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Neighboring *Deschampsia flexuosa* and *Trientalis europaea* harbor contrasting root fungal endophytic communities

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Abstract Fungal endophytic communities and potential host preference of root-inhabiting fungi of boreal forest understory plants are poorly known. The objective of this study was to find out whether two neighboring plant species, *Deschampsia flexuosa* (Poaceae) and *Trientalis europaea* (Primulaceae), share similar root fungal endophytic communities and whether the communities differ between two sites. The study was carried out by analysis of pure culture isolates and root fungal colonization percentages. A total of 84 isolates from *D. flexuosa* and 27 isolates from *T. europaea* were obtained. The roots of *D. flexuosa* harbored 16 different isolate types based on macromorphological characteristics, whereas only 4 isolate types were found in *T. europaea*. The root colonization by dark septate and hyaline septate hyphae correlated with isolate numbers being higher in *D. flexuosa* compared to *T. europaea*. The different isolate types were further identified on the basis of internal transcribed spacer sequence and phylogenetic analysis. An isolate type identified as dark septate endophyte *Phialocephala fortinii* colonized 50 % of the *T. europaea* and 21 % of the *D. flexuosa* specimens. In addition, *Meliniomyces variabilis*, *Phialocephala sphaeroides*, and *Umbelopsis isabellina* were found colonizing the grass, *D. flexuosa*, for the first time and *Mycena* sp. was confirmed as an endophyte of *D. flexuosa*. Site-specific differences were observed in the abundance and diversity of endophytic fungi in the roots of both study plants, but the differences were not as predominant as those between plant species. It is concluded that *D. flexuosa* harbors both higher amount and more diverse community of endophytic fungi in its roots compared to *T. europaea*.

Introduction

Root fungal endophytes are ubiquitous, mainly ascomycetous, intraradical associates of healthy plant roots (Rodriguez et al. 2009), their functional significance for the host plant varying from mutualistic to antagonistic (Sieber and Grünig 2006; Purahong and Hyde 2010). A subgroup of root endophytes called dark septate endophytes (DSE; Jumpponen and Trappe 1998; Addy et al. 2005), which have been under active research during last decades, are likely involved in host nutrient uptake (Newsham 2011).

Various factors are known to attribute to endophytic fungal community richness (Saikkonen et al. 2010; Botella and Diez 2011; Ghimire et al. 2011). The fungal communities of roots are affected by, for example, host plant phenology (Waid 1957), season (Wilberforce et al. 2003; Perez-Naranjo 2009), and root carbohydrate status (Hadacek and Kraus 2002). In addition, several soil factors, such as pH, soil structure, nutrient availability, and water regime, play important roles (Wilberforce et al. 2003; Summerbell 2005; Sieber and Grünig 2006; Perez-Naranjo 2009). Biotic interactions of the endophytic fungi with the host plant and within the microbial and plant communities have been shown to play a role (Ahlich and Sieber 1996; Carlsen 2002; Narisawa et al. 2002; Sieber and Grünig 2006). Although the

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prevalence of root fungal endophytes is well documented and several papers exist on the diversity of root fungal endophytes in specific host plants (e.g., Summerbell 2005; Sánchez Márquez et al. 2007, 2010; Khidir et al. 2010; Sakayaroj et al. 2010; Tejesvi et al. 2010), only limited information can be found on designed comparison of endophyte communities of adjacent host plants (but see Carlsen 2002; Grünig et al. 2004; Tejesvi et al. 2010).

The boreal forests form a characteristic vegetation zone in the northern hemisphere. The understory in these forests is dominated by ericaceous dwarf shrubs with only few herbaceous plant species being present (Nilsson and Wardle 2005). Of the herbaceous plants, the perennial grass *Deschampsia flexuosa* is omnipresent and the pseudoannual forb *Trientalis europaea* is typical, although more patchily distributed (A.L. Ruotsalainen, personal observation). These plant species often grow intermixed and their roots are inhabited by root endophytic fungi, as proven, e.g., by Zijlstra et al. (2005) and Ruotsalainen et al. (2004, 2007). Despite the ubiquity of these plant species, the fungal species inhabiting their roots are surprisingly poorly known. Two reports on root endophytic fungal species of *D. flexuosa* exist (Tejesvi et al. 2010; Zijlstra et al. 2005), whereas, to our knowledge, the root-inhabiting fungal species of *T. europaea* have not been studied to date. *D. flexuosa* and *T. europaea* are phylogenetically distant and represent different life cycle (e.g., Piqueras and Klimes 1998) and life form (Raunkiær 1934) strategies. As both the host identity and the site-specific factors affect the root endophytic community, it is warranted to ask how similar or dissimilar the root endophytic fungal communities these neighboring, but otherwise quite distinct, plants harbor.

