RESEARCH ARTICLE

Termination of belowground C allocation by trees alters soil fungal and bacterial communities in a boreal forest

Stephanie A. Yarwood[1], David D. Myrold[2] & Mona N. Högberg[3]

[1]Department of Microbiology, Oregon State University, Corvallis, OR, USA; [2]Department of Crop and Soil Science, Oregon State University, Corvallis, OR, USA; and [3]Department of Forest Ecology and Management, University of Agricultural Sciences, Umeå, Sweden

Correspondence: Stephanie A. Yarwood, Department of Microbiology, Oregon State University, Nash Hall 220, Corvallis, OR 97331, USA. Tel.: +1 541 737 1840; fax: +1 541 737 0496; e-mail: stephanie.yarwood@oregonstate.edu

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Abstract

The introduction of photosynthates through plant roots is a major source of carbon (C) for soil microbial biota and shapes the composition of fungal and bacterial communities in the rhizosphere. Although the importance of this process, especially to ectomycorrhizal fungi, has been known for some time, the extent to which plant belowground C allocation controls the composition of the wider soil community is not understood. A tree-girdling experiment enabled studies of the relationship between plant C allocation and microbial community composition. Girdling involves cutting the phloem of trees to prevent photosynthates from entering the soil. Four years after girdling, fungal and bacterial communities were characterized using DNA-based profiles and cloning and sequencing. Data showed that girdling significantly altered fungal and bacterial communities compared with the control. The ratio of ectomycorrhizal to saprobic fungal sequences significantly decreased in girdled treatments, and this decline was found to correlate with the fungal phospholipid fatty acid biomarker 18:2ω6,9. Bacterial communities also varied in the abundance of the two dominant phyla *Acidobacteria* and *Alphaproteobacteria*. Concomitant changes in fungal and bacterial communities suggest linkages between these two groups and point toward plant belowground C allocation as a key determinant of microbial community composition.

Introduction

The carbon (C) available to soil microorganisms is ultimately derived from plant photosynthesis. Thus, processes or factors that influence photosynthesis and the subsequent allocation of fixed C to plant above- and belowground parts also control the availability of soil C, microbial activity, and microbial community composition. There are two major allocation routes for fixed C from the tree canopy to soil: (1) C is allocated via the phloem directly as sugars to roots, symbiotic ectomycorrhizal fungi, and other root-associated microorganisms, or (2) C goes into organic matter that is deposited on or in the soil as litter to be decomposed by heterotrophic organisms. The effect of litter-fall on heterotrophic communities is the best known pathway (Wardle, 1992; Wardle *et al.*, 2004; Högberg & Read, 2006). Much less is known about the more direct effect of roots and fine root turnover on forest soil microorganisms and their activities (Fransson *et al.*, 2001; Brant *et al.*, 2006; Parent *et al.*, 2006; Drigo *et al.*, 2008; Högberg *et al.*, 2008), mainly because of the many methodological difficulties.

Högberg *et al.* (2003) proposed that the regulation of plant C allocation (Cannell & Dewar, 1994; Hansen *et al.*, 1997; Lambers *et al.*, 2008), in response to variations in nutrient supply, played a vital role in soil microbial community composition. The hypothesis was tested by a 'tree-girdling' experiment in a boreal Scots pine forest (Högberg *et al.*, 2001; Högberg & Högberg, 2002; Bhupinderpal-Singh *et al.*, 2003; Göttlicher *et al.*, 2006). Girdling involves stripping off the bark of trees, terminating the flow of current tree photosynthates (sugars) via the phloem to the roots. The tree-girdling treatment avoided direct physical disturbance of roots, ectomycorrhizal fungi, and associated microorganisms. The treatment caused surprisingly large (*c.* 50%) reductions in soil respiration rates within a few weeks and a loss of one-third of the microbial biomass after

1–3 months (Högberg & Högberg, 2002). Importantly, the low respiration rate was rather stable and still evident up to 4 years after the girdling treatment (Bhupinderpal-Singh *et al.*, 2003; Göttlicher *et al.*, 2006). It was suggested that the decrease in microbial biomass was mainly due to loss of ectomycorrhizal fungi. Similar results have been obtained in tree-girdling experiments elsewhere (Schulze *et al.*, 2004; Scott-Denton *et al.*, 2006; Zeller *et al.*, 2008). In another large-scale field experiment in the same area as in Högberg *et al.* (2001), tree photosynthates were labeled with $^{13}CO_2$ at ambient CO_2 for 1.5 h to trace the photosynthates into the soil system. The label peaked after 2–4 days in both the microbial cytoplasm and in soil respired CO_2, indicating a very tight coupling between roots and soil microorganisms (Högberg *et al.*, 2008). Approximately half of the soil activity in these forests appears to be supported by C from recent plant photosynthesis, which in turn may have a profound effect on the microbial community composition. The finer details of changes are not known.

We hypothesize that the long-term consequences of tree girdling, sustained disruption of root photosynthates and the decomposition of roots would continue to influence the microbial community composition 4 years after the experiment's establishment. Given that mycorrhizal and saprobic fungi compete for soil nutrients (Gadgil & Gadgil, 1971, 1975; Lindahl *et al.*, 2001), the dramatic decrease in ectomycorrhizal fungi following girdling would lead to a change in the fungal community composition in favor of saprobic fungi. The bacterial community would also be affected by decreases in C flow and resulting shifts in fungal communities. Olsson & Wallander (1998) reported differences in bacterial community composition when different species of ectomycorrhizal fungi were present, in part due to changes in soil solution chemistry. These indirect chemical effects may include lower soil pH in the presence of ectomycorrhizal fungi (Griffith *et al.*, 1994), which may lead to shifts in both fungal and bacterial composition (Blagodatskaya & Anderson, 1998; Fierer & Jackson, 2006).

Here, we determined the soil fungal and bacterial community composition in the same large-scale tree-girdling experiment as described in Högberg *et al.* (2001). Experimental treatments included control plots (not girdled), early girdling, and late girdling plots. In the original study design, trees were girdled in the early and late summer to investigate the impact of seasonal variation in plant C allocation of photosynthates to belowground parts combined with girdling.

Soil DNA was extracted and amplified to target regions of bacterial 16S rRNA gene and fungal internal transcribed spacer region (ITS). The resulting DNA was used to generate community profiles [length heterogeneity (LH)-PCR profiles of the ITS and terminal restriction fragment length polymorphism (T-RFLP) profiles of the 16S rRNA gene] and subjected to cloning and sequencing. The study enabled us to identify dominant members of the soil bacterial and fungal communities and examine the relative abundance of ectomycorrhizal fungi. Bååth *et al.* (2004) suggested that the decrease of ectomycorrhizal fungi in soil was related to the loss of the fungal biomarker phospholipid fatty acid (PLFA) 18:2ω6,9 during 1–6-month-long laboratory incubations at 15–25 °C. Högberg (2006) and Högberg *et al.* (2007) report similar decreases in the PLFA 18:2ω6,9, using the same soils that we test here. In light of these observations, we also investigate possible correlations between the relative abundance of PLFA 18:2ω6,9 as a biomarker for ectomycorrhizal fungi and ectomycorrhizal sequences.

