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Research

Peat loss collocates with a threshold in plant–mycorrhizal associations in drained peatlands encroached by trees

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Summary

- Drainage-induced encroachment by trees may have major effects on the carbon balance of northern peatlands, and responses of microbial communities are likely to play a central mechanistic role.
- We profiled the soil fungal community and estimated its genetic potential for the decay of lignin and phenolics (class II peroxidase potential) along peatland drainage gradients stretching from interior locations (undrained, open) to ditched locations (drained, forested).
- Mycorrhizal fungi dominated the community across the gradients. When moving towards ditches, the dominant type of mycorrhizal association abruptly shifted from ericoid mycorrhiza to ectomycorrhiza at *c.* 120 m from the ditches. This distance corresponded with increased peat loss, from which more than half may be attributed to oxidation. The ectomycorrhizal genus *Cortinarius* dominated at the drained end of the gradients and its relatively higher genetic potential to produce class II peroxidases (together with *Mycena*) was positively associated with peat humification and negatively with carbon-to-nitrogen ratio.
- Our study is consistent with a plant–soil feedback mechanism, driven by a shift in the mycorrhizal type of vegetation, that potentially mediates changes in aerobic decomposition during postdrainage succession. Such feedback may have long-term legacy effects upon postdrainage restoration efforts and implication for tree encroachment onto carbon-rich soils globally.

Introduction

Land-use change has profoundly altered northern peatlands, one of the largest organic carbon pools of the Earth's terrestrial biosphere. By the nineteenth century, *c.* 24 Mha (*c.* 5%) of northern peatlands had been extensively drained for agriculture and forestry practices (Greifswald Mire Centre, 2019). Historical drainage removed the anoxic constraint on decomposition, leading to peat carbon loss to the atmosphere via decomposition and more frequent and extensive wildfires (Turetsky *et al.*, 2015; Chimner *et al.*, 2017; Harris *et al.*, 2020; Krause *et al.*, 2021; Ma *et al.*, 2022; Fluet-Chouinard *et al.*, 2023). Yet, this drainage has not affected northern peatlands uniformly, mostly due to differences in peatland types, climates, and vegetation changes (Laiho, 2006; Talbot *et al.*, 2010; Urbanová & Bárta, 2016; Krause *et al.*, 2021; Kokkonen *et al.*, 2022). Where historical drainage triggered a shift in vegetation composition, there are potential biological and biogeochemical feedbacks that lead to uncertainty in decomposition dynamics (Laiho, 2006; Straková *et al.*, 2012).

Acidic peatlands of the northern hemisphere (bogs and poor fens) are dominated by *Sphagnum* mosses whose partially decayed tissues form the bulk of peat-building organic matter (Dorrepaal *et al.*, 2005). Taking root in the *Sphagnum* matrix are sedges and ericaceous shrubs, the latter of which form a mutualistic symbiosis with ericoid mycorrhizal (ErM) fungi. Growing evidence suggests that ErM shrubs and fungi promote organic matter accumulation in *Sphagnum* peatlands (Wiedermann *et al.*, 2017; Fenner & Freeman, 2020; H. Wang *et al.*, 2021), alpine tundra (Clemmensen *et al.*, 2021), and boreal forests (Fanin *et al.*, 2022). In these carbon-rich soils, both ErM shrubs and fungi produce recalcitrant tissues; the former produce tissues high in phenolic compounds (leaves and fine roots), and the latter produce tissue rich in melanin (mycelium; Joanisse *et al.*, 2009; Adamczyk *et al.*, 2019; Fernandez *et al.*, 2019; H. Wang *et al.*, 2021). In addition, ErM fungi use a broad suite of oxidative and hydrolytic enzymes to mobilize nutrients bound in soil organic matter (Martino *et al.*, 2018). Yet, they lack certain redox enzymes (e.g. ligninolytic class II peroxidases), which limits their ability to degrade lignin, lignin-like *Sphagnum* phenolics, and other complex biopolymers (Bengtsson *et al.*, 2018). Altogether, the distinct functional traits of ErM plants and of their fungal symbionts underpin soil organic matter accumulation (reviewed by Ward *et al.*, 2022).

A shift in peatland ecosystem state from a *Sphagnum* moss-dominated to a tree-dominated state can occur as drainage aerates the upper peat layers, which, overtime, enables persistent coniferous tree encroachment (Ohlson *et al.*, 2001; Pellerin & Lavoie, 2003; Holmgren *et al.*, 2015; Lõhmus *et al.*, 2015). Climate, former forest cover and peatland nutrient status all play a role in the extent and rate of stand development (Lõhmus *et al.*, 2015). Once established, trees alter peatland decomposition dynamics through above- (e.g. biomass and litter input) and belowground processes (e.g. root turnover, rhizodeposition, and mycorrhizal symbiosis; Klein *et al.*, 2022; Li *et al.*, 2022; Shi *et al.*, 2022). Across northern peatlands, however, the carbon sink capacity in response to tree encroachment may either increase (Minkkinen *et al.*, 2018; Hermans *et al.*, 2022) or decrease (Simola *et al.*, 2012; Hommeltenberg *et al.*, 2014). A mechanistic understanding of the effect of drainage-induced tree encroachment on peatland carbon balance remains elusive, in part because corresponding shifts in the structure and function of soil fungal communities have seldom been documented (Jaatinen *et al.*, 2008; Peltoniemi *et al.*, 2012, 2021; Andersen *et al.*, 2013; Kitson & Bell, 2020; Hupperts & Lilleskov, 2022).

The coniferous trees that typically encroach drained, acidic peatlands associate with ectomycorrhizal (EcM) fungi. Unlike ErM fungi, some EcM taxa have retained the ability to decay complex organic compounds using class II peroxidases (Bödeker *et al.*, 2009; Kohler *et al.*, 2015; Lindahl & Tunlid, 2015; Shah *et al.*, 2016), presumably to access nitrogen bound in soil organic matter (N-SOM; Nicolás *et al.*, 2019; Pellitier & Zak, 2021; T. Wang *et al.*, 2021). These peroxidases have some of the greatest known capacity to decay soil organic matter (Janusz *et al.*, 2017) and are necessary to access N-SOM because, in *Sphagnum* peatlands, polypeptides are rapidly complexed into protein-polyphenol complexes that are largely protected from hydrolytic enzymes (Bragazza & Freeman, 2007). Hence, following tree encroachment, a potential increase in organic matter decomposition could be a function of the EcM fungal community composition, along with saprotrophs, and fungal guild interactions (Bödeker *et al.*, 2016; Sterkenburg *et al.*, 2018; Fernandez *et al.*, 2020; Argiroff *et al.*, 2021).

The community composition of EcM fungi depends on soil fertility, which typically increases with peatland drainage as a result of organic matter mineralization (e.g. Hupperts & Lilleskov, 2022). Hence, a drainage-induced increase in nitrogen availability might favor C-strategist Basidiomycota (Competitor) with enhanced soil organic matter decay potential (mostly EcM and saprotrophs), whereas acidic soils with low nutrient availability largely constrain the fungal community to S-strategist Ascomycota (Stress-tolerator; Sterkenburg *et al.*, 2015). However, if drainage leads to high net nitrogen mineralization, the advantage of accessing N-SOM would decline and EcM taxa with greater abilities to take up inorganic nitrogen would become dominant (Lilleskov *et al.*, 2019; Argiroff *et al.*, 2021; Pellitier & Zak, 2021).

Water-table drawdown and subsequent tree encroachment typically results in intensively humified (less decomposable) peats due to oxidation and changes in plant litter quantity and quality (Blodau & Siems, 2012; Urbanová & Bárta, 2016; Kane *et al.*, 2019; Normand *et al.*, 2021; Li *et al.*, 2022; Uhelski *et al.*, 2022). Consequently, decomposition may become increasingly dependent on oxidative enzymes, as oxidation progresses during postdrainage succession. In turn, this may favor ligninolytic taxa such as the saprotroph *Galerina* and the saprotroph/root-associated *Mycena*, as well as EcM fungi with a greater genetic potential to obtain N-SOM (Lindahl & Tunlid, 2015; Argiroff *et al.*, 2021). Altogether, the functional ability of fungal taxa to decompose soil organic matter, and, indirectly, their genetic potential to produce oxidative enzymes is both a response and effect trait, because it influences the response of fungal taxa to the degree of peat decomposition, while simultaneously influencing their effect on organic matter decomposition (Koide *et al.*, 2014).