We compared the root endophytic fungal communities of *D. flexuosa* and *T. europaea* growing in a close proximity at two growth sites to gain an insight into the interdependencies of root endophyte community structures of herbaceous plants in the understory of boreal mixed forest. More specifically, we asked: (1) Do these adjacent plants have similar root fungal communities? (2) Do the communities differ between two growth sites? (3) Do the fungal isolate numbers parallel with the root fungal colonization percentages of septate hyphal types? The study was carried out by analysis of pure culture isolates and root fungal colonization percentages. The fungal isolates were identified based on internal transcribed spacer (ITS) sequencing and phylogenetic analysis. A culture-based method was chosen for this study because it allows the comparison of fungal abundance, although we are aware of the disadvantage that it is selective towards specific fungal species (Hyde and Soytong 2008; Tejesvi et al. 2010).

Materials and methods

Root sampling and isolation of the fungi

The study plants, *T. europaea* L. (Primulaceae) and *D. flexuosa* L. (Poaceae), were collected as whole, 24 specimens per plant species, with a borer of 15 cm in diameter in late autumn 2008 from two sites in Oulu, Northern Finland (65°01′ N; 25°30′ E). The plant specimens at both sites were collected from an area of 1–2 acres and they consisted of a mixed sample of different heights and flowering stages of the focal plants. Both sampling sites were *Pinus sylvestris* L.- and *Betula pubescens* Ehrh.-dominated midboreal mixed forests experiencing slight cultural impact from neighboring properties and situated at a distance of 10 km from each other. These particular sites (hereafter named as site 1 and site 2) were selected to represent two vegetationally similar, but physically separate locations. The ground layer of the sites was dominated by *D. flexuosa*, *Vaccinium myrtillus* L. (Ericaceae), and *Vaccinium vitis-idaea* L. (Ericaceae). Soil pH of site 1 was 3.9 ± 0.1 and of site 2 was 4.4 ± 0.2, conductivity was 69 ± 21 and 27 ± 14, respectively, and total nitrogen percentage was 1.50 ± 0.40 and 0.15 ± 0.13 (average$\pm$ SD), respectively ($n=5$).

The plants were brought to the laboratory and roots were washed clean of debris by subsequent washes in tap water. Roots were carefully cleaned under a dissection microscope, surface-sterilized by 3.5 % NaOCl for 15 min, and rinsed three times with sterile water. Care was taken to include only roots of good quality with no visible signs of senescence. A root sample of 10 cm in total length per study plant was cut aseptically in a laminar flow hood into 1-cm pieces, which were placed on an agar plate (9 cm in diameter). Modified 1/2 MMN medium with malt extract was used (Marx 1969) as it has been found suitable in an earlier study (Tejesvi et al. 2010). The plates were incubated at room temperature in the dark and checked frequently for fungal growth. When a newly emerged colony was observed, it was immediately transferred to a fresh plate and maintained as a pure culture in similar conditions.

Root fungal colonization assessment

The root fungal colonization of dark septate and hyaline septate type hyphae (hereafter referred to as DSE and HSE, respectively; Barrow 2003) and dark septate sclerotia (DSE sclerotia) were studied. These colonization types can be formed by several ascomycete species and can, therefore, be assumed to associate with outgrowing root endophytic community. For the study of the

intraradical colonization of roots by these fungi, and especially colorless hyphae of HSE, we used the staining method described by Phillips and Hayman (1970). Although this method is commonly used for the study of arbuscular mycorrhizal (AM) fungi, we are not reporting AM colonization because AM fungi are not culturable on artificial media (Smith and Read 2008) and, therefore, are not detected by the methodology used in this study. The staining was carried out as follows: The roots were rinsed with tap water, cleared overnight in KOH (1 % for *D. flexuosa* and 10 % for *T. europaea*) at room temperature, and rinsed with tap water. The bleaching was only performed for *T. europaea* in alkaline H_2O_2 for 15 min, after which the roots were again rinsed with tap water. Then, the root samples were acidified in 1 % HCl (1 h for *D. flexuosa* and 2.5 h for *T. europaea*) and stained in 0.01 % trypan blue in lactoglycerol for 1 h at 80°C. To obtain successful staining of fungal structures, roots of *T. europaea* required longer exposure times (Ruotsalainen et al. 2004). The colonization percentages of roots were determined under a compound microscope by using the method described by McGonigle et al. (1990) where 50 intersects per one root system were studied at the magnification of ×150–600.

Isolation of DNA from pure cultures

All pure culture isolates obtained were first classified by macromorphological characteristics (growth, color, and surface structure of the colony) into 16 isolate types. ITS sequencing and phylogenetic analysis were performed for further identification instead of micromorphological investigation. Microscopic examination of the cultures was abandoned because it would have required specialist knowledge, prolonged incubation (up to several years in cold; Ruotsalainen, personal observation) of cultures, and/or that typically root endophytic fungi do not produce identifiable conidial structures in culture (Jumpponen and Trappe 1998; Addy et al. 2005). DNA of sufficient quality was obtained for 12 isolate types, with 2 duplicates yielding in total 14 DNA samples. From five isolate types representing single isolates, DNA of sufficient quality was not obtained.

