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Research

Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests

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Summary

- Boreal forest soils store a major proportion of the global terrestrial carbon (C) and below-ground inputs contribute as much as above-ground plant litter to the total C stored in the soil. A better understanding of the dynamics and drivers of root-associated fungal communities is essential to predict long-term soil C storage and climate feedbacks in northern ecosystems.
- We used 454-pyrosequencing to identify fungal communities across fine-scaled soil profiles in a 5000 yr fire-driven boreal forest chronosequence, with the aim of pinpointing shifts in fungal community composition that may underlie variation in below-ground C sequestration.
- In early successional-stage forests, higher abundance of cord-forming ectomycorrhizal fungi (such as *Cortinarius* and *Suillus* species) was linked to rapid turnover of mycelial biomass and necromass, efficient nitrogen (N) mobilization and low C sequestration. In late successional-stage forests, cord formers declined, while ericoid mycorrhizal ascomycetes continued to dominate, potentially facilitating long-term humus build-up through production of melanized hyphae that resist decomposition.
- Our results suggest that cord-forming ectomycorrhizal fungi and ericoid mycorrhizal fungi play opposing roles in below-ground C storage. We postulate that, by affecting turnover and decomposition of fungal tissues, mycorrhizal fungal identity and growth form are critical determinants of C and N sequestration in boreal forests.

Introduction

Humus layers of northern ecosystems have accumulated carbon (C) since the last glaciation, and boreal and arctic soils presently account for almost half of the global C stored in soil and litter (Pan *et al.*, 2011). Low temperatures and low litter quality, both of which impair plant litter decomposition rates, have been invoked as principal drivers of organic matter accumulation in northern ecosystems (Brovkin *et al.*, 2012; Makkonen *et al.*, 2012). However, the dominant plant species in boreal forests allocate a substantial proportion of their C below ground to mycorrhizal fungal symbionts, and the dominant type of mycorrhizal association has been identified as an important global predictor of soil C storage (Averill *et al.*, 2014). Fungal tissues potentially represent a large C input into soil organic matter pools (Langley & Hungate, 2003; Cairney, 2012; Wallander *et al.*, 2013). Using a bomb-^{14}C model, we recently showed that at least half of the accumulated C in humus layers of boreal forested islands originated from root-derived inputs rather than from above-ground plant litter inputs (Clemmensen *et al.*, 2013). Analyses of biochemical markers and stable isotopes indicated that this accumulated C largely originated from fungal mycelium. These islands collectively represent a large post-fire successional gradient (ranging from 50 to 5000 yr since the last fire), and we concluded that impaired decomposition of organic matter derived from mycorrhizal fungi was a main driver of C accumulation in the rooting zone with increasing duration of absence of fire. Shifts in C dynamics within the root-associated fungal community thus have the potential to play a central, regulatory role in the long-term accumulation of soil C.

Differences in both fungal biomass production and turnover and necromass degradation are likely to be important determinants of long-term C accumulation and may vary considerably among both fungal species and different tissues (Fernandez & Koide, 2012; Ekblad *et al.*, 2013; Wallander *et al.*, 2013). Agerer (2006) categorized ectomycorrhizal fungal species into short- and long-distance exploration types, based on the amount and differentiation of their extramatrical mycelium. Mycelium of short-distance types is composed of simple hyphae that explore soil closer to the roots, whereas the mycelium of long-distance types differentiates into hydrophobic hyphal cords that connect mycorrhizal root tips with a more distant exploratory mycelium. This growth form framework can potentially link mycorrhizal fungal community composition to C cycling processes (Koide *et al.*, 2013).

Cord-forming types have been proposed to be the most C-demanding as a result of their extensive growth (Hobbie, 2006) and could potentially contribute large amounts of mycelial necromass to the soil organic matter pool. However, many cord formers are also equipped with a suite of extracellular enzymes that contribute to both efficient internal biomass recycling (Boddy, 1999; Falconer *et al.*, 2007) and degradation of organic complexes in the soil (Hobbie & Agerer, 2010; Hobbie *et al.*, 2013; Bödeker *et al.*, 2014), potentially leading to short residence times of C in mycelial biomass and necromass. The resistance of mycelial necromass to decomposition similarly varies among fungal species as a consequence of differences in tissue qualities, with implications for C and nitrogen (N) residence time in soil. Whereas mycelium with high chitin and N concentrations generally decomposes within weeks (Drigo *et al.*, 2012; Fernandez & Koide, 2012), mycelium with melanized cell walls, such as in many root-associated ascomycetes (Robinson, 2001; Smith & Read, 2008), may be resistant to decomposition, leading to a higher proportion of the necromass being preserved in long-term humus stores (Coelho *et al.*, 1997; Koide *et al.*, 2013; Fernandez & Koide, 2014).

Our earlier study (Clemmensen *et al.*, 2013) evaluated the role of above-ground vs below-ground inputs for long-term soil C sequestration across a natural boreal forested island chronosequence, and considered fungal communities only at the level of dominant life forms (i.e. saprotrophs and root associates). Here, we present in-depth, species-level analyses of phylogenetic and functional shifts in fungal communities across this chronosequence and characterize the spatial organization of the fungal communities in fine-scaled organic soil profiles. We test the hypothesis that shifts in species composition and growth forms within the mycorrhizal fungal community correlate with differences in C sequestration from root-associated mycelium during successional development of boreal forest. Further, we propose a conceptual framework for how to mechanistically understand observed relationships.

Materials and Methods

Sampling and DNA preparation

The study was conducted in a boreal forest chronosequence situated on 30 forested islands in two adjacent lakes, Lake Hornavan and Lake Uddjaure (65°55′N to 66°09′N; 17°43′E to 17°55′E) in northern Sweden (Wardle *et al.*, 2003, 2012a). The islands were all formed from the same parent material following the last glaciation *c.* 10 000 yr ago and represented three size classes: 10 large islands (> 1.0 ha), 10 medium islands (0.1–1.0 ha) and 10 small islands (< 0.1 ha; Supporting Information Table S1). The main disturbance regime on these islands is wildfire through lightning strike, and lightning strikes larger islands more frequently than smaller ones, meaning that larger islands burn more frequently (Wardle *et al.*, 2003). The average times since fire are 585, 2180 and 3250 yr on large, medium and small islands, respectively (Wardle *et al.*, 2003). Each island serves as an independent replicate ecosystem, with whole islands serving as the units of replication. On average, 6.2, 11.2 and 22.5 kg C m^{-2} have accumulated below ground on large, medium and small islands, respectively (Clemmensen *et al.*, 2013). Detailed accounts of soil sampling and DNA preparation are available in Clemmensen *et al.* (2013). In short, 10 soil cores (3 cm diameter) were sampled from each island down to the complete humus depth of 0.2–1.2 m. Eight cores (excluding the longest and shortest) were split into 20 cm horizons, and the uppermost 20 cm were further split into two litter (L) horizons (intact litter on the surface and more degraded intact litter in the upper *c.* 0–2 cm of the core), two fragmented litter (F) horizons (*c.* 2–7 and 7–10 cm depth) and two humus (H) horizons (*c.* 10–16 and 16–20 cm depth). Living roots and rhizomes with a diameter of > 1 mm were removed, and materials from the same horizon were pooled within each island, resulting in six to 10 horizons sampled from each of the 30 islands and a total of 233 soil samples. The samples were freeze-dried, and DNA was extracted from 50 mg of finely milled material in cetyltrimethyl ammonium bromide (CTAB) buffer (3% cetyltrimethylammonium bromide, 2 mM EDTA, 150 mM Tris-HCl and 2.5 M NaCl, pH 8). The content of total extracted DNA was measured spectrophotometrically and fungal DNA was estimated by quantitative PCR of the fungal ITS2 region using the primers fITS9 and ITS4 (Ihrmark *et al.*, 2012). ITS2 amplicons for 454-sequencing were produced using the forward primers fITS9 or gITS7 in separate PCR reactions combined with sample-tagged ITS4 reverse primers (technical triplicates, 22–35 cycles; Ihrmark *et al.*, 2012). Amplicons were sequenced by LGC Genomics GmbH (Berlin, Germany) on a GL FLX Titanium system (Roche, Basel, Switzerland).

