Determining species diversity of microfungal communities in forest tree roots by pure-culture isolation and DNA sequencing

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Abstract

Pure-culture isolation from roots was compared with transformation of total DNA from roots followed by sequence analysis of ITS 1/2 rDNA of representative clones as methods for determining the abundance and composition of microbiota in roots of *Betula pendula, Fagus sylvatica, Larix decidua, Prunus serotina* and *Quercus petraea*. The results from the two methods differed greatly, with no overlap between the taxa identified. Pure-culture isolation revealed greater species diversity (47 taxa), the most frequent fungi being *Ascomycota*, including *Penicillium* spp., *Phialocephala fortinii*, *Pochonia bulbillosa*, *Sesquicillium candelabrum* and *Trichoderma* spp. Transformation of total DNA and sequencing revealed less diversity (22 taxa), the most frequent taxa being *Basidiomycota*, including *Coprinus fissolanatus* and *Mycena* spp., and *Ascomycota*, including *Podospora–Schizothecium* spp., *Helgardia anguioides* and *Microdochium* sp. Communities characterized by either method showed slightly greater fungal diversity and less species dominance on *F. sylvatica* than on roots of other trees, whilst DNA sequencing showed least diversity and greatest species dominance on *Q. petraea*.

Keywords: Diversity, DNA sequencing, Forest trees, Fungi, Morphology, Pure-culture isolation, Roots.

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1. Introduction

Fungi and fungus-like organisms inhabiting roots play a key role in forest ecosystems and constitute an essential part of forest biodiversity. Together with the soil and rhizosphere organisms they represent a significant biological potential. They form associations with plants that are beneficial, pathogenic or neutral. They have a significant impact on plant health, growth and productivity (Peace, 1962; Wilcox, 1983; Mańka, 1998; Sinclair et al., 1987).

Most studies of fungi inhabiting trees roots have focussed on mycorrhizal species (Clapp et al., 1995; Gloud, 2000; Dahlberg, 2001; Horton and Bruns, 2001; Taylor, 2002; Allen et al., 2003; Brundrett, 2004; Bruns and Shefferson, 2004; Koide and Mosse, 2004; Simard and Durall, 2004; Menkis et al., 2005). Only a few studies have investigated other than mycorrhizal diversity. Kwaśna (1996 a, b; 1997 a, b; 2001, 2002, 2003, 2004) investigated microfungal communities on/in tree roots in relation to their involvement in resistance to root pathogens and changes in their activity resulting from different forest management practices. Microfungal communities in roots have also been studied in relation to decline or death of *Fraxinus* and *Quercus* trees in Europe (Jung et al., 1996; Przybył, 2002; Halmschlager and Kowalski, 2004), and to decay of conifer seedlings (Hamelin et al., 1996; Kernaghan et al., 2003; Menkis et al., 2006).

Information on density, diversity and functioning of fungal communities in tree roots still remains limited. Greater understanding of the processes of fungal establishment, survival, and relationships within communities, particularly in the early stages of forest successions, is required and has implications for future forest practices (Horton et al., 1999).

Effective detection and classification are important prerequisites for studies on the ecology and behaviour of microorganisms. Morphological species recognition (MSR) (Taylor et al., 2000), used in classical taxonomic classification, based on the

morphology of sexual or asexual reproductive structures, has several shortcomings. It often does not consider adequately the within–taxon variability and is useless in cases of morphological similarity, indistinctness, sterility of fungi and the presence of non-culturable organisms (Wetzel et al., 1996; Harney et al.,1997; Sieber, 2002). Therefore misclassification is possible. Despite that, the classical approach has led to recognition of the ecology and activity of some important tree-root fungi, including pathogens (Holdenrieder and Sieber, 1992; Guillaumin et al., 1993; Erwin and Ribiero, 1996; Woodward et al., 1998; Vettraino et al., 2002). Phylogenetic species recognition (PSR), with the concept of genealogical concordance, now offers additional tools for species definition and recognition (Taylor et al., 2000). PCR–based molecular methods used increasingly in studies on the diversity of root microbiota can detect latent pathogens, slow-growing endophytes and non–culturable species (Donaldson et al., 1995; Hamelin et al., 1996; Hantula et al., 2002; Kernaghan et al., 2003; Menkis et al., 2006).

Identification based on the 18S rRNA, useful in bacteria, is problematic in fungi and mostly limited to genus or family level. This is because of the lack of variation in conserved regions within 18S rRNA, resulting from the relatively short period of fungal evolution compared with that of bacteria (Hugenholz and Pace, 1996). This makes the identification of closely related members in most fungal genera impossible. Therefore reference sequences for fungal 18S rRNA in databases are limited, with interest in other sequences, such as the ITS rRNA region, increasing.

The ITS 1/2 rRNA (internal transcribed spacer) is the non-coding rRNA region located between the 18S rRNA and 28S rRNA genes and incorporating the 5.8S rRNA gene. Its advantage is a fast rate of evolution, resulting in greater sequence variation between closely related species. Fungal ITS sequences generally provide good taxonomic resolution and so are useful in studies on relationships between fungi at lower taxonomic levels (Gardes and Bruns, 1993: Cullings and Vogler, 1998; Redecker et al., 1999; Lord et al., 2002; Anderson et al., 2003 a; Roose–Amsaleg et al., 2004).

Cloning and sequencing of ITS 1/2 rDNA PCR products have shown that the alternative classical method provides only a selective view of fungal diversity in soil (Borneman and Hartin, 2000; Viaud et al., 2000; Anderson and Cairney, 2004; Hunt et al., 2004). Cloning and sequencing 18S rDNA and ITS 1/2 rDNA from fungal communities in roots of grasses showed high diversity in the mycobiota and resulted in the discovery of novel fungal lineages at higher taxonomic levels (Vandenkoornhuyse ey al., 2002; Neubert et al., 2006). All known fungal phyla were represented and a large

proportion of fungi detected could not be related to any known rDNA gene sequence. This raises questions about fungal diversity on/in roots of other plants, about the ecological implications of the occurrence and roles of novel taxa in specific ecological niches.

This prompted a study on fungal communities from tree roots in which a classical approach (isolation, culturing and identification of fungi by morphology) and a molecular approach (isolation of total DNA from roots and amplification, cloning and sequencing of ITS 1/2 rDNA) were both applied to limit the possibility of overlooking components of fungal biodiversity. The objective was to demonstrate and compare biodiversities of the mycobiota of tree roots using the two different methods. This was intended to increase our understanding of (i) relationships between plants and the mycobiota, (ii) the type of association involved, whether an active interaction resulting from nutritional preferences or passive and accidental contact, and (iii) spatial heterogeneity of mycobiota. We hypothesized that the two methods would identify two different community structures from each root system but with many species overlapping. Sampling different tree species of the same age and from one location was intended to eliminate the effects of environmental differences and ensure similarity of habitat. Implications for the ecological significance of the composition and diversity of the communities identified by each method might then be obtained.

2. Materials and methods

2.1. Sampling site

A root complex was collected from the B horizon (20–30 cm deep) from each of five 6–year–old trees of five species: *Betula pendula* Roth., *Fagus sylvatica* L., *Larix decidua* Mill., *Prunus serotina* Ehrh. and *Quercus petraea* Liebl. The trees were grown within a 4700–ha conifer–hardwood complex, after *Pinus sylvestris* L., in the Zielonka Forest District, western Poland (17^{o} 10’ E, 52^{o} 50’ N). The different species were evenly distributed among each other. The distance between sampled plants was 5–6 m. The soil profile was a podzol with very dark mineral horizon rich in organic matter (cryptopodzol). The thickness of the humus horizon was (8.5–) 10 (–12.5) cm, with a pH value in water of 4.1 and a C/N ratio of about 30. The mineral soil was a sandy loam

with particle fractions: 3% gravel 20 mm, 7% gravel 6 mm, 28% sand, 33% fine sand, 21% silt, 8% clay. Humus stains reached a depth of 40 cm and decalcification was observed below 200 cm. The sampled horizon had a weak structure and was coarsely grained with sands. The physical and chemical properties of the soil were similar under all trees sampled.

The ground–cover vegetation was mainly *Deschampsia flexuosa* (L) Trin., *Brachypodium* and *Calamagrostis epigeios* (L.) Roth.

2. 2. Root samples

Roots were shaken to remove loosely adhering soil, washed three times for 10 min in sterile distilled water and dried on blotting paper. Fifty pieces of thin, suberized roots, 1 cm long and 2–5 mm in diameter, were cut off from five root complexes of each tree species (10 pieces from each complex). Twenty-five pieces were used for DNA extraction and another 25 for fungal isolation in culture.

2. 3. DNA extraction, amplification and sequencing

Root pieces were freeze–dried and ground in liquid nitrogen. The total DNA was extracted using the method of Ward et al. (2005), followed by additional purification with SureClean (Bioline Ltd, London, Cat. No. BIO37042). PCR amplification of the ITS 1/2 rDNA was done with DNA diluted (10^{-2}) in deionized water. Primers used were: ITS 4 (5' TCC TCC GCT TAT TGA TAT GC) and ITS 5 (5' GGA AGT AAA AGT CGT AAC AAG G) (White et al., 1990). Each 25 µl PCR mixture consisted of 0.2 µM of each primer, 0.25 U of *Taq* polymerase (MBI Fermentas, St. Leon–Rot, Germany), buffer (10 mM Tris–HCl pH 8.8, 50 mM KCl, 0.08% Nonidet P–40, 0.1 mg ml^{-1} BSA, 1.5 mM $MgCl_2$), 0.2 mM deoxyribonucleoside triphosphates (dNTPs) and 2 µl (200 ng) diluted total DNA. Cycling conditions were: an initial denaturation at 94^{o}C for 10 min, followed by 30 cycles of 94^{o}C for 30 s, 42^{o}C for 1 min and 72^{o}C for 2 min, and a final extension of 72^{o}C for 10 min. The PCR products were checked by electrophoresis of 5 µl of product in a 1% agarose gel containing ethidium bromide (0.5 µg ml^{-1}) to stain the DNA. PCR products were purified using the MinElute PCR purification kit (Qiagen, Crawley, UK) and cloned into pGEM–T Easy (Promega Corporation Madison, WI, USA) following the

manufacturer's instructions. The rDNA amplicons from each tree species were used to generate individual libraries. Inserts (100 for each library) were primarily selected in blue/white screening on X–gal medium. For each insert a small amount of culture from a transfected cell was added to 300 µl 10 mM Tris pH 8, boiled for 10 min and centrifuged. Each insert was next amplified by PCR with primers ewfitsrev 1 (5' CTC CGC TTA TTG ATA TGC TTA AAC) and ITS 5 using the protocol described above. The amplified ITS 1/2 rDNA region included 600–650 bp. Representative clones were selected in two separate RFLP analyses of ITS 1/2 rDNA with *Cfo*I and *Hae*III restriction enzymes. Digestion was carried out at 37^{o}C for 20 h. The products were electrophoresed in gels (2% NuSieve agarose, Cambrex, BioScience, Wokingham, UK + 1% standard agarose) in 1 x TBE stained with 0.5 µg ml^{-1} ethidium bromide.