Temperature increases *c.* 1°C are also expected to induce encroachment by trees in northern peatlands (Heijmans *et al.*, 2013), suggesting that long-term drainage and climate warming have similar effects on vegetation in these ecosystems. Some studies on climate-induced tree encroachment into carbon-rich soils have reported a concomitant shift in the fungal community composition from root-associated Ascomycota, including the functional guild ErM fungi and the class Archaeorhizomycetes, to Basidiomycota and Mucoromycota, including the functional guilds EcM fungi and saprotrophs (Tonjer *et al.*, 2021; Hewitt *et al.*, 2022). Clemmensen *et al.* (2021), on the contrary, showed a change in the assemblage of EcM fungal functional trait and explained losses of organic matter at the transition from birch forest to heath tundra by the activity of rhizomorph-forming EcM genera with class II peroxidases, especially *Cortinarius*. Altogether, whether induced by long-term drainage or climate warming, tree encroachment into carbon-rich soils might trigger a shift in the dominance of mycorrhizal type and/or in the taxonomic and functional composition of EcM communities, with inherently complex effects on decomposition dynamics (Ritson *et al.*, 2021).

We used drainage gradients in peatlands to investigate the effect of a century-long drainage on soil fungal communities in northern Minnesota, the United States. As the peat subsided along the gradients, the vegetation transitioned from a sedge and ericaceous shrub dominance (open locations) to tree dominance (forested locations), with a concomitant decrease in soil carbon-to-nitrogen ratio (C : N) and an increase in peat humification (Hupperts & Lilleskov, 2022). We hypothesized that (H1) soil fungal community structure would shift across the drainage gradients from a higher abundance of root-associated Ascomycota to a higher abundance of Basidiomycota. We also expected the dominant type of mycorrhizal association with transition from ErM in undrained, open locations to EcM in drained, forested locations. Next, we hypothesized that (H2) as soil C : N decreases and peat humification increases along the gradients, fungal communities would increasingly be dominated by taxa with enhanced capacities to decay humified peat, indicated by a high genetic potential to produce class II peroxidases. However, we expected these taxa to decline as soil C : N continues to decrease closer to the ditches.

Materials and Methods

Drainage gradients

We selected three acidic (pH 3.1–3.7; Hupperts & Lilleskov, 2022), nutrient-poor peatlands in north-central Minnesota adjacent to ditches dug between 1916 and 1918 and separated by 5–15 km (47.083570°N, −92.839133°W; 47.116195°N, −92.796530°W and 47.168103°N, −92.694208°W; Supporting Information Fig. S1). The peatlands are within and adjacent to the Sax-Zim Bog complex, which developed following the last glacial retreat *c.* 10 000 yr BP (https://saxzim.org; accessed 08 January 2022). In each peatland, we established a single transect (*c.* 215–525 m long) from interior (undrained) to ditch (drained; for more details, see Hupperts & Lilleskov, 2022). Farther from the ditches, the ecosystems were dominated by *Sphagnum* mosses (*S. rubellum* Wilson, *S. magellanicum* Brid., and *S. fuscum* (Schimp.) Klinggr), ErM shrubs (*Chamaedaphne calyculata* L., Moench., *Kalmia polifolia* Wangenh., and *Vaccinium oxycoccus* L.), sedges (*Carex oligosperma* Michx.) and small, widely scattered black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.), and tamarack (*Larix laricina* (Du Roi) K. Koch) trees. Nearest the ditches, the ecosystems were vegetated by a dense overstory of EcM trees (predominantly, *P. mariana* and *L. laricina*) with an understory of ErM shrubs (mainly *Vaccinium myrtilloides* and *Rhododendron groenlandicum*) and a moss layer dominated by *Hylocomium splendens.*

Peat sampling and environmental variables

In September 2021, we collected 10 peat blocks (20 × 20 × 40 cm) along each transect at *c.* 20–45-m intervals with similar microtopography (i.e. lawns) using a serrated knife (sterilized with 70% ethanol). Upon collection, cores were cut in half lengthwise and sectioned into 10-cm-depth increments (0–10, 10–20, 20–30, and 30–40 cm). From each section, a representative subsample (*c.* 5 g) was collected by hand using sterile gloves, wrapped in plastic and foil, stored in liquid nitrogen for 1–3 d and stored at −80°C for *c.* 1 month until processing. The remaining peat from each section was analyzed for total carbon and nitrogen (Costech 4010 Elemental Combustion System, Costech Analytical Technologies Inc., Valencia, CA, USA), and for the degree of decomposition using the von Post scale of humification (von Post, 1924). The von Post scale consists of 10 degrees that correspond to a percentage of decomposition (e.g. H1 = 10%, H5 = 50% and H10 = 100%). The degrees H1–3 correspond to fibric peat, H4–5 to hemic peat, and H7–10 to sapric peat. In addition, we surveyed vegetation (shrub ground cover, % and tree basal area, $m^2 ha^{-1}$), measured water table depth (cm), change in peat height (m) and collected peat samples for bulk density ($g cm^{-3}$), and plant fine-root density (indicator of belowground resource allocation; $g cm^{-3}$) at each sampling location along the transects (Methods S1; Krause *et al.*, 2021). For each transect, the distance from ditch represented the average distance (m) between a given sampling location and the closest ditch(es). As such, this variable represented a cumulative ditch effect. For the remaining analyses, we used the variance of the water table depth (σ^2) calculated for each sampling location at five time points over 2 yr (Jul, Sep, and Nov 2021; Jun and Aug 2022) because the average water table depth did not change across the gradients, whereas the variance in water table depth increased from undrained to drained locations (Fig. S2).

Molecular methods

Peat for the focal dataset presented here was processed from the 10 to 20-cm and 20 to 30-cm-depth increments (60 samples total: 3 transects × 10 sampling locations × 2 depth increments). We avoided the top 10 cm, which was often dominated by green *Sphagnum* tissues. Most root biomass was consistently found in the upper 10–30-cm soil in both intact and degraded locations, except for two samples collected from the upper 0–10 cm (Fig. S3). Frozen peat samples were pulverized under liquid nitrogen using a mortar and pestle, ground to a fine powder using a sterile coffee grinder and stored at −80°C until DNA extraction. For each sample, DNA was extracted from 250 mg of frozen ground peat using the DNeasy PowerSoil Pro Kit (Qiagen) according to the manufacturer instruction and with the addition of a heating step (after vortexing the PowerBead Pro tubes, samples in the buffer CD1 were heated at 65°C for 30 min). Inhibitors of polymerase chain reactions (PCR) were removed using the DNeasy PowerClean Pro cleanup kit, and DNA was quantified using a Qubit Fluorometer (Model Q32857; Invitrogen). The fungal ITS2 gene region was amplified by PCR using the fungal-specific primers ITS4-Fun (Forward) and 5.8S-Fun (Reverse; Taylor *et al.*, 2016; Methods S2). Multiplexed libraries were sequenced on an Illumina MiSeq platform (San Diego, CA, USA) using 2 × 250 bp chemistry (MiSeq Reagent Kit V2, MS-102-2003), spiked with 20 pM denatured PhiX.

