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New Phytologist

Research

Responses of vascular plant fine roots and associated microbial communities to whole-ecosystem warming and elevated CO_2 in northern peatlands

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Summary

- Warming and elevated CO_2 (eCO_2) are expected to facilitate vascular plant encroachment in peatlands. The rhizosphere, where microbial activity is fueled by root turnover and exudates, plays a crucial role in biogeochemical cycling, and will likely at least partially dictate the response of the belowground carbon cycle to climate changes.
- We leveraged the Spruce and Peatland Responses Under Changing Environments (SPRUCE) experiment, to explore the effects of a whole-ecosystem warming gradient (+0°C to 9°C) and eCO_2 on vascular plant fine roots and their associated microbes. We combined trait-based approaches with the profiling of fungal and prokaryote communities in plant roots and rhizospheres, through amplicon sequencing.
- Warming promoted self-reliance for resource uptake in trees and shrubs, while saprophytic fungi and putative chemoorganoheterotrophic bacteria utilizing plant-derived carbon substrates were favored in the root zone. Conversely, eCO_2 promoted associations between trees and ectomycorrhizal fungi. Trees mostly associated with short-distance exploration-type fungi that preferentially use labile soil N. Additionally, eCO_2 decreased the relative abundance of saprotrophs in tree roots.
- Our results indicate that plant fine-root trait variation is a crucial mechanism through which vascular plants in peatlands respond to climate change via their influence on microbial communities that regulate biogeochemical cycles.

Introduction

Peatlands are unique ecosystems with waterlogged organic soils that mainly form due to the accumulation of slowly decomposing *Sphagnum* mosses. These ecosystems store approximately one-third of terrestrial carbon despite comprising only 3% of Earth's land surface area (Gorham, 1991; Yu *et al.*, 2010; Yu, 2012). The carbon storage potential of peatlands is derived from an imbalance between plant production and low rates of organic matter (OM) decomposition. In northern peatlands, this imbalance is largely maintained by *Sphagnum* mosses, which can produce up to 205 g carbon m^{-2} in black spruce-dominated bogs, and account for *c.* 50% of the gross primary production of these ecosystems (Walker *et al.*, 2017). In addition, *Sphagnum* is an ecosystem engineer that slows down OM decomposition by facilitating the acidic, nutrient poor and anaerobic conditions that predominate northern peatlands (Van Breemen, 1995; Bragazza *et al.*, 2013; Shao *et al.*, 2023).

Sphagnum-dominated peatlands are predominantly located at high latitudes, which are expected to experience greater temperature increases with climate change (Collins *et al.*, 2013), and the response of northern peatlands to rising temperatures threatens to release their large carbon stores (Davidson & Janssens, 2006; Bradshaw & Warkentin, 2015). Observed changes with warming in northern peatlands include *Sphagnum* moss mortality (Jassey *et al.*, 2013; Norby *et al.*, 2019; Petro *et al.*, 2023), and water table drawdowns due to enhanced evapotranspiration (Hanson *et al.*, 2020). These changes are accompanied by increased fine-root growth of vascular plants (Malhotra *et al.*, 2020; Bucher *et al.*, 2023), which may, in turn, stimulate the release of labile organic carbon into the peat (D'Andrilli *et al.*, 2010), and ultimately increase nitrogen and phosphorus availability (Laiho

et al., 2003; Munir *et al.*, 2017; Malhotra *et al.*, 2020; Iversen *et al.*, 2023). Thus, oxygenation linked to the drop in the water table may further stimulate microbial decomposition of peat OM, leading to a transition from a carbon sink to a carbon source (Wilson *et al.*, 2016; Gill *et al.*, 2017; Hopple *et al.*, 2020).

Such pronounced changes in plant communities and plant-available nutrients have important implications for belowground microbial activities (Hill *et al.*, 2014; Feng *et al.*, 2022) that mediate the fate of soil carbon (Davidson & Janssens, 2006; Bragazza *et al.*, 2013; Sjögersten *et al.*, 2016). Microbial nutrient transformations are closely associated with plants through root exudation and fine-root trait variations (Fontaine *et al.*, 2007; Shahzad *et al.*, 2015; Teng *et al.*, 2021). Root exudates may represent an underlying mechanism for enhanced peat decomposition through priming (Bragazza *et al.*, 2013; Zeh *et al.*, 2019, 2022; Hopple *et al.*, 2020; Mastný *et al.*, 2021; Yan *et al.*, 2022), whereby the exudation of labile carbon compounds stimulate microbial enzyme activity to access nitrogen (Yin *et al.*, 2013; Meier *et al.*, 2017), and facilitate the microbial utilization of complex carbon compounds (Kuzyakov, 2002; Yin *et al.*, 2013; Kuzyakov & Blagodatskaya, 2015). Along with root exudation, variations in plant fine-root functional traits may mediate the response of root and rhizosphere microbes to environmental changes (Bardgett *et al.*, 2014; Teng *et al.*, 2021; Wan *et al.*, 2021). Hence, trait-based approaches may be particularly useful to understand the responses of fine roots and associated microbes to environmental changes as fine-root traits underpin root functional capacity, and their variation informs plant strategies for soil resource acquisition. For instance, the root economic framework indicates that plant resource uptake strategies can influence microbial communities as plants either invest in fine roots to acquire soil resources or outsource this process to mycorrhizal fungi (Bergmann *et al.*, 2020).

Warming will likely influence where plants fall within the economic space (Bennett & Classen, 2020), which strongly depends on plant functional type (PFT). In northern peatlands, ericaceous shrubs, associated with ericoid mycorrhizal (ERM) fungi, whereas trees, associated with ectomycorrhizal (ECM) fungi. Recent findings suggest that ericaceous shrubs will rely more on direct resource uptake than on ERM fungi as northern peatlands become warmer (Malhotra *et al.*, 2020; Defrenne *et al.*, 2021). In upland boreal soils, using ingrowth cores and mesh bags, Leppalammi-Kujansuu *et al.* (2013) showed that soil warming stimulated the production of *Picea abies* fine roots and ECM mycelia. Similarly, Defrenne *et al.* (2021), using high-resolution minirhizotrons in an experimentally warmed peatland, showed that the abundance and growth of fungal rhizomorphs (presumably from ECM fungi), increased in warmer and drier peat. The steady rise of atmospheric CO_2 (Monnin *et al.*, 2001) can fertilize plants (Pritchard *et al.*, 1999), in addition to having indirect effects on ecosystem processes via warming. However, the potential for soil carbon sequestration by carbon-fertilized plants is thought to be nitrogen-limited in the short term (Rosbakh *et al.*, 2021), and can promote symbiotic nitrogen fixation (Yang *et al.*, 2023), or nitrogen mining by mycorrhizal fungi (Thurner *et al.*, 2023). Therefore, plants that are fertilized by elevated CO_2 (eCO_2) can considerably impact peatland biogeochemical cycles. As such, eCO_2 may offset the increase in plant-available nitrogen, typical of warmed peatlands (Iversen *et al.*, 2023; Petro *et al.*, 2023), and together, warming and eCO_2 could intensify OM degradation and accelerate carbon turnover rates (Ofiti *et al.*, 2022). Nutrient availability, and especially nitrogen, influences ECM fungal community composition (Lilleskov *et al.*, 2019), and trait-based approaches have been particularly useful to understand the response of ECM fungi to eCO_2. The exploration type of ECM fungi represents a trait that describes the distance that their hyphae extend away from the colonized roots, representing variations related to resource acquisition strategies (Agerer, 2001). Long-distance exploration types are usually favored under eCO_2 conditions (Koide *et al.*, 2014), especially in ecosystems with low inorganic nitrogen availability (Pellitier *et al.*, 2021).

Here, we leveraged the Spruce and Peatland Responses Under Changing Environments (SPRUCE) experiment, to explore the effects of a whole-ecosystem warming gradient (from +0°C to +9°C) and eCO_2 on vascular plant fine roots and their associated microbial communities. We combined trait-based approaches with the profiling of fungal and prokaryote communities in plant roots and rhizospheres, through amplicon sequencing. We tested the following hypotheses:

(**1**) (a) Warming will increase tree fine-root biomass and length, as previously shown for ericaceous shrubs (Malhotra *et al.*, 2020).