DNA sequence analyses

Sequences were quality filtered and clustered into operational taxonomic units (OTUs) that proximately correspond to the species level (1.5% distance single-linkage) using the bioinformatics pipeline SCATA (http://scata.mykopat.slu.se/) as detailed in Fig. S1. The entire UNITE database (http://unite.ut.ee; Abarenkov *et al.*, 2010) and a curated selection of sequences from the NCBI nr database (https://blast.ncbi.nlm.nih.gov) were included in the clustering procedure to provide validation of the species level and identification of some OTUs (primarily ectomycorrhizal fungi) based on the same criteria as the clustering. The globally most abundant 557 fungal OTUs (each being represented by at least 70 reads and together encompassing 92% of the total reads) were further identified through neighbour-joining analyses with reference sequences as described in detail in Fig. S1 and by Clemmensen *et al.* (2013). Identified OTUs were categorized into the fungal guilds: litter-associated saprotrophs, ectomycorrhizal fungi, ericoid mycorrhizal fungi, other root-associated fungi, moulds and yeasts, based on published literature. Fungal sequences that could not be identified to species with known function but that clustered into clades together with reference sequences derived from a particular substrate (i.e. surface-sterilized roots of ectomycorrhizal, ericoid or other hosts or litter components) were assigned to putative guilds. Thus we attempted to define fungal guilds based on their main source of C, with saprotrophic fungi being those that gain C from dead organic material and root-associated fungi

being those that gain C directly from their host plant. In cases where the closest reference sequences were derived from different substrates, the OTUs were classified as unknown with respect to guild. Ectomycorrhizal species were further ascribed to mycelial exploration types following the categories described by Agerer (2006) and implemented further by Tedersoo & Smith (2013). The relative abundances of fungal clusters in each sample were calculated after the removal of all global singletons (on average 4.3% of the reads) and nonfungal sequence reads (on average 2.1 and 15.3% for the fITS9 and gITS7 primers, respectively). Each of the 233 samples was represented by, on average, 1270 (fITS9) plus 1209 (gITS7) reads. When one of the forward primers clearly disfavoured a cluster (< 30% relative abundance of that obtained by the other primer), data from the highest yielding primer were used (< 0.5% of clusters affected). Raw molecular data are stored at The Sequence Read Archive under the accession number SRP016090 (www.ncbi.nlm.nih.gov/sra). Representative sequences for the most abundant OTUs are deposited in the UNITE database under accession codes UDB020357–UDB020913; see Table S2 for identifications and fungal guild assignments as well as links to UNITE Species Hypotheses, and Table S3 for relative abundances across all samples.

To estimate the fungal community as integrated over the whole soil profile for each island, relative abundances were first multiplied by the total number of internal transcribed spacer (ITS) copies in each sample. Communities were then summed over the complete organic profile, and new relative abundances of all OTUs calculated based on ITS copies in the complete profile. The fungal ITS copy number was significantly correlated with the fungal biomarker ergosterol across F and H layer samples, but not across the L samples, which had higher number of ITS copies per ergosterol content than did the F and H samples (Fig. S2). Thus, while our analyses of relative ITS abundances within separate horizons and fungal guilds should be largely unaffected by possible variation in ITS copy number per biomass (Baldrian *et al.*, 2013), some litter-associated fungi may be overrepresented in the fungal communities as integrated for each island. Unfortunately, no single fungal biomarker has proved to correspond most closely to real standing biomass (Baldrian *et al.*, 2013), and here we choose to use the same fungal marker (i.e. the ITS region) to characterize both the fungal communities and their relative biomass in the samples (Fig. S3).

Statistical analyses

After removing five deeper humus samples with obvious contamination by litter fungi (most probably a result of accidental vertical mixing during sampling), the complete sequence-based data set consisted of 228 samples and 4470 species-level OTUs. The entire fungal community data set was subjected to ordination analyses using CANOCO version 4.55 (Biometris Plant Research International, Wageningen, the Netherlands). Detrended correspondence analysis (DCA) was used to depict patterns extracted from all variation in fungal communities, and correlation analyses were used to explore relationships between DCA axis scores and selected environmental variables (Table S1): sample depth (cm) and below-ground C stock (g C m^{-2}) (from Clemmensen *et al.*, (2013), based on the same samples as used here), island size ($\log_{10}$ ha) and time since fire (yr) (from Wardle *et al.*, 2003), and net primary productivity (g C m^{-2} yr^{-1}) of the major tree species (*Pinus sylvestris*, *Picea abies*, *Betula pubescens*) and understorey species (*Vaccinium myrtillus*, *V. vitis-idea*, *Empetrum hermaphroditum*) (from Wardle *et al.*, 2012b). Canonical correspondence analysis (CCA) was used to analyse how much of the total variation (inertia) was explained by the environmental variables, and CCAs were followed by Monte Carlo permutation tests (9999 permutations under full model) with forward selection of explanatory variables. Because there was a strong island size × sample depth interaction term in the CCA of the full dataset, additional analyses were also performed for each soil profile layer separately (i.e. L1, L2, F1, F2, H1, H2, H3). Furthermore, species richness and Shannon's diversity and Pielou's evenness indices were calculated for each soil sample after random subsampling of the fungal communities to 200 reads (100 per primer, corresponding to the lowest read number) using the vegan (Oksanen *et al.*, 2007) and GUnifrac (Chen, 2012) packages in R, version 3.0.1 (R Core Team, 2013).

Differences in diversity indices and in relative abundance of fungal groups were analysed by generalized linear mixed models, using the GLIMMIX procedure in the SAS 9.3 package (Statistical Analysis System Institute, Cary, NC, USA). To quantify overall effects of island size and sampling layer (upper six horizons included ($n = 180$), or only rooting zone included ($n = 120$)), island 'size class' (df = 2) and 'layer' (df = 5 or 3) and their interaction (df = 10 or 6) were defined as fixed factors. To account for possible dependency between measures at different depths within individual islands, 'layer' was treated as a repeated measure with 'island' as the subject and a first-order autocorrelation structure specified through RANDOM statements. Satterthwaite-type degrees of freedom based on the Kenwood–Roger adjustment were used to calculate Wald *F*-test statistics of the fixed factors, and the results were evaluated using Tukey's adjustment for multiple comparisons with $\alpha = 0.05$. Proportional data were arcsine-transformed before analysis, and a log-normal distribution type was specified for all variables.

