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ORIGINAL PAPER

Ericaceous dwarf shrubs affect ectomycorrhizal fungal community of the invasive *Pinus strobus* and native *Pinus sylvestris* in a pot experiment

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Abstract This study aimed to elucidate the relationship between ericaceous understorey shrubs and the diversity and abundance of ectomycorrhizal fungi (EcMF) associated with the invasive *Pinus strobus* and native *Pinus sylvestris*. Seedlings of both pines were grown in mesocosms and subjected to three treatments simulating different forest microhabitats: (a) grown in isolation and grown with (b) *Vaccinium myrtillus* or (c) *Vaccinium vitis-idaea*. Ericaceous plants did not act as a species pool of pine mycobionts and inhibited the ability of the potentially shared species *Meliniomyces bicolor* to form ectomycorrhizae. Similarly, Ericaceae significantly reduced the formation of *Thelephora terrestris* ectomycorrhizae in *P. sylvestris*. EcMF species composition in the mesocosms was strongly affected by both the host species and the presence of an ericaceous neighbour. When grown in isolation, *P. strobus* root tips were predominantly colonised by *Wilcoxina mikolae*, whereas those of *P. sylvestris* were more commonly colonised by *Suillus* and *Rhizopogon* spp. Interestingly, these differences were less evident (*Suillus*+*Rhizopogon* spp.) or absent (*W. mikolae*) when the pines were grown with Ericaceae. *P. strobus* exclusively associated with *Rhizopogon salebrosus* s.l., suggesting the presence of host specificity at the intrageneric level. Ericaceous plants had a positive effect on colonisation of *P. strobus* root tips by *R. salebrosus* s.l. This study demonstrates that the interaction of selective factors such as host species and presence of ericaceous plants may affect the realised niche of the ectomycorrhizal fungi.

Keywords Plant invasions · Seedlings establishment · Ericoid mycorrhiza · *Rhizoscyphus ericae* aggregate · *Suillus* spp. · *Rhizopogon salebrosus* complex · *Meliniomyces bicolor* · Common mycorrhizal networks

Introduction

Invasive plants are among the greatest threats to species biodiversity and ecosystem functioning (Mack et al. 2000) and may have a marked economic effect (Pimentel et al. 2005). Invasiveness of plants depends on the environment and species-specific ecological traits, such as growth rate,

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escape from pathogens, production of allelochemicals, etc. (Rejmánek 1989; Keane and Crawley 2002). However, sometimes the mechanisms triggering invasiveness are unpredictable and may depend on other factors or combinations of factors including biotic interactions. In particular, plant invasions may be facilitated by the presence of compatible mutualists such as pollinators, seed dispersers and mycorrhizal fungi (Richardson et al. 2000; Klironomos 2003; Pringle et al. 2009). In their new range, invaders often lack suitable partners, but new host–mutualist combinations may emerge (Richardson et al. 2000). Soil biota may enhance plant invasions (Reinhart and Callaway 2006). Non-native plants, in turn, may alter soil microbial communities via root exudates (van der Putten et al. 2007).

Ectomycorrhizal (EcM) symbioses benefit coniferous trees by facilitating water and nutrient uptake, protecting roots from pathogens and interconnecting single plants into common mycorrhizal networks (CMNs; Selosse et al. 2006; Smith and Read 2008). Fungi that are involved in EcM symbiosis (EcMF) differ in their ability to exploit various substrates, indicating certain-level functional complementarity (van der Heijden and Kuyper 2003; Courty et al. 2005). Accordingly, plant seedlings may benefit from associating simultaneously with several EcMF particularly when growing in complex substrates (Baxter and Dighton 2001; Jonsson et al. 2001). Naturally, the great diversity of EcMF communities depends on both biotic and abiotic factors, such as host plant, soil quality, pollution, etc. (Lilleskov et al. 2002; Ishida et al. 2007; Morris et al. 2008; Tedersoo et al. 2008a, b).

In the southern hemisphere, the establishment and naturalisation of introduced *Pinus* spp. was retarded in regions that lacked compatible EcMF (Mikola 1969; Read 1998; Richardson et al. 2000; Nuñez et al. 2009). Recent studies suggest that introduced and/or invasive plants associate with fewer EcMF than the native species (Tedersoo et al. 2007; Nuñez et al. 2009; Dickie et al. 2010; Walbert et al. 2010). Native plants may share their EcMF with Pinaceae (Horton et al. 1999; Richard et al. 2009), except in exotic habitats (Tedersoo et al. 2007).