(b) Under eCO_2, trees and shrubs will increase their resource investment in mycorrhizal fungi as nitrogen will limit plant growth.

(**2**) Warming and eCO_2 will both increase the relative abundance of saprotrophs in vascular plant roots as well as the abundance of ECM fungal taxa with the medium- and long-distance exploration types (rhizomorph-forming taxa).

(**3**) Warming and eCO_2 will both increase the relative abundance of heterotrophic prokaryotic taxa in the roots and rhizosphere of vascular plants, especially those that utilize plant-derived carbon, released via plant litter and root exudates.

Materials and Methods

The SPRUCE experiment

The SPRUCE experiment is located in a forested bog at the southern boundary of the boreal region (USA, 47.30°N, 93.29°W; https://mnspruce.ornl.gov/) and consists of 10 large open-top enclosures which are 12.8 m wide and reach 7 m in height. Five enclosures span a range of warming from +0°C, +2.25°C, +4.5°C, +6.75°C, to +9°C, while another five enclosures span the same range of warming and are also exposed to eCO_2 (+500 ppm above ambient). Belowground peat warming began in June 2014, whereby an array of heating rods 3-m in depth were established within an encircling subsurface corral that hydrologically isolates each enclosure but allow natural lateral water flow. Aboveground air warming began in August 2015. Air from within the enclosure is heated and returned to the enclosure to achieve temperature targets, which is monitored at target control point +2 m in the center of the plot. The eCO_2 treatments began in 2016. Pure CO_2 is vaporized, warmed, and regulated before being delivered in the

enclosures (more details can be found in Hanson *et al.* (2017)). The forested bog is naturally dominated by two ectomycorrhizal (ECM) tree species: (i) an evergreen conifer *Picea mariana* (P. Mill.) B.S.P. (black spruce, henceforth *Picea*) and (ii) a deciduous conifer *Larix laricina* (Du Roi) K. Koch (tamarack, henceforth *Larix*), and two ERM shrub species: (i) *Rhododendron groenlandicum* Oeder and (ii) *Chamaedaphne calyculata* (L.) Moench. Herbaceous species that are rarely- or nonmycorrhizal consist of *Maianthemum trifolium* (L.) Sloboda, and a suite of graminoid species including *Rhynchospora alba* (L.) Vahl, *Eriophorum vaginatum* (L.), and *Carex* spp., which are nonmycorrhizal in wet and waterlogged environments (Muthukumar *et al.*, 2004). The *Sphagnum* moss layer is dominated by *S. angustifolium* (Russow) C.E.O. Jensen and *S. fallax* (H.Klinggr.) in a depressed hollow microtopography and *S. divinum* (Flatberg & Hassel) on a raised hummock microtopography.

Ingrowth core installation and processing

Ingrowth cores filled with moist, commercial peat were employed to capture newly grown fine roots during the growing season from June (installation) to October (collection). In 2017, paired hummock–hollow ingrowth cores were installed at two locations within each experimental enclosure (location A and B; Supporting Information Table S1). The 30 cm long cores were inserted in the hummocks to 10 cm depth below the adjacent hollow surface, so that the top 20 cm were entirely within a hummock, and in the hollows, cores were inserted down to 30 cm depth below the peat surface. For more details on ingrowth core construction and installation see Malhotra *et al.* (2020) and Table S1. Frozen cores were sectioned into 10 cm vertical depth increments. Upon removal from the peat, cores were frozen at −20°C and subsequently thawed at 4°C to allow for extraction of fine roots.

Fine-root measurements

Fine-root traits were measured from paired hummock–hollow ingrowth cores collected in 2017 from location A (Table S1). These cores were processed in 2018 as described in Malhotra *et al.* (2020). Briefly, all fine roots (root orders 1–3 and 4+, up to 2 mm in diameter) from *Larix*, *Picea*, ericaceous shrubs, and graminoids were extracted, cleaned and scanned (1400 dpi). For *Larix*, *Picea* and shrub fine roots, total root length (m) and averaged diameter (mm) were measured with WinRHIZO (Regent Instruments Inc., Québec, QC, Canada). Afterward, the roots were oven-dried for at least 3 d at 70°C and weighed.

Total root length and dry weight values for the focal dataset presented here were summed over the top 20 cm (for hummocks and hollows separately; Table S1), within each enclosure to obtain root mass density (g cm^{-3} of peat), root length density (m cm^{-3} of peat) and specific root length (m g^{-1} of dry root).

Ectomycorrhizal fungal colonization

Tree fine roots were extracted from paired hummock–hollow ingrowth cores collected in 2017 from location B (Table S1). In 2020, all these cores were split in half (lengthwise) to allow different analyses to be done on the same cores. The first halves (only the top 20 cm) were used to estimate the intensity of ectomycorrhizal fungal colonization. All fine roots were extracted from thawed sections and sorted by tree species (*Larix* and *Picea*). For each section, live fine-root branches of each tree species were carefully cleaned with distilled water and a soft brush, and the total number of fine-root tips (first-order roots) was scored under a dissecting microscope. The mycorrhizal system on colonized fine-root tips (i.e. ectomycorrhizas) was counted as one tip, and we excluded thicker, longer pioneer root tips and root primordia. To estimate the intensity of ectomycorrhizal fungal colonization for each tree species, all the root branches were laid out on a numbered grid, and grid cells were randomly selected until approximately half of the total number of root tips for a given sample was reached. The number of colonized tips was scored based on the presence of a fungal sheath (Suz *et al.*, 2008), and expressed as the percentage of the total number of root tips.

Root and rhizosphere separation

The second halves of the cores (only the top 20 cm) were used for microbial analyses (Table S1). All fine roots were extracted from thawed sections and sorted by PFT using sterilized forceps. Care was taken to leave all closely attached rhizosphere peat intact during extraction, which prevented species-level identification of the tree fine roots. The rhizosphere was separated from the roots according to Simmons *et al.* (2018) with slight modifications. Briefly, the roots and attached rhizosphere peat were transferred into 50 ml of filter-sterilized (0.2 µm pore size) epiphyte removal buffer before freezing immediately at −80°C. The samples were thawed on ice and sonicated in an ice slurry for 10 min with pulses of 150 W for 30 s, separated by pauses of 30 s. The roots were then removed with sterile forceps and transferred into a clean 50 ml microcentrifuge tube. The remaining peat in epiphyte removal buffer was analyzed as the rhizosphere peat fraction. The rhizosphere peat was centrifuged at 8000 ***g*** for 15 min at 4°C to form a pellet and the supernatant was discarded. The roots were washed three times in chilled (4°C) sterile water, flash-frozen in liquid N_2, and ground into a fine powder using a mortar and pestle. We refer to the resulting powdered sample as the root fraction, which includes both microorganisms inside the root and the organisms that were not removed by sonication, together these two fractions are the compartment of the root environment. The root and rhizosphere samples were frozen at −80°C until DNA extraction.