DNA was extracted from 0.5 to 1.0 g fresh mycelia according to the method described by Pirttilä et al. (2001). The target rDNA region including ITS1 and ITS2 regions and 5.8 S gene was amplified using primers ITS1 (TCCGTAGGT GAACCTGCGG) and ITS4 (TCCTCCGCTTATTGA TATGC) (White et al. 1990). Amplifications were performed in a total reaction volume of 25 μl containing 2 mM of each dNTP, 2.5 mM $MgCl_2$, 5 pM of each primer, 1 unit of *Taq* DNA polymerase (Dynazyme, Finnzymes, Espoo, Finland),

and 100 ng of template DNA. PCR amplifications were performed in a thermal cycler (PTC 200, MJ Research, Watertown, MA, USA) with an initial denaturing step of 94°C for 3 min, followed by 35 amplification cycles of 94°C for 60 s, 50°C for 60 s, and 72°C for 120 s and a final extension step of 72°C for 10 min. The amplification products were separated by electrophoresis at 100 V for 1 h in 1× TAE buffer on 1.4 % (*w/v*) agarose gel, which was stained with ethidium bromide (0.5 μg/ml) and visualized under 300 nm UV light and photographed. A 100-bp size marker (MBI Fermentas, Vilnius, Lithuania) was used as reference.

Sequencing of fungal ITS region

Amplification products obtained from PCR reactions with unlabeled ITS primers (ITS1 and ITS4) were used for sequencing. Sequencing reactions were performed with Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Extension products were then purified using the ethanol/EDTA precipitation protocol and analyzed on ABI 3100 Avant Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) as recommended by the manufacturer. DNA sequences obtained for each isolate from each forward (ITS1) and reverse (ITS4) primer were inspected individually for quality. Both strands of the DNA were then assembled to produce a consensus sequence for each isolate using the Sequencher 4.7 software (Gene Codes Corporation, Ann Arbor, MI, USA). The sequences were submitted to the National Center for Biotechnology and Information and accession numbers were obtained.

Molecular phylogenetic analysis

All sequences were compared with ITS sequences available in the GenBank by BLASTn search. The closest matches in the GenBank were included in a Clustal alignment and aligned using ClustalX with default settings (Thompson et al. 1997). A phylogenetic analysis was performed by the maximum parsimony method using Molecular Evolutionary Genetics Analysis (MEGA) (Tamura et al. 2007). Confidence in specific clades from the resulting topology was tested by bootstrap analysis with 1,000 replicates. Tree scores including consistency index (CI), retention index (RI), and rescaled consistency index (RCI) were also calculated for all trees. Branches corresponding to partitions reproduced in <50 % bootstrap replicates were collapsed. All positions containing gaps and missing data were eliminated from the dataset (Complete-Deletion Option).

Statistical analysis of the number of isolates and number of isolate types

The effect of host species (*D. flexuosa* and *T. europaea*) and growth site (two sites) on the total number of root fungal isolates per plant and on the number of isolate types were analyzed by using generalized linear models with Poisson error (McCullagh and Nelder 1989). The effect of host species and site on DSE hyphal colonization was studied by two-way analysis of variance (ANOVA). On HSE hyphal and DSE sclerotial colonization data, a generalized linear model with negative binomial error was applied because these were zero-inflated (Crawley 2007). The conditions were controlled for all analyses by using residual plots (Crawley 2007). Correlation between DSE hyphae and isolate number was checked with Pearson correlation coefficient separately for both plant species (for HSE and DSE sclerotia, the Pearson correlation was not applied because the conditions for these analyses could not be met due to zero inflation). Shannon–Wiener diversity index was calculated based on the number of fungal isolates for both host plants in a site-specific manner. The statistical analyses were carried out using R statistical program version 2.10.1 (Ihaka and Gentleman 1996; R Development Core Team 2009).

