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RESEARCH ARTICLE

Long-term soil warming alters fine root dynamics and morphology, and their ectomycorrhizal fungal community in a temperate forest soil

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Abstract

Climate warming is predicted to affect temperate forests severely, but the response of fine roots, key to plant nutrition, water uptake, soil carbon, and nutrient cycling is unclear. Understanding how fine roots will respond to increasing temperature is a prerequisite for predicting the functioning of forests in a warmer climate. We studied the response of fine roots and their ectomycorrhizal (EcM) fungal and root-associated bacterial communities to soil warming by 4°C in a mixed spruce-beech forest in the Austrian Limestone Alps after 8 and 14 years of soil warming, respectively. Fine root biomass (FRB) and fine root production were 17% and 128% higher in the warmed plots, respectively, after 14 years. The increase in FRB (13%) was not significant after 8 years of treatment, whereas specific root length, specific root area, and root tip density were significantly higher in warmed plots at both sampling occasions. Soil warming did not affect EcM exploration types and diversity, but changed their community composition, with an increase in the relative abundance of *Cenococcum* at 0–10 cm soil depth, a drought-stress-tolerant genus, and an increase in short- and long-distance exploration types like *Sebacina* and *Boletus* at 10–20 cm soil depth. Warming increased the root-associated bacterial diversity but did not affect their community composition. Soil warming did not affect nutrient concentrations of fine roots, though we found indications of limited soil phosphorus (P) and potassium (K) availability. Our findings suggest that, in the studied ecosystem, global warming could persistently increase soil carbon inputs due to accelerated fine root growth and turnover, and could simultaneously alter fine root morphology and EcM fungal community composition toward improved nutrient foraging.

KEYWORDS
bacterial community, climate warming, ectomycorrhiza, exploration types, fine root biomass, fine root morphology, fine root production, nutrients

1 | INTRODUCTION

Tree fine roots (<2 mm in diameter) represent less than 2% of the total biomass in forests but contribute up to 33% to the global terrestrial net primary productivity (Jackson et al., 1997; McCormack et al., 2015). Fine roots are considered a major source of plant carbon (C) input into temperate forest soils, and their biomass growth, traits, turnover, as well as their exudation of labile C are important components in soil C cycling and storage (Keller et al., 2021; Rasse et al., 2005). In temperate and boreal forests, fine roots are typically colonized by EcM mycelium (Tedersoo et al., 2012). The symbiosis of roots and EcM fungi serves as an efficient nutrient foraging strategy for trees, especially in nutrient-poor forest soils (Lõhmus et al., 2006). The mycelium of EcM fungi greatly improves access to nutrient and water resources in soils that are hardly directly accessible for fine roots (Lindahl & Tunlid, 2015; McCormack & Iversen, 2019).

Climate warming is predicted to severely affect temperate forests during the 21st century (IPCC, 2021), and thus likely also the uptake and transport of water and nutrients by fine roots. Changes in fine root functions and the diversity and functional traits of EcM fungi may alter whole ecosystem C and nutrient cycles. Developing an efficient root system through change of biomass allocation to fine roots, changes in fine root morphology and root-associated microbial communities is required to maintain plant water and nutrient uptake under changing environmental conditions (Ostonen et al., 2011). Trees can adapt their belowground surface area by modifying root biomass and fine root morphological traits to improve soil resource uptake and plant performance (Weemstra et al., 2020). FRB is driven by the balance between root growth and mortality and depends on environmental conditions, especially soil temperature and soil moisture (Salazar et al., 2020; Wang et al., 2021). Soil warming has been suggested to increase FRB due to decreased soil nitrogen (N) availability (Leppälammi-Kujansuu et al., 2013) but also to decrease FRB in temperate and boreal forests due to increased soil N availability (Dawes et al., 2015; Melillo et al., 2011; Wan et al., 2004; Zhou et al., 2011). The inconsistent findings across forest biomes illustrate the complexity of the interaction between increased soil temperature, N availability, and FRB (Wang et al., 2021). However, with ongoing high atmospheric N deposition in many forested regions, such as central Europe (Borken & Matzner, 2004; Talkner et al., 2019), where the current study took place, the availability of essential nutrients such as phosphorus (P) and potassium (K) may likely play a similar, or even a more important role, with regard to tree fine root responses to warming. Decreasing foliar P and K indicate poor availability of these nutrients in many European forests in recent decades (Jonard et al., 2015; Penuelas et al., 2020; Talkner et al., 2015), though the impact of soil warming in combination with low P and K availability on fine root systems is unknown.

Morphological traits of fine roots are indicators of trees' nutrient uptake efficiency and ecosystems' responses to changes in environmental conditions (Freschet et al., 2021; Ostonen et al., 1999). Among those, traits such as specific root length (SRL, the length of a root per unit dry mass), root tissue density (RTD, the dry mass of root per unit volume of fresh root), specific root area (SRA, the ratio of fine root surface to fine root dry mass), root area index (RAI, the surface area of roots per soil surface area), mean root diameter (D, the average of all root diameter observations of a root diameter distribution), and root tip density (RTID, the number of root tips per soil volume) are used to describe the functional characteristics of fine roots (Freschet et al., 2021; McCormack & Iversen, 2019). For example, an increase in SRL indicates morphological adaptation toward thinner and longer fine roots, which are less resistant to stress and have a shorter lifetime but are more active metabolically (McCormack et al., 2015). This is seen as a strategy to increase nutrient acquisition from the soil at low biomass production (Weemstra et al., 2020). An increase in RAI and SRA also indicates adaptation toward increased ability to explore and take up soil resources. However, only few studies have yet considered the effect of soil warming on fine root morphology in forest ecosystems (Wang et al., 2021). In their meta-analysis, Wang et al. (2021) found that SRL and fine root diameter were irresponsive to experimental warming across a wide range of terrestrial ecosystems. Of the few studies conducted in forests, Parts et al. (2019) showed that trees increased their SRL and SRA with soil warming but decreased RTD. Björk et al. (2007) reported that warming increases SRL and SRA of roots <0.5 mm, while Leppälammi-Kujansuu et al. (2013) observed no effect on RTD and diameter of first- and second-order fine roots. Hence, similar to FRB, fine root morphology can respond differently to soil warming and associated changes in nutrient availability or moisture.

Due to the interaction between fine root traits and soil biota, understanding the response of the fine root systems to soil warming also requires studying root–microbial interactions (Bennett & Classen, 2020; Weemstra et al., 2017). EcM and root-associated bacteria are particularly important because they produce extracellular enzymes that release nutrients from soil organic matter in the vicinity of fine roots (Averill & Hawkes, 2016; Averill et al., 2014). The diversity of EcM and root-associated bacteria might further increase the effectiveness of nutrient acquisition from different depths and locations in the soil (Leake, 2001). However, changes in root-associated microbial communities have received little attention in the context of soil warming. Because of the temperature dependence of soil enzymatic activities, soil warming directly influences nutrient cycling processes (Staddon et al., 2002). This may lead to shifts in EcM community composition (Solly et al., 2017; Treseder et al., 2016) and EcM diversity. Mycorrhizal associations greatly enhance the roots' surface area and, therefore, their water and nutrient uptake capacity (Smith & Read, 2008; Weemstra, 2017). EcM fungal traits like exploration types are good predictors of ecosystem processes because they integrate species functions in functionally redundant communities (Koide et al., 2014). Exploration type is a trait that connects the morphology and differentiation of ectomycorrhizal hyphae to differences in nutrient acquisition strategies (Defrenne et al., 2019). Agerer (2001) classified EcM into exploration types and assigned specific functions to those. For example, EcM with contact, short, and medium distance exploration types (low EcM biomass) might be preferred at sites with high N availability

(Hobbie & Agerer, 2010), while long-distance exploration types (high EcM biomass) might be necessary in nutrient-poor sites (Tedersoo, 2017). Thus, EcM exploration types likely are going to be affected by warming if elevated temperatures alter the availability of soil nutrients or the nutrient requirement of trees.

The effect of soil warming on fine root dynamics and root-associated bacterial–fungal communities in forests has largely remained unresolved, as most studies were short in terms of duration and differed in experimental approaches (Wang et al., 2021; Yuan et al., 2018). The long-term forest soil warming experiment at Achenkirch in the Austrian alps, where regional climate models predict an above-average increase in temperature (up to 3.3°C at the end of the 21st century) as compared to global average warming (Gobiet et al., 2014; Smiatek et al., 2009), offers an excellent opportunity to increase our understanding of how soil warming affects FRB, fine root production, fine root morphology, and root-associated bacterial and fungal communities in temperate forests. To achieve this, we studied fine roots in control and warmed (+4°C) plots in 2012 and 2019, 8 and 14 years after starting the experimental soil warming treatment, respectively. We hypothesized that (1) soil warming increases FRB after 8 or 14 years because of the globally positive response of FRB to warming (Wang et al., 2021) and the potential decline in soil P and K availability in warmed soil. We also expected (2) increases in SRL, SRA, and root tip density in warmed soil, which are linked to an increase in the absorptive capacity of fine roots. We did not expect significant changes in root-associated microbial community composition because previous results showed no soil warming effects on soil and root microbial community composition (Kuffner et al., 2012; Schindlbacher et al., 2011). However, because exploration types are directly linked to fine root morphology, we expected (3) a shift in the relative abundance of EcM exploration types toward long-distance exploration types after 14 years of soil warming.

2 | MATERIALS AND METHODS

2.1 | Study location and experimental design

This study was performed in the Achenkirch soil warming experiment located in a mountainous forest in the Austrian Alps (11°38′21″ E; 47°34′50″ N) at 910 m a.s.l. (Schindlbacher et al., 2007). The 140-year-old forest is composed mainly of Norway spruce (*Picea abies* L. H. Karst.) and few other less abundant trees species, including European beech (*Fagus sylvatica* L.) and silver fir (*Albies alba* Mill.). The mean annual air temperature and mean annual precipitation from 1988 to 2017 were 7°C and 1493 mm, respectively. The soil type was classified as a shallow Rendzic Leptosol. It consists of thin organic L and F horizons, a mineral A-horizon with about 15–20 cm thickness, with a bulk density of ~0.5 g cm^{-3}, and an underlying C-horizon deriving from dolomite. The carbonic A-horizon had a pH of ~7, a C:N ratio between 15 and 18, and stored ~120 Mg ha^{-1} of organic C. The L/F-horizons stored about 10 Mg C ha^{-1}. Based on the "Austrian bioindicator grid" analyses (https://bfw.ac.at/rz/bfwcms2.web?dok=3687), average element concentrations of live Norway spruce needles for the period 2000–2005 were 12.1 mg N g^{-1}, 0.94 mg P g^{-1}, and 3.5 mg K g^{-1}. Further site details have been published elsewhere (Herman et al., 2002; Schindlbacher et al., 2011).

The soil warming experiment comprised six blocks of paired 2 × 2 m plots (each pair consisting of one control and one warmed plot), established in 2004 (n = 3) and 2007 (n = 3). Six plots were warmed (hereafter termed warming treatment) using heating cables (Etherma, Salzburg, Austria) installed at 3 cm soil depth and at a distance of 7.5 cm. In the other six plots (hereafter termed control treatment), heating cables were installed, but not heated to account for the disturbance created by their installation. The heating system was controlled by a service unit that automatically kept a 4°C difference between the control and warming treatment throughout the snow-free period (April–December). On a half-hourly interval, soil temperature was recorded at 5 and 15 cm mineral soil depth. A detailed description of the experimental setup is given in Schindlbacher et al. (2007, 2009).

2.2 | Fine root sampling and processing

The sampling of fine roots (<2 mm in diameter) took place within one day at the end of the growing season in October 2012 and October 2019 using soil corers of 5 cm diameter and 20 cm in length. Three control and three warmed plots (warmed since 2004) were sampled in October 2012 (n = 3), whereas all 12 plots were sampled in October 2019 (n = 6). Ten soil cores were randomly taken from 0 to 10 and 10 to 20 cm soil depth in each plot. The sampling depth was less than 20 cm at a few sampling points because of the shallow soil and the dolomitic bedrock. In total, 120 samples were taken in 2012 and 240 samples in 2019. Samples were immediately transferred into zip lock plastic bags, stored in cooling boxes filled with ice packs, and transported to Bayreuth, Germany, for further processing.

In the laboratory, the soil cores were stored in a +2°C climate chamber and processed within a maximum of 3 weeks. The complete processing protocol included fine root washing, sorting into live and dead roots under a microscope, and finally scanning with a flatbed scanner. Roots were separated from the soil by wet sieving. We used a 0.63 mm sieve (Retsch Technology GmbH, 42781 Haan, Germany) to process the soil cores by hand using tap water until all soil and other impurities were removed from the fine root fraction. After washing, stones were picked using tweezers, dried in small aluminum dishes, and weighed to correct soil mass. Roots were then transferred into 500 ml glass beakers filled with water and ice and washed in an ultrasonic bath to remove residual soil particles attached to the fine roots. Right after washing, fine roots were sorted into a live and dead fraction under a binocular microscope. We used the morphological characteristics described in Wu (2000) and Burke and Raynal (1994). Herbaceous roots (white, light, and succulent) were excluded during sorting. Live roots were resilient and flexible, reddish, with several lateral root tips, while dead roots were soft,

dark, and easily breakable. After separation, the dead root fraction was dried in an oven at 60°C for 3 days, and the dry weight on a soil area basis (g m^{-2}) was determined.