Molecular methods and bioinformatics

For each PFT, DNA was extracted from *c.* 0.25 g of rhizosphere peat or root powder using the Dneasy PowerSoil Pro Kit (Qiagen) according to manufacturer's instructions. Extracted DNA was quantified with the Qubit HS assay kit (Invitrogen), and quality was assessed using a NanoDrop (NanoDrop 2000; Thermo Scientific, Waltham, MA, USA). For fungi, the nuclear ribosomal internal transcribed spacer 2 (ITS2) was amplified

using the fungal-specific primer pair 5.8S-Fun and ITS4-Fun (Taylor *et al.*, 2016). For bacteria and archaea, the 515F and 806R primer pair (Walters *et al.*, 2016) was used to amplify the V4 region of the 16S small subunit ribosomal RNA (rRNA). Details on primer pairs and polymerase chain reaction conditions are outlined in Table S2. Multiplexed libraries were quantified on the 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and sequenced on an Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) at the Georgia Institute of Technology Sequencing Core. The MiSeq reagent kit v3 with a read length of 2 × 300 bp was used for ITS2 amplicons and the MiSeq reagent kit v2 with a read length of 2 × 250 bp was used for 16S rRNA gene amplicons. Demultiplexed amplicon sequences were processed in R v.4.2.2 (R Development Core Team, 2023). Primer sequences were removed with CUTADAPT (Martin, 2011). Paired-end reads were then filtered, trimmed, merged, and grouped into amplicon sequence variants (ASVs) using DADA2 (Callahan *et al.*, 2016). For fungal ASVs, taxonomy was assigned in DADA2 which uses a I-Bayesian classifier method with the UNITE v8 dynamic database (Nilsson *et al.*, 2019). Fungal functional guilds (ECM, ERM, Orchid Mycorrhizal, Endophyte, mold, yeast, parasite, pathogen, and saprotroph) and ECM exploration types (contact, long, medium-fringe, medium-smooth, and short) were assigned using the FUNGuild database (Nguyen *et al.*, 2016), Agerer (2001), the DEEMY database (www.deemy.de), and the literature on peatland fungi (Kennedy *et al.*, 2018; Hupperts & Lilleskov, 2022; Defrenne *et al.*, 2023). For bacterial and archaeal ASVs, taxonomy was assigned with the Silva v138 database (Yilmaz *et al.*, 2014). Fungal and prokaryotic ASVs classified as 'chloroplast', 'mitochondria' or those that did not receive phylum level assignments were removed. Contaminants were removed using the R package DECONTAM (Davis *et al.*, 2018), and data were normalized using scaling with ranked subsampling (SRS(); R package SRS) normalization method for count data (Beule & Karlovsky, 2020). This method preserves ASV frequencies and alpha diversity better than species rarefaction and shows greater reproducibility.

Environmental data

Peat temperature (°C) and peat volumetric water content (cm^3 H_2O cm^{-3} peat), for the period 1 June to 1 October 2017, were obtained from Hanson *et al.* (2016). Temperature was determined by thermistor (model HMP-155; Vaisala Inc., Vantaa, Finland), and volumetric water content by 10HS FDC probes (Decagon Devices Inc., Pullman, WA, USA). For hollows, temperature is the average of soil temperatures (30 min measurements were first averaged daily) at hollow depths 0, 5, 10, and 20 cm below the level of the peat surface, and volumetric water content represents the average of data (30 min measurements were first averaged daily) from three sensors that were installed vertically into an adjacent hollow (0–20 cm depth). For all analyses, peat temperature and water content data were averaged at the plot level (i.e. averages of hummock and hollow measurements) to separate the effect of abiotic factors from that of microtopography. Plot level temperatures were averaged over the course of the growing season from when the cores were first inserted in May until their removal in September.

Statistical analysis

Statistical analyses were conducted in R v.4.1.1 (2021-08-10). To analyze how fine-root traits were related to each other and to identify the main dimensions of variation among traits, we used a principal component analysis (PCA; function PCA; FactoMineR; Lê *et al.*, 2008) on tree and shrub root traits separately. Since tree root traits were measured on roots from cores collected at different locations within each enclosure (Table S1), several cores were 'mismatched' (i.e. for a given enclosure, the core from location A would have roots whereas that from location B would not). Therefore, to ensure valid synthesis of root traits data from the two locations, 18 out of 40 samples were excluded before carrying out the PCA (this was not the case for shrub root traits or microbial community analyses). Tree root traits (specific root length, root biomass, root length density, root diameter, root tip density, and ectomycorrhizal fungal colonization) and shrub root traits (specific root length, root biomass, root length density, and root diameter) were then scaled to unit variance.

To assess whether plant fine-root trait expression was sensitive to warming and eCO_2, we fitted multiple linear regressions (lm, stats; Note that the random effect of enclosure was negligible). For each PFT and each fine-root trait, the full model included peat temperature, peat volumetric water content, eCO_2 (elevated or ambient), microtopography (hummock or hollow), and species (*Larix* or *Picea*; only for trees) as predictor variables. Before statistical analyses, response variables were transformed as needed (Tables 1, S3). For all the models, the stepAIC (direction = 'both') function (MASS, Ripley *et al.*, 2013) was used to eliminate nonsignificant predictor variables based on the Akaike information criterion, and we visually checked the model assumptions (normality of residuals and homoscedasticity) using check_model (performance; Lüdecke *et al.*, 2021). Note that, we also fitted the models with a two-way interaction between peat temperature and eCO_2, which was always nonsignificant, and thus, removed in the reported best-fit models.

For both fungal and prokaryotic communities, we fitted multiple linear regressions for each PFT with the Shannon–Wiener Species Diversity Index (α-diversity, hereafter Shannon diversity) as the response variable (lm, stats; Table S4). For trees, the full model included: peat temperature, peat volumetric water content, eCO_2, root compartments (rhizosphere and root fractions), microtopography, root length density, root tip density, root biomass, and ectomycorrhizal fungal colonization. Root traits were measured for *Picea* and *Larix* separately. We did not include specific root length and diameter in the model because our results show that morphological trait variation depends on tree species identity (this study, Table 1). Note that, to avoid multicollinearity among predictor variables, root biomass was dropped out as its variance inflation factor was above 2.5 (Zuur *et al.*, 2010). For shrubs, the full model included peat temperature, peat

Table 1 Tree fine-root trait response to environmental changes.

Best-fit model	Estimate	SE	*P*-value	Adjusted R^2 (*P*-value)
Tree root biomass				
Intercept	−0.189	0.645	0.771	**0.16 (0.014)**
Peat temperature	0.079	0.030	**0.014**	
Peat moisture	−1.384	0.668	**0.045**	
Tree root length density				
Square root transformation				
Intercept	−0.087	0.086	0.319	0.08 (0.077)
Peat temperature	0.010	0.005	0.047	
CO_2 (Ambient)	−0.033	0.019	0.097	
Tree root tip density				
Log(1 + *x*) transformation				
Intercept	−2.057	1.069	**0.062**	**0.19 (0.011)**
Peat temperature	0.145	0.059	**0.019**	
Species (*Larix*)	0.501	0.242	0.046	
Ectomycorrhizal colonization				
Intercept	28.400	6.215	**0.000**	**0.16 (0.019)**
CO_2 (Ambient)	23.832	9.494	**0.019**	
Tree specific root length				
Log_{10} transformation				
Intercept	1.379	0.049	**0.000**	**0.17 (0.012)**
Species (*Larix*)	0.175	0.065	**0.012**	
Tree root diameter				
Intercept	0.463	0.019	**0.000**	**0.39 (0.000)**
Species (*Larix*)	−0.117	0.026	**0.000**	

The root trait modeled are indicated with transformations applied for normality below. For each trait, the full model (linear regression) included peat temperature, peat volumetric water content, microtopography and tree species as predictor variables. Only the Best-fit models are shown and significant predictors are bold, note that estimates were not standardized. For the categorical predictor variables, the reference level is indicated in brackets.

volumetric water content, eCO_2, root compartments, and root length density. Among the shrub root functional trait measured, only root length density was kept as a predictor in the model since Malhotra *et al.* (2020) showed ericaceous shrubs strongly respond to warming by increasing root length production.

When analyzing fungal communities, additional multiple linear regressions were fitted for each PFT with the relative abundance of the dominant functional guilds, and the ratio of short- to long-distance ectomycorrhizal exploration type as response variables. Models for trees and shrubs included the same predictors as above, if applicable. When necessary, response variables were log-transformed to meet model assumptions (Table S5), then stepAIC was used to eliminate nonsignificant predictors, and model assumptions were visually checked using check_model (performance; Lüdecke *et al.*, 2021).

