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ORIGINAL ARTICLE

Disruption of root carbon transport into forest humus stimulates fungal opportunists at the expense of mycorrhizal fungi

Björn D Lindahl[1], Wietse de Boer[2] and Roger D Finlay[1]
[1]Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Uppsala, Sweden and [2]Department of Terrestrial Microbial Ecology, Centre for Terrestrial Ecology, Netherlands Institute of Ecology, Heteren, The Netherlands

Ectomycorrhizal fungi dominate the humus layers of boreal forests. They depend on carbohydrates that are translocated through roots, via fungal mycelium to microsites in the soil, wherein they forage for nutrients. Mycorrhizal fungi are therefore sensitive to disruptive disturbances that may restrict their carbon supply. By disrupting root connections, we induced a sudden decline in mycorrhizal mycelial abundance and studied the consequent effects on growth and activity of free living, saprotrophic fungi and bacteria in pine forest humus, using molecular community analyses in combination with enzyme activity measurements. Ectomycorrhizal fungi had decreased in abundance 14 days after root severing, but the abundance of certain free-living ascomycetes was three times higher within 5 days of the disturbance compared with undisturbed controls. Root disruption also increased laccase production by an order of magnitude and cellulase production by a factor of 5. In contrast, bacterial populations seemed little affected. The results indicate that access to an external carbon source enables mycorrhizal fungi to monopolise the humus, but disturbances may induce rapid growth of opportunistic saprotrophic fungi that presumably use the dying mycorrhizal mycelium. Studies of such functional shifts in fungal communities, induced by disturbance, may shed light on mechanisms behind nutrient retention and release in boreal forests. The results also highlight the fundamental problems associated with methods that study microbial processes in soil samples that have been isolated from living roots.
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Introduction

Filamentous fungi are classified as microorganisms because of the microscopic diameter of their hyphae that enables them to penetrate microsites within different substrates. However, many fungi, especially basidiomycetes, produce mycelia that extend over decimetre to metre distances, through which they may translocate carbohydrates and nutrients over considerable distances (Finlay and Read, 1986; Boddy, 1999). Resource reallocation enables these fungi to mobilise resources locally at one place but to use them to support growth in other, distant parts of their mycelia (Lindahl and Olsson, 2004). Ectomycorrhizal fungi support dense hyphal networks by translocating recently photosynthesised carbohydrates from the roots of their host plants, ensuring efficient nutrient assimilation in humus layers and mineral soils (Leake *et al.*, 2001; Rosling *et al.*, 2004). The dependency on translocation of photosynthetic products through roots and mycelia allows mycorrhizal fungi to act independently of locally available organic carbon in the soil, but at the same time makes them vulnerable to disruptive disturbances.

In the organic humus layers of coniferous forests, ectomycorrhizal species have been found to dominate fungal communities (O'Brien *et al.*, 2005; Lindahl *et al.*, 2007), and in laboratory microcosms, ectomycorrhizal fungi have been found to compete successfully with saprotrophs for soil space and nutrients (Lindahl *et al.*, 1999, 2001, 2002; Werner *et al.*, 2002). Being supported by carbohydrates from their host plants rather than depending on energy from organic matter, mycorrhizal fungi may exclude saprotrophs from energy-depleted substrates, to monopolise nutrients for themselves and their hosts (Lindahl *et al.*, 2007). Under nutrient poor conditions, ectomycorrhizal fungi may also increase nutrient availability by producing extracellular enzymes (Lindahl *et al.*, 2005), facilitating direct recycling of nutrients in organic forms (Lindahl

Correspondence: BD Lindahl, Department of Forest Mycology and Pathology, Swedish University of Agricultural Sciences, Box 7026, Uppsala SE-750 07, Sweden.
E-mail: Bjorn.Lindahl@mykopat.slu.se
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et al., 2002; Read and Perez-Moreno, 2003). Such tight cycling of nutrients ultimately depends on intact translocation pathways between the photosynthesising leaves, via roots to the mycorrhizal mycelium colonising organic substrates in humus and soil.

Previous long-term studies of the effects of root disruption (Siira-Pietikäinen *et al*., 2001; Brant *et al*., 2006) or tree girdling (Högberg and Högberg, 2002; Subke *et al*., 2004) have all found marked decreases in fungal biomass, because of detrimental effects on mycorrhizal taxa (Yarwood *et al*., 2009). Physical disturbance of the microbial community in boreal forest soils also frequently results in release of NH_4^+ (Yavitt and Fahey, 1984; Siira-Pietikäinen *et al*., 2001; Lavoie and Bradley, 2003; Piirainen *et al*., 2007), and elevated NH_4^+ levels have also been observed in association with repeated freeze–thaw cycles (Sulkava and Huhta, 2003) and clear-cutting (Carmosini *et al*., 2003; Lapointe *et al*., 2005). Increased N mineralisation after disturbance has been suggested to depend on a rapid turnover of dead microbial biomass (DeLuca *et al*., 1992). The transformation of disturbance-sensitive, ectomycorrhizal mycelium into a dead resource for opportunistic saprotrophs would involve complex interactions between different functional guilds of microorganisms, regarding which we still know very little.