Understorey vegetation in many boreal, temperate and Mediterranean forests consists of ericaceous dwarf shrubs that form ericoid mycorrhiza (ErM; Read 1991). ErM mycobionts from the *Rhizoscyphus ericae* (Read) Zhuang & Korf aggregate are taxonomically distinct from common EcMF, but some of them are capable of forming EcM symbiosis (Vrålstad et al. 2000, 2002). This led to hypotheses that ericaceous and ectomycorrhizal plants might be functionally interconnected with CMNs (Vrålstad 2004) and that Ericaceae could serve as an inoculum reservoir for Pinaceae. However, to date, only a single *R. ericae* aggregate strain has been demonstrated to form EcM and ErM simultaneously (Villarreal-Ruiz et al. 2004). ErM fungi (ErMF) also occur as common root endophytes in non-ericaceous hosts (Hambleton and Sigler 2005; Curlevski et al. 2009; Tedersoo et al. 2009).

Pinus strobus L. was introduced in the Czech Republic as an ornamental tree as early as in 1784, and the first plantations were established in the Elbe Sandstone Mountains (NW Czech Republic) in 1789 (Nožička 1965). However, reports of its invasion in different types of forests did not start to accumulate until as late as the early 1990s (V. Hadincová and co-workers, unpublished data). *P. strobus* is now commonly found in native forests in many sandstone areas, often regenerating within ericaceous understorey in the early stages of its invasion. Due to a combination of effective seed spread, high seedling recruitment, fast growth and enormous litter production (Hadincová et al. 2007; Kubartová 2007), *P. strobus* inhibits the growth of understorey vegetation and regeneration of native trees, especially *Pinus sylvestris* L. According to Carrillo-Gavilan and Vila (2010), *P. strobus* is the only invasive conifer in Europe. The role of mutualistic symbioses, including EcM, in the *P. strobus*, invasions remain unknown.

This study aimed to address the potential role of EcMF and ErMF in facilitating the invasion of *P. strobus*. In particular, the EcMF resistant propagule communities associated with the roots of both *P. strobus* and *P. sylvestris* were studied in order to test the hypotheses that (a) *P. strobus* associates with a less diverse and more generalist EcMF spectrum than *P. sylvestris*, (b) EcMF communities of both pine species are affected by the presence of ericaceous plants and (c) there is an increase in the abundance of ectomycorrhizal members of the *R. ericae* aggregate in the presence of ericaceous plants, due to their supposed co-association with Ericaceae.

Materials and methods

Experimental design and sampling

To test the above hypotheses, a mesocosm experiment was set up because it has advantages over a field experiment: all experimental units experience the same environmental conditions, including exposure to soil fungi and the confounding effect of spatial heterogeneity is avoided, resulting in less variation among replicates (Pickles et al. 2010). A pot experiment may alter the realisable spectrum of EcMF in favour of species dispersing predominantly by spores. However, if the EcMF spore community introduced into a pot experiment reflects, the EcMF spore community occurring in the field (e.g. the inoculation is done with a non-treated natural substrate), the differences in the realised EcMF spectrum between a pot experiment and the situation in the field should not be large. On the other hand, the mesocosm approach may select for generalists and against fungi outside their ecophysiological optimum.

In May 2005, soil (mineral, organic and litter layer) was collected at two sites in the Elbe Sandstone Mountains: (a) Babylon (50°52.20′ N; 14°22.90′ E, 310 m above sea level) and (b) Picket (50°52.84′ N; 14°20.87′ E, 380 m above sea level) that are located ca. 5 km apart. Babylon is a forest dominated by *P. sylvestris*; the closest *P. strobus* trees are 100 m away. Picket is a mixed forest dominated by *P. strobus*, *P. sylvestris* and *Picea abies* L. Karst. Both sites have a similar understorey of ericaceous shrubs (*Vaccinium myrtillus* L. and *Vaccinium vitis-idaea* L.) and podzolic soils. At both sites, 6 kg of soil was collected from five cores located approximately 20 m apart. The soils from the two localities were pooled (each layer separately), mixed and used as a substrate in 1.5-l plastic pots (diameter 15 cm). This was done to maximise the diversity of EcMF available in the substrate as an inoculum for the seedlings. The soil in each pot consisted of three layers as in the field (i.e. 1 cm *L* and 10 cm *F*+*H* layers, 5 cm sand drainage). The experiment was two-factorial: (a) *Pinus* species (the invasive *P. strobus* and native *P. sylvestris*) and (b) presence of an ericaceous neighbour (*V. myrtillus*, *V. vitis-idaea* or none). There were eight replicates of each treatment; thus, the number of mesocosms totalled 48. Thirty seeds of either *P. sylvestris* or *P. strobus* originating from the Elbe Sandstone Mountains were sown in each of the mesocosms. *P. sylvestris* seeds were sown in March 2006. As *P. strobus* seeds need cold stratification, they were sown in September 2005. The seeds germinated simultaneously in April 2006. Five healthy seedlings were left per each pot. Single branches of ericaceous shrubs along with their rhizomes, and roots were transplanted from the sites where the soil was collected. Thus, they were most likely pre-colonised by native ErMF. Ericaceous plants were collected at the same time as the soil and replanted in pots in May 2005.