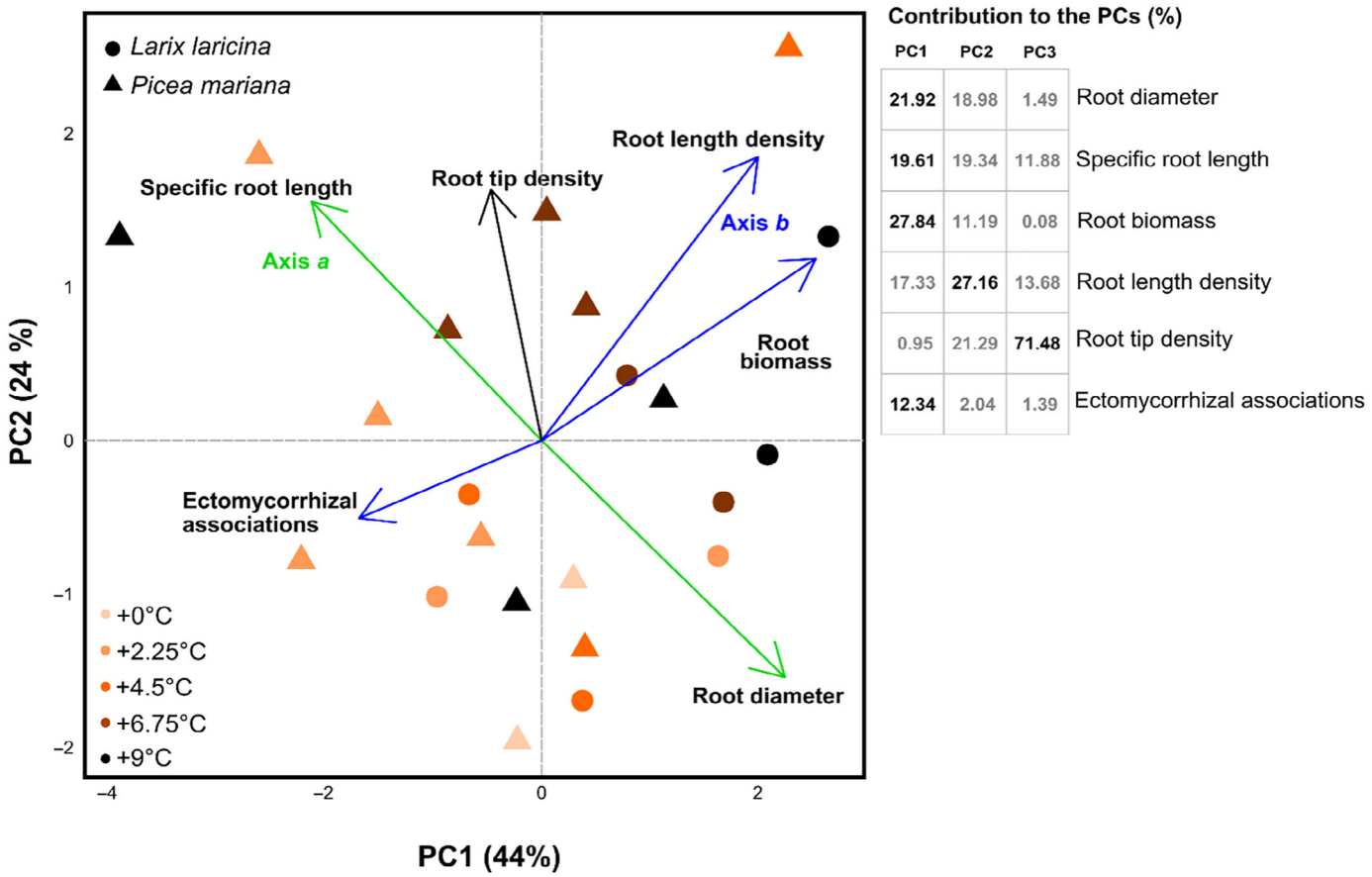
For both fungal and prokaryotic communities, distance-based redundancy analysis (dbRDA, Legendre & Anderson, 1999) was used to examine ?-diversity based on Bray–Curtis dissimilarities using dbRDA (VEGAN; Oksanen *et al.*, 2013). For each PFT, a separate model with the same predictors as in the regression models was fitted. Model selection was based on adjusted R^2 and *P*-values using *ordiR2step* (VEGAN) and assessed with a permutation test using *anova.cca* (1000 permutations; VEGAN). We partitioned the variation of the significant model variables into three categories: environmental variables (peat temperature, peat volumetric water content, and eCO2), root trait variables (root length density, root tip density, root biomass, and ectomycorrhizal fungal colonization), and experimental variables (root compartments such as rhizosphere and root fractions, and microtopography) using *varpart* (VEGAN).

Differential abundance analysis (DESEQ2; Love *et al.*, 2014) was used to identify prokaryotic and fungal taxa that varied significantly with changing environmental conditions and fine-root traits. DESEQ2 produces a negative binomial model to estimate variance–mean dependence of count data. Thus, it determines if sequence abundance, in this case, the abundance of ASV classified at the genus level, changes along the distribution of a predictor variable. We tested the effect of predictors that significantly impacted α- or β-diversity on ASV abundance (see Tables S4, S6). Amplicon sequence variants were grouped by genus using *tax_glom* (PHYLOSEQ; McMurdie & Holmes, 2013). The root compartments were analyzed separately due to their large differences in community composition (Table S6), and ASV that were disproportionally driving trends were excluded (e.g. ASV only occurring in one sample).

Results

Existence of a trade-off between resource investment in roots and in mycorrhizal associations

Fine-root traits of tree species varied along two axes (Fig. 1; axes *a*, green arrows and *b*, blue arrows), axis *a* was described by variation in morphological traits (specific root length and root diameter), and axis *b*, by variation between root soil exploitation (root biomass and length density) and ectomycorrhizal



Contribution to the PCs (%)

PC1	PC2	PC3	
21.92	18.98	1.49	Root diameter
19.61	19.34	11.88	Specific root length
27.84	11.19	0.08	Root biomass
17.33	**27.16**	13.68	Root length density
0.95	21.29	**71.48**	Root tip density
12.34	2.04	1.39	Ectomycorrhizal associations

Fig. 1 Principal component analysis of fine-root functional traits for two dominant tree species at the Spruce and Peatland Responses Under Changing Environments (SPRUCE) experiment. Symbols designate the two tree species and are color-coded according to the warming treatments. The contribution of each trait to the first three principal components (PCs) is shown at the top right corner. Tree fine-root traits varied along two axes (axes *a*, green arrows and *b*, blue arrows). Axis *a* separated the two tree species and was described by variation in morphological traits, while axis *b* separated the warming treatments and was described by variation between root soil exploitation and ectomycorrhizal associations.

associations. The two axes were related to both principal components (PCs) which together captured 68% of the variation. Axis *a* mostly separated *Picea* that constructed more numerous, thinner roots for resource acquisition from *Larix* that constructed thicker diameter roots while axis *b* separated the warming treatments. Specifically, trees in the warmest enclosures (+6.75°C and +9°C) invested resources in root length and biomass, whereas those from the unheated and colder enclosures (+2.25°C and +4.5°C) invested resources in ectomycorrhizal associations. Fine-root traits of shrubs also varied along two axes defined by the same trait trade-offs observed for trees, although ericoid mycorrhizal associations and shrub root tip density were not estimated in the present study (Fig. S1).

The trade-off between resource investment in roots and in mycorrhizal associations is responsive to warming and eCO_2

For both *Larix* and *Picea*, warming increased root soil exploitation through resource investment in root biomass and root tips (Table 1; Fig. 2). Specifically, root biomass increased by 0.1 mg cm^{-3} per °C increase in soil temperature (Table 1; Fig. 2b). By contrast, root morphological traits (i.e. specific root length and diameter) were conserved across the temperature gradient, and tree species identity was the only driver of their variation (Table 1). The eCO_2 treatment increased ECM fungal colonization by 24% for both tree species (Table 1; Fig. 2c), and there was little evidence that colonization varied with peat temperature. We note, however, that the intensity of fungal colonization appeared higher between an average peat temperature of 17°C and 19°C.

