



Predictors of taxonomic and functional composition of black spruce seedling ectomycorrhizal fungal communities along peatland drainage gradients

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Abstract

Many trees depend on symbiotic ectomycorrhizal fungi for nutrients in exchange for photosynthetically derived carbohydrates. Trees growing in peatlands, which cover 3% of the earth's terrestrial surface area yet hold approximately one-third of organic soil carbon stocks, may benefit from ectomycorrhizal fungi that can efficiently forage for nutrients and degrade organic matter using oxidative enzymes such as class II peroxidases. However, such traits may place a higher carbon cost on both the fungi and host tree. To investigate these trade-offs that might structure peatland ectomycorrhizal fungal communities, we sampled black spruce (*Picea mariana* (Mill.)) seedlings along 100-year-old peatland drainage gradients in Minnesota, USA, that had resulted in higher soil nitrogen and canopy density. Structural equation models revealed that the relative abundance of the dominant ectomycorrhizal fungal genus, *Cortinarius*, which is known for relatively high fungal biomass coupled with elevated class II peroxidase potential, was negatively linked to site fertility but more positively affected by recent host stem radial growth, suggesting carbon limitation. In contrast, *Cenococcum*, known for comparatively lower fungal biomass and less class II peroxidase potential, was negatively linked to host stem radial growth and unrelated to site fertility. Like *Cortinarius*, the estimated relative abundance of class II peroxidase genes in the ectomycorrhizal community was more related to host stem radial growth than site fertility. Our findings indicate a trade-off between symbiont foraging traits and associated carbon costs that consequently structure seedling ectomycorrhizal fungal communities in peatlands.

Keywords Ectomycorrhiza · Peatlands · Boreal · *Picea mariana* · *Cortinarius*

Introduction

Many tree species form symbiotic relationships with ectomycorrhizal (ECM) fungi that colonize the roots of their host plant, providing nutrients in exchange for photosynthetically derived carbohydrates. In contrast to saprotrophic fungi from which they evolved (Smith and Read 2008; Martino et al. 2018), ECM fungi are obligate symbionts that acquire

nearly all metabolic carbon from their hosts (Smith and Read 2008). Individual trees may host over a hundred ECM fungal species (Bruns 1995; Bahram et al. 2011), with the species mix determined by host specificity, environmental filters, and spatiotemporal factors that exert constraints on ECM communities colonizing a given host or site (Smith and Read 2008; Koide et al. 2011; Talbot et al. 2014; Alborno et al. 2016; Glassman et al. 2017).

Soil and litter quality can strongly influence ECM fungal community assembly (Guo et al. 2020; Delgado-Baquerizo et al. 2017). Low nutrient availability due to organic matter complexation and low soil pH typically favors ECM fungal communities that can hydrolyze or oxidize plant cell wall material and soil humics that are complexed with organic N (Lindahl and Tunlid 2015; Kyaschenko et al. 2017). Because ECM fungi rely on their host plant to provide carbon, they have lost many of the genes that produce enzymes for plant cell wall degradation, such as carbohydrate-active enzymes and lignin oxidoreductases (Martin et al. 2016;

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Martino et al. 2018). Several ECM lineages, however, have retained numerous enzymes from saprotrophic ancestors (Ryberg and Matheny 2012; Dickie et al. 2015; Martin et al. 2016; Martino et al. 2018). For example, some ECM fungi in Basidiomycota have genes coding for extracellular class II peroxidases such as manganese peroxidase, lignin peroxidase, and versatile peroxidase (Bödeker et al. 2009, 2014). In particular, *Cortinarius* species have class II peroxidase activity similar to some saprotrophic fungi (Bödeker et al. 2014), but the ascomycetous ECM species *Cenococcum geophilum* has very low class II peroxidase potential (Peter et al. 2016). Furthermore, many studies have found a negative correlation between *Cortinarius* species abundance and inorganic N availability (Lilleskov et al. 2011; Kyaschenko et al. 2017; Defrenne et al. 2019), suggesting that ECM species with the ability to produce class II peroxidases are favored in nutrient poor environments.

Owing to the dependence of mycorrhizal fungi on their host, ECM fungal community composition is partially driven by trade-offs between nutrient acquisition and C costs (Poznanovic et al. 2014; Fernandez et al. 2017; Rosinger et al. 2018; Defrenne et al. 2019). ECM fungal taxa are often classified by their exploration type based on the presence and length of emanating hyphae, which is useful for characterizing foraging strategy and fungal biomass (Agerer 2001), and could also affect the C cost of different fungal taxa for their host plant. Contact exploration types (e.g., *Russula* spp.) have smooth mantles with few to no emanating hyphae, while short-distance (e.g., *Cenococcum geophilum*), medium-distance (e.g., *Cortinarius* spp.), and long-distance (e.g., *Suillus* spp.) types have increasing hyphae and rhizomorph lengths that may aid in nutrient foraging by extending the host plant's zone of depletion (Agerer 2001). However, exploration types with greater rhizomorph biomass likely place a higher C demand on their hosts compared to exploration types with fewer rhizomorphs (Fernandez et al. 2017). Previous work has found that declining host photosynthetic rates in response to warming may favor short-distance exploration types over medium-distance types (Fernandez et al. 2017). Moreover, defoliation of host trees has been shown to reduce the relative abundance of medium- and long-distance exploration types (Parker et al. 2017), and other studies have demonstrated that shading decreases ECM fungal biomass (e.g., Hasselquist et al. 2016) or can shift ECM fungal communities to favor *Cenococcum* (Kummel and Lostro 2011). These works collectively suggest that host C limitations induced by canopy shading might have cascading effects on belowground fungal symbionts. Shifts in exploration types along gradients of nutrient and light availability may consequently provide valuable insight into how host trees resolve trade-offs between nutrient acquisition and C costs.

Drained peatlands present a unique opportunity to assess the effects of long-term changes in resources and

conditions on seedling ECM fungal communities along nutrient and light gradients. Globally, peatlands represent only 3–5% of terrestrial surface area yet hold approximately one-third of organic soil carbon stocks (Page et al. 2011). Northern peatlands in particular contain 500 ± 100 Gt C (Yu 2012) and continue to be vulnerable to C loss owing to climate and land use changes which often result in decreased water availability and lower water tables (Thomson et al. 2005). The water table of intact, open acidic bogs and poor fens (i.e., the least fertile sites) is often near the peat surface. Consequently, such peatlands are typically dominated by *Sphagnum* mosses, ericaceous shrubs, and nonmycorrhizal sedges (*Carex* spp.) and only sparsely colonized by trees (with sparse canopy cover) because high water tables prevent the establishment of adequate root systems (Laine et al. 1995). Moreover, low oxygen availability in peatlands with high water tables inhibits colonization of aerobic fungi, which need oxygen to survive, and is not optimum for the production of oxidative enzymes which often use oxygen as terminal electron acceptors (Philben et al. 2014; Wilson et al. 2021). However, a deeper oxic zone following drainage can support greater tree growth (Westman and Laiho 2003), and highly recalcitrant residual organic matter (e.g., *Sphagnum*) may prompt trees to form symbioses with ECM fungi that produce oxidative enzymes (Lindahl and Tunlid 2015; Kyaschenko et al. 2017). These sites (characterized by intermediate water table depth, fertility, basal area, and canopy cover) may have elevated oxygen availability that promotes more fungal growth and organic matter oxidation when compared to peatlands with high water tables (Philben et al. 2014; Leifeld et al. 2020). Sites with low water tables are likely to have high fertility, basal area, and canopy cover, because decades of higher decomposition rates can elevate nutrient availability and consequently reduce the need for trees to invest in ECM that have adapted costly traits for mobilizing organic nitrogen. In the shade of these denser forests, carbon supply to seedlings is constrained by light, which could also structure fungal communities towards taxa with lower carbon demand, as described above (Poznanovic et al. 2014; Hasselquist et al. 2016). Moreover, evidence suggests that longer-term peatland drainage reduces pH (Westman and Laiho 2003), which may favor ascomycetous ECM fungi that lack genes for oxidative enzyme (e.g., class II peroxidase) production (Sterkenburg et al. 2015). The combination of these factors could cause a decline in taxa with strong peroxidase potential under the most degraded conditions, leading to a unimodal response of these taxa over gradients of peatland degradation. While several studies have examined fungal community composition after short-term drainage (Lamit et al. 2017) or ECM fungal biomass after long-term drainage (Potila et al. 2009), we still lack a clear understanding

of the mechanisms structuring ECM fungal communities in ecosystems having undergone long-term changes in fertility, such as drained peatlands.

