (grassland and forest sites) of natural AMF soil inocula, or no AMF.

3. Lower establishment was observed in the non-mycorrhizal soil compared to AMF inoculated soils. Plant biomass, phosphorus concentration and root AMF colonization of both species were higher, and nitrogen concentration lower, in grassland as opposed to forest inoculum.

4. The common species displayed more vigorous growth than the rare counterpart in the presence of grassland inoculum. Conversely, slightly better growth of a rare species was recorded in the forest inoculum, in which plant biomass was an order of magnitude lower compared to the grassland inoculum.

5. As *Pulsatilla* spp. roots hosted site-characteristic AMF small-subunit rDNA sequence groupings, we suggest that the presence of AM fungi that are more beneficial for the common species may be one factor behind the observed differential distribution and performance of the two plant species.

Key-words: arbuscular mycorrhizal fungi, boreal forest, gaps, mycorrhizal response, seedling establishment

Introduction

Interactions between plants and microbes have a significant impact on plant performance (Bever 2003). The important role(s) played by root symbiotic arbuscular mycorrhizal fungi (AMF) in influencing plant population dynamics through their control of soil nutrient uptake, root pathogens and intra- and interplant species linkages are now being appreciated (Zobel, Moora & Haukioja 1997; Smith, Hartnett & Wilson 1999; Callaway et al. 2003; Castelli & Casper 2003).

Studies using traditional spore identification and recent molecular identification methodologies show that AMF communities in soil as spores (Merryweather & Fitter 1998; Eom, Hartnett & Wilson 2000) and in roots (Helgason et al. 1998, 2002; Daniell et al. 2001; Husband et al. 2002; Vandenkornhuyse et al. 2002; Öpik et al. 2003) are patchily distributed both among and within sites. Moreover, plants can vary widely in their response to individual AMF taxa (Klironomos 2003). Consequently, the occurrence and abundance of a vascular plant species in a particular community may be dependent on the presence of a specific AMF species or combinations of species (van der Heijden et al. 1998). Thus explanations of how natural variation of AMF communities influences the performance of plant species is of primary interest when explaining the patterns of natural plant biodiversity.

There are a number of studies where plant growth response to natural soil inoculum has been compared to that on sterile soil. However, we lack experimental...
evidence highlighting differential plant species-related growth patterns in natural soils that are likely to support dissimilar AMF communities, as the bulk of experimental studies have involved inoculations with a limited number of easily cultured AM fungi which are often poorly represented in the natural root-colonizing AMF communities (Read 2002). The only exceptions known to us are pot experiments showing differential plant growth in the presence of natural soil or root inocula of different origin (Johnson 1993; Kiers et al. 2000; Corkidi et al. 2002; Frank et al. 2003). However, the relative contribution of native AMF communities to the observed plant growth responses cannot be assessed in these studies because the AM fungi colonizing plant roots were not identified.

In order to overcome the questionable ecological relevance of experiments using a few easily cultured AMF (Read 2002), experiments coupling naturally co-occurring plant species and AMF communities are needed. At the same time, to understand the effects of native AMF communities, it is still not enough to use soil inocula either completely ‘blind’ or containing known spore communities, as long as we lack information on the actual composition of AMF communities that colonize and symbiotically interact with plant roots after exposure to soil inocula. To our knowledge, the experiment reported here is one of the first to couple these two aspects: a contrasted natural soil inoculation experiment to determine species-specific plant performance that also includes high-resolution identification of root-colonizing AMF communities of the plants investigated.