Boreal forest soils may be described as relatively stable systems. Earthworms, acting to prevent the establishment of mycelial translocation pathways by soil mixing (Butenschoen *et al*., 2007), are rare in boreal forest soils. Although seasonal changes may be pronounced, the dominant plants are evergreen and degradation of litter components is extended over several years, ensuring a continuous supply of carbohydrates to soil microorganisms via roots and litter. We hypothesise that, in this otherwise stable environment, sudden disturbances may quickly shift the balance between different functional guilds of microorganisms, with large subsequent effects on ecosystem processes. To test whether disruption of mycorrhizal translocation pathways stimulates growth of free-living saprotrophs, we studied short-term (14 days) responses of the microbial community to a sudden disturbance induced by the insertion of plastic tubes into forest humus, severing roots. Using DNA-based methods to target both fungi and bacteria, we monitored community changes at the level of species and functional groups. We also measured activities of extracellular enzymes involved in organic matter degradation.

Materials and methods

Sample collection

The field study was conducted in late September 2006 at Jädraås IhV (60°49′ N, 16°30′ E, altitude 185 m), which is a well-documented field site in central Sweden (Persson, 1980; Lindahl *et al*., 2007). The site consists of *Pinus sylvestris* L. forest with an understory of ericaceous dwarf shrubs (*Vaccinium vitis-ideae* L. and *Calluna vulgaris* (L.) Hull) and mosses (*Pleurozium schreberi* (Bridel) Mitten and *Dicranum majus* Turner), growing on a sandy podzol. Plastic tubes (150 mm long, 28 mm diameter) with sharpened edges were pushed through the litter and humus layers down into the mineral soil, using a rubber hammer. In total, 25 tubes were inserted 1 m apart along a linear transect. After 5 days, every other core was retrieved together with control samples, taken along a parallel transect 1 m from the first one. The control tubes were hammered into the soil immediately before the cores were re-collected. The remaining cores were retrieved together with new control cores after a total of 14 days of incubation. Soil cores were sub-divided immediately; mineral soil, mosses and structurally intact litter were discarded and well-fragmented litter and humus was frozen on dry ice directly on sampling. While still frozen, the samples were crushed and roughly homogenised in a mortar and sub-divided for further analyses.

Analyses

Detailed descriptions of the analysis methods are included as supplementary material (Supplementary 1).

Briefly, 4,6-diamidino-2-phenylindole (DAPI) staining bacteria and total fungal hyphal length in the organic samples were enumerated microscopically.

DNA was extracted from 50 mg of humus. The internal transcribed spacer (ITS) region of fungal ribosome-encoding genes and parts of the 16S region of bacterial ribosome-encoding genes were PCR amplified. PCR products were pooled within treatments and cloned into *Escherichia coli*, resulting in four clone libraries (two sampling times × two treatments) each for fungi and bacteria. From each clone library, 48 clones were picked, and the amplicon inserts were re-amplified and sequenced. Sequences were clustered, allowing 1% dissimilarity within clusters, and taxonomic affiliations were assigned based on phylogenetic relationships to reference sequences (Supplementary 2 and 3).

The fungal community composition in each sample was analysed by terminal restriction fragment length polymorphism (T-RFLP) based on polymorphisms in the ITS region. T-RFLP peaks in profiles obtained from humus samples were assigned to fungal taxa by comparisons with T-RFLP patterns obtained from cloned and identified genotypes (Lindahl *et al*., 2007). For each sample, the relative contribution of fungal taxa to the total community was estimated as the fluorescence of their associated T-RFLP peaks divided by the integrated fluorescence of the entire T-RFLP profile.

On the basis of the sequences obtained from cloned ITS amplicons, primers were designed to specifically amplify a group of taxa within the fungal order Helotiales with sequence affinity to

Leptodontidium anamorphs (Supplementary 3). After testing the primers for specificity, the absolute amount of *Leptodontidium* ITS template in the DNA extracts was estimated by quantitative PCR.

Activities of extracellular enzymes were assessed in humus extracts produced by adding 1 g of soil to 3 ml of deionised water and shaking vigorously for 60 min at 4 °C. After centrifugation and filtration of the extracts, activities of laccases, cellulases, manganese peroxidases and N-acetylglucosaminidases (NAGs) were estimated, using colorogenic or fluorogenic substrates.

Statistical analysis

Effects on microbial biomass estimates, amounts of extracted DNA, enzyme activities and *Leptodontidium* abundance values were tested using two-way analysis of variance (ANOVA), with root severing and sampling time as explaining variables. Hyphal lengths, cellulase activities and *Leptodontidium* abundance values were log transformed before analysis, to meet the assumptions of ANOVA. The distribution of laccase and NAG activities did not meet the assumptions of ANOVA, and effects of root severing and sampling time were tested independently using Mann–Whitney *U*-tests. Linear regression was used to test for possible correlations between microbial biomass estimates and the amount of extracted DNA or log-transformed enzyme activities. Possible correlations between *Leptodontidium* abundance and hyphal length or enzyme activities were also tested by linear regression.