To determine the drivers of ECM fungal community composition following long-term changes in site fertility, we assessed seedling root-associated fungal communities along gradients within 100-year-old drained peatlands. We focused on seedlings because this allowed us to evaluate the combined effects of nutrient availability and carbon stress on fungal communities colonizing roots of plants in the same developmental stage. We predicted the following: (1) drainage would yield better growing conditions (i.e., lower water table and lower organic matter C/N) and consequently increase tree basal area; (2) tree seedling fungal community composition would shift along the gradient and be strongly linked to total nitrogen; and (3) fungi with known genetic capacity to produce class II peroxidases would have higher relative abundance on seedling roots at intermediate site fertility and basal area. To test these hypotheses, we sampled *Picea mariana* seedlings from three drained peatland gradients in northern Minnesota, USA, and compared root-associated fungal communities to above- and belowground site variables.

Materials and methods

Site description

In August 2018, we selected three oligotrophic peatlands near Meadowlands, MN, USA (N47.07278°, W92.73167°), separated by 5 to 15 km and adjacent to longstanding ditches dug between 1916 and 1918 (St. Louis County Public Works; Fig. S1). Mean annual (1981–2010) precipitation in nearby Cloquet, Minnesota (44 km away), is 808 mm, with a mean annual temperature of 4.8 °C. Aerial photography from 1940 (University of Minnesota Libraries) illustrates incipient tree invasion along ditch corridors that has persisted into the present (Fig. S1). Intact peatland plant communities in this area are primarily composed of *Carex oligosperma* Michx., *Chamaedaphne calyculata* (L.) Moench., *Kalmia polifolia* Wangenh., *Vaccinium oxycoccos* L., *Sphagnum* species, including *S. rubellum* Wilson, *S. magellanicum* Brid., *S. fuscum* (Schimp.) Klinggr., and *Polytrichum strictum* Brid. Minor components include *P. commune* Hedw., *Eriophorum vaginatum* L., *Andromeda polifolia* L. var. *glaucophylla* (Link) DC., *Rhododendron groenlandicum* (Oeder) Kron and Judd, and *Drosera rotundifolia* L. (Potvin et al. 2015). Ditch-adjacent peatlands selected for this study are typically dominated by a dense overstory of *Picea mariana* (Mill.) B.S.P. and *Larix laricina* (Du Roi) K. Koch, with an understory composed primarily of ericaceous shrubs described above (with a higher proportion of *R. groenlandicum*) and

a small *C. oligosperma* component. *Sphagnum* declines in abundance in the areas with densest tree canopies.

Sample collection

To examine long-term effects of peatland drainage on peat properties and mycorrhizal community composition, we established one transect at each of three sites, ranging from ~215 to 525 m long, and harvested one black spruce seedling (20–50 cm height) at ~20–45 m intervals ($n = 12$ seedlings $\times$ 3 transects = 36 total seedlings; Fig. S1). Interval length and transect direction were adjusted in some cases to traverse a full gradient from open- to forested peatlands, to avoid roads and upland outcrops, and to parallel ditches from moderately to highly degraded locations, the latter associated with ditch intersections (Fig. S1). Sampling was carried out over the course of 2 days (August 13–14, 2018). To capture an accurate assessment of seedling-level mycorrhizal community composition, we harvested the entire root system of each seedling. Roots were carefully traced, removed from surrounding substrate, and placed in Whirl-Pak® bags with stem attached. Many black spruce seedlings derive from layering of a nearby tree due to *Sphagnum* accumulation over lower branches (Stanek 1961). In such cases, the primary root was severed when it was traced aboveground or merged with the primary root of another layered seedling. We did not adequately note the layering status of harvested seedlings (we estimate about half were layered), but measured stem radial growth to infer carbon gain (described under “Site variables”).

From a similar microtopographical location (e.g., hummock or lawn) within 1 m of each harvested seedling, we cut and removed a 10 $\times$ 10 $\times$ 25-cm-deep peat block with a serrated knife. Seedlings and peat blocks were transported on ice and stored at 4 °C until processed within 2 and 7 days, respectively. The ground-layer vascular plant community was assessed by recording species presence/absence on the harvested 10 cm $\times$ 10 cm peat blocks, which only included species that were less than breast height. *Carex* (sedge), *Cypripedium* (ladyslipper), and *Drosera* (sundew) were recorded to genus level, and Poaceae (grass) was recorded to family level, owing to difficulty in differentiating species. All other vascular plants were identified to species level.

Site variables

Water table depth was measured in temporary wells made using a 6.35-cm-diameter open-faced gouge auger. Canopy openness above each seedling was estimated at breast height by averaging the cover of a convex spherical densiometer from each cardinal direction. A densiometer was used in lieu of hemispherical photography due to heterogeneous cloud cover. Basal area was estimated for each tree species above

breast height in a variable radius plot surrounding each seedling using a factor 1 wedge prism ($\text{m}^2 \text{ha}^{-1}$).

Sub-capitulum bulk density was calculated by measuring the dimensions of fresh peat blocks from the top 20 cm, then oven-drying at 55 °C for at least 48 h until a constant mass was reached. To measure carbon (C) and nitrogen (N) content, foliar tissue was also oven-dried at 55 °C to a constant mass. Dried foliar and peat material was ground using a ball mill, then weighed, and analyzed for total C, N, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ on a Costech Elemental Combustion System 4010 connected to a Thermo Finnigan ConfloIII Interface and Delta^{plus} Continuous Flow-Stable Isotope Ratio Mass Spectrometer. Stable isotope signatures were measured to infer C and N sources. Peat pH was measured using a pH meter on a 1:8 dilution of oven-dried ground, homogenized peat material to deionized water after resting for 30 min. Note that pH of oven-dried ground material is typically lower than fresh peat (Lierop and MacKenzie 1977), explaining our low pH values. Peat humification was measured using the von Post humification scale (von Post 1924). Dried peat material was re-saturated with water for 5 min, then tightly squeezed, and von Post humification score was recorded.

To quantify seedling age and stem radial growth, harvested seedling stems were air-dried and a ~1-cm-thick cross section was cut from the base of the stem using a fine blade and mounted on a wooden strip. The width of each annual growth ring was measured to the nearest 0.01 mm using a manually operated Velmex UniSlide Model TA4030H1-S6 with a linear encoder and Metronics Quick-Chek digital readout, paired with a Leica boom stereo microscope.

