Adaptive root foraging strategies along a boreal-temperate forest gradient

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Summary

- Tree root-mycorhizosphere plays a key role in resource uptake, but also in adaptation of forests to changing environments.
- Adaptive foraging mechanisms of ectomycorrhizal (EcM) and fine roots of *Picea abies*, *Pinus sylvestris* and *Betula pendula* were evaluated along a gradient from temperate to subarctic boreal forest (38 sites between latitudes 48° N and 69° N) in Europe. Variables describing tree resource uptake structures and processes (absorptive fine root biomass and morphology, %N in absorptive roots, extramatrical mycelium (EMM) biomass, community structure of root-associated EcM fungi, soil and rhizosphere bacteria) were used to analyse relationships between root system functional traits and climate, soil and stand characteristics.
- Absorptive fine root biomass per stand basal area increased significantly from temperate to boreal forests, coinciding with longer and thinner root tips with higher tissue density, smaller EMM biomass per root length and with a shift in soil microbial community structure. Soil C:N ratio was found to explain most of the variability in absorptive fine root and EMM biomass, root tissue density, %N, and rhizosphere bacterial community structure.

- We suggest a concept of absorptive fine root foraging strategies involving both qualitative and quantitative changes in root-mycorhizosphere along climate and soil C:N gradients.

Introduction

Fine root foraging for water and mineral nutrients is of primary importance to ecosystem productivity and relies on a range of specific root traits to achieve its function. Characteristics such as the biomass of absorptive fine roots (Helmisaari *et al*., 2009; Ostonen *et al*., 2011), root tip morphology (Adams *et al*., 2013; Ostonen et al., 2013; Eissenstat *et al*., 2015), predisposition to ectomycorrhizal symbiosis (Trocha *et al*., 2010) and associations with rhizosphere bacterial communities (Kuzyakov & Blagodatskaya, 2015) are all critical to resource capture by trees. Despite the growing understanding of the importance of fine roots and their associated mycorrhiza and bacterial communities in the rhizosphere to carbon (C) and nutrient cycling in forests (Kuzyakov & Xu, 2013), studies of functioning and adaptability of the "root-mycorrhiza-bacteria continuum" to a range of environmental conditions are still in their infancy.

Fine roots are not homogenous; significant anatomical, morphological and physiological differentiation is present within this root category (Saljajev, 1959; Eshel & Waisel, 1996; Ostonen *et al*., 1999; Hishi, 2007; Zadworny *et al*., 2016). Following McCormack *et al*., (2015), we consider fine roots as (i) absorptive roots of first and second order or mostly mycorrhizal short roots with an intact cortex and (ii) transport roots commonly defined as thin woody roots. Fine root biomass (FRB) of both absorptive and transport roots has been found to be very similar in boreal and temperate forest ecosystems (Finér *et al*., 2007, 2011a). However, the amount of absorptive root tips per stand basal area can vary more than tenfold between these two forest biomes (Ostonen *et al*., 2011). There are known differences between the absorptive and transport fine roots in lifespan (Guo *et al*., 2008), nutrient uptake and ability to establish fungal symbiosis (Ouimette *et al*., 2013; Ostonen *et al*., 2007ab; McCormack *et al*., 2015; Zadworny & Eissenstat, 2011). These two functional fine root groups are rarely evaluated separately in current carbon-cycle models (Deckmyn *et al*., 2014; Warren *et al*., 2015).

Root tips with their symbiotic fungi and associated bacterial communities are metabolically active, making many of their traits good indicators of root system adaptability. The magnitude and the velocity of changes of morphological root traits indicate the level of root system plasticity and the adaptation potential of fine root foraging (Ostonen *et al*., 2013; Eissenstat *et al*.,

2015). A majority of trees in temperate and boreal forests extend their nutrient acquisition capacity by expanding fresh carbohydrate supply to ectomycorrhizal fungi (Read, 1992) and to rich communities of bacteria in the rhizosphere (Kuzyakov & Blagodatskaya, 2015). Extraradical mycelia of EcM fungi increase nutrient supply by exploring root-free soil pores/compartments and by translocating organic C to stimulate bacterial activity (Marupakula *et al*., 2016).

Functioning of root–mycorrhiza–bacteria continuum is critical to the performance of the root system (McNickle *et al*., 2009). Depending on the relative contribution of roots and microbionts to tree resource supply, fine root foraging strategies (Lõhmus *et al*., 2006; Ostonen *et al*., 2007a; Ostonen *et al*., 2011) have been described as: A) *an extensive fine root foraging strategy* with a predominance of absorptive fine root biomass, surface area and length, requiring greater C allocation to root formation, and B) *an intensive fine root foraging strategy* with a smaller investment to absorptive fine root biomass, but a greater reliance on root-mycorrhiza-bacteria continuum. The latter strategy, recently also termed the acquisitive resource economics strategy (Weemstra *et al*., 2016), implies greater dependence on interactions between roots, mycorrhizas and soil bacteria, possibly resulting in higher efficiency of the root system in terms of resource capture per unit C invested. However, experimental verification of this concept at the field scale is still lacking and little is known about the functional role of bi- and trilateral shifts in the root-mycorrhiza-bacteria continuum along climatic and environmental gradients.

In this study, we explore the potential of the concept of adaptive fine root foraging described in Norway spruce (*Picea abies* (L.) Karst.) forests gradient (Ostonen *et al*., 2011) to extend to other tree species, such as Scots pine (*Pinus sylvestris* L.) and silver birch (*Betula pendula* Roth.). Our main objective is to construct a conceptual multidimensional framework applicable to the description and analysis of resource capture strategies employed by fine root-mycorrhiza-bacteria communities in forest soils. We consider the adaptation potential of fine root foraging against the backdrop of a range of environmental conditions along a boreal to temperate forest gradient. We hypothesize that: (1) the pattern of absorptive fine root biomass allocation is not tree species-specific, but rather driven by environmental factors and (2) there is a causal trilateral relationships between absorptive fine roots and associated communities of ectomycorrhizal fungi and soil bacteria across an environmental gradient from northern boreal to temperate forests. We aim to link the biomass and the number of absorptive fine root tips and changes in community

structure of colonizing ectomycorrhizal fungi, and soil and rhizosphere bacteria to earlier fine root longevity estimates in our study sites to advance the concept of adaptive fine root foraging strategies.