Under drier conditions and eCO_2, shrubs constructed more numerous, thinner roots as evidence by the negative relationship between peat moisture and resource investment in root length (Table S3). Specifically, shrub root length density decreased by 92% for every increase in cm^3 of H_2O per cm^3 of peat, while increasing by 64% with eCO_2 (Table S3).

Shrub and tree root Prokaryotic Shannon diversity increased with warming and decreased with eCO2

Prokaryotic Shannon diversity in both tree and shrub root compartments (root and rhizosphere) responded to environmental

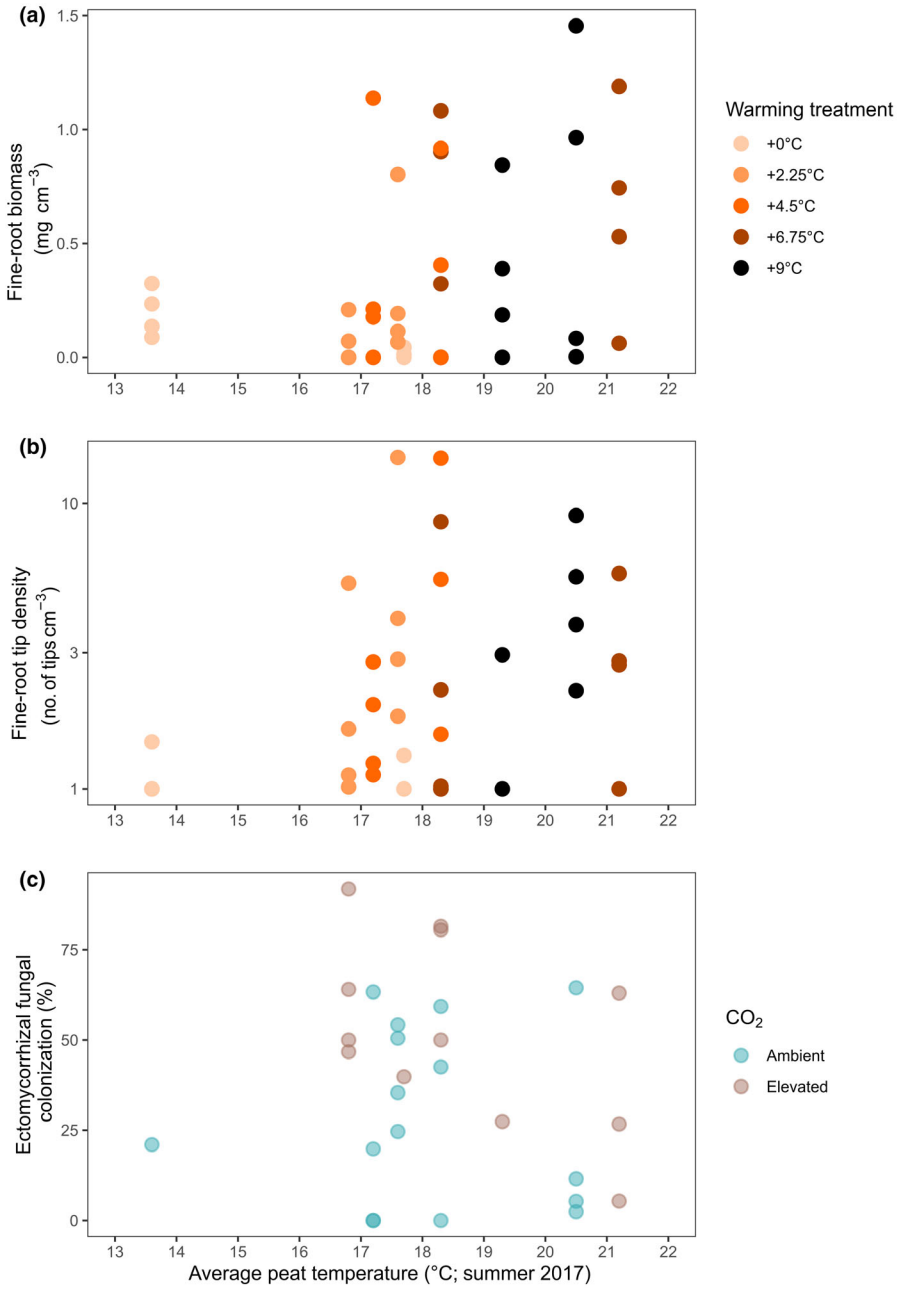


Fig. 2 Response of tree fine-root functional traits to warming and elecated CO_2 (eCO_2) at the Spruce and Peatland Responses Under Changing Environments (SPRUCE) experiment (both species were included). Model outputs can be found in Table 1. (a) Peat temperature and moisture explained 16% of the increase in tree fine-root biomass, (b) peat temperature and species identity explained 19% of the increase in tree fine-root tip density, and (c) eCO_2 explained 16% of the increase in ectomycorrhizal fungal colonization. Points are colored by peat temperature treatment (black and orange hues) and CO_2 treatment (green and brown).

changes, whereas there was little evidence it responded to plant fine-root trait variation (Table S4). Specifically, prokaryotic Shannon diversity increased with peat temperature (trees: $P = 0.038$, shrubs: $P < 0.001$; Table S4; Figs S2a, S3a) and decreased with eCO_2 (trees: $P = 0.036$, shrubs: $P = 0.004$; Table S4; Figs S2b, S3b). Prokaryotic community composition in tree and shrub roots also varied with peat temperature (trees: $P = 0.012$, shrubs: $P = 0.006$; Table S6; Figs S2c, S3c) and eCO_2 (trees: $P = 0.004$, shrubs: $P = 0.028$; Table S6; Figs S2c, S3c). Environmental variables accounted for 9.9% and 11.5% of the variation explained by the models in trees and shrubs, respectively (Tables S6, S7).

In the tree roots, an unassigned genus in the family Microbacteriaceae increased in abundance with eCO_2 (Fig. 3). In shrub roots, eCO_2 decreased the relative abundance of *Edaphobaculum* and *Rhizomicrobium*, while members of the *Coxiella* genus increased with eCO_2 (Fig. 3). Peat temperature increased the abundance of an unassigned genus in the family Gimesiaceae (Fig. S4). Peat moisture increased the richness of shrub-associated bacteria ($P = 0.010$; Table S4).

Some root traits influenced the prokaryotic community composition of tree and shrub root compartments (Table S6). In trees, root tip density ($P = 0.004$) explained 7.1% of the variation accounted for by the model, while shrub root length