The mesocosms were kept outdoors in the experimental garden of the Institute of Botany ASCR at Průhonice (49° 59.86′ N; 14°33.97′ E; 300 m above sea level; mean annual temperature 8.8°C, mean annual precipitation 565 mm). The pots were randomly placed on the ground, shaded by green plastic netting and watered when needed during hot summer days. The mesocosms were randomly harvested in September 2008. At the harvest, soil was carefully removed from the roots using running tap water, and the roots of *Pinus* spp. were carefully separated from the hairy roots of ericaceous plants. The fine roots of *Pinus* species were then cut into 5 cm lengths. For each mesocosm, 40 randomly selected lengths of roots were examined under a dissecting microscope which resulted in 2 m of root length analysed per pot. EcM root tips were counted (bifurcated and coralloid root tips were counted as a single root tip) and separated into morphotypes based on surface texture, colour and presence and type of hyphae and rhizomorphs. Morphotyping of all EcM root tips was done by the same evaluator (P. K.). For each morphotype from each pot, three to 15 of the most healthy-looking and cleanest ectomycorrhizae were placed in 0.5 ml Eppendorf tubes containing 70% ethanol and stored at 4°C until required for molecular analyses. In total, 580 root tips were individually subjected to molecular analyses. The molecular analysis indicated that each EcM morphotype corresponded to a single fungal taxon. Only the suilloid morphotype was separated into eight species of *Suillus* and *Rhizopogon* based on 200 internal transcribed spacer (ITS) sequences (Supplementary material S2).

Molecular analyses

Prior to DNA extraction, individual root tips were surface-sterilised in a 100% commercial bleach solution (4.5% available chlorine), containing 100 µl/l of Tween 20, for 30 s, followed by 30 s in 70% ethanol and 3×1-min rinses in sterile water. Root tips were then ground in liquid nitrogen. DNA was extracted from each root tip using the DNeasy Plant Mini extraction kit (Qiagen GmbH, Hilden, Germany) following the manufacturer's instructions. DNA was eluted in 75 µl of sterile ddH$_2$O and kept at −20°C. Polymerase chain reaction (PCR) amplification of the ITS region was performed using the primers ITS1F (Gardes and Bruns 1993) and ITS4 (White et al. 1990). Additionally, samples from the basidiomycetous morphotypes were amplified using a combination of primer ITS1F and Basidiomycetes-specific primer ITS4B (Gardes and Bruns 1993) or LB-W (Tedersoo et al. 2008a) in order to avoid co-amplification of ascomycetes that commonly inhabit EcM root tips (Tedersoo et al. 2009). The PCR mix included 2.5 µl of 10× PCR buffer without MgCl$_2$, 2 µl of dNTPs mixture (200 nM), 2.5 µl MgCl$_2$ (2 mM), 0.5 µl of each primer (10 mM), 1 U of *Taq* DNA polymerase (Fermentas International Inc, Burlington, ON, Canada), 15.8 µl of sterile ddH$_2$O and 8 µl of the template (DNA extract diluted 1:10 in sterile water) in a final volume of 25 µl.

Thermal cycling parameters were as follows: initial denaturation step of 4 min at 94°C, 35 cycles consisting of a denaturation step at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 70 s and a final extension at 72°C for 10 min. The length and quality/quantity of the PCR products were checked using gel electrophoresis (1% agarose). Samples that yielded double-banded PCR products were excluded from further analyses. In the case of barely visible PCR products, a semi-nested or nested PCR was performed using primers ITS1 and ITS4 with 1 µl of the ITS1F/ITS4 or ITS1F/LB-W PCR product as a template (diluted 1:100 in sterile water). PCR products were purified using the QIAquick PCR Purification Kit (Qiagen GmbH, Hilden, Germany). Each sample was separately sequenced with the primer ITS1 or ITS1F in Macrogen Inc. (Seoul, South Korea). The DNA sequences were checked for possible machine errors and edited in Sequence Scanner 1.0 (Applied Biosystems, Forest City, CA, USA). Preliminary identification of EcMF was achieved by conducting a nucleotide Basic Local Alignment Search Tool (BLASTn) search of the GenBank and UNITE (Abarenkov et al. 2010) public sequence databases.

Sequence analyses

Alignment of sequences was performed using the CLUSTAL W algorithm (Thompson et al. 1994) in BioEdit 7.0.9.0 (Hall 1999), followed by manual correction. Neighbour-joining analyses were conducted using TOPALi version 2.5 (http://www.topali.org/). To determine the phylogenetic affinities of species belonging to the suilloid morphotype and *R. ericae* aggregate, selected ITS sequences from identified fruit bodies or pure cultures were downloaded from the sequence databases. The sequences were aligned as above. Neighbour-joining analyses were performed using MEGA 4 (Tamura et al. 2007) with 1,000 bootstrap replicates. A value of 97.0% similarity in the ITS region was used as a threshold for species delimitation. Representative sequences of each species or sequence type from each experimental treatment were submitted to National Center for Biotechnology Information (accession numbers FN678889–FN678898, FN679001–FN679049, FN686777, FN686778, FN691763 and FN811647–FN811657).