Material and methods

Forest stands

A set of 38 forest stands along a climate gradient representing boreal, hemi-boreal and temperate forests was used in this study; comprising 13 Scots pine, 10 silver birch and 15 Norway spruce forests covering a latitudinal range from 69° to 48° N (Fig. 1, Table S1). IUSS Working Group WRB (2014) soil classification criteria were used to describe soils t each site (Table S2). Topsoil C:N ratio (organic layer + mineral soil up to 20 cm of soil depth) was used to describe site quality with respect to nutrient availability (Callesen *et al*., 2007; Lehtonen *et al*., 2015). We classified boreal sites as N-limited forests when N in throughfall was less than 8-10 kg N ha^{-1} yr^{-1} and hemi-boreal and temperate stands as N-enriched when N in throughfall exceeded 8-10 N kg ha^{-1} y^{-1}, following Gundersen *et al*. (2006). Stand characteristics such as mean tree height (m) and stand basal area (BA, the area of breast-high cross sections of all the trees in a stand per area unit, m^2 ha^{-1}) were either obtained from published data (Borken *et al*., 2007; Helmisaari *et al*., 2007; Merilä *et al*., 2014; Vanguelova *et al*., 2007; Varik *et al*., 2015) or measured at the time of root sample collection (Table S2). Climate, N deposition, stand and soil characteristics correlated strongly with latitude, as well as with each other (Table S3).

Root traits

FRB on 25 sites, and total root tip number and N concentration on 23 sites were established prior to this study (Ostonen *et al*., 2005; Borken *et al*., 2007; Helmisaari *et al*., 2007, 2009; Vanguelova *et al*., 2007; Leppälammi-Kujansuu *et al*., 2014a,b; Varik *et al*., 2015). On 10 of the remaining sites, FRB and tip number from the organic layer and the 0–20 cm mineral soil layer were determined from 10 to 15 soil cores per site following Ostonen *et al*. (2005). Fine root

longevity data for Norway spruce were obtained by soil core and minirhizotron methods (Table 2; Gaul *et al*., 2009; Leppälammi-Kujansuu *et al*., 2014a,b; Ostonen *et al*., 2005).

Absorptive root morphology, EcM fungal colonisers and (birch) rhizosphere microbiology were assessed by analysing 8-10 samples taken randomly from the top soil (cutting area 225 cm^2, depth of 20 cm) of all stands at the end of the growing season (September-October) once during the period from 2008 to 2012 (Table S4). Root tips were cleaned and counted under a microscope. Two or three first and second order root segments with about 20-30 tips were collected from each soil sample. The total number of root tips sampled and analysed per stand ranged from 234 to 949 in spruce, from 185 to 1330 in pine and from 239 to 1306 in birch.

Root tips were scanned at 400 dpi and analysed with WinRHIZO^{TM} Pro 2003b image analysis system (Regent Instruments Inc. 2003) to establish diameter, length and projected area. Air-dried roots were further desiccated at 70 ºC for 2–3 h to constant weight and weighed. 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 described in Ostonen *et al*. (1999). Root branching intensity was expressed as the number of root tips per 1 mg of dry mass.

Absorptive fine root biomass (aFRB, g m^{-2}) was calculated by multiplying mean root tip weight by root tip number per m^{-2}. Carbohydrate allocation to absorptive roots was established as the ratio of aFRB to total fine root biomass (FRB, g m^{-2}). Absorptive fine root biomass per stand BA (aFRB/BA, kg m^{-2}) was used as a proxy describing the functional relationship between above- and belowground parts of a forest stand. Root area index (m^2 m^{-2}) of absorptive roots was calculated as specific root area of absorptive roots multiplied by their biomass.

EcM fungal community analysis

Root tips from three additional fine root fragments (5–7 cm in length) from each root sample were sorted into morphotypes on the basis of colour and fungal mantle, hyphae and rhizomorph texture. Non-mycorrhizal root tips were found in 7 of 10 birch stands and in 2 conifer stands only, however, their proportion of the total was very low (Table S5). Dominating morphotypes, defined as those exceeding 20% of all tips in a sample, were identified and scored. Three randomly selected individual root tips of each morphotype per sample were abscised and immersed into CTAB lysis buffer [100 mM Tris-HCI (pH 8.0), 1.4 M NaCl, 20 mM EDTA, 2%

cetyl-trimethylammonium-bromide], maintained at room temperature until molecular analysis and subjected to a sequence analysis of the nuclear rDNA Internal Transcriber Spacer (ITS) region. DNA was extracted using a Qiagen DNeasy 96 Plant Kit (Qiagen, Crawley, UK) as per manufacturer's instructions. Primers, PCR conditions, product purification, sequencing and sequence processing are described in Tedersoo *et al*. (2010). Sequences were assigned to species based on a 97% ITS barcoding threshold (Tedersoo *et al*., 2003), except for *Cortinariaceae* and *Hydnangiaceae* where 99% threshold was used. For species-level identification, representative sequences of each species were subjected to a bulk megablast search against International Nucleotide Sequence Databases (INSD) as implemented in the PlutoF work-bench of the UNITE database (Abarenkov *et al*., 2010a,b). All morphotypes were also assigned to EcM exploration types (i.e. contact, short-distance, medium-distance smooth and fringe and long-distance types; cf. Agerer, 2001).

Ectomycorrhizal extramatrical mycelia biomass

Extramatrical mycelium (EMM) biomass per EcM root tip (µg cm^{-1} EcM root tip^{-1}) of each stand was calculated using biomass coefficients for different exploration types (calculations in Weigt *et al*. 2011; Weigt *et al*., 2012a,b) and frequency of dominating EcM morphotypes (percent of root samples colonised). Additional colonisation frequency data for EcM roots were acquired from the literature (Pickles *et al*., 2012; Toljander *et al*., 2006; Twieg *et al*., 2007; Jones *et al*., 2010; Deslippe *et al*., 2011; Peay *et al*., 2011; Børja & Nilsen, 2009; Karlinski *et al*., 2013; Kluber *et al*., 2012; Cox, 2010) to compare estimates of EMM biomass from different stands across the latitudinal gradient. EMM biomass was considered an indicator of (i) carbohydrate allocation to mycelia and (ii) area explored by EcM. All characteristics used in this study are presented in Table S4.