We hypothesized the following: (1) Tree girdling would result in a shift in the ratio of ectomycorrhizal to saprobic fungi. (2) The bacterial community dependent on or associated with ectomycorrhizal fungi would differ under girdling. (3) The ratio of the fungal PLFA biomarker 18:2ω6,9 to total fungal PLFAs would correlate with the relative abundance of fungal ITS sequences originating from ectomycorrhizal fungi.

Materials and methods

Site description and sample collection

The study was performed in the large-scale field experiment established in 2000 at Åheden, northern Sweden (64°14′N, 19°46′E). The experiment has been previously described in detail by Högberg *et al.* (2001). Briefly, the forest at this location is dominated by 49–59-year-old Scots pine (*Pinus sylvestris*) with underbrush communities of *Vaccinium vitis-idaea* and *Calluna vulgaris* and small seedlings of *P. sylvestris*. Tree girdling is a forestry practice applied when trees are to be killed without felling, and involves cutting through the bark (including the phloem). In 2000, this procedure was used to manipulate the direct tree belowground C allocation, terminating the flow of photosynthates to the roots and soil system. Trees were girdled in early and late summer at a breast height of 1.3 m. The experiment was a randomized block design with three replicate plots (30 × 30 m) of each of three treatments: control plots (ungirdled), early girdled plots, and late girdled plots.

Soils were collected on August 26, 2004, 4 years after trees were girdled. In each experimental plot, nine random soil cores of the organic mor layer (F+H horizons, approximately corresponding to Oe+Oa) were taken with a 0.15-m corer. Three cores were then bulked together, resulting in three composite samples per plot (Högberg *et al.*, 2007). In the field, soils were sieved and roots were sorted out. A maximum period of 3 h at 11–14 °C preceded storage on dry ice (− 78 °C). Samples were transported back to the lab on dry ice and frozen at − 20 °C until extraction of DNA.

DNA extractions and PCR amplification

DNA was extracted from soil (0.25 g) using a PowerSoil™ DNA isolation kit (MO BIO Laboratories, Carlsbad, CA) according to the manufacturer's instructions, with the modification that a FastPrep instrument was used to lyse cells (Bio 101, Carlsbad, CA). The PowerSoil™ bead-beating tubes were shaken for 45 s using the FastPrep instrument. Extracts were quantified using an ND-1000 UV–Visible Spectrophotometer (Nanodrop Technologies, Wilmington, DE) and diluted to 25 ng μL^{-1}. Three soil samples from each plot were extracted and used in separate PCR amplifications. PCR products from these three plot replicates were used to generate fungal LH-PCR and bacterial T-RFLP profiles (the mean peak areas for each sample were then used in plot-level analysis). For sequencing, an additional PCR reaction was performed on each soil extract. The three PCR products from the same plot were pooled and used in cloning reactions.

Fungal LH-PCR and bacterial T-RFLP profiles

The fungal ITS was amplified from soil DNA extracts (100 ng DNA) with primers ITS1F and ITS4 (Gardes & Bruns, 1993) under previously described conditions (Anderson *et al.*, 2003). Because this region of the fungal genome varies between 400 and 900 bp in length, LH-PCR profiles were generated. The bacterial 16S rRNA gene was amplified from soil DNA extracts (100 ng DNA) using conventional PCR reactions as described by Hackl *et al.* (2004). Primers 16S 8-F (Edwards *et al.*, 1989) and 16S 907-R (Muyzer *et al.*, 1995) were used for these reactions. The ITS1F and 16S 8-F primers contained FAM labels for sequence detection. 16S PCR products were cleaned using a Qiaquick™ PCR Purification kit (Qiagen Inc., Valencia, CA) and restricted using the restriction enzyme AluI. Restriction products were column purified and T-RFLP and LH-PCR profiles were generated by Oregon State University's Center for Genome Research and Biocomputing using an ABI 3100 capillary DNA sequencer (Applied Biosystems, Foster City, CA).

Cloning and sequencing

Clones were generated using a Topo TA cloning™ kit for sequencing (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. PCR products from three separate amplifications per plot were composited and used in one cloning reaction, resulting in a total of three cloning reactions per treatment and per gene. Approximately 500 clones were screened for each of the genes (fungal ITS and bacterial 16S rRNA gene). Those clones containing inserts were plasmid purified using QIAprep™ Spin Miniprep kit (Qiagen Inc., Carlsbad, CA) and sequenced by the High Throughput Genomics Unit (Department of Genome Science, University of Washington, Seattle, WA). A total of 288 clones containing fungal ITS genes were sequenced: 96 clones from each of the three treatments. In the case of 16S rRNA genes, 192 clones were sequenced: 96 clones from control plots, 48 clones from early girdling plots, and 48 clones from late-girdling plots.

Statistical and phylogenetic analysis

T-RFLP and LH-PCR profiles were analyzed using GENOTYPER version 3.7 (Applied Biosystems). T-RFLP and LH-PCR profiles were further analyzed according to methods described previously (Boyle *et al.*, 2006) using PC-ORD multivariate analysis of ecological data, version 4.06 (MJM software, Gleneden Beach, OR). Nonmetric multidimensional scaling (NMS) was used to visualize community composition and blocked multiresponse permutation procedures (MRPP) were used to determine treatment differences by calculating *P*-values (McCune & Grace, 2002). Blocked MRPP analysis was used to compare differences between treatments (accounting for plot variations due to blocks) by comparing the sample distance matrix to a random distribution, resulting in a *P*-value and an *A*-statistic. An *A*-statistic is a measure of within-group variability, with values > 0.1 indicative of low variability. The same distance measure was used (i.e. Bray–Curtis) for both NMS and blocked MRPP, ensuring agreement between these two analyses. Indicator species analysis was performed to determine which peaks contributed to treatment differences (McCune & Grace, 2002). In indicator species analysis, samples are identified as belonging to a particular treatment and the frequency or abundance of a single species (i.e. LH-PCR or T-RFLP peak) is compared with a random distribution. *P*-values < 0.05 were considered significant. A Mantel test was used to compare fungal and bacterial communities by creating distance matrices and comparing sample distances for both communities (McCune & Grace, 2002).

DNA sequences were aligned using CLUSTALX version 1.81 (Thompson *et al.*, 1997) and alignments were edited using BIOEDIT sequence alignment editor version 7.0.5 (Hall, 1999). All sequences were analyzed using MALLARD version 1.02 (Cardiff University, Boston, MA) to ensure that no chimeras or other sequencing anomalies occurred. Sequence identification was performed using the NCBI BLAST search engine (BLAST-N) and only exact matches were considered accurate (*E*-values = 0). Sequences were also analyzed for chimeras by taking the first and last half of each sequence and running separate BLAST searches to ensure that both halves of the sequences could also be correctly identified. Identification of 16S rRNA gene sequences was confirmed using the software and information from the Ribosomal Database Project (Cole *et al.*, 2007); here, sequences were identified to the

lowest taxonomic level by a percent similarity value of 95% or more. In the case of ITS genes belonging to ectomycorrhizal fungi, confirmation was achieved using the ectomycorrhizal fungal sequence database UNITE (Kõljalg *et al.*, 2005) (http://unite.ut.ee/index.php). All sequences were submitted to GenBank and have been assigned accession numbers FJ475352–FJ475824.

