High-resolution minirhizotrons advance our understanding of root-fungal dynamics in an experimentally warmed peatland

Camille E. Defrenne1 | Joanne Childs1 | Christopher W. Fernandez2 | Michael Taggart3 | W. Robert Nettles1 | Michael F. Allen3 | Paul J. Hanson1 | Colleen M. Iversen1

1Climate Change Science Institute and Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA
2Department of Plant and Microbial Biology, University of Minnesota, St. Paul, MN, USA
3Department of Microbiology and Plant Pathology, Center for Conservation Biology, University of California-Riverside, Riverside, CA, USA

Correspondence
Camille E. Defrenne, Climate Change Science Institute and Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA.
Email: defrennece@ornl.gov

Funding information
Biological and Environmental Research

Societal Impact Statement
Mycorrhizal fungi enable plants to thrive in the cold, waterlogged, organic soils of boreal peatlands and, with saprotrophic fungi, largely contribute to the sequestration of atmospheric carbon in peat. Hence, fungi support the contribution of peatlands to global climate regulation, on which society depends. Here we used high-resolution minirhizotrons for an unprecedented glimpse of the belowground world of a forested bog and highlighted linkages between environmental change and the abundance, dynamics, and morphology of vascular plant fine roots and fungal mycelium. These changes may have implications for peat carbon accumulation on the boreal landscape.

Summary
• Minirhizotron technology has rarely been deployed in peatlands which has limited our understanding of root-fungal dynamics in one of planet’s most carbon-dense ecosystems.
• We used novel, high-resolution minirhizotrons in a forested bog to explore temporal variation in the abundance and growth of plant fine roots and fungal mycelium with changes in peat temperature and moisture. We utilized the framework of the Spruce and Peatland Responses Under Changing Environments experiment and focused on two minirhizotron tubes installed at the coldest (+0, elevated CO2) and warmest (+9°C, elevated CO2) ends of the experimental temperature gradient, respectively.
• We found that in warmer and drier peat, ericaceous shrub roots and ectomycorrhizal fungal rhizomorphs were more abundant, and the growth of rhizomorphs and sporocarps was greater. In turn, fine roots of trees, ectomycorrhizas, and dark-colored fungal hyphae were more abundant in colder, wetter peat. Ultimately, the belowground active season for both plant roots and fungi was extended by 62 days at the warmest compared to the coldest end of the gradient, with implications for belowground carbon, water, and nutrient fluxes.
• High-resolution minirhizotrons in peatlands provided an unprecedented view of ericaceous shrub and tree fine roots and their mycorrhizal fungal partners in situ.
INTRODUCTION

Ericoid and ectomycorrhizal fungi (ERM and ECM, respectively) dominate mycorrhizal fungal communities of boreal peatlands (Read & Perez-Moreno, 2003; Thomann, 2006) and, along with saprotrophic fungi, play critical roles in peatland biogeochemical cycling. In particular, the diversity and abundance of living and dead fungal structures are key drivers of peat formation (Fernandez et al., 2019; Juan-Ovejero et al., 2020; Thomann, 2006). As changes in climate rapidly modify the boreal biome, novel challenges await fungi (Page & Baird, 2016).

Experiments manipulating climate in boreal peatlands suggest that rising temperature and a potential drawdown of the water table will convert peatlands from ecosystems gaining carbon to ecosystems rapidly losing carbon (Bragazza et al., 2016; Hanson et al., 2020; Hopple et al., 2020; Ise et al., 2008; Jassey & Signarbieux, 2019). This would critically diminish peatland contributions to ecosystem services that range from global climate regulation to more local contributions related to water flow, biodiversity protection, mitigation of natural risks, and sustenance of local economies (Page & Baird, 2016). Globally, peatland conservation is a priority for action aiming at mitigating climate change (Humpenöder et al., 2020; International Union for Conservation of Nature, 2017). Yet, generating more comprehensive conservation strategies in peatlands necessitates a better understanding of belowground processes—especially the keystone role played by fungal mycelium—now, and in response to changing environmental conditions. This is crucial as protection of intact peatlands and restoration policies may help turning the land system into a global net carbon sink by 2,100, as projected by current mitigation pathways designed to reach a predefined climate target such as limiting or returning global mean warming to 1.5°C relative to the pre-industrial base period 1850–1900 (Humpenöder et al., 2020).

Fungal taxonomic and functional diversity in high-latitude ecosystems are strongly influenced by temperature (Allen et al., 2014; Asemaninejad et al., 2018; Bennett & Classen, 2020; Salazar et al., 2020; Treseder et al., 2016). For example, experimental warming may stimulate ECM mycelial production (Leppälammi-Kujansuu et al., 2013) and impact the abundance of ECM exploration types with differing mycelial morphology, nutrient uptake strategies, and enzymatic degradation abilities (Deslippe et al., 2011; Fernandez et al., 2017; Morgado et al., 2015; Mucha et al., 2018). However, warming effects on mycelial morphology may strongly depend upon the performance of ECM plant hosts (Clemmensen et al., 2015; Fernandez et al., 2017), often via interactions with other edaphic and environmental factors including soil moisture and nitrogen mineralization.

Therefore, this technology advanced our understanding of linkages between environmental change and the abundance, morphology, and dynamics of vascular plant fine roots and fungal mycelium.

