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Acclimation of Fine Root Systems to Soil Warming: Comparison of an Experimental Setup and a Natural Soil Temperature Gradient

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ABSTRACT

Global warming is predicted to impact high-latitude and high-altitude forests severely, jeopardizing their overall functioning and carbon storage, both of which depend on the warming response of tree fine root systems. This paper investigates the effect of soil warming on the biomass, morphology and colonizing ectomycorrhizal community of spruce fine and absorptive fine roots. We compare the responses of spruce roots growing at a man-made long-term soil warming (+ 4°C) experiment to results obtained from a geothermal soil temperature gradient (+ 1 to + 14°C) extending to the forest die-off edge, to shed light on the generalizability of the warming response and reveal any thresholds in acclimation ability. Trees in warmer soils formed longer and less-branched absorptive roots with higher specific root length and area, and lower root tissue density in both spruce stands, irrespective of warming method and location. Soil warming at the experimental warming site also supported the occurrence of a more varied EcM community and an increase in the abundance of *Tomentella* spp., indicating a shift in nutrient foraging. Fine and absorptive fine root biomass decreased toward warmer soil, with a sharp reduction occurring between + 4 and + 6°C from the ambient and leading to the collapse of the fine root system at the geothermal gradient. At the experimental warming site, the applied + 4°C warming had no effect on fine and absorptive fine root biomass. The similar fine root responses at the two warming sites suggest that the observations possibly reflect general acclimation patterns in spruce forests to global warming.

HIGHLIGHTS

- Soil warming led to a shift in absorptive root morphology and ectomycorrhizal community
- Warming-induced morphological changes in man-made and natural setups were similar
- Collapse of fine root system occurred between + 4 and + 6°C from the ambient

INTRODUCTION

The rate of warming in high-latitude and high-altitude forests is predicted to exceed the global average (IPCC 2013). This warming could jeopardize the large amounts of carbon (C) stored in these ecosystems, particularly below-ground (Lal 2005; Prietzel and Christophel 2014; Schindlbacher and others 2015; Melillo and others 2017). The dynamics of soil C and the resilience of the surrounding forest ecosystems are largely dependent on developments in tree fine root systems and root-associated fungal communities (Jackson and others 1997; Averill and others 2014; Clemmensen and others 2015), which might contribute as much as 50–70% of the C stored in boreal forest soils (Clemmensen and others 2013)

Absorptive fine roots (McCormack and others 2015) constitute the most active and predominant part of the fine root system, accounting for more than 75% of total fine root length and 60% of total fine root surface (Guo and others 2004). In temperate and boreal forests, absorptive fine roots are prevalently (90–100%) colonized by ectomycorrhizal (EcM) fungi (Kraigher and others 2007; Taylor and others 2009), which in turn are associated with rhizosphere bacteria (Frey-Klett and others 2007). These trilateral interactions ensure sufficient root foraging (Ostonen and others 2017). Fine root systems have been shown to respond to changes in the growth environment with great plasticity, either through modifications in fine and absorptive fine root biomass, their absorptive surface per unit of mass (that is, specific root length (SRL) and area (SRA)), branching frequency or shifts in root-associated microbial communities (Finér and others 2007; Richardson and others

2009; Parts and others 2013; Valverde-Barrantes and others 2015; Truu and others 2017). Henceforth, we refer to the entire fine root compartment, which includes primary-structured absorptive and secondary-structured transport roots, as ''fine roots'' and to the proportion of absorptive fine roots, as ''absorptive roots.''

Fine and absorptive roots exhibit several architectural and morphological traits that determine nutrient uptake and root functioning, while providing information about root foraging strategies, demographic patterns, soil fertility and plant stress status. For example, higher investment in fine and absorptive root biomass has been shown to be common in nutrient poor forests and associated with the extensive foraging strategy (Helmisaari and others 2007; Ostonen and others 2017). In small-scale nutrient patches, extensive fine root biomass accompanied by increased root tissue density (RTD) and decreased SRL has been reported and associated with the conservative syndrome of forming long-living roots (Valverde-Barrantes and others 2015). On the other hand, high absorptive root SRL and SRA have been thought to reflect the acquisitive or intensive strategy of enhanced resource uptake at lower biomass investments, high root cost efficiency and fast growth (Comas and Eissenstat 2004; Weemstra and others 2016; Ostonen and others 2017). Depending on the circumstances, an increase in SRL and SRA can be viewed either as means of acclimation or serve as indication of plant stress (Rosenvald and others 2011). High SRL and SRA are achieved through a reduction in either RTD or root diameter (D). D has been linked to root survivorship (Wells and Eissenstat 2001; McCormack and others 2012; Weemstra and others 2016), whereas RTD has been reported to increase gradually with root age (Hajek and others 2014). Additionally, RTD is closely associated with soil nutrient availability (Ostonen and others 1999; Kramer-Walter and others 2016).

The identity of the fungal colonizer has a considerable effect on EcM absorptive root diameter, length and mass, thereby also influencing SRA and SRL (van der Heijden and Kuyper 2003; Ostonen and others 2009, 2017; Makita and others 2012), which is why changes in root morphology should not be addressed separately from developments in EcM fungal community. Besides differing in their influence on root growth and shape, EcM fungal taxa exhibit contrasting foraging strategies with varying capacities of enzymatic activities, nutrient uptake and translocation, and maintenance costs to the host plant (Gorissen and Kuyper 2000; Lilleskov and others 2002; Tedersoo and others 2012).

Therefore, EcM partners have major impact on tree nutrition both directly and indirectly by manipulating root morphology. The different ecological strategies of EcM fungi have been associated with exploration type, based on the amount and differentiation of extramatrical mycelium (Agerer 2001).

The main growth-constraining factors in high-latitude and high-altitude forests are short growing season, low temperatures and for the former, low nitrogen (N) availability (Jandl and Schindlbacher 2014). Moderate warming could therefore initially benefit tree growth through prolongation of the growing season and mobilization of nutrients (Xu and others 2012). However, excessive warming can cause depletion of soil water and nutrient supplies and result in retardation of growth and subsequently tree die-off. Presently, the majority of studies have operated with moderate warming levels and knowledge of extreme warming effects is limited (although more extensive warming systems are being developed, Hanson and others 2017). Also, the size and direction of warming effects on root biomass, morphology and the EcM community are highly dependent on nutrient and water availability in the soil (Johnson and others 2006; Leppälammi-Kujansuu and others 2013). In N-deficient boreal forests, experimental soil warming (+ 5°C) caused increased fine root production and mortality (King and others 1999; Majdi and Öhrvik 2004; Leppälammi-Kujansuu and others 2014). The possible causes for decreased fine root lifespan include enhanced root metabolism and maintenance respiration, and higher pathogen and herbivore activities (Gill and Jackson 2000; Schindlbacher and others 2009; McCormack and Guo 2014). Significantly higher fine root production and mortality and lower fine root biomass have also been witnessed in open-top chambers, where soil temperature increased only 1.2°C (Wan and others 2004), whereas Nishar and others (2017) report a significant decrease in the fine root biomass of the shrub *Kunzea tenuicaulis* toward warmer soil at a geothermal soil temperature gradient in New Zealand.