Restriction digestion patterns were compared for each library. DNA band sizes were determined by comparison with bands of a *ΦX*174 DNA *Hae*III digest and the Low DNA Mass Ladder (Invitrogen Ltd, Paisley, UK) using GeneTools gel analysis Software, (Syngene). An operational taxonomic unit (OTU) was defined as a group of clones with identical restriction digestion pattern. All members of a single OTU in an individual library were considered to belong to the same taxon. Plasmid DNA was prepared from representative clones from each OTU with QIAprep spin miniprep kit (Qiagen, Crawley, UK).

Plasmids were sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (AB Applied Biosystems, Foster City, CA 94404, USA) using primer ITS 1 (5’ TCC GTA GGT GAA CCT GCG G) and M 13 fwd (5’ GCC AGG GTT TTC CCA GTC ACG A). The purified products were run at the DNA Sequencing Facility, Oxford University, UK (http://polaris.bioch.ox.ac.uk/dnaseq/index.cfm). Each sequence was identified to the lowest taxonomic rank common to all of the top BLAST hits using the taxonomy of Kirk et al. (2001).

Chimeric sequences, which comprised at least two partial sequences resulting in cross-over artefacts, were excluded on the basis of variability introduced by them within a set of reference sequences. The reference sequences used were the closest relatives to the chimeric sequence. They were obtained from GenBank database with BLAST. A comparison of reference sequences provided variability within references. This was compared with the variability between chimeric and reference sequences. Comparisons were performed on fragments of the full-length sequences. Multiple alignments were

performed by CLUSTALW (Thompson et al., 1994) followed by manual inspection of its results. The process of alignment and chimera evaluation was automated by a program Ccode (Chimera and cross–over detection and evaluation) (http://www.irnase.csic.es/users/jmgrau/index.html).

DNA sequences were assembled using the STADEN package (Medical Research Council, Laboratory of Molecular Biology, Cambridge, UK). Representative sequences for each OTU were queried against GenBank database using BLAST. Sequences with most similarity were downloaded from GenBank (Table 1) and used for alignment using the GCG programs PILEUP (gap creation penalty = 4, gap extension penalty = 0.2) and CLUSTALX (Thompson et al., 1997). The alignments were edited manually using GENEDOC (Nicholas and Nicholas, 1997). Ambiguously aligned regions were excluded from the alignment. Phylogenetic analyses were carried out using programs in PHYLIP version 3.6 (Felsenstein, 2004). Genetic distances between pairs of fungi were calculated with the program DNADIST using the Kimura two–parameter method. Phylogenetic trees were constructed using the distance method NEIGHBOR using the original data set and 1000 bootstrapped data sets generated by the program SEQBOOT. Trees were displayed using TREEVIEW (Page, 1996).

Sequences were deposited in the Rothamsted Research database under the accession numbers of their OTUs (Table 1).

2.4. Isolation and identification by morphology of fungi in pure culture

Root fragments (25 from each tree species) were cut into 0.5 cm pieces and placed on synthetic nutrient agar (SNA: Nirenberg, 1976; 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4 \cdot 7 H_2O$, 0.5 g KCl, 0.2 g glucose, 0.2 g sucrose, 20 g agar, 1 L distilled water). SNA is a low-nutrient agar that stimulates sporulation of a vast range of fungi and fungus-like organisms and guarantees a high level of effectiveness in their detection (Bateman and Kwaśna, 1999; Kwaśna 2001, 2002, 2003). Plates were incubated at 20^{o}C for 5 days in darkness, at 20^{o}C for a further 7 days under continuous black (near-ultra violet) light, and at 20–25^{o}C for a further 30 days in natural day/night conditions. Fungi growing from the root pieces were identified on the basis of their morphology on 2% potato dextrose agar (PDA), Czapek solution agar (CzA), Czapek yeast autolysate agar (CYA), 2% malt

extract agar (MEA), 1% carrot decoct agar (CDA) and SNA using microscopy (Domsch et al., 1980; Pitt, 1980; de Hoog et al., 2000 a, b).

2. 5. Statistical analyses

Species richness and structure of fungal communities were determined for each tree species. Isolates of the same species or clones with the same OTU were grouped, and the frequency of each species or OTU was determined. A number of diversity indices (Magurran, 1988) were calculated for each community or library. These indices included three different species richness indicators: (i) the total number of species in the community or OTUs per library, (ii) Shannon's diversity index (*H'*), a general diversity index that considers both species richness and evenness, and (iii) Margalef's index (D_{Mg}), which shows richness from the ratio between number of species or OTUs and their ln function (Magurran, 1988; Hill et al., 2003). Three different indices were also calculated for evenness and dominance: (i) Shannon's evenness index (*E*), which is the ratio of Shannon's diversity index to the maximum possible value with the observed number of species or OTUs, (ii) Simpson's index (*D*), which gives the probability that two isolates or clones chosen at random will be from the same species or OTU, and (iii) Berger-Parker's index (*d*), which is the relative abundance of the most abundant species or OTU (Magurran, 1988; Hill et al., 2003). The similarity between fungal communities on roots of any two tree species was determined by calculating the qualitative Sorensen's similarity index (C_N) from the number of co-occurring species or OTUs (Magurran, 1988; McCaig et al., 1999).

3. Results

3. 1. Fungal identification by DNA cloning and sequencing

The total DNA was extracted from roots of *B. pendula, F. sylvatica, L. decidua, P. serotina* and *Q. petraea*. The ITS 1/2 rDNA was amplified from the total DNA of each tree species and cloned into pGEM–T Easy. One hundred clones from each library were analysed by RFLP, separately with two restriction enzymes: first with *Hae*III, then with *Cfo*I.

In five libraries (one for each tree species) there were 55 unique restriction digestion patterns obtained with *Cfo*I. The number of clones with unique restriction digestion pattern in one library ranged from 1 to 84 (Table 1). The number of unique restriction patterns with *Cfo*I designated to one species ranged from 1 to 5. In total, 47 clones representing 31 unique and the most frequent restriction digestion patterns with *Cfo*I were sequenced. Between six (*L. decidua*) and 19 (*F. sylvatica*) clones per library were sequenced.

The entire lengths of the 47 sequences were used as queries in BLAST searches to find related sequences (Table 1). A threshold of 97% similarity was used as an approximation to differentiate closely similar sequences at the species level. This is analogous to the practice used to distinguish bacterial species by their 16S rRNA sequences. It has also been reported that the level of intraspecific variation in the *Basidiomycota* commonly ranges from 0 to 3% (Zervakis et al., 2004).

Thirty sequences (64%) represented fungi with at least 97% similarity to database entries from other, unrelated studies. Seventeen sequences were novel, with novelty defined by the difference criterion of at least 3%. Twenty-two distinct taxa were detected by cloning and sequencing (Table 2).

Phylogenetic analysis was performed in order to insert the sequences into a molecular taxonomic framework provided by the closest matches from the databases. The analysis was restricted to 200 bp and included the 5.8S DNA and short lengths of ITS1 and ITS2. The phyla *Mycota*, including *Ascomycota* and *Basidiomycota* (larger clade)*, Protozoa* and *Oomycota* (smaller clade) were correctly separated (Fig. 1). Sequences within the same class or order grouped together. Most of the OTUs (79%) grouped closely with their BLAST database matches. Seven OTUs linked to *Podospora–Schizothecium* complex, and six OTUs to *Helgardia anguioides* (*Ascomycota*). Four and nine OTUs linked to *Mycena* spp. and *C. fissolanatus* (*Basidiomycota*). *Protozoa* with *Polymyxa graminis* and *Oomycota* with three different *Pythium* species formed two separate sub-clades with very strong bootstrap support. The *Ascomycota* and *Basidiomycota* comprised several novel sequences based on the 3% difference criterion. They include HK 413, HK 411, HK 403, HK 406, HK 388, HK 374, EW 817, HK 405 and HK 378. They were located with no context anticipated from the BLAST search, or, if located, had low similarity matrix values to the closest matches. A distinct branch for HK 405 suggests novelty at a lower taxonomic level. OTUs of

Basidiomycota were located mostly on shorter branches, relatively close to their database matches.

3. 2. Morphological identification

A total of 522 isolates was detected on SNA from the thin, suberized roots of *B. pendula, F. sylvatica, L. decidua, P. serotina* and *Q. petraea.* An average number of isolates from a root ranged from 2.8 in *Q. petraea* to 5.4 in *F. sylvatica*. The number of isolates from a single tree ranged from 69 in *Q. petraea* to 133 in *F. sylvatica*. Forty-nine distinct fungal taxa were recorded by morphotyping and morphological identification. The number of fungal taxa detected ranged from 12 in *L. decidua* to 32 in *F. sylvatica*. Most fungal isolates (86%) were identified at least to genus level. The most frequently isolated fungi were members of *Ascomycota* (*Penicillium* spp., *Phialocephala fortinii*, *Pochonia bulbillosa*, *Sesquicillium candelabrum* and *Trichoderma* spp.).

Fungal communities from different plant species had component fungi in common. Twenty-four fungal species (51%) were recorded from a single tree species, six fungal species from two tree species, and 11, four and two fungal species from three, four and five tree species, respectively (Table 3).

3. 3. Fungal community structure

Twice as many taxa (47) were identified by pure-culture isolation as by sequencing ITS 1/2 rDNA from representative clones (22) (Tables 2, 3). The total number of taxa in the combined data set was 69, of which most (85%) were identified at least to genus level. There was no overlap between taxa identified by the two methods.

The relatively small number of fungal taxa identified by the molecular method and the infrequent occurrence of most of them resulted in relatively small diversity indices based on species richness (D_{Mg}) and the proportional abundance of species (*H*') (Table 4). Species richness was less on roots of *Q. petraea* than on roots of other species. The dominance of one taxon in these communities resulted in small values for Shannon's evenness index (*E*) and high values for dominance indices (*D* and *d*). Most unevenness occurred in the community from *Q. petraea*, which was dominated by *Coprinus* and had the least diversity (Fig. 2). There was most similarity in community structure between *B.*

pendula and *L. decidua* and least similarity between *F. sylvatica* and *Q. petraea* (Table 5).