The distributions of cloned amplicons across bacterial phyla and across fungal orders were tested for independency in relation to root severing and sampling time, using χ^2 tests. Groups represented by few clones were merged, so that no events had expected frequencies lower than 4. Differences between samples in fungal community composition, as analysed by T-RFLP, were represented graphically by correspondence analysis (terBraak, 1986) using CANOCO version 4.5 for Windows (Microcomputer Power, Ithaca, NY, USA). The effects of root severing and sampling time on fungal community composition were tested for statistical significance separately by canonical correspondence analysis followed by Monte Carlo tests. The analyses were repeated with taxon abundances merged within genera. In two samples, the fungal communities were completely dominated by *Phellodon niger* and *Luellia recondita*, which were absent in all other samples. These two samples, which deviated strongly in the ordination analyses and also differed in their very thin humus layers, were excluded.

Results

Microbial biomass and enzyme activities

Average hyphal length more than doubled after root severing ($P<0.0001$) regardless of sampling time (Figure 1a). Neither bacterial counts, nor DNA amounts were significantly affected by root severing (Figures 1b and c). The total amounts of extracted DNA were higher ($P=0.048$) at the 14th-day sampling than at the 5th-day sampling, and there was a similar, nonsignificant trend for bacterial counts ($P=0.057$). The total amounts of extracted DNA correlated with bacterial counts ($P=0.011$) but not with hyphal lengths.

Laccase activities increased after root severing by an order of magnitude ($P<0.0001$) regardless of sampling time (Figure 1e), and were positively correlated with hyphal lengths ($P<0.0001$). When the root severing was included as an explaining variable, however, the correlation was reduced to a nonsignificant trend ($P=0.08$). Cellulase activities were on average fivefold higher after root severing ($P=0.024$), but only at the 5th-day sampling (interaction $P=0.023$). At the 14th-day sampling, cellulase activities were similar in both treatments and intermediate between those of disturbed and undisturbed 5th-day samples (Figure 1f). Average NAG activity was sixfold higher after root severing, but the difference was not statistically significant ($P=0.08$), because of the large variation between samples (Figure 1h). Average manganese peroxidase activities were similar regardless of root severing or sampling time (Figure 1g).

Clone libraries

From the clone libraries of bacterial 16S amplicons, 169 high-quality sequences were obtained. The sequences are deposited at NCBI (National Center for Biotechnology Information; www.ncbi.nlm.nih.gov) under accession numbers GU558922–GU559073. Eleven sequences that were attributed to plant chloroplast DNA (which is also amplified by general 16S primers) and six sequences that were identified as PCR chimeras were removed from further analyses. Five sequences could not be assigned to phyla. Actinobacteria dominated among the cloned bacterial 16S sequences followed by Alphaproteobacteria, Gammaproteobacteria, Acidobacteria and Planctomycetes (Figure 2a). The distribution of 16S clones across phyla was not significantly affected by root severing ($P=0.57$) or sampling time ($P=0.30$).

From the clone libraries of fungal ITS amplicons, 163 high-quality sequences were obtained, representing 53 genotypes (a single sequence was dismissed as a PCR chimera). The sequences are deposited at NCBI under accession numbers GU559074–GU559126. Five sequences (each represented by a single clone) could not be assigned to orders with confidence. Agaricales (mainly *Cortinarius* spp.) was the dominant order followed by Helotiales and Chaetothyriales (*Capronia* spp.) (Figure 2b). The distribution of ITS clones across orders was found to be significantly affected by root severing ($P<0.001$) but not by sampling time ($P=0.21$). The proportion of Agaricales (*Cortinarius*)