In high-latitude N-limited ecosystems, warming has increased EcM mycelial production and community diversity (Clemmensen and others 2006; Treseder and others 2016) and caused a shift toward taxa characterized by abundant extramatrical biomass and large proteolytic capacity and a reduction in taxa with high affinities for labile N (Deslippe and others 2011). On the other hand, in an alpine forest, where warming resulted in higher inorganic N availability, an increase in the relative abundance of species that are known to tolerate

high levels of N was reported (Lilleskov and others 2011; Solly and others 2017).

Besides these first indications, our knowledge on fine root adaptation strategies to global warming is still in an emerging state, especially as the number of long-term climate manipulation (warming) experiments is still low. Our objective was to determine the effects of soil warming on: (I) fine root biomass and in particular, the absorptive fine root portion; (II) absorptive root morphology, reflecting individual root level acclimation; (III) the EcM community composition colonizing the spruce roots to understand how fine root foraging adapts to soil warming. Spruce (*Picea sitchensis* Bong. Carr and *Picea abies* (L.) Karst.) was selected for this study because of its economical importance and wide distribution in temperate and boreal European forests (Caudullo and others 2016; Houston Durrant and others 2016). At the same time, the shallow root system of spruce species makes them especially vulnerable to changes in soil surface temperature. The responses of spruce roots growing at the man-made long-term soil warming experiment (a *Picea abies* stand) were compared to results obtained from the geothermal soil temperature gradient extending to the forest die-off edge (a *Picea sitchensis* stand) to shed light on the generalizability of the reactions, reveal any thresholds in acclimation ability and specify the response curve in relation to the intensity of soil warming.

We hypothesized that soil warming (1) induces a decrease in fine and absorptive root biomass, which concurs with an increase in absorptive root surface (SRA and SRL) and a shift in the community structure of EcM colonizers; (2) root response to warming is similar in the man-made experiment and the natural soil temperature gradient. In addition, we inquired whether any root traits show a sharp change at some point on the temperature gradient or whether the changes are gradual.

MATERIALS AND METHODS

Study Sites

The study was carried out on two sites: a man-made soil warming experiment in northern Limestone Alps, close to the village of Achenkirch, Austria; and a natural geothermal gradient (ForHot; www.forhot.is) close to Hveragerði in southern Iceland. Both sites are characterized by a cool humid climate. In Achenkirch, the snow-free period lasts from April/May to November/December and the growing season from May to October. At the ForHot site, snow cover is not permanent

during winter because of the mild oceanic climate. The soil typically freezes there for at least a couple of months during mid-winter, and the growing season lasts usually from late May to late August. Additional background information about the study sites and the treatments is presented in Tables 1 and 2 and more thoroughly described by Schindlbacher and others (2015, 2009) for Achenkirch and by Sigurdsson and others (2016) for ForHot.

Achenkirch Soil Warming Experiment

The studied forest was dominated by Norway spruce (*Picea abies*), inter-mixed with European beech (*Fagus sylvatica*) and silver fir (*Abies alba*). The amount of ground vegetation was negligible. In 2004, three spots were randomly selected on the site, where a warmed (W) plot and an ambient (A) plot were established. The plots had a size of 2 × 2 m each. Warmed plots were equipped with resistance heating cables (0.4 cm diameter, TE-CUTE—0.18 Ohm/m/UV, Etherma, Austria). The cables were buried in 3-cm-deep trenches and had a spacing of 7–8 cm. The soil temperature (ST) of each warmed plot was kept 4°C above that of the adjacent ambient plot during the snow-free seasons, starting in spring 2005. On ambient plots, cables were inserted that were not heated, but had inflicted the same soil disturbance as on the warmed plots, although by 2013, the cable effect can be considered irrelevant. Soil temperature, soil moisture, organic C and total N concentrations, and soil pH were measured as described in Schindlbacher and others (2009) and Schnecker and others (2016).

ForHot Natural Soil Temperature Gradient

On May 29, 2008, an earthquake with a magnitude of 6.3 on the Richter scale occurred in southern Iceland (Halldórsson and Sigbjörnsson 2009). The earthquake affected geothermal systems close to its epicenter and one such system at Reykir moved to a previously unwarmed area (Þorbjörnsson and others 2009; Sigurdsson and others 2016), where the new geothermal bedrock channels resulted in increasing temperature in the soil above by radiative heating (O'Gorman and others 2014). The recently warmed area is covered by an unthinned Sitka spruce forest (*Picea sitchensis*, provenances Seward and Homer from Alaska) that was planted in 1966–1967.

In autumn 2012, twenty-five permanent experimental plots were established on five replicate transects in the stand (Figure S1). The transects were about 50 m in length and ranged from ambient soil temperature to about + 10°C. The location of the plots was chosen aiming for the long-term warming levels (WLs) of about 0, + 1, + 3, + 5 and + 10°C warming (hereafter called levels A (unwarmed ambient), B, C, D and E, respectively), although the realized annual temperature means were slightly lower (Table 2; Sigurdsson and others 2016). Owing to the geothermal origin of soil warming, temperature also increased toward the deeper soil on average 2°C per depth interval (0–5, 5–10 and 10−20 cm; Figures 1 and S2). Ground vegetation proliferated only starting from D or E plots, which was close to the warming-induced die-off edge in the spruce stand (Figure S1; O'Gorman and others 2014).

Table 1. Background Information About the Study Sites

Site (tree species)	Achenkirch (*Picea abies*)	ForHot (*Picea sitchensis*)
Location	47.581°N, 11.639°E	64.008°N, 21.178°W
Elevation, m a.s.l.	910	83–168
Mean annual temperature, °C	6.9[a]	5.2[b]
Mean annual precipitation, mm	1506[a]	1457[b]
Stand age	130	50
Soil type[c]	Chromic Cambisol	Silandic Andosol
Bedrock	Dolomite	Volcanic (basalt)
Stand basal area[d], m² ha⁻¹	35	49
Stand density[d], trees ha⁻¹	487	4461

[a]*Measured between 1992 and 2012 at Achenkirch village (7 km away at similar altitude); data from Zentralanstalt für Meteorologie und Geodynamik (ZAMG); reported by Schindlbacher and others (2015).*
[b]*Measured between 2003 and 2015 at the closest synoptic station at Eyrarbakki (9 km south of Hveragerdi); data from Icelandic Meteorological Office; reported by Sigurdsson and others (2016).*
[c]*IUSS Working Group WBR 2015.*
[d]*Including only spruce.*