Our goal was to determine whether seedling establishment and growth of native plant species differs on natural soil inoculum originating from different sites. If a differential response was identified, a valid assumption worth investigation is that distribution of a plant species may depend, in addition to other factors, on the composition of local root symbiotic AMF communities. As a relevant model, we chose a pair of plant species with similar morphology, ecology and phylogeny: Pulsatilla patens and Pulsatilla pratensis [both (L.) Mill. Ranunculaceae]. The former is now rare with a limited distribution, while the latter is common in Estonia (Pilt & Kukk 2002). One of the major reasons for differences in local and regional abundance of plants might be related to their differential responses to the presence of (particular) soil microbes (Klironomos 2002, 2003). Seedlings of both Pulsatilla species investigated here are seldom encountered in established vegetation, as establishment takes place almost exclusively in disturbed gaps or areas of moderate disturbance (Uotila 1996; Pilt & Kukk 2002). Therefore it is probable that AMF spores are the main infection source for Pulsatilla spp. seedlings, while root colonization via functioning hyphal networks is less significant due to the lack of established vegetation in disturbed sites. Such an infection by spores may be relatively more beneficial for seedlings than inoculation via intact mycelial network, as in the latter case competitive interactions may overwhelm benefits (Kytöviita, Vestberg & Tuomi 2003).

We aimed to investigate whether different soil AM fungal communities contribute to differential establishment and growth of two Pulsatilla species in conditions simulating gapped environments. In a seedling-establishment experiment of factorial design, we used soil inoculum from two sites with distinct vegetation characteristics: a boreal forest stand, and dry grassland. Both rare and common species coexist at the forest site, but only the common P. pratensis is found in the grassland site. Unlike many earlier studies, we possess background data on AMF present in roots of the experimental Pulsatilla plants from the phylogenetic analysis of amplified and cloned small-subunit ribosomal RNA gene (SSU rDNA) sequences (Öpik et al. 2003).

Materials and methods

Soil inoculum was collected from two divergent sites in Estonia: grassland and forest. The first is a 0·5 ha remnant patch of dry perennial grassland at Pangodi in central Estonia (58°10′ N, 26°34′ E), used as pasture until agricultural activity ceased 15 years ago. There are some single, mature Scots Pines (Pinus sylvestris L.) in the area. The plant community consists of 35 species of perennial grasses and forbs, 33 of which are potentially arbuscular mycorrhizal. Helicotrichon pratense (L.) Besser, Galium verum L. and Festuca rubra L. represent the predominant species. The second site is a sparse, mature, boreal Scots Pine-dominated forest at Soomaa National Park, south-western Estonia (58°24′ N, 25°19′ E). The ectomycorrhizal Scots Pine covers 40–50% of the area; in the understorey ericoid mycorrhizal Calluna vulgaris L. predominates in the shrub layer and Hylocomium splendens (Hedw.) B., S. and G. and Pleurozium schreberi (Brud.) Mitt. in the moss layer. There are five AM herbaceous plant species in the community, with a cover of less than 5%. Among these, Festuca ovina L. is the most abundant. The distance between the sites is ≈90 km. Soils in both sites are dry arenosols with weakly differentiated horizons and rather similar properties (Table 1). Topsoil samples (3–10 cm) were collected from 10 randomly chosen sites in both target ecosystems in the second half of August 1999 and stored in the dark at 10 °C. Samples from each location were sieved to remove roots and then pooled for the experiment. Soil pH, P, N and C contents were determined according to Moore & Chapman (1986).

Pulsatilla patens and P. pratensis are perennial forbs that grow in dry grasslands and boreal forests. Pulsatilla patens is now restricted to 27 isolated populations in Estonia (Pilt & Kukk 2002). In contrast, P. pratensis is relatively abundant. Mature seeds of both species were collected in summer 1999 from three local populations. Seeds were pooled, visually examined and
selected to avoid those damaged by herbivores or pathogenic fungi.

Plant seeds were sown on 8 February 2000 onto a 1:1 mixture of the two natural soils, with one of the soils being autoclaved (40 min at 121 °C). We did not detect any significant differences in soil characteristics between soil mixtures where either the soil from grassland, soil from forest, or both soils were autoclaved (Table 1). A mixture of both autoclaved soils served as a non-mycorrhizal control treatment. Hereafter, the soil inoculum treatments are referred to as grassland inoculum (mixed with sterilized forest soil); forest inoculum (mixed with sterilized grassland soil); and sterile soil (both soils sterilized). All pots received 2 ml filtered washing of respective mixed soil inoculum to correct for possible differences in soil bacterial and fungal communities (Koide & Li 1989).