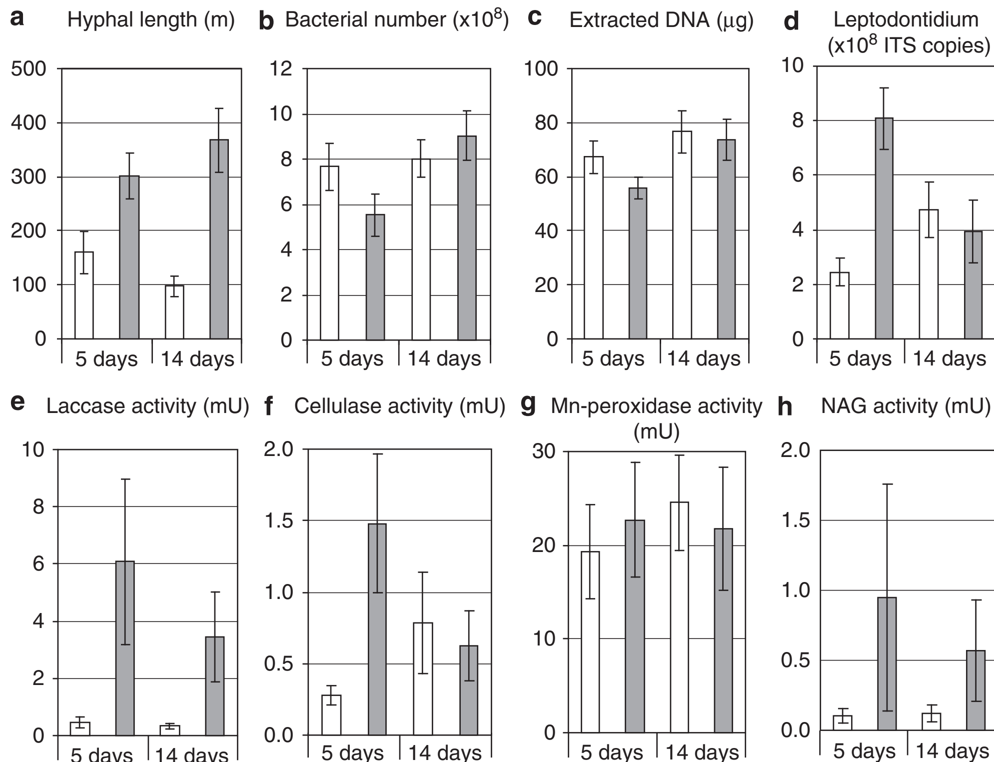

Figure 1 Estimates of microbial biomass (**a-d**) and enzyme activities (**e-h**) in humus samples from a *Pinus sylvestris* forest. Non-disturbed samples (open bars) are compared with samples in which root connections were severed (shaded bars) 5 or 14 days before collection. All estimates are reported per gram dry weight of humus with error bars representing ± 1 s.e.

clones decreased after root severing, whereas the proportion of Helotiales and Chaetothyriales (*Capronia*) clones increased. At the 5th day sampling, the proportion of clones attributed well-established mycorrhizal taxa was 87% in non-disturbed samples but only 54% in samples with severed roots. The corresponding figures for the 14th day sampling were 67% and 35%.

Terminal restriction fragment length polymorphism

The entire set of reference T-RFLP patterns derived from the sequenced clones contained six groups of 2–4 genotypes and 34 single genotypes, which all yielded unique T-RFLP peaks from at least some of the primer–enzyme combinations. Of these, 22 were identified among the T-RFLP profiles from the soil samples, whereas 18 genotypes failed to reach the cut-off threshold (5% of the height of the strongest peak in the sample) in one or more of the primer–enzyme combinations and were recorded as absent. Taxon abundances according to their average share of the T-RFLP fluorescence correlated reasonably well with the representation of taxa in the clone libraries ($R^2 = 0.82$). Four additional taxa were detected in the samples as T-RFLP patterns, although they were not present among the sequenced clones. The T-RFLP patterns of these taxa were obtained from sequenced clones from a previous study at the same site (Lindahl *et al.*, 2007) and belong to a subphylum within the Ascomycota, hitherto described on the basis of DNA sequences only (clone group I; Porter *et al.*, 2008). Considering the high abundance of these taxa in the T-RFLP profiles; on average 14% of the fluorescence, it is surprising that they did not occur among the sequenced clones. The identified taxa accounted for 30–93% (average 65%) of the total fluorescence in the T-RFLP profiles, the rest being attributed to genotypes with low abundance, together with erroneous non-ITS PCR products (Figure 3).

In the correspondence analysis, approximately half of the samples with severed roots were found outside the space occupied by the early, non-disturbed samples, indicating a difference in fungal community composition. Some of the undisturbed samples from the late sampling also deviated in community composition in the same direction (Figure 4). Canonical analysis indicated that the effect of root severing on community composition was marginally insignificant when analysed at the species level ($P = 0.062$), but clearly significant when taxa were merged within genera ($P = 0.012$). There was no significant effect of sampling time on community composition ($P = 0.15$).

In the following presentation, average taxon abundances are presented as fractions of the total identified community, assuming that most of the