Sequences were further analyzed by calculating richness estimates and examining the numbers of operational taxonomic units (OTUs) using the DOTUR program (Schloss & Handelsman, 2005). A 95% similarity value was used for both fungal ITS and bacterial 16S rRNA gene sequences. Sugita *et al.* (1999) found that cultured representatives of the same fungal species had $>$ 95% similarity in both regions of the ITS, and 95% similarity in bacterial 16S rRNA genes has been found to roughly correspond to a species grouping (Acinas *et al.*, 2004). TREECLIMBER (Schloss & Handelsman, 2007) was used to test for differences among clone libraries. TREECLIMBER calculates a parsimony-based test statistic to determine phylogenetic-based variation between two or more subpopulations. In order to complete TREECLIMBER analysis, phylogenetic trees were constructed using MRBAYES version 3.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) and confirmed using PHYLIP version 3.2 (Felsenstein, 1989). MRBAYES was run using an omega variation model (M3), a codon model that calculates the likely rate of variation at each site. The model was run for 1 million iterations to ensure convergence at a stable value (Hall, 2001). Means are reported $\pm$ 1 SE.

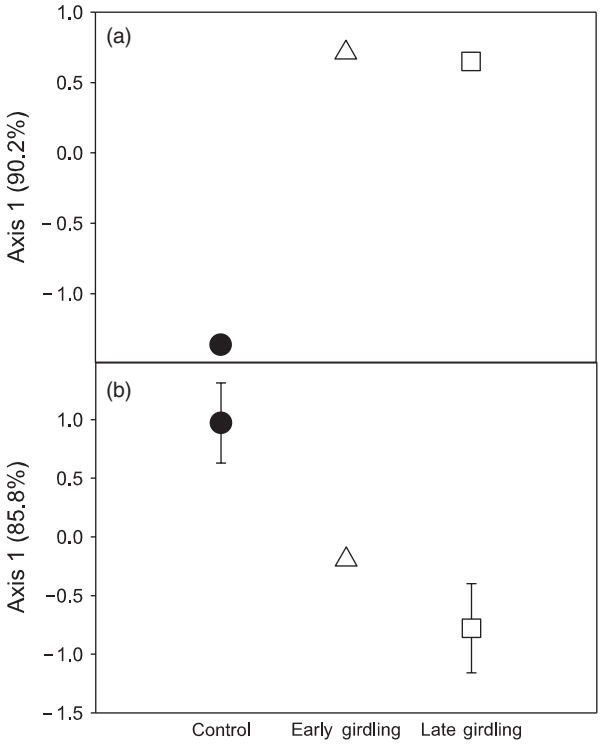


Fig. 1. NMS ordinations of (a) fungal ITS LH-PCR profiles and (b) bacterial 16S T-RFLP profiles. NMS analysis resulted in a one-dimensional solution shown here as axis 1. The percent of data explained is given in parentheses. Data are the means with SEs ($n = 3$). In some cases, SE bars are smaller than symbols.

Results

Community profiles

Seventy-five unique fragment lengths were identified from all three treatments of fungal ITS LH-PCR profiles. The majority of these fragments occurred only once. Any one sample contained an average of 15 full-length fragments. The numbers of fragments were not significantly different among treatments and there were no observed differences in Shannon's diversity index within the data set. When blocked MRPP was used to test for differences between all three treatments, fungal community composition did differ significantly among all treatments (P-value = 0.031, A-statistic = 0.4). Results of NMS ordinations showed that control plots clustered apart from the two girdling treatments, but early and late girdling did not separate (Fig. 1a). Therefore, pairwise comparisons using blocked MRPP were also performed: the control plots were different from early- (P-value = 0.0062, A-statistic = 0.4) but not late- (P-value = 0.062, A-statistic = 0.3) girdling treatments. There was no significant difference between early- and late-girdling profiles (P-value = 0.911, A-statistic = $-$ 0.06). Indicator species analysis identified seven fragments that were significant treatment indicators. Of these peaks, four fragments comprised small percentages of the total populations (each peak averaged 1–5% of the total fluorescence signal) and were subsequently not detected in clone libraries. The remaining three peaks could be identified through sequencing. Two of these peaks were tentatively identified as *Cortinarius* sp. and were more abundant in control treatments (P-values = 0.033 and 0.032). The other peak was also more prevalent in control soils and closely matched other environmental fungi in the family *Atheliacaea* (P-value = 0.032).

In the case of 16S T-RFLP profiles, all samples had a total of 44 terminal restriction fragments (T-RFs) and samples contained an average of 24 T-RFs. Similar to the fungal profiles, 16S rRNA gene profiles did not differ based on Shannon's diversity index, but blocked MRPP analysis showed that bacterial community composition was significantly different among the three treatments (P-value = 0.023, A-statistic = 0.3). In ordinations, treatments separated along axis one (Fig. 1b). In pairwise comparisons, there were no significant differences between control plots and early (P-value = 0.063, A-statistic = 0.5)- or late (P-value = 0.063, A-statistic = 0.3)-girdling plots. Similarly, early- and late-

girdling plots were not significantly different (P-value = 0.41, A-statistic = 0.0). Indicator species analysis identified nine T-RFs that significantly contributed to differences between control and girdling treatments. Similar to the fungal indicators, the majority of 16S T-RFs composed only a small percentage of the total community profiles (1–4%) and were not detected in the clone libraries. The exceptions were four peaks that matched to dominant bacterial phyla: *Actinobacteria* (P-value = 0.004), *Acidobacteria* (two peaks, P-values = 0.022, 0.030), and *Proteobacteria* (class *Alphaproteobacteria*) (P-value = 0.030).

Fungal community composition

After screening sequences for chimeras and other anomalies, four ITS sequences were excluded from the final analysis, leaving 95 sequences each for the control and early-girdling treatments and 94 sequences for the late-girdling treatment. Using a 95% similarity cutoff, the control plot contained 30 unique OTUs, the early girdling contained 51 OTUs, and the late girdling contained 58 OTUs. These OTUs contained representatives from 32 known fungal families with the largest number of sequences matching members of six families: *Dermateaceae*, *Helotiaceae*, *Magnaporthaceae*, *Atheliaceae*, *Cortinariaceae*, and *Ganodermataceae* (Table 1). Additionally, sequences from early-girdling treatments matched *Polyscytalum* sp., an ascomycete not assigned in a known order or family, and several sequences from early- and late-girdling treatments matched to environmental clones tentatively assigned to the order *Sebacinales*. The control plots had an average of 6 ± 1 fungal families; early- and late-girdling sequences averaged 9 ± 1 and 8 ± 2 families, respectively. A total of 32 sequences did not match known fungal groups, but were highly similar to other environmental clones recovered from soils and root tips. The majority of unknown sequences were found in early (9)- and late (21)-girdling treatments.