**KEYWORDS**

- dynamics
- fine roots
- minirhizotron
- mycorrhizal fungi
- peatland
- phenology
- warming

The timing, or phenology of fungal activity, like that of fine roots, has received little attention in terrestrial ecosystems underlain by organic soils (Iversen et al., 2012). This is a serious shortcoming because fine-root and fungal mycelial phenology is critical to our understanding of the dynamics of carbon fluxes of the planet’s most carbon-dense ecosystems (Iversen et al., 2018; Juan-Ovejero et al., 2020; Schwieger et al., 2019). Mixed evidence on fine-root phenology from North American and European peatlands shows that there tends to be an offset of leaf and fine-root growth, but that root growth can occur in single or multiple flushes in the spring and autumn, and that the importance of peat temperature and water table depth varies among sites (Iversen et al., 2018; Schwieger et al., 2019). Studies in boreal forests suggest that mycelial growth peaks during autumn and coincides with periods of maximum fine-root growth (Wallander et al., 2001). In addition, the abundance of medium- and long-distance exploration types was reported to be lower during leaf flush (Hupperts et al., 2017).

Our limited knowledge of fine-root and fungal dynamics in peatlands has largely been based upon extrapolation from destructive methods such as sequential soil coring or ingrowth cores (reviewed by Iversen et al., 2012). However, non-destructive technology such as minirhizotrons remains one of the best methods to understand belowground dynamics in natural ecosystems. Yet, the deployment of minirhizotron technology in peatlands has been limited because of the environmental challenges these ecosystems pose (e.g., poor drainage and an accumulation of thick organic horizons) and because of common misconceptions regarding the effectiveness of minirhizotrons in peatlands (Iversen et al., 2012).

Here we peered into the unseen belowground world of a water-saturated peat bog using novel, automated, high-resolution minirhizotron (AMR) technology (Rhizosystems, LLC; Figure 1). This robotic technology provided an unprecedented view of the physical interactions among ericaceous shrub and tree fine roots and their ERM and ECM fungal partners in situ (images showcased at INSIDE THE SPRUCE BOG), which, to the best of our knowledge, the first of its kind. Prior to the installation of AMRs in a peat bog, several AMRs were previously deployed in a meadow ecosystem (Hernandez & Allen, 2013), a neotropical forest (Swanson et al., 2019) and in Californian mixed forest ecosystems (Allen & Kitajima, 2014; Taniguchi et al., 2018; Vargas & Allen, 2008), where findings highlighted the importance of the dynamics of fungal mycelium in response to changing environmental conditions.

Our goal was to explore how AMR technology may advance our understanding of belowground dynamics in peatland ecosystems.
In particular, we investigated temporal variations in the abundance and growth of plant fine roots and fungal mycelium with changing environmental conditions (peat temperature and moisture) in a forested bog. Our investigation was conducted against the backdrop of the Spruce and Peatland Responses Under Changing Environments (SPRUCE) experiment, in which five enclosures spanned a range of warming from +0, +2.25, +4.5, +6.75, to +9°C, while another five enclosures spanned the same range of warming and were also exposed to elevated CO$_2$ (e[CO$_2$]; +500 ppm above ambient), therefore creating a wide set of environmental conditions. Here we focused on two enclosures: the unheated (+0, e[CO$_2$]) and warmest (+9°C, e[CO$_2$]) because (a) they are at the two extremes of the experimental temperature gradient, (b) both temperature and CO$_2$ are manipulated in these enclosures, which are interdependent climate change factors and (c) both enclosures had the longest and best AMR data records.

To date, findings on belowground dynamics at SPRUCE obtained using litter bags, root ingrowth cores, and ion-exchange resins suggest that whole-ecosystem warming and associated peat drying have increased mycorrhizal necromass decomposition rates (Fernandez et al., 2019), ericaceous shrub root growth, partly due to an extended belowground active season (Malhotra et al., 2020), of which the ECM communities are dominated by highly melanized Cenococcum geophilum (Fernandez et al., 2019), (c) has decreased Sphagnum moss annual productivity (Norby et al., 2019), and (d) has increased ericaceous shrub annual net primary productivity (Hanson et al., 2020). Building on these results, we zoomed in on the plant-fungal symbiosis to investigate linkages between belowground dynamics and changing environmental conditions both within (seasonal patterns) and between the two enclosures selected. We hypothesized that: (a) the abundance of vascular plant fine roots and fungal rhizomorphs would be higher in autumn and with warmer peat temperatures (e.g., in the warmed enclosure), especially at depth, as plants may have more aerobic peat available for colonization under drier conditions, (b) the abundance of dark-colored ECM fungal structures would be higher in the unheated enclosure due to the decline of black spruce with warming (which has roots mostly colonized by melanized fungi), (c) fungal growth would be offset from fine-root production because the growth of mycorrhizal fungi is mainly dependent on newly produced photosynthates, and (d) the belowground active season for both plant roots and fungi would be extended in the warmed enclosure compared to the unheated enclosure.

