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PLANT MICROBE INTERACTIONS

Temperate Forests Dominated by Arbuscular or Ectomycorrhizal Fungi Are Characterized by Strong Shifts from Saprotrophic to Mycorrhizal Fungi with Increasing Soil Depth

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Abstract

In temperate and boreal forests, competition for soil resources between free-living saprotrophs and ectomycorrhizal (EcM) fungi has been suggested to restrict saprotrophic fungal dominance to the most superficial organic soil horizons in forests dominated by EcM trees. By contrast, lower niche overlap with arbuscular mycorrhizal (AM) fungi could allow fungal saprotrophs to maintain this dominance into deeper soil horizons in AM-dominated forests. Here we used a natural gradient of adjacent forest patches that were dominated by either AM or EcM trees, or a mixture of both to determine how fungal communities characterized with high-throughput amplicon sequencing change across organic and mineral soil horizons. We found a general shift from saprotrophic to mycorrhizal fungal dominance with increasing soil depth in all forest mycorrhizal types, especially in organic horizons. Vertical changes in soil chemistry, including pH, organic matter, exchangeable cations, and extractable phosphorus, coincided with shifts in fungal community composition. Although fungal communities and soil chemistry differed among adjacent forest mycorrhizal types, variations were stronger within a given soil profile, pointing to the importance of considering horizons when characterizing soil fungal communities. Our results also suggest that in temperate forests, vertical shifts from saprotrophic to mycorrhizal fungi within organic and mineral horizons occur similarly in both ectomycorrhizal and arbuscular mycorrhizal forests.

Keywords Fungal guilds · Soil physico-chemistry · Podzolic soil · Vertical segregation · *Acer saccharum* · *Fagus grandifolia*

Introduction

Soil fungi drive the biogeochemical cycling of carbon (C) and nutrients in terrestrial ecosystems. Free-living saprotrophic fungi are major decomposers of soil organic matter, but mycorrhizal fungi also play an important role [1–3]. In northern temperate forests, there are two major types of root-associated fungi: arbuscular mycorrhizal (AM) and ectomycorrhizal (EcM) fungi [4, 5]. Mycorrhizal fungi acquire C via plant hosts and many EcM fungi possess the enzymatic capacity to directly degrade organic matter, potentially competing with free-living saprotrophs for organic nutrients such as nitrogen (N), which promote soil C accumulation [6–8]. By contrast, AM fungi have limited degrading abilities and therefore might compete less strongly with saprotrophic fungi for nutrients [9–11]. Such interactions among saprotrophic and mycorrhizal fungi could have far-reaching implications for the C cycle, especially in northern forests where a large fraction of global soil C is stored [3, 12, 13]. In particular, it has been suggested that these interactions might help to explain differences in the amount and vertical distributions of soil C between ectomycorrhizal- and arbuscular mycorrhizal-dominated forests [7, 14, 15].

A first step toward understanding of interactions among saprotrophic and mycorrhizal fungi and their functional consequences is to identify their co-occurrence patterns in soils [e.g., 16]. Different groups of fungi can compete with each

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other for soil resources because of overlapping niches [7, 16–18]. In particular, fungal types and taxa differ in their vertical distribution, especially in well-stratified soil [19–21]. In EcM-dominated ecosystems such as boreal forests, strong vertical segregation of fungal guilds occurs in the soil profile, where the litter layer is dominated by saprotrophic fungi and in older and deeper layers are increasingly dominated by EcM fungi [21–23]. However, it remains unclear whether this spatial separation reflects niche differentiation or competitive exclusion of saprotrophic fungi by EcM fungi [7, 17]. Competitive interactions for nutrients among these fungal groups could promote organic matter accumulation [24–26]. In AM-dominated forests, interactions and distribution patterns may be different because AM fungi might not compete as strongly with saprotrophic fungi than EcM fungi. However, studies of fungal vertical distribution in AM-dominated ecosystems have largely focused on grasslands and crop systems [27–29] but not forests. To better understand the impacts of global and land use changes on forest functioning, there is a crucial need to take different mycorrhizal types fungi into consideration simultaneously [6, 7, 30], especially the AM strategy given its importance in temperate forests [10].

A general hypothesis on vertical segregation among mycorrhizal types suggests that, when they co-occur, EcM fungi and ericoid mycorrhizal (ErM) will dominate organic horizons while AM fungi will predominantly occupy mineral horizons or soils [31, 32]. This view is supported by studies based on (i) root colonization patterns in environments where mycorrhizal types co-occur [e.g., 33], (ii) root patterns and isotopic measurements of plants of different mycorrhizal types [e.g., 32, 34], (iii) root colonization patterns in "dual mycorrhizal" plants [35–37], (iv) the different nutritional benefits of fungal symbionts and their enzymatic capacity [31, 32], and (v) global patterns of mycorrhizal distribution [31, 38]. However, to our knowledge, this hypothesis about vertical distribution of distinct mycorrhizal types (e.g., EcM and AM) across horizons has not been supported by detailed fungal community analyses. For example, mycorrhizal fungal distribution does not always follow root distribution (e.g., presence of AM fungi in the litter horizon [39]), and to focus on roots or rhizosphere sampling overlooks at long extraradical hyphae of mycorrhizal fungi that penetrate far from root surfaces. Few studies have studied vertical distribution at spatial scales that are fine (i.e., cm) and functional (i.e., by horizons). To our knowledge, the vertical distribution of soil fungi in neighboring forest stands dominated by different mycorrhizal types has not been reported. Therefore, it is not clear whether EcM or AM fungi show similar vertical niches [32].