The experiment was conducted in the greenhouse of the Viikki Biocentre at Helsinki University. Seeds were sown at a uniform density (1.2 seeds cm⁻²) into pots (9 × 12 cm, depth × diameter). Pots were carefully watered with tap water as required. Each treatment was replicated 10 times. Plants were grown in full daylight (daylength 16 h) for 14 weeks. At both harvests the plants were still in at the juvenile stage, and there were no major differences in plant phenology between the two harvests.

Plant establishment rate was recorded daily until no new germlings were detected, then plants were thinned to three individuals per pot (at maximum equidistant positions to avoid competition). Two seedlings from each pot were harvested carefully 5 weeks later (10 for biomass analysis; five for root staining; five for molecular analysis) and the third was allowed to grow on further for 9 weeks. The 14-week-old plants were harvested to prevent plants from becoming root-bound in pots. Shoots and roots were separated, dried at 85 °C for 24 h, and weighed. A correction in root biomass values at second harvest was made using differences between fresh and dry weight of root sample (for AMF determination) per treatment. Phosphorus and nitrogen concentrations in 14-week-old plants were determined following Kjeldahl digestions of dried plant tissues. Due to the limited plant material available, the P concentration of plants in sterile soil treatment could not be determined.

The percentage AMF colonization (root length colonized, %) was estimated on the basis of the full root system of five seedlings (first harvest), or 1–2 g (FW) randomly selected root segments (second harvest). Root samples were stained (Koske & Gemma 1989) and the percentage of colonization was determined (Rajapakse & Miller 1992).

The AMF community composition in the roots of four to five seedlings from each treatment was determined from randomly selected root samples (total length 5 cm), or the entire root system if under this length, as described for a related study by Öpik et al. (2003). Briefly, a ~550 bp fragment located in the middle section of the SSU gene was amplified with the AMF-specific primer pair NS31/AM1 (Simon, Lalonde & Bruns 1992; Helgason et al. 1998). The PCR products were separated by denaturing gradient gel electrophoresis (DGGE) and individual bands excised, cloned and sequenced. The fungal sequence groups identified in the seedlings roots of this study comprise part of a larger AMF community survey of native plants and trap seedlings of P. patens and P. pratensis in various Estonian locations (Öpik et al. 2003).

For analysis of the plant establishment rate, a repeated-measures ANOVA was conducted, with plant species and soil inoculum as fixed factors and time as the repeated-measures factor. Biomass, root AMF colonization rate, and plant P and N concentrations (presented as percentage of plant dry biomass) data were subjected to ANOVA. Biomass and percentage AMF colonization data were, respectively, log- and arcsine-transformed prior to statistical analysis. All analyses were conducted with the Windows version of STATISTICA (StatSoft, Inc., 2000, Tulsa, OK, USA). Similarities of AM fungal communities, calculated on the basis of the fungal sequence groups’ presence/absence in a root system, were analysed by multivariate cluster analysis (Ward’s linkage method and Euclidean distance measure) implemented in PC-ORD ver. 4 01 (McCune & Mefford 1999).

Table 1. Characteristics of natural soils (data from Pilt & Kukk 2002 for 30 cm topsoil layer), and of soil mixtures used in the current experiment: forest inoculum – forest soil mixed with sterilized grassland soil; grassland inoculum – grassland soil mixed with sterilized forest soil; sterile soil – both soils sterilized and mixed.