Bioinformatics

Analyses of amplicon sequences were carried out with forward reads only because there was insufficient high-quality sequence of reverse reads. While relevant information is lost when discarding reverse reads, this approach has been shown to accurately estimate fungal abundances in complex communities. For example, Nguyen *et al.* (2015) found that using single highest quality read direction (forward reads) provided a more accurate picture of a mock community of Basidiomycota and Ascomycota species than applying a paired sequence approach. Taylor *et al.* (2016) developed lineage-specific primer (used in the present study) and were able to accurately estimate fungal abundance in complex mock communities by carrying out analyses with forward reads only. Similarly, Pauvert *et al.* (2019) showed that the use of single forward sequences with DADA2 (Callahan *et al.*, 2016) was a good option for fungal community characterization.

The forward reads were denoised, dereplicated, trimmed, and truncated using the DADA2 plugin in QIIME2 on unmerged paired-end reads (Callahan *et al.*, 2016; Bolyen *et al.*, 2019). In particular, the large subunit (28S) flank (31 bases) was manually trimmed from the 5′ end of the reads, and the 3′ end of the reads was truncated to 200 bases (based on quality scores; note that the primers were not part of the reads because of the library layout). We assigned taxonomy to the resulting amplicon sequence variants (ASVs) using a Naïve–Bayesian classifier trained with the UNITE QIIME release for eukaryote database (v.8.3; 97–99% sequence similarity; Abarenkov *et al.*, 2021).

Fungal guilds and class II peroxidase potential

The primary and secondary lifestyle of each ASV was identified using the FungalTraits database according to the author's instructions (Flores-Moreno *et al.*, 2019; Põlme *et al.*, 2020). We then used the three variables from the FungalTraits file (Primary_lifetsyle, Secondary_lifestyle and Comment_on_lifetsyle) in combination with the Mycocosm database (DOE Joint Genome Institute, Berkeley, CA, USA; https://mycocosm.jgi.doe.gov; accessed 12 April 2022) and the literature to classify the ASVs into seven guilds: EcM; root endophyte; ErM (or putative ErM); ErM/EcM; saprotroph (litter, wood, soil, or unspecified); saprotroph/root-associated; and unassigned (ASVs that did not fit into the above categories). Note that ASVs in the genus *Hyaloscypha* that were not identified at the species level were classified as 'unassigned' (Vohník *et al.*, 2007; Fehrer *et al.*, 2019). The ASVs assigned to the species *Hyaloscypha hepaticicola* (deprecated synonyms: *Pezoloma ericae* and *Rhizoscyphus ericae*) were classified as ErM, and the ASVs assigned to the species *Hyaloscypha variabilis* (deprecated synonym *Meliniomyces variabilis*) were classified as ErM/EcM (Vohník *et al.*, 2007; Fehrer *et al.*, 2019). The ASVs assigned to the family Serendipitaceae and to the genera *Oidiodendron* and *Serendipita* were classified as putative ErM after blasting the representative ITS sequences (blastn against the UNITE database) to ensure they came from a habitat with ericaceous shrubs (Weiß *et al.*, 2016). The ASVs assigned to the species *Clavaria sphagnicola* (the only detected species in the genus *Clavaria*) were classified as putative ErM (Straker, 1996; Birkebak *et al.*, 2013; Olariaga *et al.*, 2015), and those assigned to the genus *Mycena* were classified as saprotroph/root-associated (Thoen *et al.*, 2020; Harder *et al.*, 2021). For the remaining of the text, we refer to the putative ErM taxa as 'ErM' to facilitate interpretation.

Plant ASVs or those unidentified at the kingdom level were removed (function subset_taxa, R package phyloseq; McMurdie & Holmes, 2013; 386 ASVs removed). The count data were then normalized by scaling with ranked subsampling (Beule & Karlovsky, 2020), aggregated at the genus level (function tax_glom, phyloseq), and transformed to relative abundance (function transform_sample_counts, phyloseq). Note that the genus *Hyaloscypha* was primarily classified as ErM because the ErM ASVs had, on average, a higher relative abundance across samples. Indeed, the genus *Hyaloscypha* comprised 18 ErM ASVs (relative abundance of 0.60%, on average), 21 ErM/EcM ASVs (relative abundance of 0.09%, on average), and 47 unassigned ASVs (relative abundance of 0.20%, on average).

We assessed the class II peroxidase potential within the sampled fungal community by calculating the average number of Auxiliary Activity 2 (AA2) genes for fungal genera in our dataset using published values from the MycoCosm database and multiplying them by the relative abundance of the respective genus in each sample (Table S1). This approach was used to assess the potential of individual fungal genera to decay lignin and phenolic compounds by combining their genetic potential to produce class II peroxidases with their relative abundance. To obtain the average AA2 genes for each fungal genus, we had the following criteria: genera without published genomes and/or for which no guild was assigned were excluded. Although *P. sphaerosporum* does not have a published genome, we used its genome as it was the only species of *Piloderma* in our dataset (authorization to use the genome was granted by the principal investigator of the project); it is important to point out that the AA2 gene family comprises the ligninolytic class II peroxidases (lignin, manganese and versatile peroxidases) as well as three nonligninolytic peroxidases, including general, cytochrome-C, and ascorbate peroxidases. Since ligninolytic class II peroxidases evolved within Agaricomycetes (Nagy *et al.*, 2016; Floudas *et al.*, 2020; Ruiz-Dueñas *et al.*, 2021), we disregarded AA2 genes in taxa outside this class (e.g. *Cenococcum*, *Hyaloscypha*). In addition, to ensure that we only targeted class II peroxidases, we manually verified that the best hit (under protein ID) was indeed a class II peroxidase.

Statistical analyses

Statistical analyses were conducted in R v.4.2.1 (R Core Team, 2022). We identified multicollinearity between environmental variables measured along the drainage gradients by performing a principal component analysis (PCA). For this analysis, we used Spearman's rank correlation coefficients because the relationships between variables were typically monotonic but not consistently linear (Fig. S4). The PCA was performed on nine rank-ordered variables including distance from ditch, water table depth σ^2, tree basal area, shrub ground cover, peat density, tree and shrub fine-root density, soil C : N, and peat humification (function *rda*, package VEGAN). The first PCA axis (first principal component) was used as a composite variable in subsequent analyses because it captured the majority of the environmental changes across the gradients (shifts in above- and belowground vegetation structure and peat physicochemical properties; Fig. 1a).

To test our first hypothesis related to soil fungal community structure across the drainage gradients, we first calculated the relative abundance of fungal phyla and guilds at each sampling location by aggregating data by transect and depth. We then carried out a partial distance-based redundancy analysis (db-RDA) at the genus level (function *capscale*, package VEGAN). We used Bray–Curtis distance measures and the argument *Condition* to account for the potential variation associated with differences among transects. The ordination axes were constrained by the first PCA axis and tree fine-root density, because the latter was the only variable strongly correlated with the second PCA axis (Fig. 1a). The significance of constraints was assessed using a permutation test (function anova.cca, package VEGAN). To accommodate the hierarchical structure of our data (depth nested within sampling location, nested within transect), we restricted