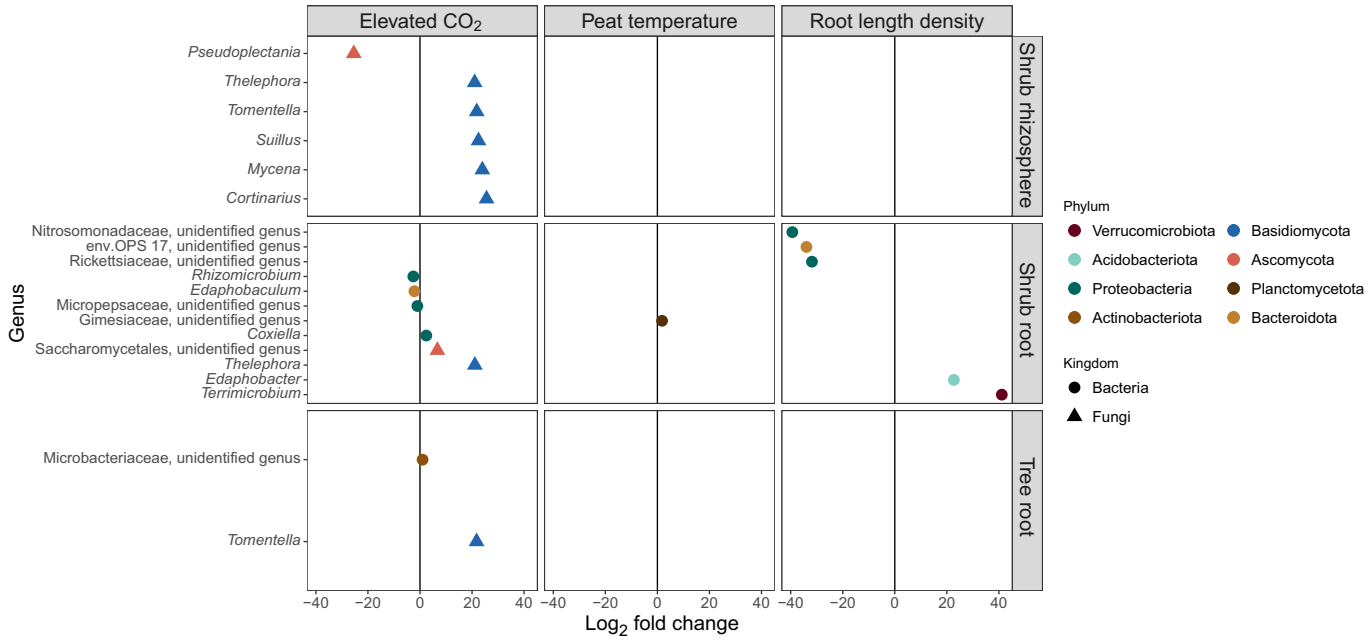


Fig. 3 Shifts in root microbiome taxa in response to elevated CO_2 treatment, peat temperature, or root length density (identified in grey above each column). Change in the abundance of each amplicon sequence variant (ASV) in response to increases in the predictor variable expressed as fold changes along the *x*-axis. The ASVs are separated along the *y*-axis according to their assigned genus, symbols indicate the kingdom, which are colored by their assigned phylum. Values are grouped by rows based on their sample origin (the shrub root and rhizosphere, and the tree root only).

density ($P = 0.038$) explained 3.3% of the variation accounted for by the model (Table S6). Differential abundance analysis revealed that the abundance of several prokaryotic taxa varied with shrub root length density (Fig. S4). These included members of the *Edaphobacter* and *Terrimicrobium*, which increased in abundance at higher root length densities, as well as genera in the family Nitrosomonadaceae, Rickettsiaceae, and the order Sphingobacteriales, which were more abundant at low root length densities (Fig. S4). Details on the microbial community composition of both prokaryotic and fungal ASVs in tree and shrub root compartments, and an analysis of the impact of microtopography on microbial community composition can be found in the Notes S1.

Fine-root traits and eCO_2 as drivers of the changes in the fungal community composition in plant root compartments

The community of fungi colonizing tree and shrub roots responded to environmental and PFT-specific root traits (Table S6). In tree roots, peat moisture ($P = 0.032$; Table S6) and eCO_2 ($P = 0.002$; Table S6) explained 11.0% of the variation accounted for by the dbRDA (Fig. S2e). Differential abundance analysis indicated that, in tree roots, the fungal genus *Tomentella* increased in abundance with eCO_2 (Fig. 3). Additionally, there was strong evidence that the fungal community composition in tree roots was related to ECM fungal colonization ($P = 0.004$; Table S6) and tree root tip density ($P = 0.022$; Table S6), which together accounted for 11.2% of the variance explained by the model (Fig. S2e).

In shrub roots, eCO_2 ($P = 0.032$; Table S6) was kept as part of the model, yet it explained only 2.3% of the variation in the community composition of fungi that was captured by the model (Fig. S2d). In the shrub roots, the genus *Thelephora* was positively related with eCO_2 (Fig. 3). In the shrub rhizosphere, the genera *Tomentella*, *Suillus*, *Thelephora*, and *Cortinarius*, and *Mycena* increased with eCO_2, while the relative abundance of the genus *Pseudoplectania* decreased with eCO_2 (Fig. 3).

Divergent responses of mycorrhizal and saprotrophic fungi to warming and eCO_2

Of the 736 fungal ASVs in tree roots and rhizosphere, 52.0% were not assigned a functional guild using FUNGuild. The fungal ASVs that could be functionally identified consisted predominantly of saprotrophs (24.0%), ECM fungi (10.8%), endophytes (2.6%), putative animal and plant pathogens (2.2%), and ERM or ERM-ECM fungi (1.2% and 1.9%, respectively). The remaining 5.3% of ASVs were classified as yeasts, molds, epiphytes, parasites, and orchid mycorrhizal fungi. In shrub roots, we found 796 ASVs, 55.8% of which were not assigned to a functional guild. Saprotrophs were the dominant guild (22.5%), although ECM (8.9%), ERM (1.5%), and ERC-ECM fungi (1.4%) were also present.

The relative abundance of ECM in tree roots was positively related to ECM fungal colonization as measured via microscopy ($P = 0.006$; Fig. 4a; Table S5). The unheated and colder enclosures (+2.25°C and +4.5°C) showed investment in ECM via an increase in colonization (Fig. 1). These results indicate that