Statistical analyses

The influence of EcM host plant (*P. sylvestris* or *P. strobus*) and cultivation treatment (presence/absence of ericaceous plants) on EcM colonisation and EcMF species richness was analysed using the non-parametric Kruskal–Wallis test in software package STATISTICA 8 (StatSoft, Inc., Tulsa, OK, USA). The EcM colonisation was expressed as a number of EcM root tips per 2 m of the root length. To determine the effects on each EcMF species separately, Fisher's exact test based on their occurrence patterns in mesocosms was used. Presence–absence data per pot were used instead of abundance because it was not possible to quantify species of *Rhizopogon* and *Suillus* within the suilloid morphotype; thus, absence of a suilloid species may equally indicate that its abundance was below the molecular detection limit.

The relative effect of EcM host plant, presence of ericaceous shrubs and their interaction on the perceived EcMF community structure were further tested using the ADONIS routine and visualised using the non-metric multidimensional scaling (NMDS) in the Vegan package of R (R Core Development Team 2007). Species occurrence and Bray–Curtis distance measure were used in both analyses.

Results

EcM fungi species richness and diversity

In all treatments, nearly all pine roots were ectomycorrhizal. In total, approximately 11,500 root tips from *Pinus* spp. seedlings were analysed by morphotyping (Table 1 and Supplementary material S1), and 580 EcM root tips were consequently used for DNA extraction and PCR. However, only 330 root tips produced a sequence of sufficient quality that could be matched to an EcM fungus. According to the Kruskal–Wallis test, the total number of EcM root tips per 2 m root length in each mesocosm was similar in the two *Pinus* spp. and three ericaceous plant treatments (Table 1).

In total, 13 fungal taxa were identified. Seven species of EcMF (*Cenococcum geophilum* Fr., *Inocybe* sp. Fr., *Rhizopogon luteolus* Fr., *Rhizopogon roseolus* s.l. (Corda) Th. Fr., *Rhizopogon salebrosus* s.l. A.H. Sm., *Thelephora terrestris* Ehrh. ex Fr. and *Wilcoxina mikolae* Yang & Wilcox) were identified based on their closest ITS BLAST match (Table 2). The remaining six were members of the genus *Suillus* and of the *R. ericae* aggregate and were identified to species level based on phylogenetic analyses (Fig. 1; Supplementary material S3).

There were 11 and 10 fungal species associated with the root tips of *P. sylvestris* and *P. strobus*, respectively, of which eight were shared. Of all root tips, 46% and 72% of those of *P. sylvestris* and *P. strobus*, respectively, were colonised by *Ascomycota*. There were no significant effects of pine species or ericaceous neighbours on EcMF species richness ($p>0.05$).

Community composition of EcM fungi

The ascomycete *W. mikolae* was the most abundant EcMF species associated with the roots of both pine species in all treatments (on average 53.1%), except where *P. sylvestris* was grown in isolation (Fig. 2). *Meliniomyces bicolor* Hambl. & Sigler (a member of the *R. ericae* aggregate) was almost exclusively detected in treatments where the pine species were grown in isolation (Fisher's exact test: $p<0.001$; Table 3), colonising on average 47.2% of the root tips. Similarly, *T. terrestris* was mainly recorded when there were no ericaceous neighbours ($p=0.022$). Conversely, *R. salebrosus* s.l. was significantly more frequent in treatments with an ericaceous neighbour ($p=0.048$). More suilloid fungi (six subspecies, 37.4% of all root tips) were associated with *P. sylvestris* when it was grown in isolation. In contrast, four species of suilloid fungi colonised only 3.5% of root tips of *P. strobus* grown without an ericaceous neighbour. The ascomycetes *W. mikolae* and *M. bicolor* colonised more than 95% of all EcM root tips in this treatment.

Similarly, host plants had a strong effect on the occurrence of individual EcMF species (Table 3). In particular, *Suillus*

Table 1 Total numbers of EcM root tips per treatment and numbers of EcM root tips colonised by different morphotypes in each treatment per 2 m root length (means±SD; $n=8$)

	Suilloid	*M. bicolor*	*W. mikolae*	*Inocybe* spp.	*T. terrestris*	*C. geophilum*	Total no. of EcM root tips	Nm
Sy	89±42.8	111±53	0	0	35±20.5	7±15.8	246±42	0
SyVm	166±109.4	2±5	124±124	2±5.6	2±4.6	0	296±42	0
SyVt	73±32.4	0	75±77.4	0.3±0.7	1±3.9	0	165±45	0
St	12±23.8	163±157.7	166±124.1	0	0	0	341±42	0.8±1.2
StVm	35±35.1	0.4±1.1	178±61.1	8±15.9	1±2.8	0	236±42	1±1.8
StVt	95±61	0.1±0.4	104±127.4	0	0	0	230±45	2±2.4