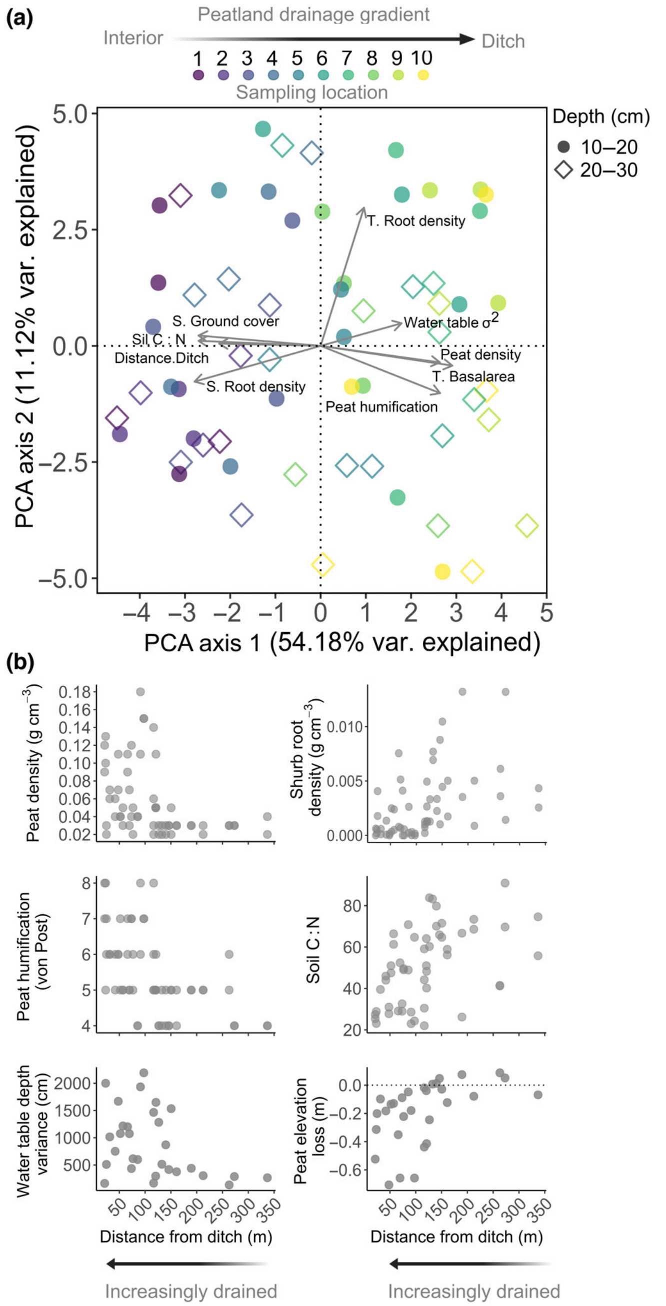

Fig. 1 Interrelated shifts in above- and belowground vegetation structure and peat physicochemical properties across peatland drainage gradients. (a) Principal component analysis of environmental variables measured along transects from undrained, open locations to drained, forested locations (60 samples total: 3 transects × 10 sampling locations × 2 depth increments). Note that this analysis was performed using Spearman's rank correlation coefficients. (b) Relationship between the distance from ditch and selected environmental variables (Supporting Information Fig. S4). S. ground cover, shrub ground cover (%); Soil C : N, soil carbon-to-nitrogen ratio; Distance.Ditch, for each transect, the average distance (m) between a given sampling location and the closest ditch(es); S. root density, shrub fine-root density (g cm^{-3}); T. root density, tree fine-root density (g cm^{-3}); Water table σ^2, variance of the water table depth (cm); Peat density, peat bulk density (g cm^{-3}); T. basal area, tree basal area (m^2 ha^{-1}); Peat humification, degree of peat decomposition (von Post scale).

the permutation design using the argument *permutations* (with the function *how*, package PERMUTE; Simpson, 2022). The samples were permuted at the level of depth (function *within*, package PERMUTE), or at the level of sampling locations (function *Plots*, package PERMUTE).

To examine the relationship between mycorrhizal guilds and environmental changes, we used linear mixed effects models (function lmer, package LME4). We fitted two separate models, one for the relative abundance of EcM genera and one for that of ErM genera. For both models, the first PCA axis and depth were added as fixed effects and sampling location nested within transect was added as a random effect. The best fitting models included the first PCA axis as a first- and second-order term (quadratic models) based on Akaike information criterion and marginal R^2 (represents the variance of the fixed effect; function r2_nakagawa, package performance). For both models, we visually checked model assumptions (function check_model, package performance), and assessed the significance of the first PCA axis and depth with an analysis of variance (function anova, package STATS).

We explored responses of individual fungal genera using Threshold Indicator Taxa ANalysis (TITAN; package TITAN2; Baker & King, 2010). This analysis can reveal potential nonlinear transitions in community data and detect concomitant changes in taxa distributions along a continuous variable (i.e. an environmental gradient). For every taxon frequency and abundance along a continuous variable, TITAN identifies the optimum change point (defined by the sum of *z*-score maximum) using the indicator species analysis approach. Data were averaged by depth to account for spatial autocorrelations, genera that occurred in fewer than three samples were removed (106 genera removed out of 195 genera total), and distance from ditch was used to represent the drainage gradients for ease of interpretation and because it was strongly negatively correlated with the first PCA axis (Fig. 1a).

To test our second hypothesis, related to the relationship between peat physicochemical properties and fungal functional composition, we implemented a db-RDA of class II peroxidase potential within fungal communities. Axes were constrained by peat humification and tree fine-root density, the argument *Condition* was used to account for the potential variation associated with differences among transects, and we assessed the significance of constraints using the same methods as above (db-RDA at the genus level).

Results

Environmental changes across the drainage gradients

Using PCA, we identified two dimensions of variation among environmental variables, which jointly explained 65% of the overall variation (Fig. 1a). The first PCA axis accounted for *c.* 54% of the total variation and, as expected, represented a gradient from undrained, shrub-dominated locations, with a high soil C : N, to drained, tree-dominated locations, where the peat was denser, more humified and water table fluctuations were larger. In particular, the first PCA axis had a strong positive loading for tree basal area (0.40; Table S2) and a comparably strong negative loading for shrub fine-root density (−0.38; Table S2). The second PCA axis (*c.* 11% of total variation) was highly positively correlated with tree fine-root density (loading of 0.89; Table S2), and separated samples collected from the upper peat layers (10–20 cm) from those collected at 20–30 cm depth, where the fine-root density of trees was generally lower (Fig. S3). Interestingly, tree fine-root density did not align with the main environmental gradient because it was unrelated to tree basal area ($\rho = 0.38$; Fig. S4).

By contrast with aboveground vegetation structure, belowground variables including peat properties, water table variability, and plant fine-root density did not vary linearly across the gradients (Figs 1b, S4). Rather, belowground variables exhibited an abrupt shift between 100 and 150 m from the ditches, which corresponded to the onset of peat subsidence (Fig. 1b).

Fungal community structure

Diverse fungal communities were recovered through amplicon sequencing. We obtained an average of 15 410 (±9729) reads per sample (76 ± 5%), ranging from 2716 to 35 468 reads. The reads were sorted into 1081 fungal ASVs (136 EcM, 81 ErM, and 289 saprotrophs and saprotrophs/root associated) and 195 fungal genera, of which 24 were EcM, four ErM, and 88 were saprotrophs and saprotrophs/root-associated (Table S1). The EcM and ErM genera predominantly belonged to Agaricales (Basidiomycota) and Helotiales (Ascomycota) and accounted for 49.3% and 32.4% of the reads, respectively (Table S1).

In support of H1, fungal communities were largely dominated by Ascomycota in samples collected from undrained locations (Fig. 2a; locations 1–3), whereas Basidiomycota made up most of the community in samples collected from increasingly drained locations (locations 4–10). Fungi in the phyla Mortierellomycota, including *Mortierella* (saprotroph) and Mucoromycota, including *Umbelopsis* (saprotroph) accounted for less than 15% of the community at each sampling location and their relative abundance slightly increased from undrained (1–3) to increasingly drained locations (4–10; Figs 2a, S5). The vast majority of Ascomycota were ErM fungi, whereas most Basidiomycota were EcM fungi (Fig. 2b). Farther from the ditches, the EcM fungal community was dominated by *Cenococcum* (Ascomycota) and *Piloderma* (Basidiomycota), while *Cortinarius* and *Amanita* made up most of the community in samples collected from increasingly drained locations (Fig. 2b).