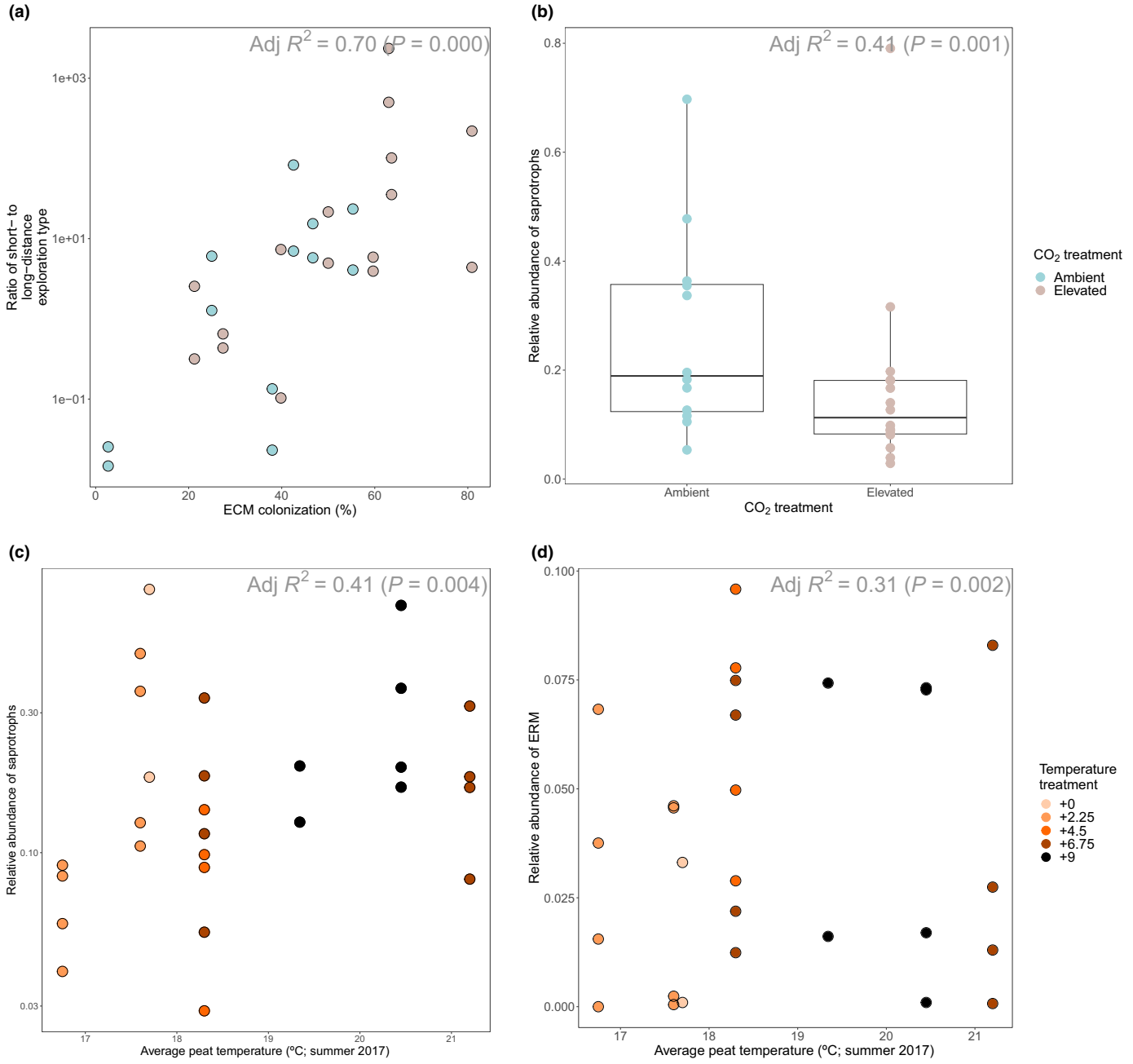


Fig. 4 Climate change drivers alter the functional composition of fungal communities in tree and shrub roots. Elevated CO_2 stimulates ectomycorrhizal fungal colonization (see Table 1), thereby (a) indirectly increasing the ratio of short- to long-distance ectomycorrhizal fungi exploration type while directly (b) decreasing the relative abundance of saprotrophs. Points represent samples from enclosures with elevated CO_2, or ambient CO_2. Peat temperature increased (c) the logged relative abundance of saprobes (d) and ericoid mycorrhizal fungi. Circles are colored by peat temperature treatment (black and orange hues) and CO_2 treatment (green and brown). Adjusted R^2 values for the full models (Table S5) and the P-value for the specific variable, as tested via analysis of variance, are identified in the top right of the figure.

this decrease in colonization is accompanied by a decrease in ECM diversity. When aggregated at the genus level, 70% of the ECM fungal genera were assigned an exploration type. The ECM fungi with the medium- and short-distance exploration types were the most abundant, with 28 and 20 genera, respectively. Only 12 ECM fungal genera were identified as long-distance type, and 11, the contact type. The ratio of short- to long-distance exploration types increased with increasing tree root colonization ($P < 0.001$; Table S5). Short-distance types increased by 6.8% ($P < 0.001$) in relative abundance with each % increase in ECM colonization of the roots. However, these same conditions suppressed long-distance exploration types by 7.1% per % increase in ECM colonization ($P = 0.010$) (Fig. 4a; Table S5).

In tree roots, the relative abundance of saprotrophs decreased by 77.4% with eCO_2 ($P = 0.001$; Fig. 4b; Table S5), while increasing by 18.7% ($P = 0.006$) per °C increase in peat temperature (Fig. 4c; Table S5). Saprobes were 284.2 times more abundant with each additional cm^3 H_2O cm^{-3} peat ($P = 0.043$; Figs S5, S6; Table S5). The final, best-fit model explained 41% of the variation in the relative abundance of saprobes. Peat temperature also increased the relative abundance of ERM fungi (Fig. 4d). In fact, for each °C increase in peat temperature, there was a −0.02 unit decrease in relative abundance of ERM fungi in shrub roots, explaining 31% of the variation in the relative abundance of ERM fungi of shrub roots (Table S5).

Discussion

Warming and eCO_2, will profoundly impact peatland ecosystem processes, as evidenced by pronounced impacts observed during the ongoing SPRUCE experiment including the rapid loss of *Sphagnum* (Norby *et al.*, 2019), the spread of vascular plants (Malhotra *et al.*, 2020; McPartland *et al.*, 2020), an increase in nitrogen and phosphorous availability (Iversen *et al.*, 2023), a change in OM quality toward increased availability of labile substrates (Wilson *et al.*, 2021), and the loss of carbon from the surface layer of the peat through respiration (Wilson *et al.*, 2016; Gill *et al.*, 2017; Hopple *et al.*, 2020). We found that these changes are accompanied by a rapid response in vascular plant root microbiomes, both directly and indirectly through variations in their host's fine-root traits.

According to our hypothesis (H1a), warming and associated drier conditions lead trees and shrubs to rely on direct resource uptake, as evidenced by higher allocation to root biomass and tips (trees), and reduced mycorrhizal fungi associations (Figs 2, 4d; Tables 1, S3, S5). Accompanying the rapid growth of shrub roots with warming, the relative abundance of ERM fungi decreases dramatically (Table S5). Further research estimating the degree of ERM fungal colonization is needed to enhance our understanding of shrub resource acquisition strategies. The loss of reliance on mycorrhizal fungi by trees and shrubs could be linked to the warming-induced rise in nutrient availability, a widespread phenomenon observed in a range of ecosystem types (Fernandez *et al.*, 2017; León-Sánchez *et al.*, 2018; Querejeta *et al.*, 2021). This has profound implications for ecosystems vulnerable to climate change, as mycorrhizal fungi can influence the dynamic of soil OM formation (Frey, 2019), particularly in boreal ecosystems where the main contributors of stored carbon are roots and root-associated microbes (Clemmensen *et al.*, 2013).

Conversely, although eCO_2 treatments began a year before our data collection, CO_2 fertilization is already increasing plant nitrogen demand, favoring ECM associations in trees and partly confirming hypothesis H1b (Tables 1, S5; Fig. 2). At SPRUCE, Malhotra *et al.* (2020) reported that eCO_2 could be causing nitrogen to become limiting to plant growth, as demonstrated by elevated C : N ratios and $\delta^{15}N$ values in shrub roots from eCO_2 enclosures. At the same site, Ofiti *et al.* (2022) observed increased nitrogen demand under eCO_2 and linked this phenomenon to the potential rise in plant and microbial activity associated with CO_2 fertilization. Furthermore, inorganic plant-available nitrogen was shown to be in limited in eCO_2 plots by 2018, a year following our experiment (Iversen *et al.*, 2023). Thus, an increase in ECM colonization of roots has the potential to further limit the supply of N to saprobes. The earlier discussion on the stimulation of saprotrophs by warming anticipates an increase in overall heterotrophic respiration and greenhouse gas emissions (Hopple *et al.*, 2020; Wilson *et al.*, 2021; Ofiti *et al.*, 2022), but the interplay of climate change drivers results in complex ecosystem-level responses. We suggest that the contrasting response (stimulation vs suppression) of saprotrophs to climate drivers is linked to changing relationships between plants and their symbiotic fungi, with implications for belowground carbon and nitrogen cycling (Iversen *et al.*, 2023; Ofiti *et al.*, 2022).

Contrary outcomes were observed for shrubs and trees, with CO_2 fertilization leading to a 64% increase in shrub root length density. This implies that, akin to warmer conditions, elevated CO_2 levels promote self-reliance in resource acquisition among shrubs. Under eCO_2, we anticipate dense distribution of shrub rhizosphere soil, encompassing the mycelium of rhizomorph-forming genera like *Suillus* and *Cortinarius*, due to their numerous, thinner roots (Table S3). Fungal rhizomorphs explore the soil away from the tree roots they colonize to mine the dense patches of peat in-between shrub roots. This may explain why these ECM fungal genera increase in abundance in the rhizosphere of shrubs under eCO_2 (Fig. 3), even if their relative abundance in tree roots does not increase with eCO_2 (Fig. 3; Table S5).

The relative abundance of saprotrophs increased with warming (Fig. 4c), which is consistent with our hypothesis H2, and with findings in northern peatlands and other systems (Maheshwari *et al.*, 2000; Romero-Olivares *et al.*, 2017; Allison *et al.*, 2018; Asemaninejad *et al.*, 2018; Looby & Treseder, 2018; Pec *et al.*, 2021; Querejeta *et al.*, 2021). One of the major mechanisms by which root exudates accelerate nutrient cycling is by stimulating the extracellular microbial enzymes that break down amino acids, amino sugars, and recalcitrant carbon pools (Meier *et al.*, 2017; Lei *et al.*, 2023). In fact, plant root traits such as root biomass, which we have shown increases with temperature (Table 1; Fig. 2), are tightly correlated to root exudation rates (Williams *et al.*, 2022; Rathore *et al.*, 2023). The increase in readily available carbon compounds from plants fuels both aerobic and anaerobic carbon respiration (Wilson *et al.*, 2021), which in parallel supports the activity of saprotrophic fungi. Under current climate conditions, peatlands already host a variety of saprotrophic taxa, particularly degraders of simple sugars and complex carbon compounds such as lignin and polyphenols, that are major contributors to the degradation of peat in the aerobic layer (Thormann, 2006, 2011; Asemaninejad *et al.*, 2017; Juan-Ovejero *et al.*, 2020). Our findings suggest that warming enhances fungal and saprobe diversity, likely mediating its impact on carbon storage over short and long time scales (Fanin *et al.*, 2022). Direct measurements of biomarkers at the SPRUCE site indicate higher rates of OM degradation associated with warming in the surface peat (Ofiti *et al.*, 2022). In turn, this enhances heterotrophic respiration and greenhouse gas emissions

(Hopple *et al.*, 2020; Querejeta *et al.*, 2021; Wilson *et al.*, 2021; Fanin *et al.*, 2022; Ofiti *et al.*, 2022).