2 | MATERIALS AND METHODS

2.1 | Site description

The backdrop of our zoomed-in investigation of root-fungal dynamics in a bog was the SPRUCE experiment, located in the forested S1...
bog at the southern boundary of the boreal region (USA, 47.30°N, 93.29°W; SPRUCE website). At SPRUCE, 10 large open-top enclosures, 12.8-m wide and reaching to 7 m in height, are used to explore the effects of whole-ecosystem warming (above- and belowground) on peatland ecological and biogeochemical processes (Figure S1). More details on the technology used to provide a gradient of warming, combined with e\(\text{CO}_2\), can be found in Hanson et al. (2017). Species that dominate the bog vascular plant community include (a) two ECM tree species: an evergreen conifer \textit{Picea mariana} (Mill.) Britton, Stens & Poggenb. (black spruce, henceforth \textit{P. mariana}) and a deciduous conifer \textit{Larix laricina} (Du Roi) K. Koch (larch, henceforth \textit{L. laricina}), and (b) two ERM shrub species: \textit{Rhododendron groenlandicum} Oeder and \textit{Chamaedaphne calyculata} (L.) Moench. Pre-treatment investigation of the bog mycorrhizal fungal community suggests a dominance of \textit{Cenoccoccum geophilum}. on \textit{P. mariana}, Suillus spp. and Tomentella spp. on \textit{L. laricina}, and Heliotales and Sebacinales spp. on \textit{Rhododendron} (Kennedy et al., 2018; P. Kennedy, personal observation). Rarely or non-mycorrhizal herbaceous species consist of \textit{Maianthemum trifolium} (L.) Sloboda, and a variety of graminoid species including \textit{Rhynchospora alba} (L.) Vahl, \textit{Eriophorum vaginatum}, and \textit{Carex} spp. (sedges). Generally, sedges are non-mycorrhizal in wet and waterlogged environments (Muthukumar et al., 2004). The \textit{Sphagnum} moss layer is dominated by \textit{S. angustifolium} and \textit{S. fallax} in depressed hollow microtopography and \textit{S. magellanicum} on raised hummock microtopography.

### 2.2 | Automated minirhizotron installation

The AMR system (Rhizosystems, LLC) is a newly developed technology that provides an unprecedented glimpse at root-fungal dynamics over time and throughout the peat profile; our study is the first time this technology has been used in a bog ecosystem. The AMRs are each composed of a USB-port microscope (ProScope camera) connected to a local computer and placed on a sled that moves within a clear acrylic minirhizotron tube that is 157 cm long × 10.8 cm wide (Figure 1; INSIDE THE SPRUCE BOG). An AMR tube is imaged in full in ~24 hr and a full-tube scan consists of 33,784 individual images that measure 3.01 × 2.26 × 0.125 mm (\(x, y, z\) = depth of field; 640 × 480 pixels at 100 × magnification). The AMR system is fully sealed, and a pump system cycles air through a desiccant to avoid condensation on the inside of the tube, which could obscure images (Figure 1). Images are transmitted to a server through a wired...
network and assembled into mosaics using RootView software (http://205.149.147.131:8010/index.php). In 2014, each of the ten SPRUCE enclosures received one AMR, installed at an ~45° angle into a pre-made hole in raised hummock microtopography. Only one AMR was installed in each enclosure due to the high production cost of this technology (as the market is limited) and its prototype status; we focused on raised hummock microtopography because previous investigation of the spatial distribution of fine roots across the S1 bog found that raised, drier hummocks had the greatest root standing crop (Iversen et al., 2018). The calculated depth of each image or group of images accounts for the angle of installation and we considered “0” cm depth to be where the tube met the peat surface of the hummock. Full tube scans were archived weekly or bi-weekly beginning in early June 2014 and will continue to be archived for the duration of the SPRUCE experiment.

2.3 | Image analyses

Here we focused on two AMR tubes, one in the unheated (+0, e[CO$_2$]) and one in the warmest (+9°C, e[CO$_2$]) enclosure. Full tube scans were collected weekly or bi-weekly from autumn 2018 to spring 2020. In both enclosures, _P. mariana_ was the nearest tree species to the AMRs (the unheated enclosure had 13 _P. mariana_ trees and one _L. laricina_ tree and the warmed enclosure had 18 _P. mariana_ trees and six _L. laricina_ trees; see Childs et al., 2020; data citation).

To deal with the overwhelming number of images (33,784 images per tube per imaging date), we followed two complementary approaches: (a) tube-level assessments and (b) patch-level assessments (Figure S2; Ripley, 2005).

2.3.1 | Tube-level assessments

To examine shifts in root and fungal abundance with changing environmental conditions, we surveyed 48 sub-mosaics of 36 images each for both the unheated and the warmest enclosures from autumn 2018 to spring 2020 (1,728 images per imaging date). We imposed a regular sampling grid on a given tube mosaic (from 0 to ~30 cm peat depth) in order to sample belowground patches proportionally to their area in the tube mosaic (Ripley, 2005; Figure S2A). For each sub-mosaic, at each imaging session, we estimated the abundance of five belowground classes: (a) fine roots of vascular plants, including ericaceous shrubs (0.1–0.7 mm root diameter), sedges (0.3–0.5 mm root diameter), and trees (0.4–1.0 mm root diameter), (b) ectomycorrhizas (0.3–1 mm diameter), (3) fungal hyphae (4–6 µm diameter), (4) fungal rhizomorphs (0.1–0.3 mm diameter), and (5) fungal sporocarps (Figure 3; SHRUB ROOTS TO MUSHROOMS). Shrub roots were differentiated from tree roots based on their smaller diameter, lighted color, absence of ectomycorrhizas, and distinct branching pattern.