Table 1. Dominant phyla and families of fungi included in the sequences from control, early-girdling, and late-girdling treatments

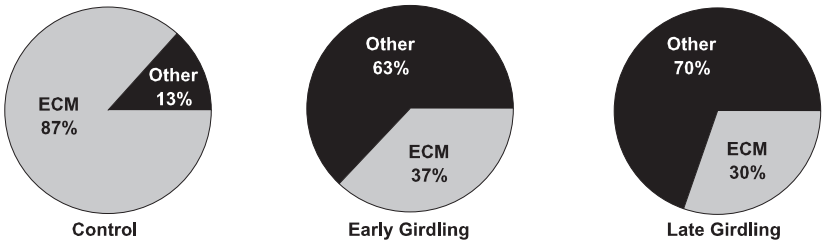
Fungal group	Control	Early girdling	Late girdling
Ascomycota			
Dermateaceae	0	2	7
Helotiaceae	5	10	5
Magnaporthaceae	0	1	5
Polyscytalum sp. (family *incertae sedis*)	0	14	0
Basidiomycota			
Atheliaceae	45	0	0
Cortinariaceae	29	13	0
Ganodermataceae	0	5	0
Environmental clone (order *Sebacinales*)	0	11	19
Unknown	5	13	27

Numbers show the total sequences recovered for each fungal family. (Note: only groups containing more than five sequences are shown here.) Sequences identified in the order *Sebacinales* did not match known families, but were highly related to each other, suggesting similarity at least at the family level. 'Unknown' sequences did not match any known fungal families.

Fungal families were placed into two groups: those that contain known ectomycorrhizal symbionts and those families containing other fungi where ectomycorrhizal associations are not known. Excluding those sequences that only matched unknown environmental fungal clones, the proportion of ectomycorrhizal to other fungal sequences was calculated for each treatment (Fig. 2). The percentage of ectomycorrhizal fungal sequences averaged $87 \pm 2\%$ of the total sequences recovered in the control plots, but the proportion of sequences of ectomycorrhizal fungi was significantly lower (P-value < 0.001) in early girdling, averaging $37 \pm 5\%$, and late girdling, averaging $30 \pm 9\%$ (Fig. 2).

Phylogenetic analysis using TREECLIMBER supported differences between the control and the girdling treatments (Table 2). When all three treatments were compared with each other, parsimony tests using TREECLIMBER showed that the clone libraries were significantly different. Control plots were also significantly different from late-girdling plots, but in the case of control vs. early-girdling treatment, the clone libraries were not significantly different. Both the control and early-girdling clone libraries contained a large number of fungal sequences belonging to the family *Cortinariaceae* and this may explain the higher P-value. Interestingly, although both treatments contained the family *Cortinariaceae*, the control plot sequences matched the genus *Cortinarius*, whereas the early-girdling sequences primarily matched the genus *Gymnopilus*. Clone libraries did not differ between early girdling and late girdling (Table 2).

Presumptive LH-PCR fragment lengths were calculated for each sequence and compared with ITS LH-PCR profiles. Several of the sequence lengths matched fragments from the ITS profiles. These included fragment lengths of 617 and 683 bp. A total of six sequences had a length of 617 bp. Three of these sequences closely matched *Polyscytalum* sp. and three exactly matched *Phialophora* sp. clone aurim 712, a described saprobic fungus (accession number DQ069046). There was an increased relative abundance of fragment 617 in both early (14%)- and late-girdling (17%) treatments compared with the control (5%). Profiles from girdling plots had a significantly greater proportion of this indicator (P-value = 0.004) (Fig. 3). Fragment 683 had previously been identified as an indicator species for control soils. Forty-five sequences were 683 bp in length and belonged to *Atheliaceae*. Some members of the family *Atheliaceae* are known to form ectomycorrhizal associations and all sequences matched



Other fungal families		Ectomycorrhizal families
Dermateaceae	*Cystofilobasidiaceae*	*Atheliaceae*
Helotiaceae	*Ganodermataceae*	*Bankeraceae*
Herpotrichiellaceae	*Hyaloriaceae*	*Cortinariaceae*
Magnoporthaceae	*Psathyrellaceae*	*Clavulinaceae*
Mycosphaerellaceae	*Stereaceae*	*Suillaceae*
Myxtrichaceae	*Strophariaceae*	
Rhytismataceae	*Trechisporaceae*	
Trichocomaceae	*Tricholomataceae*	
Verturiaceae	*Typhylaceae*	
Verrucariaceae	*Mortierellaceae*	
Polyscytalum sp. (family incertae sedis)		

Fig. 2. Pie charts showing the distribution of ectomycorrhizal (ECM) and nonectomycorrhizal (other) fungal families for ITS sequences. Numbers represent the percentage of sequences found in each treatment: control ($n = 90$), early girdling ($n = 81$), and late girdling ($n = 66$). Fungal families in the ectomycorrhizal and nonectomycorrhizal grouping are listed in the table.

Table 2. Comparison of fungal ITS clone libraries using parsimony tests of phylogenetic trees

Comparisons	*P*-value
Control vs. early girdling vs. late girdling	0.003
Control vs. girdling	0.007
Control vs. early girdling	0.068
Control vs. late girdling	0.031
Early girdling vs. late girdling	0.160

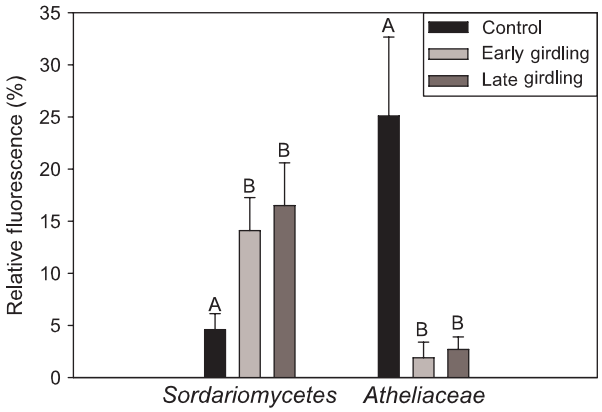


Fig. 3. Abundance of selected LH-PCR fragments matching clone library sequences. LH-PCR fragment 617 contained members of the class *Sordariomycetes* (family unknown), a group of saprobic ascomycetes. Fragment 683 was identified as an indicator for the family *Atheliaceae*, which has known ectomycorrhizae. Bars are the mean relative fluorescence for each treatment with SEs ($n = 3$). Different letters indicate significant treatment effects.

closely to environmental fungi recovered from root tips of various plant species including roots from *P. sylvestris* and *Tsuga canadensis*. The abundance of fragment 683 was the highest in control plots (25%) and the lowest in early-girdling plots (2%) (Fig. 3). The relative abundance of fragment 683 was significantly higher in control plots compared with both girdled treatments (*P*-value = 0.003).

Bacterial community composition

Two 16S rRNA gene sequences from the control treatments were excluded from analysis because of unexplained anomalies resulting in a total of 94 control sequences, 48 early-girdling sequences, and 48 late-girdling sequences for the final analysis. Using a 95% similarity cutoff, control treatments contained 42 OTUs and early girdling and late girdling contained 26 and 28, respectively. These sequences belonged to nine phyla of bacteria, including a large number of sequences matching known *Proteobacteria* and *Acidobacteria*. The abundances of sequences from these phyla were highly similar among the three treatments (Fig. 4). Within the phylum *Proteobacteria*, *Alphaproteobacteria* were the most abundant sequences in the control treatment clone library ($34 \pm 8\%$) and also made up high proportions of sequences in the early girdling ($25 \pm 3\%$) and late girdling ($28 \pm 8\%$). The order represented by each alphaproteobacterial sequence was also identified. In the case of control treatments, sequences matched known representatives in the orders *Caulobacteriales*, *Rhizobiales*, and *Rhodospirillales*.