At the genus level, *c.* 17% of the variation in fungal taxonomic composition across samples was captured by the first PCA axis and tree fine-root density (Fig. 2c; transects contributed an additional 4%). Most of the variation in fungal community composition was explained by the environmental changes across the drainage gradients (PCA axis 1; score = 0.98 on CAP1; $F = 9.60$, $P = 0.001$). On the contrary, tree fine-root density only explained 2% of the total variation (score = 0.93 on CAP2) and was not a robust predictor of fungal taxonomic composition ($F = 1.57$, $P = 0.053$). Fungal communities in undrained, open locations (interior) were dominated by ErM genera (ErM and putative ErM), including *Hyaloscypha* (Ascomycota), *Clavaria* (*sphagnicola*; Basidiomycota) and, to a lesser extent, *Serendipita* (Basidiomycota; Fig. 2c), while the EcM community was dominated by *Cenococcum* (Ascomycota) and *Piloderma* (Basidiomycota). At the tree-dominated end of the drainage gradients (ditch), EcM genera such as *Cortinarius*, *Lactarius*, and *Amanita* (all Basidiomycota) accounted for most of the community. Saprotrophs and saprotroph/root-associated genera including *Mortierella*, *Umbelopsis*, and *Mycena* tended to be slightly more abundant in drained, forested locations (Figs 2c, S5).

Consistent with H1, and the aforementioned results, there was strong evidence that the relative abundance of EcM and ErM genera was positively and negatively associated with the environmental changes across the drainage gradients (PCA axis 1), respectively (Fig. 3). For both models, marginal $R^2 = 0.70$, $P < 0.001$ for PC1, $P = 0.002$ for $(\mathrm{PC1})^2$ and $P = 0.879$ for depth. The dominant type of mycorrhizal association shifted from ErM to EcM at *c.* 120 m from the ditches (corresponding to slightly > −2 on PCA axis 1; Fig. S6).

Fungal community threshold

The indicator fungal genera had a significant change point at 81 m from the ditches, for the declining genera [fsumz−], and 121 m for the increasing genera [fsumz+] (Fig. 4a). Out of the 14 indicator fungal genera, two of the most dominant ErM genera (ErM and putative ErM), *Hyaloscypha* and *Clavaria* (*Sphagnicola*), contributed the most to the threshold for the increasing genera since their greatest shift in relative abundance occurred at *c.* 120 m from the ditches (Fig. 4b). Similarly, the greatest shift in relative abundance for the EcM genus *Cortinarius*, and to a lesser extent *Lactarius*, also occurred at or near this distance (small blue peak on Fig. 4a). Consequently, the threshold in community structure that occurred at 120 m from the ditches was mostly driven by mycorrhizal fungi. The free-living saprotrophs *Talaromyces* (Ascomycota), *Penicillium* (Ascomycota), and the genus *Archaeorhizomyces* (Ascomycota) also markedly contributed to this threshold (Fig. 4b). By contrast, *Cenococcum* and *Piloderma*, two of the dominant EcM genera across the gradients, as well as the saprotroph/root-associated *Mycena*, were not identified as indicator genera.

Fungal functional composition

In support of H2, the degree of peat decomposition (i.e. peat humification) explained 9% of the variation in class II peroxidase potential within the sampled fungal community (score = 0.87 on CAP1; $F = 4.50$, $P = 0.001$; Fig. 5a). Fungal communities in strongly decomposed, sapric peat (with C : N values between 20 and 50) were dominated by *Mycena* and *Cortinarius*, which together represented 16% of the read abundance and had an average genetic potential of *c.* 110% (average relative abundance across samples multiplied by the average number of AA2 genes; 141% for *Cortinarius* and 78% for *Mycena*; Fig. 5b; Table S1).

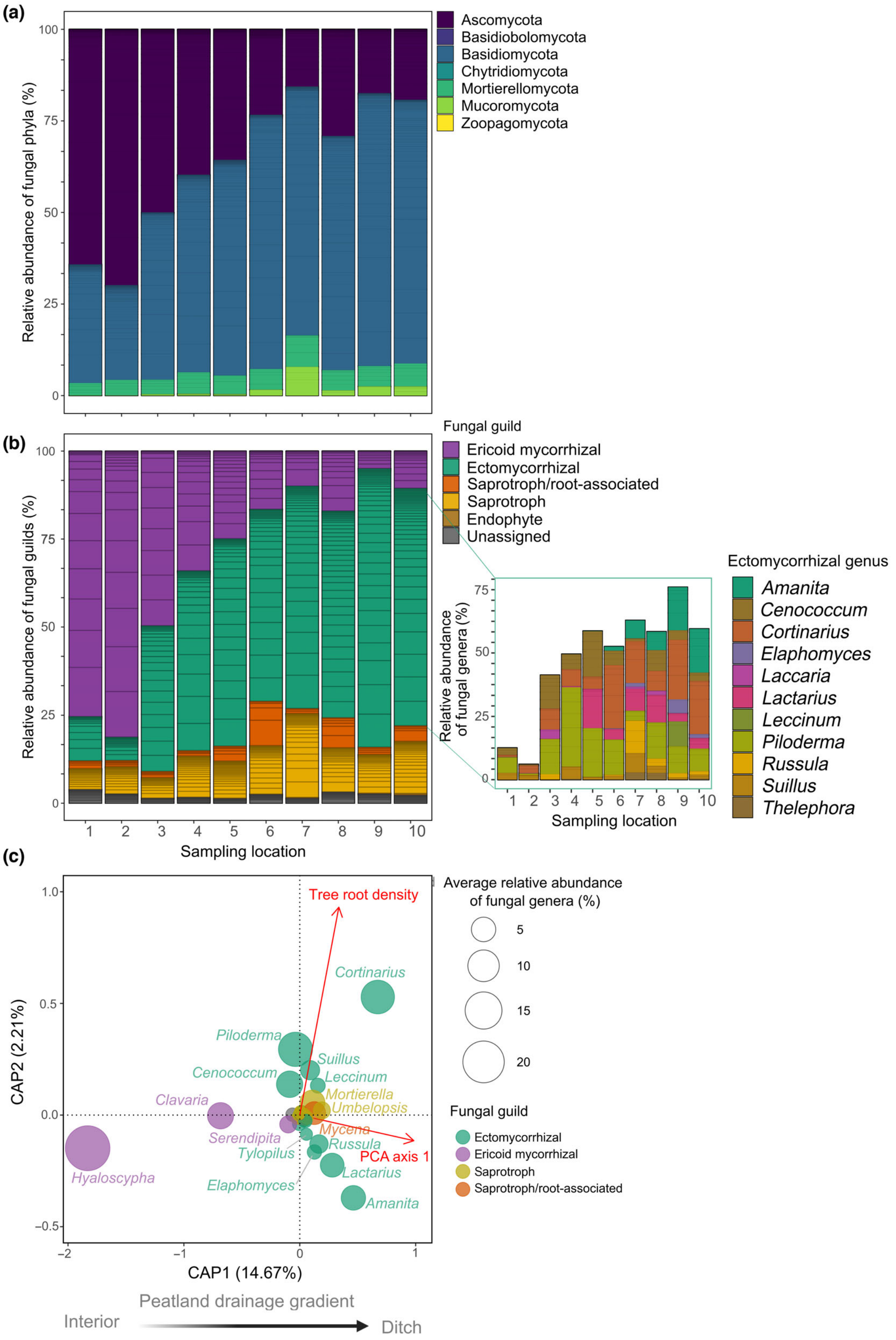

Fig. 2 Fungal community structure across peatland drainage gradients. (a) Relative abundance of fungal phyla and (b) guilds (all and ectomycorrhizal) in peat samples collected along transects from undrained, open locations to drained, forested locations. Data were aggregated by transects (three transects) and depths (two depth increments). Only the most abundant ectomycorrhizal genera were included in the inset figure for visualization purposes (11 out of 24 genera for which the sum of the relative abundance values across samples was at least 30%). (c) Plot of fungal genera from a distance-based redundancy analysis of 195 fungal genera in 60 peat samples. Tree root density, tree fine-root density (g cm^{-3}); PCA axis 1, first axis of the PCA (Fig. 1a). In (b, c), the ericoid mycorrhizal guild also includes genera classified as putative ericoid mycorrhizal including *Oidiodendron*, *Serendipita*, and *Clavaria*. In (c), the fungal genera are color-coded by their functional guild and are sized according to their relative abundance averaged across samples.