Elevated CO_2 enhances plant associations with ECM and alters functional composition of the ECM fungal communities. We expected (H2) a greater prevalence of taxa with the long-distance exploration type under eCO_2, due to an increase in plant demand for nitrogen (Koide *et al.*, 2014; Defrenne *et al.*, 2021; Ofiti *et al.*, 2022), especially in an oligotrophic peatland, but found the opposite. Long-distance exploration types, such as members of the genus *Suillus*, identified in our dataset, have been shown to produce phenol oxidases (Agerer, 2001) and access OM bound nitrogen (Koide *et al.*, 2014; Shah *et al.*, 2016; Argiroff *et al.*, 2022). Taxa with short-distance strategies, such as members of the genus *Tomentella*, which we show to be stimulated by CO_2 fertilization, have greater abilities to take up inorganic nitrogen (Hobbie & Agerer, 2010; Defrenne *et al.*, 2019; Lilleskov *et al.*, 2019). Altogether, CO_2 fertilization promotes the reliance of trees on mycorrhizal associations for resource acquisition, whereby trees mostly associated with taxa that do not form rhizomorphs (hydrophilic, short-distance exploration types, that preferentially use labile forms of soil N), which is consistent with findings at the global scale (Terrer *et al.*, 2016).

Changes in ECM associations in response to climate change drivers have far reaching implications for peatland ecosystem function, particularly for respiration in the rooting zone, which is predicted to increase with warming (Wilson *et al.*, 2016; Gill *et al.*, 2017). Furthermore, the competition between saprotrophs and mycorrhizal fungi for soil resources such as nitrogen has been hypothesized to slow decomposition rates, a phenomenon called the Gadgil effect (Gadgil & Gadgil, 1975). In ecosystems where nutrients are scarce, as is the case at the SPRUCE experimental site (Hobbie *et al.*, 2017), fungi compete for limited labile carbon and nitrogen supplies (Kaye & Hart, 1997; Schimel & Weintraub, 2003). Thus, when nitrogen is limited, the presence of ECM can decelerate decomposition by suppressing saprotrophs (Averill & Hawkes, 2016; Mayer *et al.*, 2023). As ECM are supplied carbon by their host plants, they are shown to allocate more resources to nitrogen acquisition (Terrer *et al.*, 2016; Pellitier *et al.*, 2021). In accordance with this hypothesis, the rise in EMF diversity in response to eCO_2 is accompanied by a significant decline in saprobes (Fig. 4b). We propose that, as trees invest in greater mycorrhizal associations under eCO_2 conditions, the competition between ECM and saprobes also intensifies. This interaction has the potential to dampen the positive effect of warming on decomposition rates for the surface layers of peat.

Warming increased prokaryotic alpha diversity in tree and shrub roots as hypothesized (H3, Fig. S2). However, eCO_2 suppressed prokaryotic diversity, contrary to our H3 (Fig. S3). Considering the observed taxonomic shifts in prokaryotes with warming and eCO_2, or associated plant root traits, root-associated bacterial responses reflected the discussed changes in fungal functional composition, with warming stimulating and eCO_2 suppressing decomposers, respectively. In fact, in warmer enclosures, chemoorganoheterotrophs from the genera *Edaphobacter* (Koch *et al.*, 2008) and *Terrimicrobium* (Qiu *et al.*, 2014) were stimulated by increased root length density of shrubs, indicating an elevated carbon degradation potential in the root zone of vascular plants. The suppression of heterotrophs genera *Edaphobaculum* (Cao *et al.*, 2017) and *Rhizomicrobium* (Kodama & Watanabe, 2011) by eCO_2 (Fig. 4) suggests that bacteria undergo a similar change in function as fungi, and as they face competition with mycorrhizal fungi for nutrients.

Conclusion

High-latitude ecosystems are warming faster than those at lower latitudes (Bekryaev *et al.*, 2010), and northern peatlands will have to adapt to significant changes in water and nutrient regimes linked to climate feedbacks (Hanson *et al.*, 2020; Iversen *et al.*, 2023; Ofiti *et al.*, 2022; Petro *et al.*, 2023). Climate change drivers (warming, eCO_2) are having profound impacts on the microbial communities intimately associated with vascular plant roots, both directly and indirectly through their effects on plant root traits.

As peatlands experience warmer drier conditions, vascular plants increase their carbon allocation to root biomass and reduce associations with ECM and ERM. These changes are accompanied by an increase in the relative abundance of saprophytic fungi and heterotrophic bacteria. On the contrary, CO_2 fertilization appears to increase the demand for nitrogen (Ofiti *et al.*, 2022; Iversen *et al.*, 2023), favoring ECM associations in trees and suppressing saprobes. We propose that alterations in plant root traits and associated microbial communities are linked to the acceleration in belowground nutrient cycling at SPRUCE observed by Iversen *et al.* (2023) and Ofiti *et al.* (2022). Given the central role of microbial communities in decomposing soil OM in peatlands, their climate change responses could have far reaching implications for peatland carbon storage.

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Competing interests

None declared.

Author contributions

Experimental design: PJH and CMI; Environmental monitoring: PJH; Field sampling: CMI, AM and JC; Root data: AM, JC and CMI; Ectomycorrhiza data: CED, JC and CMI; Microbial data: KD, CP and JEK; Data analysis: KD, CED and JAMM; Writing: KD and CED and JEK. All authors commented on and edited the manuscript.

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Data availability

The raw amplicon sequences can be found under accession no. PRJNA907736 in the BioProject database (http://ncbi.nlm.nih.gov/bioproject). The remaining data that support the findings of this study are available from the SPRUCE long-term data repository (https://mnspruce.ornl.gov/datasets/). Specifically, the environmental data is at doi: 10.3334/CDIAC/spruce.032, and the root data is at doi: 10.25581/spruce.077/1607860.

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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 Principal component analysis of shrub fine-root traits.

Fig. S2 Overall diversity trends in tree root and rhizosphere microbiome.

Fig. S3 Overall diversity trends in shrub root and rhizosphere microbiome.

Fig. S4 Differential abundance of bacterial genus with factors influencing the species richness and composition of shrub roots.

Fig. S5 Predicted logged relative abundance of saprobes over the range of moistures while holding all other variables in the model equal (Table S7).

Fig. S6 Microbial community structure of the fine roots and rhizospheres of trees and ericaceous shrubs at the SPRUCE experiment.

Notes S1 Supplementary results detailing the general microbial community composition and response of the microbial community to microtopography.

Table S1 Division of ingrowth core A and B, and measurements taken on each core section.

Table S2 Primers and PCR conditions used for amplicon analysis.

Table S3 Best-fit model of the impact of climate change drivers on root traits of shrubs.

Table S4 Best-fit models of the impact of root traits and climate change drivers on bacterial and fungal richness in trees and shrubs.

Table S5 Best-fit models of the impact of root traits and climate change drivers on fungal mycorrhiza, their exploration types, and saprotrophs.

Table S6 Best-fit distance-based redundancy analysis of microbial community composition.

Table S7 Best-fit model of the microbial diversity differences between trees and shrubs.

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