Table 2. Main Site and Soil Characteristics per Treatment in 2013

Site, treatment (mean 2013 warming levels[a], ΔT)	Average soil temperature during vegetation period[a,b], °C (ΔT)	Soil temperature during sampling, °C (ΔT)[c]	Stand basal area, m² ha⁻¹	Topsoil C/N ratio	Topsoil NO_3^- availability, µg 10 cm⁻² 89 days⁻¹[d]	Volumetric water content, %[e]	pH-$_{H2O}^f$
AK A	10.5	8.0	35	14.9	NE	47.2	∼ 7
AK W (+4)	14.6 (+ 4.1)	12.1 (+ 4.1)	35	16.0	NE	43.5	∼ 7
FH A	8.2	5.8	48	17.3	0.58	31.2	5.3
FH B (+ 1)	8.9 (+ 0.7)	7.1 (+ 1.3)	56	22.3	0.98	31.2	5.1
FH C (+ 2)	10.2 (+ 2.0)	7.7 (+ 1.9)	50	19.5	1.22	31.3	5.1
FH D (+ 4)	11.7 (+ 3.5)	8.7 (+ 2.9)	35	17.1	2.84	30.7	5.0
FH E (+ 8)	15.9 (+ 7.7)	13.7 (+ 7.9)	27	16.6	82.2	45.0	5.2

AK Achenkirch warming manipulation, FH ForHot geothermal gradient, A ambient, W warming at Achenkirch, B–E warming levels at ForHot, NE not estimated.
[a]*Soil temperature (for long-term averages) was measured at 10 cm.*
[b]*Vegetation period at ForHot from May to August, vegetation period at Achenkirch from May to October.*
[c]*For Achenkirch, mean October 2013 soil temperature is given.*
[d]*Measured from 0 to 10 cm depth throughout the growing season.*
[e]*Measured from 0 to 5 cm depth from April to June 2013.*
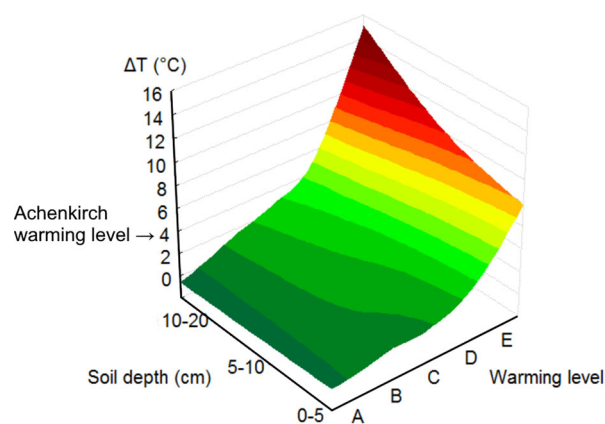[f]*Measured from 0 to 10 cm of mineral soil in July 2013.*

Figure 1. Increase in soil temperature horizontally toward warmer plots (A → E) and depthwise toward warmer soil layers (0 → 20cm) at the ForHot site. The temperature values presented are not absolute values, but the mean increase in °C from the ambient.

In addition to repeated soil temperature measurements, there were comprehensive data of topsoil macro- and microelement availability (measured with exchange membranes), soil pH and soil water content taken during the vegetation period of 2013 available for the ForHot gradient (Table 2). For full description of how the measurements were taken, see Sigurdsson and others (2016). Soil organic C and total N concentrations had also been analyzed for the same depth intervals as mentioned above on sieved (2 mm) and ball-milled samples by dry combustion (Macro Elemental Analyser, model vario MAX CN, Hanau, Germany).

Fine and Absorptive Root Biomass and Morphology

Sampling for absorptive root morphological studies and fine root biomass measurements (FRB, < 2 mm) was conducted in September 2013 at the ForHot site and in October 2013 and 2014 at Achenkirch. The samples were collected with a soil corer (8.8 cm in diameter for 0−10 cm depth and 3.8 cm for 10−20 cm depth at ForHot and 5 cm in diameter for the whole 0−20 cm profile at Achenkirch). During root sampling, we also measured soil temperature from three depths (0−5, 5−10 and 10–20 cm) in each plot at the ForHot site (Figures 1 and S2).

At ForHot, one soil core was taken per plot (5 cores per warming level, altogether 25 soil cores from the whole experimental area). For morphological studies, the cores were further cut into three soil layers: 0–5, 5–10 and 10–20 cm, because of the approximately 2°C increase in soil temperature per layer, as stated above. Each of the 75 samples was then divided into three subsamples, to facilitate work under the microscope. Care was taken to ensure that the subsamples originated from different root fragments. All morphological parameters were measured and calculated for the subsample level, that is, $N = 9$ per plot (45 per warming level) for morphology and $N = 1$ per plot (5 per warming level) for biomass estimations at ForHot.

At Achenkirch, five soil cores were taken per plot for morphological analyses (altogether 15 cores per treatment) and three cores per plot for biomass assessment (9 cores per treatment). The soil cores

from Achenkirch were not further divided, because cable placement ensured maximum homogeneity in soil warming (Schindlbacher and others 2009). The samples were again divided into three sub-samples (comprising of 6–40 root tips) for morphological measurements, that is, $N = 15$ per plot for morphology and $N = 3$ per plot for fine root biomass evaluation at Achenkirch.

Spruce roots were separated from understory roots and washed. To determine the number of absorptive root tips per m^2, the root tips of two or three fine root fragments (which accounted for at least 1/5 of a sample) were counted. Fine roots were then dried at 65°C for 48 h to constant weight and weighed to ± 0.05 mg accuracy. Absorptive fine root biomass (aFRB, g m^{-2}) was calculated by multiplying the mean absorptive root tip weight by the root tip number per m^2. The FRB and aFRB were presented per stand basal area (BA) to describe the functional relationship between the above- and below-ground parts of a forest stand.

For morphological studies, first-to-third-order absorptive roots (altogether > 2600 root tips from the ForHot site and > 1800 root tips from the Achenkirch site) were analyzed after separating them from the woody fine roots, cleaning and counting under the microscope. The length (L_S), diameter (D) and surface area (SA) of the absorptive roots were measured by the WinRHIZO™ Pro 2003b image analysis system (Regent Instruments Inc., Quebec). The roots were dried at 70°C for 2–3 h to constant weight and weighed with an accuracy of ± 0.05 mg. Root tissue density (RTD, kg m^{-3}), specific root area (SRA, m^2 kg^{-1}) and specific root length (SRL, m g^{-1}) were calculated as M_S/V_S, SA/M_S and L_S/M_S, respectively, where V_S is the volume, L_S is the length, and M_S is the dry mass of all absorptive roots in a subsample (Ostonen and others 2007b). Branching frequency was expressed as number of root tips per unit of length (B_L) and unit of weight (B_W). Mean root tip weight (W, mg) was calculated as the dry mass of all the absorptive roots in a subsample divided by the respective number of root tips. Similarly, mean root tip length (L, mm) was calculated as the length of all the absorptive roots in a subsample divided by the number of root tips (Ostonen and others 1999).