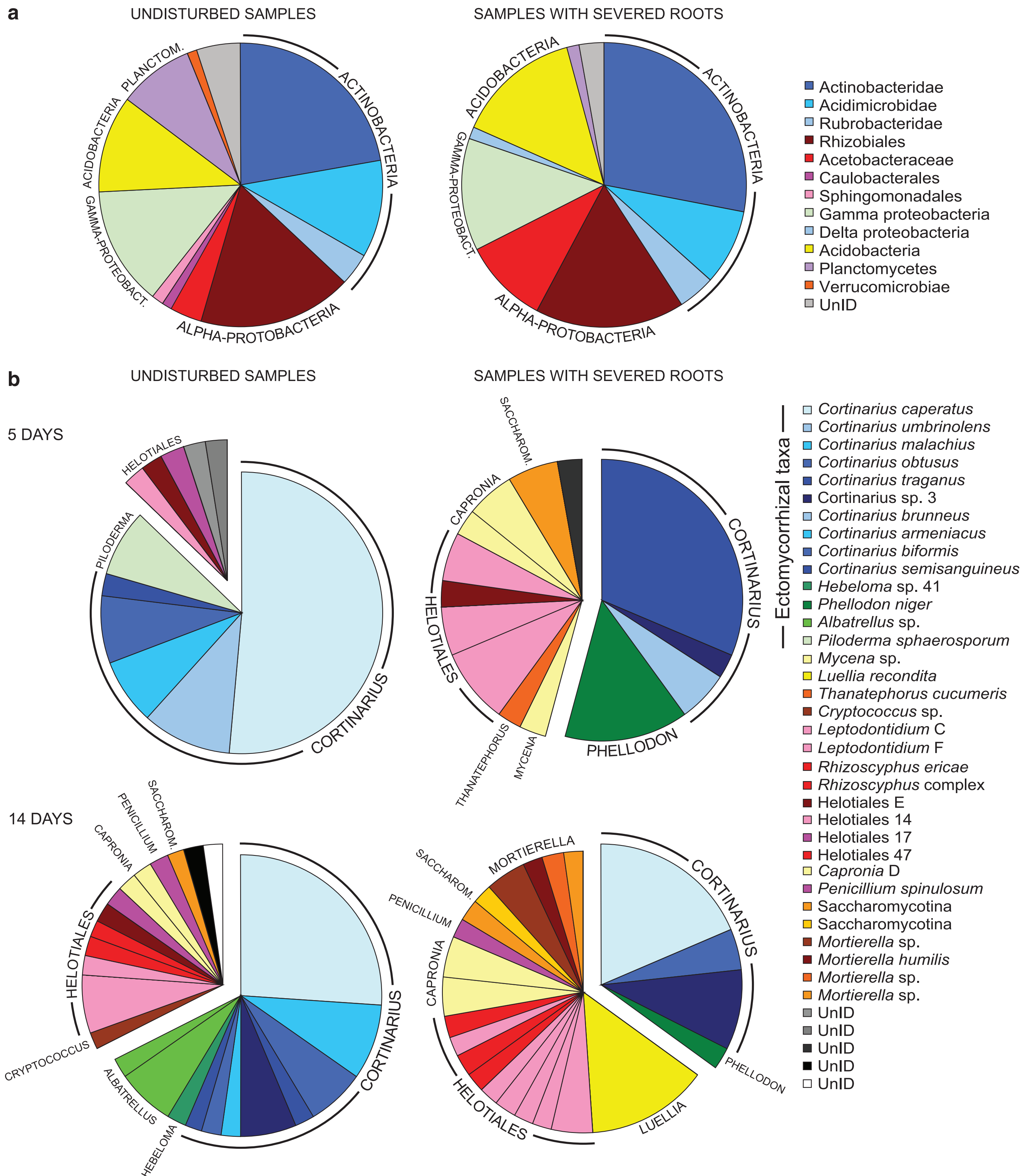

Figure 2 Distribution of (**a**) bacterial 16S clones and (**b**) fungal ITS clones across bacterial groups and fungal species in humus samples from a *Pinus sylvestris* forest where some samples had root connections severed 5 or 14 days before collection. Bacterial 16S data from the two sampling times were merged. Ectomycorrhizal fungal taxa are represented by blue–green colours, while other functional groups of fungi are shown in yellow–red–brown.

unidentified fluorescence in the T-RFLP analyses may be attributed to erroneous non-ITS PCR products. According to the T-RFLP analysis, *Cortinarius* on average constituted 69% of the fungal community in non-disturbed samples, but only 33% after root severing at the 5th day sampling. The corresponding figures for the 14th day sampling were 48% and 25%. The most common species