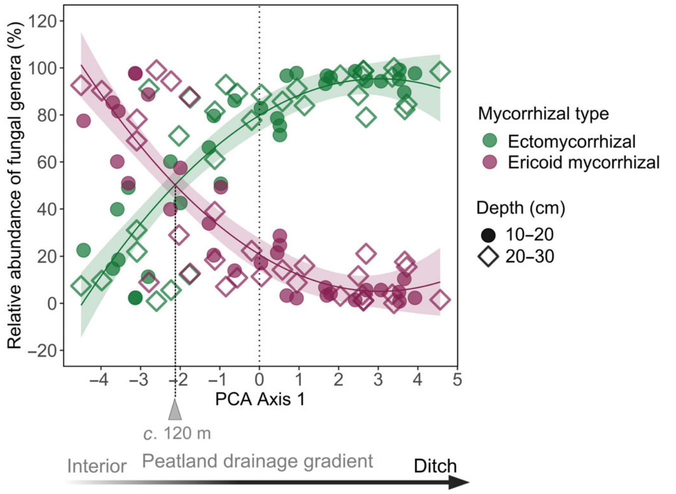

Fig. 3 Shift in the dominant type of mycorrhizal association across peatland drainage gradients. The relative abundance of ectomycorrhizal (green) and ericoid mycorrhizal (purple) fungal genera in relation to the first axis of the PCA (PCA axis 1; see Fig. 1a). Note that, for this figure, the relative abundance was calculated with mycorrhizal genera only, and that the ericoid mycorrhizal type also includes genera classified as putative ericoid mycorrhizal including *Oidiodendron*, *Serendipita*, and *Clavaria*. For both linear mixed effects models (quadratic models), marginal $R^2 = 0.70$. Shading associated with the linear smooths represent 95% confidence intervals. See Supporting Information Fig. S6 for the relationship between PCA axis 1 and distance from ditch.

The average class II peroxidase potential of fungal communities in moderately decomposed, hemic peat (with C : N values between 50 and 80) was eight times lower than that in sapric peat and was mostly driven by *Piloderma* (potential of *c.* 25%), and, to a lesser extent, *Galerina* (potential of *c.* 3%), which jointly made up *c.* 12% of the read abundance (Fig. 5b; Table S1). Similar to findings on taxonomic composition, tree fine-root density explained only a small portion of the variation in fungal functional composition (2% for class II peroxidase potential; $P = 0.011$ Fig. 5a).

Discussion

The undrained end of the gradients is dominated by ericoid mycorrhizal fungi

Farther from the ditches, the above- and belowground dominance of shrubs in the Ericaceae, along with the higher soil C : N, may largely explain the greater proportion of ErM Ascomycota relative to Basidiomycota. In particular, the ErM fungal community was dominated by the genus *Hyaloscypha* (Leotiomycetes; Fig. 2), and mostly by the species *H. hepaticicola*, which inhabit the roots of ericaceous shrubs world-wide (Fehrer *et al.*, 2019), and is common in *Sphagnum* peatlands (Kennedy *et al.*, 2018; Lamit *et al.*, 2021). *Hyaloscypha* species harbor traits related to

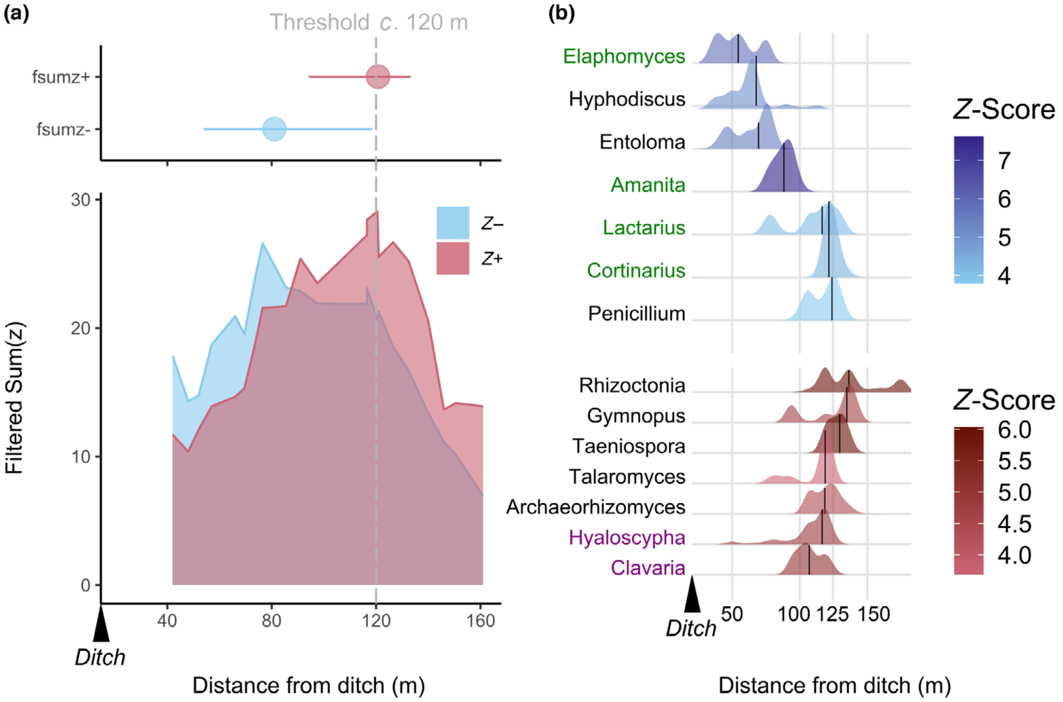

Fig. 4 Fungal community threshold detected using genus-level Threshold Indicator Taxa Analysis (TITAN). Only pure (purity ≥ 0.95) and reliable (reliability ≥ 0.95) genera were considered (filtered *z*-scores). Samples were averaged by depth to account for spatial autocorrelations. (a) The upper panel shows the change points corresponding to increasing [fsumz+, red] and declining genera [fsumz−, blue]. Change points are shown as circles with 95th percentile of their distribution as horizontal lines. The lower panel shows the magnitude of change among increasing and declining genera. Peaks in the values indicate distances from the ditch(es) at which large amount of change in community composition occur. (b) Change points of indicator fungal genera that contribute to the sum(z) scores (declining genera, top panel, blue curves and increasing genera, bottom panel, red curves). Within each panel, genera change points are visualized as one or multiple peak(s) representing location(s) of the greatest shift(s) in relative abundance. Ectomycorrhizal and ericoid mycorrhizal genera (including putative ericoid) are shown in green and in purple, respectively.