Results

Depth and island size effects on fungal community composition

The first axis of the DCA of the complete dataset identified vertical location in the organic profiles as the major determinant of fungal community composition, whereas the second axis described differences in fungal communities that were related to island size and associated variables (Fig. 1). A CCA with sampling depth included as a covariate indicated a significant effect of island size, and an interactive effect of island size and depth, on fungal community composition ($P = 0.0001$, Table S4a). When testing layers in the soil profile separately, island size affected fungal communities in all layers ($P < 0.01$ for all, Table S4b). The overall effect of island size on fungal

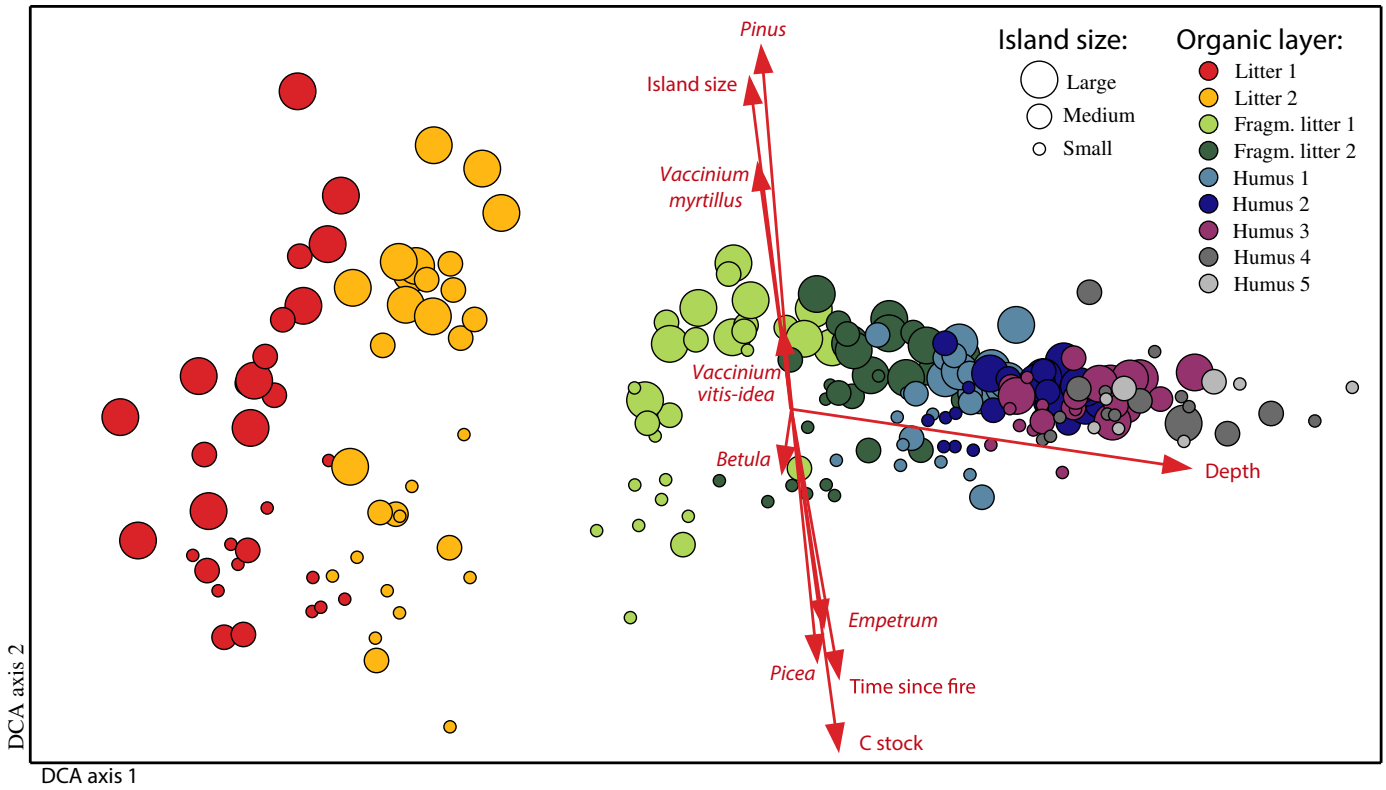

Fig. 1 Sample plot based on a detrended correspondence analysis (DCA) of fungal communities including all 228 samples (each represented by a separate dot) and 4470 fungal operational taxonomic units. The total inertia of the DCA was 15.33 and the first two axes explained 5.1 and 2.0% of inertia, respectively. Island size class is indicated by dot size and organic layer by colour. The vectors indicate direction and degree of correlation between the two first DCA axes and ecosystem characteristics. Fragm., fragmented; C, carbon.

communities was further confirmed through CCAs of fungal communities integrated across all soil horizons ($F = 1.728$, $P = 0.0001$; total inertia was 3.91 of which 5.8% was explained by island size). Forward selection of explanatory variables identified C stock ($P = 0.0001$) and *Picea* ($P = 0.0016$), *Betula* ($P = 0.0348$) and *Empetrum* ($P = 0.029$) productivity as the factors most strongly related to fungal community composition, together accounting for 19.7% of total inertia (Table S5a). With C stock removed from this analysis, the significant explanatory variables were *Picea* productivity ($P = 0.0003$), island size ($P = 0.0001$) and *Betula* productivity ($P = 0.022$), which together explained 16.0% of total inertia (Table S5b). Island size also significantly affected community composition within each of the five fungal guilds, explaining 6–9% of total inertia (Table S6a). Forward selection of explanatory variables identified *Empetrum* and all three tree species as most strongly related to the ectomycorrhizal fungal community. These variables collectively explained 28.7% of total inertia, whereas C stock was the factor that was most strongly related to the variation in both ericoid mycorrhizal and other root-associated fungal communities (Table S6b). Island size and *Picea* productivity were the variables most strongly related to communities of litter saprotrophs, whereas *Picea* productivity and C stock explained most of the variation in the mould and yeast communities (Table S6b).

Effects on fungal diversity, phylogenetic groups and fungal guilds

Fungal species richness, Shannon's diversity and Pielou's evenness index displayed similar patterns across soil profiles, and the first two estimates were significantly higher on small and medium islands than on large islands, whereas evenness was higher on medium islands than on large islands (Figs 2a, S4; Tables S7, S8). However, the effect of island size was eliminated when any of the estimates were expressed per standing stock of either ITS copies or ergosterol (not shown). On all islands, diversity peaked in the L2 and F1 layers, corresponding to the highest contribution by Basidiomycota and the lowest contribution by Ascomycota (Fig. 2a–c). Fungi belonging to Basidiomycota were most abundant (20–35% of the amplified ITS sequences) in the upper 10 cm, whereas Ascomycota increased in relative abundance and accounted for over 80% of the sequences below 10 cm depth. Relative abundances of Basidiomycota, Ascomycota and Mucoromycotina were stable across the island gradient (Figs 2b–d, S5; Tables S7, S8).