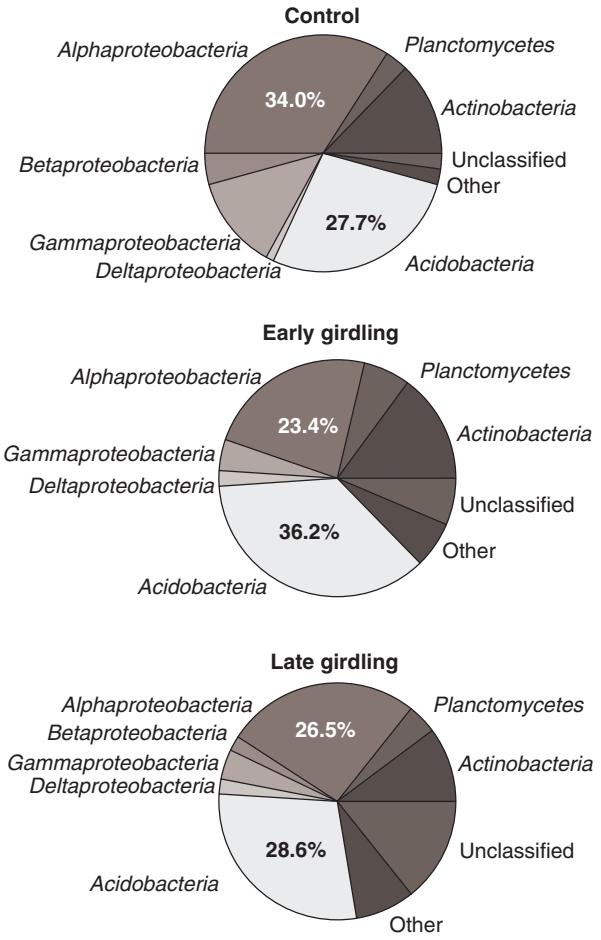


Fig. 4. The abundance of 16S rRNA gene sequences across bacterial phyla for the three treatments: control ($n = 94$), early girdling ($n = 48$), and late girdling ($n = 48$). Phylum *Proteobacteria* is further divided into classes: *Alpha-*, *Beta-*, *Gamma-*, and *Deltaproteobacteria*. Phyla containing fewer than five sequenced representatives were combined into 'Other' phyla; these included *Bacteroidetes*, *Firmicutes*, *Cyanobacteria*, *Gemmatimonadetes*, and *Verrucomicrobia*. All sequences were identified as bacterial 16S rRNA genes, but a total of 12 sequences could not be classified into known phyla and so are listed as unclassified bacteria. Percentages show the abundance of *Alphaproteobacteria* and *Acidobacteria* in each clone library.

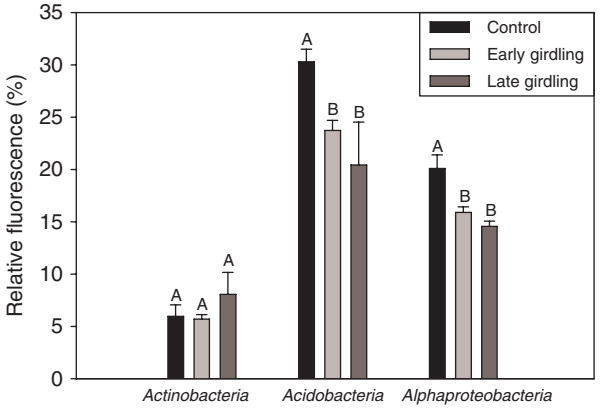


Fig. 5. Abundance of selected T-RFs matching clone library sequences. *Actinobacteria* are the sum of T-RF lengths 137, 231, and 243 bp, *Alphaproteobacteria* are the sum of T-RFs 140 and 207–208 bp, and *Acidobacteria* are the sum of 152 and 721 bp. Bars are the mean relative fluorescence for each treatment with SEs ($n = 3$). Different letters indicate significant treatment effects.

Sequences from both girdling treatments belonged to *Rhizobiales* and *Rhodospirillales*. Across all treatments, sequences from the order *Rhodospirillales* matched within the family *Acetobacteraceae*.

T-RF fragment lengths were identified for each sequence using the BIOEDIT program and compared with 16S T-RFLP profiles. T-RFs specific for three bacteria phyla were identified: *Actinobacteria*, *Proteobacteria* (specifically *Alphaproteobacteria*), and *Acidobacteria*. Sequences from *Actinobacteria* contained cut sites 137, 231, and 243 bp, alphaproteobacterial sequences cut at 140 and 207–208 bp, and *Acidobacteria* T-RFs were identified at 152 and 721 bp. Each of these T-RFs had three or more sequenced representatives. Previous indicator species analysis had also identified T-RFs 137 (P-value = 0.004), 207 (P-value = 0.030), and 152 (P-value = 0.022) as significant indicators. These T-RFs were compared with T-RFLP profiles and summed to approximate the contribution of each bacterial phylum to community composition (Fig. 5). *Actinobacteria* composed on average 11% of the bacterial community and did not significantly vary among treatments (P-value = 0.507). The contributions of acidobacterial and alphaproteobacterial indicator peaks were higher in control plots with means of $31 \pm 1\%$ and $21 \pm 1\%$, respectively. In early-girdling treatments, *Acidobacteria* indicator fragments made up an average of $24 \pm 4\%$ and *Alphaproteobacteria* represented $16 \pm 1\%$ of the community, and in late-girdling treatments, *Acidobacteria* indicator peaks averaged $21 \pm 4\%$ and *Alphaproteobacteria* indicator peaks averaged $15 \pm 4\%$ of the community. The abundance of *Acidobacteria* and *Alphaproteobacteria* did not differ between early and late girdling, but control treatments had significantly greater abundance of indicator fragments for *Acidobacteria* (P-value = 0.016) and *Alphaproteobacteria* (P-value = 0.049). No differences were observed between clone libraries in parsimony comparisons using TREECLIMBER (data not shown).

Rarefaction curves indicated that neither the ITS nor the 16S rRNA gene sequencing efforts were sufficient for capturing the total community diversity (data not shown). This inability to saturate fungal (Horton & Bruns, 2001; O'Brien *et al.*, 2005) and bacterial (Roesch *et al.*, 2007) rarefaction curves has also been found by others. A Mantel test was used to test for correlations between fungal LH-PCR profiles and

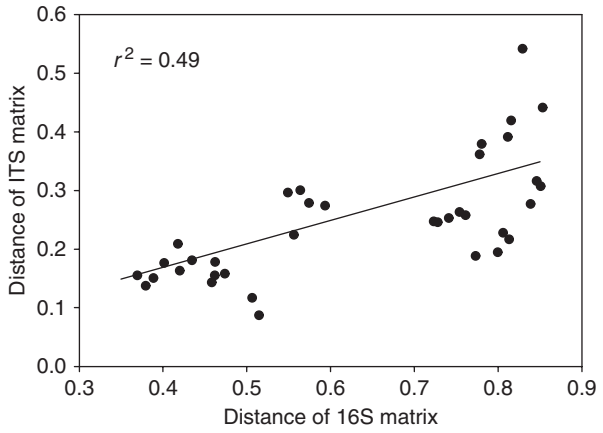


Fig. 6. Correlation of fungal and bacterial communities as determined by a Mantel test distance matrix. Each point represents the calculated distance of ITS and 16S rRNA gene profiles for each row and column of the distance matrix.