Each row represents a single treatment

Sy P. sylvestris grown in isolation, *SyVm P. sylvestris* gown with *V. myrtillus*, *SyVv P. sylvestris* grown with *V. vitis-idaea*, *St P. strobus* grown in isolation, *StVm P. strobus* grown with *V. myrtillus*, *StVv P. strobus* grown with *V. vitis-idaea*, *Nm* non-mycorrhizal

bovinus (Pers.) Roussel ($p=0.023$) and *T. terrestris* ($p=0.002$) were significantly more frequently, but not exclusively, associated with *P. sylvestris*. Conversely, *R. salebrosus* s.l. was only associated with *P. strobus* ($p<0.001$). According to Fisher's exact test, *W. mikolae* was significantly more frequently associated with *P. strobus* ($p=0.008$). This result was probably due to the complete absence of *W. mikolae* on the eight seedlings of *P. sylvestris* grown in isolation. These results were supported at the community level, where the host plant (ADONIS: $F_{1,\ 42}=14.38$; $p<0.001$), ericaceous neighbour ($F_{2,\ 42}=13.03$; $p<0.001$) and their interaction ($F_{2,\ 42}=4.59$; $p<0.001$), respectively, explained 15.7%, 28.44% and 10.02% of total variation in the EcMF species distribution (Fig. 3).

Discussion

EcM communities associated with the native vs. invasive pine

In this mesocosm study of a resistant propagule community, there were no differences in species richness of EcMF colonising the native and introduced species of *Pinus*. This study along with those of Nuñez et al. (2009) and Tedersoo et al. (2007) indicates that introduced or invasive EcM trees may host a comparable number of symbionts when a suitable species pool is present. However, the mesocosm approach used in our study might select for generalists and against fungi outside their ecophysiological amplitude.

The ascomycetes *W. mikolae* and *M. bicolor* and suilloid fungi were the most abundant EcMF associated with both pine species. This observation accords with previous studies on resistant propagule and nursery communities of conifer seedlings (Iwanski et al. 2006; Rusca et al. 2006). Leski et al. (2010) showed that *P. sylvestris* seedling survival is negatively correlated with the relative abundance of *W. mikolae* but positively correlated with the relative abundance of suilloid mycorrhizae. This indicates that EcMF taxa may significantly differ in their effects on host fitness and therefore have different roles in the pine invasion processes. Interestingly, Ericaceae supported formation of both *W. mikolae* (in *P. sylvestris*) and suilloid (in *P. strobus*) ectomycorrhizae at the expense of *M. bicolor*, underlining the differential effect of surrounding vegetation on the native vs. introduced pine (see below).

In particular, host species had a strong effect on the frequency of several EcMF species, which contrasts with the results of most previous studies on intergeneric EcMF selectivity (Molina and Trappe 1994; Walker et al. 2005; Tedersoo et al. 2008a). However, Morris et al. (2008, 2009) demonstrated that host species is an important factor

Table 2 Identification of EcM fungi based on a BLASTn search of the public sequence databases GenBank and UNITE

EcM species	EMBL accession number	Best BLASTn species match: accession number, similarity (%), species identity
Cenococcum geophilum	FN686778	AY880936 (98%) *Cenococcum geophilum*
Inocybe sp.	FN679046	AM882710 (99%) *Inocybe jacobi*
Rhizopogon luteolus	FN679020	EU784397 (99%) *Rhizopogon luteolus*
Rhizopogon roseolus s.l.	FN679014	EU784401 (98%) *Rhizopogon roseolus*
Rhizopogon salebrosus s.l.	FN679024	FJ197209 (98%) *Rhizopogon salebrosus*
Thelephora terrestris	FN679049	AF272921 (100%) *Telephora terrestris*
Wilcoxina mikolae	FN679042	DQ093774 (99%) *Wilcoxina mikolae*