A different community structure was identified by morphology, resulting in different diversity and evenness indices (Table 4). Species richness was smaller on roots of *L. decidua* than on roots of other species. No dominance of one taxon resulted in high values for Shannon's evenness index (*E*) and low values for dominance indices (*D* and *d*). There was most unevenness in communities from *Q. petraea* and *L. decidua*, which were dominated by *Penicillium* spp. There was most similarity between communities from *L. decidua* and *P. serotina* and least similarity between those from *L. decidua* and *Q. petraea* (Table 5). *Fagus sylvatica* tended to have the most diverse and most even communities according to both methods.

4. Discussion

Microbiota from roots of five species of 6-year-old forest trees were characterized by (i) cloning of ITS 1/2 rDNA PCR products isolated from root-associated organisms and sequencing of representative clones, and (ii) pure-culture isolation from individual root pieces, which involved morphotyping and identification on the basis of morphology. Similar numbers of clones and isolates were characterized by each method (approx. 500 per method).

The two methods detected 69 fungal species that were mostly root saprotrophs. The number of taxa detected by the molecular method was 22 and by pure-culture isolation was 47. The detection of a greater number of fungal species by culturing and morphotyping than by the PCR-based molecular method is consistent with other studies (Burke et al., 2005; Allmer et al., 2006; Menkis et al., 2006).

The total number of species recorded seems to relate to the age of the trees. Greater species richness has been found in mature trees: 90-year-old *Pseudotsuga menziesii* (Mirb.) Franco and *Tsuga heterophylla* (Raf.) Sarg., and 350-400-year-old *Abies* yielded 200 and 80 morphotypes, respectively (Luoma et al., 1997; Bidartondo et al., 2000). Younger trees usually yield fewer than half the numbers of fungi that occur in mature trees (Baxter et al., 1999).

There were large differences in the abundance and composition of microbiota determined by molecular method and the pure-culture isolation, and there were no species common to both communities. In other studies, only two species of fungi from soil were common to 67 taxa culturable on agar and 51 taxa detected by PCR–RFLP of ITS 1/2 rDNA (Viaud et al., 2000). Six years later, 10.7% of taxa from decayed conifer seedling roots were common for communities identified using classical and molecular approaches (Menkis et al., 2006). This suggests that as more sequence information becomes available in the public databases the species overlap between communities found by classical and molecular approach is being increased. Our results do not support this trend, however. The absence of overlapping species may indicate inefficiency in both techniques. In pure-culture isolation, this may result from the specificity of microbiota colonizing roots of trees. It is often dominated by endophytes and ectomycorrhizal species that are difficult to culture, have specific, often unknown, nutritional and environmental preferences and are often suppressed by more vigorous and aggressive taxa. Inefficiency of PCR amplification may result from incompatibility of primers unable to amplify DNA with complicated structure or heterogeneous nature. The problem posed by heterogeneous groups of fungi has been countered by using degenerate primers (Jacobsen et al., 2005) or including degenerate nucleotides in the primer sequences (Landgraf, 2006).

Cloning and sequencing representative clones from root-associated organisms resulted in the detection of members of *Ascomycota, Basidiomycota*, *Oomycota, Protozoa*, but no *Zygomycota* were found.

Most diversity and differentiation was found in the *Ascomycota. Podospora glutinans, H. anguioides* and *Microdochium* sp. from, respectively, the orders *Sordariales, Helotiales* and *Xylariales* were the most common. *Cladophialophora* sp., *Lewia infectoria*, *Ophiosphaerella* sp. and *Tetracladium furcatum* occurred rarely and only in roots of one tree species. *Podospora glutinans* was detected in roots of *B. pendula, F. sylvatica* and *L. decidua*, suggesting that it occurs commonly, at least in the area of study. *Schizothecium miniglutinans* was also detected in roots of *F. sylvatica*. The *Podospora–Schizothecium* complex includes coprophilous fungi reported so far only from opossum and rabbit dung in New Zealand and Australia (CBS 113105) (Bell and Mahoney, 1997) and bearberry (*Arctostaphylos uva-ursi* (*Ericaceae*) in Switzerland (CBS 134.83). The current record is the first for these fungi in trees tissúes. *Helgardia anguioides* and *Microdochium* sp. were detected in roots of *B. pendula, F. sylvatica, L.*

decidua and *Q. petraea*. These fungi were so far known only from cereals and grasses. Their occurrence in tree roots may have resulted from their growth in the proximity of roots of grasses in the ground-cover vegetation. Such a phenomenon is not unknown. Kennedy et al. (2003) showed that formation of mycorrhizal networks between the canopy trees and understorey plants in an evergreen forest is possible and even common. According to Johnson et al. (2003) and Hawkes et al. (2006) invading plants may strongly influence the diversity of endophytes and mycorrhizas in roots of native plants. Competition between fungal species invading from bordering regions and the indigenous fungal population may alter the overall structure of a fungal community and influence the nutrient cycling processes. The other, less frequently occurring *Ascomycota* have also never been recorded as specialist colonists of forest tree tissues. *Cladophialophora* sp., although apparently predominant in tropical and subtropical regions (de Hoog et al., 2000 b), can also occur in colder habitats. Clones matching *Cladophialophora* sp. have been found in the receding forefront of Lyman glacier, North Cascade Mountains, USA (Jumpponen, 2003). *Lewia* and *Ophiosphaerella* sp. are grass pathogens and *T. furcatum* is an aquatic fungus.

The most frequently occurring fungi were *Basidiomycota*. Between one and three species of *Basidiomycota* from *Agaricales* and *Ceratobasidiales* were recorded in roots of a single tree. *Coprinus fissolanatus* was the most common, particularly in *Q. decidua* (92% of OTUs) and *B. pendula* (61% of OTUs). The occurrence of *C. fissolanatus* within sterile fungal morphotypes detected in *Pinus tabulaeformis* Carr., studied using rDNA sequencing, has been suggested (Wang et al., 2005). *Coprinus disseminatus* (Pers.) Gray and *Coprinus* sp. were reported from roots of *Pinus sylvestris* and *Picea abies* (L.) H. Karst seedlings in Lithuania (Menkis et al., 2006). Before being detected in forest tree roots *Coprinus* species were known to be free-living saprotrophs, particularly common in beech forest stands in central and eastern Europe (Burel, 2004; Fukiharu et al., 2005; Quere et al., 2006). *Mycena* and *Arrhenia obscurata* were detected less frequently. Two species of *Mycena* were also reported from roots of *P. sylvestris* seedlings in Lithuania (Menkis et al., 2006). So far, the genus was known to be abundant in forest soil, particularly under mixed hardwood in the temperate region of the USA (O'Brien et al., 2005).

Oomycota was represented by three different species of *Pythium.* One of them colonized roots of *B. pendula* and the other two were in roots of *F. sylvaticus*. *Pythium* spp. are important root pathogens, causing damping-off and decay. The efficiency of

their detection without *Pythium*–specific primers is significant. Menkis et al. (2005) suggested that detection of *Pythium* with fungal consensus rDNA primers is difficult. Only the molecular method detected *Protozoa*, represented by *P. graminis* from *Plasmodiophorales*. It could not be detected earlier using morphological procedures because it is non-culturable *in vitro* by known methods. Generally, it is recognized as an obligately biotrophic, non-pathogenic colonizer of plant roots (Simpson and Roger, 2004: Adl et al., 2005). So far it was considered as almost entirely restricted to the *Poaceae,* with a reservoir in *w*ild grasses. It is of considerable agronomic importance because of its ability to acquire and transmit a range of plant viruses pathogenic on cereal crops (Kanyuka et al., 2003). This is the first record of its occurrence in tree roots and a forest habitat. The extent to which it colonizes trees and the degree of host specialization are largely unknown. In this study it was recorded on *F. sylvatica* and *Q. petreaea* roots.

Cloning and sequencing detected neither *Penicillium* nor *Trichoderma* spp. (A*scomycota, Eurotiales, Hypocreales*), which are common fungi in tree roots (Kwaśna 1996 a, b; 1997 a, b; 2001, 2002, 2003, 2004). Neither genus was recorded by oligonucleotide fingerprinting of rRNA (Valinsky et al., 2002), direct isolation of SSU and ITS rRNA by PCR and high-throughput sequencing of cloned fragments (O'Brien et al., 2005) or direct sequencing of ITS rDNA (Menkis et al., 2006). Anderson et al. (2003 b) was able to detect *Eupenicillium* and *Penicillium* species in ITS analysed by DGGE (denaturing gradient gel electrophoresis). Methods in which the 18S rDNA region was analysed with fungal-automated rRNA intergenic spacer analysis (F–ARISA), and cloning and sequencing, were more effective for their detection (Jumpponen, 2003; Hansgate et al., 2005). It seems, so far, that only the arbuscular mycorrhizal (AM) fungi have the sufficient variation in the 18S rRNA gene for discrimination between species and below species level, because of the early radiation of *Glomeromycota* (Vandenkoornhuyse and Leyval, 1998).

Pure-culture isolation allowed detection of at least 40 species of *Ascomycota* (mostly *Fusarium* spp., *Penicillium* spp.*, Phialocephala fortinii*, *Trichoderma* spp. from *Eurotiales, Helotiales* and *Hypocreales*), three species of *Zygomycota* (*Mortierella* spp.) but no *Basidiomycota*. The majority of *Ascomycota* detected are ubiquitous saprotrophs and root endophytes. They are successful competitors culturable on artificial media and easy to detect by isolation and morphotyping (Grünig et al., 2006). Identification of some, e.g. *P. fortinii*, was made possible by the use of SNA, which stimulates

sporulation and helps identification by morphology. In earlier studies, *P. fortinii* was categorized as a dark septate endophyte (DSE) (Jumpponen, 2001). It is usually detected on roots of younger trees (Chlebicki, 2004; Menkis et al., 2005, 2006) and it is thought that under some conditions *P. fortinii* is capable of forming mutualistic associations functionally similar to mycorrhiza (Chlebicki, 2004; Jumponnen et al., 1998).