**FIGURE 3** Images of fine roots and fungal mycelium in a forested bog where the Spruce and Peatland Responses Under Changing Environments (SPRUCE) experiment is located. Images were captured using automated, high-resolution minirhizotrons installed in the unheated (+0, elevated [CO$_2$]) and in the warmest (+9°C, elevated [CO$_2$]) SPRUCE enclosures (in raised hummock microtopography). The first row represents (a) a tree fine root (_Picea mariana_ or _Larix laricina_), (b) a shrub root (_Rhododendron groenlandicum_ or _Chamaedaphne calyculata_), and (c) a sedge root (_Carex_ sp.). The second row represents (d) a dark ectomycorrhiza (likely of the genus _Cenococcum_), (e) a light ectomycorrhiza, and (f) dark fungal hyphae. The last row represents (g) light fungal hyphae, (h) fungal rhizomorphs, and (i) a fungal sporocarp. More images can be found at INSIDE THE SPRUCE BOG.
In addition, we attributed the shade "light" or "dark" to ectomycorrhizas and fungal hyphae as a proxy of the level of melanization (Fernandez et al., 2013; fungal rhizomorphs were exclusively light-colored). Fine roots and ectomycorrhizas were not separated by tree species or fungal species and fungal classes were not separated by fungal guilds (saprotrophs or mycorrhizal) because this was impossible using images only. The abundance of each of our defined classes was expressed as the percent of the sub-mosaic surface where the class was present (Childs et al., 2020; data citation).

### 2.3.2 | Patch-level assessments

To examine root and fungal phenology, for each tube, five sub-mosaics of 36 images were arbitrarily chosen to encompass one belowground patch where one or more class(es) were present (DYNAMICS; Figure S2B). Out of the five sub-mosaics for the +9, e[CO$_2$] enclosure, one was within the 0–10 cm peat depth range, three within the 10–20 cm peat depth range and one within the 20–30 cm peat depth range. For the +O, e[CO$_2$] enclosure, four sub-mosaics were within the 0–10 cm peat depth range and one within the 10–20 cm peat depth range (Childs et al., 2020). All sub-mosaics were analyzed by the same person to obtain length per individual root or fungal structure (except fungal hyphae that did not grow linearly but rather increased in areal coverage) per sub-mosaic area using RooTracker (Duke University). Growth phenology (October 2018–March 2020) was calculated as the length extension of each structure (e.g., root, rhizomorph etc.) at each imaging session divided by the surface area of the sub-mosaic and the number of days between imaging session ($\mu$m cm$^{-2}$ day$^{-1}$). To assess the growth phenology of fungal hyphae, we measured the surface area covered by the hyphae at each imaging session using ImageJ (Fiji) and divided it by the surface area of the sub-mosaic. We averaged the growth phenology of each class across the five sub-mosaics per tube (in some cases, averaging in "0" growth where no growth was observed), and treated negative production as zero (Childs et al., 2020; data citation).

### 2.4 | Environmental variables

Given our focus on just two experimental endpoints within the SPRUCE experiment, we considered root and fungal dynamics in relation to the quantifiable effects of experimental warming and e[CO$_2$] on edaphic conditions in the surrounding peat, using SPRUCE as a proxy for changing environmental conditions. Hummock temperature (°C) and peat volumetric water content (cm$^3$ H$_2$O/cm$^3$ peat) were obtained from Hanson et al. (2016). Hummock temperature (measured with thermistors, model HMP-155; Vaisala, Inc.) is the average of the soil temperatures (30-min measurements were first averaged daily) at hummock heights 0, +10, and +20 cm above the level of the hollow (which was defined as "0 cm") and volumetric water content is the average of data (30-min measurements were first averaged daily) from three sensors (10HS; Decagon Devices Inc.) that were installed laterally at about +15 to +20 cm in a hummock. Water table height with respect to mean hollows (m ± hollow = 0) was measured using sensors (2,000 mm TruTrack Water and Temperature Voltage Output) installed within well casings and was obtained from Hanson et al. (2016).

### 2.5 | Data analyses

Data analyses were conducted in R version 3.6.1 (R CoreTeam, 2019). Due to the absence of replication in our study (one tube per enclosure), we focused our data analyses on the responses of root-fungal dynamics to quantified changes in peat temperature and moisture over time and across tubes. We averaged continuously collected environmental data to obtain one value for each imaging date and to test the effects of temperature and peat volumetric water content on the abundance and growth of root and fungal mycelium, both within and between enclosures together, we fitted generalized linear mixed models using adaptive gaussian quadrature (R package ‘GLMMadaptive’; Rizopoulos, 2020). This package is useful for analysis of over-dispersed data with an excess of zeros and repeated measures. For each belowground class, we fitted zero-inflated negative binomial model using the "mixed_model" function. This model is a mixture of a count distribution (negative binomial part) and a binary distribution (zero-inflated part). Temperature and volumetric water content data were centered and scaled using the "scale" function and both added as fixed effects in both the negative binomial and zero-inflated parts. Time was added as a random effect only in the negative binomial part and the three depths were not separated. Models had the following structure: belowground class ~ temperature+moisture, random = 1|date, family (zero-inflated negative binomial), zero-inflated (factors) = temperature + moisture. We used the DHARMA package (Hartig, 2020) to evaluate the goodness of fit of the models; here we report both statistically significant results at p < .05 and marginally significant results at p < .1.