Fungal identification

Seedling roots were gently washed over a 1.2-mm sieve to remove soil and debris, then placed in 50-mL Falcon tubes with 95% ethanol, and stored at –20 °C. For each seedling, fine *Picea* roots were then separated from coarse- and non-*Picea* roots, cut into ~2-cm fragments, placed in a gridded petri dish filled with 95% ethanol, and mixed to homogenize. At 50 marked grid intersections, the nearest root tip was removed, blotted dry, and placed in a 1.5-mL PCR-clean microcentrifuge tube containing 400 µL Buffer AP1 from the Qiagen DNeasy Plant Mini Kit (Qiagen). Fungal DNA (from the 50 root tips per seedling) was then extracted according to the Qiagen protocol, followed by purification with the Qiagen Powerclean Pro Cleanup Kit. Following purification, extracted DNA was quantified on a Qubit 4 Fluorometer (Thermo Fisher Scientific, Waltham, USA). The ITS region was then amplified using the fungal-specific 5.8S-Fun and ITS4-Fun primer pair with (in 5' to 3' direction) a 24- to 29-bp Illumina sequencing adapter, 12-bp indexing barcode (5.8S-Fun only), 10-bp primer pad, 2-bp linker, and 21- to 27-bp core primer (5.8S-Fun: 5'-CAAGCAGAAGACGGC

ATACGAGAT-NNNNNNNNNN-AGTCAGTCAG-GG-AACTTTYRCAAYGGATCWCT-3' and ITS4-Fun: 5'-AATGATACGCGACCACCGACT ACAC-TATGGT AATT-AA-AGCCTCCGCTTA TTGATATGCTTAART-3') (Taylor et al. 2016). Polymerase chain reactions were conducted with an initial denaturation step at 95 °C for 3 min followed by 24 cycles × (95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s) and a final extension at 72 °C for 5 min. Each 25 µL reaction contained 12.5 µL 5 PRIME HotMasterMix (5 Prime, Inc. Gaithersburg, MD, USA), 0.5 µL 5.8S-Fun primer with Illumina overhang adapter (10 µM), 0.5 µL ITS4-Fun primer with Illumina overhang adapter (10 µM), 6.5 µL PCR-grade H₂O, and 5.0 µL DNA template. Multiplexed libraries were quantified on an Illumina Eco qPCR and then sequenced with a 2 × 250-bp paired-end chemistry on an Illumina MiSeq platform (Illumina, Inc., USA) spiked with PhiX control oligonucleotides for base diversity (Taylor et al. 2016). Template-free negative controls were included in all steps (i.e., extraction, amplification, and sequencing). Raw sequences are deposited at the NCBI Sequence Read Archive (BioProject PRJNA653874).

FastQ files were demultiplexed with ea-utils (Aronesty 2011) and imported into QIIME v2 (Bolyen et al. 2019). Primers were removed with Cutadapt (Martin 2011), and ITS2 sequences extracted with ITSxpress and HMMer with a quality filter threshold of $q = 20$ (Rivers et al. 2018; www.hmmer.org). We then used the DADA2 plugin on paired-end reads to denoise reads, remove chimeras, and merge reads into amplicon sequence variants (ASVs) (Callahan et al. 2016). Taxonomy was assigned using a trained Naïve-Bayesian classifier with the UNITE v8 dynamic database (97–99% sequence similarity; Nilsson et al. 2019). Genus-level (or higher order if not identified to genus) ASV feature tables were converted into relative abundance per sample for all statistical analyses. ASVs not identified to Kingdom Fungi were removed from further analyses.

Ecological guilds were assigned using the FUNGuild database (Nguyen et al. 2016). Ectomycorrhizal exploration types were assigned using FUNGuild, Agerer (2001) and the DEEMY database (www.deemy.de), supplemented by literature characterization for *Piloderma sphaerosporum*, which deviates from other *Piloderma* species in exploration type (Clemmensen et al. 2015; Hagenbo et al. 2018, Supplementary Table S1). To assess the relative abundance of class II peroxidase gene copies within the sampled ECM community, we calculated the average number of Auxiliary Activity Family 2 (AA2, which contains class II peroxidases) genes for ECM genera in our dataset using published values (Supplementary Data). Genera without published genomes were excluded. For the purposes of this study, we assumed that genus-level copy number was representative of the members of the genus present in our study, recognizing that this will not always be the case but is our best estimate given the

breadth of genome sequencing. Fourteen of the 25 ECM genera have published genome data for at least one species in the genus (Table S1), representing 90.5% of the read abundance in this study. We then multiplied the mean AA2 value per genus by the relative abundance of the respective genus in each sample as an approximate indicator of class II peroxidase gene potential within the ECM community.

Statistical analyses

To test our first hypothesis, the relationships among site variables were assessed by calculating Pearson correlation coefficients among all measured site variables ($n = 36$). Total stand basal area and canopy openness had strong covariance but are both ecologically important when assessing plant and fungal communities. We consequently collapsed them into a single composite variable (“canopy density”) by using ordination scores from the first axis of a principle component analysis (PCA, $n = 36$), which explained 95% of variation of the two variables. Due to multicollinearity of many other variables, we selected seedling stem radial growth (average of three years preceding collection), peat pH, and peat % N as predictor variables in all fungal community analyses based on our hypotheses and ecological relevance (e.g., Lilleskov et al. 2002; Poznanovic et al. 2014; Glassman et al. 2017).

To test our second hypothesis, the relationship among canopy density, peat pH, peat % N, and plant communities was assessed using distance-based redundancy analyses (dbRDA). Three statistical outliers were removed (final $n = 33$). We then tested the relationship among seedling stem radial growth, peat pH, peat % N, and fungal communities using dbRDA. We first tested the relationship among site variables and the composition of the total fungal community (two statistical outliers removed, final $n = 34$) and then focused specifically on the composition of the ectomycorrhizal fungal community (two statistical outliers removed, final $n = 34$), root-associated saprotrophic fungal community ($n = 36$), and ectomycorrhizal (ECM) exploration types (one statistical outlier removed, final $n = 35$). Analyses were conducted using the *capscale* function from the “vegan” package in R 3.6.2 software (Oksanen et al. 2018; Team 2018), with Bray–Curtis distance measures.

To test our third hypothesis, we first assessed the relationships among ECM functional groups and site variables (seedling stem radial growth, peat pH, and peat % N) using multiple regression. For functional groups, we selected medium-distance fringe exploration type ($n = 36$), short-distance exploration type ($n = 36$), and relative abundance of class II peroxidases genes ($n = 36$). Medium distance fringe and short-distance exploration types were the two most frequently occurring exploration types. We also explored the use of mixed models to account for a random site effect, but this rarely improved model fit so we proceeded with

regular multiple regressions. We then tested the relationships between the three most frequent fungal taxa with seedling stem radial growth, peat pH, and peat % N using multiple regression ($n = 36$). In addition to linear regression, we tested the use of log scale and second-order polynomial regression and selected the best fitting regression based on AIC and adjusted R^2 values. All regressions were conducted in R 3.6.2 software, and all regression figures were created using the “ggplot2” package in R software (Wickham 2016). The relationships between seedling stem radial growth, pH, peat % N, and the relative abundance of *Mycena* (a genus known for high class II peroxidase production; Kellner et al. 2014) and *Lactarius* (a short-distance exploration type ECM genus) were tested with Spearman’s rank correlation due to low frequency (i.e., violation of regression assumptions).

To further examine how lowered water table influences the mechanisms which may structure the relative abundance of root-associated fungi, we utilized path analytic (i.e., structural equation modeling) techniques. The theoretical framework guiding our path analysis was based on our hypotheses, along with previous studies (Fig. S2). Original predictor variables included water table depth, canopy density, seedling stem radial growth, peat pH, and peat % N (Fig. S2). All predictor variables were standardized prior to analysis (Rosseeel 2012). Path analyses were conducted using the *sem* function in the “lavaan” package of R software (Rosseeel 2012). Final path analyses were determined using fit indices.