Soil and root chemistry

Bulk soil samples for microbiological (stored in a -20 C°) and chemical analyses (pH-KCl, N, soluble P, Ca, Mg, K, loss of ignition; methods described in Table S2) were taken from the same soil core as the root samples. Root fragments were gently shaken to separate the rhizosphere

fraction from the soil particles adhering to roots. Total C and N content in the absorptive roots were determined using a CHN analyzer (Perkin Elmer 2400/SII).

Bacterial community analyses

In order to assess the role of soil bacterial community in fine root foraging strategy, a pilot study was conducted in birch stands. PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Inc., USA, manufacturer's protocol) was used to extract DNA from bulk and rhizosphere soil samples. The only modification was at the cell lysis and homogenisation step, which was performed for 20 s at 5,000 rpm using homogenizator Precellys 24 (Bertin Technologies). The abundance of bulk soil bacterial communities was evaluated by 16S rRNA gene fragment copy numbers and applying quantitative PCR (qPCR). The forward (5`-GAACGCGARGAACCTTACC-3`) and reverse (5`-ACAACACGAGCTGACGAC-3`) primers were used to amplify a bacteria-specific V6 hypervariable region of the 16s rRNA gene (Gloor *et al*., 2010). All amplifications and calculations were performed as described by Ligi *et al*. (2015).

Bacterial community profiling was performed using Illumina® HiSeq 2000 (Illumina Inc., San Diego, CA, USA) by sequencing combinatorial sequence-tagged PCR products using the same primers as described in qPCR. The forward and reverse primers with 6 bp length barcodes were used in PCR. Sample PCR reaction conditions and library preparation for sequencing are described by Ligi *et al*. (2014).

The paired-end reads were assembled into composite reads using PEAR (Zhang *et al*., 2013). The total initial number of sequences after assembling paired-end reads was 3,934,542. The assembled reads were analysed using Mothur version 1.33.3 (Schloss *et al*., 2009), following modified standard operating procedure guidelines, apart from the clustering step which was carried out with the external programme CROP (Hao *et al*., 2011). Low quality sequences (containing ambiguous bases or more than six homopolymers, minimum read length of 70 bp, or an average sequencing quality score less than 35 over a 25-bp sliding window) were discarded. In total 3,667,727 usable reads were obtained (the total of unique reads was 268,673). The remaining sequences were aligned to the SILVA-compatible reference alignment (Pruesse *et al*., 2007) to screen out overlapping sequences from resulting multiple sequence alignment for clustering.

The sequences were also classified using Mothurs internal version of RDP classifier (Wang *et al*., 2007) using Greengenes (DeSantis *et al*., 2006) reference database and these sequences that remained unclassified at kingdom or phylum level, or were classified as other than bacterial sequences, were removed. Suitable sequences (3,006,517 – 47,988 of them unique) were clustered with CROP into operational taxonomic units (OTUs) at 95% similarity level. In the final step the samples were normalised to the smallest sample size (29,635 reads) by random re-sampling to make them statistically comparable with each other in Mothur. The taxonomic identity of each phylotype was determined by referring to the Greengenes reference database. All assembled reads were deposited in the European Nucleotide Archive under the accession number PRJEB12905.

Statistical analyses

Variables describing EcM root traits were tested for normality of distribution using Lilliefors and Shapiro–Wilk tests, homogeneity of variance was tested using F and Levene tests. Multiple comparisons of means were carried out using Tukey's test for unequal sample sizes with 95% confidence intervals. Forward selection simple regression models were used to analyse relationships between root traits and environmental factors (n=38). Spearman rank correlation coefficients were used to describe EcM exploration types (ranked from 1 to 5 starting from contact type, n=372 for pine; n=317 for birch) as affected by root traits and environmental factors (STATISTICA 7.0: StatSoft, Sweden). GLM (Type III SS) was used to assess the effect of tree species and forest zone (boreal, hemi-boreal, temperate forests) on root traits; climate, soil and stand factors were used as covariates.

Redundancy analysis (RDA, CANOCO; ter Braak & Šmilauer, 2002) was used to describe relationships between root morphological characteristics and sites and morphotypes as descriptive factors separately for all tree species. The significance of RDA results was tested with a permutation test ($p<0.01$).

Inverse Simpson Indexes (ISI) for bacterial communities of the bulk soil and rhizosphere were calculated from OTU data. Kendall rank correlation coefficients were calculated to test the relationships between bacterial community diversity parameters (OTU number and ISI) and soil

and root morphology parameters and to test the relationship between the OTU abundances and stand geographic location (distance from equator).

Hellinger transformation (HTM) was used to transform OTUs relative abundances for both soil fractions and then used in RDA. The non-metric multidimensional scaling (NMDS), based on the HTM, was applied to bulk soil and rhizosphere samples to explore and visualise differences between studied stands. Phylogenetic molecular ecological networks (pMENs) based on bacterial OTU data were constructed for birch stand bulk soil and rhizosphere by applying the Molecular Ecological Network Analyses Pipeline (MENAP) (Deng *et al*., 2012). Topological properties of the empirical phylogenetic molecular ecological networks of microbial communities and their associated random phylogenetic molecular ecological networks for bulk soil and rhizosphere samples were calculated (Table S6). Relationships of environmental factors (soil variables, root morphological parameters) with obtained networks modules were analysed using modules HTM and applying RDA. In case of network modules that were related to the stand distance from the equator according to Mantel test the correlation of module OTU relative abundances to the stand distance from the equator was tested using linear regression analysis. Procrustes analyses using ordinations of the bacterial (whole community and pMEN modules of the rhizosphere and bulk soil) and EcM fungal community (at functional group level) were applied to explore the relationships between bacterial and EcM fungal community structure in birch stand soils.