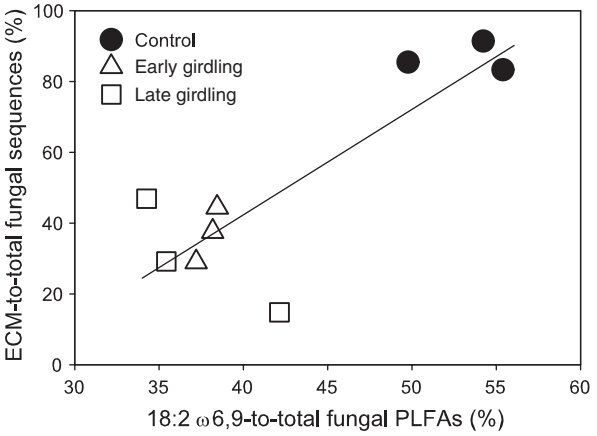


Fig. 7. Correlation between the percentage ectomycorrhizal fungal sequences out of total fungal sequences and the PLFA biomarker 18:2ω6,9 out of total fungal biomarkers for the three treatments: control, early girdling, and late girdling.

bacterial T-RFLP profiles. Results showed that the two communities were highly correlated ($P < 0.001$, $r^2 = 0.49$) (Fig. 6).

Discussion

LH-PCR profiles clearly indicated a difference in fungal community composition between control and girdled treatments (Fig. 1), and sequence identification suggests that the fraction of ectomycorrhizal fungi significantly decreased following girdling (Fig. 2). Also, fungal clone libraries suggested changes in the community (Table 2), which complimented LH-PCR profiles. Dominant fungal lineages in control plots included *Cortinarius* sp. and a number of environmental samples belonging to the family *Atheliaceae*. Of those clones matching *Atheliaceae* sequences, matches were made to environmental sequences tentatively identified as *Piloderma* sp. (accession numbers AY097053, DQ474722, and DQ474729). Both ectomycorrhizal fungi, *Cortinarius* sp. and *Piloderma* sp., have been previously described in *P. sylvestris* forests (Shaw *et al.*, 2003; Lindahl *et al.*, 2007; Stankeviciene *et al.*, 2008). An *Atheliaceae*-specific LH-PCR fragment was significantly higher in control vs. girdled treatments (Fig. 3), and indicated that this fungal family represents at least one-quarter of the total fungal abundance in undisturbed *P. sylvestris* forest soil.

Högberg (2006) and Högberg *et al.* (2007) reported a 45% decrease in the PLFA 18:2ω6,9 in girdling compared with control plots and suggested that this drop was likely due to a reduction in ectomycorrhizal fungi following girdling. In contrast, the PLFA 18:1ω9 did not correlate to girdled treatments, suggesting that this biomarker can be used as a more general fungal indicator. This previously reported decrease in the PLFA ratio 18:2ω6,9 to the sum of 18:2ω6,9 and 18:1ω9 correlated to a decline in the abundance of ectomycorrhizal sequences ($r^2 = 0.70$) (Fig. 7). Hence, both variables are influenced by tree belowground C allocation. These observations support Bååth *et al.*'s (2004) contention that the 18:2ω6,9 is a good biomarker for ectomycorrhizal fungi and supports the conclusions drawn by Högberg *et al.* (2003) that ectomycorrhizal fungi contribute a large proportion of total fungal biomass in this type of forest. The highly significant correlation we found should be evaluated with known ectomycorrhizal and saprobic fungi.

In contrast to control soils, fungal communities in girdled plots were composed of ericoid mycorrhizal and saprobic fungi. Clone libraries from girdled plots contained a large number of sequences belonging to the order *Sebacinales* (Table 1), a group of fungi known to form ericoid mycorrhiza (Selosse *et al.*, 2007). Clones matched unidentified environmental sequences, but in all cases, the closest sequence matches came from roots of ericaceous shrubs such as *C. vulgaris* and *Vaccinium myrtillus*. Clone libraries for early- and late-girdling treatments also contained members of the family *Helotiaceae*, and these sequences were most closely matched to *Rhizoscyphus ericae*, another known ericoid mycorrhizal fungus (Bougoure *et al.*, 2007). Again the information in clone libraries supports previous speculation that following girdling, ericoid mycorrhizal fungi compose a significant fraction of fungal biomass (Högberg *et al.*, 2007). The current study cannot determine whether ericoid mycorrhizal population increased in response to decreasing ectomycorrhizal fungi or remained unchanged. Future research is

needed to determine the extent to which ericoid and ectomycorrhizal fungi compete for nutrients. Perhaps not surprising, under girdled trees, soils also contained saprobic fungi including *Gymnopilus sapineus*, a member of the order *Agaricales* known to degrade wood (Guzmán-Dávalos, 1996). The observed increase in the diversity of fungi associated with girdling suggests that under normal forest conditions, ectomycorrhizal fungi dominate and perhaps outcompete pathogenic or saprobic fungi (Lindahl *et al.*, 2001). Research has shown that ectomycorrhizal fungi compete with other fungal species for space and nutrients (Gadgil & Gadgil, 1975; Kennedy *et al.*, 2007a, b), but further research is needed to determine the extent to which ectomycorrhizal fungi influences fungal community structure through direct and indirect competition.

Bacterial T-RFLP profiles differed between control and girdling treatments (Fig. 1). Among all treatments, clone libraries were dominated by bacteria in the phyla *Acidobacteria*, *Proteobacteria*, and *Actinobacteria*. Across all three clone libraries, most acidobacterial sequences matched with members of *Acidobacteria* subdivision 1. The function of this subdivision is not known, but is thought to be metabolically diverse (Barns *et al.*, 1999; Eichorst *et al.*, 2007). Between 27% and 36% of clones were identified as *Acidobacteria*, and T-RFs identified as acidobacterial made up a higher proportion of community profiles in control plots compared with girdling (Fig. 5). Sait *et al.* (2006) reported an increased abundance of *Acidobacteria* subdivision 1 sequences in soils with pH < 6. The pH of Åheden soils significantly differed between control and girdling treatments: control soils had a pH 3.7 and girdled plots had a pH 4.1 (Högberg *et al.*, 2007). These differences in pH and a decrease in DOC and low-molecular-weight organic acids (Giesler *et al.*, 2007) may have contributed to the different proportions of *Acidobacteria* among treatments.

Clones belonging to the *Alphaproteobacteria* also matched with acidophilic genera and groups previously identified as ectomycorrhization helper bacteria. Clones from the control treatments included *Acidisphaera* sp. and *Acidocella* sp., both of which have been described as acidophilic bacteria ubiquitous in environments such as forest soil and acid mine drainage (Hallberg & Johnson, 2001). Interestingly, these two genera were not represented in clone libraries of girdled plots. Clone libraries from control and girdling treatments contained numerous sequences of *Bradyrhizobium*. *Bradyrhizobium* sp. have been identified in clone libraries from similar soil types and it has been suggested that these bacteria may be part of a large group of ectomycorrhization helper bacteria (Frey-Klett *et al.*, 2007; Kataoka *et al.*, 2008). Unfortunately, we were unable to identify a TR-F specific to *Bradyrhizobium* that would allow us to observe changes in abundance between control and girdling, but the high number of sequences, their presence among all treatments, and previous observations (Frey-Klett *et al.*, 2007) suggest that *Bradyrhizobium* populations may significantly contribute to soil function.