Fungal communities in the litter layers were clearly dominated by litter-associated saprotrophic species, with fewer, more dominant ascomycetes in the uppermost litter layer (L1) and a larger number of litter-degrading basidiomycetes (mainly *Mycena* spp.) in the lower litter layer (L2; Figs 2a–c,e,f, 3). Litter-associated species also contributed to the community in the upper rooting

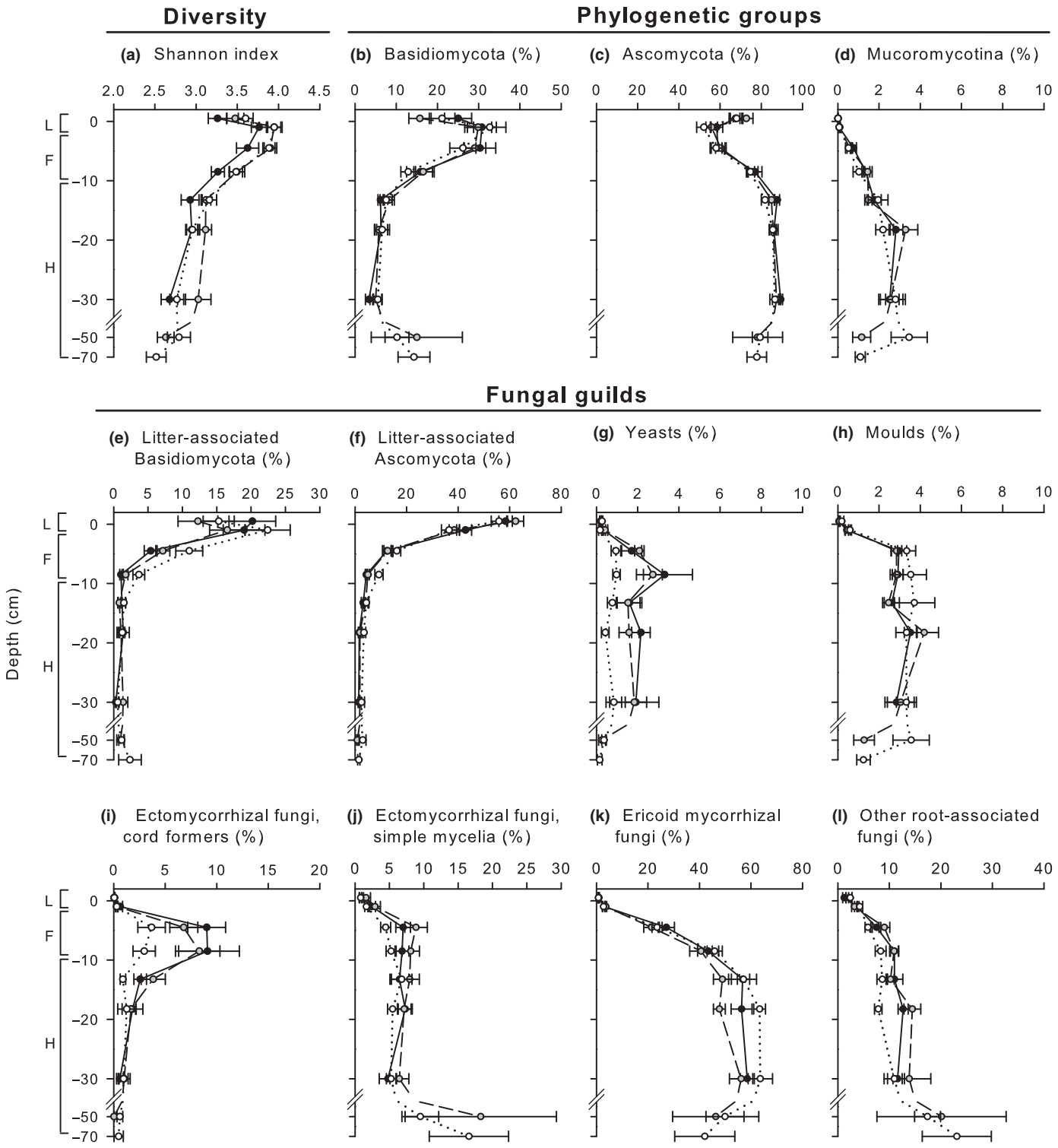

Fig. 2 Fungal species diversity (a) and relative abundances of fungal phylogenetic groups (b–d; % of total fungal reads) or fungal guilds (e–l; % of total fungal reads) in organic soil profiles (L, litter; F, fragmented litter; H, humus) on large (solid lines), medium (dashed lines) and small (dotted lines) forested islands. All data are means ± 1 SE ($n = 10$, except $n = 4$–8 for the lowest horizons). Statistical analyses are presented in Supporting Information Tables S7 and S8. Shannon's diversity index (a) is based on random subsampling of each sample to 200 reads. The most abundant 557 (out of a total of 4470) fungal operational taxonomic units were included in analyses (b–l), covering on average 85–95% of the total reads per layer; < 3% of included reads could not be assigned to phylogenetic group and, on average, 13% could not be assigned to fungal guild.

zone (F1; Fig. 4b), and their summed relative abundance was significantly higher on small islands than on large islands (Fig. 2e,f; Table S7).

Cord-forming ectomycorrhizal fungi (mainly *Suillus variegatus*, *Cortinarius* and *Piloderma* spp.) primarily occupied the upper rooting zone (F1 and F2), and their relative abundance was twice

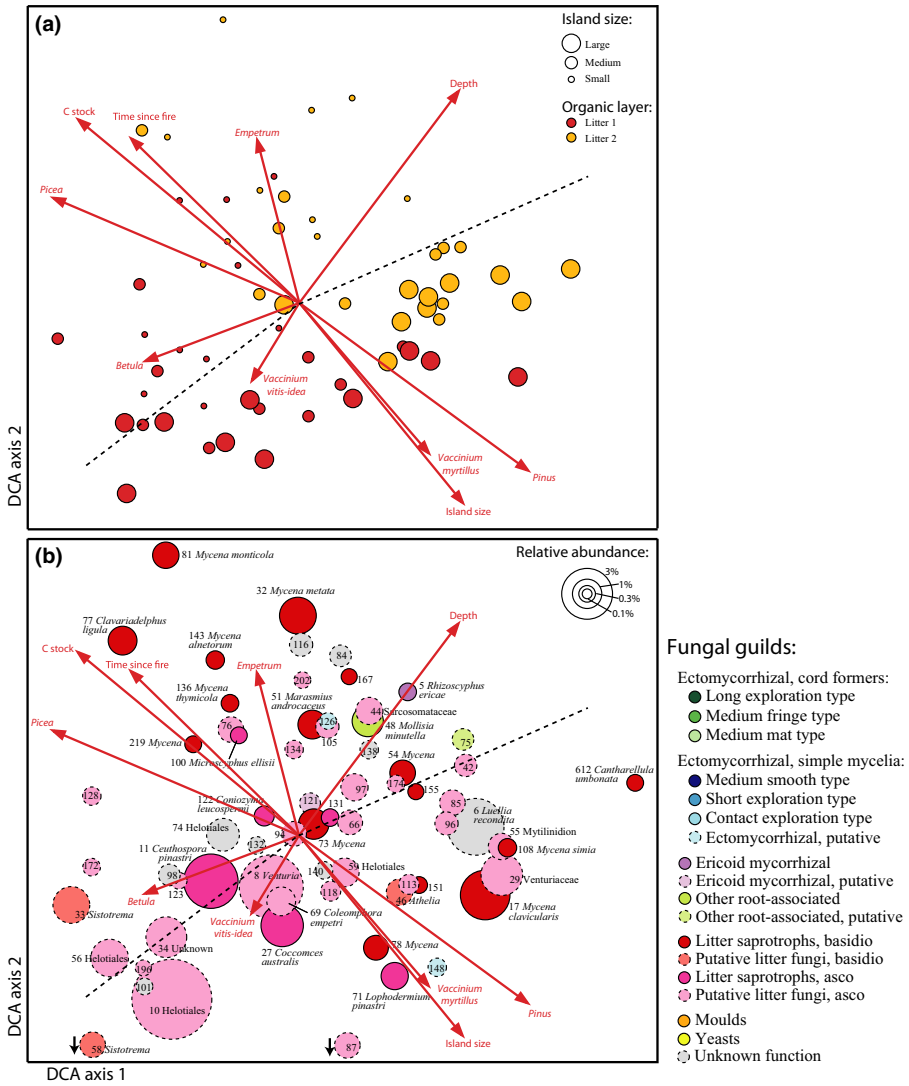

Fig. 3 Sample (a) and species (b) plots derived from a detrended correspondence analysis (DCA) of fungal communities in the two litter layers (litter layers 1 and 2) with the two first axes shown and accounting for 5.6 and 4.4% of the total inertia of 7.27. The DCA is based on all fungal operational taxonomic units (OTUs) present (2258), but only the 67 OTUs that, on average, represented at least 0.3% of the total amplicon, together adding up to 65% of the reads, are shown. In (a), island size class is indicated by dot size and organic layer by colour. In (b), OTU relative abundance is indicated by dot size and fungal guild by colour. The broken line approximately delimits large and small island communities. The vectors indicate direction and degree of correlation between the two first DCA axes and ecosystem characteristics. Numerical prefixes identify all OTUs that are further detailed in Supporting Information Table S2.