Fine root production was measured using ingrowth cores. In October 2019, after the soil coring described above, five ingrowth polypropylene mesh tubes per plot (5 cm diameter, 20 cm long, 6 mm × 4 mm mesh) were inserted into the cored soil holes. They were filled with root-free mineral sieved soil deriving from the same study site. Soil bulk density was adjusted to ~0.5 g cm^{-3} (see above) by alternating filling and compaction of the soil using a funnel and a piston. After 12 months, the ingrowth cores were sampled, and roots grown within a year were processed as described above. Fine root production was calculated on a soil area basis (g m^{-2} $year^{-1}$). We also estimated absorptive fine root biomass (aFRB) by multiplying mean root tip weight (see next paragraph for detailed information) by root tip number per m^2 (Ostonen et al., 2017).

2.3 | Fine root morphology

The live fine root fraction was scanned in 20 × 25 cm transparent trays filled with cold water using a flatbed scanner (EPSON Perfection V700; SEIKO EPSON CORP, Japan) with the following setting: scanning resolution of 400 dpi; pixel classification method based on grey level and dark root on white background. Care was taken to avoid root overlap within the tray, and images were analyzed using the software WinRhizo™ Reg 2008 (Regent Instruments Inc., Canada). Due to the many replicates, we scanned on average 80% of fine roots collected at 0–10 cm depth, while the complete sample collected at 10–20 cm depth was scanned. Images were analyzed for fine root length (cm), average diameter of the fine roots (mm), fine root volume (cm^3), the number of root tips, and surface area (cm^2). Based on those basic parameters, the following morphological fine root traits were calculated: specific root length, expressed as the ratio of root length to dry mass (SRL; m g^{-1}); specific root area, expressed as the ratio of root surface to dry mass (SRA; cm^2 g^{-1} d.w); root area index, expressed as the ratio of root surface to soil surface (RAI; m^2 m^{-2}); root branching intensity, expressed as the number of root tips per root length (RBI; tips cm^{-1} root length) and root tip density, the number of root tips per soil volume (RTID; tips m^{-3} soil). We recalculated root volume as the sum of all diameter class' averages (Freschet et al., 2021; Rose, 2017) and estimated RTD as the ratio of root dry mass to root volume (RTD; g cm^{-3}). Right after scanning, root tips were excised and dried at 60°C for 2 days to determine root tip weight (RTW; mg). Mycorrhizal root colonization was determined under the microscope by random examination of intact root fragments. The roots to be examined were placed in a petri dish, and using different magnification levels allowed careful examination of the presence of emanating hyphae and rhizomorphs. Roots colonized by EcM were sampled using scalpels (ca. 20–30 root tips per samples), transferred into 5 ml Eppendorf® tubes, and stored at −24°C until DNA extraction. The live fine root fraction was finally dried in an oven at 60°C for 3 days to determine fine root mass. Root biomass was expressed on a soil area basis (g m^{-2}).

2.4 | Fine root chemistry

Dried fine root samples were ground using a ball mill (MM 400; Retsch Technology GmbH). Fine root chemistry was assessed on fine roots sampled in 2019. Element concentrations of P, Na, K, Ca, Mg, Fe, and Mn were determined after 65% HNO_3 digestion in a microwave device (Mars 5, CEM, Germany). For the digestion, 8 ml 65% HNO_3 was added to 100 mg dried and ground fine roots. Digests were then transferred to 50 ml volumetric flasks, diluted with deionized water, and filtered through nylon filters (0.45 µm). Element concentrations in the extracts were determined using ICP-OES (Optima 3200 xl; Perkin Elmer, Germany) and AAS (SpectraA 220 Z; Varian, USA). The organic C and total nitrogen (TN) concentrations were analyzed with an elemental analyzer (EA1110; CE Instrument, Italy).

2.5 | DNA extraction and amplicon barcoding

We extracted DNA from 48 samples collected during the second fine root sampling in 2019 (two technical replicates per plot and soil depth, 10 root tips per sample) using the ChargeSwitch® gDNA plant kit (Invitrogen™; Carlsbad, USA) following the manufacturer's instructions. Amplicon libraries were prepared in two consecutive PCR runs using a liquid handling workstation (epMotion® 5075; Eppendorf, Hamburg) and a thermocycler with a high-pressure lid (peqstar 384X HPL). Amplicon libraries were prepared of the V3 and V4 regions of the 16S rRNA gene as recommended by Illumina (2013) and otherwise processed as detailed in the following for fungi. The fungal ITS2 region was first (PCR1) amplified from the DNA extracts using the primers gITS7 (Ihrmark et al., 2012) and ITS4g ("CGCTTATTGATATGCTTAAGT", as modified after Schlegel et al. (2018)). The PCR1 primers ("nGS grade," Metabion, Planegg, Germany) included the fungus-specific sequences prepended by "Tags" (barcode sequences for sample identification of 4–7 bp in length, Table S3) and the recommended overhang adapter sequences (https://support-docs.illumina.com/SHARE/AdapterSeq/illumina-adapter-sequences.pdf). The reactions were conducted in a total volume of 4 µl, including the primers (370 nM, each), 2 µl OneTaq® 2X Master Mix (NEB, Frankfurt am Main), and 0.2 µl DNA extract. The thermocycler kept the prevailing 95°C for another 2 min after the reaction mixes were inserted. Amplification was achieved in 25 cycles of 94°C for 20 s, 45°C for 40 s, and 68°C for 55 s, followed by a final extension at 68°C for 7 min. Purification of the PCR1 products was achieved by adding 3.1 µl of a mixture of Exonuclease (3.2 U) and Shrimp Alkaline Phosphatase (0.32 U, both NEB, Frankfurt am Main) to each PCR1 product and incubating the total volume of 7.1 µl at 37°C for 25 min, followed by 80°C for 15 min. Index (barcode sequences of 8 bp in length) and adapter sequences (P5 and P7, respectively) were appended to the PCR1 products in the second

PCR (PCR2), utilizing the overhang adapter sequences (Table S4). The reactions were conducted in a total volume of 8 μl, including the primers (270 nM, each), 4 μl OneTaq® 2X Master Mix, and 2.5 μl of purified PCR1 product. Equal volumes (1 μl) of root-derived fungal and bacterial PCR2 products were pooled separately. The two pools were purified using CleanPCR® Nucleic acid Clean up (CleanNA, GC biotech B.V.) as recommended by the manufacturer. To discriminate against short fragments, such as primer dimers, CleanPCR® beads were applied in a volume corresponding to 70% of the volume of the pooled PCR2 products. The sequencing service of the Faculty of Biology at LMU Munich, Germany, assessed the DNA concentration (Qubit® 2.0 fluorometer; Life Technologies) and the amplicon size distribution (Bioanalyzer 2100; Agilent Technologies GmbH & Co. KG), before sequencing using an Illumina MiSeq® sequencer (Illumina, Inc.) with 2 × 300 bp paired-end sequencing (MiSeq Reagent Kit v3 Chemistry; Illumina, Inc.). Sequence reads were deposited in the European Nucleotide Archive (http://www.ebi.ac.uk/ena/data/view/PRJEB48843).

2.6 | Sequence data processing

Sequence reads were pre-sorted by the sequencer according to the inserted dual index sequences, that is, each combination including a unique forward and a unique reverse index. Within index combinations, reads were assigned to samples according to dual index combinations, using the open-source software QIIME version 1.9.0 (Caporaso et al., 2010). During demultiplexing, only reads with at most one ambiguity base were retained, and a quality filter (threshold phred 19) was applied: reads were truncated after nine consecutive low-quality base calls and only retained if at least 35% of the entire sequence consisted of consecutive high-quality base calls. Only the R1 reads were further processed. Reads were grouped according to the lengths of the tag-sequences and length adjusted using the FastX toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). CD-HIT-OUT (Li & Chang, 2017) was used for de novo clustering sequence reads to operational taxonomic units (OTUs) based on 97% sequence similarity. Sequences representing an OTU were assigned to taxa using QIIME and the UNITE database v8 (Kõljalg et al., 2013) as a reference. Initially, unassignable OTUs were assigned as detailed by Peršoh et al. (2010). A table coding the read counts per sample and OTU was generated and rarefied to 1646 reads per sample using the *rrarefy* function of the R package *vegan* (Oksanen et al., 2019). For improved comprehensibility, the OTU identification number is prepended by the assigned genus name in the following.

According to their taxonomic affiliation, fungal OTUs were assigned to functional guilds, as indicated by Agerer (2006), Cannon and Kirk (2007), and Kirk et al. (2011) and the Faces of Fungi (Jayasiri et al., 2015) and FUNGuild (Nguyen et al., 2016) databases. Taxonomic and functional assignments were verified by species-level identification or sequence comparison for abundant and discriminative OTUs, in particular for OTUs assigned to the genus *Sebacina*. Assignment and sequences of the OTUs from identified mycorrhiza taxa are provided in Table S7. Subsequent analyses were exclusively based on this (re-standardized) subset of mycorrhizal fungi. From the bacterial dataset, sequences of plant plastids were removed before normalization.

2.7 | Statistical analysis

All statistical analyses and graphs were performed using R version 4.0.3 (R Core Team, 2020). The R packages *ggplot2* (Wickham, 2016) and *gridExtra* (Auguie, 2017) were used for data visualization. Potential outliers in the data set were first identified visually with boxplots and then tested with Rosner's test using the package *EnvStats* (Millard, 2013). Root traits were checked for normality using the Shapiro–Wilk test and, when needed, were square-root transformed before analysis to meet the assumption of normality. Because of the layout of our control and warmed plots in pairs, we tested the effect of warming on FRB, necromass, morphological traits, and nutrient contents with paired *t* tests ($\alpha = 0.05$ was used as the significance level).

We used Fisher's alpha, Shannon–Wiener, and Pielou's evenness to characterize EcM fungal and root-associated bacterial communities at the two soil depths. Fisher's alpha is a parametric diversity index that assumes that the species abundance distribution follows a log series distribution. It was calculated as $S = a \times \ln(1 + n/a)$; with S, the number of taxa, n is the number of individuals, and a is the Fisher's alpha. The Shannon–Wiener index considers the number of individuals as well as the number of taxa. It varies from 0 for communities with a single taxon to high values for communities with many taxa. It was calculated as $H' = -\sum \left[P_i \log\left(P_i\right)\right]$, where P is the proportion of the total count arising from the ith species. Pielou's evenness, finally, measures the diversity along with species richness, that is, how evenly the individuals are distributed amongst species, and was calculated as follows: $J' = H'/\log(S)$; where S is the number of species and H' is the Shannon–Wiener index. It varies from 0 (no evenness) to 1 (complete evenness). A detailed description of those diversity measures is given in Clarke et al. (2014).

We used linear mixed-effects models to test the effect of treatment and soil depth on fungal and bacterial diversity using the package *lme4* (Bates et al., 2015). Treatment and soil depth were fixed factors and plot, inserted as random factor. Post-hoc pairwise comparisons among groups were conducted using the package *emmeans* version 1.5.4 (Lenth, 2021). To test the effect of treatment and soil depth on fungal and bacterial communities, we used permutational multivariate analysis of variance (PERMANOVA) based on Bray–Curtis dissimilarity implemented in the *adonis* function of the package *vegan*, version 2.5-6 (Oksanen et al., 2019). The contribution of individual OTUs to the dissimilarity between control and warming treatments was evaluated using the similarity percentages breakdown (SIMPER) procedure implemented in the *simper* function of the package *vegan*. We further grouped EcM fungal OTUs into different exploration types, based on Agerer (2001, 2006): contact; contact or medium-distance; short distance; contact, short or medium

distance; medium distance and long distance. Because contact or medium and contact, short or medium distance, were rare, we reclassified exploration types into short distance (contact; short distance) and long distance (medium distance and long distance) types. Paired *t* tests were performed to test for differences in exploration types between control and warming treatments.

We used the classification method program (CLAM) implemented in the package *vegan* to evaluate the preference of OTUs for either control or warming treatments. The CLAM statistical approach uses a multinomial model to classify OTUs in either generalist (i.e., well distributed between the two treatments) or specialist species (preference in one treatment) based on their relative abundance in the different treatments (Chazdon et al., 2011).

We carried out principal component analysis (PCA) to assess the interrelation between fine root traits, soil nutrients, EcM exploration types, and bacterial and fungal diversity indices using the R package *FactoMinerR* (Lê et al., 2008) and *factoextra* (Kassambara, 2017). The variation of the EcM fungal community with treatment and soil depth was visualized with non-metric dimensional scaling (NMDS) using the function *metaMDS* of the package *Vegan*.

3 | RESULTS

3.1 | Fine root biomass, necromass, and fine root production

Total FRB increased by 17% (from 355 to 414 g m^{-2}) by soil warming in 2019 (Figure 1). The effect of soil warming on FRB was stronger at 10–20 cm (+34%) than at 0–10 cm soil depth (+12%); however, the absolute increase was greater at 0–10 cm soil depth. Soil warming decreased fine root necromass by 14% in 2019 (from 85 to 74 g m^{-2} for control and warmed plots, respectively). Absorptive fine root biomass measured in 2019 increased by 22% in warmed plots (76.6 and 93.4 g m^{-2} in control and warmed plots, respectively) and represented approximately 23% of the total FRB at 0–20 cm soil depth, irrespective of treatment (Table 1).