The difficulties associated with identifying the microorganisms directly involved in soil biogeochemical cycling such as fungal saprotrophs and mycorrhizal fungi though their extraradical hyphae has been a major obstacle to understand their impacts and the importance of their interactions. Specific biomarkers can be used as proxy to quantify fungal biomass in soils such as phospholipid fatty acid [e.g., 40], but they are common in many fungal groups and cannot discriminate between free-living saprotrophic fungi and EcM fungal lineages because EcM symbiosis has arisen independently and persisted numerous times in the Basidiomycetes, Ascomycetes, and Zygomycetes [41]. Also, the mycelia of some fungi does not contain ergosterol [42]. With advances in high-throughput amplicon sequencing [43], we are able to identify community members and their corresponding guilds [44–46]. Determining the taxonomic composition of fungal communities is important because different species within the same fungal guild can vary in their effects on C and nutrient cycling [e.g., 47, 48]. Using such sequencing methods, fungal community composition has been found to vary markedly across large spatial scales, driven by broad-scale changes in climate and soil properties [49, 50]. However, the mechanisms shaping distribution of fungal community and fungal groups such as free-living and root-associated at small spatial scales remain comparatively little studied, and high-throughput amplicon sequencing will allow to understand their potential impact on ecosystem functioning [19, 51, 52].

To determine the vertical distribution of fungal communities and guilds among temperate forests, we characterized soil fungi and chemistry in adjacent forest patches dominated by trees that form AM or EcM or a mixture of both strategies. Specifically, we used the natural co-occurring distribution of *Acer saccharum* and *Fagus grandifolia* that associates exclusively with AM and EcM fungal symbionts respectively [53]. These two co-occurring tree species share similar ecological strategies that they are both deciduous and shade-tolerant and can dominate the canopy in adjacent forest patches in northeastern North America [54, 55]. Their natural co-occurrence patterns provide an opportunity to compare vertical distribution of fungal community composition in different forest mycorrhizal types, under similar environmental conditions, thus minimizing variation in other important factors such as climate, parent material, or topography. Using this natural experimental design, we assessed how the fungal community, guilds, and root colonization vary across soil horizons along an AM-EcM gradient, and determined to which extent this variability was linked with changes in soil chemical properties. We expected the shift from saprotrophic to mycorrhizal fungi to occur deeper in AM forests compared to EcM forests, and at an intermediate depth in forests containing a mixture of both strategies.

Materiel and Methods

Study Area

The study was conducted at the University of Montréal's field station (Station de biologie des Laurentides, Saint-Hippolyte,

Québec, Canada). The field station is representative of temperate forests of the Lower Laurentians and the Canadian Shield. The soil has a sandy loam texture derived from well-drained rocky glacial till on a bedrock of Precambrian anorthosite [56, 57]. The soils are ferro-humic and gleyed humo-ferric podzols with moder humus forming the forest floor [57–59]. The mean annual temperature is 4.3 °C and total annual precipitation is 1195 mm, with ~ 25% falling as snow (based on 1981–2010 data, meteorological station no. 7037310, Saint-Hippolyte). The study area is located within the sugar maple-yellow birch domain [60]. Most of the forest regrew following a major fire that occurred around 1923 [61]. Mesic sites are composed mostly of a mosaic of *Acer saccharum* and *Fagus grandifolia*, with *Betula alleghaniensis*, *Populus grandidendata*, and *Acer rubrum* also common [57]. The understory comprised various small tree species (e.g., *Acer pensylvanicum*) and shrubs (e.g., *Vaccinium* spp., *Viburnum* spp.).

Selection of Forest Plots

Plots were selected based on the dominance of different mycorrhizal tree types: AM-dominated stands (> 80% relative basal area by AM trees; mainly *Acer saccharum*) and EcM-dominated stands (generally > 80% relative basal area by EcM trees except one plot at 63%; mainly *Fagus grandifolia*), and mixed stands (approximately equal basal area of AM and EcM trees, mainly *A. saccharum maple* and *F. grandifolia*). Tree basal area was based on all trees ≥ 5-cm diameter at breast height (DBH) within a plot. Plots were 20 m × 20 m in size. We selected five blocks, each containing one plot of each corresponding to one of the three mycorrhizal types (i.e., EcM, AM, mixed), for a total of 15 plots (Fig. S1). Plots were selected as to minimize variation in environmental conditions (i.e., altitude, slope, aspect, total basal area; Table S1) among plots within a block, and to be as close as possible from each other (< 400 m). For each plot, precise geographic coordinates, altitude, topographic location, slope, and orientation were measured (Table S1).

Soil Sampling

Soil sampling was conducted in July and August 2015. In each plot, 10 samples were taken along two oriented north-south transects (five samples per transect). Samples were collected to 20-cm depth using PVC cores (7.5 cm in diameter). Samples were kept in coolers with ice and transported to the laboratory to be processed within 96 h of sampling. The PVC cores were split open to measure horizon thickness then separated by the following: litter (L), where original structures are easily distinguishable; fragmented (F), where there had been partial decomposition where structures were difficult to recognize; and humus (H), comprised of highly decomposed organic matter, where original structures are indistinguishable (see Fig. S2). The mineral horizons were Ae, as characterized by leaching/eluviation of clay; Fe, Al, or organic matter; and B, as characterized by illuviation/enrichment in organic matter [62]. The 10 samples per plot were pooled by horizon. One sub-sample per horizon per plot was immediately frozen for subsequent DNA extraction. Fine roots (< 2 mm in diameter) were set aside for mycorrhizal colonization analyses and a sub-sample of soil was air-dried for chemical analyses.

Soil Analysis

Air-dried soils were analyzed for pH, total carbon (C), total nitrogen (N), total phosphorus (P), organic P, inorganic P, and labile P. The pH was determined in 10 mM $CaCl_2$ in a 1:2 soil to solution ratio with a glass electrode. Total C and N were determined simultaneously by automated combustion and gas chromatography with thermal conductivity detection on a Flash EA112 analyzer (CE Elantech, New Jersey, USA). After NaOH-EDTA extraction, inorganic P in the extraction material was determined by molybdate colorimetry at 880 nm with a 1-cm path length. Total P in the NaOH-EDTA extracts was determined by molybdate colorimetry at 880 nm with a 1-cm path length, following acid-persulfate digestion at 80 °C overnight in sealed glass tubes. Organic P was calculated as the difference between NaOH-EDTA total P and NaOH-EDTA P_i. Labile (plant-available) P was determined by Bray-1 extraction, with phosphate detected using automated molybdate colorimetry on a Lachat Quikchem 8500 (Hach Ltd., Loveland, CO). Exchangeable cations were determined by extraction in 0.1 M $BaCl_2$ (2 h, 1:30 soil to solution ratio) and detection by inductively coupled plasma optical-emission spectrometry (ICP–OES) with an Optima 7300 DV (Perkin-Elmer Ltd., Shelton, CT, USA). Total exchangeable bases (TEBs) was calculated as the sum of the charge equivalents of Ca, K, Mg, and Na. Effective cation exchange capacity (ECEC) was calculated as the sum of the charge equivalents of Al, Ca, Fe, K, Mg, Mn, and Na. Base saturation was determined as TEB / ECEC × 100.