as high on large islands as on small islands (Figs 2i, 4c; Table S8). Yeasts (including Saccharomycetales and Tremellales) and ascomycete moulds (including *Penicillium* spp.) were also primarily found in the upper rooting zone, whereas Mucoromycotina mould species (mainly *Mortierella* and *Umbellopsis*) increased in relative abundance in deeper humus layers (Figs 2d,g,h, 4b; Table S8). Yeasts were significantly more abundant on large and medium islands (Figs 2g, 4b, 5b; Table S8). Ericoid mycorrhizal and other root-associated fungi increased in dominance with depth, but while the total relative abundance of ericoid mycorrhizal fungi was not affected by island size, other root-associated fungi (including *Phialocephala* spp. and some *Oidiodendron* spp.) were more abundant on large islands (Figs 2k,l, 4d, 5b; Table S8). Ectomycorrhizal fungi with simple mycelia made up a rather constant proportion of the communities across humus layers and island sizes, although they, together with other root-associated fungi such as Archaeorhizomycetes, increased in relative abundance in the deepest humus layers of medium and small islands, which were not included in the mixed model analyses (Figs 2j,l, 4c,d, 5b, S5; Table S8).

Discussion

It has recently been shown in the studied chronosequence that 50–70% of the C stored in humus during the past 100 yr originated from C allocated to roots and associated fungi rather than from above-ground plant litter, and that accelerating accumulation of root-derived C could explain the increasing rate of C sequestration with time since fire (Clemmensen *et al.*, 2013). Here we present data documenting successions in fungal communities, both vertically (i.e. from surface litters to deeper humus layers) and with time since fire. We observed distinct patterns in species composition within each of the major fungal guilds across the chronosequence, and identified a specific root-associated fungal community in the particular humus layers where ^{14}C-based estimates indicated high rates of C deposition. The composition of this fungal community was found to correlate with differences in C dynamics between islands of contrasting fire histories. In multivariate analyses, forward selection of explanatory variables suggested that the effect of island size, and thus time since fire, on fungal communities, is indirect and mediated by

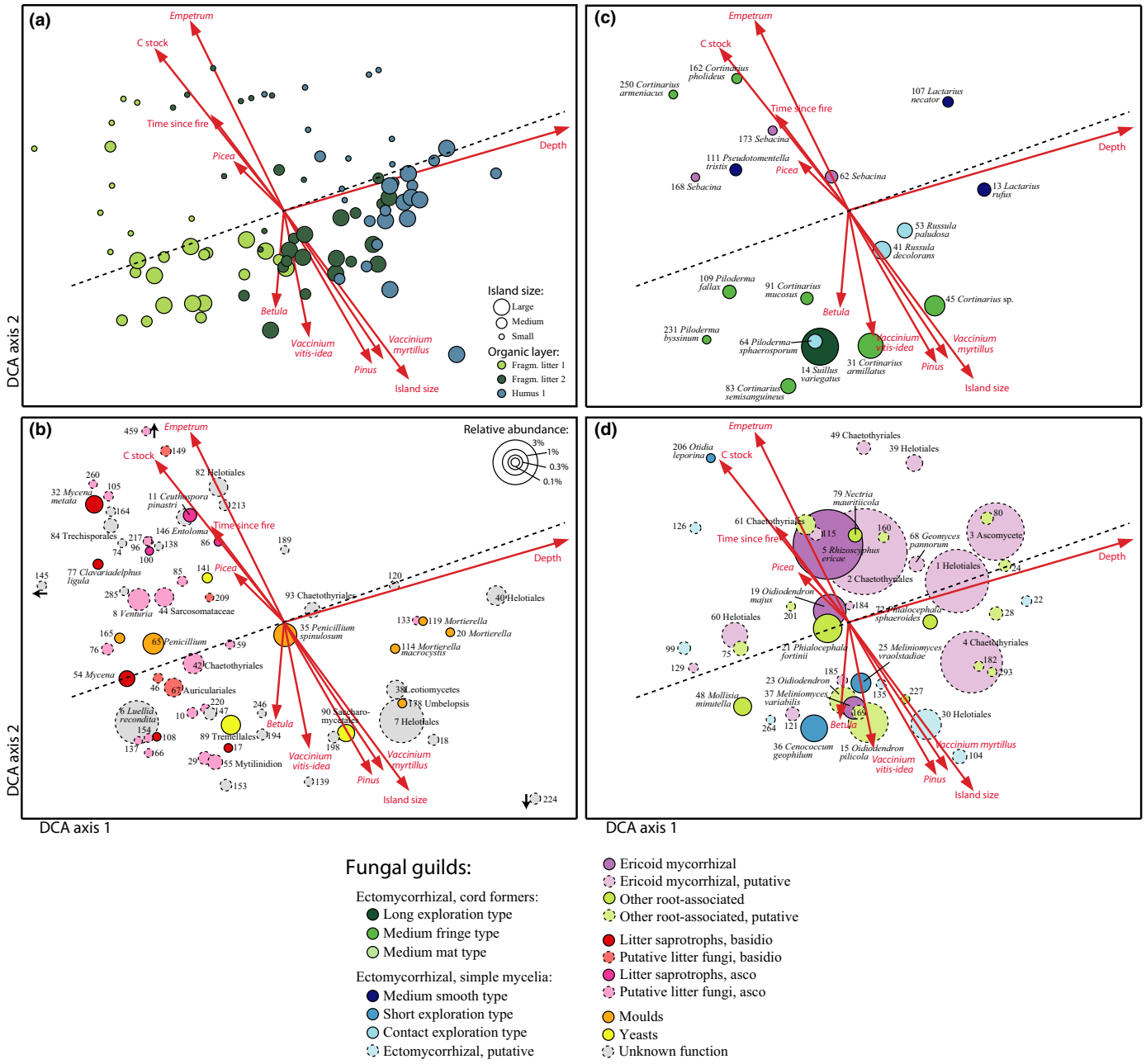

Fig. 4 Sample (a) and species (b–d) plots derived from a detrended correspondence analysis (DCA) of fungal communities in the main rooting zone (fragmented litter layers 1 and 2 and humus layer 1) with the two first axes shown and accounting for 5.3 and 2.9% of the total inertia of 7.60. The DCA is based on all fungal operational taxonomic units (OTUs; 3155), but only the 128 OTUs, each representing at least 0.1% of the total community, and adding up to 80% of the reads, are shown. Fungal OTUs are divided into free-living saprotrophs and species with unknown function (b), root-associated basidiomycetes (c) and root-associated ascomycetes (d). In (a), island size class is indicated by dot size and organic layer by colour. In panels (b)–(d), OTU relative abundance is indicated by dot size and fungal guild by colour. The broken line approximately delimits large and small island communities, and vectors indicate direction and degree of correlation between the two first DCA axes and ecosystem characteristics. Numerical prefixes identify all OTUs that are further detailed in Supporting Information Table S2. Fragm., fragmented.