During the first sampling campaign in 2012, mean FRB amounted to 513 and 582 g m^{-2} in control and warmed plots, respectively. Soil warming had no significant effect, although there was a trend to higher root biomass (+13%) in the warmed plots (Figure 1). We also found that FRB decreased with soil depth on both occasions. In comparison to 2019, soil warming did not affect fine root necromass in 2012 (Table 1).

In the warmed plots, fine root biomass production in ingrowth cores increased strongly, by 128% from 99 to 225 g m^{-2} $year^{-1}$ between October 2019 and October 2020 (Figure 2). While newly produced roots showed no changes in average diameter, SRL and SRA, their root tissue density and root tip density increased by 11% and 168%, respectively, in the warmed plots (Figure 2).

3.2 | Fine root morphology

In 2019, morphological analyses of fine root samples revealed that soil warming increased SRL, SRA, RAI, RTID, and RTW. The average diameter of fine roots was also affected, especially at 0–10 cm (Table 1). There was an average increase in SRL by 29% under warming conditions. SRA showed a similar trend and increased in warmed plots (311 and 368 cm^2 g^{-1}, under control and warming conditions, respectively, for both depths). An increase in SRA from 156 to 193 and 163 to 177 cm^2 g^{-1} was observed at 0–10 cm and 10–20 cm soil depth in the control and warming treatment, respectively. Soil warming increased RAI significantly by 21% at both soil depths (from 4.7 to 5.7 m^2 m^{-2}). Mean fine root diameter increased by soil warming at 0–10 cm depth (0.51 and 0.60 mm, in control and warmed

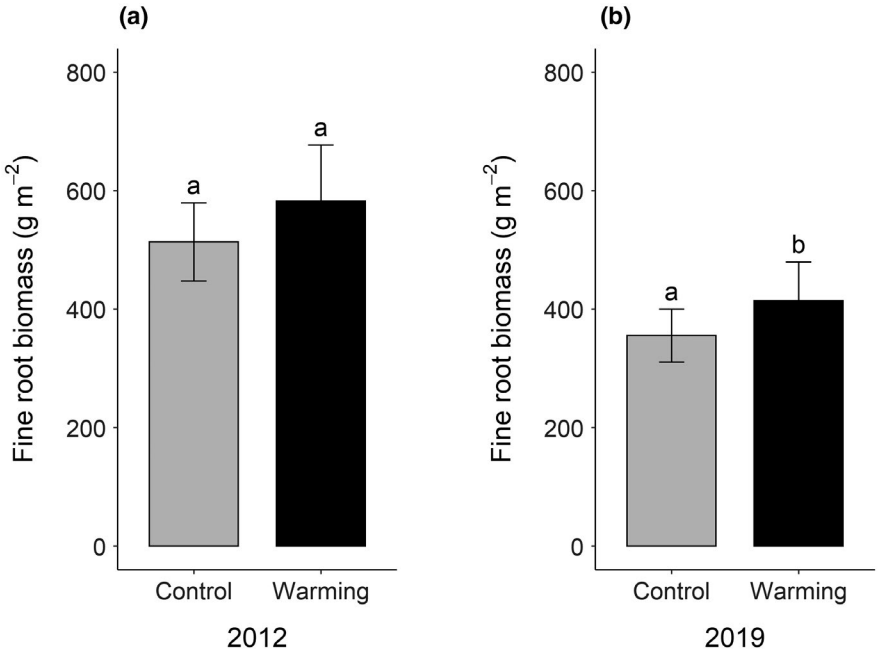

FIGURE 1 Mean (± SE) fine root biomass at 0–20 cm soil depth in the control and the warming treatments in (a) October 2012 ($n = 3$) and (b) 2019 ($n = 6$). Lowercase letters indicate significant differences between treatments (*t* test; $p < .05$)

TABLE 1 Means ± SE of absorptive fine root biomass, fine root necromass, and morphological traits of live roots at 0–10 and 10–20 cm soil depth in control and warmed plots in 2012 ($n = 3$) and 2019 ($n = 6$). Different letters indicate significant differences between control and warming treatments, tested separately for each soil depth (t test; $p < .05$)

		2012		2019	
Fine root traits	**Depth**	**Control**	**Warming**	**Control**	**Warming**
aFRB (g m^{-2})	0–10 cm	–	–	64.2 ± 18.8 a	80.2 ± 10.6 b
	10–20 cm	–	–	12.3 ± 3.9 a	13.12 ± 3.3 a
FRN (g m^{-2})	0–10 cm	294 ± 13 a	293 ± 29 a	67 ± 12 a	45 ± 6 b
	10–20 cm	96 ± 16 a	114 ± 58 a	18 ± 4 a	29 ± 7 b
Diameter (mm)	0–10 cm	0.46 ± 0.06 a	0.46 ± 0.07 a	0.51 ± 0.02 a	0.60 ± 0.07 b
	10–20 cm	0.44 ± 0.08 a	0.40 ± 0.02 a	0.49 ± 0.02 a	0.47 ± 0.03 a
SRL (m g^{-1})	0–10 cm	14 ± 1 a	16 ± 3 b	11 ± 1 a	14 ± 1 b
	10–20 cm	14 ± 1 a	17 ± 3 b	12 ± 2 a	15 ± 3 a
RTD (g cm^{-3})	0–10 cm	1.5 ± 0.8 a	0.9 ± 0.2 b	0.42 ± 0.04 a	0.43 ± 0.04 a
	10–20 cm	1.3 ± 0.4 a	1.5 ± 0.3 a	0.43 ± 0.04 a	0.41 ± 0.02 a
SRA (cm^2 g^{-1})	0–10 cm	124 ± 46 a	174 ± 16 b	156 ± 17 a	193 ± 21 b
	10–20 cm	68 ± 28 a	172 ± 33 b	163 ± 24 a	177 ± 27 b
RAI (m^2 m^{-2})	0–10 cm	5.2 ± 2.1 a	6.0 ± 0.7 a	3.7 ± 0.5 a	4.4 ± 0.2 b
	10–20 cm	1.2 ± 0.3 a	1.5 ± 0.6 a	1.1 ± 0.2 a	1.4 ± 0.4 b
RTID (10^5 × tips m^{-3})	0–10 cm	154 ± 55 a	190 ± 35 b	80 ± 11 a	136 ± 18 b
	10–20 cm	39 ± 7 a	47 ± 16 b	21 ± 4 a	28 ± 7 b
RBI (Tips cm^{-1})	0–10 cm	3.0 ± 1.1 a	3.7 ± 0.2 b	3.3 ± 0.2 a	3.6 ± 0.1 b
	10–20 cm	3.0 ± 1.0 a	3.7 ± 0.3 b	3.0 ± 0.1 a	3.2 ± 0.3 a
RTW (mg)	0–20 cm	–	–	0.063 ± 0.009 a	0.053 ± 0.005 b

Abbreviations: aFRB, absorptive fine root biomass; FRN, fine root necromass; RAI, root area index; RBI, root branching intensity; RTD, root tissue density; RTID, root tip density; RTW, root tip weight; SRA, specific root area; SRL, specific root length.

plots, respectively), but no effect was observed at 10–20 cm soil depth. RBI and RTID also increased in warmed plots (Table 1). The most significant increase of the latter two was observed at 0–10 cm soil depth. RTD was not affected at 0–10 cm depth (0.42 and 0.43 g cm^{-3}, in the control and warming treatment, respectively). Similar fine root morphological patterns were also observed in 2012. SRL, SRA, and RTID significantly increased by 11%, 43%, and 23%, respectively. On the other hand, fine root diameter and RAI were not affected by soil warming in 2012 (Table 1).

We observed an increase in the proportion of fine root length in the 0–0.2 mm diameter class in the warming treatment (Figure S1). We also noted a decrease in the proportion of fine root length with increasing diameter of fine roots, where warming became non-significant. Root length of the first diameter class (0–0.2 mm) accounted for up to 52% of the total root length, irrespective of treatment and soil depth. The first three diameter classes (0–0.2, 0.2–0.4, and 0.4–0.6 mm) contributed about 86% to the total fine root length in both treatments (Figure S1).

3.3 | Nutrient concentrations in fine roots

The concentration of the macronutrients N, P, K, and Mg did not differ between control and warmed plots, although they showed a tendency to decrease with soil warming (Table 2). However, C and Ca concentrations significantly decreased by 5% and 28% with soil warming at 0–10 and 10–20 cm soil depth, respectively. The concentration of the micronutrients Fe and Mn was not affected by soil warming, except Na, which decreased by 40% at 10–20 cm soil depth.

3.4 | Response of ectomycorrhizal fungal and bacterial communities to warming

A total of 92 EcM fungal OTUs were detected across root tips of all samples. *Hysterangium*, *Sebacina*, *Tricholoma*, and *Russula* were the most abundant genera in fine roots from the upper soil layer of the control plots. *Cortinarius*, *Sebacina*, *Lactarius*, *Helvellosebacina*, *Pachyphlodes*, *Trichophaea*, and *Inocybe* were most abundant at 10–20 cm depth (Figure S2). Soil warming increased the relative abundance of *Sebacina*, *Amphinema*, and *Cenococcum* at 0–10 cm depth, while *Sebacina* and *Boletus* largely dominated at 10–20 cm depth. In all, 12 EcM fungal OTUs accounted for >50% (i.e., 73%) of the overall difference between fungal community composition in control and warmed plots in the upper soil layer (Figure 3). Only six OTUs accounted for >50% (i.e., 60%) of the difference in fungal community composition in the deeper soil (Figure 4).

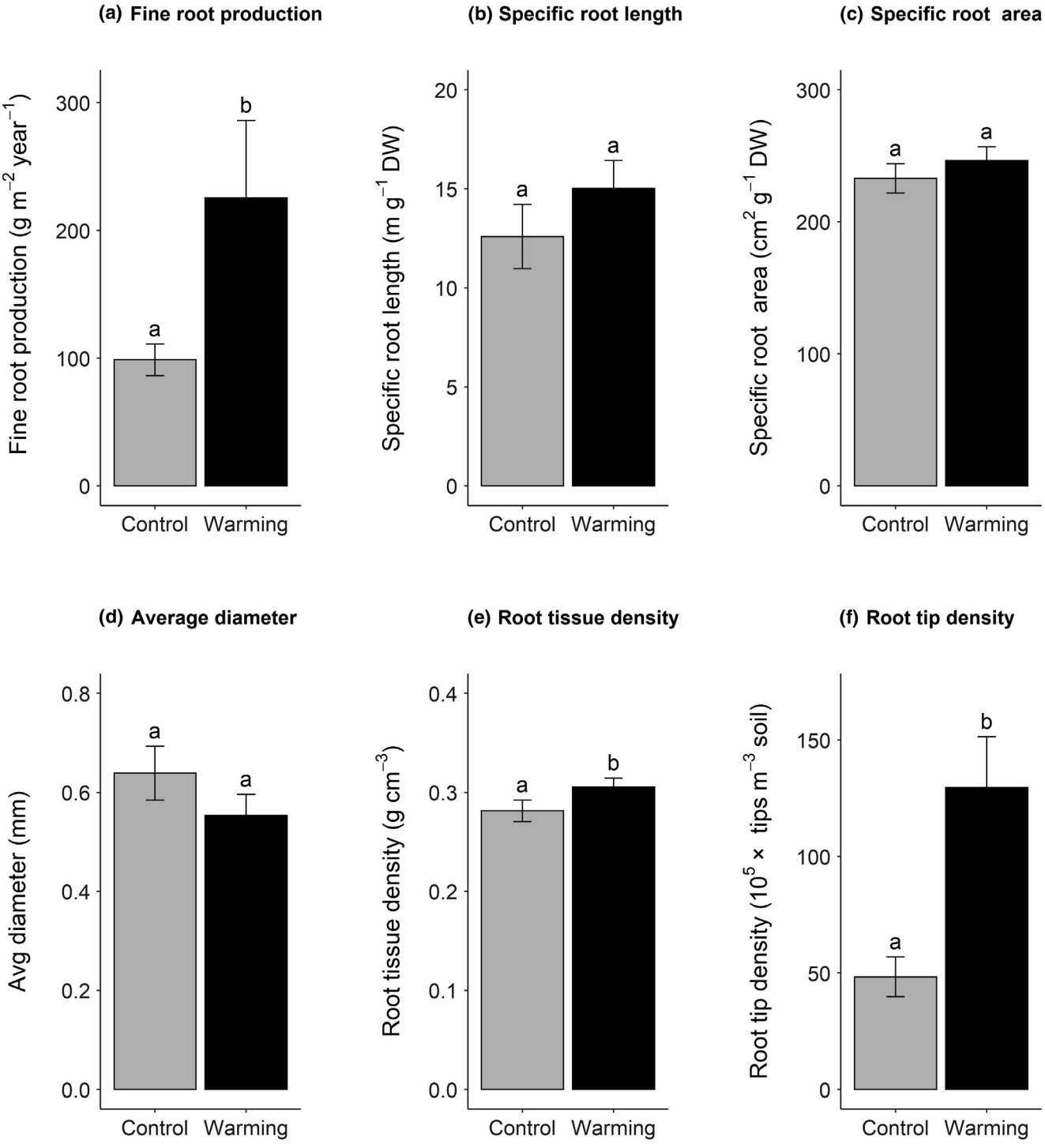

FIGURE 2 Mean (± SE) fine root biomass production (a) and morphological traits of fine roots from ingrowth cores in control and warmed plots between October 2019 and October 2020 ($n = 6$): specific root length (b), specific root area (c), average diameter (d), root tissue density (e), and root tip density (f). Different letters indicate significant differences between control and warming treatments (t test; $p < .05$)

The linear mixed-effects models showed that warming, but not soil depth, significantly affected bacterial diversity. Root-associated bacterial communities were altered in warmed plots, as indicated by the Shannon–Wiener diversity index and Pielou's evenness (Table S1). For EcM fungi, not treatment but soil depth significantly affected all diversity measures (Table S2). PERMANOVA revealed a significant effect of soil depth ($p = .003$) and warming treatment ($p = .011$) on EcM fungal community composition (Table 3), as also indicated by the NMDS ordination (Figure S7). Soil depth and treatment explained approximately 34% of the total variation observed in the EcM community at the site. Bacterial community composition was significantly affected by depth ($p = .021$), but not by treatment (Table 3).