Neither of the methods detected ectomycorrhizal species. There are no reports on a mycorrhizal habit of *Coprinus, Mycena* or *A. obscurata.* Mycorrhizas were absent probably because thin (2–5 mm diam.) suberized roots were used and they were from a young (6-year-old) stand. Suberized roots are usually non–mycorrhizal (Smith and Read, 1997). Disturbance of soil while planting the trees 5 years earlier may have led to insufficient time for mycorrhizal establishment. A complex community of mycorrhizal fungi usually develops over many years (Deacon and Fleming, 1992). Mycorrhizas may develop earlier in fast-growing trees such as *Populus* and *Salix* (Heijden et al., 1999; Baum and Makeschin, 2000; Khasa et al., 2002). Greater frequency of mycorrhizal fungi is often not associated with greater diversity; only one mycorrhizal species, *Rhizopogon rubescens* (Tul. & C. Tul.) Tul. & C. Tul. was found in 55% of root tips of *Pinus pinea* L. seedlings (El–Karkouri et al., 2002).

Among the microbiota detected, 21% of OTUs did not have matches in the current EMBL/GenBank database at the species threshold of 97% similarity. This is likely to be a consequence of poor taxon coverage in the database, even though the ITS rDNA is the best-represented locus for the *Mycota* and the most commonly used marker in studies on fungi. Novel sequences were found more often within *Ascomycota* (nine OTUs) than *Basidiomycota* (two OTUs).

Most fungal species were recorded too infrequently to draw general conclusions about their distribution. Identification by cloning and sequencing indicated, however, considerable species dominance (Fig. 2). In the situation of low community diversity in *Q. petraea*, some specificity for the dominant *C. fissolanatus* was indicated. Similar dominance by a single taxon was recorded in the community from roots of *Q. ilex* in old-growth Mediterranean forest (Richard et al., 2005) and in roots of healthy reed (Neubert et al., 2006). By contrast, identification by morphology suggested a relatively even distribution of species. The different tree species had similar evenness indices, providing no information on host-specificity among the fungi. There was no apparent effect of tree species on the structure of microbial communities. There was no apparent

specialization for resources, host defence reaction or competition by other fungi, or other factors that differentiated the communities. The most common and most frequent fungal species (49% of the total number of isolates) were usually shared by two to five tree species. A similar pattern of colonization was reported for mycorrhizal fungi in a mixed *Pinus contorta* Dougl. and *Picea engelmannii* Parry ex Engelm. forest in the USA (Cullings et al., 2000). Communities identified by morphology showed greater diversity among and within tree species.

This study showed that each method provides only a selective view of diversity of the biota in a natural environment. The two methods differed more in their indication of diversity (taxon detected) than density (number of individual taxon per site). The molecular methods effectively detected fungi of the *Basidiomycota,* which usually form important mycorrhizal associations, and *Oomycota*, which are often pathogenic on young roots. Pure-culture isolation effectively detected saprotrophs and endophytes, particularly within the *Ascomycota*. The results emphasize that only a combination of morphological and molecular techniques provides an effective approach for assessment of diversity and density of root biota. Advantages and limitations of molecular and morphological methods for determining the abundance and composition of biota in plant and soil have also been discussed elsewhere (Menkis et al., 2005, 2006; O'Brien et al., 2005; Tiquia, 2005; Allmer et al., 2006; Girlanda et al., 2006).

Acknowledgements

This work was supported partly by an EU INCO fellowship awarded to Hanna Kwaśna. Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the UK. We very much appreciate the help of anonymous reviewers. Their critical reading of the manuscript and valuable comments and suggestions are gratefully acknowledged.

References

Adl, SM., Simpson, AGB., Farmer, MA., Anderson, RA., Anderson, OR., Barta, JR., Bowser, SS., Brugerolle, G., Fensome, RA., Fredericq, S., James, TY., Karpov, S.,

527 Kugrens, P., Krug, J., Lane, CE., Lewis, LA., Lodge, J., Lynn, DH., Mann, DG.,
528 Mccourt, RM., Mendoza, L., Moestrup, O., Mozley–Standridge, SE., Nerad, TA.,
529 Shearer, CA., Smirnov, AV., Spiegel, FW., Taylor, MFJR. 2005. The new higher
530 level classification of eukaryotes with emphasis on the taxonomy of protists. J. Euk.
531 Microbiol. 52, 399–451.
532 Allen, MF., Swenson, W., Querejeta, JI., Egerton-Warburton, L., Treseder, KK. 2003.
533 Ecology of mycorrhizae: a conceptual framework for complex interactions among
534 plants and fungi. Ann. Rev. Phytopath. 41, 271–303.
535 Allmer, J., Vasiliauskas, R., Ihrmark, K., Stenlid, J., Dahlberg, A. 2006. Wood–inhabiting
536 fungal communities in woody debris of Norway spruce (*Picea abies* (L.) Karst.), as
537 reflected by sporocarps, mycelial isolations and T–RFLP identification. FEMS
538 Microb. Ecol. 55, 57–67.
539 Anderson, IC., Cairney, JWG. 2004. Diversity and ecology of soil fungal communities:
540 increased understanding through the application of molecular techniques. Environ.
541 Microbiol. 6, 769–779.
542 Anderson, IC., Campbell, CD., Prosser, JI. 2003 a. Potential bias of fungal 18S rRNA and
543 internal transcribed spacer polymerase chain reaction primers for estimating fungal
544 biodiversity in soil. Environ. Microbiol. 5, 36–47.
545 Anderson, IC., Campbell, CD., Prosser, JI. 2003 b. Diversity of fungi in organic soils under
546 a moorland–Scots pine (*Pinus sylvestris* L.) gradient. Environ. Microbiol. 5, 1121–
547 1132.
548 Bateman, GL., Kwaśna, H. 1999. Effects of number of winter wheat crops grown
549 successively on fungal communities on wheat roots. Appl. Soil. Ecol. 13, 271–282.
550 Baum, C., Makeschin, F. 2000. Effects of nitrogen and phosphorus fertilization on
551 mycorrhizal formation of two poplar clones (*Populus trichocarpa* and *P. tremula* x
552 *tremuloides*). J. Plant Nut. Soil Sci. 163, 491–497.

553 Baxter, JW., Pickett, STA., Carreiro, MM., Dighton, J. 1999. Ectomycorrhizal diversity and
554 community structure in oak forest stands exposed to contrasting anthropogenic
555 impacts. Can. J. Bot. 77, 771–782.

556 Bell, A., Mahoney, DP. 1997**.** Coprophilous fungi in New Zealand. II. *Podospora* species
557 with coriaceous perithecia. Mycologia 89, 908–915.

558 Bidartondo, MI., Kretzer, AM., Pine, EM., Bruns, TD. 2000. High root concentration and
559 uneven ectomycorrhizal diversity near *Sarcodes sanguinea* (Ericaceae): a cheater
560 that stimulates its victims? Amer. J. Bot. 87, 1783–1788.

561 Borneman, J., Hartin, RJ. 2000. PCR primers that amplify fungal rRNA genes from
562 environmental samples. Appl. Environ. Microbiol. 66, 4356–4360.

563 Brundrett, M. 2004. Diversity and classification of mycorrhizal associations. Biol. Rev. 78,
564 473–495.

565 Bruns, TD., Shefferson, RP. 2004. Evolutionary studies of ectomycorrhizal fungi: recent
566 advances and future directions. Can. J. Bot. 82, 1122–1132.

567 Burel, J. 2004. Profiles of species from *Coprinus* subsection *Setulosi*. Mykol. Sbor. 81,
568 94–97.

569 Burke, DJ., Martin, KJ., Rygiewicz, PT., Topa, MA. 2005. Ectomycorrhizal fungi
570 identification in single and pooled root samples: terminal restriction fragment length
571 polymorphism (TRFLP) and morphotyping compared. Soil Biol. Bioch. 37, 1683–
572 1694.

573 Chlebicki A. 2004. Od pasożytnictwa do mutualizmu. Konsekwencje długotrwałych
574 interakcji. (From parasitism to mutualizm, long term interactions consequence.)
575 Kosmos, Problemy Nauk Biologicznych, 53, 33–38.

576 Clapp, JP., Young, JPW., Merryweather, JW. 1995. Diversity of fungal. symbionts in
577 arbuscular mycorrhizas from a natural community. New Phytol. 130, 259–265.

578 Cullings, KW., Vogler, DR. 1998. A 5.8S nuclear ribosomal RNA gene sequence
579 database: applications to ecology and evolution. Mol. Ecol. 7, 919–923.

580 Cullings, KW., Vogler, DR., Parker, VT., Finley, SK. 2000. Ectomycorrhizal specificity in a
581 mixed *Pinus contorta* and *Picea engelmannii* forest in Yellowstone National Park.
582 Appl. Environ. Microbiol. 66, 4988–4991.

583 Dahlberg, A. 2001. Community ecology of ectomycorrhizal fungi: an advancing
584 interdisciplinary field. New Phytol. 150, 555–562.

585 Deacon, JW., Fleming, LV. 1992. Interaction of ectomycorrhizal fungi. In: Allen, MJ., (Ed.),
586 Mycorrhizal functioning, Chapman and Hall, New York, USA, pp. 249–300.

587 de Hoog, GS., Guarro, J., Gené, J., Figueras, MJ. 2000 a. Atlas of clinical fungi, 2nd ed.
588 Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands and University
589 Rovira i Virgili, Reus, Spain.

590 de Hoog, GS., Queiroz–Telles, F., Haase, G., Fernandez–Zeppenfeldt, G., Angelis, DA.,
591 den Ende van, A., Matos, T., Peltroche–Llacsahuanga, H., Pizzirani–Kleiner, AA.,
592 Rainer, J., Richard–Yegres, N., Vicente, V., Yegres, F. 2000 b. Black fungi: clinical
593 and pathogenic approaches. Med Mycol. 38, 243–250.
594 Domsch, KH., Gams, W., Anderson, T–H. 1980. Compendium of soil fungi. Academic
595 Press, London.
596 Donaldson, RM., Ball, LA., Axelrood, P., Glass, NL. 1995. Primer set developed to amplify
597 conserved genes from filamentous ascomycetes are useful in differentiating
598 *Fusarium* species associated with conifers. Appl. Environ. Microbiol. 61, 1331–1340.
599 El–Karkouri, K., Martin, F., Mousain, D. 2002. Dominance of the mycorrhizal fungus
600 *Rhizopogon rubescens* in a plantation of *Pinus pinea* seedlings inoculated with
601 *Suillus collinitus*. Ann. For. Sci. 59, 197–204.
602 Erwin, DC., Ribiero, O.K. 1996. *Phytophthora* diseases world–wide. American
603 Phytopathological Society, St Paul, .pp. 1–562.
604 Felsenstein, J. 2004. *PHYLIP: phylogeny inference package. Version 3.6.* Seattle, WA,
605 USA: Department of Genome Sciences and Department of Biology, University of
606 Washington. Distributed by the author.
607 Fukiharu, T., Takarada, K., Hosoya, T. Kinjo, N. 2005. Three *Coprinus* species occurred
608 on the animal dungs collected at Yatsugatake range, central Honshu, Japan. Bull.
609 Nat. Sci. Mus. (Botany) 31, 117–126.
610 Gardes, M., Bruns, TD. 1993. ITS primers with enhanced specificity for basidiomycetes:
611 application to the identification of mycorrhiza and rusts. Mol. Ecol. 2, 113–118.
612 Girlanda, M., Selosse, MA., Cafasso, D., Brilli, F., Delfine, S., Fabbian, R., Ghignone, S.,
613 Pinelli, P., Segreto, R., Loreto, F., Cozzolino, S., Perotto, S. 2006. Inefficient
614 photosynthesis in the Mediterranean orchid *Limodorum abortivum* is mirrored by
615 specific association to ectomycorrhizal Russulaceae. Mol. Ecol. 15, 491–504.