<table>
<thead>
<tr>
<th>Soil</th>
<th>pH (H₂O)</th>
<th>Total N (%)</th>
<th>Mobile P (mg per 100 g soil)</th>
<th>Total C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural soils</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pangodi grassland</td>
<td>7·7</td>
<td>0·059</td>
<td>10·1</td>
<td>ND</td>
</tr>
<tr>
<td>Soomaa forest</td>
<td>8·6</td>
<td>0·109</td>
<td>9·8</td>
<td>ND</td>
</tr>
<tr>
<td>1 : 1 Soil mixture used in the current experiment (n = 5, mean ± SE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forest inoculum</td>
<td>6·9 (± 0·01)</td>
<td>0·08 (± 0·001)</td>
<td>9·0 (± 0·07)</td>
<td>2·3 (± 0·12)</td>
</tr>
<tr>
<td>Grassland inoculum</td>
<td>6·8 (± 0·01)</td>
<td>0·07 (± 0·001)</td>
<td>8·9 (± 0·08)</td>
<td>2·21 (± 0·08)</td>
</tr>
<tr>
<td>Sterile soil</td>
<td>6·8 (± 0·01)</td>
<td>0·07 (± 0·000)</td>
<td>9·1 (± 0·04)</td>
<td>2·17 (± 0·07)</td>
</tr>
</tbody>
</table>

ND, not determined.


Results

PLANT ESTABLISHMENT

Seed germination commenced 17 days after sowing and continued for 12 days. The establishment rate of P. patens was higher than that of P. pratensis (F = 10·58, P = 0·002), with the final rates on AMF inoculum-containing soils being 51 and 30%, respectively. Soil inoculum had a significant effect on establishment (F = 14·19, P < 0·001) – establishment on sterile soil (28% P. patens, 21% P. pratensis) was significantly lower than that on AMF inoculum-containing soil. The significant interaction between plant species and time (F = 18·03, P < 0·001) stressed the early
common establishment rates of the two species, later followed by a higher establishment of *P. patens* over *P. pratensis*. The interaction between soil inoculum and time was also significant (*F* = 7.99, *P* < 0.001).

From the fifth day of germination, establishment on the sterile soil remained significantly lower than on the other two test soils.

**PLANT BIOMASS**

Total plant biomass did not differ between the two *Pulsatilla* species (Table 2). Grassland inoculum supported higher plant biomass production than the forest and sterile soils (Fig. 1a,b). Hierarchical patterns of interactions between factors were resolved (Table 2). The soil-inoculum treatment had no effect on biomass at the first harvest, but differences appeared at the second harvest (Fig. 1a,b). Compared with sterile soil, the rare *P. patens* grew to a higher biomass in the forest inoculum while the common *P. pratensis* did not, although no significant difference was detected in the final biomass of *P. patens* and *P. pratensis* plants.

On grassland inoculum, plants grew larger than on the two other soils; the absolute biomass values differed by an order of magnitude. Overall, the *P. pratensis* seedlings produced the largest total biomass on grassland inoculum compared with the *P. patens*.

Shoot biomass variation was the main cause of total biomass variation between the two plant species, shown by the same significant main effects and interactions in the analyses of shoot and total biomass (Table 2). Root biomass did not differ between the species, but the soil inoculum effect was significant (Table 2). The greatest root production was observed on grassland inoculum, followed by plants grown on forest and sterile soil. Unlike in the case of total plant biomass, there were no significant interactions between plant species and other factors. Shoot-to-root ratio decreased with time on grassland inoculum and sterile soil, but not on forest inoculum (Table 2). Rapid shoot growth was favoured during the first 5 weeks, with a later switch to more intensive development of roots.

**AM Fungal Colonization of Roots**

No host species-related differences were detected in percentage mycorrhizal fungal colonization of roots (Table 2). Plant roots were not colonized by AMF on sterile soil. The colonization rate remained moderate on forest inoculum, while on grassland inoculum plant roots were highly colonized by AMF (Fig. 1c). AMF root colonization increased with time and was positively correlated with plant total biomass (*r* = 0.630, *P* = 0.007, Pearson’s correlation).