Results

Colonization frequency of root fungal endophytes

One hundred and eleven pure culture isolates were obtained altogether; 84 from *D. flexuosa* and 27 from *T. europaea*. The total number of isolates per study plant was higher for *D. flexuosa*, but there was also a significant interaction between the host species and the study site. The number of isolates in *D. flexuosa* was lower at site 2, whereas in *T. europaea*, it was higher at site 2 (Figs. 1 and 2; Table 1). The
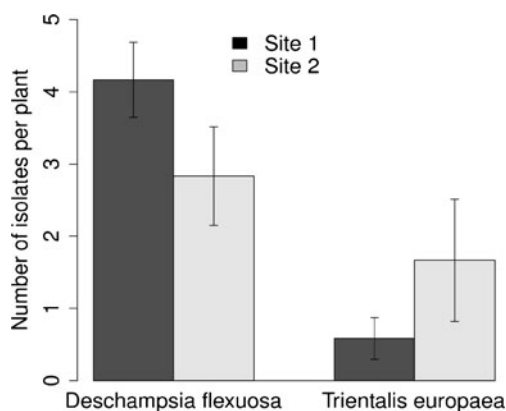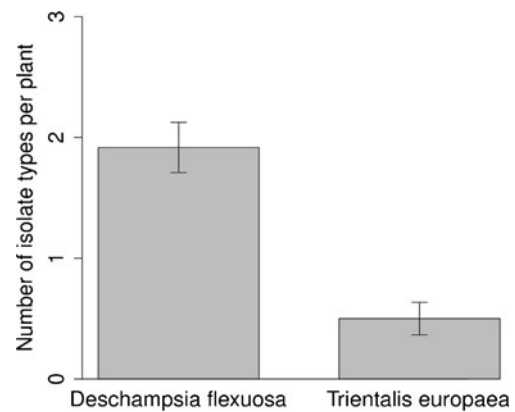

Fig. 2 Number of fungal isolate types (±SE) in *D. flexuosa* and *T. europaea*

colonization percentages of root endophytic fungi in the study plants, determined by the number of DSE and HSE hyphae, were higher in *D. flexuosa* (Fig. 3). In contrast, DSE sclerotia were more common in *T. europaea*, but there was also an interaction with the study sites (Fig. 4). There was no correlation between DSE hyphal colonization and the total isolate number in either of the study plants (*D. flexuosa* $r=-0.19$, $N=22$, $p=0.374$ and *T. europaea* $r=0.07$, $N=22$, $p=0.730$).

DNA-based identification and phylogenetic analysis

Out of the 14 sequences which were BLAST-searched (Table 2), six sequences could be identified to the species level, namely, two isolates of *Phialocephala fortinii* (DF7 and TE14), *Meliniomyces variabilis* (TE2), *Phialocephala sphaeroides* (TE5), *Umbelopsis isabellina* (DF10), *Mycena sanguinolenta* (DF11), *Mycena galopus* (DF4), and *Umbelopsis* sp. (DF3). Six isolates (DF1, DF6, DF8, TE9, DF13, and DF15) were classified at the family level, all belonging to ascomycetes.

The phylogenetic analysis was done using MEGA4. Some of the GenBank sequences that were closely related to the endophytic fungi of the present study were included in the phylogenetic analysis. In the phylogenetic tree, altogether 39 strains, which included 14 root endophytic fungi of *T. europaea* and *D. flexuosa* and 25 sequences from GenBank, were analyzed. Base frequencies across taxa (mean frequencies: $A=0.242$; $T=0.286$; $C=0.233$; $G=0.239$), as determined by MEGA4, were homogeneous. There were a total of 317 positions in the final dataset, out of which 164 were parsimony informative. The most parsimony rooted tree of the 14 most parsimonious unrooted trees (CI=0.732; RI=0.904; RCI=0.662) is shown in Fig. 5. Similar results were obtained using neighbor-joining analyses (data not shown).

Fig. 1 Number of root fungal isolates (±SE) in *D. flexuosa* and *T. europaea* roots at two studied sites

Table 1 Impact of host plant species (*D. flexuosa* and *T. europaea*) and study site on total number of fungal isolates and the number of different isolate types and colonization parameters

Source of variation	Total number of isolates[a]		Number of isolate types[a]		DSE hyphal colonization[b]		HSE colonization[a]		DSE sclerotial colonization[a]	
	F	P	F	P	F	P	χ^2	P	χ^2	P
Host species	30.72	<0.001	21.27	<0.001	24.33	<0.001	24.68	<0.001	2.59	0.107
Site	0.08	0.776	0.00	1.000	0.09	0.764	0.23	0.631	1.16	0.280
Host species×site	9.51	0.002	0.42	0.516	0.14	0.706	1.77	0.183	4.32	0.038

The residual *df* for the host species=46, site=45, and host species×site=44

DSE dark septate endophyte, *HSE* hyaline septate endophyte

[a] Analysis of deviance

[b] ANOVA

Most of the endophytic fungi isolated in this study were distributed across the phylogram into five clades. *Acephala applanata* was used as an outgroup. The first cluster consisted of 13 fungi distributed into different subgroups. DF11 and DF4 were grouped together with *M. galopus*, *M. sanguinolenta*, *Mycena haematopus*, *Mycena purpureofusca*, and *Mycena rubromarginata*, whereas DF10 and DF3 grouped with *U. isabellina* and another *Umbelopsis* species. DF15 did not cluster with any genera or species included in the study. TE9, DF1, and DF8 that were only classified at the family level as ascomycetes, grouped with other two ascomycetes species in the second clade. *M. variabilis* (TE2) grouped with other *M. variabilis* strains (EF093178 and EF093172) in the third clade with a high confidence. DF13 and DF6 that were classified as ascomycetes species grouped with other ascomycetes species strains in clade 4, whereas *P. sphaeroides* (TE5) grouped together with

another *P. sphaeroides* strain from GenBank. *P. fortinii* (DF7 and TE14) clustered together with two *Phialocephala subalpina* (EF093161), one *P. europaea*, and six *P. fortinii* strains from GenBank in the fifth clade of the phylogram.