EcM Fungal Community

To determine the composition of the EcM fungal community at the Achenkirch site, 30 root samples were collected from the three warmed and the three ambient plots (five root samples per plot as described in the morphology section) in October 2013. The roots of Norway spruce were separated from the roots of understory vegetation and cut into 5 cm fragments, and two or three fragments from each sample were subjected to morphotyping based on mantle color, texture and the presence of emanating hyphae and rhizomorphs, as described in Agerer (1991). The relative abundance of each morphotype was estimated, and all the morphotypes were classified into exploration types based on visual observation. Categorization into exploration types was verified, if possible, using the relevant literature (Agerer 2006; the DEEMY information system). At least two individual root tips of each morphotype per sample were immersed into CTAB lysis buffer [100mM Tris-HCI (pH 8.0), 1.4 M NaCl, 20mMEDTA, 2% cetyl-trimethyl-ammonium-bromide] and maintained at room temperature until molecular analyses. One or two representative root tips of each morphotype per sample were subjected to DNA analysis. Fungal taxa were identified by use of sequence analysis of the nuclear rDNA internal transcriber spacer (ITS) region. The DNA was extracted using a Qiagen DNeasy 96 Plant Kit (Qiagen, Crawley, UK) according to the manufacturer's instructions. Primers, PCR conditions, product purification, sequencing and sequence processing are described in Tedersoo and others (2010). Sequence processing was carried out in Sequencher 4.0 (GeneCodes Corp., Ann Arbor, MI, USA). Sequences were assigned to operational taxonomic units (OTUs) based on a 97.0% ITS barcoding threshold (Tedersoo and others 2003). In order to identify the EcM fungi and possible contaminants, representative sequences of each OTU were subjected to a megablast search against International Nucleotide Sequence Databases (INSD) and UNITE (Abarenkov and others 2010), a ''species hypothesis'' code (an SH code) was assigned to the sequences with more than 97% identity over the blast alignment (Kõljalg and others 2013) and deposited under accessions UDB034264-UDB034324. The above-described separation into morphotypes before sequencing served our aim to identify dominating root colonizers and shifts in their proportions, while we acknowledge that this method underestimates the true OTU richness at Achenkirch. Fungal community composition was not assessed at ForHot.

Statistical Analyses

Statistical analyses were carried out using STATISTICA 7.1 (StatSoft 2005), PERMDISP (Anderson 2004), CANOCO (ter Braak and Šmilauer 2002) and EstimateS 9.1.0 (Colwell 2013) programs. The

sample size for analyses carried out with absorptive and fine root biomass was 25 (plot level) for ForHot and 18 (3 samples per plot) for Achenkirch. All analyses for morphology were done on the sub-sample level ($N = 187$ for ForHot and $N = 90$ for Achenkirch), besides partial correlations and multiple regressions performed between absorptive root morphology, fine root biomass and soil properties of the ForHot site, which were done on the plot level ($N = 22$) and in the 0–10 cm depth. Although at ForHot 25 plots were sampled, there were not enough living absorptive roots present in three E plots for morphological measurements, which decreased sample size for morphological analyses on the plot level to 22. For testing the effect of warming on the abundances of specific EcM genera or exploration types at Achenkirch, analyses were carried out on the sample level ($N = 29$, 4–5 samples per plot). The value of $\alpha = 0.05$ was used as the significance level for all the analyses.

Absorptive root variables were checked for normality using the Kolmogorov–Smirnov test. The effect of warming on absorptive root morphological traits was checked with the t test at the Achenkirch site and using simple linear regression analysis and one-way ANOVA on the ForHot soil temperature gradient. General linear models (type III decomposition) were used to affirm that experimental plots did not have any temperature-independent effect on absorptive root morphology at the ForHot site. After confirming that our manually measured soil temperatures correlated highly with the soil temperature means of the vegetation period and of the whole year of 2013 (Sigurdsson and others 2016; Figure S2, Tables 2 and S3), we used the manually measured soil temperature values from the different soil depth intervals for root morphological analyses, as these values depicted the surrounding conditions during root sampling most precisely.

The effect of warming on fine and absorptive root biomass was checked with the t test (Achenkirch data) and simple linear regression analysis (ForHot data). The warming response of FRB/BA, aFRB/BA and RTD was modeled by the Gompertz function, which was fitted on the average biomass values of each temperature elevation step (calculated from the average vegetation period soil temperatures and rounded to whole numbers).

Redundancy analysis (RDA) (CANOCO program; ter Braak and Šmilauer 2002) was used to visualize relationships between absorptive root morphological characteristics and soil temperature on both experimental sites. The significance of RDA results was tested with the Monte Carlo permutation

procedure in CANOCO. In the ForHot fine root biomass regression analyses, we used soil temperature averages of the 2013 vegetation period taken from Sigurdsson and others (2016).

To determine which soil properties influenced absorptive root morphology and fine root biomass in addition to soil temperature at ForHot, partial correlations were examined and all soil parameters that correlated with a given root trait with a significance of $p < 0.1$ were included in stepwise multiple regression analyses.

For the EcM community analyses, OTU accumulation (rarefaction) curves and the minimum richness estimators, Chao1, Jackknife1 and ACE were calculated. These functions were used to evaluate the sufficiency of sample size and to estimate the proportion of unseen OTUs at the site. When analyzing the effect of soil temperature on the EcM community, all singletons (that is, OTUs present only in one sample) were excluded from analysis, besides for estimating the OTU richness and the Shannon diversity indices. To evaluate the effect of warming and illustrate the dissimilarities in fungal communities (OTU relative abundance), the canonical correspondence analysis (CCA) was employed in CANOCO. The Monte Carlo permutation test was used to decide whether soil temperature had a significant effect on the EcM community composition. The differences in colonization percentages of dominant genera and exploration types between the treatments were checked with the Mann-Whitney U Test. The centroids and the dispersions of the two fungal communities were compared using PERMDISP (Anderson 2001). Differences in OTU richness and Shannon diversity indices between the treatments were tested with the Mann–Whitney U test.

RESULTS

Soil Warming Effects on Fine and Absorptive Root Biomass

At the ForHot site, soil warming reduced both fine root biomass (FRB) and absorptive fine root biomass (aFRB) significantly from 753 ± 171 (mean $\pm$ SE) and 100 ± 28 g m^{-2} at the ambient level, respectively, to 50 ± 27 and 8 ± 7 g m^{-2} in the warmest plots (E; + 8°C), respectively (R^2 varied from 0.17 to 0.42, p varied from <0.001 to <0.05; Table S1 and S3). At Achenkirch, no significant temperature effect on neither FRB nor aFRB was revealed, although there was a similar trend of smaller biomass values in warmed plots: mean ambient FRB was 451 ± 94 ($\pm$ SE) g m^{-2}

and aFRB was 134 ± 23 g m^{-2}, mean warming FRB was 392 ± 87 g m^{-2} and aFRB was 113 ± 38 g m^{-2}. The proportion of absorptive roots among fine roots fluctuated insignificantly along the soil temperature gradient, the means of the warming levels ranging from 0.14 to 0.25 in ForHot and 0.29–0.36 in Achenkirch.