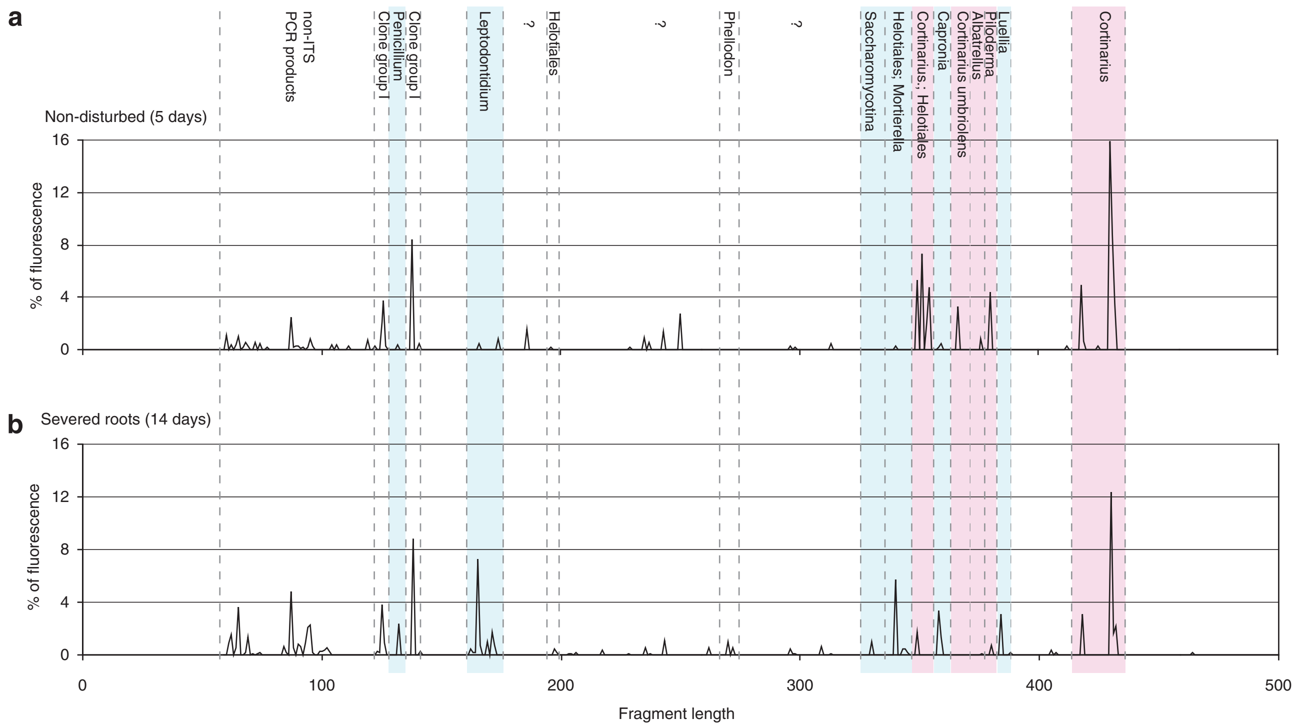

Figure 3 T-RFLP profiles based on the average fluorescence associated with different fragment lengths. The profiles were obtained from humus samples from a *Pinus sylvestris* forest and represent (**a**) non-disturbed samples (**b**) samples collected 14 days after severing of roots. The profiles represent fungal ITS amplicons after digestion with the restriction enzyme *Cfo*I, with the ITS1f primer fluorescently labelled. Taxa that decrease in relative abundance in response to root severing are indicated by red shading, whereas taxa that increase in relative abundance after root severing are indicated by green shading.

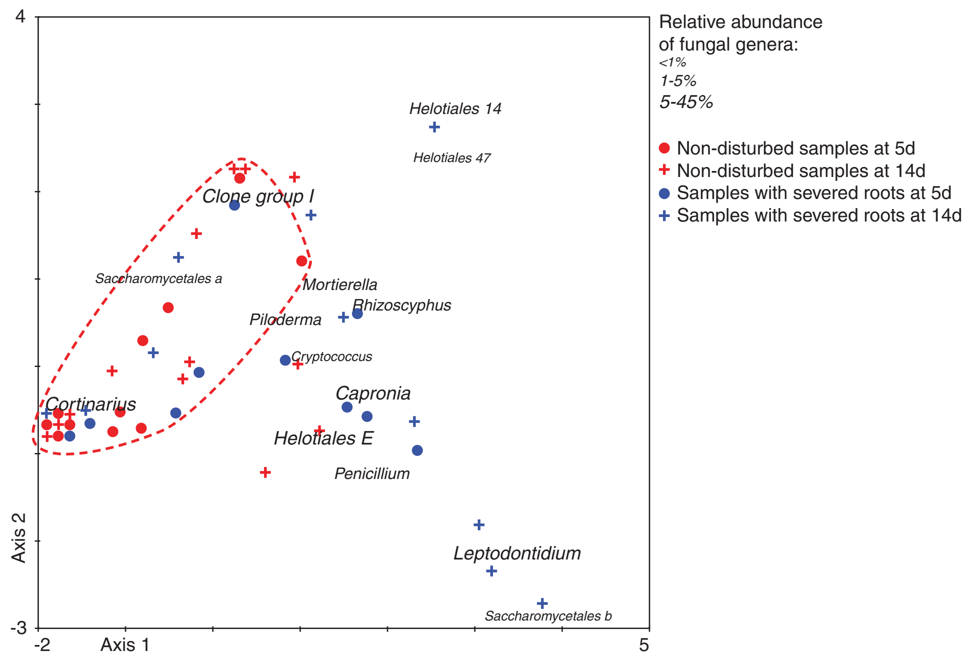

Figure 4 Correspondence analysis based on the relative abundances of fungal genera in humus samples from a *Pinus sylvestris* forest, as estimated by T-RFLP. Axes 1 and 2 together account for 45% of the total inertia of the data.

(at the start of the experiment) was *Cortinarius caperatus*, which constituted 36% of non-disturbed communities, but only 3% after root severing at the 5th day sampling. At 14 days, however, it accounted for 16–17% of both disturbed and undisturbed communities. Taxa within the Helotiales constituted

6% of non-disturbed communities, but 21% after root severing (13% and 30% after 14 days). The same pattern was observed for other ascomycetes; *Capronia* species constituted 1% of non-disturbed communities, but 10% after root severing (2% and 6% at 14 days) and *Penicillium spinulosum* was not detected in non-disturbed samples, but constituted 6% in samples with severed roots (4% in both treatments at 14 days). *Mortierella* species were not detected in non-disturbed samples but constituted 1% of the community 5 days after root severing and 4% after 14 days. The ascomycetous clone group I seemed unaffected by root severing, constituting 16% in undisturbed samples and 14% after root severing (32% and 19% at 14 days) (Figure 3).

Quantitative PCR on Leptodontidium species

The primers we used specifically amplified taxa within the *Leptodontidium* complex. Taken together, the two different reverse primers amplified all cloned ITS products within the group without overlap in target range. PCR efficiencies ranged between 74% and 79% and inhibition ranged between 0% and 70% with an average of 18%.

The numbers of *Leptodontidium* ITS copies per gram humus (results of both reverse primers added) were significantly higher after root severing ($P = 0.025$) but not generally affected by sampling occasion ($P = 0.38$). The interaction between root disturbance and sampling time was, however, highly significant ($P = 0.001$). At 5 days after root severing, the numbers of *Leptodontidium* ITS copies were, on average, more than three times higher in samples with severed roots compared with undisturbed samples. After 14 days, the levels had decreased in samples with severed roots and increased in non-disturbed samples and were similar in the two treatments (Figure 1d). The amount of *Leptodontidium* DNA in the humus correlated positively with cellulase activity ($P < 0.0001$), and the relationship remained when root severing, sampling time and their interaction were included as explaining variables.