Results

Biomass allocation into absorptive roots

The proportion of absorptive root biomass (aFRB) out of the total FRB along the latitudinal gradient increased towards the northern boreal forests in all tree species (Table 1), the rate of increase did not differ between species (difference test, $p<0.05$; Fig. S1). The absorptive fine root biomass per stand BA increased exponentially from the temperate to the boreal zone (Fig. 2), with a significant forest zone effect on aFRB/BA (GLM; $F=74.8$, $p<0.0001$, $n=31$, Fig. 2). An increase of 10° latitude from temperate to hemi-boreal forests means an increase of aFRB/BA by 9.0, 12.7 and 16.1 kg m^{-2} in pine, spruce and birch stands, respectively. A further increase of 10° latitude from hemi-boreal to northern boreal forests adds an additional 40.5, 44.7 and 27.9 kg m^{-2}

of absorptive FRB per stand BA in pine, spruce and birch stands, respectively (Table 2, Fig. 2). Stepwise regression analyses comparing climatic, soil and stand factors indicate that aFRB/BA was related to soil C:N ratio and to mean tree heights ($y=0.753(C:N)-0.686$ (height); $R^2=0.81$; $p<0.0001$). Root area index was up to 5-fold higher in the northern forests, mainly due to higher biomass of absorptive roots (Table 2) and was related to soil C:N ratio (stepwise regression analysis $R^2=0.69$; $p<0.01$, $n=30$).

Absorptive FRB per stand BA in relation to soil C:N ratio and %N of root tips

Soil C:N ratio was the main factor describing the variability of absorptive FRB per stand BA along the climatic gradient (GLM, Type III SS; whole model $R^2=0.90$, $p<0.001$), with a significant difference between birch and conifers (Fig. 3a). Soil C:N ratio varied from 12 to 23 in birch stands compared to a range of 18 to 49 in coniferous stands (Table S2). In birch, aFRB/BA was five times higher at the northern sites, with soil C:N ratio from 19 to 23, than at the southern stands where it declined below 17.

Absorptive FRB per stand BA was negatively correlated with nitrogen content (%N) of absorptive roots both in pine ($r=-0.66$, $p=0.018$, $n=12$) and in spruce ($r=-0.71$, $p=0.015$, $n=11$). Soil C:N ratio was the main environmental parameter driving absorptive root %N ($R^2=0.57$, $p<0.000$, $n=34$; Fig 3b). The threshold of a root %N at what the drastic change in the absorptive FRB per stand BA occurs was <2.5% for birch and <1.5 % for conifers (Table 2). Fine root longevity in the spruce stands was, on average, 1.99 years in the north and 0.66 years in the south (t-test, $p=0.012$, $n=4$).

Root morphology

The total absorptive fine root biomass per stand BA was related to mean SRL and length of root tips ($R^2=0.43$; $p<0.001$; $F_{2,29}=10.89$), indicating a link between biomass allocation and morphology of root tips. Morphological traits of absorptive roots varied across the latitudinal gradient and among tree species (Fig. 4; Table S7). On the basis of the length of correlation vectors, the highest proportion of variation in root traits was explained by latitude (correlation matrix is not shown). Tree species and geographical location of the stands explained 41% of the

variation in absorptive root morphology ($p<0.001$, RDA, Fig. S2). Root morphology of birch and pine exhibited similar pattern of increasing SRL towards the north (Fig. 4). The increase in SRL was mainly determined by the variation of diameter (by 61% in birch and by 52 % in pine; $p<0.01$). Absorptive roots in spruce adjusted to the environmental gradient by modifying root branching intensity, which was higher in temperate stands and was determined by a variation of root tip length (41%; Ostonen *et al*., 2013). The length of an absorptive root tip in conifers was positively correlated with latitude ($r=0.75$; $p<0.000$); the average absorptive root tip was 2.1 times longer in spruce and 1.7 times longer in pine in the northern sites compared to the southern forests (Fig. 4; Table S7).

Branching intensity and root tip length of birch and pine were not affected by soil chemistry, while root tissue density, diameter and SRL related significantly to %N (R^2 varied from 0.55 to 0.59; $p<0.05$) and Mg content (R^2 varied from 0.28 to 0.51; $p<0.05$) in the soil. RTD was species-specific (tree sp as random factor) and determined by soil C:N ratio ($F=8.29$; $p<0.01$). RTD of absorptive roots (Fig. 4) of all tree species, as well as RTD of non-colonised root tips in birch (data not shown) was significantly higher (Tukey test, $p<0.05$, $n_{bor}=6$ and $n_{temp}=7$) in northern low-N forests.

Ectomycorrhiza

Community structure of dominating EcM explained most of the morphological variability of absorptive roots in all tree species. Based on the redundancy analysis, the dominating morphotypes explained 46.7% of the variation in spruce (Ostonen *et al*., 2011), 63.2% and 57.0% of variation in pine and birch absorptive root morphology, respectively (Monte Carlo permutation test, $p<0.05$; $n=48$ in spruce, $p<0.001$; $n=46$ in pine and $p<0.001$; $n=56$ in birch, respectively).

In spruce (Ostonen *et al*., 2011) and birch forests, the largest number of EcM fungal species was assigned to contact and short-distance exploration types, while the medium-fringe exploration type was prevalent in pine forests (Table S5). An increasing presence of long-distance exploration types was observed in both coniferous species in southern forests, but not in birch (Table S5; data for spruce from Ostonen *et al*., 2011).

Biomass of EcM mycelia.

Biomass of EcM extramatrical mycelia (EMM; µg cm^{-1} EcM root tip^{-1}) of dominating morphotypes varied from 107 to 1417 µg cm^{-1} EcM root tip^{-1} in all stands, increased towards lower latitudes and was similar in all tree species (Fig. 5). EMM biomass of dominating morphotypes was related to latitude, fine root biomass, absorptive FRB per stand BA and soil C:N ratio (R^2=0.65, $F_{5,21}$=7.74; p<0.001; n=27), however it was not directly affected by N-deposition (p<0.36).