616 Gloud, AB. 2000. Mycorrhizal endosymbiosis. In: Bacon, CW., White, JE. (Eds),
617 Microbial Endophytes, Marcel Dekker, Inc., New York, Basel, pp.1–487.

618 Grünig, CR., Duò, A., Sieber, TN. 2006. Population genetic analysis of *Phialocephala*
619 *fortinii* s.l. and *Acephala applanata* in two undisturbed forests in Switzerland and
620 evidence for new cryptic species. Fung. Gen. Biol. 43, 410–421.

621 Guillaumin, J.–J., Mohammed, C., Anselmi, N., Courtecuisse, R., Gregory, S.C.,
622 Holdenrieder, O., Intini, M., Lung, B., Marxmüller, H., Morrison, D., Rishbeth, J.,
623 Termorshuizen, A.J., Tirro, B., Van Dam, B. 1993. Geographical distribution and
624 ecology of the *Armillaria* species in Western Europe. Eur. J. For. Pathol. 23. 321–
625 341.

626 Halmschlager, E., Kowalski T. 2004. The mycobiota in nonmycorrhizal roots of healthy
627 and declining oaks. Can. J. Bot. 82, 1446–1458.

628 Hamelin, R., Bérubé, P., Gignac, M., Bourassa, M. 1996. Identification of root rot fungi in
629 nursery seedlings by nested multiple PCR. Appl. Environ. Microbiol. 62, 4026–4031.

630 Hansgate, AM., Schloss, PD., Hay, AG., Walker, LP. 2005. Molecular characterization of
631 fungal, community dynamics in the initial stages of composting. FEMS Microbiol.
632 Ecol. 51, 209–214.

633 Hantula, J., Lilja, A., Veijalainen, AM. 2002. Polymerase chaín reaction primers for the
634 detection of *Ceratobasidium bicorne* (uninucleate *Rhizoctonia*). For. Pathol. 32, 231–
635 239.

636 Harney, SK., Rogers, SO., Wang, CJK. 1997. Molecular characterization of dematiaceous
637 root endophytes. Mycol. Res. 101,1397–1404.

638 Hawkes, CV., Belnap, J., D'Antonio, C., Firestone, MK. 2006. Arbuscular mycorrhizal
639 assemblages in native plant roots change in the presence of invasive exotic grasses.
640 Pl. Soil. 281, 369–380.

641 Heijden, EW van der., de Vries, FD., Kuyper, TW. 1999. Mycorrhizal associations of
642 *Salix repens* L. communities in succession of dune ecosystems. I. Above–ground
643 and below–ground views of ectomycorrhizal fungi in relation to soil chemistry. Can.
644 J. Bot. 77, 1821–1832.

645 Hill, CJ., Walsh, KA., Harris, JA., Moffett, BF. 2003. Using ecological diversity measures
646 with bacterial communities. FEMS Microbiol. Ecol. 43, 1–11.

647 Holdenrieder, O., Sieber, TN. 1992. Fungal asssociations of serially washed healthy
648 nonmycorrhizal roots of *Picea abies*. Mycol. Res. 96, 151–156.

649 Horton, TR., Bruns, TD. 2001. The molecular revolution in ectomycorrhizal ecology:
650 peeking into the black–box. Mol. Ecol. 10, 1855–1871.
651 Horton, TR., Bruns, TD., Parker, VT. 1999. Ectomycorrhizal fungi associated with
652 *Arctostaphylos* contribute to *Pseudotsuga menziesii* establishment. Can. J. Bot. 77,
653 93–102.
654 Hugenholz, P., Pace, NR. 1996. Identifying microbial diversity in the natural environment:
655 a molecular phylogenetic approach. Trends Biotechnol. 14, 190–197.
656 Hunt, J., Boddy, L., Randerson, PF., Rogers, HJ. 2004. An evaluation of 18S rDNA
657 approaches for the study of fungal diversity in grassland soils. Microbiol. Ecol. 47,
658 385–395.

659 Jacobsen, J., Lydolph, M., Lange, L. 2005. Culture independent PCR an alternative
660 enzyme discovery strategy. J. Microbiol. Met. 60, 63–71.

661 Johnson, D., Vandenkoornhuyse, PJ., Leake, JR., Gilbert, L., Booth, RE., Grime, JP.,
662 Young, JPW., Read, DJ. 2003. Plant communities affect arbuscular mycorrhizal
663 fungal diversity and community composition in grassland microcosms. New Phytol.
664 161, 503–515.

665 Jumpponen, A. 2001. Dark septate endophytes – are they mycorrhizal? Mycorrhiza, 11,
666 207–211.
667 Jumpponen, A. 2003. Soil fungal community assembly in a primary successional glacier
668 forefront ecosystem as inferred from rDNA sequence analyses. New Phytol. 158,
669 569–578.

670 Jumpponen, A., Mattson, KG., Trappe, JM. 1998. Mycorrhizal functioning of *Phialocephala*
671 *fortinii* with *Pinus contorta* on glacier forefront soil: interactions with nitrogen and
672 organic matter. Mycorrhiza, 7, 261–265 .
673 Jung, T., Blaschke, H., Neumann, P. 1996. Isolation, identification and pathogenicity of
674 *Phytophthora* species from declining oak stands. Eur. J. For. Path. 26, 253–272.
675 Kanyuka, K., Ward, E., Adams, MJ. 2003. *Polymyxa graminis* and the cereal viruses it
676 transmits: a research challenge. Mol. Pl. Pathol. 4, 383–406.
677 Kennedy, PG., Izzo, AD., Bruns, TD. 2003. High potential for common mycorrhizal
678 networks between understorey and canopy trees in a mixed evergreen forest. J.
679 Ecol. 91, 1071–1080.

680 Kernaghan, G., Siegler, L., Khasa, D. 2003. Mycorrhizal and root endophytic fungi of
681 containerized *Picea glauca* seedlings assessed by rDNA sequence analysis.
682 Microbiol. Ecol. 45, 128–136.
683 Khasa, PD., Chakravarty, P., Robertson, A., Thomas, BR., Dancik, BP. 2002. The
684 mycorrhizal status of selected poplar clones introduced in Alberta. Biom. Bioen. 22,
685 99–104.
686 Kirk, PM., Cannon, PF., David, JC., Stalpers, JA. 2001. *Ainsworth and Bisby's*
687 *dictionary of the fungi*. 9th ed. Wallingford, UK: CAB International.
688 Koide, RT., Mosse, B. 2004. A history of research on arbuscular mycorrhiza. Mycorrhiza
689 14, 145–163.
690 Kwaśna, H. 1996 a. Mycobiota of birch roots and birch stump roots and their possible
691 effect to the infection by *Armillaria* spp. (Romagn) Herink growth. part I. Acta
692 Mycol. 31, 101–110.

693 Kwaśna, H. 1996 b. Mycobiota of birch roots and birch stump roots and their possible
694 effect to the infection by *Armillaria* spp. (Romagn) Herink growth. part II. Acta
695 Mycol. 31, 111–122.

696 Kwaśna, H. 1997 a. Antagonistic effect of fungi communities from Scots pine fine roots
697 on *Heterobasidion annosum* (Fr.) and *Armillaria ostoyae* (Romagn.) Herink growth.
698 Phytopathol. Pol. 1, 133–146.

699 Kwaśna, H. 1997 b. Antagonistic effect of fungi from Scots pine stump roots on *Heterobasidion*
700 *annosum* and *Armillaria ostoyae*. Acta Mycol. 32, 369–381

701 Kwaśna, H. 2001. Fungi in the rhizosphere of common oak and its stumps and their possible
702 effect on infection by *Armillaria.* Appl. Soil Ecol. 17, 215–227.
703 Kwaśna, H. 2002. Changes in microfungal communities in roots of *Quercus robur* stumps
704 and their possible effect on colonization by *Armillaria*. J. Phytopat. 150, 403–411.
705 Kwaśna, H. 2003. The effect of felling on the occurrence of microfungi stimulatory to
706 *Armillaria* rhizomorph formation in thin roots of *Quercus robus*. J. Phytopat. 151,
707 185–189.
708 Kwaśna, H. 2004. Natural shifts in communities of rhizosphere fungi of common oak
709 after felling. Pl. Soil, 264, 209–218.
710 Landgraf, M. 2006. Detection of food-relevant filamentous fungi by real time PCR. PhD
711 thesis. Technischen Universität Berlin. pp. 1–126.

712 Lord, N.S., Kaplan, C.W., Shank, P., Kitts, C.L., Elrod, S.L. 2002. Assessment of fungal
713 diversity using terminal restriction fragment (TRF) pattern analysis: comparison of
714 18S and ITS ribosomal regions. FEMS Microbiol. Ecol. 42, 327–337.
715 Luoma, DL., Eberhart, JL., Amaranthus, MP. 1997. Biodiversity of ectomycorrhizal types
716 from southwest Oregon. In: Wilson, MV., (Ed), Conservation and management of
717 native plants and fungi. Native Plant Society of Oregon, Corvallis, USA, pp. 249–253.
718 Magurran, AE. 1988. Ecological diversity and its measurement. Princeton University
719 Press , Princeton, NJ, USA.
720 Mańka, K. 1998. Fitopatologia leśna. Państwowe Wydawnictwo Rolnicze i Leśne,
721 Warszawa, Poland.
722 McCaig, AE., Glover, LA., Prosser, JL. 1999. Molecular analysis of bacterial community
723 structure and diversity in unimproved and improved upland grass pastures. Appl.
724 Environ. Microbiol. 65, 1721–1730.