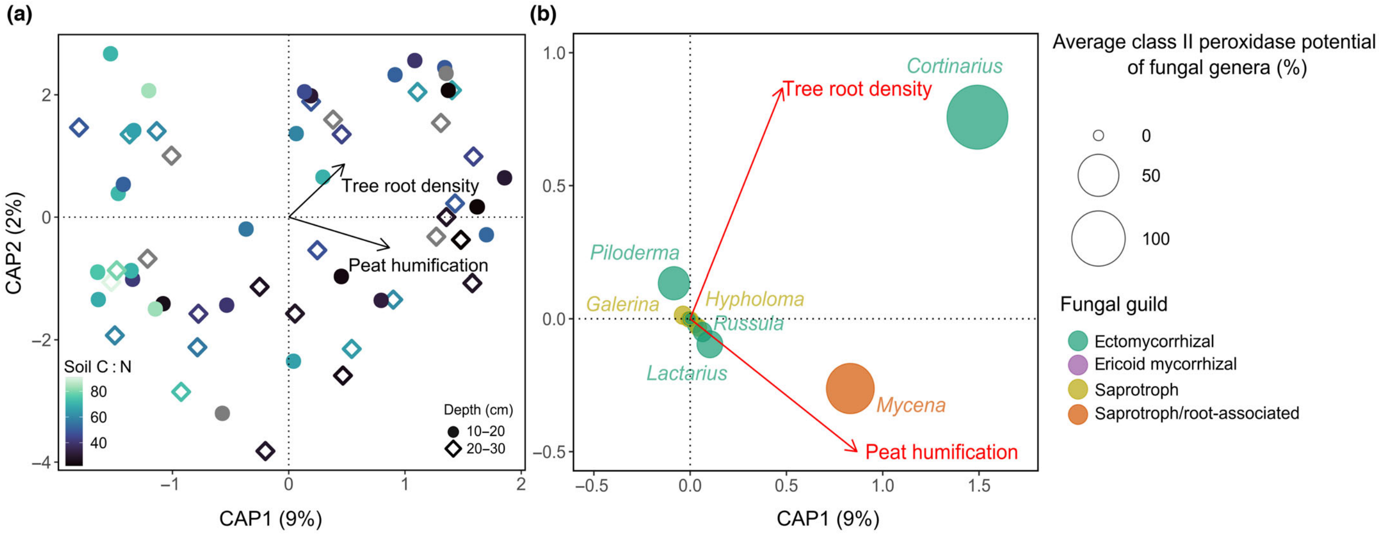

Fig. 5 Distance-based redundancy analysis of class II peroxidase potential within fungal communities in soil samples collected along peatland drainage gradients (based on a sample × genera matrix of class II peroxidase potential). (a) Plot of samples and (b) plot of genera. Class II peroxidase potential was calculated for 50 out of 195 fungal genera (representing 85% of the read abundance; Supporting Information Table S1). For each genus, the average number of class II peroxidase genes from publicly available genomes was multiplied by the relative abundance in each sample. Genera with a class II peroxidase potential equal to zero were included in the analysis. In (a), grey points represent missing soil C : N data and in (b), the fungal genera are color-coded by their functional guild and are sized according to their class II peroxidase potential averaged across samples. The ericoid mycorrhizal guild also includes genera classified as putative ericoid mycorrhizal including *Oidiodendron*, *Serendipita*, and *Clavaria*. Peat humification was assessed using the von Post scale of humification; Tree root density, tree fine-root density (g cm^{-3}). Note the different scales for (a, b).

stress-tolerance, such as melanized cell walls, slow growth rate, and long life span, which are commonly associated with harsher environmental conditions (e.g. nutrient-poor, acidic soils; Tedersoo *et al*., 2014; Sterkenburg *et al*., 2015; Kennedy *et al*., 2018; Peltoniemi *et al*., 2021; Tonjer *et al*., 2021). Of particular note are the free-living saprotrophs, which represented only a small proportion of the broader fungal community in undrained locations, and were mostly nonligninolytic decomposers such as *Mortierella* (Mortierellomycota *sensu* Tedersoo *et al*., 2018; formerly Zygomycetes; Figs 2, S5). Several mechanisms may explain their low abundance in peatlands dominated by Ericaceae. First, because of their distinct structural and biochemical traits, both ErM plants and fungi reinforce the nitrogen-limited, acidic environments created by *Sphagnum* mosses, under which ErM fungi are better competitors for soil nutrients than free-living saprotrophs (van Breemen, 1995; Bengtsson *et al*., 2018; Ward *et al*., 2022). Second, it was recently proposed that ErM plants and fungi can form association with *Sphagnum* mosses, in which nutrients are internally recycled, which would further impede the growth of saprotrophs (Shao *et al*., 2022).

Drainage-induced tree encroachment favors fungal genera with a greater class II peroxidase potential

Drainage-induced tree encroachment was associated with more humified peat, where Basidiomycota dominated over ErM Ascomycota, and were mainly assigned to genera with a greater class II peroxidase potential, including *Mycena* and *Cortinarius* (Figs 2, 5). The litter basidiomycete *Mycena* was also recovered from spruce seedling roots in a previous study across the same gradients (Hupperts & Lilleskov, 2022). This suggest that *Mycena* may exploit different nutritional models in northern peatlands as previously suggested for arctic and alpine areas (Thoen *et al*., 2020; Harder *et al*., 2021). Although *Mycena* is commonly restricted to the uppermost litter layers (Lindahl *et al*., 2007; Peltoniemi *et al*., 2012; Clemmensen *et al*., 2015), it appears to extend deeper into the peat in the drained locations (Fig. S7a), perhaps reflecting a functional shift from litter to more decayed peat, which may parallel a trophic shift from saprotrophy to biotrophy. As such, it may have a strong impact on peroxidase activity at greater depth, and on the decomposition of humified soil organic matter (Kellner *et al*., 2014; Bödeker *et al*., 2016; Kyaschenko *et al*., 2017a,b).

Interestingly, *Cortinarius* had a class II peroxidase potential almost twice as large as that of *Mycena* (Fig. 5). Since EcM fungi have access to host-derived photosynthates, they have a competitive advantage in humified peat because host sugars cover the large metabolic cost of using oxidative enzymes to mine for organically bound nutrients, especially nitrogen (Lindahl *et al*., 2007, 2021; Sterkenburg *et al*., 2018; Argiroff *et al*., 2021). By contrast, saprotrophs are constrained by both carbon quality and nutrient availability (Lindahl & Tunlid, 2015). Our findings highlight the key role that *Cortinarius* may play in nutrient mobilization from humified organic matter in drained, forested peatlands, as recently observed in other ecosystems (Clemmensen *et al*., 2021; Lindahl *et al*., 2021; Pellitier & Zak, 2021). However, we acknowledge that class II peroxidase production is not a general feature of all *Cortinarius* species (Bödeker *et al*., 2014; Lindahl *et al*., 2021).

Unexpectedly, there was no evidence of a decline in the abundance of *Cortinarius* with decreasing soil C : N (Figs 2c, 5, S7a), unlike in studies over nitrogen deposition gradients (Lilleskov *et al*., 2011, 2019). In addition, its class II peroxidase potential tended to increase with soil fertility (Fig. S7b). This suggests that soil C : N remained high enough (> 20) for EcM Basidiomycota with class II peroxidases and a greater sensitivity to nitrogen deposition to persist (Sterkenburg *et al*., 2015; Kyaschenko *et al*., 2017b; Argiroff *et al*., 2021; Pellitier & Zak, 2021).

While assessing the genetic ability of fungal communities to access specific substrates is critical to understand their contribution to carbon cycling in peatlands, this approach has limitations. First, possession of a gene does not necessarily imply that the gene is expressed or translated, although Bödeker *et al.* (2014) revealed a clear link between peroxidase activity and DNA from *Cortinarius* species. Second, copy number of a given gene might not directly predict the magnitude of expression of that gene. Third, our estimates of class II peroxidase potential are based on genomes matching only 50 out of the 195 fungal genera in our study. Although the 50 genera represented 85% of the read abundance, the genetic potential of genera for which no genome was available might have been underestimated. Nonetheless, our results provide support for a relationship between peat organic chemistry and fungal enzyme systems, in which humified peat with a larger proportion of complex organic compounds favor oxidative extracellular enzymes (Xu *et al*., 2021; Xue *et al*., 2021).