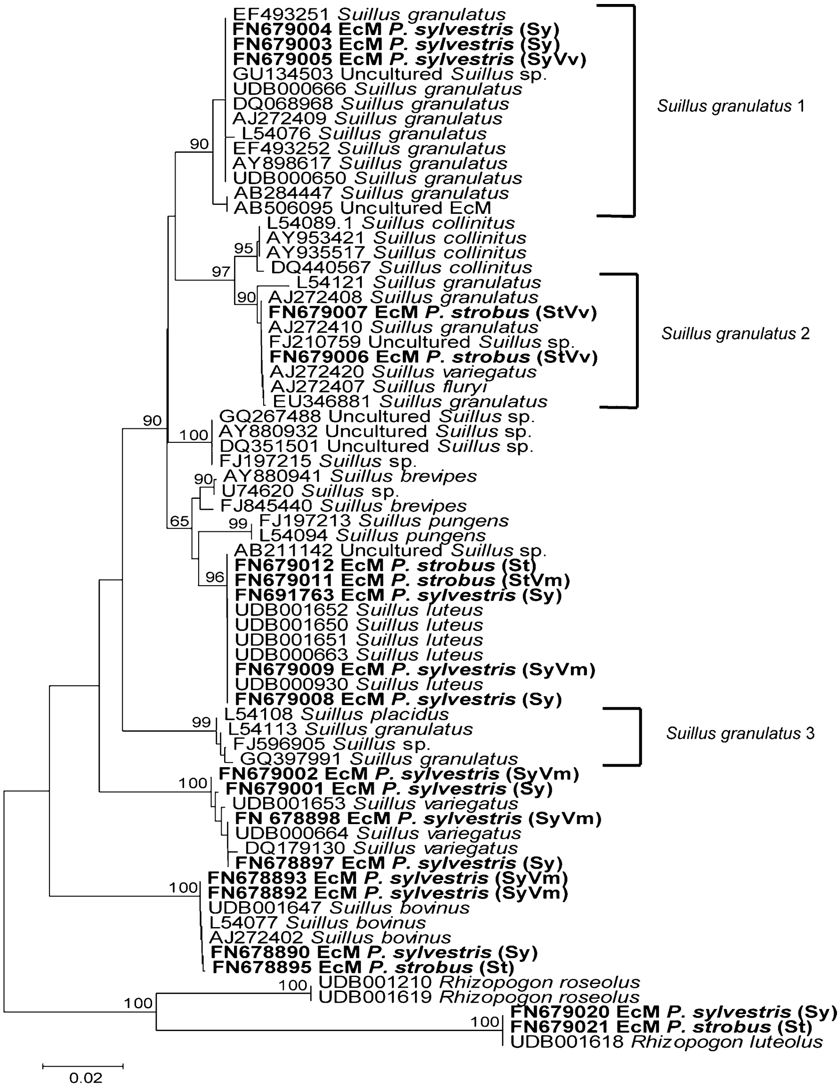

Fig. 1 Phylogenetic tree of a part of the genus *Suillus* based on a neighbour-joining analysis of 494 characters of ITS1, 5,8S rDNA and part of the ITS2 sequences. The *numbers above branches* denote neighbour-joining bootstrap values from 1,000 replications. The tree was rooted using sequences of *R. luteolus* and *R. roseolus*. Sequences obtained in the present study are shown in *bold*. They are labelled with the database accession number, the host plant species from which they were obtained and the cultivation treatment (*Sy P. sylvestris* grown in isolation, *SyVm P. sylvestris* gown with *V. myrtillus*, *SyVv P. sylvestris* grown with *V. vitis-idaea*, *St P. strobus* grown in isolation, *StVm P. strobus* grown with *V. myrtillus*, *StVv P. strobus* grown with *V. vitis-idaea*). The *parentheses* show the delimitation of the fungal taxa

affecting the EcMF community composition in ecosystems of two co-occurring *Quercus* species. Congruently, Jacobson and Miller (1992) recorded the host specificity among cryptic species of *Suillus granulatus* (L.) Roussel for the subgenera *Pinus* and *Strobus*. It is probable that incompatibility between invasive host plants and indigenous EcMF could shift communities of EcMF associating with a mature forest dominated by invasive pines. These changes could result in a large impact on functional traits in soil as well as extinctions of crucial ecosystems components, e.g. indigenous EcMF. However, more detailed studies are needed to evaluate the impact of invasive pines on indigenous EcMF communities.

We detected several EcMF that have not been found in Europe based on sequenced fruit-body data. However, the phylogenetic analyses indicate that the closest relatives of *S. granulatus* 2 originated in Europe (Fig. 1). Similarly, a sequence derived from *Arctostaphylos uva-ursi* (L.) Spreng. from the Alps (Krpata et al. 2007) nested in our sequences of *R. salebrosus* s.l. (Supplementary material S4). Thus, this taxonomically overlooked species may be derived from the communities of *Pinus cembra* L., which belongs to *Pinus* subgenus *Strobus* and is a native of the Alps. It is speculated here that *R. salebrosus* s.l. may have switched subsequently to *P. strobus*, following its invasion. The only known host plant of *R. salebrosus* s.l. in Europe is *A. uva-ursi*, which is also an understorey plant in The Elbe Sandstone Mountains. Thus, *A. uva-ursi* may have facilitated the host switch from *P. cembra* to *P. strobus* (Horton et al. 1999; Krpata et al. 2007).