Diversity of isolate types in host plants

The number of isolate types per study plant was higher in *D. flexuosa* than in *T. europaea* (Figs. 1 and 6; Table 1). *D. flexuosa* roots also had higher total species richness than *T. europaea* roots, as they harbored 16 different isolate types in total, whereas only 4 types were found in *T. europaea* (Fig. 6). According to the Shannon–Wiener index (*H*), both *D. flexuosa* and *T. europaea* had higher fungal diversity at site 2 (*H*=2.50 and 1.21, respectively) compared to site 1 (*H*=2.21 and 0.67, respectively). In addition, the sequencing revealed inconsistencies resulting from visual typification. One isolate type of *D. flexuosa* consisted of two separate fungal species, identified as *M. galopus* and *M. sanguinolenta* (Fig. 6). Similarly, another isolate type of *D. flexuosa* consisted of two separate fungal taxa based on
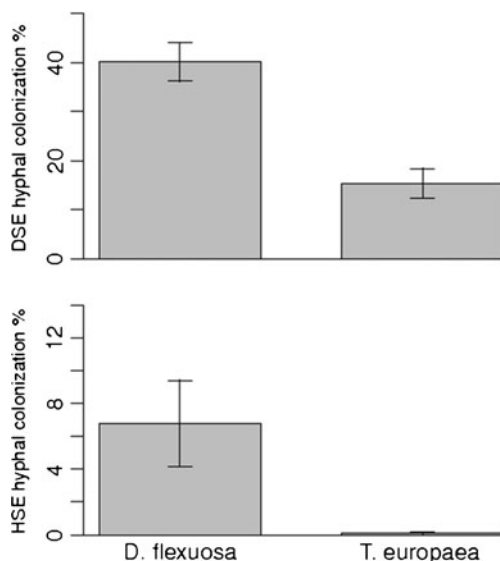
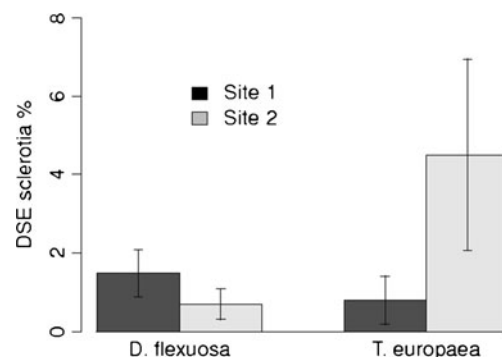**Fig. 3** The colonization percentages of DSE and HSE in roots of *D. flexuosa* and *T. europaea*

Fig. 4 The colonization percentage of DSE sclerotia in roots of *D. flexuosa* and *T. europaea* at two studied sites

Table 2 Culturable endophytic fungal ITS sequences obtained from *D. flexuosa* (DF) and *T. europaea* (TE) closely matching to the GenBank sequences

Sl. no.	Fungal isolate	Accession numbers	Closest match in the BLAST incl. score	Score	Accession numbers	E' value	Sequence coverage (%)	Maximum identity (%)
1	DF1	HQ856902	Ascomycete sp. 1	867	DQ004265	0.0	96	98
2	TE2	HQ856903	*Meliniomyces variabilis*	957	EF093178	0.0	99	100
3	DF3	HQ856904	*Umbelopsis* sp.	983	HQ637323	0.0	95	97
4	DF4	HQ856905	*Mycena galopus*	1,077	HM240534	0.0	94	98
5	TE5	HQ856906	*Phialocephala sphaeroides*	845	EU434852	0.0	92	99
6	DF6	HQ856907	Ascomycete sp. 2	841	GU062244	0.0	90	98
7	DF7	HQ856908	*Phialocephala fortinii*	942	EF446148	0.0	99	100
8	DF8	HQ856909	Ascomycete sp. 3	869	DQ004265	0.0	98	98
9	TE9	HQ856910	Ascomycete sp. 4	828	DQ004265	0.0	99	96
10	DF10	HQ856911	*Umbelopsis isabellina*	922	AB193546	0.0	97	96
11	DF11	HQ856912	*Mycena sanguinolenta*	643	FJ596764	0.0	80	88
12	DF13	HQ856913	Ascomycete sp. 5	1,053	FM207642	0.0	88	98
13	TE14	HQ856914	*Phialocephala fortinii*	883	EU314701	0.0	99	98
14	DF15	HQ856915	Ascomycete sp. 6	835	FN178471	0.0	88	95

sequencing, identified as ascomycete species 5 and 6 (Fig. 6).