Although EMM biomass per length unit of EcM root tip was significantly higher in N-enriched southern stands (Fig. 5), taking into account the higher number of longer root tips in the north, the estimated extramatrical mycelium was 2-4 times higher in the north than in the south, e.g. 93, 96 and 113 g m^{-2} in boreal pine, birch and spruce forests, respectively. Estimates for temperate pine, birch and spruce forests were 25, 35 and 62 g m^{-2}, respectively

Bacterial community structure in soils of silver birch forests

The bacterial 16S rRNA gene abundance varied between 8.26×10^9 and 8.64×10^{10} copies g^{-1} DW in the bulk soils of the studied birch stands (Table S8) and this variation was not related to the distance between the stands or to distance from the equator. The bacterial community diversity index (ISI) was the lowest in both bulk soil and rhizosphere in the northernmost (Kivalo, Syktyvkar) and southernmost (Risley Moss) stands (Table S8), with no relationship between diversity indicators (OTUs numbers, ISI) and stand distance from the equator. The bulk soil bacterial communities were dissimilar in geographically more distant stands than in closer stands (Mantle test, r=0.51, p<0.01). Rhizosphere bacterial communities were grouping similarly to the bulk soil communities (Procrustes analyses, r=0.83, p<0.001), based on differences in relative abundances of bacterial groups at different taxonomic level, i.e. phyla *Acidobacteria* and *Bactroidetes*, classes *Acidobacteria* and *Spartobacteria*, order *Acidobacterials* (Table S9). Rhizosphere bacterial communities of the southern-most (Risley Moss) and the northern-most site (Kivalo) were distinctive from other sites on the NMDS ordination plots (Fig. S3a,b; Table S9).

The application of Molecular Ecological Network Analyses Pipeline on the OTU data resulted in two distinct phylogenetic molecular ecological networks (pMEN) for bulk soil and rhizosphere

bacterial communities, consisting of eight and nine related modules, respectively (Fig. S4). All the modules had a unique phylotypic composition (Table S10). A substantial part of phylotypes from both soil fractions (about 56% in bulk soil and 74% in rhizosphere) were not involved in these networks. The stand distance from the equator was a significant predictor only in the case of one bulk soil module (H: $r=0.58$, $p<0.05$). The species from phyla *Actinobacteria* and *Proteobactera* dominated (16 and 10 OTUs from 36, respectively), but there were also representatives from phyla *Acidobacteria*, *Bacterioidetes, Firmicutes*, *Clamydiae*, *Spirochaetes* and *Verrucomicrobi*. Relative abundances of four bacterial phylotypes from this module were negatively related to the distance from the equator; however, two phylotypes in Risley Moss appeared to be deviant from the general pattern (Table S10; Fig. S5).

Soil characteristics had a strong effect on the bacterial community structure in birch forest soils (Table 3), describing 47.53% of the bulk soil and 51.06% of the rhizosphere bacterial community variations ($p<0.001$ in both cases). pH and P content were the driving soil factors - the numbers of phylotype (OTUs) and diversity indices (ISI) in both soil factions were correlated to soil pH (Kendall correlations $\tau= 0.6$ to $\tau=0.69$; $p<0.05$ in all cases). Soil C:N ratio correlated significantly with the number of OTUs in the rhizosphere ($r=-0.64$, $p=0.044$, $n=10$). Soil K content was related to rhizosphere bacterial community diversity index values (Kendall correlations $\tau=-0.51$, $p<0.05$).

Root-mycorrhiza-bacteria continuum in birch forests

Strong relationships between absorptive root morphology, EcM fungal community structure and bacterial community structure were found in bulk soil and rhizosphere in birch stands (Fig. 6).

There was a significant correlation between dominant fungal lineages, and the whole rhizosphere bacterial community structure (Procrustes analysis, $p<0.05$). This relationship was statistically significant also in case when absorptive root morphology or soil chemical parameters were used in the analysis as covariables. In addition, diversity and proportions of dominant linages of EcM fungi correlated with the structure of rhizosphere phylogenetic molecular ecological network modules J and M (Fig.S4, Fig 6).

The relationship between birch absorptive root morphology and soil bacterial community structure was stronger in the rhizosphere than in bulk soil. Significant correlations between root tip weight and bacterial diversity index ($\tau=-0.51$; $p<0.05$), and between root branching intensity

and phylotype numbers (τ=0.54, $p<0.05$) in rhizosphere were revealed from the analyses. The structure of rhizosphere pMEN module N was also affected by root tip weight. In bulk soils, the proportions of bacterial phylotypes in module E were related to root tissue density and tip weight of absorptive roots (Fig. 6).

Discussion

Fine root foraging strategies

Tree fine root system forms a continuum with soil microbial communities for acquiring nutrients from the soil. Since it is not possible to isolate individual groups of organisms when studying their contribution to tree nutrition, we propose a multidimensional conceptual framework for fine root nutrient foraging strategies to advance the ecological gradient-related theory of adaptive plant economic spectrum (Freschet *et al*., 2010; Prieto *et al*., 2015). Birch, spruce and pine all grow an extensive mass of absorptive roots when growing in the N-poor subarctic soils close to their northernmost natural distribution limit. At the other end of the N availability scale, however, their fine root systems appear to switch to intensive foraging, resulting in a smaller absorptive root biomass per stand BA in temperate forests. The mechanisms employed to optimise the efficiency of absorptive root foraging are thought to include changes in root morphology, in mycelial biomass per root tip length unit and shifts in soil and rhizosphere bacterial community structure. We found significant complementarity in adaptive changes within the continuum of root-mycorrhiza-bacteria of birch and within the root-mycorrhiza continuum of pine and spruce driven by similar biomass allocation pattern in all studied tree species (Fig. 7).

Response curves of most root traits along the gradient were strongly related to the soil C:N ratio, which is a good indicator of soil organic matter quality as it determines how much N could potentially be mineralized per unit of C respired (Lehtonen *et al*., 2015). Our analysis of bulk soil bacterial community structure as a function of distance from the equator indicates lower macromolecules degradation activity potential in soils from northern birch stands. A smaller proportion of two species belonging to the cellulose degrading family *Chitinophagaceae* (Bailey *et al*., 2013) may indicate a slowdown of litter decomposition and a subsequent decrease of nutrient availability.