Results

Site variables

Transects captured a drainage gradient from interior (i.e., undrained) to ditch (i.e., drained); water table depth was correlated with all measured site variables except pH (Table 1; Table S2; Table S3; Fig. 1; Fig. S3). Specifically, water table depth was positively correlated with total basal

Table 1 Pearson correlation coefficients among site variables in plots along drained peatland gradients near Meadowlands, MN, USA ($n = 36$). Sub-capitulum peat tissue was harvested from the top 20 cm. Bold values denote significant correlation ($p < 0.05$); italic values denote marginal significance ($0.05 < p < 0.10$). Water table depth is included as a positive value. N, nitrogen

	Water table depth	Canopy density	pH	Peat % N
Canopy density	0.65			
pH	−0.03	−0.11		
Peat % N	0.63	0.68	0.15	
Seedling stem radial growth	−0.19	−0.48	<i>0.31</i>	−0.14

area ($r=0.61$, $p=0.0001$; Table S3; Fig. S3), bulk density ($r=0.78$, $p<0.0001$; Table S3; Fig. S3), and the von Post score ($r=0.60$, $p=0.0002$; Table S3; Fig. S3), which all increased from interior to ditch (Table S2; Fig. S3). Consequently, water table depth was negatively correlated with canopy openness ($r=-0.66$, $p<0.0001$; Table S3; Fig. S3) which ranged from 99.8 (± 0.0)% in the interior to $15.9 \pm 5.4\%$ near the ditch (Table S2; Fig. S3). Peat % N was positively correlated with water table depth ($r=0.63$, $p<0.0001$; Table 1; Fig. 1). Consequently, peat C/N declined along the gradient, ranging from 60.4 (± 7.5) in the interior to 34.1 (± 6.9) near the ditch (Table S2; Table S3; Fig. S3) and was positively correlated with foliar $\delta^{13}\text{C}$ ($r=0.45$, $p=0.006$; Table S3). In contrast, peat C/N was negatively correlated with peat $\delta^{13}\text{C}$ ($r=-0.74$, $p<0.0001$; Table S3), peat $\delta^{15}\text{N}$ ($r=-0.75$, $p<0.0001$; Table S3), and foliar $\delta^{15}\text{N}$ ($r=-0.636$, $p<0.0001$; Table S3). Peat pH was not correlated with any site variable and remained unchanged from interior to ditch (Table 1; Fig. 1).

Canopy density (i.e., basal area–canopy openness composite variable) was positively correlated with water table depth ($r=0.65$, $p<0.0001$; Table 1; Fig. 1). Basal area of *Picea mariana* increased from interior to ditch-adjacent plots, ranging from 0 to $31 \text{ m}^2 \text{ ha}^{-1}$ and comprising more than 75% of stand basal area in 24 of 36 plots (Fig. S4). Less abundant and frequent tree species included *Larix laricina* ($0\text{--}14 \text{ m}^2 \text{ ha}^{-1}$), *Betula papyrifera* ($0\text{--}5 \text{ m}^2 \text{ ha}^{-1}$), and *Pinus strobus* ($0\text{--}1 \text{ m}^2 \text{ ha}^{-1}$).

Water table depth did not directly affect seedling age ($r^2=-0.03$, $p=0.94$), which ranged from 5 to 21 years (Table S2), or recent stem radial growth (i.e., mean stem radial growth of three years preceding collection; Table 1;

Fig. 1). However, recent seedling stem radial growth was negatively correlated with seedling age (Table S3). Recent seedling stem radial growth was also negatively related to canopy density ($r^2=0.30$, $t=-3.988$, $p=0.0003$), suggesting that increasing canopy density (induced by a lowered water table) was negatively linked to seedling carbon gain. We did not adequately note the layering status of harvested seedlings, but recent stem radial growth had no relation to layering status of seedling that were adequately noted ($r^2=-0.04$, $p=0.585$, $n=19$).

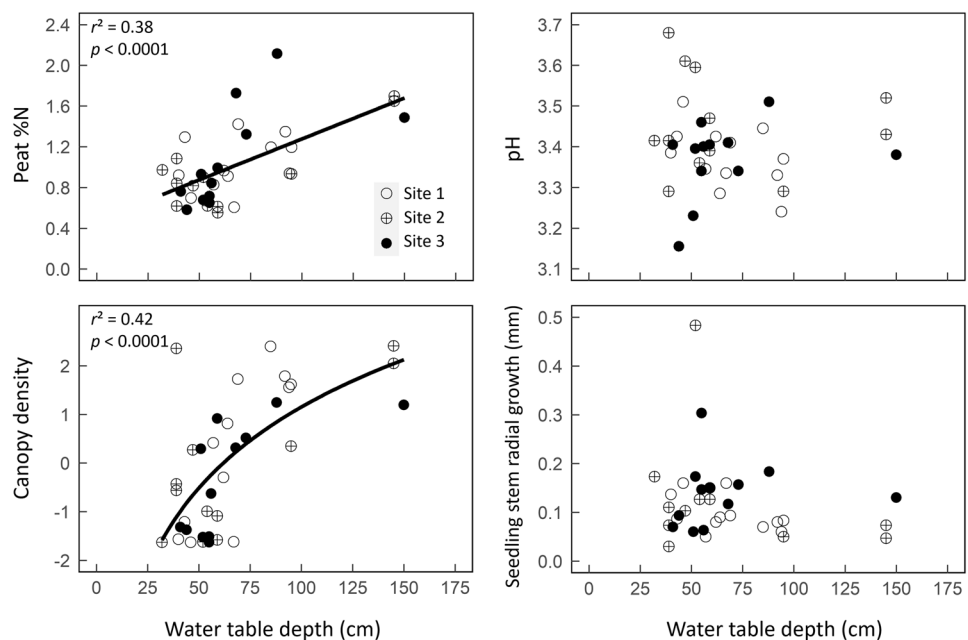
Ground-layer vascular plant community

Canopy density (i.e., basal area–canopy openness composite variable) was the strongest predictor of the vascular plant community in $10 \times 10\text{-cm}$ plots ($F_{1,27}=3.340$, $p=0.001$; Table S4; Fig. S5). Ordinations indicate that canopy density was negatively correlated with the presence of *Vaccinium oxycoccos* and *Chamaedaphne calyculata*, but positively correlated with the presence of *Rhododendron groenlandicum* and *Gaultheria hispida* (Fig. S5).

Root-associated fungal community composition

Sequencing yielded an average of $8222 (\pm 319.6)$ reads per sample ($n=36$), ranging from 4707 to 13,135, composed of 490 amplicon sequence variants (ASVs) and 142 genera (including unidentified and unassigned genera) following quality filtering (Supplementary Data). The five most abundant genera accounted for 86.3% of reads and were all ectomycorrhizal: *Cortinarius* ($40.4 \pm 8.7\%$ of reads per sample), *Piloderma* ($26.7 \pm 8.1\%$ reads per sample, which were all

Fig. 1 Relationship between site variables and water table depth among plots along drained *Picea mariana* — dominated peatland gradients near Meadowlands, MN, USA. Peat tissue was harvested from the top 20 cm. Canopy density represents the first axis of a PCA (accounting for 95% of variation) using basal area and canopy openness



P. sphaerosporum), *Cenococcum* ($12.9 \pm 5.7\%$ of reads per sample), *Hebeloma* ($3.0 \pm 4.5\%$ of reads per sample), and *Lactarius* ($2.8 \pm 4.1\%$ of reads per sample). Four of the five most frequently occurring genera were at least potentially ectomycorrhizal: *Cortinarius* (ectomycorrhizal, 36 of 36 samples), *Cenococcum* (ectomycorrhizal, 34 of 36 samples), *Piloderma* (ectomycorrhizal, 32 of 36 samples), *Mortierella* (saprotrophic, 24 of 36 samples), and *Mycena* (potentially ectomycorrhizal, 23 of 36 samples). *Mycena* has both free-living saprotrophic and recently identified ectomycorrhizal roles (Thoen et al. 2020).