Root Colonization by Fungi

Fungal colonization was determined on fine roots (< 2-mm diameter) of F, H, Ae, and B horizons (no roots in the L). Roots were cleared in 10% w/v KOH, then stained in an ink and vinegar solution for 5 min at 90 °C [63–65]. Roots were then rinsed in slightly acidified tap water for 30–40 min to remove excess ink, after which they were placed in a 50% (v/v) lacto-glycerol solution for storage until colonization could be evaluated. The gridline intersection method was performed under stereomicroscope to quantify the length of roots

colonized by AM and EcM fungi [63, 66]. Due to magnification limits, some structures of ericoid mycorrhizal fungi might have been included in the AM colonization percentage.

Fungal Community Characterization

The fungal community was characterized by amplicon sequencing. Soil DNA was extracted using the PowerSoil DNA Isolation Kit (no. 12888-100—Mo-Bio Laboratories Inc., Carlsbad, USA) following the instructions of the manufacturer. Around 100 mg of soil for organic horizons (L, F, and H) and 200 mg for mineral horizons (Ae and B) were used for the extraction.

Soil amplification of the Internal Transcribed Spacer of the ribosomal RNA was performed by Genome Québec (Montréal, Canada) with the ITS3_KYO2 and ITS4 primer pair [67]. This pair of primer limits coverage bias toward Ascomycetes or Basidiomycetes and is also known to amplify Glomeromycetes [e.g., 68]. The final reaction mix contained 0.02 U μl^{-1} Taq Roche HiFi polymerase, 1 × Buffer 10 × with 18 mM $MgCl_2$, 5% DMSO, 0.2 mM of each dNTP, and 0.5 μM of each primer and DNA sample diluted at 1/100. Thermal cycling was done in an Eppendorf Mastercycler Gradient (Eppendorf, Hamburg, Germany) with the following cycling conditions: 2-min initial denaturation at 94 °C; 40 cycles of 30-s denaturation at 94 °C, 30-s annealing at 55 °C, and 30-s elongation at 72 °C; and a 7-min final elongation at 72 °C. The PCR products were loaded on 1% agarose gels with 1 × sodium borate buffer run at 220 V, and visualized after ethidium bromide staining (1 μg ml^{-1}).

Soil amplicon sequencing was performed by using the MiSeq Illumina technology by Genome Québec (Montréal, Canada). The final concentration of the reaction mix contained 0.025 U μl^{-1} Taq Roche HiFi polymerase, 1 × Buffer 10 ×, 1.8 mM of $MgCl_2$, and 5% DMSO. Sequencing was done in an MiSeq Illumina with the following conditions: 10-min initial denaturation at 95 °C; 15 cycles of 15-s denaturation at 95 °C, 30-s annealing at 60 °C, and 1-min elongation at 72 °C; and a 3-min final elongation.

Bioinformatics

The fungal community was determined by filtering, denoising, and assigning taxonomy to paired amplicons using a customized script (https://github.com/alexiscarter/Fungal_com_SBL/tree/master/dada2) adapted from the DADA2 pipeline [69]. In brief, using the *filterAndTrim* function, reads were truncated at 280 bp and discarded if they had more than three expected errors or a quality score lower than six. Then, amplicon sequence variants (ASVs) were inferred for each sample with the *dada* function. Forward and reverse reads were merged using the *mergePairs* function with a minimum overlap of 12 bp. Potentially, chimeric sequences were identified by the pooled method of the *removeBimeras* function. The amplicon sequence variant approach was used instead of the classical operational taxonomic as proposed by Callahan et al. [70] and others [71]. This method does not use a particular threshold for classifying sequences into operational taxonomic units, as no threshold appears to be universally applicable for fungi [72]. Instead, it used the divisive amplicon denoising algorithm aimed at finding ASV that refer back to original biological sequences [69, 73]. The taxonomy of the ASV was assigned with the UNITE database, version 7.2 [74]. ASV that belong to the same species were grouped together. The functional information for ASV was obtained from the online FUNGuild database [44].

Statistical Analyses

To describe the fungal community and assess the effects of environmental parameters, we used ordination approaches and multivariate analyses of variance. The community matrix was composed of the number of sequences per ASV of 75 soil samples from five soil horizons in each of 15 plots (one sample of L horizon in an EcM plot was excluded due to poor amplification). Due to some inherent limitations of the approach, either biological (e.g., varying number of DNA copies per organism) or technical (varying sequencing depth, extraction, and amplification biases among samples), the number of sequence reads is not a direct measure of taxa abundance in the environment, but comparisons among samples remain useful as they can be considered semi-quantitative [75, 76]. Explanatory variables for each sample were classified into three groups: (i) soil chemistry, (ii) soil horizon (L, F, H, Ae, or B), and (iii) forest type (AM, EcM, or mixed).

Differences in soil properties, root colonization, guild abundance, and richness among horizons and forest type were tested using linear mixed effect models; block was treated as random factor in these analyses. Model assumptions were assessed by visual inspections of residuals. Comparisons were determined using post hoc Tukey tests which were used to determine significant differences.

In β-diversity analyses, we used the Bray-Curtis dissimilarity index for the community structure and its binary version, the Sørensen index, for the community composition [77]. These asymmetrical coefficients do not consider double zeroes and can therefore be used with raw abundances or counts [77].

To visualize differences in fungal community composition and abundance among samples, we used non-metric multidimensional scaling (NMDS). To test for differences

between samples across horizons and forest types, we used permutational multivariate analysis of variance (PERMANOVA). *P* values for pairwise tests were adjusted using the Benjamini-Hochberg method [78]. Because the PERMANOVA method is sensitive to differences in multivariate dispersions among groups, the homogeneity of dispersion was tested to assess differences and tested for significance by permutations [79].