**Phosphorus and Nitrogen Concentration of Plant Tissues**

The P and N concentrations in plant tissues differed between plants grown in different soil inocula (Fig. 1d,e) but not between the two *Pulsatilla* species (Table 2). Higher root tissue P and total plant P concentrations were observed in plants on grassland inoculum than on forest inoculum. Whereas lower root and total plant N concentrations occurred in plants on grassland inoculum than on forest inoculum, shoot N concentration was lower on grassland than on forest inoculum. Plant total biomass and

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Table 2. ANOVAs for plant total biomass characters, percentage arbuscular mycorrhizal fungal colonization of roots, and N and P tissue concentrations of *P. patens* and *P. pratensis* (species, sp.) grown on three soil inocula at two harvest times (time)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Shoot biomass</th>
<th>Root biomass</th>
<th>Total biomass</th>
<th>Shoot : root ratio</th>
<th>Mycorrhizal colonization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
<td>Species</td>
<td>1</td>
<td>4.6</td>
<td>0.034</td>
<td>0.09</td>
<td>0.770</td>
</tr>
<tr>
<td>Inoculum</td>
<td>2</td>
<td>64.1</td>
<td>0.000</td>
<td>29.86</td>
<td>0.000</td>
</tr>
<tr>
<td>Time</td>
<td>1</td>
<td>802.8</td>
<td>0.000</td>
<td>35.57</td>
<td>0.000</td>
</tr>
<tr>
<td>Species × inoculum</td>
<td>2</td>
<td>10.3</td>
<td>0.000</td>
<td>0.63</td>
<td>0.535</td>
</tr>
<tr>
<td>Species × time</td>
<td>1</td>
<td>4.4</td>
<td>0.038</td>
<td>0.08</td>
<td>0.776</td>
</tr>
<tr>
<td>Inoculum × time</td>
<td>2</td>
<td>635.0</td>
<td>0.000</td>
<td>287.48</td>
<td>0.000</td>
</tr>
<tr>
<td>Sp. × inoc. × time</td>
<td>2</td>
<td>10.2</td>
<td>0.000</td>
<td>1.33</td>
<td>0.269</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phosphorus concentration*</th>
<th>Shoot</th>
<th>Root</th>
<th>Total</th>
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<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
<td>Species</td>
<td>1</td>
<td>1.22</td>
<td>0.277</td>
</tr>
<tr>
<td>Inoculum</td>
<td>1</td>
<td>0.23</td>
<td>0.633</td>
</tr>
<tr>
<td>Species × inoculum</td>
<td>1</td>
<td>3.82</td>
<td>0.059</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nitrogen concentration</th>
<th>Shoot</th>
<th>Root</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
<td>Species</td>
<td>1</td>
<td>0.37</td>
<td>0.543</td>
</tr>
<tr>
<td>Inoculum</td>
<td>1</td>
<td>5.2</td>
<td>0.009</td>
</tr>
<tr>
<td>Species × inoculum</td>
<td>1</td>
<td>3.82</td>
<td>0.059</td>
</tr>
</tbody>
</table>

*Not determined in plants grown on control soil due to small plant biomass.
The percentage of AMF root colonization were positively correlated with plant total P concentration ($r = 0.384$, $P = 0.019$; $r = 0.669$, $P = 0.003$, Pearson’s correlation) and negatively correlated with plant total N concentration ($r = -0.587$, $P < 0.001$; $r = -0.750$, $P = 0.050$, Pearson’s correlation). The same correlation pattern was observed when shoot and root biomasses were analysed separately.

**AM Fungal Communities in Roots**

Root-colonizing AM fungal sequence groups were determined following PCR amplification, DGGE, cloning and sequencing (Öpik et al. 2003). Cluster analysis of AM fungal communities in each root system, based on presence/absence data for the sequence groups identified, distinguished two main clusters predominantly based on the origin of soil inoculum (forest or grassland) and not on host-plant species that appeared intermixed on the dendrogram (Fig. 2). The prevalence of root-colonizing sequence groups G1, G2 and G5 characterized the forest soil communities, while G3, G13 and G14 were more abundant in grassland communities. Seven fungal sequence groups were specifically detected in the roots of plants grown on grassland inoculum, including the most common