Seedling stem radial growth was the strongest predictor of the root-associated fungal community ($F_{3,30} = 6.139$, $p = 0.001$; Table 2a; Fig. 2a). When classified by ecological guild, mycorrhizal (ecto- and ericoid) fungal composition was also strongly influenced by seedling stem radial growth, as indicated by distance-based redundancy analysis ($F_{3,30} = 6.356$, $p = 0.001$; Table 2b; Fig. 2b) and path analysis ($z = 5.050$, $p < 0.001$; Table S5; Fig. 3) but unrelated to pH

or peat % N. Root-associated saprotrophic fungal composition was marginally related to seedling stem radial growth (Table 2c; Fig. 2c). However, the average relative abundance of root-associated saprotrophs per sample ($2.3 \pm 0.8\%$, $n = 36$) was substantially less than that of mycorrhizal fungi ($93.9 \pm 1.2\%$, $n = 36$). The composition of ectomycorrhizal exploration types was also related to seedling stem radial growth ($F_{3,30} = 6.144$, $p = 0.001$; Table 2d; Fig. 2d), but unrelated to pH or peat % N.

ECM functional and taxonomic responses

Medium distance fringe exploration types, which include *Cortinarius* and *Tricholoma*, were positively related to seedling stem radial growth, but negatively related to peat % N, as indicated by multiple regression (Table S6a; Fig. 4) and path analysis (Table S5; Fig. 3). Medium distance fringe types were also negatively related to pH, as indicated by multiple regression (Table S6a; Fig. 4), but pH was removed from path analysis due to poor fit. Short distance exploration types, which include *Cenococcum*, *Hebeloma*, and *Elaphomyces*, were somewhat negatively related to seedling stem radial growth but somewhat positively related to peat % N, as indicated by multiple regression (Table S6a; Fig. 4), while path analysis indicated a significant effect of both factors (Table S5; Fig. 3). Short distance types were unrelated to pH. The relative abundance of class II peroxidase gene copies in ECM taxa was positively related to seedling stem radial growth and negatively related to peat % N, as indicated by multiple regression (Table S6b; Fig. 4) and path analysis (Table S5; Fig. 3). However, stem radial growth had the strongest effect in both analyses. Multiple regression found a negative relationship between class II peroxidase gene copies and pH (Table S6b; Fig. 4), but pH was removed from the path analysis due to poor fit.

Shifts in fungal community composition from open interior to forested ditch margins were driven by a threefold decline in the relative abundance of *Cortinarius*, which was by far the most abundant genus. Multiple regression (Table S7; Fig. 5) and path analysis (Table S5; Fig. 3) both indicated that *Cortinarius* was positively related to seedling stem radial growth and negatively related to peat % N, but the effect of stem radial growth was stronger than that of peat % N. Multiple regression also indicated that *Cortinarius* was negatively related to pH (Table S7; Fig. 5). However, pH was removed from path analysis due to poor fit. *Piloderma*, the second most abundant genus, was negatively related to seedling stem radial growth, as indicated by multiple regression (Table S7; Fig. 5) and path analysis (Table S5; Fig. 3). Multiple regression also indicated that *Piloderma* was marginally negatively related to peat % N (Table S7; Fig. 5), but path analysis did not indicate a relationship. *Cenococcum*, the third most abundant genus, was negatively related to

Table 2 Distance-based redundancy analysis (dbRDA) of the relationship between site properties along drained peatland gradients near Meadowlands, MN, USA, and the composition of *P. mariana* seedling (a) total root-associated fungal community, (b) mycorrhizal fungal community, (c) root-associated saprotroph community, and (d) ectomycorrhizal (ECM) exploration types, using Bray–Curtis dissimilarity. Seedling stem radial growth represents the mean stem radial growth of 3 years preceding collection. Exploration types were classified using FUNGuild (Nguyen et al. 2016), Agerer (2001), Clemmensen et al. (2015), and Hagenbo et al. (2018). Bold values denote significance ($p < 0.05$); italicized values denote marginal significance ($0.05 < p < 0.10$). N, nitrogen; DF, degrees of freedom

Predictor	DF	F-value	p value
a) Total fungal community ($n = 34$)			
Seedling stem radial growth	1	6.139	0.001
pH	1	1.273	0.216
Peat % N	1	0.929	0.442
Residuals	30		
b) Mycorrhizal fungal community ($n = 34$)			
Seedling stem radial growth	1	6.356	0.001
pH	1	1.271	0.177
Peat % N	1	0.860	0.499
Residuals	30	6.276	
c) Root-associated saprotroph community ($n = 36$)			
Seedling stem radial growth	1	1.566	0.051
pH	1	0.907	0.572
Peat % N	1	0.953	0.478
Residuals	32	11.359	
d) Ectomycorrhizal (ECM) exploration types ($n = 34$)			
Seedling stem radial growth	1	6.144	0.001
pH	1	1.290	0.180
Peat % N	1	1.144	0.254
Residuals	31	7.273	