Several isolate types were specific for *D. flexuosa* (Fig. 6). The only isolate type found frequently from *T. europaea* was the DSE *P. fortinii*, which was present in seven specimens (in 50 % of *T. europaea* samples; Fig. 6). In contrast, *P. fortinii* was found in 21 % of *D. flexuosa* samples (Fig. 6). In addition to *P. fortinii*, one ascomycete species (isolate number 4) was found from both host plant species at both sites (Fig. 6). *P. sphaeroides*, which belongs to the DSE fungi, was specific for site 2, being relatively abundant there in both study plants (Fig. 6).

Discussion

Markedly higher numbers of root fungal endophytic isolates were obtained from *D. flexuosa* than from *T. europaea*. This finding was supported by the higher colonization percentage of hyphae of septate endophyte types in the roots of *D. flexuosa*. In earlier studies, the DSE-type colonization percentage of *T. europaea* has been more or less higher in field conditions, although variations were found between sites (Ruotsalainen et al. 2004). DSE hyphal colonization percentage of *D. flexuosa* has earlier been reported, on average, to be 20–50 % of the root length (Zijlstra et al. 2005; Ruotsalainen et al. 2007), which corresponds well with the present results. These results suggest that *D. flexuosa* has a higher number of root fungal endophytes than *T. europaea*, estimated both by root colonization percentage and fungal outgrowth.

The number of different fungal isolate types was higher in *D. flexuosa* than in *T. europaea*, which is in accordance with the results on total fungal isolate numbers and root fungal colonization percentage. Similarly, the diversity, estimated as the Shannon–Wiener index, was also higher in *D. flexuosa*. These results altogether indicate that the root fungal community of *D. flexuosa* is more diverse compared to neighboring *T. europaea*. Based on the sequencing results, our isolate morphotypes may include multiple, unrelated species and, therefore, the absolute numbers of fungal taxa given in this study should be treated as indicative. The root endophyte communities of *D. flexuosa* have earlier been studied by Vrålstad et al. (2002) and by Zijlstra et al. (2005). Although those studies reported several helotialean fungi in the roots of *D. flexuosa*, the same fungal taxa were not found in our study, except for *P. fortinii*, a fungus belonging to the DSE complex (Jumpponen and Trappe 1998). This can be partly due to markedly increased accuracy of fungal endophyte identification by sequences available in the GenBank today or because of differences in methodology, as culturing methods are always selective (Hyde and Soytong 2008). On the other hand, *P. fortinii* dominates the root endophyte community of *D. flexuosa* when analyzed by a culture-based method (Tejesvi et al. 2010).

Besides differences in root fungal abundance between the plant species studied, the number of fungal isolates and the percentage of dark septate fungal sclerotia in roots were affected by the sampling site. These differences were most distinctive for *T. europaea*. Based on general vegetation analysis, the sampling sites were relatively identical (A.L. Ruotsalainen,
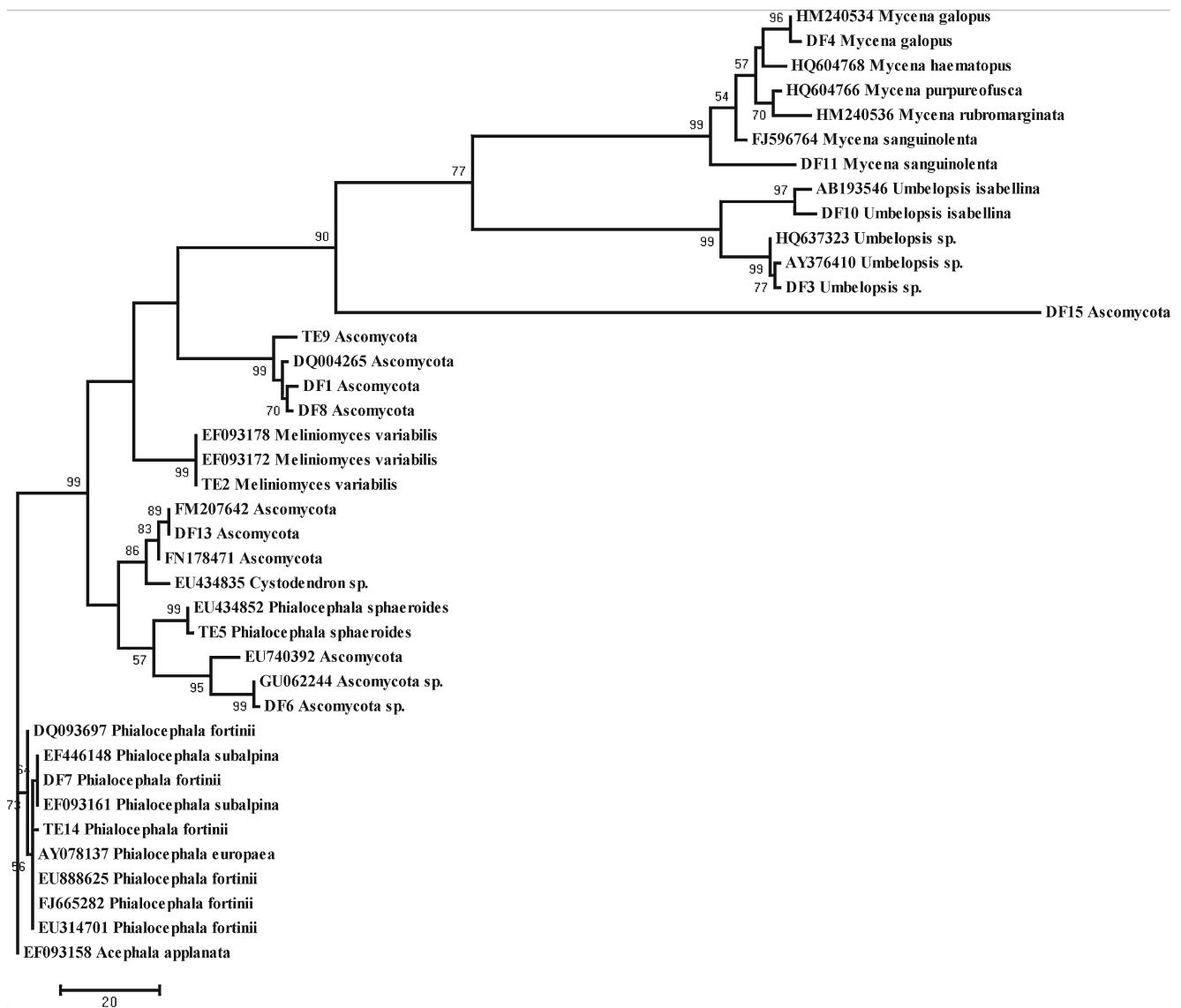
Fig. 5 Phylogenetic analysis of ITS and 5.8 S rDNA sequences of root endophytic fungi of *D. flexuosa* and *T. europaea*. The tree was derived from 14 endophytic fungal sequences and 25 sequences were retrieved from GenBank. Branch lengths are scaled in terms of expected numbers of nucleotide substitutions per site, number at branches are bootstrap values (1,000 replicates, values below 50 % are not shown). The sequences from GenBank are given with accession numbers followed by names