The effects of ericaceous plants

There were no statistically significant effects of ericaceous plants on colonisation and richness of EcMF in the mesocosm experiment. In field studies, ericaceous plants inhibit EcM colonisation (Walker et al. 1999; Collier and

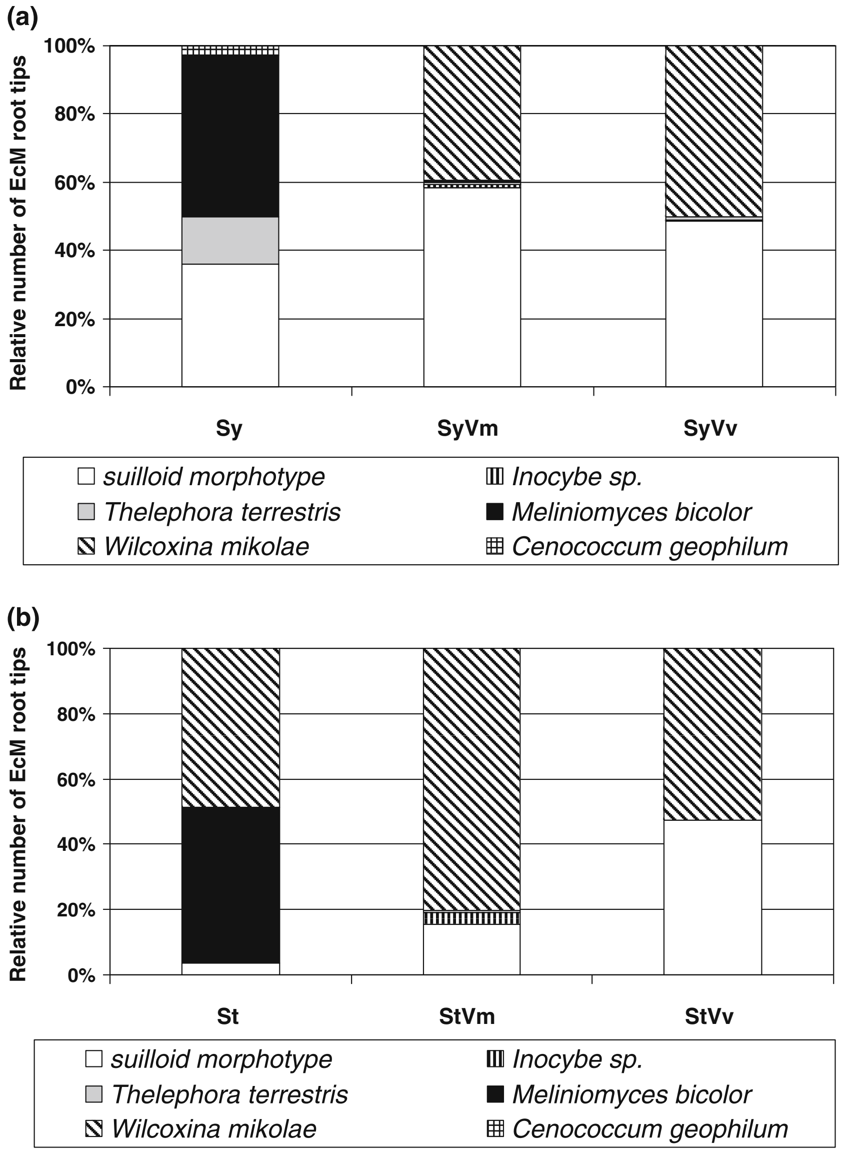

Fig. 2 **a** Relative number of EcM morphotypes associated with the roots of *P. sylvestris* grown in isolation (*Sy*) or grown with *V. myrtillus* (*SyVm*) or *V. vitis-idaea* (*SyVv*). **b** Relative number of EcM morphotypes associated with the roots of *P. strobus* grown in isolation (*St*) or grown with *V. myrtillus* (*StVm*) or *V. vitis-idaea* (*StVv*)

Bidartondo 2009) and/or reduce the number of EcMF species (Collier and Bidartondo 2009). Nevertheless, in our study, ericaceous plants had the strongest effect on EcMF community composition.

The differential effect of ericaceous plants on the EcMF communities of the two *Pinus* species can only partly be attributable to host specificity. For example, *W. mikolae* did not form an association with *P. sylvestris* but did with *P. strobus* when it was grown in isolation. However, it heavily colonised both pines when grown with ericaceous shrubs. As *W. mikolae* is regarded as a poor competitor (Mikola 1969), the fungi preferring *P. sylvestris* may have outcompeted *W. mikolae* in favourable conditions in the absence of ericaceous neighbours.

This indicates that factors such as host, surrounding vegetation and soil conditions may interact to create niches for EcMF. At the species level, ericaceous plants promoted the proliferation of one EcMF species (*R. salebrosus* s.l.) and inhibited two EcMF species (*T. terrestris* and *M. bicolor*). The selective effect of ericaceous dwarf shrubs on EcMF is intriguing and deserves further investigation because in some pine habitats, Ericaceae form dense understorey and may therefore shift EcMF communities available for pine seedlings. EcMF species that are not inhibited by the presence of ericaceous shrubs and ErMF are probably adapted to poor soil conditions (Nilsson et al. 1993; Genney et al. 2000) and form an important symbiont pool for seedling establishment on heathlands (Collier and Bidartondo 2009) and forest ground.