TABLE 2 Mean ± SE element concentrations; C:N, N:P, N:K ratios of live fine roots in control and warmed plots at 0–10 and 10–20 cm soil depth in 2019 ($n = 6$). Different letters within each row indicate significant differences between control and warming treatments tested separately for each soil depth (t test; $p < .05$)

	Control		Warming	
Parameters	**0–10 cm**	**10–20 cm**	**0–10 cm**	**10–20 cm**
C (%)	48.5 ± 0.6 a	48.9 ± 0.8	46.1 ± 0.2 b	47.8 ± 0.5
N (%)	0.85 ± 0.05	0.73 ± 0.04	0.80 ± 0.04	0.74 ± 0.04
P (mg g^{-1})	0.51 ± 0.02	0.39 ± 0.04	0.50 ± 0.03	0.38 ± 0.04
K (mg g^{-1})	1.56 ± 0.09	1.14 ± 0.09	1.42 ± 0.05	1.20 ± 0.09
C:N	58.2 ± 3.9	68.0 ± 4.0	58.5 ± 2.3	65.4 ± 3.2
N:P	16.9 ± 0.9	19.7 ± 2.0	16.0 ± 0.7	20.4 ± 1.6
N:K	5.6 ± 0.6	6.5 ± 0.4	5.6 ± 0.3	6.3 ± 0.5
Na (mg g^{-1})	0.20 ± 0.04	0.24 ± 0.04 a	0.14 ± 0.01	0.15 ± 0.02 b
Mg (mg g^{-1})	1.27 ± 0.07	1.41 ± 0.10	1.27 ± 0.09	1.25 ± 0.15
Ca (mg g^{-1})	11.45 ± 1.35	14.35 ± 1.40 a	10.25 ± 0.44	10.36 ± 0.73 b
Mn (mg g^{-1})	0.005 ± 0.007	0.041 ± 0.004	0.067 ± 0.007	0.029 ± 0.002
Fe (mg g^{-1})	0.51 ± 0.08	0.45 ± 0.06	0.71 ± 0.09	0.41 ± 0.03

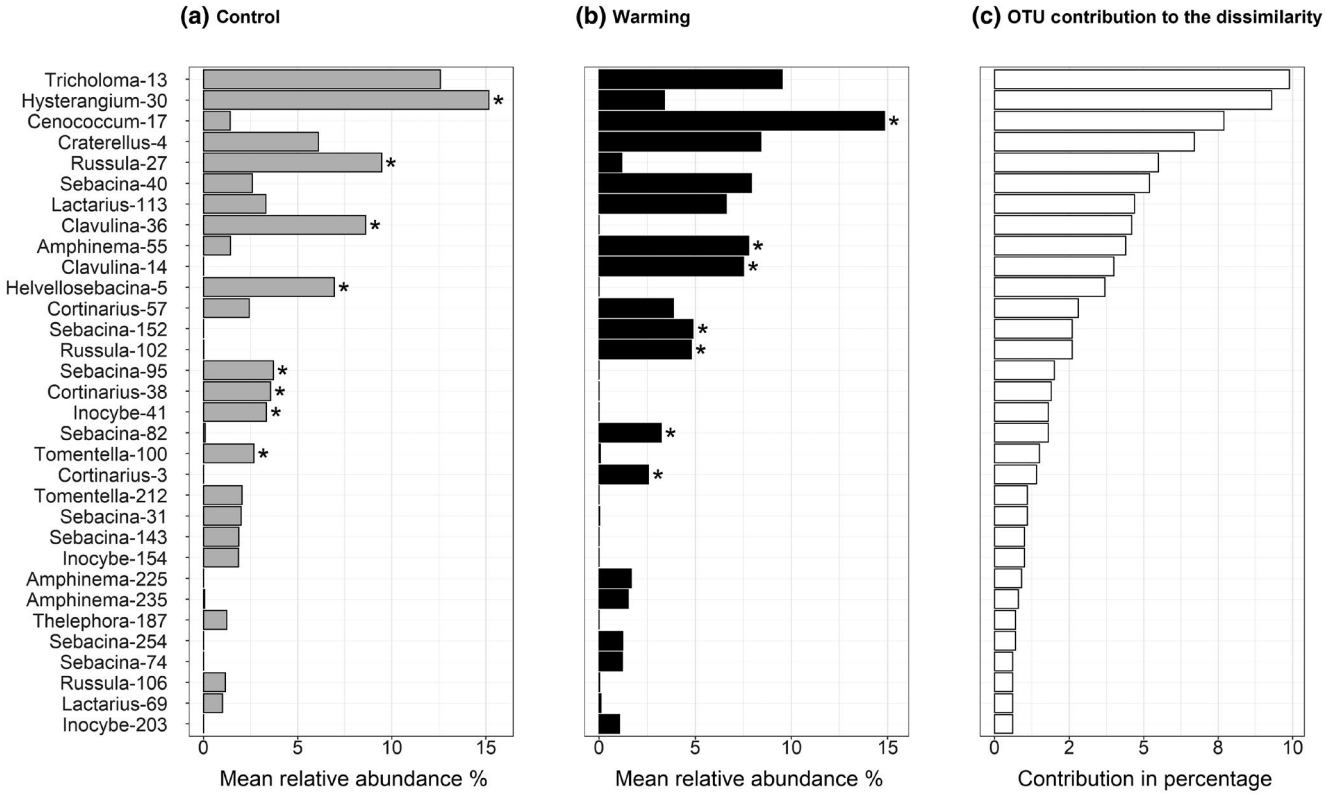

FIGURE 3 Abundance of ectomycorrhizal fungal genera with >1% relative abundance across all samples in control (a) and warmed (b) plots at 0–10 cm soil depth and their contribution to dissimilarity (c) determined by SIMPER analysis. Numbers behind genera of fungus are OTU numbers. Asterisks indicate a significantly higher abundance in either control or warmed plots

According to the classification method program (CLAM), soil warming affected EcM fungal OTUs differently (Figures 3 and 4). In the upper soil layer, *Cenococcum*-17, *Clavulina*-14, *Amphinema*-55, *Sebacina*-152, *Russula*-102, *Sebacina*-82, and *Cortinarius*-3 had higher relative abundances in warmed plots, while *Hysterangium*-30, *Russula*-27, *Clavulina*-36, *Helvellosebacina*-5, *Sebacina*-95, *Cortinarius*-38, *Inocybe*-41, and *Tomentella*-100 (numbers behind genera of fungus are OTU numbers) contributed more to the overall communities in control plots (Figure 3). At 10–20 cm soil depth, *Cortinarius*-38, *Cortinarius*-3, *Helvellosebacina*-5, *Trichophea*-134, *Lactarius*-1, *Pachyphlodes*-336, *Inocybe*-41, *Sebacina*-40, *Sebacina*-175, *Inocybe*-159, and *Sebacina*-31 had higher relative abundances in control plots, while *Sebacina*-28, *Boletus*-2, *Trichophea*-37, *Amphinema*-55, *Inocybe*-39, and *Tomentella*-24 had higher abundances in warmed plots (Figure 4). Proportions of the different EcM exploration types were not significantly different between treatments (Figure 5).

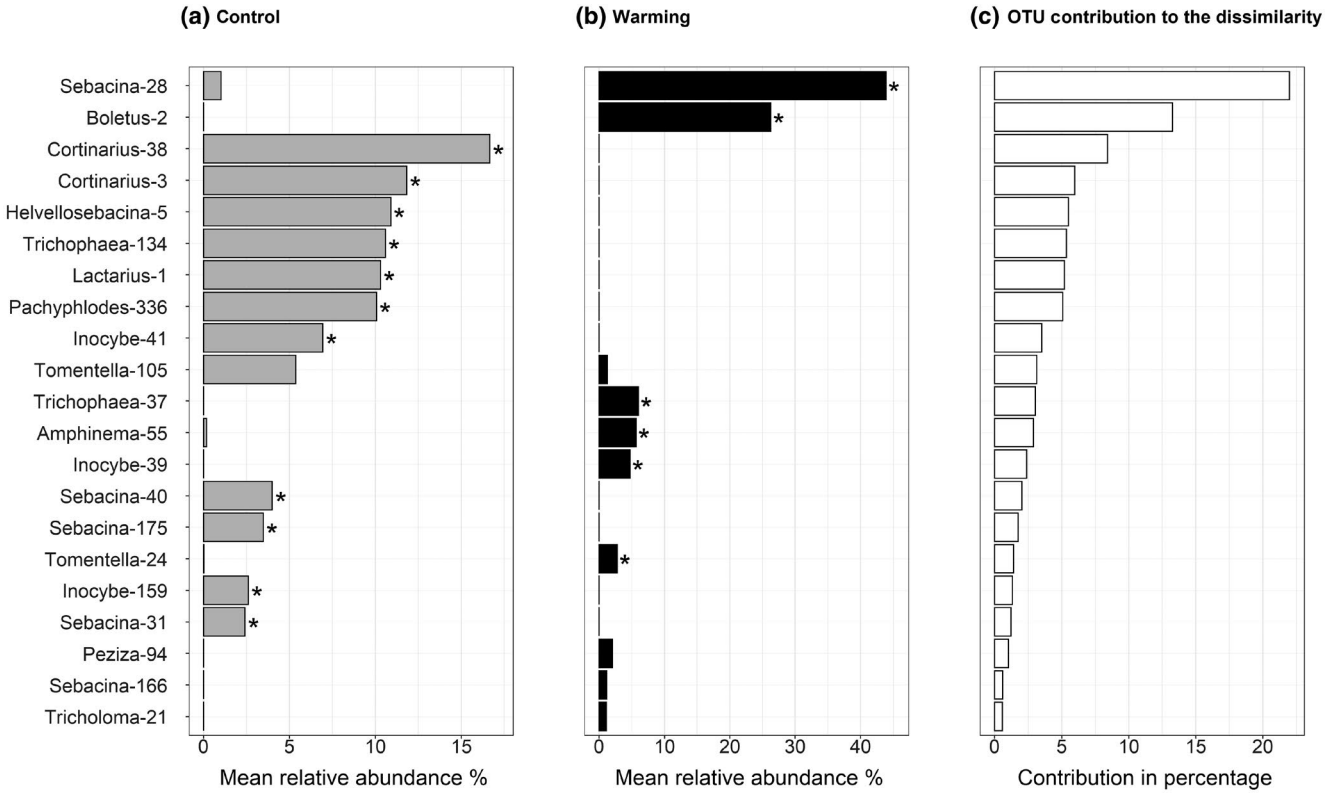

FIGURE 4 Abundance of ectomycorrhizal fungal genera with >1% relative abundance across all samples in control (a) and warmed (b) plots at 10–20 cm soil depth and their contribution to dissimilarity (c) determined by SIMPER analysis. Numbers behind genera of fungus are OTU numbers. Asterisks indicate a significantly higher abundance in either control or warmed plots

TABLE 3 Results of PERMANOVA based on Bray–Curtis dissimilarity analysis showing the effects of treatment (T: control and warming treatments), depth (D: 0–10 and 10–20 cm soil depth) on fungal and bacterial communities

	Ectomycorrhizal fungal community						Bacterial community					
Source	*df*	SS	MS	Pseudo-*F*	*p* (perm)	% Var	*df*	SS	MS	Pseudo-*F*	*p* (perm)	% Var
Depth	1	8107	8107	1.88	.003**	17.8	1	4193.6	4194	2.18	.021*	15.7
Treatment	1	7376	7376	1.71	.011**	16	1	2435.6	2436	1.27	.181	7.4
D × T	1	5208	5208	1.21	.204	12.2	1	1748.8	1749	0.91	.578	−6.2
Residual	20	86,228	4311			65.7	15	28,891	1926			43.9
Total	23	106,840					18	37,206				

Note: Significance level given as $^{**}p < .01$; $^{*}p < .05$.

Abbreviations: *df*, degrees of freedom; MS, the mean sum of squares; Pseudo-*F*, *F* value by permutation; *p* (perm), p values based on more than 9000 permutations; SS, the sum of squares; % Var, the percentage of variation explained.