Trees are thought to down-regulate their belowground C allocation in favour of aboveground growth in response to high N supply as fewer roots are needed to maintain sufficient N uptake (Vanninen & Mäkelä, 1999). A higher amount of fine roots and EcM tips per needle biomass (Helmisaari *et al*., 2007, 2009), or up to 11 times more absorptive root biomass per stand BA (Ostonen *et al*., 2011), is needed at higher latitudes (> 65° N) on sites with high soil C:N ratio. In this study, absorptive root biomass per unit stand BA in the subarctic stands when compared to temperate stands was up to 12-times higher in pine and 6-times on birch. Even taking into account faster fine root turnover in temperate forests, the investment to absorptive root biomass per stand BA in boreal forests is still more than 4 times higher on average. These results are consistent with the previously proposed functional equilibrium theory (Brouwer, 1983), optimal partitioning theory (Bloom *et al*., 1985), resource economic spectrum (Weemstra *et al*., 2016), as well as with the recent development of process-based growth models recognising belowground C allocation (Mäkelä *et al*., 2016). All studied tree species preferentially allocate more biomass to fine roots and EcM under N deficiency, the observed increase in root absorptive area in northern N-limited forests might be a reflection of that.

Our study provides evidence that the morphology of absorptive roots is closely related to biomass allocation to root tips. Irrespective of tree species, an increase in absorptive root biomass at stand level coincides with (i) longer and thinner roots with higher root tissue density and (ii) higher degree of colonisation by short-distance EcM types. Morphological adaptation was shown to be critical in stressful environments such as the northern boreal forests (Ostonen *et al*., 2013), tree species-specific differences in absorptive root morphology were smaller in temperate forests (Fig. 4).

Root morphology and structural shifts of root associated microbial communities

Our results for birch suggest a strong relationship between absorptive fine root morphology and the structure of EcM and bacterial communities in the rhizosphere and bulk soil (Fig. 6). The role of each associated partner organism in resource uptake is modified by environmental conditions, e.g. soil C:N ratio across the latitudinal climate gradient. Further, these relationships are linked to biomass allocation patterns of absorptive roots observed between the northern N-poor and the southern N-rich forests. Our results are in good agreement with Högberg *et al*. (2007),

demonstrating an increase of fungi-to-bacteria ratio and higher C allocation to belowground in N-limited forests with high soil C:N and with shifts in mycorrhizal and bacterial community structure. We show an effect of soil organic matter quality on bacterial community structure in the rhizosphere of birch absorptive roots. Where the number of bacterial phylotypes in the rhizosphere increased at lower soil C:N ratios, we saw a predominance of a bacterial consortium (module H) containing *Fluviicola* in soils with higher N content. Bacteria from this genus prefer rich soils and are able to degrade persistent organic molecules in plant root rhizosphere (Song *et al*., 2016). Similarly, the share of *Tomentella* sp among the dominating EcM fungal colonisers increased, whereas *Cortinarius* sp colonization rate decreased towards richer soils of temperate forests. This is in good accordance with the results of Kranabetter *et al*., (2009), who showed a similar pattern of these morphotypes along productivity gradients in a southern boreal forest. Furthermore, the rate of ammonium uptake of *Tomentella* spp was shown to be over three times that of *Cortinarius* spp (Kranabetter *et al*., 2015), supporting our hypothesis of higher efficiency of absorptive roots in temperate forests. EcM community structure affects root-associated bacterial communities (Korkama *et al*., 2007; Simard *et al*., 2013) and bacteria may assist mycorrhiza formation as well (Frey-Klett et al., 2007). We found that two bacterial consortiums in the rhizosphere of birch absorptive roots were related to the diversity of dominating colonizing EcM fungi. Our study across a gradient of birch forests revealed that bacterial network consortiums (classified at order level) in both bulk and rhizosphere soil can be linked to various types of phosphatases and phosphorous transport systems (Bergkemper *et al*., 2016). *Rhizobiales*, *Solibacteriales*, *Acidobacteriales* and *Rhodospirillales* were all represented in several bacterial network consortiums, with the structure of some of these (M) directly related to the dominant EcM community. The presence of the root-mycorrhiza-bacteria continuum discussed in this paper hints at interactions and feedback between root growth promotion mechanisms (e.g. phytostimulation via hormones) or direct physiological and metabolic mechanisms (e.g. production of hydrolytic enzymes and root metabolites) that enable acquisition of soil phosphorus (Richardson & Simpson, 2011). The role of EcM fungi in P acquisition is well known (Plassard & Dell, 2010). In temperate spruce (Ostonen *et al*., 2011) and temperate pine forests, the proportion of root tips colonised with mycelium-rich EcM fungi forming rhizomorphs with long exploration morphotypes significantly increased. This supports our hypothesis of higher efficiency of an average root tip due to the enlargement of the explored soil volume through a

mycelium-rich EcM fungal partner (Fig. 5) and related qualitative shift in the soil and rhizosphere bacterial communities in temperate stands, where a smaller absorptive fine root biomass is supporting the same forest basal area unit.

Absorptive root tissue density was found to correlate with rhizosphere bacterial network structure, highlighting the direct impact of root physiological traits on rhizosphere bacteria. Furthermore, significant correlations between bacterial phylotype numbers and root branching intensity, as well as between bacterial diversity index and root tip weight, suggest that a higher number of bacterial species were more evenly distributed, particularly around younger root tips probably due to the better substrate supply from root (Folman *et al*., 2001). In birch forests subjected to the climate change manipulation, the changes in the structure of soil bacterial community and root morphology were complementary to each other (Truu *et al*., 2017). Root tissue density has been shown to correlate with root tip lifespan (Ryser, 1996; Ostonen *et al*., 2013), where resource uptake rates decline with increasing root age (Yanai *et al*., 1995). Up to a 1.5-fold increase in RTD of absorptive roots towards the boreal spruce forests coincides with a threefold increase of fine root longevity. Older mycorrizal root tips are more likely to support only limited extramatrical mycelium activity and lowered availability of transferable nutrients in the fungus (Cairney & Alexander, 1992). This is consistent with our hypothesis of absorptive roots with lower efficiency in the north.