Distance-based redundancy analysis (RDA) was used to quantify the extent to which changes in fungal community structure were related to soil chemistry, horizon, and forest type [77]. Soil chemistry data were standardized and linear dependencies were explored using variance inflation factors and avoided if > 10 [80]. To test how much variance was independently explained by the explanatory matrices, variation partitioning was performed using partial RDA [pRDA, 81]. In RDA and pRDA, coefficients of determination were adjusted (i.e. adjusted-R^2 values) to take into account the number of explanatory variables in the model [82, 83].

Analyses were performed and visualized using the R software [84] with the following main packages: *dada2* [69], *dplyr* [85], *emmeans* [86], *ggplot2* [87], *ggpubr* [88], *nlme* [89], *phyloseq* [90], and *vegan* [91]. Code for bioinformatical and statistical analyses are available at https://doi.org/10.5281/zenodo.3631982. Sequence and chemistry data can be accessed at https://doi.org/10.5281/zenodo.3631861.

Results

Soil Chemistry Variation Across Horizons and Forest Types

All soil chemical properties varied significantly across horizons (Fig. 1), and these differences were consistent across forest types (soil horizon × forest type interaction, $P > 0.05$; except for pH where $P = 0.026$). The pH of the L horizon declined from pH ~4 (in 0.01 M $CaCl_2$) to ~3.25 in the H horizon, but this decline was not as pronounced for AM forests than for EcM or mixed forests (Fig. 1(a)). The pH then increased from the H to the B horizon in all forests. Effective cation exchange capacity and base saturation declined with increasing depth (Fig. 1(b, c)), except for ECEC in the Ae horizon. Organic C generally declined with depth, but AM forests tended to have lower organic C concentration in the H horizons than EcM or mixed forests (Fig. 1(d)). By contrast, total N increased from the L to the Ae horizon and then declined in the B horizon (Fig. 1(e)). As a result, the C:N ratio decreased with increasing depth from the L to the Ae horizon (Fig. 1(f)). Inorganic and organic P increased in deeper horizons while labile (Bray) P decreased (Fig. 1(g–i)).

Forest types differed significantly in their pH, C:N ratio, NaOH-EDTA total P, NaOH-EDTA organic, and inorganic P concentrations ($P < 0.05$). AM-dominated forest plots tended to have higher pH, total P, inorganic P, and organic P but lower C:N ratio compared to EcM-dominated forest plots.

Root Colonization by Mycorrhizal Fungi

Colonization of fine roots by AM and EcM fungi was significantly different among mycorrhizal type ($P < 0.0001$, Fig. 2) but only differs across horizons in the EcM-dominated forest ($P = 0.007$). Fine roots in AM forest were more strongly colonized by AM fungi than those from mixed and EcM forests ($P < 0.05$, Fig. 2(a)). By contrast, fine roots in EcM forests were more strongly colonized by EcM fungi compared to those from AM forests ($P < 0.05$, Fig. 2(b)). Root colonization by EcM fungi tended to decrease with soil depth in EcM forest down to ~20% in the B horizon (Fig. 2(b)). In mixed and AM forests, EcM colonization was highest in the H or Ae horizons but always lower than 30%.

Overall Fungal Community

We found 781 fungal taxa (at the species level or below) from a total of 2521 ASV detected using high-throughput amplicon sequencing across all horizons and plots. Fungal ASV richness tended to decrease with soil depth regardless of the forest type (Fig. S3). The highest fungal ASV richness was found in L horizons of the AM forests.

Fungal Guilds

Saprotrophic and symbiotrophic (EcM, AM, and ErM) guilds showed distinct vertical distributions among horizons and across forest types (Fig. 2(c–f)). Saprotrophic fungal taxa dominated the upper horizons (especially L and F; Fig. 2(c)), and mycorrhizal fungi were almost absent in the L horizon (Fig. 2(d–f)). Fungal taxa assigned to the saprotrophic guild were slightly more abundant in the organic horizons of the AM and mixed forests compared to EcM forest (Fig. 2(c)). Abundance of saprotrophic fungi was significantly different among forest types ($P < 0.031$) but differences were not significant across horizons of different forest types (soil horizon × forest type, $P = 0.325$). In deeper horizons, sequences attributed to mycorrhizal fungi were more abundant (Fig. 2(d–f)). Sequences of AM (i.e., Glomeromycetes) fungi were much more abundant in the AM forest (Fig. 2(d)), and the opposite was true for EcM fungi (Fig. 2(f)). Both AM and EcM taxa were well represented in the mixed forests (Fig. 2(d, e)). Sequences of ericoid mycorrhizal (ErM) fungi were less abundant in AM forest except for the F horizon where their abundance was high in all forests (Fig. 2(f)). Richness patterns of fungal guilds tended to follow abundance data (Fig. S4). Saprotrophic fungi had the higher number of taxa followed by EcM, ErM, and AM fungi. Saprotrophic fungal richness was highest in the upper horizons and decreased with depth.