3.5 | Relationship between fine root traits, soil nutrients, EcM exploration types, and fungal and bacterial diversity

At 0–10 cm soil depth, the first and second principal components (PC1 and PC2) explained 40% and 22% of the variance, respectively (Figure 6). PC1 was significantly correlated with SRL ($r = .90$), RTID ($r = .84$), aFRB ($r = .82$), soil temperature ($r = .61$), soil N ($r = -.73$), and RTD ($r = -.80$). PC2 was significantly correlated with EcM short-distance exploration type ($r = .79$), EcM diversity ($r = .65$), FRB ($r = .64$), and EcM long-distance exploration type ($r = -.71$). At 10–20 cm soil depth, PC1 and PC2 explained 34% and 27% of the variance, respectively. PC1 was significantly correlated with FRB ($r = .93$), aFRB ($r = .88$), RTID ($r = .81$), RTD ($r = .75$), D ($r = .62$), and SRL ($r = -.83$). PC2 was positively correlated with bacterial diversity ($r = .72$) and soil temperature ($r = .68$) and negatively associated with the long-distance exploration type ($r = -.60$), soil P ($r = -.65$), and soil N ($r = -.80$).

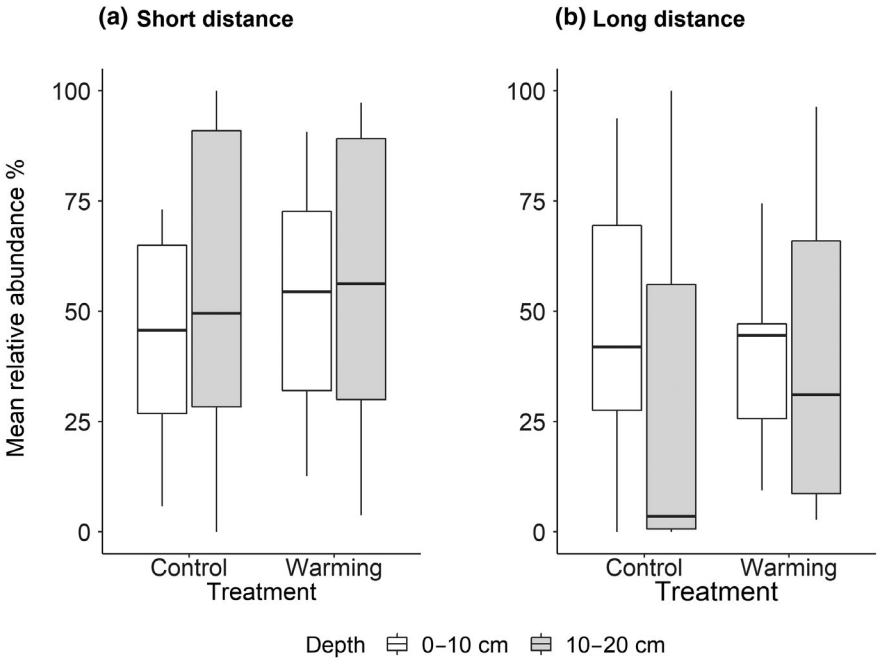

FIGURE 5 Box plots showing mean relative abundances (%) of ectomycorrhizal fungi grouped into short-distance (a) and long-distance (b) exploration types in control and warmed plots at 0–10 and 10–20 cm soil depth

4 | DISCUSSION

We explored how long-term soil warming impacts the fine root system and associated ectomycorrhizal fungi and root-associated bacterial communities in a temperate mountain forest. We found that FRB, aFRB, and fine root production were consistently higher in warmed plots, indicating greater root litter input. Soil warming also changed the morphology of fine roots, including higher RAI, SRL, SRA, and RTID, which increased the absorptive surface of the tree root systems for nutrient and water uptake. The community composition of ectomycorrhizal fungi was also affected by soil warming. Overall, our results suggest that the fine root system responded to warmer soil temperatures, although soil warming did not affect N availability. The low availabilities of P and K in the soil and low contents in roots and needles indicate a strong limitation of these nutrients in this temperate forest. Decreases in soil P and K availability have likely contributed to the changes of the root system in the warmed plots.

4.1 | Effects of soil warming on fine root biomass

In agreement with our first hypothesis, our results showed that soil warming increased FRB, and this increase was significant after 14 years of soil warming. Absorptive fine root biomass also increased with warming by 22%. Since there are no indications that soil warming changed soil N availability (Heinzle et al., 2021), we postulate that the increase in FRB and aFRB with soil warming in our study could be linked to the low P and K availability in the warmed plots, as explained by the optimal partitioning theory (Bloom et al., 1985). We found a tendency of increasing FRB and aFRB in warmed plots with decreasing soil P and K (Figure 6). Mean P concentration in fine roots (0.4 mg g^{-1} in warmed plots) was below the global mean fine root P concentration (0.9 mg g^{-1}) (Gordon & Jackson, 2000). This P deficiency is further supported by the high N:P ratios of fine roots, indicating an imbalance between N and P in root tissues. Needle P concentration at the field site (0.9 mg g^{-1}) also indicated P deficiency, being below the critical P concentration of 1.2 mg g^{-1} for Norway spruce needles (Ilg et al., 2009). Average fine root K concentration (1.3 mg g^{-1}) was also below the global mean K concentration (2.8 mg g^{-1}) in fine roots (Gordon & Jackson, 2000). The few existing studies on nutrient levels in fine roots of Norway spruce (0.7–1.7 mg P g^{-1} and 2.2–4.4 mg K g^{-1}; Borken et al., 2007; Brunner et al., 2002; Genenger et al., 2003) illustrate the strong P and K deficiency at the study site.

At Achenkirch, the K concentration in soil solutions was persistently below the detection limit of 0.25 mg L^{-1} in all plots during the growing season (unpublished data), suggesting that K availability is strongly limiting, and K nutrition relies on K input by atmospheric deposition and ecosystem recycling. By contrast, Ca and Mg concentrations were very high in fine roots and soil solutions, resulting from the weathering of the underlying dolomite bedrock. Phosphorus is mainly supplied through weathering, desorption, and organic matter mineralization and is immobilized by sorption, precipitation, and microbial uptake processes (Bünemann, 2015). Phosphorus limitation causes strong plant–microbe competition for labile P resources, and the low P availability suggests that P might be a key player in driving the increase in FRB and fine root biomass production, especially at 0–10 cm soil depth. Recent findings at our site showed that long-term soil warming decreased total P in both organic and inorganic forms (Ye Tian et al., unpublished data). One would expect that soil warming increases the availability of P and K due to the increasing mineralization of litter and soil organic matter. However, those nutrients might become depleted in the long run due to high turnover rates, increased leaching losses, or intense competition for uptake between trees and microbes (Dawes et al., 2017). The availability of

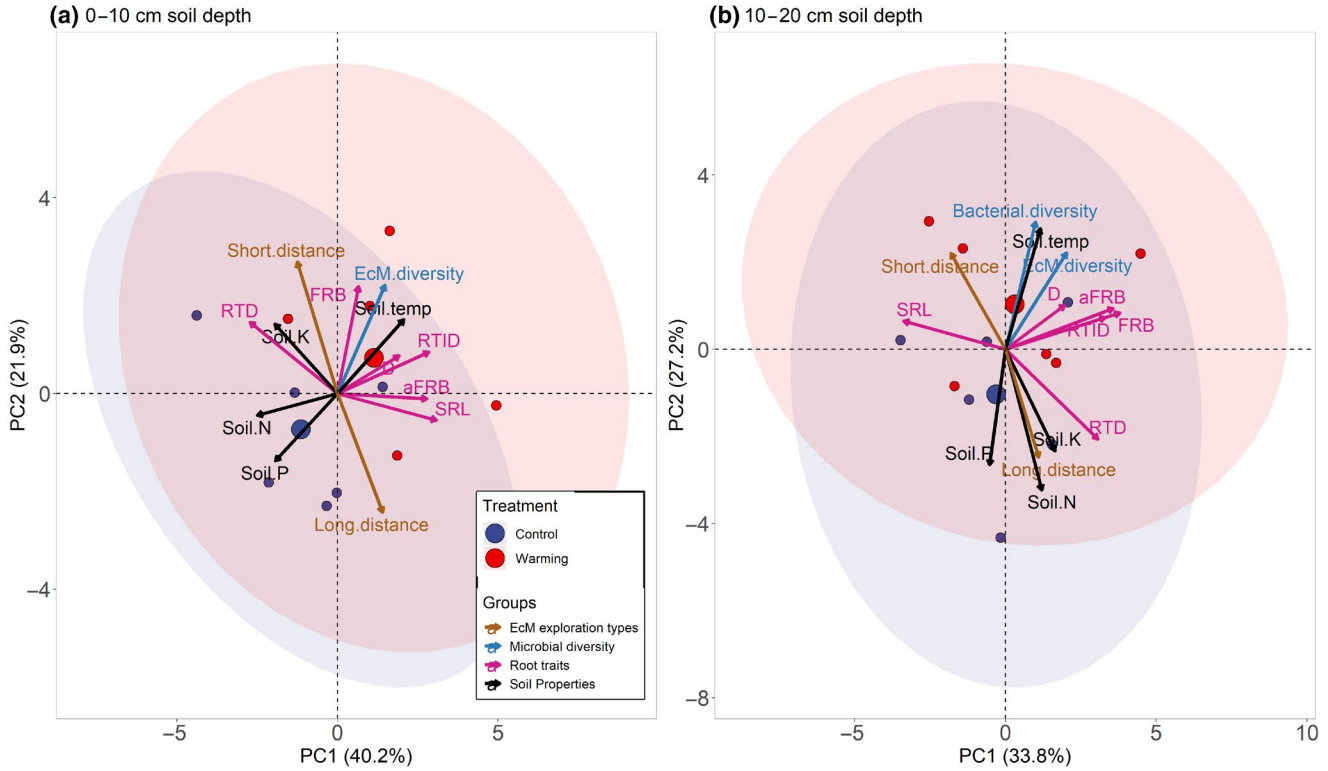

FIGURE 6 Principal component analysis (PCA) of fine root traits, EcM exploration types, bacterial and fungal diversity, and soil nutrient measured in 2019 at 0–10 cm (a) and 10–20 cm (b) soil depths. Principal component scores of samples in both treatments are represented by symbols (red and blue cycles), and arrows represent loadings of variables. Positively correlated variables are groups together, while negatively correlated variables are positioned on opposite sides of the plot origin. The distance between variables and the plot origin measures their importance on the respective principal component. Grey arrows represent EcM exploration types (short distance and long distance); blue arrows represent root-associated microbial diversity (Fischer diversity of bacteria and EcM fungi); red arrows represent fine root traits measured (aFRB, absorptive fine root biomass; D, fine root diameter; FRB, fine root biomass; RTD, root tissue density; RTID, root tip density; SRL, specific root length) and black arrows are soil properties (Soil P, total soil phosphorus; soil N, total soil N; soil K, soil K^+). Due to their strong correlation with SRL, SRA, and RAI were removed from the plots. At 0–10 cm, bacterial diversity was not important for both PCs, and therefore removed for graph visualization. 95% Ellipses are shown

these elements was low in the warmed plots, and the plant–microbe competition for these limiting nutrients therefore was likely strong. Increasing the FRB and the absorptive surface (see below) strengthens the competitiveness for nutrient uptake against other plants and non-root-associated soil microorganisms. Therefore, increased FRB and fine root production might be a key plant strategy to efficiently take up P and K, which seems to have become more limiting for trees in warmed plots.

Our result has been confirmed globally in a meta-analysis, demonstrating that soil warming increases fine root biomass production and FRB by 30% and 9%, respectively (Wang et al., 2021). However, they attributed the increase in FRB to the stimulation of growth due to high photosynthetic rates and an increase in soil N mineralization. The response might, however, also be due to decreased soil moisture in the warmed plots, which is a common phenomenon in soil warming experiments (Xu et al., 2013), but which has not consistently been shown at the Achenkirch site, as the high site-level precipitation frequently resets any decrease in soil moisture in warmed plots, back to the levels in control soils. Therefore, while soil moisture certainly is a driver of tree fine root biomass and production, at the Achenkirch site, increased plant demands for soil P and K seem to be the major trigger of the fine root responses as observed in the warmed plots.

4.2 | Effects of soil warming on fine root morphology

In agreement with our second hypothesis, we found significant increases in SRL, SRA, and RTID in warmed plots at both sampling occasions. This suggests a tree strategy to form long roots with a large surface area and short lifespan (Weemstra et al., 2020), which improve nutrient and water uptake as well as soil exploration in warmed plots. This aligns with an acquisitive resource plant strategy (McCormack & Iversen, 2019; Weemstra et al., 2017). There was also a tendency toward a decrease in RTD in warmed plots, indicating lower costs for production of fine roots, which then, however, are less stress-resistant, but have faster metabolic and growth rates (Birouste et al., 2014). SRL and RTID tended to be negatively correlated with soil nutrients at both soil depths. RTD and soil K were positively correlated, indicating a resource conservation strategy

for soil K (Figure 6). As mentioned earlier, the low availability of soil P and K may have contributed to the above changes observed in fine root morphology. With decreasing nutrient availability, long thin roots with a high surface area might be preferred to acquire soil resources more efficiently. By enlarging the absorptive surface by 29%, fine roots improved their ability to compete with other soil organisms for limited soil nutrients. Increases in SRL and the number of root tips were also shown in warmed plots by Leppälammi-Kujansuu et al. (2013) on first- and second-order roots, while Parts et al. (2019) found significant increases in SRL and SRA due to warming in fine roots <2 mm in diameter. However, our estimates of RAI are smaller than the global estimate of 9.8 m^2 m^{-2} for temperate deciduous forests, as reported by Jackson et al. (1997). Björk et al. (2007) reported an increase in SRL and SRA, but no effect on RTD of fine roots <0.5 mm in a soil warming experiment in a boreal forest in Northern Sweden. These changes in fine root morphology were also linked to increasing plant nutrient uptake efficiency. However, changes in fine root morphological traits observed in this study differ from the meta-analysis of Wang et al. (2021), who found no response to warming. They explained this non-response by the limited number of studies and the high variability of morphological traits.