personal observation), but the soil parameters indicate subtle differences. Generally, substrate pH and nutrient availability strongly affect the performance of fungi (Deacon 2006). High nutrient availability has been reported to decrease the diversity of root endophytic community (Wilberforce et al. 2003). According to Postma et al. (2007), DSE colonization increased in relation to AM colonization along a decreasing pH gradient. The DSE *P. fortinii* is also known to prefer acidic soils (Ahlich et al. 1998). Therefore, such soil factors may contribute to differences observed in the fungal communities at each site, which also were more profound in *T. europaea* than in *D. flexuosa*. However, as the number of sampling sites was only two in our

study, this conclusion must be interpreted cautiously. Therefore, there is preliminary evidence that the growth site has a modifying impact on intraradical fungal communities and more distinct differences can be found between adjacent plant species within these sites.

To our knowledge, there are no earlier studies on root endophyte communities of *T. europaea*, although root fungal colonization rates have been reported earlier (Ruotsalainen et al. 2002). No fungal isolates specific only for *T. europaea* were identified in this study, when compared to *D. flexuosa*. The fungal isolates of *T. euro-paea* were identified as *M. variabilis*, *P. fortinii*, *P. sphaeroides*, and ascomycete species 4 and they were

Fig. 6 Number of host plant
specimens which yielded fungal
outgrowth at studied sites 1 and
2. Isolate names are based on
the closest match of ITS in
GenBank, isolates U1–U5 not
identified due to poor DNA
quality. *M. galopus* and *M.
sanguinolenta* were visually
classified into one isolate type
but could be separated by
sequencing. Ascomycete
species 5 and 6 were visually
classified as one isolate type,
but sequencing indicated
heterogeneity within this type,
which cannot be separated in
the figure
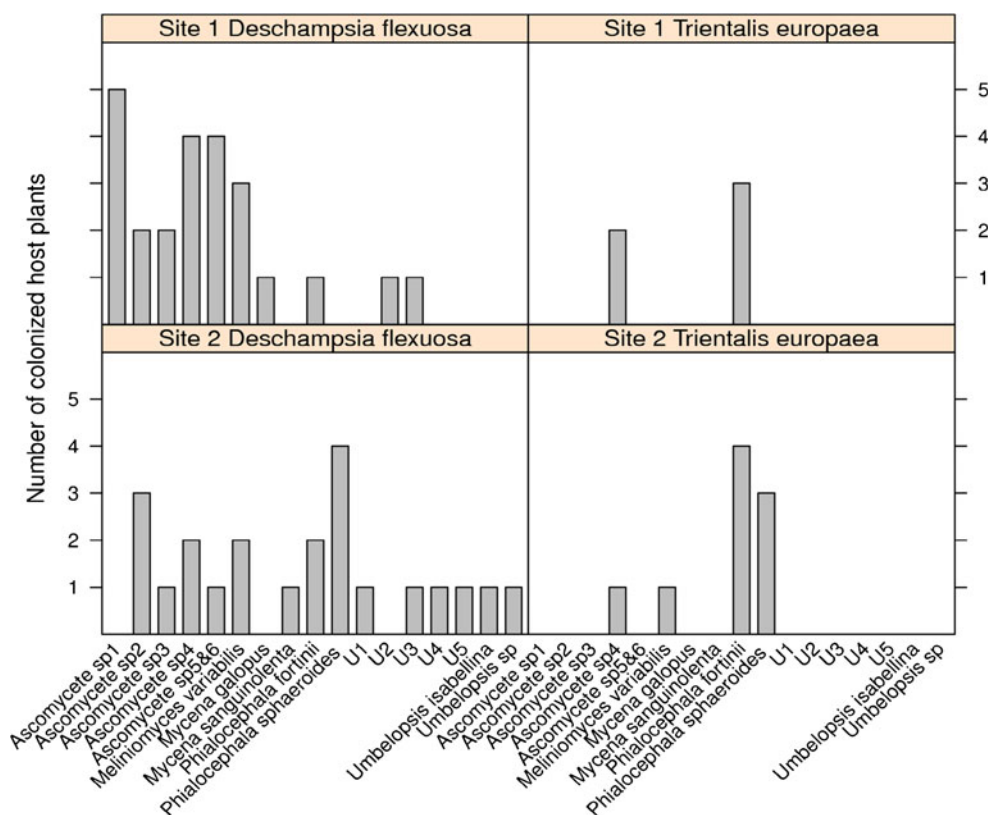
found in both study plant species, and *P. fortinii* and
ascomycete species 4 were also present at both study
sites. *P. fortinii* is an ubiquitous DSE fungus found in
plants of wide taxonomic range (Jumpponen and Trappe
1998, Addy et al. 2005; Tejesvi et al. 2010).

A few strains were isolated from *D. flexuosa* in this study for
the first time. *M. variabilis* has earlier been reported to colonize
several plant hosts (Ohtaka and Narisawa 2008), especially
ericaceous plants (Hambleton and Sigler 2005), but to our
knowledge, this is the first report from a grass. Similarly, *P.
sphaeroides*, which is considered to belong to the DSE group,