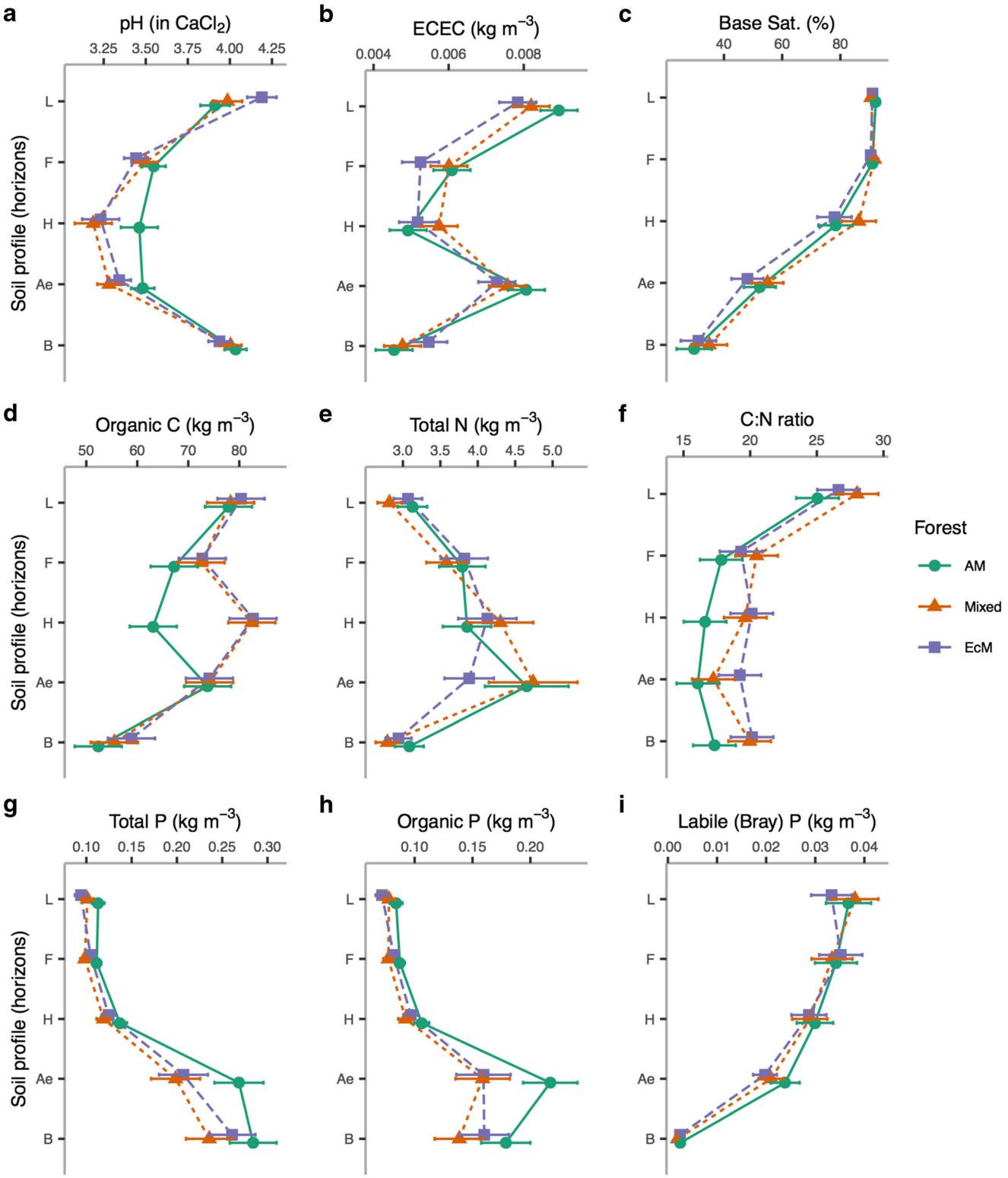

Fig. 1 Soil physico-chemical characteristics from organic-to-mineral horizons (L, F, H, Ae, B) in each mycorrhizal forest type (AM, arbuscular mycorrhizal; EcM, ectomycorrhizal; Mixed, mixture of AM and EcM): (a) pH (in $CaCl_2$), (b) effective cation exchange capacity, (c) base saturation, (d) organic carbon, (e) total nitrogen, (f) carbon over nitrogen ratio, (f) total phosphorus, (g) organic phosphorus, and (h) labile (Bray) phosphorus. All data are means ± 1 SE ($n = 5$)