### 3 | RESULTS

#### 3.1 | Tube-level assessments (abundance)

In both enclosures, the abundance of ericaceous shrub and tree roots was significantly related to seasonal patterns in hummock temperature and water content (Figure 4; Table S1A). Shrub root abundance decreased when the peat became wetter in the spring and tree root abundance decreased when the peat became warmer in the summer. Fungal mycelial structures were more abundant when temperature and peat water content started to drop, in autumn, in both enclosures (Figure 4). Accordingly, mixed-model analysis suggested that temperature negatively predicts light and dark hyphae abundance, although it did not significantly predict the abundance of fungal rhizomorphs. Lastly, the presence (but not the abundance)
DEFRENNÉ ET AL.

of sporocarps was significantly negatively related to peat water content (Table S1A).

For the entire time period, ericaceous shrub roots were mainly constrained to shallow peat and were about five times less abundant in the unheated than in the warmed enclosure. In particular, shrub roots were present on 4% and 19% of the sub-mosaic surface on average, in the 0–10 cm peat layer for the unheated and warmed enclosure, respectively (Figure 4). By contrast, tree fine roots and ectomycorrhizas appeared to be evenly distributed throughout the peat profile (from 0 to ~30 cm peat depth) and their abundance increased with hummock temperature between enclosures. Over the whole peat profile, tree fine roots and ectomycorrhizas (dark and light) were present on 7% and 0.6% of the sub-mosaic surface respectively in the unheated enclosure compared to 2% and 0.3% of the sub-mosaic surface, respectively in the warmed enclosure. Ectomycorrhizas present in the unheated enclosure were also darker than the ones present in the warmed enclosure. In turn, both the abundance of light and dark fungal hyphae sharply decreased with hummock temperature: hyphae were present on about 40% of the sub-mosaics surface in the unheated enclosure while they were present on about 0%-10% of the sub-mosaics surface in the warmed enclosure. In the SPRUCE bog, Helotiales and Meliniomyces spp. dominate the ERM fungal community (Fernandez et al., 2019; Kennedy et al., 2018) and form non-aggregated mycelia of dark hyphae with thick melanized cell walls (Clemmensen et al., 2015; Martino et al., 2018). It is thus plausible that a sizeable fraction of the dark hyphae we observe on the unheated SPRUCE enclosure are of ERM origin as well as from Cenococcum geophilum (Fernandez et al., 2013, 2019). Fungal rhizomorph and sporocarp abundance increased with hummock temperature between enclosures, especially in deeper peat. Fungal sporocarps were absent in the unheated enclosure, and when found in the warmed enclosure, were constrained to 10–20 cm depth.

3.2 | Patch-level assessments (phenology)

For both enclosures, daily growth of tree and ericaceous shrub roots was significantly, negatively related to daily changes in peat temperature and to peat water content, for shrub roots only (Table S1B), while daily rhizomorph production was significantly positively related to daily temperature. Furthermore, temperature was a good indicator of the occurrence of rhizomorph production as predicted by the zero-inflated portion of the mixed-model (Table S1B).

In both enclosures, tree and shrub roots had a bimodal pattern of production (focused in the spring and autumn), with a higher growth in the spring (Figure 5). Tree and shrub root production reached 200 and 100 µm cm⁻² day⁻¹ respectively in the unheated enclosure, but reached 300 µm cm⁻² day⁻¹ and almost 400 µm cm⁻² day⁻¹ respectively in the warmed enclosure. The percent cover of light and dark fungal hyphae peaked in the spring and autumn (similar to tree and shrub roots) and was strongly reduced in the +9°C, elevated [CO₂] enclosure (no dark hyphae was produced in the warmed enclosure; Figure 5). In contrast to fine-root and fungal hyphal dynamics, ectomycorrhizas, fungal rhizomorphs, and sporocarps had a unimodal pattern of production (autumn), which was offset from root phenology. The growth of rhizomorphs was higher in the warmed enclosure, reaching 1,000 µm cm⁻² day⁻¹ in September, while belowground sporocarps...
were only produced in the warmed enclosure. Furthermore, the belowground active season (duration of root and mycelium production) increased from c. 181 days in the unheated enclosure to c. 243 days in the warmed enclosure.

4 | DISCUSSION

We used high-resolution minirhizotrons to peer belowground in a boreal, forested bog that is the location of one of the world’s largest peatland warming experiments. Despite the lack of replication in our study, high-resolution minirhizotrons highlighted striking differences in the abundance and phenology of plant fine roots and fungal mycelium between the two extremes of the experimental temperature gradient at SPRUCE (+0°C, elevated [CO₂]) vs. +9°C, elevated [CO₂]). At the warmer and drier end of the temperature gradient, ericaceous shrub roots and ectomycorrhizal fungal rhizomorphs were more abundant and the growth of rhizomorphs and sporocarps was greater than in the colder, wetter, end of the gradient which was characterized by a higher abundance of tree fine roots, dark-colored ectomycorrhizas and fungal hyphae (Figure 2). In turn, the belowground active season for both plant roots and fungi was extended by 62 days in the warmed enclosure compared to the unheated enclosure.