According to the Gompertz function, a sharp decline in FRB/BA started slightly below + 4°C (D plots), followed by a rapid decrease in aFRB/BA from + 4°C onwards and had reached a plateau by + 6°C (E plots), where spruces could not sustain sufficient fine and absorptive root biomass anymore (Figure 2), suggesting a threshold value of + 4°C in warming endurance at ForHot. In respect of above-ground growth, stand basal area followed a similar reduction starting from + 3°C onwards, which was due to increased tree mortality (Table 2).

Regarding correlations between fine and absorptive root biomass and morphology, both FRB/BA and aFRB/BA were most strongly correlated with root tissue density (RTD) at ForHot (R^2=0.51 for FRB/BA and R^2= 0.40 for aFRB/BA, $p < 0.001$; Table S3). The decrease in RTD started at about + 6°C onwards (that is, E plots), whereas the response of other morphological root parameters (SRL, SRA, L and B_L) to soil warming was more gradual and no abrupt changes could be distinguished (discussed below). At Achenkirch, FRB/BA was most strongly correlated with root tip weight (W) (R^2= 0.88, $p < 0.01$) and aFRB/BA to specific root length (SRL) (R^2= 0.77, $p < 0.05$).

Other soil properties besides temperature that significantly influenced FRB and aFRB (both per BA and stand area) in the 0–10 cm depth at ForHot were soil P, Fe, water content and soil pH (Table S5 regression results).

Soil Warming Effects on Absorptive Root Morphology

Soil warming significantly affected absorptive root morphology at both sites and in the same direction: in warmed soils, absorptive roots had greater length (L), specific root length (SRL), specific root area (SRA), but lower RTD and branching frequency per unit of length (B_L) (Achenkirch: p varied from < 0.001 to < 0.01; ForHot: R^2 varied from 0.38 to 0.70, p from < 0.01 to < 0.0001) (Figures 3 and S3, Table S1). At Achenkirch, absorptive root diameter (D) was significantly larger in ambient plots, which was partly because of certain EcM colonizers present in ambient plots that form especially large morphotypes (*Lactarius scrobiculatus*). At ForHot, we did not detect a significant warming effect on root diameter.

At both sites, branching, root tip length and weight (W) experienced the largest variation among the morphological traits (CV varied from 47 to 27), followed by SRL and SRA (CV from 29 to 16). RTD and D varied the least (CV from 15 to 14).

Compared to root morphology, the effects of warming were largest for FRB/BA and aFRB/BA in the warmest plots (E), which was due to the pau-
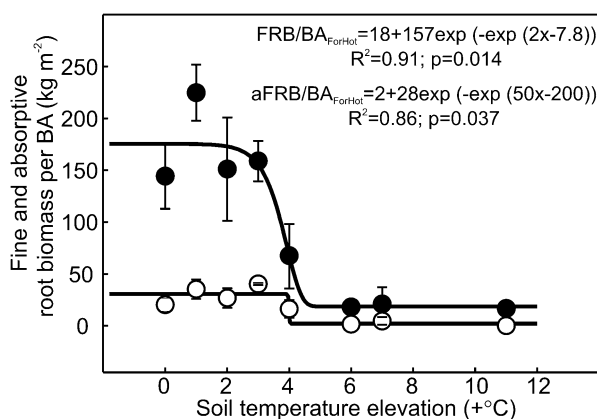

Figure 2. The effect of soil warming on fine (filled circles) and absorptive root biomass (open circles) per stand basal area (kg m^{-2} for both) at the ForHot soil temperature gradient (means $\pm$ SE, $N = 8$). The soil temperature elevations for the ForHot site are calculated from the average soil temperatures of the 2013 vegetation period.
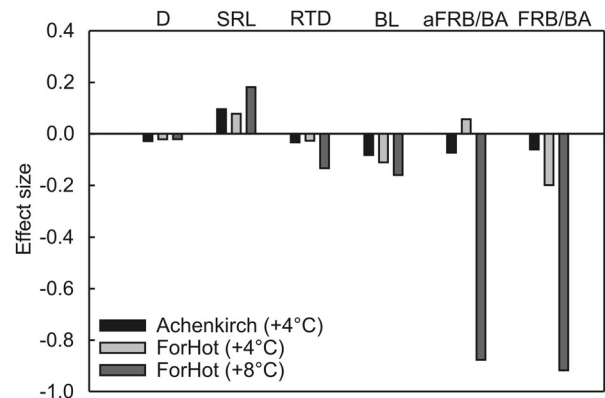

Figure 3. The effect sizes (magnitude of response to soil warming presented in log scale) of absorptive root morphological parameters and fine and absorptive root biomass at the Achenkirch and ForHot sites. *D* absorptive root diameter (mm), *SRL* specific root length (m g^{-1}), *RTD* root tissue density (kg m^{-3}), *B_L* branching per unit of length (no mm^{-1}), *aFRB/BA* absorptive root biomass per stand basal area (kg m^{-2}), *FRB/BA* fine root biomass per stand basal area (kg m^{-2}).

city of roots there, illustrating the proximity of the die-off edge (Figure 3).

At the ForHot site, the warming response of absorptive RTD, L, W and their reciprocals—B_L and B_W—varied with soil depth (Figure 4, Table S1). In the upper part of our studied profile, which experienced more moderate soil temperature elevations, warming reduced root tip proliferation (that is, decreased B_L and increased L), while RTD remained unchanged. At the same time, in the deeper soil that experienced most intense warming (D and E plots), root shape remained unchanged, while RTD decreased. Absorptive root weight and B_W had no overall significant response to warming at ForHot, because counterdirectional changes in different soil depths canceled each other out (Figure 4).

At ForHot, soil depth also had a significant independent (not temperature associated) effect on absorptive root morphology, with the decrease in RTD and branching frequency (both per length and weight) and the increase in SRA and W toward deeper soil layers (GLM type III SS; $p < 0.05$).