Although fine root lifespan has been shown to be longer in boreal than in temperate forests (Finér *et al*., 2011b), existing fine root longevity data are not yet sufficient to evaluate tree species-specific patterns on a broad spectrum of soil C:N ratios. Some evidence of higher fine root longevity in soils with high C:N ratio is available for spruce (Ostonen *et al*., 2005; Gaul *et al*., 2009; Leppälammi-Kujansuu *et al*., 2014a,b) and for birch (Varik *et al*., 2015; Uri *et al*., 2017). The observed increase in absorptive root biomass per stand BA towards the north is complementary with a decrease in N concentration of absorptive roots (Fig. 7), both related to an increase in soil C:N ratio. %N of roots is asymptotically approaching the physiological limit (Wang *et al*., 2014) in low-N subarctic stands matching with the northernmost extension of studied tree species. Root tip %N might be a good predictor for the absorptive fine root biomass. A switch to a larger absorptive root biomass occurs when the average N concentration reaches <1.5% in conifers and <2.5% in birch (Fig. 3b). Trees increase absorptive root biomass to ensure sufficient nutrient uptake, this often coincides with two- to fourfold increase in the amount of

connected mycelia (irrespective of fungal community structure). Although ectomycorrhizal N uptake is more cost-efficient for the individual trees at low soil N availability, purely mycorrhizal strategy may cause immobilisation and decline of N in the soil at the stand level (Näsholm et al., 2013; Franklin *et al*., 2014). This theory is supported by our results of a low %N level of root tips and high C investment to root and mycelial biomass in boreal forests. The critical mass of absorptive roots per stand BA for transition of the foraging strategy in all three studied tree species seems to be close to 20 kg absorptive roots per m^2 (Fig. 2), despite the difference in absolute root %N values between conifers and birch.

Our concept of fine root foraging strategies puts forward the notion that quantitative differences in absorptive fine root biomass per stand BA are concurrent with changes in root morphology. At the same time, a foraging strategy involves a qualitative shift in multitrophic interactions in the rhizosphere involving host trees, EcM fungi and associated bacteria. The variety of alternatives within root-mycorrhiza-bacteria continuum enables adaptive root foraging in both northern subarctic boreal and southern temperate forests. We envisage a trilateral relation between the morphological traits of absorptive fine roots, exploration types of colonising EcM fungi and rhizosphere and bulk soil bacterial community structure. Thus, qualitative shifts in roots associated microbial communities affect biomass partitioning of trees, which in turn can lead to a switch in the fine root foraging strategy and to a change in belowground C pathways.

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Author contributions

I.O., M.T., J.T. and K.L. designed the study with contributions from H-S. H. (Finland), W.B. and U.Z. (Germany), D.G. and E.V. (UK), K.A. (Lithuania); M.T., J.T., J-K. P. carried out the analyses of soil and rhizosphere bacteria, I.O. morphotyped and L.T. carried out molecular analysis of EcM fungi; I.O., K.R., K.P., M.K., U.Z, performed morphological studies and determined fine root biomass for some of the stands; D.G. and M.L. conducted field work in Syktyvkar and Risley Moss; J.A., M.V. and V.U. were responsible for measuring stand characteristics in Estonia and P.N. for Finland; A-J.L., P.M., Ü.N., J.F., N.K., K.A. were responsible for climatic and soil characteristics in Finnish, Estonian and Lithuanian stands. J. L-K. conducted field work and provided data for Flakaliden. I.O., K.L., J.T., L.T. and J-K.P. carried out statistical analyses. All authors discussed the results; I.O. oversaw the study and drafted the manuscript; I.O., M.L., M.T., J.T., H-S.H., E.V., W.B., D.G., K.R. and L.T. co-wrote the paper.

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877 **Legends of the Figures**

878 **Fig. 1** Study sites in European boreal and temperate *Picea abies* (red dots), *Pinus sylvestris*
879 (green), *Betula pendula* stands (yellow). Blow-up box shows sites in Estonia due to their close
880 proximity.

881 **Fig. 2** The absorptive fine root biomass per stand basal area (aFRB/BA, kg m^{-2}) in birch, pine and
882 spruce stands along the latitudinal gradient.

883 **Fig. 3** The relationship between (a) absorptive fine root biomass of birch, pine and spruce stands
884 and respective soil C:N ratio and (b) %N of absorptive roots in birch (open circles), pine
885 (triangles) and spruce (filled circles) stands along the soil C:N ratio gradient.
886
887 **Fig. 4** (a) Mean diameter (mm), (b) mean length (mm) of absorptive root tips and (c) root tissue
888 density (RTD, kg m^{-3}), (d) root branching intensity (No of tips mg^{-1}) and specific root length
889 (SRL, m g^{-1}) of the absorptive roots in birch (open circles), spruce (filled circles) and pine
890 (triangles) stands along the latitudinal gradient.
891
892 **Fig. 5** The change of specific ectomycorrhizal extramatrical mycelial biomass (EMM biomass;
893 µg cm^{-1} EcM root tip^{-1}) of dominating morphotypes along the latitudinal gradient for all stands;
894 open circles represent data calculated from the literature.
895
896 **Fig. 6** A scheme showing statistically significant relationships between the structure of
897 rhizosphere and bulk soil bacterial communities, dominant ectomycorrhizal (EcM) fungal
898 community and absorptive root morphology in studied birch stands soils. Capital letters denote
899 modules of bacterial phylogenetic molecular ecological networks (pMENs). Arrows indicate
900 RDA relationships direction, bacterial community or morphology variation percentages explained
901 by factors variations within the groups are shown above the arrows. Procrustes relationships are
902 indicated by simple lines with p values indicated by asterisks (*$p<0.05$, **$p<0.01$, ***$p<0.001$).
903 The relationships between whole community and particular subunits or factor sets are indicated
904 with solid lines. The information about exploration types of EcM fungi and OTUs taxonomy are
905 given in Tables S5 and S10, respectively. Abbreviations for absorptive root morphological

characteristics: RTD - root tissue density, kg m^{-3}, SRL and SRA - specific root length, m g^{-1} and area, m^2 kg^{-1}.