It is clear that ericaceous plants play an important role in the formation of EcMF communities associated with the

Table 3 Fisher's exact test based on the pattern of each EcMF occurrence in the microcosms

EcMF	Host plant effect (p)	Understorey effect (p)
Suillus bovinus	0.023	>0.999
Suillus variegatus	nd	nd
Suillus granulatus 1	nd	nd
Suillus granulatus 2	nd	nd
Suillus luteus	0.666	0.143
Rhizopogon roseolus s.l.	0.740	0.173
Rhizopogon luteolus	nd	nd
Rhizopogon salebrosus s.l.	<0.001	0.048
Meliniomyces bicolor	>0.999	<0.001
Wilcoxina mikolae	0.008	0.075
Inocybe sp.	nd	nd
Thelephora terrestris	0.002	0.022
Cenococcum geophilum	nd	nd

Species which were not abundant (less than six pots) were excluded from the analyses

nd not determined

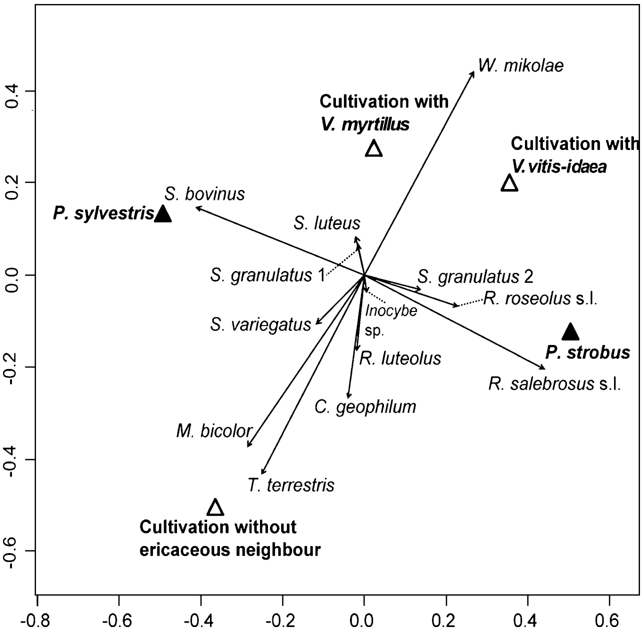

Fig. 3 NMDS ordination of EcM community data from 48 mesocosms, illustrating the effect of host trees (*filled triangles*) and cultivation treatments (*open triangles*). For better visualisation, mesocosms are not shown

roots of *P. sylvestris* seedlings. However, the effect of these changes on seedling survival and physiology needs further investigation and evaluation. Besides host specificity (Jacobson and Miller 1992), differential spore dormancy (Bruns et al. 2009) and competitive hierarchy (Kennedy et al. 2009), ericaceous plants may provide a niche for the differentiation of suilloid fungi.

The hypothesis that there would be a greater abundance of *M. bicolor* (a member of the *R. ericae* aggregate) and other ErMF as ectomycorrhizal symbionts of the pines in the Ericaceae treatments was not supported. Surprisingly, *M. bicolor* was detected as forming ectomycorrhizae almost exclusively when the pines were grown in isolation. Thus, our results suggest that rather than being unable to form ectomycorrhizal symbiosis, the competitive abilities of the respective *M. bicolor* strains were inhibited by ericaceous plants and/or ErMF. Alternatively, *M. bicolor* might display a hitherto overlooked preference for ericaceous roots.

EcM species of the *R. ericae* aggregate are among the dominant fungi in both young and mature boreal forests in northern Europe (Genney et al. 2006; Toljander et al. 2006; Korkama et al. 2007; Tedersoo et al. 2008b). Some of its members are able to form ericoid mycorrhizae and ectomycorrhizae (Vrålstad et al. 2002; Vohník et al. 2007a, b; Grelet et al. 2009) or simultaneously both types in vitro (Villarreal-Ruiz et al. 2004). Our findings are congruent with those of Collier and Bidartondo (2009) and Richard et al. (2009) who recorded no ectomycorrhizae formed by the *R. ericae* aggregate in roots of EcM seedlings in areas dominated by Ericaceae. This suggests that if any mycelial links between ectomycorrhizal and ericoid mycorrhizal plants exist under natural conditions, they are limited rather to fungi outside the *R. ericae* aggregate.

Conclusions

Our findings reveal that both host plant species identity and surrounding vegetation may influence EcMF assemblages in roots of establishing pine seedlings. Contrary to our hypotheses, the invasive and native pines associated with a comparably species-rich EcMF community and the ericaceous plants did not act as a species pool of pine mycobionts. On the contrary, the ericaceous plants inhibited ectomycorrhizae formed by *M. bicolor*, a mycobiont that is potentially shared between ectomycorrhizal plants and Ericaceae. Such changes in EcMF communities might be explained by selective stimulation/inhibition of some EcMF by Ericaceae, or a preference of some mycobionts for ericaceous to coniferous roots.

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