At 0–10 cm soil depth, the mean diameter of fine roots increased in warmed plots in 2019, while no change was observed in 2012. This contradicts the assumption that SRL and fine root diameter are negatively correlated (Bergmann et al., 2020). However, the proportion of root length in the 0–0.2 mm diameter class tended to increase (+38% in both soil depths) in the warmed plots (Figure S1), supporting the optimization theory. Low diameter fine roots represent the most active part of the root system, which is highly relevant for plant nutrient and water uptake from soils. It is also this absorptive root size fraction that shows little secondary development, high metabolic activity, and high mycorrhizal colonization, which make them most responsive to changes in soil environmental conditions (McCormack et al., 2015). On the other hand, fine roots in the higher diameter classes were less affected, likely due to their high content of non-structural carbohydrates, making them more resistant to warming (Eissenstat et al., 2000; McCormack et al., 2015). These changes in fine root morphology imply that warming may profoundly alter tree nutrient and water uptake.

4.3 | Effects of soil warming on root-associated fungal and bacterial communities

Contrary to our third hypothesis, soil warming did not affect the relative abundance of EcM exploration types. With the observed changes in FRB and their morphology, one could expect a shift in the differentiation of extraradical hyphae, as fine roots are the primary source of C for EcM fungi (Koide et al., 2014). EcM fungi are usually patchily distributed in soils due to the heterogeneity in the distribution of soil nutrients (Luis et al., 2005). This micro-heterogeneity and patchiness might make it hard to detect significant responses of the EcM community at the species level. Fungal traits might contribute more clearly to shifts in ecosystem processes in the context of soil warming. For example, at 0–10 cm soil depth, we observed a 15% increase in the relative abundance of the EcM genus *Cenococcum* in warmed plots (Figure 3). This wide host and habitat range (Trappe, 1962) short-distance exploration type fungus associates well with all tree species present at our study site. Its high melanin content makes it more drought stress tolerant (Koide et al., 2014; LoBuglio, 1999) and might reduce eventual drought stress for the host tree species. *Cenococcum* mainly acquires NH_4^+ as an N-source but also has well-developed proteolytic abilities (LoBuglio, 1999). However, at 10–20 cm soil depth, *Sebacina-28* and *Boletus-2* were most abundant in warmed plots (Figure 4). An increase in the relative abundance of *Sebacina* in warmed plots, a hydrophilic EcM genus with a short-distance exploration type, which requires low plant energy investment, might be beneficial for the host to satisfy its increased demand for water and nutrient uptake at low C investment. This fits well to changes in the fine root morphology as discussed above (increases in SRL, SRA, RAI, and RTID), because alterations in host–plant nutrition might induce a direct shift in host–tree C allocation to EcM fungi (Lilleskov & Bruns, 2001; Treseder, 2004). The increase in the relative abundance of the genus *Boletus*, a long-distance exploration EcM species, appears conflicting, but it is very long and highly differentiated hydrophobic mantles and rhizomorphs, which avoid hyphal water and nutrient leakage when transported over long distances (Agerer, 2006), may indicate increasing plant demand for nutrients such as P and K. The increase in the relative abundance of long-distance EcM, like *Boletus* at 10–20 cm soil depth in warmed plots, was also observed in other soil warming studies (Defrenne et al., 2021), although it was mainly related to increasing water uptake from deeper soil layers.

Similar to others studies (Fernandez et al., 2017; Mucha et al., 2018; Parts et al., 2019), soil warming did not affect EcM fungal diversity. Fernandez et al. (2017) attributed the lack of a significant effect of experimental warming on fungal diversity to the high density of boreal and temperate host species in their experimental site, while Mucha et al. (2018) highlighted the dominance of generalist EcM species in their study. However, an increase in EcM fungal diversity with warming was reported in the arctic (Deslippe et al., 2011) and a boreal forest (Allison & Treseder, 2008). In our study, EcM communities were dominated by host-generalist taxa, which are known to be less sensitive to changes in environmental conditions (Mucha et al., 2018). The dominance of these host-generalist taxa might be the reason why we did not find an effect of soil warming on EcM fungal diversity. In addition, relatively high atmospheric N deposition at the site, about 12 kg ha^{-1} $year^{-1}$ (Herman et al., 2002), may have potentially shifted the competitive capabilities of the EcM fungi or decreased the tree dependence on mycorrhizal N acquisition (Clemmensen et al., 2006; Lilleskov & Bruns, 2001; Treseder, 2004).

Root-associated bacterial community diversity (Pielou's evenness and Shannon–Wiener index) increased with soil warming. Greater bacterial diversity is beneficial for the ecosystem as a whole, because it promotes metabolic activities and efficient nutrient mineralization (Nautiyal & Dion, 2008). A more diverse and

evenly distributed bacterial community might have greater resilience and functional stability in relation to warming (Cleland, 2011). This indicates that, in this forest ecosystem, root-associated bacterial communities may have maintained their ability to perform ecosystem multifunctionality with soil warming and fits well with the observed sustained increase in soil respiration in warmed plots since the beginning of the experimental warming manipulation at the site (Schindlbacher et al., 2011, 2015). Soil depth, related to a strong change in physicochemical properties, which greatly influences soil microorganisms, affected bacterial community composition in our study. A wide range of edaphic factors such as soil nutrients, the quality and quantity of litter inputs, and root-derived C could affect the composition of soil and root-associated bacterial communities (Baldrian, 2017). Because those factors change with soil depth, a corresponding shift in the root-associated bacterial community is expected. In our study, one of the most evident changes through the soil profile was the decrease in FRB with soil depth, which may affect root exudation, a crucial C source for root-associated bacteria.

It has to be noted that the experimental warming setup had some limitations. Only a limited area of soil was warmed, whereas the above-ground parts of the tree vegetation remained unaffected and experienced ambient temperatures. Thus, we cannot exclude varying fine root responses if the whole rooting area of individual trees or the whole forest would have warmed. For instance, if soil warming increases nutrient availability, one can anticipate root (in)growth from the surrounding soil into the warmed plots. This would result in an overestimation of the warming effect on FRB growth as well as stocks. Under the preconditions in our study, such an artefact can rather be excluded since it is unlikely that trees invest into root in-growth in warmed plots and progressively nutrient-depleted soils at the expense of ingrowth in unwarmed soil with higher nutrient availability. A general limitation of soil warming studies is that we could not predict how climate warming and the associated above-ground physiological responses will affect below-ground C allocation, fine roots, and EcM dynamics. Despite these constraints, we provide important insights into long-term warming effects on tree fine root dynamics and the connected soil C and nutrient dynamics.

In conclusion, our findings suggest that soil warming profoundly changed FRB, fine root production, root morphology, and the community composition of EcM fungi, which may have strong implications on fine root functions in temperate forests. The response of fine root systems to soil warming is linked to the availability and acquisition of soil nutrients which can differ among forests. The limited soil P and K availability align well with the observed responses in fine root biomass and morphology, though other more general physiological responses to warming, like faster growth (Pregitzer et al., 2000), may have contributed to the observed changes. Compared to the strong warming response of fine roots, the effects on root-associated microbial communities seem limited, at least for the parameters measured in this study. This is surprising because the root system is seen as a continuum of roots, symbiotic fungi, and bacteria (Freschet et al., 2021; Ostonen et al., 2017). More plots and seasonal replicates seem necessary to increase the statistical power of microbial community analyses and to assess their potential effects on the fine root systems. The long-term warming response of tree fine roots may have strong implications on ecosystem C dynamics. Assuming steady-state conditions between production and mortality, fine root C input to soil is around 106 g C m^{-2} $year^{-1}$ in warmed plots versus 48 g C m^{-2} $year^{-1}$ in control plots, based on fine root production in ingrowth cores and fine root C concentration (Table 2). Because of the disturbances associated with ingrowth cores, the estimated C input only represents an approximation to the surrounding soil. Other long-term soil warming studies showed that the stimulatory effect of temperature on soil CO_2 efflux decreased over time (Melillo et al., 2011, 2017). However, this was not yet the case at the Achenkirch site (Schindlbacher et al., 2009), indicating that the increase in FRB and fine root production increased the root system C input into the warmed soils by root exudation and fine root turnover. How mechanistically the continued increase in soil CO_2 efflux is linked to FRB, turnover, production, exudation, and morphological changes is currently under investigation, using field root exudation experiment and fine root radiocarbon dating methods. The consistent patterns of root responses during more than a decade of intensive soil warming indicate that changes in the fine root system are not of transient nature but likely persistently affect tree and soil C dynamics.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Dryad at https://doi.org/10.5061/dryad.jwstqjqbj.

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REFERENCES

Agerer, R. (2001). Exploration types of ectomycorrhizae. A proposal to classify ectomycorrhizal mycelial systems according to their patterns of differentiation and putative ecological importance. *Mycorrhiza*, *11*, 107–114. https://doi.org/10.1007/s005720100108

Agerer, R. (2006). Fungal relationships and structural identity of their ectomycorrhizae. *Mycological Progress*, *5*(2), 67–107. https://doi.org/10.1007/s11557-006-0505-x

Allison, S. D., & Treseder, K. K. (2008). Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. *Global Change Biology*, *14*(12), 2898–2909. https://doi.org/10.1111/j.1365-2486.2008.01716.x

Auguie, B. (2017). *gridExtra: Miscellaneous Functions for 'Grid' graphics*. Retrieved from https://CRAN.R-project.org/package=gridExtra

Averill, C., & Hawkes, C. V. (2016). Ectomycorrhizal fungi slow soil carbon cycling. *Ecology Letters*, *19*(8), 937–947. https://doi.org/10.1111/ele.12631

Averill, C., Turner, B. L., & Finzi, A. C. (2014). Mycorrhiza-mediated competition between plants and decomposers drives soil carbon storage. *Nature*, *505*(7484), 543–545. https://doi.org/10.1038/nature12901

Baldrian, P. (2017). Microbial activity and the dynamics of ecosystem processes in forest soils. *Current Opinion in Microbiology*, *37*, 128–134. https://doi.org/10.1016/j.mib.2017.06.008

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Bennett, A. E., & Classen, A. T. (2020). Climate change influences mycorrhizal fungal-plant interactions, but conclusions are limited by geographical study bias. *Ecology*, *101*(4), e02978. https://doi.org/10.1002/ecy.2978

Bergmann, J., Weigelt, A., van der Plas, F., Laughlin, D. C., Kuyper, T. W., Guerrero-Ramirez, N., Valverde-Barrantes, O. J., Bruelheide, H., Freschet, G. T., Iversen, C. M., Kattge, J., McCormack, M. L., Meier, I. C., Rillig, M. C., Roumet, C., Semchenko, M., Sweeney, C. J., van Ruijven, J., York, L. M., & Mommer, L. (2020). The fungal collaboration gradient dominates the root economics space in plants. *Science Advances*, *6*(27), eaba3756. https://doi.org/10.1101/2020.01.17.908905

Birouste, M., Zamora-Ledezma, E., Bossard, C., Pérez-Ramos, I. M., & Roumet, C. (2014). Measurement of fine root tissue density: A comparison of three methods reveals the potential of root dry matter content. *Plant and Soil*, *374*(1–2), 299–313. https://doi.org/10.1007/s11104-013-1874-y

Björk, R. G., Majdi, H., Klemedtsson, L., Lewis-Jonsson, L., & Molau, U. (2007). Long-term warming effects on root morphology, root mass distribution, and microbial activity in two dry tundra plant communities in northern Sweden. *New Phytologist*, *176*(4), 862–873. https://doi.org/10.1111/j.1469-8137.2007.02231.x

Bloom, A. J., Chapin, F. S., & Mooney, H. A. (1985). Resource limitation in plants—An economic analogy. *Annual Review of Ecology and Systematics*, *16*(1), 363–392. https://doi.org/10.1146/annurev.es.16.110185.002051

Borken, W., Kossmann, G., & Matzner, E. (2007). Biomass, morphology and nutrient contents of fine roots in four Norway spruce stands. *Plant and Soil*, *292*(1–2), 79–93. https://doi.org/10.1007/s11104-007-9204-x

Borken, W., & Matzner, E. (2004). Nitrate leaching in forest soils: An analysis of long-term monitoring sites in Germany. *Journal of Plant Nutrition and Soil Science*, *167*(3), 277–283. https://doi.org/10.1002/jpln.200421354

Brunner, I., Brodbeck, S., & Walthert, L. (2002). Fine root chemistry, starch concentration, and 'vitality' of subalpine conifer forests in relation to soil pH. *Forest Ecology and Management*, *165*(1–3), 75–84. https://doi.org/10.1016/S0378-1127(01)00633-8

Bünemann, E. K. (2015). Assessment of gross and net mineralization rates of soil organic phosphorus—A review. *Soil Biology and Biochemistry*, *89*, 82–98. https://doi.org/10.1016/j.soilbio.2015.06.026

Burke, M. K., & Raynal, D. J. (1994). Fine root growth phenology, production, and turnover in a northern hardwood forest ecosystem. *Plant and Soil*, *162*(1), 135–146. https://doi.org/10.1007/BF01416099

Cannon, P. F., & Kirk, P. M. (2007). *Fungal families of the world*. CAB International. ISBN 978 0 85199 827 5.