725 Menkis, A., Vasiliauskas, R., Taylor, AFS., Stenlid, J., Finlay, R. 2005. Fungal communities
726 in mycorrhizal roots of conifer seedlings in forest nurseries under different cultivation
727 systems, assessed by morphotyping, direct sequencing and mycelial isolation.
728 Mycorrhiza 16, 33–41.
729 Menkis, A., Vasiliauskas, R., Taylor, AFS., Stenstrom, E., Stenlid, J., Finlay, R. 2006.
730 Fungi in decayed roots of conifer seedlings in forest nurseries, afforested clear–cuts
731 and abandoned farmland. Pl. Pathol. 55, 117–129.
732 Neubert, K., Mendgen, K., Brinkmann, H., Wirsel, SGR. 2006. Only a few fungal species
733 dominate highly diverse mycofloras associated with the common reed. Appl. Environ.
734 Microbiol. 72, 1118–1128.
735 Nicholas, KB., Nicholas, HB. 1997. GeneDoc, a tool for editing and annotating multiple
736 sequence alignments. Distributed by the authors.
737 Nirenberg, HI. 1976. Untersuchungen über die morphologische und biologische
738 Differenzierung in der *Fusarium*–Section *Liseola.* Mitt. Biol. Bund. Land– und
739 Forstwirtsch. Berlin–Dahlem 169, 1–117.

740 O'Brien, HE., Parrent, JL., Jackson, JA., Moncalvo, J–M., Vilgalys, R. 2005. Fungal
741 community analysis by large–scale sequencing of environmental samples. Appl.
742 Environ. Microbiol. 71, 5544–5550.

743 Page, RDM. 1996. TREEVIEW: An application to display phylogenetic trees on personal
744 computers. Comp. App. Bioscie. 12, 357–358.
745 Peace, TR. 1962. Pathology of trees and shrubs. Oxford University Press, Oxford, UK.
746 Pitt, JI. 1980. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and
747 *Talaromyces*. Academic Press, New York, pp. 1–634.
748 Przybył, K. 2002. Mycobiota of thin roots showing decay of *Fraxinus excelsior* L. young
749 trees. Dendrobiology 48, 65–69.
750 Quere la, A., Eriksen, KA., Rajashekar, B., Schützendübel, A., Canbäck, B., Johansson,
751 T., Tunlid, A. 2006. Screening for rapidly evolving genes in the ectomycorrhizal
752 fungus *Paxillus involutus* using cDNA microarrays. Mol. Ecol.15, 535–550.
753 Redecker, D., Hijri, M., Dulieu, H., Sanders. IR, 1999. Phylogenetic analysis of a dataset
754 of fungal 5.8S rDNA sequences shows that highly divergent copies of internal
755 transcribed spacers reported from *Scutellospora castanea* are of ascomycete
756 origin. Fun. Gen. Biol. 28, 238–244.
757 Richard, F., Millot, S., Gardes, M., Selosse, M–A. 2005. Diversity and specificity of
758 ectomycorrhizal fungi retrieved from an old–growth Mediterranean forest
759 dominated by *Quercus ilex*. New Phytol. 166, 1011–1023.
760 Roose–Amsaleg, C., Brygoo, Y., Harry, M. 2004. Ascomycete diversity in soil–feeding
761 termite nests and soils from a tropical rainforest. Environ. Microbiol. 6, 462–469.
762 Sieber, TN. 2002. Fungal root endophytes. In: Waisel, Y., Eshel, A., Kafkafi, U. (Eds),
763 Plant Roots: the hidden half. Marcel Dekker, New York, Basel, pp. 887–917.
764 Simard, SW., Durall, DM. 2004. Mycorrhizal networks: a review of their extent, function,
765 and importance. Can. J. Bot. 82, 1140–1165.
766 Simpson, AG., Roger, AJ. 2004. The "real" kingdoms of eukaryotes. Cur. Biol. 14, 693–
767 696.
768 Sinclair, WA., Lyon, HH., Johnson, WT. 1987. Diseases of trees and shrubs. Constock
769 Publishing Associates, Ithaca, NY, USA.
770 Smith, SE., Read, DJ. 1997. Mycorrhizal symbiosis. Academic Press, Harcourt Brace &
771 Company, San Diego, CA, USA.

772 Taylor, AFS. 2002. Fungal diversity in ectomycorrhizal communities: sampling effort and
773 species detection. Pl. Soil 244, 19–28.

774 Taylor, JW., Jacobson, DJ., Kroken, S., Kasuga, T., Geiser, DM., Hibbett, DS., Fisher, MC.
775 2000. Phylogenetic species recognition and species concepts in fungi. Fung. Genet.
776 Biol. 31, 21–32.

777 Thompson, JD., Higgins, DG., Gibson, TJ. 1994. CLUSTAL W: improving the
778 sensitivity of progressive multiple sequence alignment through sequence
779 weighting, position specific gap penalties and weight–matrix choice. Nuc. Acids
780 Res. 22, 4673–4680.

781 Thompson, JD., Gibson, TJ., Plewniak, F., Jeanmougin, F., Higgins, DG. 1997. The
782 ClustalX windows interface: flexible strategies for multiply sequence alignment aided
783 by quality analysis tools. Nuc. Acids Res. 24, 4876–4882.

784 Tiquia, SM. 2005. Microbial community dynamics in manure composts based on 16S and
785 18S rDNA T–RFLP profiles. Environ. Tech. 26, 1101–1113.
786 Valinsky, L., Della Vedova, G., Jiang, T., Borneman, J. 2002. Oligonucleotide
787 fingerprinting of rRNA genes for analysis of fungal community composition. Appl.
788 Environ. Microbiol. 68, 5999–6004.
789 Vandenkoornhuyse, P., Leyval C. 1998. Intraspecific diversity of *Glomus mosseae*
790 revealed by microsatellite–primed PCR, SSU rDNA PCR-sequencing, Mycologia
791 90, 791–797.
792 Vandenkoornhuyse, P., Baldauf, SL., Leyval, C., Straczek, J., Peter, J., Young, W.
793 2002. Extensive fungal diversity in plant roots. Science 295, 2051–2051.
794 Vettraino, AM., Barzanti, GP., Bianco, MC., Ragazzi, A., Capretti, P., Paoletti, E., Luisi,
795 N., Anselmi, N., Vannini, A. 2002. Occurrence of *Phytophthora* species in oak
796 stands in Italy and their association with declining oak trees. For. Path. 32, 19–28.
797 Viaud, M., Pasquier, A., Brygoo, Y. 2000. Diversity of soil fungi studied by PCR–RFLP
798 of ITS. Mycol. Res. 104, 1027–1032.
799 Wang, YU., Guo, LD., Hyde, KD. 2005. Taxonomic placement of sterile morphotypes of
800 endophytic fungi from *Pinus tabulaeformis* (*Pinaceae*) in northeast China based on
801 rDNA sequences. Fun. Diver. 20, 235–260.

802 Ward, E., Kanyuka, K., Motteram, J., Kornyukhin, D., Adams, MJ. 2005. The use of
803 conventional and quantitative real–time PCR assays for *Polymyxa graminis* to

examine host plant resistance, inoculum levels and intraspecific variation. New Phytol. 165, 875–885.

Wetzel, HC., Dernoeden, PH III., Millner, PD. 1996. Identification of darkly pigmented fungi associated with turfgrass by mycelial characterization and RAPD–PCR. Pl. Dis. 80, 359–364.

White, TJ., Bruns, T., Lee, S., Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, MA., Gelfand, DH., Sninsky, JJ., White, TJ., (Eds), PCR protocols. A guide to methods and applications. Academic Press, San Diego, USA, pp. 315–322.

Wilcox, HE. 1983. Fungal parasitism of woody plants roots from mycorrhizal relationship to plant diseases. Ann. Rev. Phytopat. 21, 221–242.

Woodward, S., Stenlid, J., Karjalainen, R., Hüttermann, A. (Eds) 1998. *Heterobasidion annosum*: Biology, Ecology, Impact and Control. CAB International, Wallingford. pp. 235–258.

Zervakis, GI., Moncalvo, JM., Vilgalys, R. 2004. Molecular phylogeny, biogeography and speciation of the mushroom species *Pleurotus cystidiosus* and allied taxa. Microbiology 150, 715–726.

Descriptions

Fig. 1 – Phylogenetic tree obtained by NEIGHBOR analysis of the ITS 1/2 rDNA showing relationships between organisms from roots of deciduous trees. Bootstrap values based on 1000 replicates are indicated above the branches. Sequences from the study are labelled **HK**.

Fig. 2 – Diversity in communities of microbiota from tree roots

Table 1 – Database typing of ITS sequences

Table 2 – Frequency of microbiotal taxa estimated by the molecular method

Table 3 – Frequency of fungi estimated by the pure–culture isolation method

Table 4 – Diversity indices for microbiota communities from roots of five tree species calculated from cloned OTUs and isolates produced by pure–culture isolation

Table 5 – Similarity coefficients based on pairwise comparisons of community structures of microbiota on roots of five tree species

Table 1 – Fungal sequences from GenBank showing most similarity to OTUs from roots of *B. pendula*, *F. sylvatica*, *L. decidua*, *P. serotina* and *Q. petraea.*