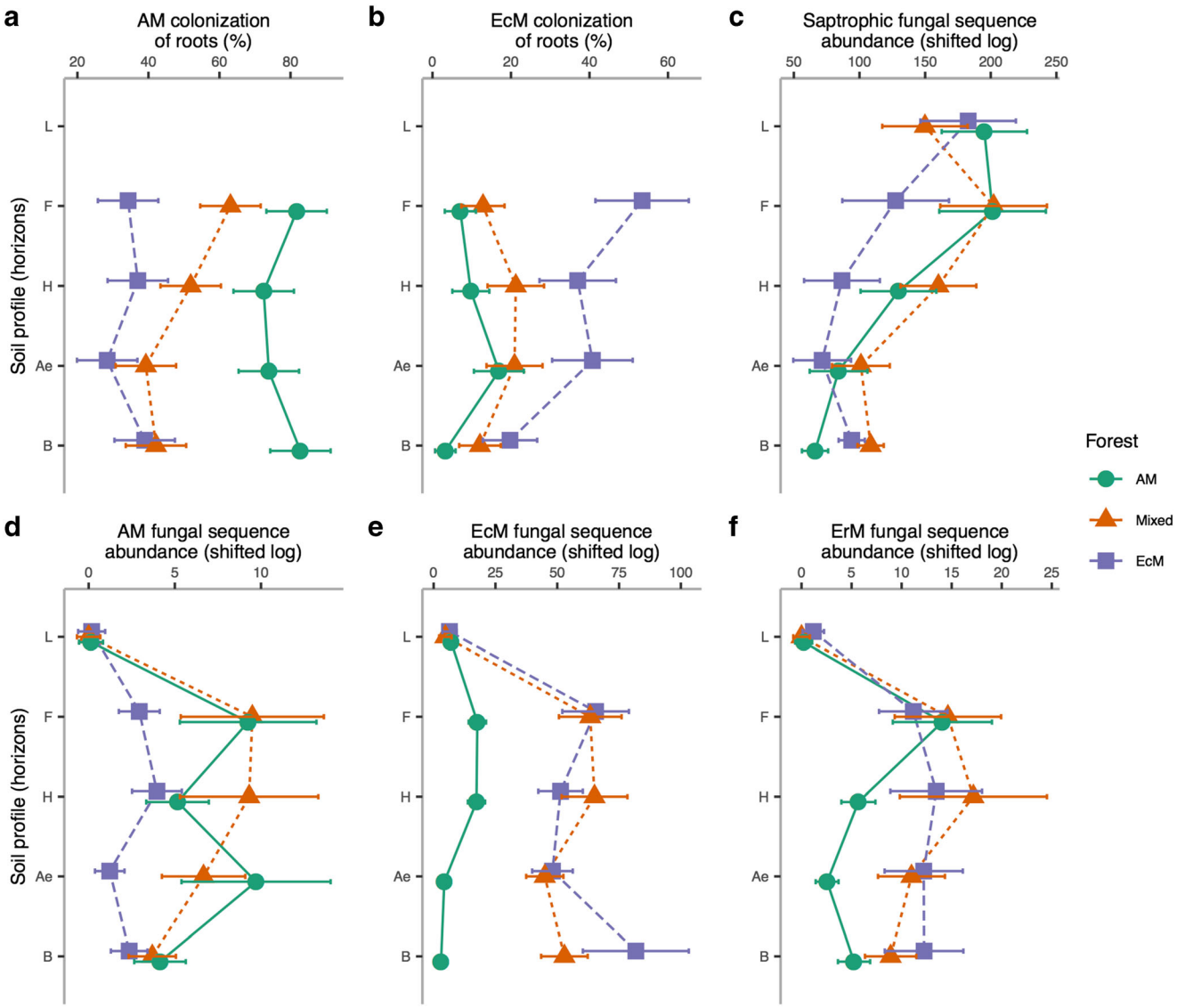

Fig. 2 Soil profiles from organic-to-mineral horizons (L, F, H, Ae, B) on each mycorrhizal forest type (AM, arbuscular mycorrhizal; EcM, ectomycorrhizal; Mixed, mixture of AM and EcM) showing variations in: root colonized by (a) AM fungi, (b) EcM fungi, and abundances (on shifted log data) of sequences belonging to (c) saprotrophic fungi, (d) AM fungi, (e) EcM fungi, and (d) ericoid mycorrhizal (ErM) fungi. Upper organic horizon (L) had no roots so colonization was set to zero. All data are means ± 1 SE ($n = 5$, except $n = 4$ for the L horizon in EcM forest)

There was a higher richness of EcM fungi in EcM and mixed forests and very few EcM taxa in the L horizon.

Fungal Community Structure

Soil horizons had the strongest influence over fungal community structure (includes abundance data) in the three forest types, as shown by the NMDS ordination (Fig. 3). The composition (based on presence-absence data) of the fungal community showed similar patterns (Fig. S5), suggesting that results primarily reflected changes in ASV composition rather than relative abundance. Differences in multivariate dispersions with Bray-Curtis and Sørensen measures were not significant among forest types ($P > 0.05$) but were significant among horizons ($P < 0.05$), with the L horizon showing the lowest multivariate dispersions. In other words, fungal communities from the L horizons were more similar to each other than fungal communities from the other horizons. Fungal community composition and abundance significantly differed among all horizons but also among forest types ($P < 0.001$, Table S2). However, the differences among horizons did not depend on forest type and vice versa (soil horizon × forest type interaction not significant; Table S2). Pairwise comparisons revealed that fungal community composition and abundance in AM and EcM forests significantly differed from each other, but not from mixed forests (Fig. 3).

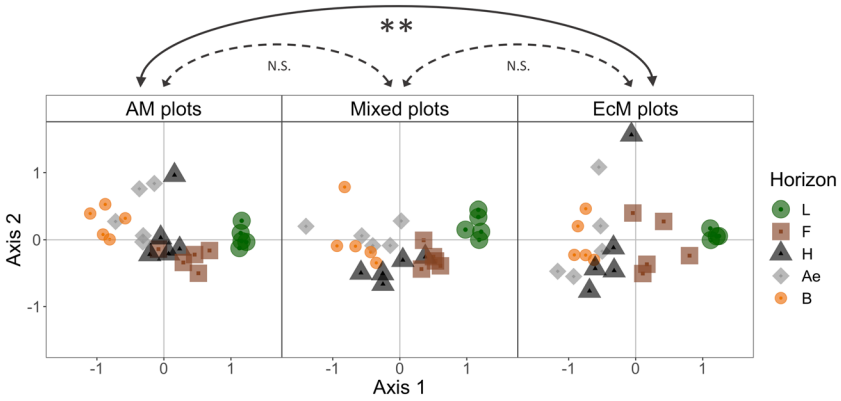

Fig. 3 Ordination of the fungal community composition (Bray-Curtis dissimilarities) plotted in the different forest types using a non-metric multidimensional scaling with two dimensions and a stress of 0.17. ** indicates difference in fungal community structure between arbuscular mycorrhizal (AM) and ectomycorrhizal (EcM) plots ($P \leq 0.01$), N.S. indicates non-significant differences (see Table S2 for details)

Edaphic Drivers of Fungal Community Structure

Variation in soil chemistry explained a large fraction of the total variation in fungal community structure (adjusted-R^2 = 23.3%, $P = 0.001$, see Table S3 for results of the constrained ordinations). In the L horizons, fungal communities were associated with higher pH, ECEC, labile L, and C:N ratio (Fig. 4). Fungal communities in mineral horizons (Ae and B) were associated with high organic and inorganic P but low labile P (Fig. 4). Between L and mineral horizons, fungal communities were associated with low pH (H horizon) and high labile P (F horizon).

Forest mycorrhizal type explained a lower but still significant amount of variation (adjusted-$R^2 = 2.7\%$, $P = 0.006$). There was a clear difference in the fungal community structure of AM and EcM forests, whereas the mixed forests were intermediate or more similar to EcM forest (Fig. 5).

Abiotic and biotic variables together explained ~ 35% ($P = 0.001$) of the total variation in the fungal community structure. Variation in fungal community structure depended on horizons and forest mycorrhizal types, and was also influenced by soil chemistry (Fig. 6). Within forest types, fungal communities were not significantly different among blocks. Horizon, forest type, and soil chemistry still explained a significant fraction of the variation in the fungal community structure when considering the effects of the other variables (Table S3). Most of the explained variation was shared between soil chemistry and horizon (Fig. 6). However, forest type still had a unique and significant impact on the variation of the fungal community. A small fraction of variation was shared between soil chemistry and forest type (Fig. 6).