4.1 | Trade-offs between shrub roots and ericoid fungi

Ericaceous shrub fine-root abundance was higher in hummock microtopography at the warmest end of the SPRUCE temperature gradient which partially supports our first hypothesis that vascular plant fine roots would be more abundant in the warmed enclosure. This is in line with Malhotra et al. (2020), who utilized the whole temperature gradient at SPRUCE and found that shrub fine-root production increased linearly by 1.2 km m⁻² year⁻¹ per degree increase in soil temperature at SPRUCE, using root ingrowth cores in which fungal mycelium were not examined. These results suggest that ericaceous shrubs may dominate the belowground plant community in warmer boreal peatlands, potentially leading to a decline in peat carbon accumulation because shrub roots exudate labile carbon compounds that prime the rhizosphere and thus increase heterotrophic decomposition (Bragazza et al., 2013; Lin et al., 2014; Gavazov et al., 2018; but see Basiliko et al., 2012). Ericaceous shrubs may also dominate the aboveground plant community in warmer peatlands, as observed at SPRUCE—for example, shrub annual net primary productivity was ~111.3 g C m⁻² year⁻¹ in the unheated enclosure compared to ~140.6 g C m⁻² year⁻¹ in the warmest enclosures in 2018 (Hanson et al., 2020) and in Canadian and European peatlands (Bragazza et al., 2016; Chong et al., 2012). This could further
reduce peat formation because growing shrubs shade out peat-forming *Sphagnum* mosaics (Bragazza et al., 2016; Chong et al., 2012; Norby et al., 2019).

Given that ericaceous shrub root production, biomass, length and abundance are higher at the warmest end of the SPRUCE gradient (Malhotra et al., 2020; this study; Figure 2), we expect that shrubs will rely more on direct root resource uptake than on ERM fungal mycelium as peatlands become warmer (as hypothesized at a global level by Bergmann et al., 2020). Indeed, we found that the abundance of dark fungal hyphae—which could be ERM in origin and from *Cenococcum geophilum*—was lower in the warmed enclosure (Figure 4). Our expectation is consistent with the model of Baskaran et al. (2017), which predicts that plants rely more on direct root uptake under conditions of high nitrogen availability, and Iversen et al. (unpublished data; Iversen CI, Latimer JL, Brice DB, Childs JC, Vander Stel HV, Griffiths NG, Malhotra AM, Graham JG, Norby RN, Olehieser KO, Sebestyen SS, Salmon VS, Phillips JP, Hanson PH) showed that warming increased plant-available nitrogen and phosphorus throughout the peat profile. Furthermore, our expectation concurs with Van Geel et al. (2020) who showed that soil phosphorus content was negatively related to ERM fungal richness in European bogs. Taken together, changes in belowground shrub dynamics in the warmed enclosure may lead to a decline in peat accumulation, at least as facilitated by fungal interactions because of the increase in ericaceous shrub root abundance and the loss of ERM fungi that produce necromass known to be highly recalcitrant (Clemmensen et al., 2015; Fernandez et al., 2019; Thor Mann, 2006) and that potentially produce mycelial networks (Grelet et al., 2010).

### 4.2 Rhizomorph abundance

While molecular analyses would be required to know the taxonomic identity of fungi producing the rhizomorphs in the SPRUCE bog, in general, a large fraction of the rhizomorph mass in soils is thought to be mycorrhizal and not saprotrophic (Godbold et al., 2006; Read, 1992; Vargas & Allen, 2008). Besides, the high carbon cost of rhizomorph production and maintenance is likely paid by plants (Agerer, 2001; Clemmensen et al., 2015; Hobbie, 2006; Hupperts et al., 2017; see minirhizotron images at FUNGAL MYCELIUM). Therefore, our results may reflect a shift to more carbon-demanding ECM fungi as observed in other warming experiments in cold ecosystems (Deslippe et al., 2011; Morgado et al., 2015; Salazar et al., 2020).

The abundance of fungal rhizomorphs was higher deep in the peat profile of the warmed enclosure, further confirming our first hypothesis. Associating with rhizomorph-forming ECM fungi may also be a strategy for plant hosts to acquire water, especially at depth. Consistent with this idea, we have observed drying of the peat with elevated temperatures, indicating that there may be a larger volume of aerobic peat for roots and fungi to colonize (Figure 4). We thus expect ECM rhizomorphs to facilitate plant water uptake in warmer peatlands because (a) they form vessel-like, hydrophobic elements that can efficiently transport water over long distances (Agerer, 2001; Brownlee et al., 1983) and (b) they form common mycorrhizal networks that may provide a pathway for the transfer of hydraulically lifted water between plants (Egerton-Warburton et al., 2007; Warren et al., 2008).

Observed differences in mycelial morphology between the two ends of the temperature gradient at SPRUCE may be related to ECM host performance (Fernandez et al., 2017). At SPRUCE, multiple data streams indicate that *L. laricina* individuals may be better acclimating to warming than *P. mariana* individuals (Dusenge et al., 2020; Peters et al., unpublished data; Peters JP, Warren JW, Ward EW, Guha AG, Childs JC, McLennan DM, Brice DB, Wullschleger SW, Hanson PH). Given that the ECM communities of *L. laricina* are dominated by the rhizomorph-forming ECM genus *Suillus* (Kennedy et al., 2018), we suspect that portions of the rhizomorphs in the warmed enclosure are from *Suillus* spp., as previously postulated by Fernandez et al. (2019). Accordingly, ECM rhizomorph formers may recycle mycelium to minimize nitrogen immobilization in their biomass, thereby providing the nitrogen surplus to their plant hosts (Clemmensen et al., 2015). This mechanism could partly explain the increase in *L. laricina* leaf nitrogen content and the higher rate of mycorrhizal fungal necromass decomposition with elevated temperatures at SPRUCE (Fernandez et al., 2019).