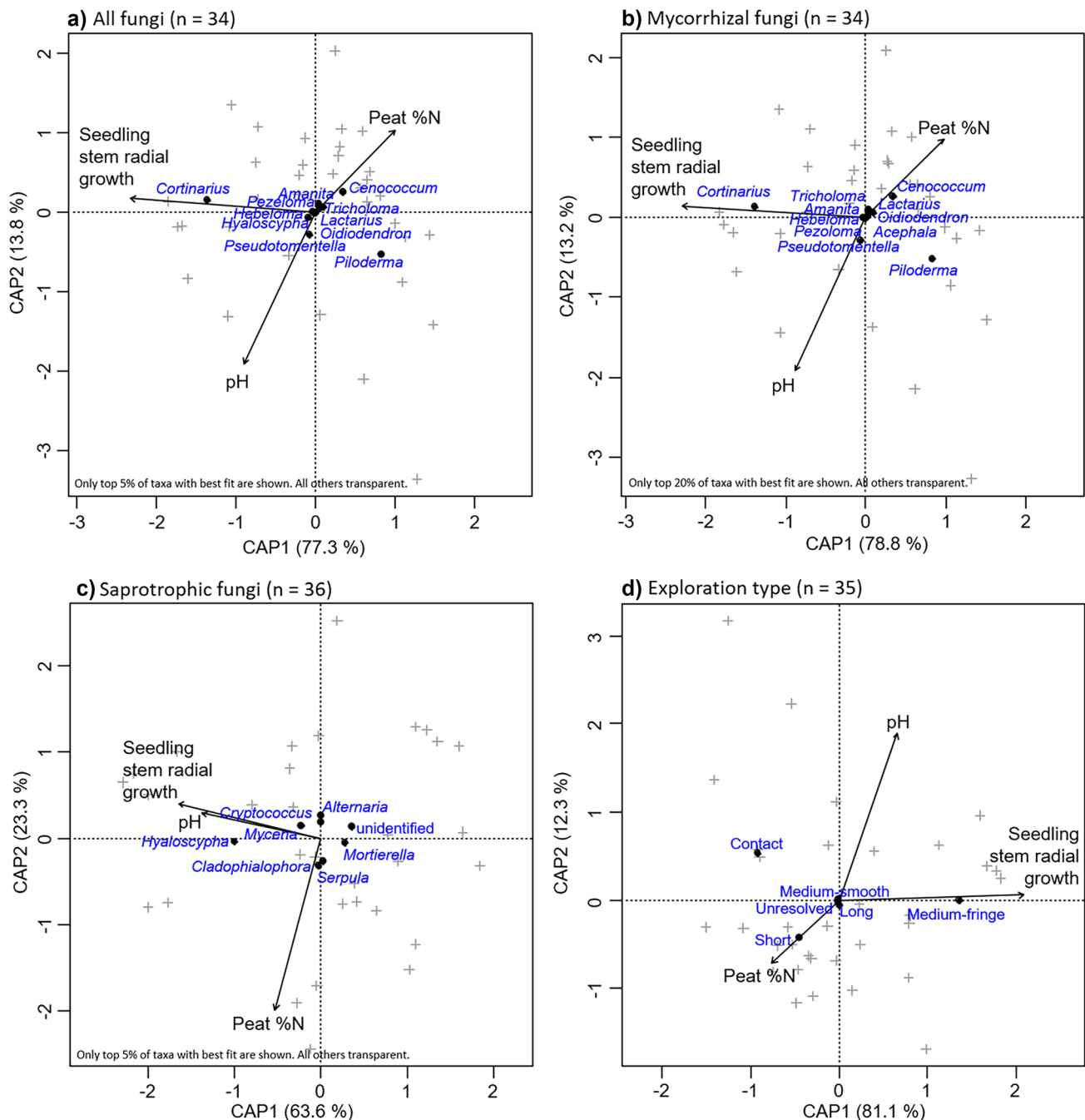


Fig. 2 Canonical analysis of principal coordinates of fungal communities on root tips of *P. mariana* seedlings collected from drained peatland gradients near Meadowlands, MN, USA. Ordinations depict (a) all fungal genera ($n=34$, only top 5% of taxa with best fit are shown, all others transparent), (b) fungal genera classified as mycorrhizal ($n=34$, only top 20% of taxa with best fit are shown, all others

transparent), (c) fungal genera classified as saprotrophic ($n=36$, only top 5% of taxa with best fit are shown, all others transparent), and (d) ectomycorrhizal (ECM) exploration types ($n=35$) derived from FUNGuild (Nguyen et al. 2016), Agerer (2001), Clemmensen et al. (2015), and Hagenbo et al. (2018)

seedling stem radial growth, as indicated by multiple regression (Table S7; Fig. 5) and path analysis (Table S5; Fig. 3). *Cenococcum* was unrelated to pH or peat % N. The relative abundance of *Mycena*, a genus known for high class II peroxidase production (Kellner et al. 2014), ranged from only 0

to 5% of sequence reads per sample but was positively correlated with peat % N, based on Spearman's rank correlation ($\rho=0.36$, $p=0.029$). The relative abundance of *Lactarius*, a short-distance exploration type ECM, ranged from 0 to 26.3% of sequence reads per sample and was also marginally

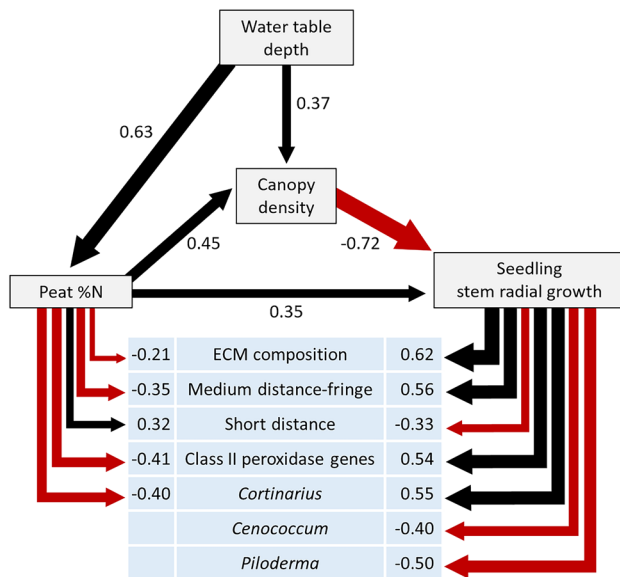


Fig. 3 Results of path analyses for ECM community composition (represented by the first PCA axis of mycorrhizal genera, accounting for 61.3% of variance), medium-distance fringe exploration type, short-distance exploration type, relative abundance of class II peroxidase gene copies (calculated by multiplying the relative abundance of a given genus with the average number of published gene copies for that genus; see Supplementary Data), *Cortinarius*-relative abundance, *Cenococcum geophilum*-relative abundance, and *Piloderma sphaerosporum* relative abundance, collected from fine roots of *P. mariana* seedlings along drained peatland gradients near Meadowlands, MN, USA ($n=36$). Only significant ($p < 0.05$) paths were retained. Values indicate standardized path coefficients, and arrow width is proportional to the standardized path coefficient; black arrows indicate positive coefficients while red arrows indicate negative coefficients

positively correlated with peat % N, based on Spearman's rank correlation ($\rho = 0.29$, $p = 0.081$). Both *Mycena* and *Lactarius* were unrelated to seedling stem radial growth or pH.

Discussion

One century of ditching altered the peatland properties along a water table gradient that were paralleled by shifts in the root-associated fungal community of black spruce seedlings. We predicted that site fertility (i.e., peat % N) would be the best predictor of ectomycorrhizal (ECM) fungal community shifts, but instead found that the ECM community was more strongly linked to host seedling stem radial growth, which was negatively related to canopy density. The relative abundance of the dominant genus in our gradient, *Cortinarius*, known for relatively high fungal biomass coupled with elevated class II peroxidase potential, declined in tandem with seedling stem radial growth. These findings suggest that host seedlings may struggle to subsidize costly ECM symbionts in low light environments, despite the potential for common

mycorrhizal networks with canopy species or layering from nearby trees. Ultimately, we surmise that the assembly of seedling ECM communities along peatland drainage gradients is driven by trade-offs between ECM foraging ability and potential carbon costs.

Drainage effects on site variables

Supporting our first hypothesis, lower water tables induced by drainage promoted greater decomposition of organic matter, as evidenced by declining peat C/N with simultaneous increases in the von Post humification scale, bulk density, peat % nitrogen (N), and $\delta^{15}\text{N}$ values. Increasing peat % N from interior to ditch-adjacent plots indicates that ditching improved site fertility over a 100-year timescale. Similarly, increasing peat $\delta^{15}\text{N}$ indicates greater microbial fractionation of nitrogen sources, and parallel increases in foliar $\delta^{15}\text{N}$ confirm that seedlings are accessing more mineralized N and perhaps less reliant on ECM for N (Garten 1993; Hobbie and Högberg 2012). These findings support earlier work demonstrating that an experimentally lowered water table allows decomposition of previously inaccessible organic matter (Wiedermann et al. 2017). On the other hand, some laboratory studies have suggested that altered environmental conditions may sometimes inhibit decomposition following peatland drainage (Ivarson 1977; Bergman et al. 1999; Toberman et al. 2010). Specifically, lower peat surface temperatures under high canopy cover, coupled with lower pH due to declining groundwater flow through high bulk density soil, may potentially attenuate an increase in decomposition (Ivarson 1977; Bergman et al. 1999). In the present study, however, consistently increasing peat and foliar $\delta^{15}\text{N}$ along the gradient provides evidence of greater net decomposition with increased drainage, even one century after initial ditching, whereas we found no evidence of pH decline with ditching.