Taxon	Order	Accession No.	Depositor	No. of clones in OTU	*Cfo*I RFLP pattern	Host species	OTU
Arrhenia obscurata (D.A. Reid) Redhead, Lutzoni, Moncalvo & Vilgalys	*Agaricales*	U66448	Lutzoni, F. Aug 1997[a]	4	350	*F. sylvatica*	HK 378
Ascomycete sp.		AY787739	Lygis, V., Vasiliauskas, R., Larsson, K.-H. Nov 2004[b]	6	200,180,120,100	*F. sylvatica*	HK 374
Cladophialophora sp.	*Chaetothyriales*	AY781217	Vasiliauskas, R., Larsson, E., Larsson, K.-H., Stenlid, J. Dec 2004[b]	1	300,220	*Q. petraea*	HK 399
Coprinus fissolanatus Kemp	*Agaricales*	AF345812	Park, DS., Shin, HS., Moncalvo, JM. Feb 2001[c]	1	600	*Q. petraea*	EW 816
				2	600,150,100	*Q. petraea*	HK 366
				60	400,300	*B. pendula*	HK 367
				17	400,300	*F. sylvatica*	HK 373, HK 387
				45	400,300	*L. decidua*	HK 433
				84	400,300	*Q. petraea*	HK 365
				2	400,250,150,140	*Q. petraea*	HK 402
				3	320,300,100	*Q. petraea*	HK 404
Helgardia anguioides (Nirenberg) Crous & W. Gams	*Helotiales*	AY266144	Stewart, EL., Liu, Z., Crous, P., Szabo, L.J. Mar 2003[d]	4	410,280	*B. pendula*	HK 407
				2	380	*F. sylvatica*	HK 385, HK 386
				6	280,100,80	*F. sylvatica*	HK 376
				2	280,100,80	*L. decidua*	HK 391, HK 435
Lewia infectoria (Fuckel) M.E. Barr & E.G. Simmons	*Pleosporales*	Y17066	McKay, GJ. Apr 1998[e]	2	280,180,140,100	*B. pendula*	HK 408
Microdochium sp.	*Xylariales*	AJ279481	Wirsel, SGR. Dec 1999[f]	5	300,180	*B. pendula*	HK 406
				6	300,180	*F. sylvatica*	HK 379
				1	280,180	*B. pendula*	HK 411
				2	280,180	*Q. petraea*	HK 403
Mycena epipterygia (Scop.) Gray	*Agaricales*	AY805613	Menkis, A., Allmer, J., Vasiliauskas, R., Lygis, V., Stenlid, J., Finlay, R. Nov 2004[b]	5	380,350	*B. pendula*	HK 405
				8	380,350	*F. sylvatica*	HK 377
				5	380,350	*L. decidua*	HK 434
Mycena aff. *murina* Murrill	*Agaricales*	AF335444	Berbee, ML., Inderbitzin, P., Zhang, G. Jan 2001[g]	1	320,200,180,100	*L. decidua*	HK 437
				1	320,200,180,100	*Q. petraea*	HK 401

Ophiosphaerella sp.	*Pleosporales*	AJ246157	Carter, JP. Jan 1999[h]	2	350,180,150	*F. sylvatica*	HK 380
Podospora glutinans (Cain) Cain	*Sordariales*	AY615207	Krug, JC., Schulz, MJ., Jeng, RS. Apr 2004[i]	5	230,120,80	*B. pendula*	EW 818
				17	230,120,80	*F. sylvatica*	HK 375, HK 382
				4	230,120,80	*L. decidua*	HK 436
				4	230,220,120,100,80	*F. sylvatica*	HK 392
Polymyxa graminis Ledingham	*Plasmodiophorales*	Y12826	Ward, E., Adams, MJ. Apr 1997[j]	1	380,250	*Q. petraea*	HK 400
				4	350,80,70	*F. sylvatica*	HK 381
Pythium arrhenomanes Drechsler	*Pythiales*	AF330180	Heelan, LA., Croft, BJ., Dietzgen, RG., Maclean, DJ. Dec 2000[k]	2	600,280,75	*F. sylvatica*	HK 383
Pythium insidiosum De Cock, L. Mend., A.A. Padhye, Ajello & Kaufman	*Pythiales*	AY151179	Schurko, AM., Mendoza, L., Levesque, CA., Desaulniers, NL., de Cock, AW., Klassen, GR. Sep 2002[l]	6	700,180,120	*B. pendula*	EW 819
Pythium sylvaticum W.A. Campb. & F.F. Hendrix	*Pythiales*	AY598645	Levesque, CA., de Cock, AWAM. Apr 2004[m]	2	400,110,90,70	*F. sylvatica*	HK 384
Schizothecium miniglutinans (J.H. Mirza & Cain) N. Lundq.	*Sordariales*	AY515362	Debuchy, R., Bell, AE., Mahoney, DP. Dec 2003[n]	1	400,240,80	*F. sylvatica*	HK 389
Tetracladium furcatum Descals	*Incertae sedis*	AF411026	Nikolcheva, LG., Baerlocher, FJ. Aug 2001[o]	2	380,300	*B. pendula*	HK 409
Thanatephorus cucumeris (A.B. Frank) Donk	*Ceratobasidiales*	AY154300	Kuramae, EE., Buzeto, AL., Ciampi, MB., Souza, NL. Sep 2002[p]	1	390	*B. pendula*	HK 410
Tricladium splendens Ingold	*Helotiales*	AY204635	Baschien, C., Marvanova, L., Manz, W., Szewzyk, U. Dec 2002[r]	15	190,120	*F. sylvatica*	HK 388
				1	190,120	*Q. petraea*	EW 817
Mycorrhizal ascomycete of *Rhododendron*		AB089660	Usuki, F., Abe, J., Kakishima, M. Aug 2002[s]	1	200,170,120	*F. sylvatica*	HK 390
Uncultured fungus		AF504837	Anderson, IC., Campbell, CD., Prosser,JI. Apr 2002[t]	3	600	*B. pendula*	HK 412
Uncultured Zygomycete		AY969893	O'brien, HE., Parrent, JL., Jackson, JA., Moncalvo, JM., Vilgalys, R. Mar 2005[u]	1	700,400,300	*B. pendula*	HK 413

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Table 2 – Frequency of microbiotal taxa estimated by the molecular method.

	Taxa	*Betula pendula*	*Fagus sylvatica*	*Larix decidua*	*Prunus serotina*	*Quercus petraea*
		% of OTUs				
1.	*Arrhenia obscurata*	1.0	4.0	3.0	3.0	
2.	Ascomycete sp.		6.0		8.0	
3.	*Cladophialophora* sp.					1.0
4.	*Coprinus fissolanatus*	61.0	19.0	45.0	36.0	92.0
5.	*Helgardia anguioides*	4.0	9.0	10.0	2.0	
6.	*Lewia infectoria*	2.0				
7.	*Microdochium* sp.	6.0	6.0	17.0	3.0	2.0
8.	*Mycena epipterygia*	5.0	8.0	5.0	14.0	
9.	*Mycena* aff. *murina*	1.0		3.0	6.0	1.0
10.	*Ophiosphaerella* sp.		2.0		1.0	
11.	*Podospora glutinans*	5.0	21.0	9.0	8.0	
12.	*Polymyxa graminis*		4.0		3.0	1.0
13.	*Pythium arrhenomanes*	1.0	2.0		8.0	
14.	*Pythium insidiosum*	6.0		4.0	3.0	2.0
15.	*Pythium sylvaticum*		2.0			
16.	*Schizothecium miniglutinans*	1.0	1.0			
17.	*Tetracladium furcatum*	2.0				
18.	*Thanatephorus cucumeris*	1.0			2.0	
19.	*Tricladium splendens*		15.0	2.0	3.0	1.0
20.	Zygomycete sp.	1.0				
21.	Mycorrhizal ascomycete of *Rhododendron*		1.0	2.0		
22.	Uncultured fungus	3.0				
	Number of clones studied	100	100	100	100	100
	Number of taxa detected	15	14	10	14	7
	Number of taxa per root	0.6	0.6	0.4	0.6	0.3

Table 3 – Frequency of fungi estimated by the pure–culture isolation method.

	Taxa	*Betula pendula*	*Fagus sylvatica*	*Larix decidua*	*Prunus serotina*	*Quercus petraea*
		% of the total number of isolates				
1.	*Arthrinium phaeospermum* (Corda) M.B. Ellis				2.9	
2.	*Aspergillus fumigatus* Fresenius		0.8			
3.	*Cladosporium cladosporioides* (Fresenius) G.A. de Vries					1.5
4.	*Coniothyrium fuckelii* Saccardo			7.1		
5.	*Cylindrocarpon didymum* (Harting) Wollenweber		1.5			
6.	*Fusarium avenaceum* (Fries : Fries) Saccardo		1.5			1.5
7.	*Fusarium oxysporum* Schlechtendal : Fries		1.5	1.0	1.9	
8.	*Fusarium redolens* Wollenweber				0.9	
9.	*Fusarium* sp.	1.7				
10.	*Geotrichum candidum* Link ex Leman		1.5			
11.	*Gliocladium roseum* Bainier				3.9	
12.	*Humicola grisea* Traaen				1.9	
13.	*Mortierella elongata* Linnem.		1.5			
14.	*Mortierella gamsii* Milko		3.0			
15.	*Mortierella humilis* Linnem. ex W. Gams		1.5			2.9
16.	*Paecilomyces farinosus* (Holmskjold : Fries) A.H.S. Brown & G. Smith		3.8			
17.	*Papulaspora* sp.				0.9	
18.	*Penicillium adametzii* Zaleski	6.9	1.5	10.1	13.5	20.0
19.	*Penicillium canescens* Sopp		1.5	1.0		
20.	*Penicillium cyclopium* Westling	3.4	3.0		1.9	
21.	*Penicillium daleae* Zaleski	18.8	12.0	3.1	13.5	21.7
22.	*Penicillium herquei* Bainier & Sartory				1.9	
23.	*Penicillium janczewskii* Zaleski	23.9	9.1		2.9	18.8
24.	*Penicillium restrictum* J.C. Gilman & E.V. Abbott				1.9	
25.	*Penicillium simplicissimum* (Oudem.) Thom	10.3	0.8	28.4	17.3	
26.	*Penicillium steckii* Zaleski	12.0			1.9	4.3
27.	*Penicillium vinaceum* Gilman et Abbott		9.1	17.2	14.4	
28.	*Penicillium* spp.		0.8			
29.	*Phialocephala fortinii* C.J.K. Wang & H.E. Wilcox		3.0	8.0		4.3

30.	*Phialophora botulispora* Cole & W.B. Kendr.		0.8			
31.	*Phialophora richardsiae* (Nannf.) Conant		1.5			1.5
32.	*Phoma* cf. *lingam* (Tode) Desm.					2.9
33.	*Pochonia bulbillosa* (W. Gams & Malla) Zare & W. Gams	5.1	6.0	8.0		1.5
34.	*Sesquicillium candelabrum* (Bonorden) W. Gams		3.0	2.0	1.9	5.8
35.	*Sporothrix schenckii* Hectoen et Perkins		4.5		1.9	
36.	*Tolypocladium inflatum* W. Gams		4.5			2.9
37.	*Trichoderma aureoviride* Rifai				4.9	
38.	*Trichoderma harzianum* Rifai		0.8			
39.	*Trichoderma koningii* Oudemans		5.3	11.0	9.7	
40.	*Trichoderma viride* Pers. ex Gray		5.3	3.1		1.5
41.	*Trichoderma* sp.	9.4				
42.	*Varicosporium elodeae* W. Kegel					1.5
43.	*Verticillium* sp.		2.2			
44.	non–sporulating, black-mycelium	3.4	2.2			1.5
45.	non–sporulating, brown-mycelium	1.7	2.2			1.5
46.	non–sporulating, grey-mycelium	1.7	2.2			2.9
47.	non–sporulating, white-mycelium	1.7	2.2			1.5
	Number of isolates	117	133	99	104	69
	Number of taxa detected	13	32	12	19	19
	Number of taxa per root	0.5	1.3	0.5	0.8	0.8
	Number of isolates per inoculum	2.4	2.7	2.0	2.2	1.4
	Number of isolates per root	4.8	5.4	4.0	4.4	2.8

Table 4 – Diversity indices for microbiotal communities from roots of *B. pendula*, *F. sylvatica*, *L. decidua*, *P. serotina* and *Q. petraea* calculated from cloned OTUs and isolates produced by pure–culture isolation.