At the ForHot site, soil moisture and total N, NO_3^-, Mg, K and Al concentrations significantly correlated with soil temperature and absorptive root morphology (Table S2). Nevertheless, soil temperature was the prime predictor of variance for SRA, SRL, L and B_L, as the correlations between the above-mentioned soil chemical components and root morphological parameters were not significant, when the effect of soil temperature was excluded. Also, soil temperature was the first component in the multiple regression models for these parameters. For RTD, other soil characteristics that appeared in the regression model in addition to soil temperature were soil C/N ratio, pH and K concentration. The variance in absorptive root diameter related most strongly to variation in soil
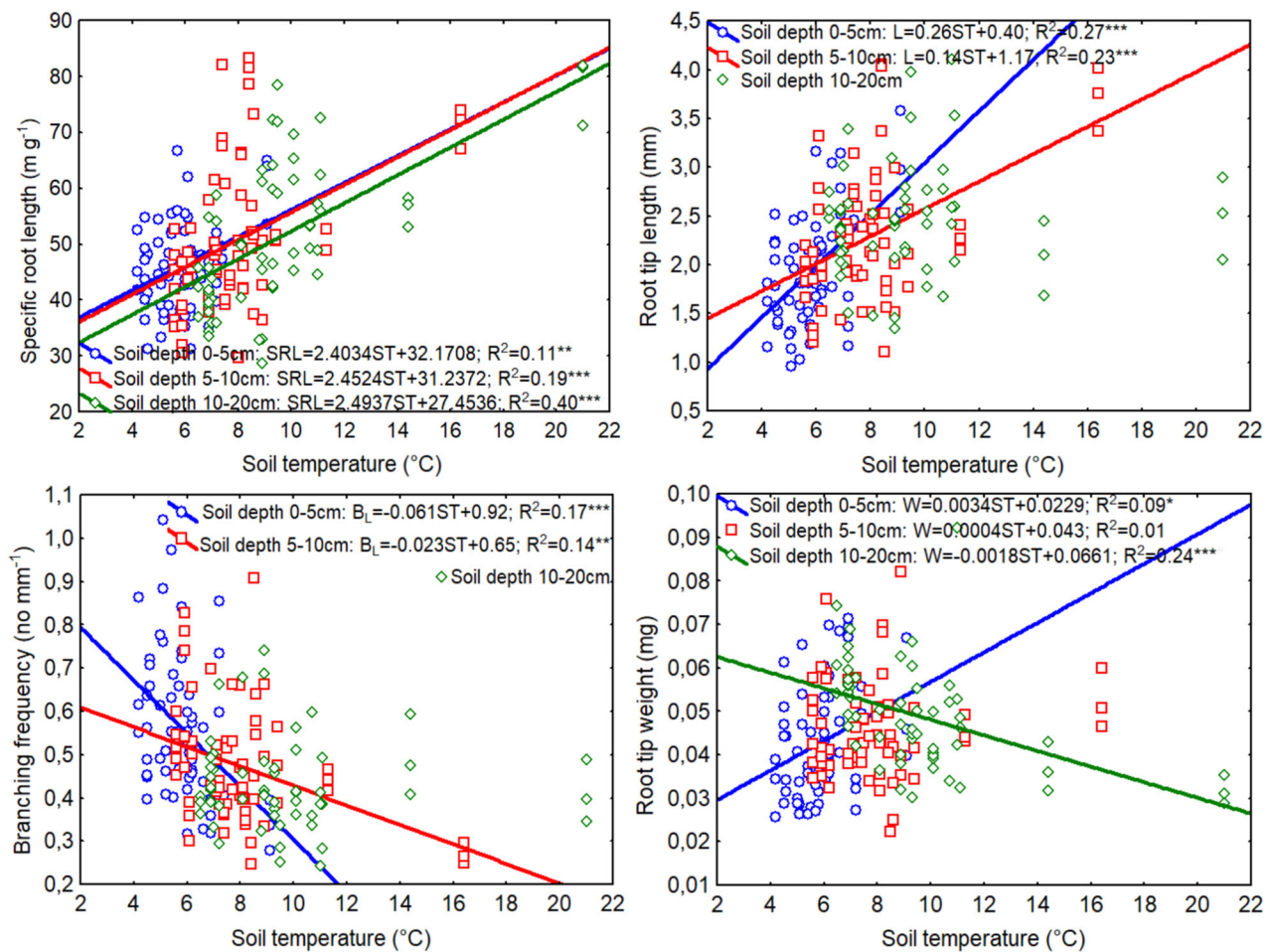

Figure 4. The effect of soil temperature on root morphology in different soil depths at ForHot. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. *ST* soil temperature, *SRL* specific root length, *L* root tip length, *B_L* branching frequency per length, *W* root tip weight.

Al, K, Mn and P concentrations, but not soil temperature (see Table S4 for regression results).

Soil Warming Effects on the EcM Fungal Community

At the Achenkirch site, sequence analysis revealed 61 ectomycorrhizal fungal OTUs (operational taxonomic units), 52 of which were assigned to predefined SHs (species hypothesis, the proxy for species; see Kõljalg and others 2013) at 97% sequence similarity level (Table S6). Of these 61 OTUs, 40 occurred in only one sample. Altogether, the OTU richness of control plots was 33 (including 19 singletons) and 41 in warmed plots (21 singletons). Minimum richness estimates for the whole site ranged from 63 (ACE) to 129 (Jackknife2) (Figure S4). However, the large proportion of single occurrences and the fact that neither the rarefaction curve nor the minimum richness estimate curves approached an asymptote suggest underestimation of the true OTU richness at the site.

Overall, the most frequent OTUs were *Cenococcum geophilum* (SH196545.06FU), *Tomentella* sp (SH219860.06FU) and *Inocybe nitidiuscula* (SH236998.06FU). The 61 OTUs belonged to 15 EcM fungal lineages. The /tomentella–thelephora was the most OTU-rich lineage—represented by *Tomentella* spp., which was significantly more abundant in the warmed plots in terms of overall colonization percentage (Figure 5; $p = 0.029$, Mann–Whitney U test). The *Tomentella* genus was the only group where a significant difference between the treatments was found.
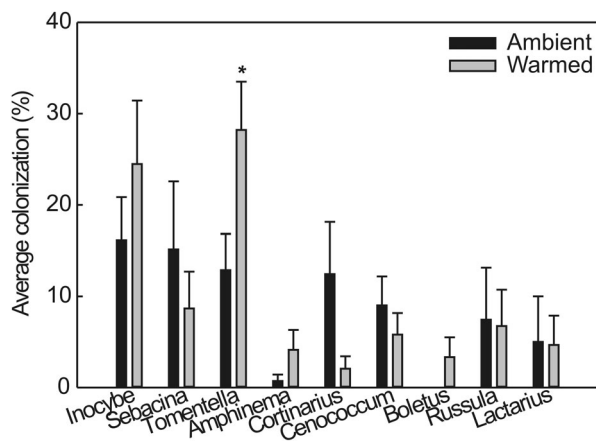
Morphotypes forming long-distance exploration type (represented by *Boletus luridus*) were present only in the warmed plots, although in low abundance (Figure 5). There was a trend of larger colonization percentages of contact, short- and medium-distance smooth exploration types in warmed plots, owing to the larger abundance of *Tomentella* spp., and smaller colonization percentage of medium fringe type in warmed plots, albeit no significant differences between the abundances of the exploration types.