Fungal and bacterial community composition was highly correlated among treatments, suggesting close links between fungi and bacteria. Other researchers have reported similar links between arbuscular or ectomycorrhizal fungi and bacteria (Olsson & Wallander, 1998; Singh *et al.*, 2008), suggesting that mycorrhizal communities may influence bacterial community composition across a wide variety of soil types. Although we observed a significant shift in composition of fungal communities (i.e. significant reductions in ectomycorrhizal fungi), the response of bacterial communities was more subtle. Lauber *et al.* (2008) proposed that the factors affecting fungi and bacteria will vary due to scale and metabolic capability. For example, several studies have noted correlations in bacterial community composition and soil pH (Fierer & Jackson, 2006; Lauber *et al.*, 2008; Singh *et al.*, 2008), but Singh *et al.* (2008) did not observe a correlation between fungal community composition and pH. Högberg *et al.* (2001) and Högberg & Högberg (2002) reported a dramatic decrease in ectomycorrhizal fungi just months after girdling, supporting the idea that fungal communities may be closely linked to vegetation types and plant C allocation. More research is needed to fully understand the extent to which fungal and bacterial communities interact, but our research shows that the disruption of plant photosynthate supply to the soil results in shifts in both communities and that these effects persist even after 4 years.

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References

Acinas SG, Klepac-Ceraj V, Hunt DE, Pharino C, Ceraj I, Distel DL & Polz MF (2004) Fine-scale phylogenetic architecture of a complex bacterial community. *Nature* **430**: 551–554.

Anderson IC, Campbell CD & Prosser JI (2003) Potential bias of fungal 18S rDNA and internal transcribed spacer polymerase chain reaction primers for estimating fungal biodiversity in soil. *Environ Microbiol* **5**: 36–47.

Bååth E, Nilsson LO, Göransson H & Wallander H (2004) Can the extent of degradation of soil fungal mycelium during soil incubation be used to estimate ectomycorrhizal biomass in soil? *Soil Biol Biochem* **36**: 2105–2109.

Barns SM, Takala SL & Kuske CR (1999) Wide distribution and diversity of members of the bacterial kingdom

Acidobacterium in the environment. *Appl Environ Microb* **65**: 1731–1737.

Bhupinderpal-Singh, Nordgren A, Ottosson-Löfvenius A, Högberg MN, Mellander P-E & Högberg P (2003) Tree root and soil heterotrophic respiration as revealed by girdling of boreal Scots pine forest: extending observations beyond the first year. *Plant Cell Environ* **26**: 1287–1296.

Blagodatskaya EV & Anderson TH (1998) Interactive effects of pH and substrate quality on the fungal-to-bacterial ratio and QCO_2 of microbial communities in forest soils. *Soil Biol Biochem* **30**: 1269–1274.

Bougoure DS, Parkin PI, Cairney JWG, Alexander IJ & Anderson IC (2007) Diversity of fungi in hair roots of *Ericaceae* varies along a vegetation gradient. *Mol Ecol* **16**: 4624–4636.

Boyle SA, Rich JJ, Bottomley PJ, Cromack Jr K & Myrold DD (2006) Disturbance effects on denitrifying community composition and activity at forest and meadow sites in the Cascade Mountains of Oregon. *Soil Biol Biochem* **38**: 870–878.

Brant JB, Myrold DD & Sulzman EW (2006) Root controls on soil microbial community structure in forest soils. *Oecologia* **148**: 650–659.

Cannell MGR & Dewar RC (1994) Carbon allocation in trees – a review of concepts for modeling. *Adv Ecol Res* **25**: 59–104.

Cole JR, Chai B, Farris RJ, Wang Q, Kulam-Syed-Mohideen AS, McGarrell DM, Bandela AM, Cardenas E, Garrit GM & Tiedje JM (2007) The ribosomal database project (RDP-II): introducing *myRDP* space and quality controlled public data. *Nucleic Acids Res* **35** (database issue): D169–D172 DOI: 10.1093/nar/gkl889.

Drigo B, Kowalchuk G & van Veen J (2008) Climate change goes underground: effects of elevated atmospheric CO_2 on microbial community structure and activities in the rhizosphere. *Biol Fert Soils* **44**: 667–679.

Edwards U, Rogall T, Blöcker H, Emde M & Böttger EC (1989) Isolations and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucleic Acids Res* **17**: 7843–7852.

Eichorst SA, Breznak JA & Schmidt TM (2007) Isolation and characterization of soil bacteria that define *Terriglobus* gen. nov., in the phylum *Acidobacteria*. *Appl Environ Microb* **73**: 2708–2717.

Felsenstein J (1989) PHYLIP – phylogenetic inference package (version 3.2). *Cladistics* **5**: 164–166.

Fierer N & Jackson RB (2006) The diversity and biogeography of soil bacterial communities. *P Natl Acad Sci USA* **103**: 626–631.

Fransson PMA, Taylor AFS & Finlay RD (2001) Elevated atmospheric CO_2 alters root symbiont community structure in forest trees. *New Phytol* **152**: 431–442.

Frey-Klett P, Garbaye J & Tankka M (2007) The mycorrhiza helper bacteria revisited. *New Phytol* **176**: 22–36.

Gadgil RL & Gadgil PD (1971) Mycorrhiza and litter decomposition. *Nature* **233**: 133.

Gadgil RL & Gadgil PD (1975) Suppression of litter decomposition by mycorrhizal roots of *Pinus radiata*. *New Zeal J For Sci* **5**: 33–41.

Gardes M & Bruns TD (1993) ITS primers with enhanced specificity for basidiomycete – application to the identification of mycorrhizae and rusts. *Mol Ecol* **2**: 113–118.

Giesler R, Högberg MN, Strobel BW, Richter A & Högberg P (2007) Current tree photosynthates drive production of dissolved organic carbon and low-molecular weight organic acids in soil solution. *Biogeochem* **84**: 1–12.

Göttlicher SG, Steinmann K, Betson NR & Högberg P (2006) The dependence of soil microbial activity on recent photosynthates from trees. *Plant Soil* **287**: 85–94.

Griffith RP, Baham JE & Caldwell BA (1994) Soil solution chemistry of ectomycorrhizal mats in forest soil. *Soil Biol Biochem* **26**: 331–337.

Guzmán-Dávalos L (1996) New records of the genus *Gymnopilus* (Agaricales, Cortinariaceae) from Mexico. *Mycotaxon* **59**: 61–78.

Hackl E, Zechmeister-Boltenstern S, Bodrossy L & Sessitsch A (2004) Comparison of diversity and compositions of bacterial populations inhabiting natural forest soils. *Appl Environ Microb* **70**: 5057–5065.

Hall BG (2001) *Phylogenetic Trees Made Easy*. Sinauer Associates, Sunderland, MA.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Sym Ser* **41**: 95–98.

Hallberg KB & Johnson DB (2001) Biodiversity of acidophilic prokaryotes. *Adv Appl Microbiol* **49**: 37–84.