Shifts in root-associated fungal communities

In contrast to our second hypothesis, both mycorrhizal and total fungal communities were best predicted by seedling stem radial growth rather than site fertility. As basal area increased along the gradient due to lower water tables and higher peat N (Watt and Heinselman 1965), seedling-layer light availability subsequently declined. Because light-sufficient trees partition up to 22% of net primary production to mycorrhizal symbionts (Hobbie 2006), there is a potentially large pool of carbohydrates to support ECM with high C demand in open sites. In contrast, host carbon assimilation rates in shaded environments can have a large impact on belowground C flux and non-structural carbohydrate pools (e.g., Weber et al. 2018), likely influencing seedling mycorrhizal communities in the shaded conditions

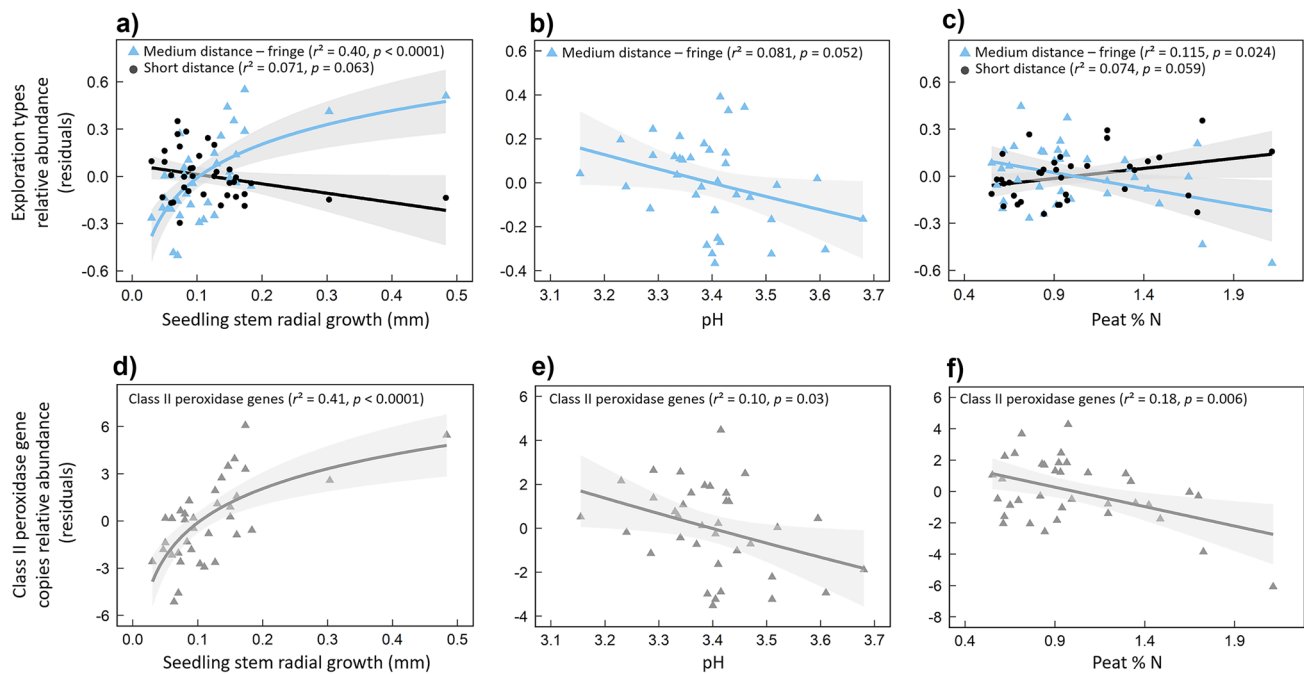


Fig. 4 The relative abundance (residuals of multiple regression) of (a) medium-distance fringe and short-distance ectomycorrhizal exploration types as a function of seedling stem radial growth; (b) medium-distance fringe exploration types as a function of pH; (c) medium-distance fringe and short-distance exploration types as a function of peat % N; (d) class II peroxidase gene copies in the ectomycorrhizal community (calculated by multiplying the relative abundance of a given genus with the average number of published gene copies found for that genus; see

Supplementary Data) as a function of seedling stem radial growth; (e) class II peroxidase gene copies as a function of pH; (f) class II peroxidase gene copies as a function of peat % N. *Picea mariana* seedlings were collected from drained peatlands near Meadowlands, MN, USA ($n=36$ samples). Exploration types were classified using FUNGuild (Nguyen et al. 2016), Agerer (2001), Clemmensen et al. (2015), and Hagenbo et al. (2018)

near ditches. Shaded seedlings may consequently be dominated by fungal symbionts with low C demands as found by Poznanovic et al. (2014), or have less fungal tissue colonizing fine roots (Hasselquist et al. 2016).

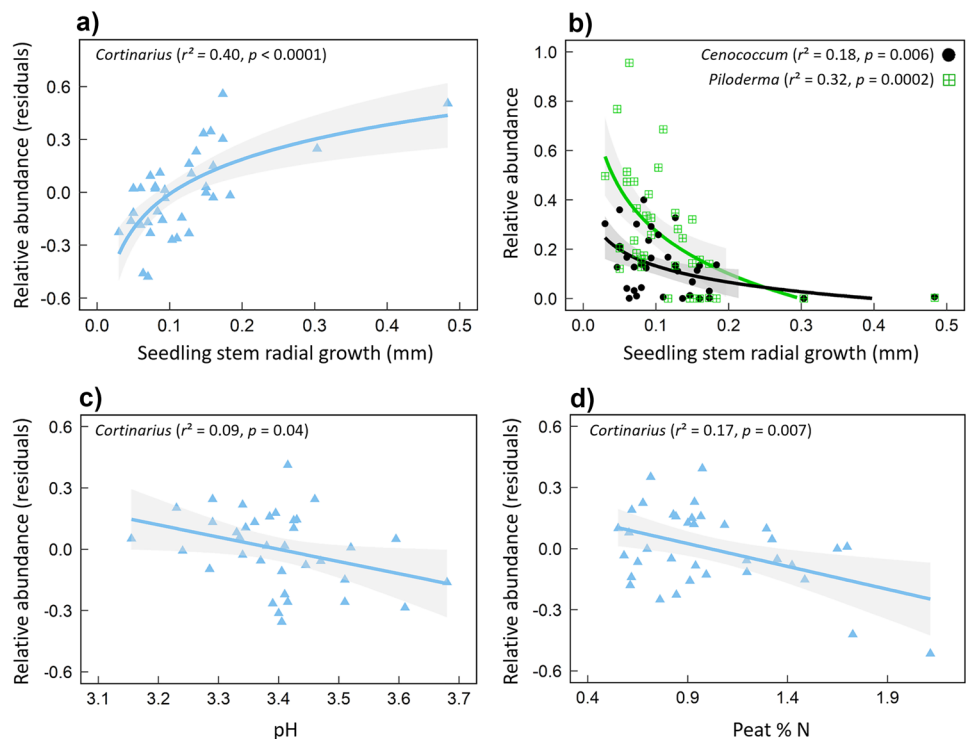
In the absence of a basal area or light gradient, site fertility is well known to structure root-associated fungal communities (e.g., Lilleskov et al. 2011; Sterkenburg et al. 2015; Glassman et al. 2017). The gradient in our study, however, suggests that root-associated fungal communities on seedlings in drained peatlands are at least as strongly linked to host stem radial growth as site fertility, pointing to the importance of host resources in constraining ECM fungal communities. It also is possible that the effects of low light and increased peat N could be additive in affecting C available to seedlings, because belowground C allocation can decline under more nutrient-sufficient conditions, potentially structuring ECM communities (Lilleskov et al. 2019). Lastly, we found that host stem radial stem growth was negatively correlated with seedling age, meaning that fast-growing seedlings (in the three years preceding collection) tended to be younger than slow-growing seedlings, which may have contributed toward ECM community assembly.

Trends of ECM functional and taxonomic groups

Our third hypothesis was partially supported; we did not observe the predicted unimodal response along the gradient, but the observed shifts in ECM exploration types and taxa with class II peroxidase potential suggest trade-offs between nutrient acquisition and C costs. Medium distance fringe types (e.g., *Cortinarius* and *Tricholoma*) were positively linked to host stem radial growth and negatively linked to site fertility, while short-distance types (e.g., *Cenococcum geophilum*, *Hebeloma*, and *Elaphomyces*) tended to have the opposite (though non-significant) response. This finding supports the hypothesis that medium-distance exploration types, which may be more effective nutrient foragers but have relatively more biomass, are not favored by either host selection or in competition with other fungi when C costs outweigh mineral nutrient compensation, such as when site fertility is high and/or light availability is low (Lilleskov et al. 2011, 2019; Poznanovic et al. 2014; Fernandez et al. 2017; Rosinger et al. 2018; Defrenne et al. 2019).