Warming did, however, clearly affects the EcM fungal community structure (CCA of non-singleton OTUs, $p < 0.01$; Figure 6). The EcM community of warmed (W) plots was more variable than that of the ambient plots ($p < 0.01$; PERMDISP), that is, the average distances of OTUs from the group centroid were significantly larger in warmed plots.
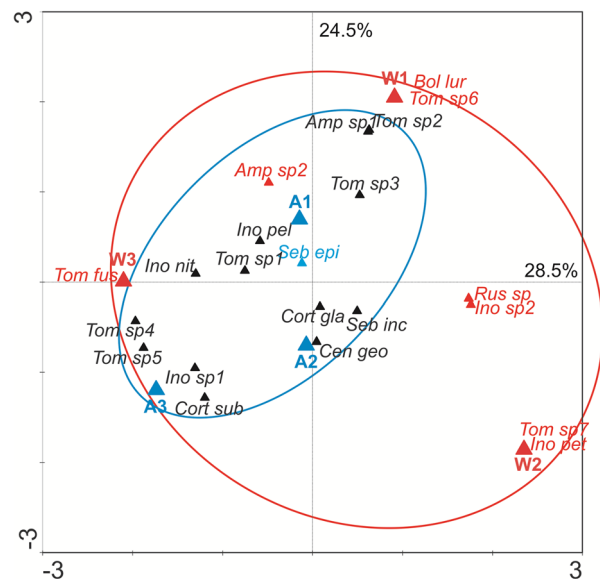

Figure 6. The species-environmental variables biplot of canonical correspondence analysis (CCA), which illustrates the effect of warming on the EcM fungal community (singletons excluded) (Monte Carlo permutation test, $p < 0.01$). Altogether, the plots explained 87% of variation in the EcM community structure. Red font color signifies SHs that were found only in warmed plots, and blue font color represents SHs present only in the ambient plots. The black SHs were present in both treatments. Large triangles depict the experimental plots. *W* warming, *A* ambient, *Cen geo Cenococcum geophilum*, *Ino pet Inocybe petiginosa*, *Ino pel Inocybe pelargonium*, *Ino nit Inocybe nitidiuscula*, *Ino sp Inocybe* sp., *Bol lur Boletus luridus*, *Tom fus Tomentella fuscocinerea*, *Tom sp Tomentella* sp., *Cort sub Cortinarius subsertipes*, *Cort gla Cortinarius glaucopus* var *olivaceus*, *Seb epi Sebacina epigaea*, *Seb inc Sebacina incrustans*, *Amp sp Amphinema* sp., *Rus sp Russula* sp.

Figure 5. The average colonization percentages of dominant genera in warmed and ambient plots at the Achenkirch site. *$p < 0.05$; Mann–Whitney U test. Whiskers signify standard errors.

At the same time, there were no significant differences in Shannon diversity indices nor cumulative species richness between the treatments (Figure S5).

DISCUSSION

This study is the first to compare the effects of soil warming on fine root traits in a man-made experiment (Achenkirch: 9 years of warming) and along a natural soil temperature gradient (ForHot: 6 years of warming). Root system acclimations were viewed simultaneously on different organizational levels: fine and absorptive fine root biomass, absorptive root morphology and the colonizing EcM community. Spruces in warmer soils formed longer and less-branched absorptive roots with higher specific root length and area, and lower root tissue density —a morphological response, which was uniform both at the 47-year-old Sitka spruce stand and the 130-year-old Norway spruce alpine forest, irrespective of warming method and location (Figures 3, 4 and S3). Artificial + 4°C warming also supported the occurrence of a more varied EcM community and an increase in the abundance of *Tomentella* spp. at Achenkirch, indicating a shift in nutrient foraging (Figures 5 and 6). Soil warming led spruces to grow less fine and absorptive root biomass, with a significant decline occurring between + 4 and + 6°C from the ambient and leading to the collapse of the fine root system at the ForHot site (Figure 2).

At the ForHot geothermal gradient, approximately + 4°C from the ambient emerged as a threshold in soil warming endurance, from which onwards Sitka spruces could not sustain sufficient
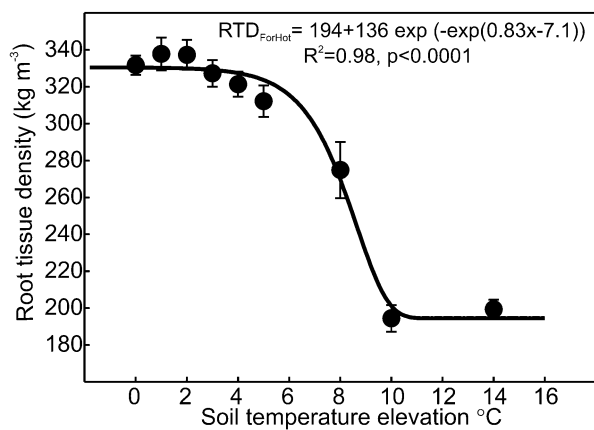

Figure 7. Dynamics of root tissue density (RTD) along the ForHot soil temperature gradient. Means (± SE, $N = 9$) of the whole 0–20 cm profile were plotted against soil temperature elevations measured during sampling.

levels of neither absorptive nor fine root biomass per stand basal area. Regarding above-ground growth at the ForHot gradient, warming induced a rapid decrease in living spruce basal area from + 3°C, which was due to increased tree mortality. On absorptive root level, root tissue density followed a similar decreasing pattern as fine and absorptive root biomass (Figure 7) and could be indicative of faster root turnover as estimated by W. Borken and others (unpublished data) for Achenkirch, based on ^{14}C signatures. Other soil warming studies also report a decrease in fine root biomass and/or an increase in fine root mortality in warmed soils (Majdi and Öhrvik 2004; Wan and others 2004; Nishar and others 2017), agreeing with our findings.

The decline in fine root biomass between + 4 and + 6°C might partly explain the absence of a significant biomass response at the Achenkirch site, where the warming level was + 4°C above the ambient. At ForHot, there were also no significant differences in fine and absorptive root biomass between the ambient and D plots (+ 4°C) yet. The other possible reason arises from the experimental setup at Achenkirch, where only parts of the individual tree fine root system were exposed to warming, which again demonstrates the sensitivity of absorptive root morphology compared to fine root biomass in responding to environmental changes of small spatial scales. Warming experiments that have reported no effect on fine root production or standing crop, also operated with low temperature elevations, which might explain the lack of response (in addition to nutrient limitation) (Johnson and others 2006; Rinnan and others 2007; Xu and others 2012). As emphasized by Beier and others (2012), a limitation of manipulation experiments is that they rarely apply such extreme treatment levels that lead to ecosystem collapse, whereas the geothermal warming gradient allowed us to pinpoint the mortality threshold of Sitka spruce.