Discussion

We hypothesised that a rapid decline in the abundance of living mycorrhizal mycelium, in response to root severing, would lead to increased abundance of opportunistic saprotrophs. Rapid growth of fungi occurred within 5 days of root severing, as indicated by the doubled density of microscopically distinguishable fungal hyphae in the humus (Figure 1a). The results of the molecular analyses indicate that certain groups of ascomycetes within the order Helotiales and the genera *Capronia* and *Penicillium*, together with mucoromycetes (*Mortierella* species), increased their share of the fungal DNA pool (Figures 2b, 3 and 4), and it seems likely that the increased hyphal densities may be attributed to these groups. Quantitative PCR confirmed that the amount of *Leptodontidium* DNA 5 days after root disruption had increased compared with controls, not only in relative terms, but also in absolute abundance (Figure 1d). After 14 days, the relative abundance of *Leptodontidium* remained high in root-severed samples, but the absolute amount had decreased to half of the level detected at 5 days and to a similar level as in the controls. Between 5 and 14 days, it thus seems as if the total fungal DNA pool decreased, implying that the relative decrease of ectomycorrhizal taxa corresponds to an even more dramatic decline in absolute terms. The increasing share of other fungi in the community during this period has to be considered in relation to the mycorrhizal decline.

Root disruption, thus, seemed to cause a major reduction in the amount of ectomycorrhizal DNA concomitant with a pulse of hyphal production by certain ascomycetes and, later, also mucoromycetes. The disturbance made new resources available in the form of senescing mycorrhizal mycelium, which may have been used by the proliferating, opportunistic saprotrophs. In addition, root disruption may have relaxed mycorrhizal interference competition, allowing free-living saprotrophs to increase in abundance. This field-based finding is in accordance with previous observations of mycorrhizal interference competition in laboratory microcosms (Lindahl *et al.*, 1999, 2001, 2002; Werner *et al.*, 2002). The relative importance of reduced mycorrhizal interference and substrate enrichment is, however, difficult to establish. These concepts overlap in that the living mycorrhizal mycelium constitutes a potential resource for saprotrophs and parasites, which is defended by the mycorrhizal fungi through antagonistic interactions. This study addressed short-term effects of disrupted translocation. In a longer term perspective, tree girdling has been shown to increase the relative abundance of non-ectomycorrhizal fungi (Yarwood *et al.*, 2009), and increased decomposition rates have been observed in trenched plots (Gadgil and Gadgil, 1971). However, long-term effects on the absolute abundance and activities of saprotrophs remain uncertain.

PCR products with 99–100% sequence similarity to the most commonly detected *Leptodontidium* genotype have also been amplified from coniferous forest soils in Canada, Alaska and eastern USA, as well as from hair roots of ericaceous shrubs in Scotland (Bougoure *et al.*, 2007). The ecology of *Capronia* species is uncertain; pathogenic, mycoparasitic as well as lichen forming taxa have been described, but their capacity as primary saprotrophs of plant litter has been questioned (Untereiner and Malloch, 1999). Some *Capronia* strains have been confirmed to form mycorrhiza-like structures within roots of ericaceous shrubs (Allen *et al.*, 2003). It is not impossible that ericoid symbionts could have been favoured by root disruption, as *Vaccinium*

plants were often rooted inside isolated cores. However, the rapid decline of the *Leptodontidium* group during the later phase of the experiment, together with the positive correlation with cellulase activity and the relative rarity of the group under undisturbed conditions, suggest that this group consists of free-living opportunists. One of the more common *Mortierella* genotypes was assigned to *M. humilis*, which has been shown to degrade cellulose when colonising straw (Varnaitė *et al.*, 2008). None of the species that responded positively to root severing was detected in decomposing needles collected at the same research site (Lindahl *et al.*, 2007), indicating that they are not regular litter saprotrophs.

The high abundance of Actinobacteria and Acidobacteria, in combination with the absence of Betaproteobacteria and Bacteriodetes, indicates a bacterial community adapted to low carbon availability (Fierer *et al.*, 2007). In contrast to the dramatic responses observed in the fungal community, the bacterial community did not respond significantly to root disruption (Figures 1b and 2a). A plausible explanation is that the saprotrophic fungi, which prospered in response to mycorrhizal senescence, suppressed the bacterial community, replacing the mycorrhizal fungi as dominants. Antagonism against bacteria is well established for many ascomycetes (Gloer, 2007).