Existence of a threshold in plant–mycorrhizal associations

The dominant type of mycorrhizal association abruptly shifted from ErM to EcM at *c*. 120 m from ditches (Fig. 3). Among the genera decreasing with distance from ditch, *Cortinarius* was the only EcM genus whose shift in relative abundance coincided with the community threshold, suggesting that *Cortinarius* was the most responsive EcM genus to the environmental changes occurring at *c*. 120 m from the ditches. At this distance, there was no marked shift in shrub ground cover or tree basal area; rather, these aboveground variables steadily decreased and increased across the gradients, respectively (Fig. S4). This suggest that host plant abundance might not be tightly associated with the sharp change in the dominant type of mycorrhizal association. By contrast, belowground variables, including peat properties, water table variability, and host plant fine-root density, exhibited a marked shift at *c*. 120 m from the ditches (Fig. 1). This distance corresponded to the onset of peat subsidence and to the ‘ditch

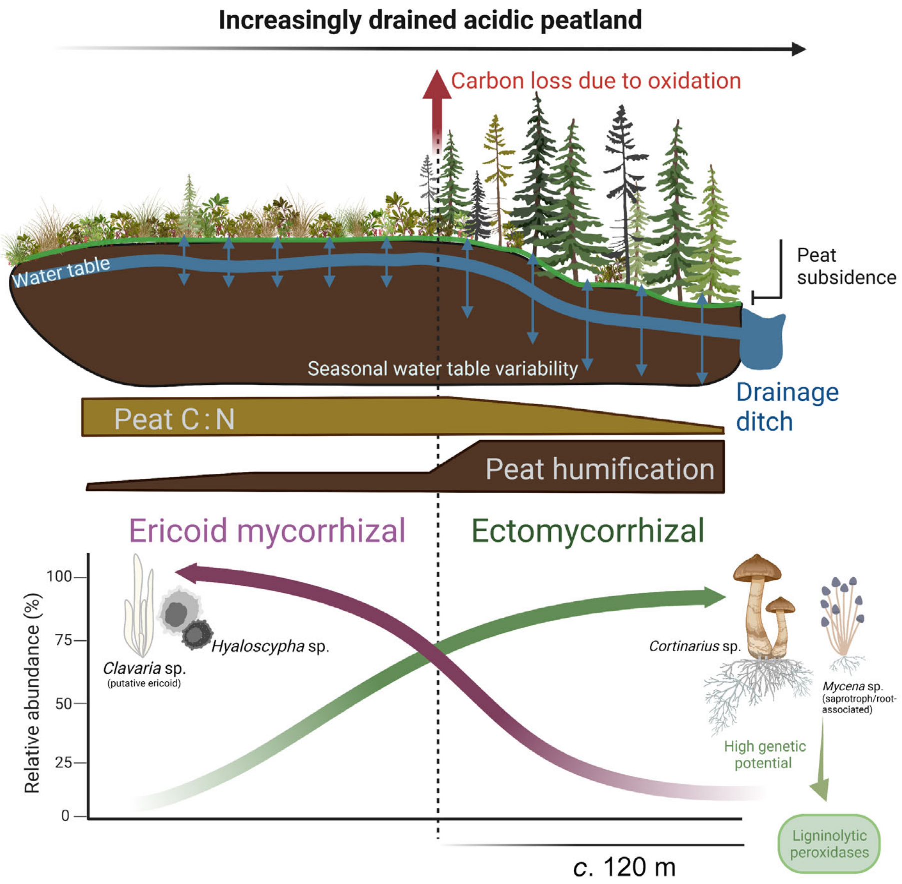

Fig. 6 Conceptual diagram of peatland drainage gradient. The dominant type of mycorrhizal association shifted from ericoid mycorrhiza to ectomycorrhiza at *c*. 120 m from the ditches and collocated with the onset of peat subsidence and marked changes in belowground abiotic variables. In the long term, the decline of slow-growing, melanized ericoid fungi combined with the increase in fungal genera with a relatively higher genetic potential to decay humified peat may hamper the accumulation of soil organic matter in drained peatlands. Created with BioRender.com.

effect zone', where ditches have a detectable effect on peat volume and carbon loss (Krause *et al.*, 2021). Taken together, our findings suggest that the abrupt shift in plant–mycorrhizal associations reflected ecological thresholds belowground. However, experimental manipulations of plant community composition along with water table variability are necessary to disentangle the effect of individual variables and directly establish causality. Nonetheless, postdrainage succession is a slow process and observations along environmental gradients is required in combination with manipulative studies to better understand how initial transitional changes affect long-term trajectories of peatland development (Laiho, 2006).

The threshold in plant–mycorrhizal associations collocates with increased peat loss

The dominant type of mycorrhizal association, which shifted from ErM to EcM at *c.* 120 m from the ditches, collocated with the onset of peat subsidence, from which more than half may be attributed to oxidation (Krause *et al.*, 2021). Indeed, the three peatlands selected in the present study were included in the study of Krause *et al.* (2021), which was aimed to assess the historical impact of drainage ditches on the peatlands of northern Minnesota. Using a model predicting the relative contribution of oxidation to subsidence from years since drainage, Krause *et al.* (2021) predicted that approximately half of the peat loss was attributable to carbon loss via oxidation, while the other half was attributable to peat compaction.

Although our experimental design does not enable us to directly assess causality between the fungal community threshold and peat subsidence, we propose a functional link between the shift from ErM to EcM dominance and carbon loss via peat oxidation (Fig. 6). In particular, *Cortinarius* may play a key role because it was the most abundant EcM genus in drained locations and the only EcM genus with class II peroxidases whose shift in relative abundance collocated with the onset of peat subsidence (Figs 1, 4). Along with a potential role of *Cortinarius*, *Mycena* likely contributed to carbon loss via peat oxidation because it was the only ligninolytic saprotroph/root-associated genus in increasingly drained areas (Fig. S5), and because saprotrophic Agaricomycetes are considered more efficient organic matter decayers than EcM fungi with peroxidases (Kyaschenko *et al.*, 2017b).

The drained locations in our study are still a net carbon sink (data not shown); yet, the potential decay of humified peat by Agaricomycetes *Mycena* and *Cortinarius*, and the loss of ErM plants and fungi may hamper soil organic matter accumulation in the long term, especially as trees represent a weaker long-term carbon sink than peat. Furthermore, the by-products of organic matter decomposition by oxidative enzymes (e.g. quinones derived from phenol oxidation) serve as important electron acceptors in anaerobic metabolism (Kellner *et al.*, 2014). In turn, these compounds may sustain anaerobic respiration and competitively suppress methanogenesis, thereby increasing the release of CO_2 to the atmosphere while reducing that of CH_4 (Trettin *et al.*, 2006; Yrjälä *et al.*, 2011; Blodau & Siems, 2012; Kane *et al.*, 2019).

The drainage gradients stretching from open to forested locations within acidic peatlands represented a corresponding shift in belowground fungal communities, from ErM to EcM dominance. Our findings show the interrelated alterations in above- and belowground vegetation structure, peat properties, and soil fungal communities that can occur when drainage induces encroachment by trees in northern peatlands. Although experimental manipulations are needed to assess causality between these alterations and peat carbon loss, our results suggest a key role of *Mycena* and of the ectomycorrhizal genus *Cortinarius*, as recently highlighted in other ecosystems. We identified a threshold in plant–mycorrhizal associations where a shift in peatland ecosystem state occurs, potentially decreasing belowground sink strength for CO_2 in drained peatlands. Although the relative importance of increased saprotrophic decomposition vs ectomycorrhizal fungal decay under peatland drainage has yet to be determined, the spatial link between drainage, increased *Cortinarius* abundance, and stimulated decomposition is striking, justifying continued investigation of this novel decay mechanism in peatland carbon cycling.

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Competing interests

None declared.

Author contributions

ESK, RKK, RAC, JKK and EAL designed the study. CLT implemented the gradient treatments and environmental sampling. CED collected, processed, and analyzed fungal community data and wrote the manuscript with contributions from JAMM, CLT, LJL and EAL.

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Data availability

The sequence data have been submitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive under accession no. PRJNA923675 (released on 2023-04-01). Environmental data are available through Pangea (environmental variables measured across peatland drainage gradients in Minnesota, the United States).

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 Transect locations in three peatlands in Minnesota, the United States.

Fig. S2 Variance and average of the water table depth in relation to distance from ditch.

Fig. S3 Relationship between distance from ditch and vascular plant fine-root density.

Fig. S4 Relationships between environmental variables.

Fig. S5 Relative abundance of saprotroph and saprotroph/root-associated genera.

Fig. S6 Relationship between distance from ditch and PCA axis 1.

Fig. S7 Linkages between ectomycorrhizal fungi and soil C : N.

Methods S1 Environmental data collection.

Methods S2 Detailed PCR protocol.

Table S1 Fungal genera in peat samples collected along peatland drainage gradients.

Table S2 Principal component analysis of environmental variables.