Our results from the soil temperature gradient demonstrate the sensitivity of absorptive root morphology, which responded already to low temperature elevations, compared to more delayed changes in fine and absorptive root biomass. In contrast to RTD, which had significantly decreased at + 4°C at Achenkirch and started declining from + 6°C onwards at ForHot, significant changes in the other studied morphological parameters (SRL, SRA, L and B_L) appeared already from lower warming levels (+ 3°C onwards). We attribute the decrease in B_L and increase in L to diminished root tip proliferation, while the reduction in RTD suggests re-

duced lifetime of absorptive roots, but also higher metabolic activity (Wahl and Ryser 2000; Ostonen and others 2011; Hajek and others 2014). Therefore, considering SRL and SRA as stress indicators is quite appropriate, at least in relation to the extreme temperature increases experienced at the ForHot gradient. In Achenkirch, similar changes in root morphology correspond to a previously measured increase in microbial stress biomarkers and enhanced metabolic activity (Schindlbacher and others 2011; Liu and others 2017). Co-occurring shifts in absorptive root morphology and rhizosphere microbes have also been shown for birch forests in other climate change simulations (Ostonen and others 2017; Truu and others 2017).

The fact that the above-described morphological differences were evident even after 9 years of warming in a mature forest, suggests persistent change. The morphological parameters exhibiting largest variation for spruce were root branching (per length and weight) followed by absorptive root length, agreeing with the observations of Ostonen and others (2013), who consider high plasticity to reflect higher relevance in the acclimation process.

Leppälammi-Kujansuu and others (2013) also reported an increase in SRL with warming, while RTD decreased and D increased only when warming was accompanied by fertilization in the boreal forest of Flakaliden. Extensive association between RTD and absorptive root diameter, on the one hand, and soil nutrient availability, on the other hand, was evident also at the ForHot site, which distinguished these parameters from the other morphological traits that were influenced by mainly soil temperature. RTD of Norway spruce has been shown to be more sensitive to soil nutrients than other morphological parameters, decreasing toward soils of higher fertility (Ostonen and others 1999, 2007a).

The + 4°C increase in soil temperature at the Achenkirch site led to the formation of a more varied EcM community and a significant increase in the colonization of *Tomentella* spp. in warmed plots—a genus characterized by contact, short- or medium-distance smooth exploration type specialized for nutrient uptake in close vicinity of the roots and an affinity toward labile inorganic forms of nitrogen (Agerer 2001; Cox and others 2010; Kranabetter and others 2015). Because Achenkirch is a N-rich site with high N deposition, soil warming did not result in any significant differences in soil N availability (ammonium, nitrate, free amino acid), mineralization or microbial N between the treatments (Schindlbacher and others 2015; Schnecker and others 2016). Therefore, nitrogen demand can

be ruled out in explaining the shift in the fungal community. Instead, the need for other nutrients (P, K) might have been driving the changes in the EcM community.

Nutrient cycling was presumably faster in the warmed plots at Achenkirch because of intensified fine root turnover (W. Borken and others, unpublished data). *Tomentella* spp. have been demonstrated to exhibit high activities of cellobiohydrolase, *β*-glucosidase and laccase, but also produce acid phosphatase and leucine aminopeptidase (the latter at high pH), the amount varying with species, habitat patch, soil horizon and pH (Courty and others 2005; Buée and others 2007; Tedersoo and others 2012). These enzymes are essential in fresh litter degradation and phosphorus acquisition. *Tomentella* spp. also have high chitinase activities, indicating their ability to degrade saprotrophic fungal tissues for obtaining nutrients (Courty and others 2005; Buée and others 2007). Indeed, significantly higher *β*-glucosidase activity, although lower chitinase and leucine aminopeptidase activities have been recorded at the warmed soils of Achenkirch (Schindlbacher and others 2015).

Contrary to our EcM community study conducted at a temperate alpine forest, soil warming at sub-arctic Iceland showed no significant influence on total (*Amphinema* dominated) fungal community or basidiomycete EcM community composition in incubated mesh bags, according to an earlier study within the same Sitka spruce stand at ForHot (Rosenstock and others 2018). However, they found a significant influence of soil warming on saprotrophic fungal community composition and observed an increase in the abundance of ascomycetous EcM fungi with soil warming. This increase was mainly through changes in the ascomycetes *Wilcoxina rehmii* and *Trichophaea hybrida*, which responded positively to warming above ca. 2.5°C. It has been proposed that the contrasting findings of warming experiments from different ecosystems might not only be caused by variation in soil nutrient form and quantity (being more concentrated into organic form in the arctic), but also differences in soil drought-risk, host species and photosynthetic performance (Cox and others 2010; Lilleskov and others 2011; Pold and DeAngelis 2013; Fernandez and others 2017). Moreover, the numerous biotic and abiotic factors could be simultaneously selecting for taxa with contrasting foraging strategies, which provide complementarity in accessing different resources and coping with different environmental stresses at the same time (reviewed by Courty and others 2010).

In this study, we joined forces and assessed fine root traits at the man-made soil warming experiment in Achenkirch, where soil was heated via buried cables, and the geothermal soil temperature gradient experiment of ForHot. Artificial soil warming experiments enable us to study any ecosystem (or soil type) of interest and identify causal effects. At the same time, the amount of treatment levels and the spatial and temporal span are usually limited, which prevents the detection of more complex reactions. Also at Achenkirch, only parts of the individual tree fine root system are exposed to warming, which potentially enables unwarmed parts of the root system to compensate for any resource imbalances. Geothermal gradients, on the other hand, allow us to investigate large and gradual temperature increases over relatively small distances, while keeping other confounding environmental factors (climate, elevation, soil type) unchanged. Their large temperature ranges encompass the entire set of climate change predictions, which helps to reveal the shape of the warming response and potential thresholds in acclimation ability (Leblans and others 2017). However, geothermal studies are confined to volcanic environments. The main limitation of both the warming-cable method and geothermal gradients is that they do not imitate global warming to the full, as heat originates from the ground, leaving the air mostly unwarmed (O'Gorman and others 2014; Leblans 2016).

We conclude that soil warming affected all organizational levels of tree fine root systems, and results obtained from an experimental setup proved to be similar to developments on a natural soil warming gradient. The hypotheses found confirmation—soil warming caused a shift toward the intensive strategy of nutrient foraging (substantial decline in fine and absorptive root biomass complemented with morphological acclimation and associated shift in colonizing EcM community) before the fine root system collapsed. It needs to be emphasized that, in both experiments, warming was restricted to soil, whereas above-ground tree biomass was exposed to ambient air temperatures (and ambient atmospheric CO_2 concentrations). Accordingly, the real fine root responses to global change are likely more complex; for example, they could be additionally triggered by increasing labile C flow from above- to below-ground (Norby and Luo 2004) or changes in soil nutrition (Peñuelas and others 2013; Niu and others 2016). Nevertheless, the similar fine root responses at the two warming sites, which were situated in different climate zones and stocked with different spruce species, suggest that the observations are likely to reflect general acclimation patterns to global warming.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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