The shifts in the fungal community were associated with increased cellulase, laccase and NAG activities (Figures 1e–g), although the latter was highly variable between samples. Cellulase activity correlated with *Leptodontidium* abundance, suggesting a causal relationship. Increased activities of enzymes involved in hydrolysis and oxidation of organic substrates support the picture of a shift from a mycorrhizal community, independent of the need to acquire energy from dead organic matter, to a saprotrophic community, acquiring energy from the degradation of organic substrates through the action of extracellular enzymes. Laccases may be used to degrade phenolic compounds in organic matter, but are also frequently produced during competitive interactions between saprotrophic fungi (Baldrian, 2004). NAGs are enzymes involved in chitin degradation, and are produced at increased rates when fungi overgrow dead mycelium of other fungi (Lindahl and Finlay, 2006). The trend of increased NAG activity, in response to root severing, supports the idea that dead, mycorrhizal mycelium could be an important substrate for the responding saprotrophs (Lindahl *et al.*, 2001). However, increased cellulase activity indicates that plant-derived organic matter was also used. Dying roots could, potentially, constitute a new substrate in the disturbed samples, although the decline in cellulase activity after 14 days suggests that other, more rapidly depleted substrates were mainly used. The activity of Mn-peroxidases, which are enzymes capable of oxidation of the most recalcitrant compounds in soil, such as large humic complexes and lignin, did not change in response to root severing (Figure 1f). The lack of increase in peroxidase activity is consistent with the view that these enzymes are primarily produced by basidiomycetes, and that the responding ascomycetes and mucoromycetes act mainly as opportunists, using more easily degradable substrates. It has been suggested that ectomycorrhizal fungi may be the main decomposers of humus, as they possess Mn-peroxidase genes (Bödeker *et al.*, 2009) and are the only basidiomycetes that occur abundantly in humus (Lindahl *et al.*, 2007). This hypothesis is supported by the relatively high Mn-peroxidase activities observed in the undisturbed samples, in which mycorrhizal fungi dominated the community, but disagrees with the sustained activities in spite of root disruption. The persistently high Mn-peroxidase activities after root severing may indicate a slow turnover rate of these enzymes in the humus.

After 14 days, some of the control samples showed resemblance to root-severed samples, both with respect to fungal community composition (Figures 1d, 2b and 4) and raised cellulase activities (Figure 1f). Possibly, trampling during establishment of the experiment and the first sample collection, together with the disruption of roots associated with collection of other samples, also caused some disturbance of the fungal community in the control samples. After 14 days, *Cortinarius caperatus* had increased in abundance in root-severed samples relative to a very low prevalence at 5 days. This could be explained by re-colonisation of the cores from below, because this species was present in the mineral soil (Lindahl *et al.*, 2007).

Litter saprotrophs in the boreal forest are nitrogen limited (Boberg *et al.*, 2008) and little net release of nitrogen seems to take place during the early, saprotrophic stages of decomposition (Berg *et al.*, 1982). Instead, nutrients are recycled to plants from more degraded litter and humus (Melillo *et al.*, 1989; Lindahl *et al.*, 2007). As previously shown, fungal communities in undisturbed humus layers of boreal forests are dominated by mycorrhizal fungi, and nutrients are likely to be recycled to plants directly by mycorrhizal fungi rather than via mineralisation by saprotrophs (Lindahl *et al.*, 2002, 2007; Schimel and Bennet, 2004). However, disturbance of translocation pathways from leaves, via roots to mycorrhizal mycelium reduces the dominance of mycorrhizal fungi and permits free-living fungi to proliferate in the humus. The opportunistic saprotrophs presumably live on dead mycorrhizal mycelium, as well as on other compounds that become easily available because of the disturbance. Having no access to other, external carbon sources, such as living roots or fresh litter, the opportunists are more likely to experience carbon limitation and release nutrients in an inorganic form. We have, thus, shown a plausible mechanism behind the raised levels of inorganic nitrogen that have been observed

in association with disturbance in boreal forests (Siira-Pietikäinen *et al*., 2001; Carmosini *et al*., 2003; Lavoie and Bradley, 2003; Sulkava and Huhta, 2003; Lapointe *et al*., 2005; Piirainen *et al*., 2007). However, because of the potential removal of NH_4^+ by roots in the undisturbed systems, we were not able to directly address the question of whether the changes in fungal community composition actually led to increased N mineralisation rates. This problem highlights the difficulties in measuring mineralisation rates in systems that are sensitive to root disruption. In fact, our results cast a shadow of doubt over all methods used to study microbial processes in isolated forest soil samples, in which root connections have been severed. Clearly, such measurements are conducted on a microbial community that is functionally very different from that of the undisturbed soil. By excluding mycorrhizal fungi, measurements are likely to underestimate microbial respiration and growth. In contrast, because of triggering of opportunistic saprotrophs, nutrient mineralisation rates are likely to be vastly overestimated.

The functional shift in the microbial community shown in this study was related to changes in relative dominance of functional groups of fungi, highlighting the insufficient resolution of methods that target all fungi as a single functional entity. Molecular community analyses provide the resolution required to identify species and functional groups. Methodological development in molecular fungal ecology is currently undergoing a boom (Hibbett *et al*., 2009), and in combination with careful field manipulations and measurements of environmental and functional parameters, such as elemental concentrations and enzyme activities. DNA-based methods may enable us to elucidate environmental drivers of fungal community assembly, as well as the functional roles of fungal communities in ecosystems. Our results suggest that repression of free-living fungi by mycorrhizal fungi may be a significant factor in shaping fungal communities. Disruption of carbon translocation pathways because of natural or anthropogenic disturbances may induce dramatic shifts in fungal community composition, potentially reducing nutrient retention in fungal mycelial networks.

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