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., … Knight, R. (2010). Qiime allows analysis of high-throughput community sequencing data. *Nature Methods*, *7*(5), 335–336. https://doi.org/10.1038/nmeth.f.303

Chazdon, R. L., Chao, A., Colwell, R. K., Lin, S.-Y., Norden, N., Letcher, S. G., Clark, D. B., Finegan, B., & Arroyo, J. P. (2011). A novel statistical method for classifying habitat generalists and specialists. *Ecology*, *92*(6), 1332–1343. https://doi.org/10.1890/10-1345.1

Clarke, K. R., Gorley, R. N., Somerfield, P. J., & Warwick, R. M. (2014). *Change in marine communities: An approach to statistical analysis and interpretation* (3rd ed.). PRIMER-E.

Cleland, E. E. (2011). Biodiversity and ecosystem stability. *Nature Education Knowledge*, *3*(10):14.

Clemmensen, K. E., Michelsen, A., Jonasson, S., & Shaver, G. R. (2006). Increased ectomycorrhizal fungal abundance after long-term fertilization and warming of two arctic tundra ecosystems. *New Phytologist*, *171*(2), 391–404. https://doi.org/10.1111/j.1469-8137.2006.01778.x

Dawes, M. A., Philipson, C. D., Fonti, P., Bebi, P., Hättenschwiler, S., Hagedorn, F., & Rixen, C. (2015). Soil warming and CO_2 enrichment induce biomass shifts in alpine tree line vegetation. *Global Change Biology*, *21*(5), 2005–2021. https://doi.org/10.1111/gcb.12819

Dawes, M. A., Schleppi, P., Hättenschwiler, S., Rixen, C., & Hagedorn, F. (2017). Soil warming opens the nitrogen cycle at the alpine treeline. *Global Change Biology*, *23*(1), 421–434. https://doi.org/10.1111/gcb.13365

Defrenne, C. E., Childs, J., Fernandez, C. W., Taggart, M., Nettles, W. R., Allen, M. F., Hanson, P. J., & Iversen, C. M. (2021). High-resolution minirhizotrons advance our understanding of root-fungal dynamics in an experimentally warmed peatland. *Plants, People, Planet*, *3*(5), 640–652. https://doi.org/10.1002/ppp3.10172

Defrenne, C. E., Philpott, T. J., Guichon, S. H. A., Roach, W. J., Pickles, B. J., & Simard, S. W. (2019). Shifts in ectomycorrhizal fungal communities and exploration types relate to the environment and fine-root traits across interior Douglas-fir forests of western Canada. *Frontiers in Plant Science*, *10*, 643. https://doi.org/10.3389/fpls.2019.00643

Deslippe, J. R., Hartmann, M., Mohn, W. W., & Simard, S. W. (2011). Long-term experimental manipulation of climate alters the ectomycorrhizal community of *Betula nana* in arctic tundra. *Global Change Biology*, *17*(4), 1625–1636. https://doi.org/10.1111/j.1365-2486.2010.02318.x

Eissenstat, D. M., Wells, C. E., Yanai, R. D., & Whitbeck, J. L. (2000). Building roots in a changing environment: Implications for root longevity. *New Phytologist*, *147*(1), 33–42. https://doi.org/10.1046/j.1469-8137.2000.00686.x

Fernandez, C. W., Nguyen, N. H., Stefanski, A., Han, Y., Hobbie, S. E., Montgomery, R. A., Reich, P. B., & Kennedy, P. G. (2017). Ectomycorrhizal fungal response to warming is linked to poor host performance at the boreal-temperate ecotone. *Global Change Biology*, *23*(4), 1598–1609. https://doi.org/10.1111/gcb.13510

Freschet, G. T., Pagès, L., Iversen, C. M., Comas, L. H., Rewald, B., Roumet, C., Klimešová, J., Zadworny, M., Poorter, H., Postma, J. A., Adams, T. S., Bagniewska-Zadworna, A., Bengough, A. G., Blancaflor, E. B., Brunner, I., Cornelissen, J. H. C., Garnier, E., Gessler, A., Hobbie, S.

E., … McCormack, M. L. (2021). A starting guide to root ecology: Strengthening ecological concepts and standardising root classification, sampling, processing and trait measurements. *The New Phytologist*, *232*(3), 973–1122. https://doi.org/10.1111/nph.17572

Genenger, M., Zimmermann, S., Hallenbarter, D., Landolt, W., Frossard, E., & Brunner, I. (2003). Fine root growth and element concentrations of Norway spruce as affected by wood ash and liquid fertilisation. *Plant and Soil*, *255*(1), 253–264. https://doi.org/10.1023/A:1026118101339

Gobiet, A., Kotlarski, S., Beniston, M., Heinrich, G., Rajczak, J., & Stoffel, M. (2014). 21st century climate change in the European Alps—A review. *The Science of the Total Environment*, *493*, 1138–1151. https://doi.org/10.1016/j.scitotenv.2013.07.050

Gordon, W. S., & Jackson, R. B. (2000). Nutrient concentrations in fine roots. *Ecology*, *81*(1), 275. https://doi.org/10.2307/177151

Heinzle, J., Wanek, W., Tian, Y., Kengdo, S. K., Borken, W., Schindlbacher, A., & Inselsbacher, E. (2021). No effect of long-term soil warming on diffusive soil inorganic and organic nitrogen fluxes in a temperate forest soil. *Soil Biology and Biochemistry*, *158*, 108261. https://doi.org/10.1016/j.soilbio.2021.108261

Herman, F., Smidt, S., Englisch, M., Feichtinger, F., Gerzabek, M., Haberhauer, G., Jandl, R., Kalina, M., & Zechmeister-Boltenstern, S. (2002). Investigations of nitrogen fluxes and pools on a limestone site in the alps. *Environmental Science and Pollution Research International*, *9*(S2), 46–52. https://doi.org/10.1007/BF02987478

Hobbie, E. A., & Agerer, R. (2010). Nitrogen isotopes in ectomycorrhizal sporocarps correspond to below-ground exploration types. *Plant and Soil*, *327*(1–2), 71–83. https://doi.org/10.1007/s11104-009-0032-z

Ihrmark, K., Bödeker, I. T. M., Cruz-Martinez, K., Friberg, H., Kubartova, A., Schenck, J., Strid, Y., Stenlid, J., Brandström-Durling, M., Clemmensen, K. E., & Lindahl, B. D. (2012). New primers to amplify the fungal ITS2 region-evaluation by 454-sequencing of artificial and natural communities. *FEMS Microbiology Ecology*, *82*(3), 666–677. https://doi.org/10.1111/j.1574-6941.2012.01437.x

Ilg, K., Wellbrock, N., & Lux, W. (2009). Phosphorus supply and cycling at long-term forest monitoring sites in Germany. *European Journal of Forest Research*, *128*(5), 483–492. https://doi.org/10.1007/s10342-009-0297-z

Illumina. (2013). *16S Metagenomic sequencing library preparation preparing 16S ribosomal RNA gene amplicons for the Illumina MiSeq system*. Illumina. Retrieved from https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf

IPCC. (2021). *Climate change 2021: The physical science basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* [V. Masson-Delmotte, P. Zhai, A. Pirani, S. L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M. I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J. B. R. Matthews, T. K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, & B. Zhou (Eds.)]. Cambridge University Press. In press.

Jackson, R. B., Mooney, H. A., & Schulze, E. D. (1997). A global budget for fine root biomass, surface area, and nutrient contents. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(14), 7362–7366. https://doi.org/10.1073/pnas.94.14.7362

Jayasiri, S. C., Hyde, K. D., Ariyawansa, H. A., Bhat, J., Buyck, B., Cai, L., Dai, Y.-C., Abd-Elsalam, K. A., Ertz, D., Hidayat, I., Jeewon, R., Jones, E. B. G., Bahkali, A. H., Karunarathna, S. C., Liu, J.-K., Luangsa-ard, J. J., Lumbsch, H. T., Maharachchikumbura, S. S. N., McKenzie, E. H. C., … Promputtha, I. (2015). The Faces of Fungi database: Fungal names linked with morphology, phylogeny and human impacts. *Fungal Diversity*, *74*(1), 3–18. https://doi.org/10.1007/s13225-015-0351-8

Jonard, M., Fürst, A., Verstraeten, A., Thimonier, A., Timmermann, V., Potočić, N., Waldner, P., Benham, S., Hansen, K., Merilä, P., Ponette, Q., de La Cruz, A. C., Roskams, P., Nicolas, M., Croisé, L., Ingerslev, M., Matteucci, G., Decinti, B., Bascietto, M., & Rautio, P. (2015). Tree mineral nutrition is deteriorating in Europe. *Global Change Biology*, *21*(1), 418–430. https://doi.org/10.1111/gcb.12657

Kassambara, A. (2017). *Practical guide to principal component methods in R* (1st ed.). CreateSpace Independent Publishing Platform.

Keller, A. B., Brzostek, E. R., Craig, M. E., Fisher, J. B., & Phillips, R. P. (2021). Root-derived inputs are major contributors to soil carbon in temperate forests, but vary by mycorrhizal type. *Ecology Letters*, *24*(4), 626–635. https://doi.org/10.1111/ele.13651

Kirk, P. M., Cannon, P. F., Minter, D. W., Stalpers, J. A., Ainsworth, G. C., & Bisby, G. R. (2011). *Ainsworth & Bisby's dictionary of the fungi: By P.M. Kirk… [et al]* (10th ed./with the assistance of T.V. Andrianova … [et al]. CABI.

Koide, R. T., Fernandez, C., & Malcolm, G. (2014). Determining place and process: Functional traits of ectomycorrhizal fungi that affect both community structure and ecosystem function. *New Phytologist*, *201*(2), 433–439. https://doi.org/10.1111/nph.12538

Kõljalg, U., Nilsson, R. H., Abarenkov, K., Tedersoo, L., Taylor, A. F. S., Bahram, M., Bates, S. T., Bruns, T. D., Bengtsson-Palme, J., Callaghan, T. M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G. W., Hartmann, M., Kirk, P. M., Kohout, P., … Larsson, K.-H. (2013). Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology*, *22*(21), 5271–5277. https://doi.org/10.1111/mec.12481

Kuffner, M., Hai, B., Rattei, T., Melodelima, C., Schloter, M., Zechmeister-Boltenstern, S., Jandl, R., Schindlbacher, A., & Sessitsch, A. (2012). Effects of season and experimental warming on the bacterial community in a temperate mountain forest soil assessed by 16S rRNA gene pyrosequencing. *FEMS Microbiology Ecology*, *82*(3), 551–562. https://doi.org/10.1111/j.1574-6941.2012.01420.x

Lê, S., Josse, J., & Husson, F. (2008). FactoMineR: An R package for multivariate analysis. *Journal of Statistical Software*, *25*(1), 1–18. https://doi.org/10.18637/jss.v025.i01

Leake, J. R. (2001). Is diversity of ectomycorrhizal fungi important for ecosystem function? *New Phytologist*, *152*(1), 1–3. https://doi.org/10.1046/j.0028-646X.2001.00249.x

Lenth, R. V. (2021). *emmeans: Estimated marginal means, aka least-squares means*. Retrieved from https://CRAN.R-project.org/package=emmeans

Leppälammi-Kujansuu, J., Ostonen, I., Strömgren, M., Nilsson, L. O., Kleja, D. B., Sah, S. P., & Helmisaari, H.-S. (2013). Effects of long-term temperature and nutrient manipulation on Norway spruce fine roots and mycelia production. *Plant and Soil*, *366*(1–2), 287–303. https://doi.org/10.1007/s11104-012-1431-0

Li, W., & Chang, Y. (2017). CD-HIT-OTU-MiSeq, an improved approach for clustering and analyzing paired end MiSeq 16S rRNA sequences. *bioRxiv*. https://doi.org/10.1101/153783

Lilleskov, E. A., & Bruns, T. D. (2001). Nitrogen and ectomycorrhizal fungal communities: What we know, what we need to know. *New Phytologist*, *149*(2), 156–158. https://doi.org/10.1046/j.1469-8137.2001.00042-2.x

Lindahl, B. D., & Tunlid, A. (2015). Ectomycorrhizal fungi—potential organic matter decomposers, yet not saprotrophs. *New Phytologist*, *205*(4), 1443–1447. https://doi.org/10.1111/nph.13201

LoBuglio, K. F. (1999). Cenococcum. In J. W. G. Cairney & S. M. Chambers (Eds.), *Ectomycorrhizal fungi key genera in profile* (pp. 287–309). Springer. https://doi.org/10.1007/978-3-662-06827-4_12

Lõhmus, K., Truu, J., Truu, M., Kaar, E., Ostonen, I., Alama, S., Kuznetsova, T., Rosenvald, K., Vares, A., Uri, V., & Mander, Ü. (2006). Black alder as a promising deciduous species for the reclaiming of oil shale mining areas. In C. A. Brebbia & Ü. Mander (Eds.), *WIT transactions on ecology and the environment, Brownfield sites iii: Prevention, assessment, rehabilitation and development of brownfield sites* (pp. 87–97). WIT. https://doi.org/10.2495/BF060091

Luis, P., Kellner, H., Zimdars, B., Langer, U., Martin, F., & Buscot, F. (2005). Patchiness and spatial distribution of laccase genes of

ectomycorrhizal, saprotrophic, and unknown basidiomycetes in the upper horizons of a mixed forest Cambisol. *Microbial Ecology*, *50*(4), 570–579. https://doi.org/10.1007/s00248-005-5047-2