compositional changes in plant communities. Among the environmental variables, C stock was singled out as the variable most clearly related to overall fungal community composition, but regarding the causality of this correlation we postulate that C storage depends on the composition of fungal communities, not the other way around. Based on observed correlations, we propose a conceptual framework for how shifting community composition of mycorrhizal symbionts during long-term succession of boreal forests might affect N and C dynamics and therefore soil C sequestration. While natural experiments like this boreal forest chronosequence enable ecological processes to be studied over far greater spatial and temporal scales than is possible with manipulative experiments, natural experiments rely more on correlative approaches to identify mechanistic

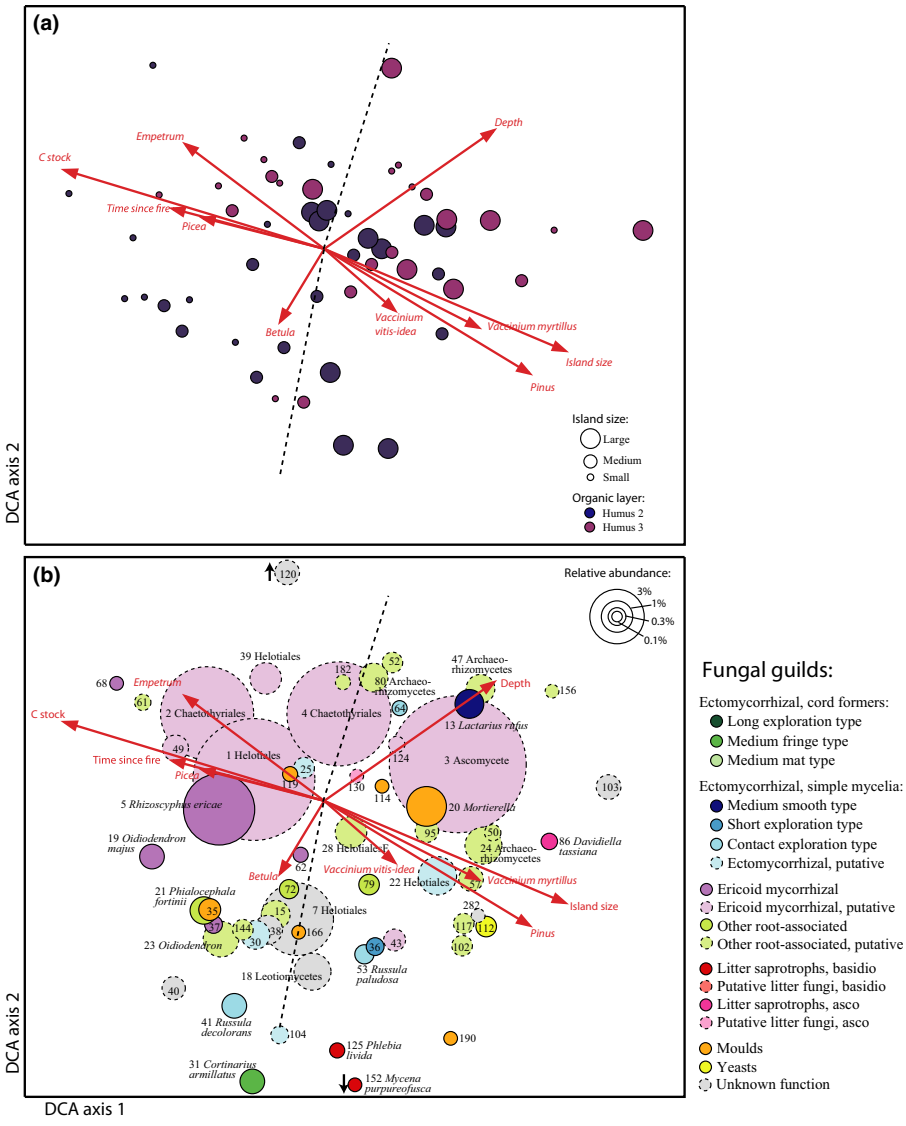

Fig. 5 Sample (a) and species (b) plots derived from a detrended correspondence analysis (DCA) of fungal communities in the deeper humus present across the complete island gradient (humus layers 2 and 3), with the two first axes shown and accounting for 6.5 and 4.0% of the total inertia of 3.91. The DCA is based on all fungal operational taxonomic units (OTUs) present (1728), but only the 61 OTUs that, on average, represented at least 0.15% of the total amplicon, together adding up to 86% of the reads, are shown. In panel (a), island size class is indicated by dot size and organic layer by colour. In panel (b), OTU relative abundance is indicated by dot size and fungal guild by colour. The broken line approximately delimits large and small island communities. The vectors indicate direction and degree of correlation between the two first DCA axes and ecosystem characteristics. Numerical prefixes identify all OTUs that are further detailed in Supporting Information Table S2.

relationships. The large-scale relationships identified in this study thus call for finer-scaled manipulative experiments to further explore mechanistic underpinnings and causal relationships.

Cord-forming ectomycorrhizal fungi may restrict C sequestration

The most obvious difference in fungal community composition between the early successional-stage forests on large islands and the late successional-stage forests on small islands was the higher contribution by cord-forming ectomycorrhizal basidiomycetes (*Suillus variegatus*, *Cortinarius* and *Piloderma* spp.) on the large islands (Figs 2i, 4c). Cord-forming ectomycorrhizal fungi were primarily located in the fragmented litter and uppermost humus layers, corresponding to the zone where ^{14}C analyses indicated a lower accumulation of root-derived C on large than on small islands (Clemmensen *et al.*, 2013). The lower ergosterol and chitin concentrations below this zone on large islands (Clemmensen *et al.*, 2013) suggest that mycelial production is counteracted by biomass turnover and necromass degradation, preventing fungal remains from accumulating to the same extent as on small islands. Cord-forming basidiomycetes form extensive soil mycelia (Agerer, 2006) characterized by a dense front of exploratory hyphae that rapidly disintegrate as the mycelium differentiates, leaving behind a sparse network of long-lived, hydrophobic hyphal cords that serve as transport conduits to the mycorrhizal roots (Finlay & Read, 1986). Through their growth form, ectomycorrhizal cord formers presumably also utilize nutrients, such as N, more efficiently by transforming and recycling their own biomass through autolytic processes in a manner that has been well described for saprotrophic basidiomycetes (Dowson *et al.*, 1989; Boddy, 1999; Falconer *et al.*, 2007). Ectomycorrhizal cord formers in the boreal forest may recycle mycelium particularly to minimize N immobilization in their biomass and thereby provide an N surplus that can be transferred to the plant host (Abuzinadah *et al.*, 1986). We postulate that such a strategy would act to reduce long-term C and N sequestration despite a high rate of mycelial production in systems where cord-forming

ectomycorrhizal fungi are abundant (Fig. 6). The degree to which such a strategy acts to minimize competition between the symbiotic partners in different ecosystems may explain why mycorrhizal fungi in some cases (such as in our late successional-stage forests) appear to aggravate N limitation of their plant hosts (Alberton *et al.*, 2007; Näsholm *et al.*, 2013), whereas in other cases (such as in our early successional-stage forests) act to increase both C and N cycling through soil pools (Drake *et al.*, 2011; Phillips *et al.*, 2012).