has been described quite recently from trees and forbs from
Sphagnum-dominated, acidic wetland sites (Wilson et al.
2004). To our knowledge, our finding is the first from a grass.
The presence of fungal isolates identified as *M. galopus* and *M.
sanguinolenta* in *D. flexuosa* roots is somewhat surprising, as
the members of this basidiomycete genus are generally consid-
ered saprophytic (Emmett et al. 2008). However, Sánchez
Márquez et al. (2006) reported a *Mycena* sp. as an endophyte
in the grass *Dactylis glomerata*, and Tejesvi et al. (2010) also
found a *Mycena* species (*Mycena galericulata*) as the root
endophyte in *D. flexuosa*. Some *Mycena* species can form an
association with nonphotosynthetic orchids, being parasitized
in this case (Ogura-Tsujita et al. 2009). These observations
support our finding of *Mycena* species being a root endophyte
and may indicate a new, yet undiscovered, association between
this species and *D. flexuosa*. Two strains were identified as

Umbelopsis (*Mortierella*) *isabellina*, which also is a soil sap-
rophyte belonging to Mucorales (Meyer and Gams 2003), but
earlier, Summerbell (2005) has reported *U. isabellina* as a root
endophyte *Picea mariana* (Pinaceae). It is also worth mention-
ing that the interpretation of our results at a detailed taxon level
should be done with slight caution, as the names of sequences
in GenBank may be erroneous (Ko Ko et al. 2011).

To conclude, our data show that *D. flexuosa* and *T. euro-
paea* living in close proximity within a relatively small area
harbor root fungal endophytic communities which differ in
diversity and richness and are, to some extent, affected by the
growth site. Grasses and forbs have major differences in their
life cycles and survival strategies (Raunkiær 1934). For ex-
ample, their adaptive responses to herbivory and to the suc-
cessional changes in the environment have marked differences
(Grime 1979; Tilman 1982). The variability in growth
responses may be related to species-specific differences in
the plant metabolism and phenology, which are, in turn,
known to affect the endophytic community (Hadacek and
Kraus 2002; Wilberforce et al. 2003). For example, grasses
depend more on sugar accumulation, whereas forbs depend on
both sugar and K+ for osmotic adjustments, which may also
influence the presence of various fungal endophytes inside the
plant (Barnes et al. 2007). Furthermore, *T. europaea* has a
pseudoannual life cycle (Taylor et al. 2002), which means that
the fungal inoculation of new emerging roots must be received
via surrounding soil and roots.

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