Fig. 7 A conceptual scheme of fine root foraging strategy related to latitudinal climate and soil C:N gradient from boreal to temperate forests. Soil C:N ratio increases from left to right, from N-rich temperate forests to N-poor northern boreal forests. Foraging strategies are based on adaptation of biomass allocation to absorptive fine roots associated with fine root turnover rate, fine root morphology and changes of root associated EcM fungi and rhizosphere bacterial communities. EXTENSIVE strategy refers to investment in larger absorptive fine roots biomass per forest basal area (kg m^{-2}), while INTENSIVE strategy denotes the tendency to establish smaller absorptive root biomass, associated with functional changes in root morphology and a larger reliance on EcM and bacterial communities in the rhizosphere. Note that the presented trends for root tip number, absorptive fine root biomass and morphology, %N and EcM mycelium are based on data of all three studied tree species, while trend in fine root turnover is based on spruce stands data and supported by literature data for birch stands (Varik *et al*., 2015; Uri *et al*., 2017) and for general tendencies along biomes (Finér *et al*., 2011b). The trilateral relationships between roots, EcM fungi and soil and rhizosphere bacteria and trend in number of bacterial phylotypes from boreal to temperate forests are based on pilot study across birch forests.

925 **Table 1** The proportion of ectomycorrhizal absorptive fine root biomass (aFRB) in the total fine
926 root biomass (FRB) (%, ± SE) for Norway spruce, Scots pine and silver birch forests in different
927 forest zones. Different letters denote significant differences between forest zones (Tukey test,
928 $p<0.05$).

Forest zone/tree sp	Spruce(n=15)	Pine (n=12)	Birch (n=6)
Boreal	28 ± 2^{a}	23 ± 2^{a}	17 ± 8^{a}
Hemi-boreal	18 ± 5^{ab}	23 ± 3^{a}	12 ± 2^{a}
Temperate	11 ± 3^{b}	9 ± 3^{b}	7^{a}

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Table 2 Absorptive fine root biomass (aFRB), root area index and N concentration (%) and C:N ratio of absorptive roots (first and second order, mostly ectomycorrhizal roots) in Norway spruce, silver birch, Scots pine forests across a latitudinal gradient (from 69˚ to 48˚ N). * aFRB, root area index, %N and C:N ratio have been published in Ostonen *et al*., 2011. Fine root longevity estimations are published in: a – Leppälammi-Kujansuu *et al*., 2014b; b- Leppälammi-Kujansuu *et al*., 2014a; c – Ostonen *et al*., 2005; d - Gaul *et al*., 2009.

Stand	**aFRB, g m^{-2}**	**Root area index, m^2 m^{-2}**	**%N**	**C:N of root tips**	**Longevity, yr**
		Picea abies			
Pallasjärvi*	69.9	3.69	1.30	38.3	-
Kivalo*	132.1	4.07	1.59	31.7	1.85[a]
Flakaliden	138.1	6.73	-	-	2.13[b]
Uusikaarlepyy*	58.0	2.35	1.77	26.8	-
Juupajoki*	65.2	2.44	1.63	28.7	-
Tammela*	57.2	2.94	1.30	37.0	-
Voore*	20.3	0.84	2.79	17.1	0.63[c]
Saarejärve	94.7	-	-	-	-
Tõravere	19.9	1.02	-	-	-
Järvselja*	-	-	1.79	24.8	-
Waldstein*	15.9	0.74	2.14	23.0	0.80[d]
Goldkronach*	20.1	0.86	2.25	21.9	-
Flössenburg*	49.8	2.06	1.95	25.4	-
Höglwald*	26.9	1.51	2.15	22.5	-
Altötting*	24.1	1.09	2.50	20.0	-
		Betula pendula			
Kivalo	96.9	5.23	2.27	21.2	-
Syktyvkar 1	-	-	1.82	26.7	-
Syktyvkar 2	-	-	1.86	25.2	-
Syktyvkar 3	-	-	1.62	28.5	-
Punkaharju	-	-	2.77	16.8	-

Olkiluoto	19.7	0.97	2.10	22.8	-
Alatskivi 1	8.2	0.50	3.00	14.7	-
Alatskivi 2	27.7	1.42	2.54	18.4	-
Erastvere	40.8	1.84	2.39	19.6	-
Risley Moss	2.7	0.15	3.12	15.2	-
		Pinus sylvestris			
Sevettijärvi	71.1	3.76	1.37	36.1	-
Kivalo	99.5	5.72	1.29	38.8	-
Ylikiiminki	77.1	5.24	1.21	41.1	-
Juupajoki	33.2	2.15	1.65	28.7	-
Tammela	29.1	1.86	1.77	27.6	-
Saarejärve	54.7	2.67	1.69	29.4	-
Vilsandi	52.4	2.45	2.86	16.6	-
Sõmerpalu	30.1	1.95	1.65	30.1	-
Kačerginė	70.4	3.71	1.94	25.4	-
Thetford	21.2	1.39	2.68	18.6	-
Alice Holt	-	-	2.72	18.0	-
Altdorf	11.6	0.56	2.08	23.7	-
Dinkelsbühl	8.4	0.38	1.61	31.2	-

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Table 3 Statistically significant relationships between bulk soil and rhizosphere bacterial phylogenetic molecular ecological network' (pMEN) modules and soil chemical parameters according to RDA analysis. Percentages of bacterial community variations explained by individual chemical parameters are given in brackets. *p<0.05; ** p<0.01; ***p<0.001

Module	**Soil chemical parameters**	**Variation explained %**
	Bulk soil	
All	pH(33.1%)+P(47.5%)***	47.5
B	P(35.9%)+pH(23.8%)***	49.8
C	P**	33.7
D	P**	26.2
E	pH(43.7%)+K(6.8%)**	59.9
F	pH(50.7%)+Mg(20.8%)+Ca(14.4%)+P(33.5%)***	84.8
G	pH	31.2
H	pH(27.8%)+P(23.2%)***	49.8
	Rhizosphere	
All	pH(33.9%)+P(30.7%)***	51.1
I	C/N(20.7%)+K(19.5%)**	42.7
J	pH**	31.5
K	P*	33.4
L	pH(38.2%)+ P(17.1%)**	62.1
M	P(27.6%)+N(16.8%)**	45.5
N	pH**	33.3
O	pH**	48.7
Q	P(24.6%)+N(19.8%)***	45.6
R	pH(38.8%)+P(38.0%)***	56.3