Hansen J, Türk R, Vogg G, Heim R & Beck E (1997) Conifer carbohydrate physiology: updating classical views. *Trees – Contributions to Modern Tree Physiology* (Rennenberg H., *et al.*, eds), pp. 97–108. Backhuys Publishers, Leiden, the Netherlands.

Högberg MN (2006) Discrepancies between ergosterol and the phospholipid fatty acid 18:2ω6,9 as biomarkers for fungi in boreal forest soils. *Soil Biol Biochem* **38**: 3431–3435.

Högberg MN & Högberg P (2002) Extramatrical ectomycorrhizal mycelium contributes one-third of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. *New Phytol* **154**: 791–795.

Högberg MN, Bååth E, Nordgren A, Arnebrant K & Högberg P (2003) Contrasting effects of nitrogen availability on plant carbon supply to mycorrhizal fungi and saprotrophs – a hypothesis based on field observations in boreal forest. *New Phytol* **160**: 225–238.

Högberg MN, Högberg P & Myrold DD (2007) Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? *Oecologia* **150**: 590–601.

Högberg P & Read D (2006) Towards a more plant physiological perspective on soil ecology. *Trends Ecol Evol* **21**: 548–554.

Högberg P, Nordgren A, Buchmann N, Taylor AFS, Ekblad E, Högberg MN, Nyberg G, Ottosson-Löfvenius M & Read DJ (2001) Large-scale forest girdling shows that current photosynthesis drives soil respiration. *Nature* **411**: 789–792.

Högberg P, Högberg MN, Göttlicher SG *et al.* (2008) High temporal resolution tracing of photosynthate carbon from the tree canopy to forest soil microorganisms. *New Phytol* **177**: 220–228.

Horton TR & Bruns TD (2001) The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Mol Ecol* **10**: 1855–1871.

Huelsenbeck JP & Ronquist F (2001) MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**: 754–755.

Kataoka R, Taniguchi T, Ooshima H & Futai K (2008) Comparison of the bacterial communities established on the mycorrhizae formed on *Pinus thunbergii* root tips by eight species of fungi. *Plant Soil* **304**: 267–275.

Kennedy PG, Bergemann SE, Hortal S & Bruns TD (2007a) Determining the outcome of field-based competition between two *Rhizopogon* species using real-time PCR. *Mol Ecol* **16**: 881–890.

Kennedy PG, Bergemann SE, Hortal S & Bruns TD (2007b) Competitive interactions among three ectomycorrhizal fungi and their relation to host plant performance. *J Ecol* **95**: 1338–1345.

Kõljalg U, Eberhardt U, Erland S *et al.* (2005) UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. *New Phytol* **166**: 1063–1068.

Lambers H III, Chapin FS & Pons TL (2008) Effects on growth rate, net assimilation rate, and specific leaf area. *Plant Physiological Ecology*, pp. 321–367. Springer-Science/Business Media, New York.

Lauber CL, Strickland MS, Bradford MA & Fierer N (2008) The influence of soil properties on the structure of bacterial and fungal communities across land-use types. *Arch Microbiol* **164**: 165–172.

Lindahl B, Stenlid J & Finlay R (2001) Effects of resource availability on mycelial interactions and ^{32}P transfer between a saprotrophic and an ectomycorrhizal fungus in soil microcosms. *FEMS Microbiol Ecol* **38**: 43–52.

Lindahl BD, Ihrmark K, Boberg J, Trumbore SE, Högberg P, Stenlid J & Finlay RD (2007) Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *New Phytol* **173**: 611–620.

McCune B & Grace JB (2002) *Analysis of Ecological Communities*. MjM Software, Gleneden Beach, OR.

Muyzer G, Teske A, Wirsen CO & Jannasch HW (1995) Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. *Soil Biol Biochem* **40**: 2507–2415.

O'Brien HE, Parrent JL, Jackson JA, Moncalvo JM & Vilgalys R (2005) Fungal community analysis by large-scale sequencing of environmental samples. *Appl Environ Microb* **71**: 5544–5550.

Olsson P-A & Wallander H (1998) Interactions between ectomycorrhizal fungi and the bacterial community in soils amended with various primary minerals. *FEMS Microbiol Ecol* **27**: 195–205.

Parent JL, Morris WF & Vilgalys R (2006) CO_2-enrichment and nutrient availability alter ectomycorrhizal fungal communities. *Ecology* **87**: 2278–2287.

Roesch LF, Fulthorpe RR, Riva A, Casella G, Hadwin AKM, Kent AD, Daroub SH, Camargo FA, Farmerie WG & Triplett EW (2007) Pyrosequencing enumerates and contrasts soil microbial diversity. *ISME* **1**: 283–290.

Ronquist F & Huelsenbeck JP (2003) Mr. Bayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.

Sait M, Davis KER & Janssen PH (2006) Effect of pH on isolation and distribution of members of subdivision 1 of the phylum *Acidobacteria* occurring in soils. *Appl Environ Microb* **72**: 1852–1857.

Schloss PD & Handelsman J (2005) Introducing DOTUR, a computer program for defining operational taxonomic units and estimating species richness. *Appl Environ Microb* **71**: 1501–1506.

Schloss PD & Handelsman J (2007) Introducing TreeClimber, a test to compare microbial community structures. *Appl Environ Microb* **72**: 2379–2384.

Schulze WX, Gleixner G, Kaiser K, Guggenberger G, Mann M & Schulze ED (2004) A proteomic fingerprint of dissolved organic carbon and of soil particles. *Oecologia* **142**: 335–343.

Scott-Denton LE, Rosenstiel TN & Monson RK (2006) Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. *Glob Change Biol* **12**: 205–216.

Selosse M-A, Setaro S, Glatard F, Richard F, Urcelay C & Weiß M (2007) *Sebacinales* are common mycorrhizal associates of *Ericaceae*. *New Phytol* **174**: 864–878.

Shaw PJA, Kibby G & Mayes J (2003) Effects of thinning treatment on an ectomycorrhizal succession under Scots pine. *Mycol Res* **107**: 317–328.

Singh BK, Nunan N, Ridgway KP, McNicol J, Young JPW, Daniell TJ, Prosser JI & Millard P (2008) Relationship between assemblages of mycorrhizal fungi and bacteria on grass roots. *Environ Microbiol* **10**: 534–541.

Stankeviciene D, Kasparavicius J, Rudawska M & Iwanski M (2008) Studies of ectomycorrhizal fungi above- and below-ground in the 50-year-old *Pinus sylvestris* L. forest. *Balt Fores* **14**: 7–15.

Sugita T, Nishikawa A, Ikeda R & Shinoda T (1999) Identification of medically relevant *Trichosporon* species based on sequences of internal transcribed spacer regions and construction of a database for *Trichosporon* identification. *J Clin Microbiol* **37**: 1985–1993.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F & Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **24**: 4876–4882.

Wardle DA (1992) A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. *Biol Rev* **67**: 321–358.

Wardle DA, Bardgett RD, Klironomos JN, Setala H, van der Putten WH & Wall DH (2004) Ecological linkages between aboveground and belowground biota. *Science* **304**: 1629–1633.

Zeller B, Liu J, Buchmann N & Richter A (2008) Tree girdling increases soil N mineralisation in two spruce stands. *Soil Biol Biochem* **40**: 1155–1166.