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**Fig. 1.** Total dry weight of (a) 5-week-old and (b) 14-week-old plants; (c) arbuscular mycorrhizal root colonization; and total tissue (d) phosphorus and (e) nitrogen concentrations (14-week-old plants) of *Pulsatilla patens* (shaded bars) and *Pulsatilla pratensis* (open bars) seedlings grown in the presence of forest or grassland soil inoculum, or on non-AMF-containing (sterile) soil. Different lower case letters above bars indicate a significant difference ($P \leq 0.05$) between AM fungal inoculations according to Tukey’s HSD test. Note the different scales in (a) and (b). Values presented are means ± 1 SE.
sequence group in the grassland AMF community, G3. Four rare sequence groups were detected only in roots of plants grown in the forest inoculum. Group G5 was the most frequent, but this group also occurred in grassland inoculum plants. In total 12 and eight AMF sequence groups were detected in grassland and forest inoculum plants, respectively.

**Discussion**

**EFFECT OF SOIL INOCULUM SOURCE ON PLANT ESTABLISHMENT AND GROWTH**

Clear response patterns of the rare and common *Pulsatilla* species seedlings to soil inocula from two different sites were identified in this study. The presence of natural soil containing AM fungi significantly improved seedling establishment compared with the sterile soil control treatment. These data provide strong support for a beneficial role of soil microbiota (including AMF) during establishment. With no differences between the two natural soils, the establishment rate of the rare species *P. patens* was higher than that of a common species, *P. pratensis*. Establishment aside, the natural soils imparted an obvious differential impact on subsequent plant growth. The grassland inoculum supported seedling growth an order of magnitude greater than that of the forest inoculum. Earlier studies (van der Heijden et al. 1998; Bray, Kitajima & Sylvia 2003) identified plant species-specific growth responses to different combinations of cultured AM fungi, and hypothesized that there is also a differential effect of native AMF communities. Our results show for the first time, to our knowledge, the differential growth response of native plants in the presence of divergent root-colonizing natural AMF communities, identified by high-resolution SSU-based molecular analysis. We conclude that the performance of particular plant species in natural habitats may depend on the composition of local AMF communities.

**ROOT AMF COLONIZATION AND TISSUE P AND N CONTENT**

The positive growth response of plants to AMF inoculation can be related to either improved P nutrition or avoidance of root infection by root pathogens (Newsham, Fitter & Watkinson 1995). In our experiment, improved P nutrition was the most probable explanation behind the growth response, as the increased growth coincided with increased AMF root colonization and higher P content in plant tissues.

The rate and extent to which AM fungi colonized plant roots appeared to be strongly dependent on the origin of inoculum. The high colonization levels of seedlings exposed to grassland inoculum at 5 weeks,
which in forest inoculum seedlings was reached only after 14 weeks, was capitalized in better growth compared with forest inoculum seedlings at 14 weeks. In natural plant communities, successful establishment of seedlings may be strongly influenced by the availability and colonization ability of AMF diaaspores (Hart & Reader 2002; Hart, Reader & Kironomos 2003). The presence of sufficient numbers of fungal diaaspores that are potentially able to rapidly colonize and promote a good sustained growth response could explain early seedling colonization and subsequent promotion of plant growth with the grassland inoculum.

Differences in root AMF colonization levels could partially explain the differential plant growth on the two natural soil incoula, given that higher colonization levels and tissue P concentration accompanied higher plant biomass on grassland inoculum. Positive relationships between root AMF colonization rate and plant biomass response have been reported for plant species with higher responsiveness to AMF (van der Heijden et al. 1998; Wilson & Hartnett 1998). Mycorrhizal colonization levels have been positively associated with plant tissue P concentrations in manipulated and native field studies (Merryweather & Fitter 1995, 1996).

In support of the recent findings of Miller et al. (2002) on mycorrhiza-mediated control of plant nutrient status, the inverse relationship between plant tissue N concentration with P concentration and biomass was highlighted in the present study. As lower N concentration in leaves may indicate higher N-use efficiency (Berendse & Aerts 1987), it is conceivable that one of the mechanisms behind the differential positive effect of grassland inoculum on plant performance may be the improved P nutrition and associated increase in N-use efficiency, possibly mediated by specific AMF in that soil.