Some of the dominant cord formers in our system and in the boreal forest in general – the *Cortinarius* species – may also be particularly efficient at mobilizing organic N from complex organic polymers (Hobbie & Agerer, 2010; Hobbie *et al.*, 2013). This is probably a consequence of their production of oxidative exoenzymes, such as class II manganese (Mn) peroxidases, in response to low N availability (Bödeker *et al.*, 2014). Hence, although all *Cortinarius* species appear to be obligate biotrophs and depend on their host for easily available sugars, they may still act to degrade organic matter in their search for N (see also Lindahl & Tunlid, in press). Our data also indicate that communities of putative mycelium degraders and opportunists, including various yeasts and moulds, thrive in the rooting zone of large islands, and their activity could contribute to a higher turnover of dead mycelium and decomposition residues produced on these islands. An overall consequence of the higher abundance of cord-forming ectomycorrhizal fungi thus seems to be that a major part of the C allocated to mycelial growth is turned over rapidly, reducing C inputs to long-term storage pools in the soil and facilitating more rapid recycling of N to the plant biomass (Fig. 6). This may seem counterintuitive, as mycelial cords have been found to persist for long time periods in the soil (Treseder *et al.*, 2005; Pritchard *et al.*, 2008). However, the cords themselves only account for a minor fraction of mycelial production in cord-forming fungi and the majority of production consists of diffuse exploratory mycelium which turns over much more rapidly than the sparse network of remaining cords (Finlay & Read, 1986). A greater efficiency of mobilization and transfer of N to the plant hosts is also reflected by the steeper $\delta^{15}N$ gradient and higher C : N ratio in the humus profiles of large islands (Clemmensen *et al.*, 2013).

Ericoid mycorrhizal fungi may mediate C sequestration

Ericoid mycorrhizal fungi constituted a major and stable proportion of the amplicon communities across all islands (Fig. 2k), although the best-characterized ericoid mycorrhizal fungus (*Rhizoscyphus ericae*; Smith & Read, 2008) was clearly more abundant on small islands (Figs 4d, 5b). Total stocks of both ergosterol and ITS copy numbers were higher in the deeper

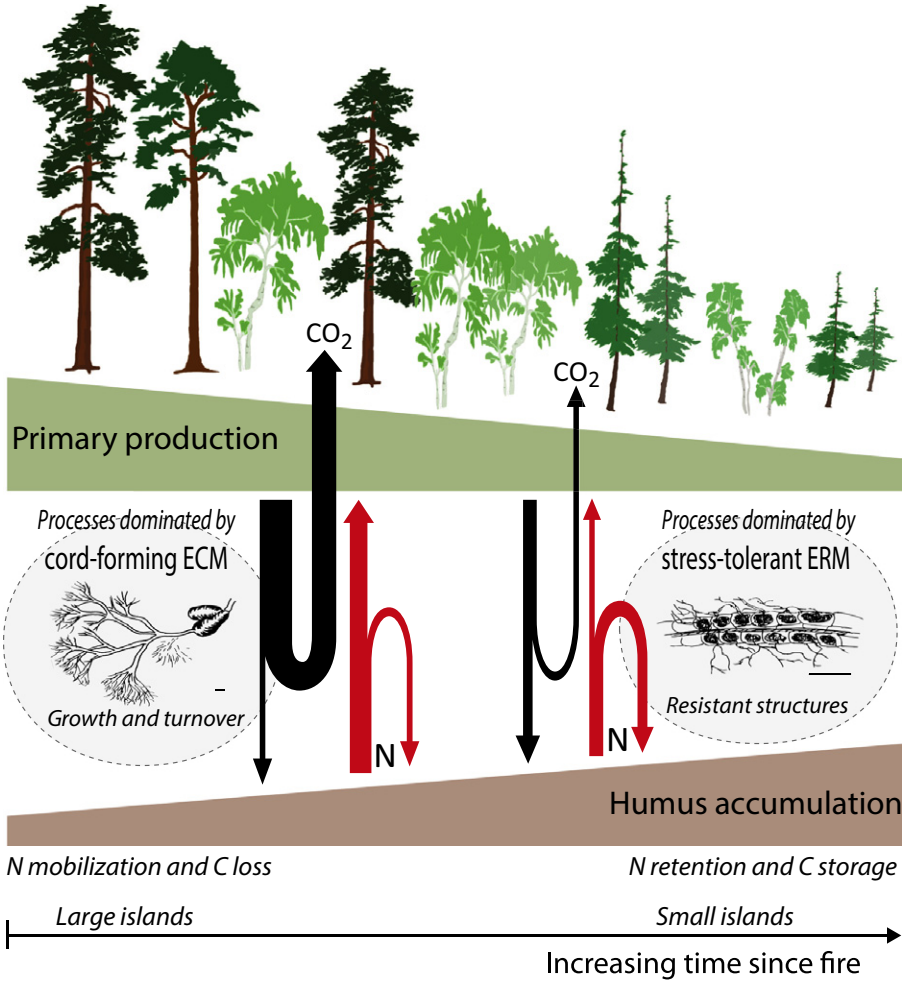

Fig. 6 Conceptual framework depicting how shifts in mycorrhizal symbionts in a 5000 yr boreal forest succession after wildfire affect nitrogen (N) and carbon (C) dynamics. In the succession, *Pinus* dominates early stages and *Picea* dominates late stages, whereas *Betula* has the highest abundance at intermediate stages. The understorey is dominated by *Vaccinium myrtillus*, *Vaccinium vitis-idea* and *Empetrum hermaphroditum* at early, intermediate and late stages, respectively. Above-ground primary production by both trees and ericoid dwarf shrubs decreases, while below-ground C sequestration increases, with time since fire. At earlier successional stages, C allocation (black arrows) to mycorrhizal fungi is high, and cord-forming ectomycorrhizal (ECM) basidiomycetes (e.g. *Suillus*, *Cortinarius* and *Piloderma*) dominate root zone processes. Through rapid growth and turnover of their exploratory mycelium, they facilitate N mobilization (red arrows) to the host plants but restrict the amount of C and N transferred to long-term humus pools. At later successional stages, less C is allocated to mycorrhizal symbionts, and stress-tolerant, root-associated ascomycetes (e.g. ericoid mycorrhizal species, ERM) dominate C and N dynamics in the root zone. By building biomass structures resistant to decomposition, they facilitate N retention and long-term C storage in the humus. The scale bars within the dashed ellipses represent 0.1 mm.

humus layers of small islands (Fig. S3b,d) where ericoid fungi dominated, suggesting that the standing biomass of these communities was larger on small islands. Most of the root-associated ascomycetes in boreal forests belong to the Helotiales or Chaetothyriales and form short-ranging, nonaggregated mycelia of dark hyphae with thick, melanized cell walls that are well protected against adverse environmental conditions, such as desiccation and fluctuating temperatures (Butler & Day, 1998; Robinson, 2001; Grelet *et al.*, 2010; Fernandez & Koide, 2013). These hyphae are likely to be characterized by slow growth (Robinson, 2001; Smith & Read, 2008) and long life span (Koide *et al.*, 2013). Melanized hyphae are also likely to have cell walls that are more resistant to degradation (Coelho *et al.*, 1997; Butler & Day, 1998) than the exploratory hyphae produced by most ectomycorrhizal basidiomycetes. We postulate that these characteristics lead to a larger proportion of fungal necromass being preserved in long-term humus stores on small islands, in which root-associated ascomycetes dominate root zone processes (Fig. 6). Previously, we have shown that C accumulation at late successional stages depends on accumulation of mycelial remains, as indicated by high concentrations of the fungal cell wall component chitin in the humus (Clemmensen *et al.*, 2013). However, chitin is relatively labile under both laboratory and field conditions (Drigo *et al.*, 2012; Fernandez & Koide, 2012; Russell, 2014), and instead differences in melanin concentration influence variation in mycelial recalcitrance to decomposition (Fernandez & Koide, 2014). Fungal melanins have been found to associate with more labile components of fungal cell walls, such as proteins and chitin, and may form physical and chemical complexes that may be well protected against decomposition (Coelho *et al.*, 1997; Butler & Day, 1998). Hence, we propose that stress-tolerant, root-associated ascomycetes play a central role in the formation of boreal forest humus.