Discussion

In this study, we determined vertical shifts in soil fungal community composition across soil horizons and forest mycorrhizal types (AM, EcM, and mixed AM/EcM) and compared how saprotrophic fungal dominance extends to deeper horizons in AM vs. EcM forests. Although there was a tendency for lower abundance of saprotrophic fungi in organic F and H horizons in EcM forests than in AM or mixed forests, all three forest types showed a similar saprotrophic-to-mycorrhizal shift in fungal composition with increasing soil depth. This shift in fungal dominance was most pronounced in organic horizons. Moreover, we found that changes in fungal community composition were largely driven by differences in soil chemistry, which were far stronger across horizons (i.e., depth) within a single forest than across forest mycorrhizal types for the same horizon. Our results highlight the importance of considering soil vertical structure and associated changes in chemistry when characterizing soil fungal communities. They also suggest that, at least in northern forests, AM fungi are not being restricted where inorganic nutrients predominate and might have more similar edaphic vertical

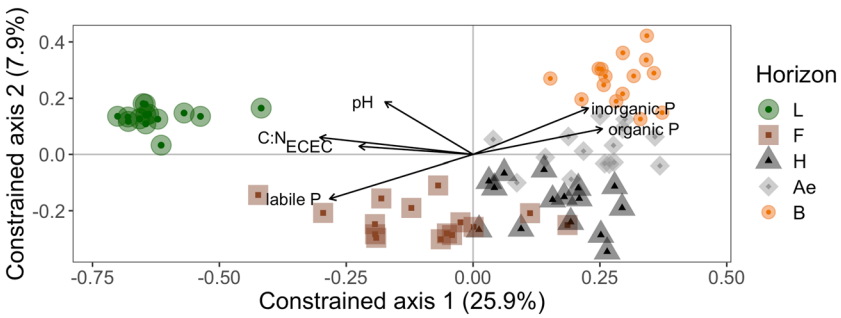

Fig. 4 Constrained ordination of the overall fungal community by soil chemistry variables using a distance-based redundancy analysis with Bray-Curtis dissimilarities. Horizons are shown in different shape and colors. The two first constrained axes explaining most variation are drawn. Adjusted-$R^2 = 23.3\%$, $P = 0.001$

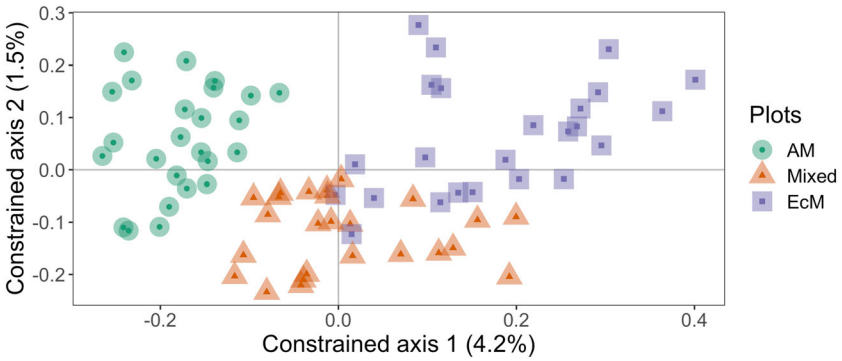

Fig. 5 Constrained ordination of the fungal community structure depending on the forest mycorrhizal type (AM, arbuscular mycorrhizal; EcM, ectomycorrhizal; Mixed, mixture of AM and EcM) using a distance-based redundancy analysis with Bray-Curtis dissimilarities. Forest types are shown in different shapes and colors. The two constrained axes are shown. Adjusted-R^2 = 2.7%, P value = 0.006

niches with EcM fungi than what has been suggested in the literature [31, 32, 35].

Fungal communities were strongly stratified with depth along the soil profile, being most distinct in the L horizon (composed of recently fallen leaves). Litter of the EcM, AM, and mixed forests had high fungal richness and distinct fungal communities that were dominated by saprotrophic fungi. This has also been observed in forests of tropical, temperate, and boreal biomes dominated by EcM trees [19, 21, 22, 92, 93]. Dominance by saprotrophic fungi in the most superficial litter layer has also been observed in other AM-dominated ecosystems [29, 94], as we have found in this northern temperate forest. Our results therefore provide further evidence of this general pattern whereby the L horizon possesses a distinct fungal community dominated by fungal saprotrophs, compared to deeper horizons in which mycorrhizal fungi are more abundant.

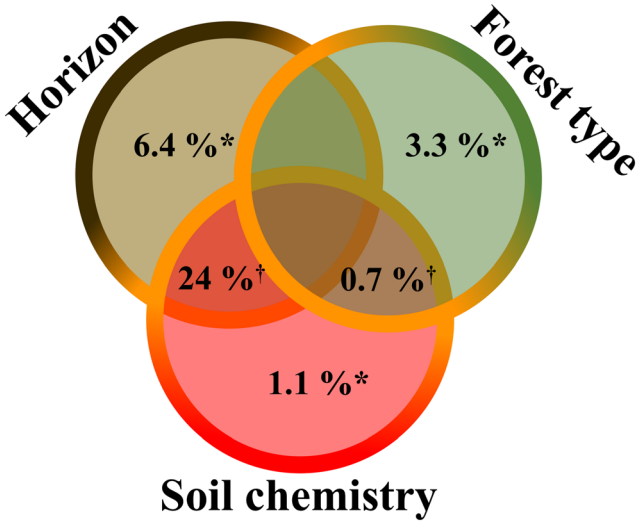

Fig. 6 Venn diagram displaying the amount of variation (i.e., adjusted-R^2) of the fungal community explained by horizon, soil chemistry, and forest mycorrhizal type or a combination of them. Values < 0.1% are not shown. Ellipses are not drawn to scale. Only variables with significant redundancy analysis (RDA) results were tested for partial-RDA and included in this diagram. Overall adjusted-R^2 = 34.8%, * indicates $P < 0.05$ and † indicates non-testable portion. For more details, see Table S3

As suggested by Bahram et al. [51], studies that have reported weak vertical segregation of fungal communities have often excluded the most superficial L horizon from their analyses [e.g., 16, 49]. The L horizon of the EcM, AM, and mixed forests tended to have higher C:N ratio, pH, concentration of cations, and labile P than deeper horizons. While this pattern seems generalizable for pH [e.g., 21, 93], it remains uncertain or unexplored for the other chemical variables. Our results suggest that the L horizon which is characterized by the presence of organic matter in which the original structures can be visually distinguished [62] should be considered separately in future studies of fungal community composition, given its chemical, microbial, and functional distinctiveness.