Tree species		Species richness indices		Evenness or dominance indices		
	No. of OTUs/ isolates	Margalef's index (D_{Mg})	Shannon's index (*H*')	Shannon's evenness index (*E*)	Simpson's index (*D*)	Berger-Parker's index (*d*)
	Community identified by molecular method					
Betula pendula	100	3.04	1.61	0.59	0.38	0.61
Fagus sylvatica	100	2.83	2.27	0.86	0.12	0.21
Larix decidua	100	1.96	1.75	0.76	0.25	0.45
Prunus serotina	100	2.83	2.15	0.81	0.17	0.36
Quercus petraea	100	1.30	0.37	0.19	0.85	0.92
	Community identified by morphology					
Betula pendula	117	2.52	2.21	0.86	0.13	0.24
Fagus sylvatica	133	6.34	3.17	0.92	0.05	0.12
Larix decidua	99	2.40	2.11	0.85	0.14	0.28
Prunus serotina	104	3.88	2.52	0.86	0.10	0.17
Quercus petraea	69	4.25	2.32	0.79	0.23	0.22

Table 5 – Similarity coefficients based on pairwise comparisons of community structures of microbiota on roots of *B. pendula*, *F. sylvatica*, *L. decidua*, *P. serotina* and *Q. petraea*.

Tree species	Sorensen's similarity index (C_N)			
	B. pendula	*F. sylvatica*	*L. decidua*	*P. serotina*
	Community identified by molecular method			
F. sylvatica	0.42			
L. decidua	0.71	0.54		
P. serotina	0.58	0.52	0.65	
Q. petraea	0.66	0.23	0.51	0.43
	Community identified by morphology			
F. sylvatica	0.40			
L. decidua	0.27	0.38		
P. serotina	0.37	0.39	0.58	
Q. petraea	0.48	0.49	0.24	0.40

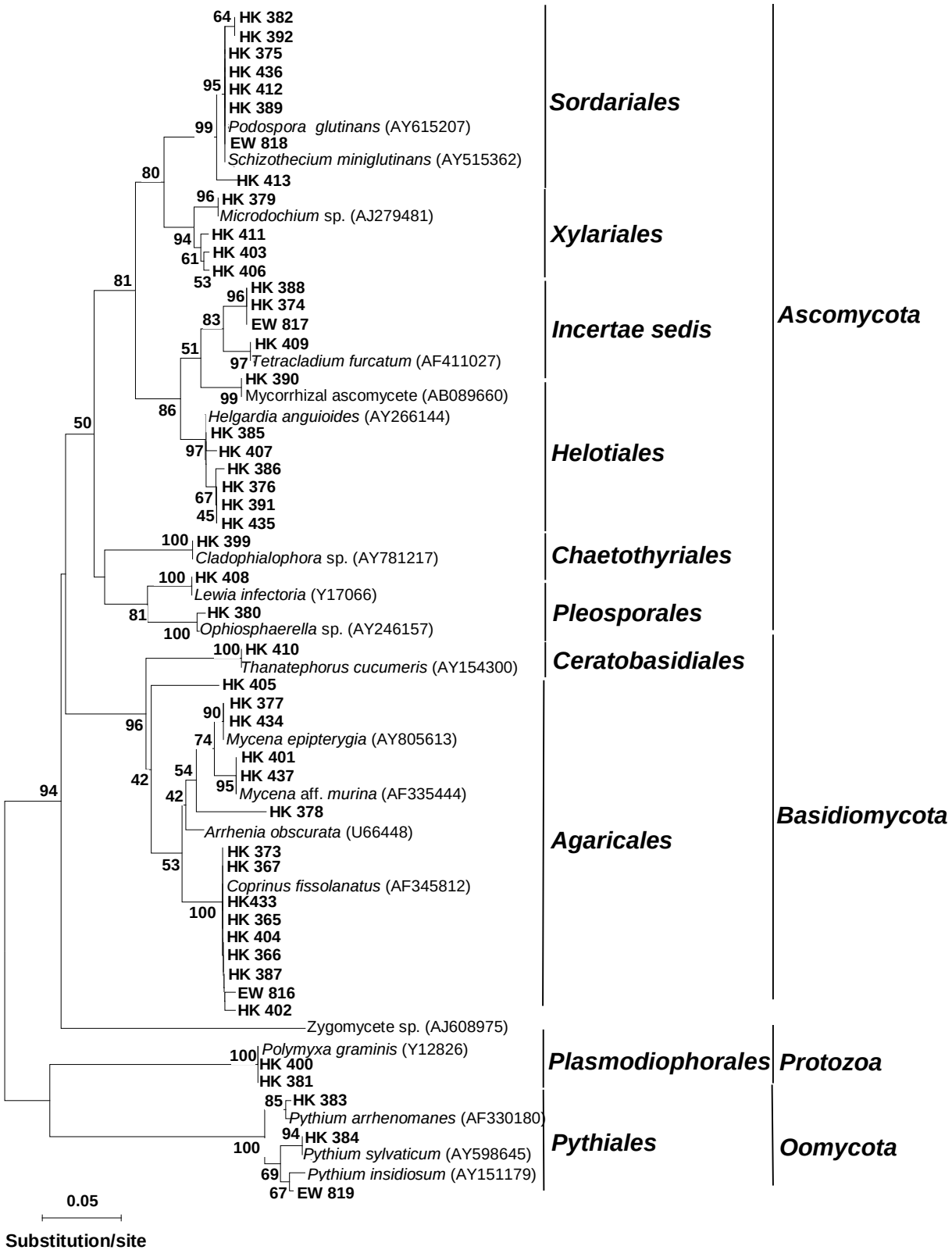

Fig. 1 – Phylogenetic tree obtained by NEIGHBOR analysis of the ITS 1/2 rDNA showing relationships between organisms from roots of *B. pendula*, *F. sylvatica*, *L. decidua*, *P. serotina* and *Q. petraea*. Bootstrap values based on 1000 replicates are indicated above the branches. Sequences from the study are labelled **HK** or **EW**.

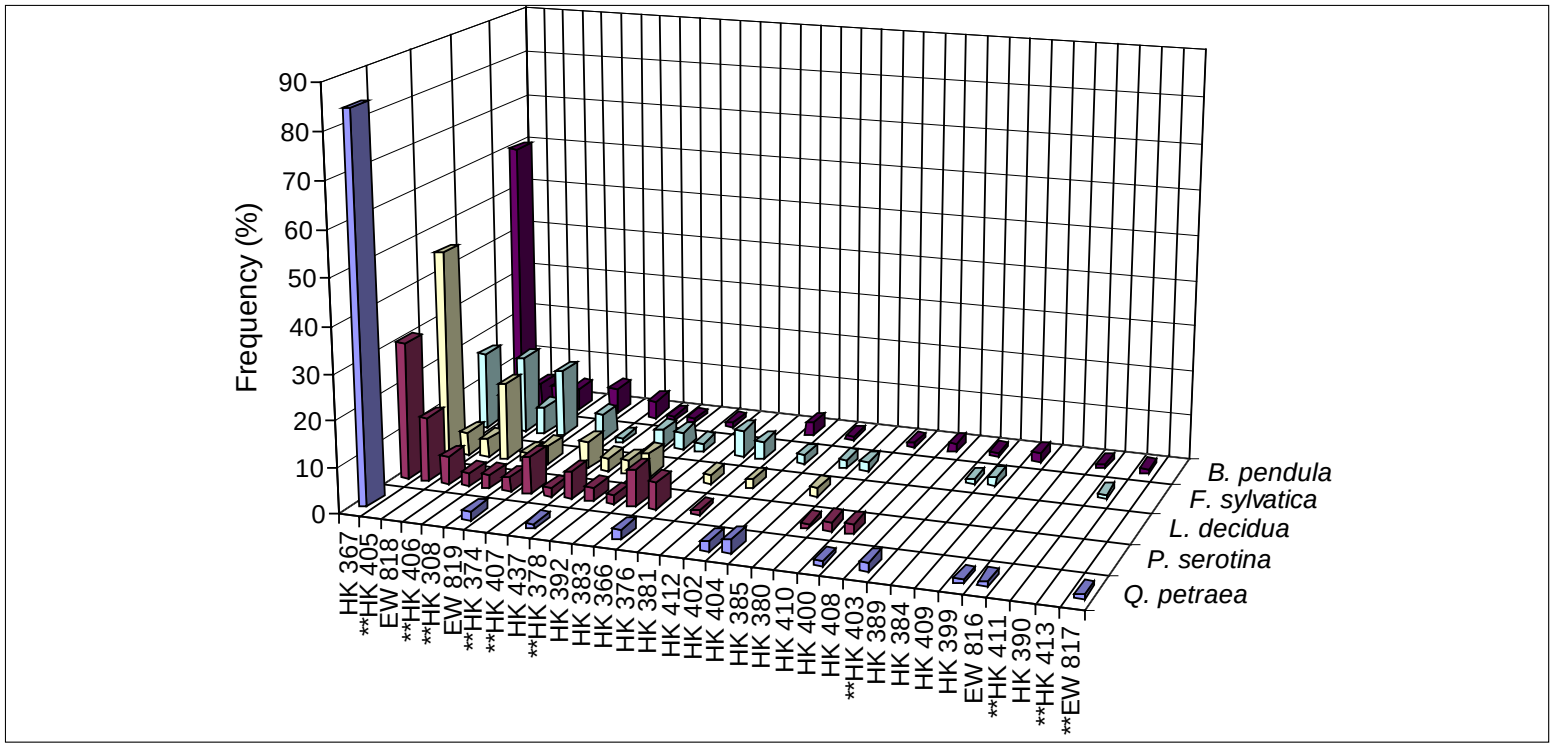

Fig. 2 – Frequency of microbiotal taxa in roots of *B. pendula*, *F. sylvatica*, *L. decidua*, *P. serotina* and *Q. petraea* estimated by the molecular method.