McCormack, M. L., Dickie, I. A., Eissenstat, D. M., Fahey, T. J., Fernandez, C. W., Guo, D., Helmisaari, H.-S., Hobbie, E. A., Iversen, C. M., Jackson, R. B., Leppälammi-Kujansuu, J., Norby, R. J., Phillips, R. P., Pregitzer, K. S., Pritchard, S. G., Rewald, B., & Zadworny, M. (2015). Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *The New Phytologist*, *207*(3), 505–518. https://doi.org/10.1111/nph.13363

McCormack, M. L., & Iversen, C. M. (2019). Physical and functional constraints on viable below-ground acquisition strategies. *Frontiers in Plant Science*, *10*, 1215. https://doi.org/10.3389/fpls.2019.01215

Melillo, J. M., Butler, S., Johnson, J., Mohan, J., Steudler, P., Lux, H., Burrows, E., Bowles, F., Smith, R., Scott, L., Vario, C., Hill, T., Burton, A., Zhou, Y.-M., & Tang, J. (2011). Soil warming, carbon-nitrogen interactions, and forest carbon budgets. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(23), 9508–9512. https://doi.org/10.1073/pnas.1018189108

Melillo, J. M., Frey, S. D., DeAngelis, K. M., Werner, W. J., Bernard, M. J., Bowles, F. P., Pold, G., Knorr, M. A., & Grandy, A. S. (2017). Long-term pattern and magnitude of soil carbon feedback to the climate system in a warming world. *Science (New York, N.Y.)*, *358*(6359), 101–105. https://doi.org/10.1126/science.aan2874

Millard, S. P. (2013). *EnvStats: An R package for environmental statistics*. Springer. Retrieved from https://www.springer.com

Mucha, J., Peay, K. G., Smith, D. P., Reich, P. B., Stefański, A., & Hobbie, S. E. (2018). Effect of simulated climate warming on the ectomycorrhizal fungal community of boreal and temperate host species growing near their shared ecotonal range limits. *Microbial Ecology*, *75*(2), 348–363. https://doi.org/10.1007/s00248-017-1044-5

Nautiyal, C. S., & Dion, P. (2008). *Molecular mechanisms of plant and microbe coexistence. Soil biology: v. 15*. Springer. https://doi.org/10.1007/978-3-540-75575-3

Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., Schilling, J. S., & Kennedy, P. G. (2016). FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*, *20*, 241–248. https://doi.org/10.1016/j.funeco.2015.06.006

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., & Wagner, H. (2019). *vegan: Community ecology package*. Retrieved from https://CRAN.R-project.org/package=vegan

Ostonen, I., Helmisaari, H.-S., Borken, W., Tedersoo, L., Kukumägi, M., Bahram, M., Lindroos, A.-J., Nöjd, P., Uri, V., Merilä, P., Asi, E., & Lõhmus, K. (2011). Fine root foraging strategies in Norway spruce forests across a European climate gradient. *Global Change Biology*, *17*(12), 3620–3632. https://doi.org/10.1111/j.1365-2486.2011.02501.x

Ostonen, I., Lõhmus, K., & Lasn, R. (1999). The role of soil conditions in fine root ecomorphology in Norway spruce (*Picea abies* (L.) Karst.). *Plant and Soil*, *208*(2), 283–292. https://doi.org/10.1023/A:1004552907597

Ostonen, I., Truu, M., Helmisaari, H.-S., Lukac, M., Borken, W., Vanguelova, E., Godbold, D. L., Lõhmus, K., Zang, U., Tedersoo, L., Preem, J.-K., Rosenvald, K., Aosaar, J., Armolaitis, K., Frey, J., Kabral, N., Kukumägi, M., Leppälammi-Kujansuu, J., Lindroos, A.-J., … Truu, J. (2017). Adaptive root foraging strategies along a boreal-temperate forest gradient. *New Phytologist*, *215*(3), 977–991. https://doi.org/10.1111/nph.14643

Parts, K., Tedersoo, L., Schindlbacher, A., Sigurdsson, B. D., Leblans, N. I. W., Oddsdóttir, E. S., Borken, W., & Ostonen, I. (2019). Acclimation of fine root systems to soil warming: Comparison of an experimental setup and a natural soil temperature gradient. *Ecosystems*, *22*(3), 457–472. https://doi.org/10.1007/s10021-018-0280-y

Penuelas, J., Fernández-Martínez, M., Vallicrosa, H., Maspons, J., Zuccarini, P., Carnicer, J., Sanders, T. G. M., Krüger, I., Obersteiner, M., Janssens, I. A., Ciais, P., & Sardans, J. (2020). Increasing atmospheric CO_2 concentrations correlate with declining nutritional status of European forests. *Communications Biology*, *3*(1), 125. https://doi.org/10.1038/s42003-020-0839-y

Peršoh, D., Melcher, M., Flessa, F., & Rambold, G. (2010). First fungal community analyses of endophytic ascomycetes associated with Viscum album ssp. Austriacum and its host Pinus sylvestris. *Fungal Biology*, *114*(7), 585–596. https://doi.org/10.1016/j.funbio.2010.04.009

Pregitzer, K. S., King, J. S., Burton, A. J., & Brown, S. E. (2000). Responses of tree fine roots to temperature. *New Phytologist*, *147*(1), 105–115. https://doi.org/10.1046/j.1469-8137.2000.00689.x

R Core Team. (2020). *R: A language and environment for statistical computing*. Retrieved from https://www.R-project.org/

Rasse, D. P., Rumpel, C., & Dignac, M.-F. (2005). Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. *Plant and Soil*, *269*(1–2), 341–356. https://doi.org/10.1007/s11104-004-0907-y

Rose, L. (2017). Pitfalls in root trait calculations: How ignoring diameter heterogeneity can lead to overestimation of functional traits. *Frontiers in Plant Science*, *8*, 898. https://doi.org/10.3389/fpls.2017.00898

Salazar, A., Rousk, K., Jónsdóttir, I. S., Bellenger, J.-P., & Andrésson, Ó. S. (2020). Faster nitrogen cycling and more fungal and root biomass in cold ecosystems under experimental warming: A meta-analysis. *Ecology*, *101*(2), e02938. https://doi.org/10.1002/ecy.2938

Schindlbacher, A., Rodler, A., Kuffner, M., Kitzler, B., Sessitsch, A., & Zechmeister-Boltenstern, S. (2011). Experimental warming effects on the microbial community of a temperate mountain forest soil. *Soil Biology & Biochemistry*, *43*(7), 1417–1425. https://doi.org/10.1016/j.soilbio.2011.03.005

Schindlbacher, A., Schnecker, J., Takriti, M., Borken, W., & Wanek, W. (2015). Microbial physiology and soil CO_2 efflux after 9 years of soil warming in a temperate forest—No indications for thermal adaptations. *Global Change Biology*, *21*(11), 4265–4277. https://doi.org/10.1111/gcb.12996

Schindlbacher, A., Zechmeister-Boltenstern, S., Glatzel, G., & Jandl, R. (2007). Winter soil respiration from an Austrian mountain forest. *Agricultural and Forest Meteorology*, *146*(3–4), 205–215. https://doi.org/10.1016/j.agrformet.2007.06.001

Schindlbacher, A., Zechmeister-Boltenstern, S., & Jandl, R. (2009). Carbon losses due to soil warming: Do autotrophic and heterotrophic soil respiration respond equally? *Global Change Biology*, *15*(4), 901–913. https://doi.org/10.1111/j.1365-2486.2008.01757.x

Schlegel, M., Queloz, V., & Sieber, T. N. (2018). The endophytic mycobiome of European ash and sycamore maple leaves—Geographic patterns, host specificity and influence of ash dieback. *Frontiers in Microbiology*, *9*, 2345. https://doi.org/10.3389/fmicb.2018.02345

Smiatek, G., Kunstmann, H., Knoche, R., & Marx, A. (2009). Precipitation and temperature statistics in high-resolution regional climate models: evaluation for the European alps. *Journal of Geophysical Research*, *114*(D19), https://doi.org/10.1029/2008JD011353

Smith, S. E., & Read, D. J. (2008). *Mycorrhizal symbiosis* (3rd ed.). Elsevier/Acad: Press.

Solly, E. F., Lindahl, B. D., Dawes, M. A., Peter, M., Souza, R. C., Rixen, C., & Hagedorn, F. (2017). Experimental soil warming shifts the fungal community composition at the alpine treeline. *New Phytologist*, *215*(2), 766–778. https://doi.org/10.1111/nph.14603

Staddon, P. L., Heinemeyer, A., & Fitter, A. H. (2002). Mycorrhizas and global environmental change: Research at different scales. *Plant and Soil*, *244*(1/2), 253–261. https://doi.org/10.1023/A:1020285309675

Talkner, U., Meiwes, K. J., Potočić, N., Seletković, I., Cools, N., de Vos, B., & Rautio, P. (2015). Phosphorus nutrition of beech (*Fagus sylvatica* L.) is decreasing in Europe. *Annals of Forest Science*, *72*(7), 919–928. https://doi.org/10.1007/s13595-015-0459-8

Talkner, U., Riek, W., Dammann, I., Kohler, M., Göttlein, A., Mellert, K. H., & Meiwes, K. J. (2019). Nutritional status of major forest tree species in Germany. In N. Wellbrock & A. Bölte (Eds.), *Ecological studies, 0070-8356: Volume 237. Status and dynamics of forests in Germany: Results of the National Forest Monitoring* (Vol. 237, pp. 261–293). Springer Open. https://doi.org/10.1007/978-3-030-15734-0_9

Tedersoo, L. (2017). *Biogeography of mycorrhizal symbiosis* (Vol. 230). Springer International Publishing. https://doi.org/10.1007/978-3-319-56363-3

Tedersoo, L., Bahram, M., Toots, M., Diédhiou, A. G., Henkel, T. W., Kjøller, R., Morris, M. H., Nara, K., Nouhra, E., Peay, K. G., Põlme, S., Ryberg, M., Smith, M. E., & Kõljalg, U. (2012). Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. *Molecular Ecology*, *21*(17), 4160–4170. https://doi.org/10.1111/j.1365-294X.2012.05602.x

Trappe, J. M. (1962). *Cenococcum graniforme-its distribution, ecology, mycorrhiza formation, and inherent variation*. University of Washington. Retrieved from https://digital.lib.washington.edu/researchworks/handle/1773/5550

Treseder, K. K. (2004). A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. *New Phytologist*, *164*(2), 347–355. https://doi.org/10.1111/j.1469-8137.2004.01159.x

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*(10), 3395–3404. https://doi.org/10.1111/gcb.13238

Wan, S., Norby, R. J., Pregitzer, K. S., Ledford, J., & O'Neill, E. G. (2004). CO_2 enrichment and warming of the atmosphere enhance both productivity and mortality of maple tree fine roots. *New Phytologist*, *162*(2), 437–446. https://doi.org/10.1111/j.1469-8137.2004.01034.x

Wang, J., Defrenne, C., McCormack, M. L., Yang, L., Tian, D., Luo, Y., Hou, E., Yan, T., Li, Z., Bu, W., Chen, Y., & Niu, S. (2021). Fine-root functional trait responses to experimental warming: A global meta-analysis. *New Phytologist*, *230*(5), 1856–1867. https://doi.org/10.1111/nph.17279

Weemstra, M. (2017). *Below-ground uptake strategies: How fine-root traits determine tree growth*. Wageningen University. https://doi.org/10.18174/400247

Weemstra, M., Kiorapostolou, N., Ruijven, J., Mommer, L., Vries, J., & Sterck, F. (2020). The role of fine-root mass, specific root length and life span in tree performance: A whole-tree exploration. *Functional Ecology*, *34*(3), 575–585. https://doi.org/10.1111/1365-2435.13520

Weemstra, M., Sterck, F. J., Visser, E. J. W., Kuyper, T. W., Goudzwaard, L., & Mommer, L. (2017). Fine-root trait plasticity of beech (*Fagus sylvatica*) and spruce (*Picea abies*) forests on two contrasting soils. *Plant and Soil*, *415*(1–2), 175–188. https://doi.org/10.1007/s11104-016-3148-y

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer-Verlag. Retrieved from https://ggplot2.tidyverse.org

Wu, K. (2000). Fine root production and turnover and its contribution to nutrient cycling in two beech (*Fagus sylvatica*) forest ecosystems. *Ber Forsch Zentr Waldökosysteme*, *A170*, 1–130.

Xu, W., Yuan, W., Dong, W., Xia, J., Liu, D., & Chen, Y. (2013). A meta-analysis of the response of soil moisture to experimental warming. *Environmental Research Letters*, *8*(4), 44027. https://doi.org/10.1088/1748-9326/8/4/044027

Yuan, Z. Y., Shi, X. R., Jiao, F., & Han, F. P. (2018). Changes in fine root biomass of *Picea abies* forests: Predicting the potential impacts of climate change. *Journal of Plant Ecology*, *11*(4), 595–603. https://doi.org/10.1093/jpe/rtx032

Zhou, Y., Tang, J., Melillo, J. M., Butler, S., & Mohan, J. E. (2011). Root standing crop and chemistry after six years of soil warming in a temperate forest. *Tree Physiology*, *31*(7), 707–717. https://doi.org/10.1093/treephys/tpr066

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