### 4.3 Tree fine-root and ectomycorrhiza abundance

We hypothesized that tree fine roots would also be more abundant at the warmest end of the temperature gradient, as Malhotra et al. (2020) showed that vascular plant fine-root length increased linearly with temperature across the experimental temperature gradient. Here focusing on the two extremes of the gradient, we found that tree roots and ectomycorrhizas were less abundant in the warmed enclosure compared to the unheated enclosure. In addition, in the warmed enclosure, ectomycorrhizas were only light-colored forms (see minirhizotron images at MYCORRHIZAS; Figure 4, which supports our second hypothesis that the abundance of dark-colored ECM fungal structures would be higher in the unheated enclosure. This implies that the contribution of tree root- and mycorrhizal-derived carbon inputs to the total carbon stored in peat may decrease at the warmest end of the gradient, because non-melanized necromass inputs are highly labile (Fernandez et al., 2019).

Decreases in tree fine-root abundance in the warmed enclosure could explain the increase in rhizomorph abundance because they generally dominate areas of low root density (Mucha et al., 2018; Peay et al., 2011). The observed decreases in tree fine-root abundance and increases in rhizomorph abundance at the warmest end of the temperature gradient suggest that trees may adopt an opposite strategy to the increasingly “do-it-yourself” resource acquisition of ericaceous shrubs by “outsourcing” to ECM fungal mycelium in a warmer future (e.g., Bergmann et al., 2020).
4.4 | Belowground phenology

Our third hypothesis that fine-root phenology would be offset from that of fungi was supported as fine-root production peaked in March and in February, while that of fungi peaked in August and September, in the unheated and warmed enclosures, respectively. The peak in fungal growth was largely driven by rhizomorphs. Therefore, ECM fungi may have greatly contributed to the autumn peak in rhizomorph production (Högberg et al., 2010; Hupperts et al., 2017). This is consistent with the increase in rhizomorph growth with elevated temperatures. Indeed, whole-ecosystem warming extends the aboveground active season (Richardson et al., 2018) and increases vascular plant photosynthetic capacity (especially that of L. laricina, Dusenge et al., 2020). As a result, ECM hosts may allocate higher amounts of newly fixed or stored carbon to their mycorrhizal partners. Furthermore, the peak in rhizomorph growth in the warmed enclosure corresponded to the highest seasonal temperatures and lowest peat water contents, which supports our expectation that associating with rhizomorph-forming ECM fungi may be a strategy for plant hosts to acquire water in drying peat soil (Vargas & Allen, 2008).

Sporocarps were produced belowground in the warmed enclosure only, in autumn 2018 and 2019, when temperatures were the highest (Figures 4 and 5). In addition, sporocarp abundance linearly increased with warming in autumn 2019 as illustrated by Figure S3. These results could be explained by a potential increase in ECM host-derived carbon allocation belowground, because, in boreal forests, mushrooms from ECM origin are known to fruit toward late autumn (Boddy et al., 2014; Högberg et al., 2010; Kauserud et al., 2012), however, more information is needed on ECM fruiting body phenology in peatlands.

We found that the belowground active season increased from c. 181 days in the unheated enclosure to c. 243 days in the warmed enclosure which supported our fourth hypothesis that the belowground active season for both plant roots and fungi would be extended in the warmed enclosure compared to the unheated enclosure. This may greatly increase root and fungal resource uptake and belowground respiration. Yet, these processes also depend on root and fungal turnover, which were not estimated here but will be a focus of future work. In addition, future work should focus on understanding root and fungal dynamics across hummock and hollow microtopography (Asemaninejad et al., 2017; Fernandez et al., 2019; Malhotra et al., 2020) as well as linking minirhizotron observations and molecular techniques to fungal ecological strategies (i.e. fungal guilds) to better understand fungal guild-specific responses to climate changes.

5 | CONCLUSIONS

Climate changes are rapidly modifying the boreal biome, yet, whether fungi will hinder plant resilience or provide a buffer against changes in temperature and atmospheric CO₂ concentrations remains to be answered. Tackling this question is crucial in order to quantify the future contribution of boreal peatlands to global climate regulation. The development of new minirhizotron technology in recent years enables observation of narrow-diameter extra-radical fungal hyphae directly at an unprecedented resolution. Here we used this technology to peer belowground in a boreal, forested bog that hosts one of the world’s largest peatland warming experiments. Despite the lack of replication in our study, high-resolution minirhizotrons highlighted striking differences in the abundance and phenology of plant fine roots and fungal mycelium between the two extremes of an experimental temperature gradient. We showed that warmer and drier environmental conditions were associated with shifts in the abundance of vascular plant fine roots, alterations in the morphology of mycorrhizal and saprotrophic fungal mycelium and lengthening of the belowground active season. These changes represent a loss of fungal functional diversity and may reduce peat carbon accumulation. Protecting boreal peatland belowground biodiversity may be crucial for action aiming at mitigating climate change.