The most intensively studied ericoid mycorrhizal fungal species, *R. ericae*, grows well on fungal necromass as a sole N source (Kerley & Read, 1998), and ericoid fungi have long been thought to be more efficient mobilizers of organic N relative to ectomycorrhizal fungi (Read *et al.*, 2004). However, lower C : N and $^{15}N : {}^{14}N$ ratios but higher chitin content of the humus on small islands indicate that less efficient recirculation of N to plants is linked to mycelial necromass build-up (Clemmensen *et al.*, 2013), supposedly by ericoid mycorrhizal fungi. Thus, in contrast to Read *et al.* (2004), we propose that ericoid mycorrhizal fungi may lock up more C and N than they release from the long-term soil organic matter pool, primarily as a result of impaired decomposition of their necromass. There was a distinct transition in community composition within the guilds of putative ericoid mycorrhizal and other root-associated ascomycetes across the island gradient (Fig. 4d). It is possible that these root-associated ascomycetes cover a wide spectrum of lifestyles from facultative to obligate mycorrhizal biotrophs, and that they form structurally and functionally different associations with various host species (e.g. Vohnik *et al.*, 2007; Grelet *et al.*, 2010; Vohnik & Albrechtova, 2011). For example, the two melanized ascomycetes *Cenococcum geophilum* and *Meliniomyces vraolstadiae* were found preferentially in early successional forests, probably because their ectomycorrhizal habit link their abundance to the production of their host trees. Interspecific differences in melanin production and mycelial dynamics may be important for C sequestration and deserve particular future research attention.

Shifting vertical distribution of litter-associated fungi

Litter layers were clearly dominated by saprotrophs, with a succession from a few dominant ascomycete litter endophytes (e.g. *Ceutospora pinastri*, *Lophodermium pinastri*) in the younger litters, to basidiomycete saprotrophs (mainly *Mycena* spp.) in the more decomposed litters (Fig. 3). Saprotrophic communities were largely confined to the litter layers, supporting previous studies and showing that vertical separation of saprotrophic and root-associated communities is a widespread phenomenon in ectomycorrhizal-dominated ecosystems (Lindahl *et al.*, 2007; Baldrian *et al.*, 2012; McGuire *et al.*, 2013). However, both litter-associated basidiomycetes and ascomycetes were more abundant at greater depths on small islands than on large islands (Fig. 2e,f; Table S8), corresponding to the slower decomposition of above-ground litter components on small islands (Wardle *et al.*, 2003). Additionally, reduced competition from ectomycorrhizal cord formers in the fragmented litter layers on the small islands may have allowed the litter fungi to persist at greater depths, in a manner similar to the competitive release of saprotrophic communities shown to occur after root trenching to exclude roots of ectomycorrhizal tree species (Gadgil & Gadgil, 1971). In contrast to the litter layers, saprotrophic fungal communities in the humus layers were dominated by yeasts and moulds, particularly on large islands. Wood decay fungi appear to be confined mainly to discrete patches of woody debris in this system.

Coordinated above-ground and below-ground successions

The most abundant cord-forming ectomycorrhizal fungi in our system (Fig. 4c) live exclusively in symbiosis with *Pinus* (i.e. *Suillus variegatus*, *Cortinarius semisanguineus*, *C. mucosus*) or *Betula* (i.e. *C. armillatus*). *Pinus* and *Betula* dominate the earlier succession stages after wildfire (Wardle *et al.*, 2003) and could thereby contribute to the higher abundance of these cord-forming species on larger islands. The increasing relative contribution of ericaceous dwarf shrubs to total net primary productivity with time since fire (Wardle *et al.*, 2003) might explain the sustained high relative abundance of ericoid mycorrhizal ascomycetes as island size decreases (Fig. 2k). Conversely, fungal successions following wildfire may also play a central role in driving changes in the plant community. The increasing importance of stress-adapted traits during long-term succession in the prolonged absence of disturbances such as fire, and with declining soil fertility, is probably highly coordinated between plant and fungal communities. The greater fungal species diversity in the late successional-stage forests matches observations of plant diversity (Wardle *et al.*, 2012a), further highlighting the parallel development of above-ground and below-ground community responses.

The declining net primary productivity associated with prolonged absence of fire in our study system (Wardle *et al.*,

2012a) suggests that absolute C allocation to root-associated fungi would be largest early in the succession. Cord-forming ectomycorrhizal fungi have been proposed to be the most C-demanding of all mycorrhizal types, owing to their extensive mycelial production (Hobbie, 2006). Their supposedly high production of enzymes would lead to a high energy demand, and their maintenance in the fungal community may depend on sufficient quantities of C being allocated below ground. The high demand for host-derived C by these cord-forming fungi would be matched by a more efficient supply of nutrients back to their hosts, leading to a positive feedback between the two partners and a higher primary production (Fig. 6). Thus, both vegetation composition and productivity are likely to be important drivers of the observed differences in root-associated fungal communities between the large and small islands.

Taken together, our observations provide evidence that long-term successional development and accompanying humus build-up in the forest chronosequence occurs in concert with phylogenetic and morphological shifts in the mycorrhizal fungal community with potential consequences for C and N cycling (Fig. 6). We propose that these fungal community changes play an important role in explaining the large accumulation of organic matter below ground that is often observed in older ecosystems. While stress-adapted, root-associated ascomycetes generally seem to promote biochemical stabilization of C and N in organic matter derived from mycelium, the higher abundance of certain cord-forming ectomycorrhizal fungi at earlier successional stages implies efficient recycling of N and C from both recently produced fungal mycelium and older humus. Long-term ecosystem succession thus involves impairment of mycorrhizal N recirculation and, consequently, progressive nutrient limitation and compositional changes in the vegetation with time since fire (Alberton *et al.*, 2007; Wardle *et al.*, 2012a). Changes in plant community composition in turn influence total below-ground allocation of C and relative C allocation to different fungal symbionts, as a consequence of specificity in the plant–fungal interactions. These feedbacks result in increasing C and N accumulation in the humus layer and reduced plant production over time – a pathway that can only be reset by major disturbances, such as wildfire.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Flowchart detailing sequence data processing.

Fig. S2 Relations among ergosterol, DNA and ITS copies.

Fig. S3 Total stocks of SOM, ergosterol, DNA and ITS copies.

Fig. S4 Fungal species richness and evenness in soil profiles.

Fig. S5 Relative abundance of fungal taxonomic classes in soil profiles.

Table S1 Characteristics of the island study system

Table S2 OTU representative sequence accessions, identities and guild assignments

Table S3 Relative abundance of fungal OTUs in all samples

Tables S4–S6 CCA analyses of data presented in Figs 1 and 3–5

Tables S7 and S8 Mixed model statistics of results in Figs 2 and S4

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