**AM Fungal Communities**

AM fungi that are easily cultivable via trap plants and frequently used in experiments are, however, rarely or (more often) not found in roots of native plants in field surveys (Clapp et al. 2002; Helgason et al. 2002). Thus ecologically relevant studies need to be carried out to test differential plant performance in response to AMF. It should be noted that in earlier experimental studies using soil or root inocula, no information has been given on the AMF species actually colonizing plant roots.

Here, SSU sequence-based analyses identified 12 and eight AM fungal sequence groups in grassland and forest soil inoculum treatments. Four of these are related to known species: G3 to *Glomus intraradices*; G10 to *Glomus caledonium*; G11 to *Glomus mosseae*; and S2 to *Scutellospora nodosa*; several have been detected in other studies (Öpik et al. 2003). The somewhat smaller number of AM fungi might be related to lower host-plant density in the forest site which can influence mycorrhizal colonization (Genney, Hartley & Alexander 2001) and possibly fungal propagule and species density in soil.

Community analysis of root-colonizing fungi separated root samples almost exclusively based on soil inoculum origin, showing that plants grown on the two natural soil inocula hosted fungal communities differing in composition and in dominant sequence groups. A number of unique AMF sequence groups were identified from the grassland soil. These fungi may potentially contribute to the observed positive effect on plant growth because of their specific, yet unknown, functional characteristics. The most abundant grassland-specific group, G3, is related to *G. intraradices*, some isolates of which show rapid root-colonization ability (Hart & Reader 2002) which might be decisive for seedling establishment success. Fungal sequence groups characteristic to forest soil, G2 and G5, show high similarity to an undescribed *Glomus* sp. (isolate UY 1225) known to confer some benefit to a relatively broad range of plant hosts, and to a sequence group *Glomus* sp. Glo2 of unknown properties (Helgason et al. 2002), respectively.

Another aspect revealed by the AMF community analysis was the tendency for a reduction in AMF sequence groups in 5-week-old vs 14-week-old seedlings. This difference may indicate the successional replacement of AMF species during plant ontogenesis (Husband et al. 2002).

**Performance of Two Plant Species**

The two *Pulsatilla* species differed in their growth response in the two test inocula, as the common *P. pratensis* performed better on grassland inoculum than the rare counterpart *P. patens*, while *P. patens* did slightly better on forest inoculum. However, the differences among species were relatively small when compared with differences induced by the two natural soil inocula. In general, soil inoculum had a pronounced effect on plant performance, and the common *P. pratensis* gained relatively more from the grassland inoculum.

Fragmentation of *P. patens* populations in Estonia has evidently resulted in strong dispersal limitation (Pilt & Kukk 2002). As both *Pulsatilla* species rarely regenerate in established vegetation, and mostly do so following soil disturbance (Uotila 1996; Pilt & Kukk 2002), then even if plant diaaspores are available, mycorrhizal growth promotion of seedlings and juvenile plants in conditions where most of the AMF infection is evidently spore-borne may be an important factor determining the fate of plant individuals. The composition of a local AMF community is, without doubt, an important determinant of the establishment success of both *Pulsatilla* species. Differential performance of *Pulsatilla* species on different soil inocula – a common species grew significantly better on grassland inoculum, and a rare species slightly better on a forest inoculum – refers to the possibility that growth response
to particular AMF community may be one factor behind the observed differential distribution pattern.

Returning to our initial working hypotheses, we conclude that establishment and growth of native Pulsatilla spp. is influenced by the composition of native AM fungal communities, and the growth of congeneric plant species differs due to their response to specific AMF communities. Further targeted ecophysiological experimentation is needed to determine the functional roles of the observed divergent AM root colonization (Öpik et al. 2003; this study) on seedling establishment and subsequent plant performance, individually and in mixed-species communities.

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