ACKNOWLEDGEMENTS

We thank Deanne Brice, Kristen Holbrook, Les Hook, Nathan Thorp, and Jordan Woodward for image processing and data availability. The Spruce and Peatland Responses Under Changing Environments experiment is supported by the Office of Biological and Environmental Research in the United States Department of Energy’s Office of Science. This manuscript has been authored by UT-Battelle, LLC under Contract No. DE-AC05-00OR22725 with the U.S. Department of Energy. A portion of this work was performed by the Universities of Minnesota and California-Riverside. The publisher, by accepting the article for publication, acknowledges that the United States Government retains a non-exclusive, paid-up, irrevocable, world-wide license to publish or reproduce the published form of this manuscript, or allow others to do so, for United States Government purposes. The Department of Energy will provide public access to these results of federally sponsored research in accordance with the DOE Public Access Plan (http://energy.gov/downloads/doe-public-access-plan). The DOI link for the dataset used in this paper can be accessed at https://doi.org/10.25581/spruce.081/1637336.

AUTHOR CONTRIBUTIONS

C.M.I., J.C., M.T., and W.R.N. installed and maintained the minirhizotron under the supervision of P.H. The study was planned and designed by C.M.I. and C.E.D., the manuscript was written by C.E.D., the images were analyzed by C.E.D. and J.C. and C.E.D. carried out the data analyses. C.E.D., C.M.I., C.W.F., and M.F.A. contributed to the data interpretation and edited the manuscript. All authors contributed critically to the drafts and gave final approval for submission.

ORCID

Camille E. Defrenne https://orcid.org/0000-0003-2767-4892
Christopher W. Fernandez https://orcid.org/0000-0002-6310-6027
Salazar, A., Rousk, K., Jónsdóttir, I. S., Belling, J.-P., & Andrésson, Ö. S. (2020). Faster nitrogen cycling and more fungal and root biomass in cold ecosystems under experimental warming: A meta-analysis. *Ecology, 101*, e02938. https://doi.org/10.1002/ecn.2938

Schwieger, S., Blume-Werry, G., Peters, B., Smiljanić, M., & Kreyling, J. (2019). Patterns and drivers in spring and autumn phenology differ above- and belowground in four ecosystems under the same macroclimatic conditions. *Plant and Soil*. https://doi.org/10.1007/s11104-019-04300-w

Swanson, A. C., Schwendenmann, L., Allen, M. F., Aronson, E. L., Artavia-León, A., Dierick, D., Fernandez-Bou, A. S., Harmon, T. C., Murillo-Cruz, C., Oberbauer, S. F., Pinto-Tomás, A. A., Rundel, P. W., & Zelikova, T. J. (2019). Welcome to the Atta world: A framework for understanding the effects of leaf-cutter ants on ecosystem functions. *Functional Ecology, 33*, 1386–1399. https://doi.org/10.1111/1365-2435.13319

Taniguchi, T., Kitajima, K., Douhan, G. W., Yamanaka, N., & Allen, M. F. (2018). A pulse of summer precipitation after the dry season triggers changes in ectomycorrhizal formation, diversity, and community composition in a Mediterranean forest in California, USA. *Mycorrhiza, 28*, 665–677. https://doi.org/10.1007/s00572-018-0859-3

Thormann, M. N. (2006). Diversity and function of fungi in peatlands: A carbon cycling perspective. *Canadian Journal of Soil Science, 86*, 281–293. https://doi.org/10.4141/S05-082

Treseder, K. K., Marusenko, Y., Romero-Olivares, A. L., & Maltz, M. R. (2016). Experimental warming alters potential function of the fungal community in boreal forest. *Global Change Biology, 22*, 3395–3404. https://doi.org/10.1111/gcb.13238

Van Geel, M., Jacquemyn, H., Peeters, G., Acker, K., Honnay, O., & Ceulemans, T. (2020). Diversity and community structure of ericoid mycorrhizal fungi in European bogs and heathlands across a gradient of nitrogen deposition. *New Phytologist, 228*(5), 1640–1651. https://doi.org/10.1111/nph.16789

Vargas, R., & Allen, M. F. (2008). Dynamics of fine root, fungal rhizomorphs, and soil respiration in a mixed temperate forest: Integrating sensors and observations. *Vadose Zone Journal, 7*, 1055–1064. https://doi.org/10.2136/vzj2007.0138

Wallander, H., Nilsson, L. O., Hagerberg, D., & Baath, E. (2001). Estimation of the biomass and seasonal growth of external mycelium of ectomycorrhizal fungi in the field. *New Phytologist, 151*, 753–760. https://doi.org/10.1046/j.0028-646x.2001.00199.x

Warren, J. M., Brooks, J. R., Meinzer, F. C., & Eberhart, J. L. (2008). Hydraulic redistribution of water from Pinus ponderosa trees to seedlings: Evidence for an ectomycorrhizal pathway. *New Phytologist, 178*, 382–394. https://doi.org/10.1111/j.1469-8137.2008.02377.x

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Defrenne CE, Childs J, Fernandez CW, et al. High-resolution minirhizotrons advance our understanding of root-fungal dynamics in an experimentally warmed peatland. *Plants, People, Planet*. 2020;00:1–13. https://doi.org/10.1002/ppp3.10172