From the F to the B horizon, fungal communities showed strong turnover across soil horizons, with distinct fungal communities in each horizon. The fungal composition, abundance, and guilds tended to progressively change among horizons in the soil profile but these changes were less pronounced than with the L. This was also observed in other study systems [21, 93, 95]. There are reports of evenly distributed guilds among the organic and mineral horizons [e.g., 16], but vertical segregation of fungi and especially root-associated fungi is often strongly impacted by determinant factors such as soil chemistry and host plants [19, 20, 51]. In our study, there was major variation in the vertical distribution of soil fungi that was largely driven by soil chemical characteristics, with these changes being observed in all three forest mycorrhizal types. Our results further support those of other studies that have found the vertical variability of mycorrhizal and saprotrophic fungal communities across different soil horizons to be much larger than horizontal or temporal variability [51, 96]. Studies that focus on ecosystem topsoil processes in terrestrial environments should consider the strong physical, chemical, and biological heterogeneity that occurs within the first few centimeters, by sampling distinct soil horizons separately.

We showed that underground fungal community structure varied significantly between neighboring forest dominated by AM or EcM trees. As expected, AM forests showed higher abundance of AM fungi, whereas EcM forests showed higher abundance of EcM fungi. Direct observation of fungal colonization in roots confirmed these patterns. Forests with a mix of both strategies supported intermediate communities between the two extremes of the gradient, as reported in a study focusing on ecosystem processes [e.g., 97]. It is worth noting that fungal saprotrophs tended to be more abundant in organic horizons of mixed and AM forests compared to EcM forests. Together with higher pH and lower organic C in these AM forests, this result might indicate a tendency toward a more "inorganic nutrient economy" compared to the studied forests dominated by EcM fungi. The latter would represent a more "organic nutrient economy," associated with a slower turnover of plant-derived C due to lower abundance of free-living saprotrophs [10]. These small differences observed at local scale may be responsible for observed patterns found at the ecosystem scale [14]. It has been found elsewhere that forests dominated by different species of broadleaf trees of the same mycorrhizal strategy can also show differences in fungal community structure [98]. However, in our study, fungal composition, abundance, and guilds tended to differ between EcM and AM forests. Such a distinction has previously been reported in a study comparing very distinctive EcM forests of broadleaf trees vs. conifers [99]; the effect of mycorrhizal type was relatively small but nonetheless present, and could also be linked to differences in nutrient availability.

Our study design provides a useful system for exploring the relative importance of mycorrhizal type on soil biogeochemical cycling. The soil profile in these northern temperate forests have low vertical mixing, resulting in podzols with high stratification, as commonly encountered in boreal soils. Soil horizons were easily identifiable mainly through their color and such sampling may allow for better association between DNA sequences and soil chemistry as well as more valuable comparison across sites [100]. Variation in important factors such as parent material, topography, and regional climate were minimized but other factors (e.g., productivity, soil texture) could still co-vary with mycorrhizal dominance at the plot scale. Importantly, this study system allowed us to study different mycorrhizal types within the same site [7, 30, 51] and across a gradient of mycorrhizal dominance [15]. The observed differences in soil chemistry among forests could be linked with dominant mycorrhizal strategies. Higher saprotrophic fungal diversity has been observed in the upper soil layers of AM-dominated tropical forests compared to EcM forests [101]. Our study provides further evidence that, in a temperate system, host plants are an important factor controlling mycorrhizal community composition [51, 102]. To some extent, this was expected given that AM and EcM fungi are obligate symbionts with their host plants [32]. As such, considering tree mycorrhizal strategies and their interactions with saprotrophs may help to better predict carbon storage at small and global scale [8].

Our use of high-throughput amplicon sequencing approach allowed us to assess the distribution of the soil fungal community and to discriminate among AM, EcM, and saprotrophic fungi. However, result from high-throughput sequencing approaches need to be interpreted with caution because of unavoidable biases at different levels [43, 103]. For example, how to adequately normalize for taxa abundance among samples remains unresolved [104, 105]. Furthermore, although we acknowledge that soil and root compartments might host different fungal communities [e.g., 106], sampling bulk soil allows to capture the potential free-living saprotrophs as well as root-associated fungi and their extraradical hyphae. Finally, our choice of the primers might have resulted in an under-representation of some fungal groups such as Glomeromycetes, but comparisons in taxa abundance between samples remain relevant [76].Using specific primers targeting Glomeromycetes [107, 108] and plants using DNA from the root tissue [68, 109] would certainly allow to further understand the importance of these underground interactions and the vertical segregation among root and fungi of different mycorrhizal types.

Our results show that fungal communities in horizons vertically separated by a few centimeters are very different from each other in terms of composition and abundance. This contributes to high fungal and functional diversity in the topsoil. Moreover, our work suggests that the forest mycorrhizal type influences the overall and saprotrophic fungal community, advancing our current understanding of the potential impacts of mycorrhizal strategies on the distribution of key organisms for ecosystem functioning such as C and nutrient cycling [10]. We also reported for the first time that broad patterns of vertical fungal distribution across the upper five horizons in AM-dominated northern forest are comparable to neighboring EcM-dominated or mixed forests. This result challenges the traditional view that AM fungi have a more restricted niche toward mineral soils compared to EcM fungi due to their incapability to directly decompose organic matter [31]. Our study suggests that the ecological and functional roles of AM fungi in organic horizons of temperate forests, including recently deposited litter, deserve more attention [39].

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Code Availability Custom code for bioinformatical and statistical analyses are available at https://doi.org/10.5281/zenodo.3631982.

Authors' Contributions EL and AC conceived the ideas and designed methodology; AC, BT, SJ, and MB collected the data; AC analyzed the data; AC and EL interpreted the results; AC led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data Availability Sequence and chemistry data can be accessed at https://doi.org/10.5281/zenodo.3631861.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

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