Similar to our findings, previous work has found that short and medium-distance types have contrasting responses to site fertility, with some exceptions. Sterkenburg et al.

Fig. 5 (a) The relative abundance (residuals of multiple regression) of *Cortinarius* spp. as a function of host seedling stem radial growth; (b) the relative abundance (proportion) of *Cenococcum geophilum* and *Piloderma sphaerosporum* as a function of host seedling stem radial growth; (c) the relative abundance (residuals of multiple regression) of *Cortinarius* spp. as a function of pH; and (d) the relative abundance (residuals of multiple regression) as a function of peat % N, on fine roots of *P. mariana* seedlings from drained peatland gradients near Meadowlands, MN, USA ($n = 36$)



(2015) found an increase of short-distance types in fertile *Picea abies* forests of central Sweden, and Lilleskov et al. (2011) found declining medium-distance fringe types in high N environments. However, low N availability may not always favor medium-distance exploration types if C supply is limited. Despite extremely low nutrient availability, Poznanovic et al. (2014) found that the ECM community on shaded seedlings was dominated by short-distance types typically found in high N environments, suggesting that C costs of medium-distance exploration types may constrain their ability to colonize roots of C-stressed hosts, whether that stress is caused by shading or high N availability. Moreover, Karst et al. (2021) found that 13 years of N fertilization doubled the abundance of high-biomass exploration types on boreal *Populus tremuloides* roots (but did not affect *Cortinarius* abundance), again suggesting that N availability does not always predict the composition of presumed ECM fungal traits.

In addition to biomass production, class II peroxidase production represents an ostensibly high C cost associated with some medium-distance fringe types (Stuart and Plett 2020; Zheng et al. 2020). In our study, the relative abundance of class II peroxidase gene copies in the ECM community was positively related to host stem radial growth and negatively related to site fertility. The C cost of class II peroxidase production coupled with biomass production may account for the threefold decline in *Cortinarius*, a medium-distance fringe type that has among the highest number of class II peroxidase gene copies of ECM taxa in this study

(Chen et al. 2001; Bödeker et al. 2009, 2014; Grigoriev et al. 2014; Kohler et al. 2015). We predicted that site fertility would drive *Cortinarius* to respond unimodally to the gradient because intermediate water table depths would increase oxygen availability and consequently promote decomposition of recalcitrant *Sphagnum* tissue by ECM species possessing class II peroxidases, such as *Cortinarius*. Instead, our path analysis suggests that *Cortinarius* relative abundance was most strongly linked to host stem radial growth, rather than peat % N. Regardless, these findings indicate that *Cortinarius* relative abundance is simultaneously linked to aboveground and belowground factors. For example, Parker et al. (2017) found lower *Cortinarius* abundance in defoliated birch stands when compared to non-defoliated stands, suggesting a host C limitation, while Lilleskov et al. (2001, 2002) found that *Cortinarius* abundance declined with increasing N deposition in Alaska, suggesting a negative effect of soil N availability.

In contrast to *Cortinarius*, *Piloderma sphaerosporum* (the only *Piloderma* species found in this study) and *Cenococcum geophilum* were both negatively related to host stem radial growth and unrelated to peat % N. Sequenced *Piloderma* and *Cenococcum* genomes have fewer class II peroxidase gene copies, and hence putatively less class II peroxidase activity than *Cortinarius* species (Chen et al. 2001; Bödeker et al. 2009, 2014; Kohler et al. 2015), which, when combined with the low-biomass exploration types of these taxa, suggests that these taxa might be less C demanding than *Cortinarius* and may explain their inverse

relationship with host stem radial growth. In congruence with our study, Kummel and Lostroh (2011) found a higher relative abundance of *Cenococcum*-like morphotypes on shaded versus unshaded *Abies balsamea* seedlings, both in natural light gradients and experimental shading. The authors concluded that *A. balsamea* seedlings were first colonized by *Cenococcum*-like ECM, which were then outcompeted on new root tips by other ECM species as the seedling increased its growth rate. Such priority effects may similarly help explain trends in our study. For example, spruce seedlings in our study may be initially colonized by *Cenococcum geophilum* or *Piloderma sphaerosporum*, but as open-grown seedlings increased their growth rate and C budget, new roots tips became colonized by *Cortinarius*.

Taken together, our findings suggest that seedlings growing in low N soils may harbor ECM symbionts that possess elevated class II peroxidase ability or are more effective nutrient foragers under N limitation in the presence of organic N (i.e., *Cortinarius* or medium-distance exploration types), but only when host plants have presumably adequate C budgets. Despite the potential for shared mycorrhizal networks between canopy and understory trees (Kennedy et al. 2003; Richard et al. 2005), it is possible that host C status would drive a divergence of canopy and understory ECM communities. Consequently, seedlings under strong shading, and therefore lower stem radial growth, may not have the ability to take full advantage of all members of the ECM community in common mycorrhizal networks (Selosse et al. 2006). If *Cortinarius* root colonization is limited by C supply, but canopy trees have ample light availability and thus C supply, we would hypothesize a higher abundance of *Cortinarius* on roots of canopy trees when compared to shaded understory seedlings, simply because *Cortinarius* might receive more C in exchange for N when colonizing the roots of canopy trees compared to understory seedlings. In another Minnesota peatland complex, for example, Kennedy et al. (2018) found a high abundance of *Cortinarius* spp. on roots under mature black spruce trees.

Alternative mechanisms may also be contributing to our findings. For example, previous work has shown that seedlings growing along forest edges often harbor greater ECM fungal diversity when compared to isolated individuals beyond the forest edge (Dickie and Reich 2005; Peay et al. 2010). Similarly, in our study, seedlings growing in open sites with high water tables may simply be more isolated from common mycorrhizal networks when compared to seedlings growing along forested ditch margins, consequently yielding higher dominance of *Cortinarius* on seedlings in open sites. It is also likely that peat % N and seedling stem radial growth equally influenced the ECM community in our study, but their strong covariance masked a stronger effect of peat % N. Alternatively, the causal relationship that we speculate here might be the inverse direction (i.e.,

Cortinarius actually increases the stem radial growth of its host), but such a relationship requires further investigation. Lastly, seedlings derived from layering may have access to more C reserves than seedlings derived from germination. Though we did not adequately track the layering status of harvested seedlings, stem radial growth provides a suitable indication of recent C gain (Babst et al. 2014), regardless of layering status.

Conclusions

Century-long drainage induced shifts in peat properties and overstory dynamics that had cascading effects on seedling ectomycorrhizal fungal communities. We predicted that spruce seedling ECM fungal community composition would be strongly linked to site fertility and driven by shifting relative abundances of species with ability to produce class II peroxidases, such as *Cortinarius*. Instead, the relative abundances of class II peroxidase gene copies or ECM fungal groups characterized by high fungal biomass were best explained by a positive relationship with host stem radial growth, which was in turn best predicted by canopy density. Furthermore, we found that stem radial growth was the best predictor of ECM fungal community composition, ECM exploration type composition, and *Cortinarius* relative abundance. Our findings suggest a possible trade-off between the C cost of fungal symbionts and their ability to forage for nutrients in highly organic soils. Seedlings with more access to light (and consequently higher stem radial growth) appear to favor medium-distance fringe exploration types and, in particular, those that produce class II peroxidases for the degradation of plant cell wall material. Shaded seedlings, on the other hand, may be relatively C starved and are therefore unable to subsidize high abundances of costly fungal symbionts. Our findings warrant future work to experimentally test this trade-off that structures belowground fungal communities, and how it affects canopy-understory common mycorrhizal network structure and strength of interactions.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00572-021-01060-3>.

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Declarations

Conflict of interest The authors declare no competing interests.

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