Scots pine fine roots adjust along a 2000-km latitudinal climatic gradient

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Summary

- Patterns of plant biomass allocation and functional adjustments along climatic gradients are poorly understood, particularly belowground. Generally, low temperatures suppress nutrient release and uptake, and forests under such conditions have a greater proportion of their biomass in roots. However, it is not clear whether ‘more roots’ means better capacity to acquire soil resources.
- Herein we quantified patterns of fine-root anatomy and their biomass distribution across Scots pine (Pinus sylvestris) populations both along a 2000-km latitudinal gradient and within a common garden experiment with a similar range of populations.
- We found that with decreasing mean temperature, a greater percentage of Scots pine root biomass was allocated to roots with higher potential absorptive capacity. Similar results were seen in the common experimental site, where cold-adapted populations produced roots with greater absorptive capacity than populations originating from warmer climates.
- These results demonstrate that plants growing in or originated from colder climates have more acquisitive roots, a trait that is likely adaptive in the face of the low resource availability typical of cold soils.

Introduction

Fine roots are responsible for nutrient and water acquisition. However, very little is known regarding their role in plant adaptation to large-scale climatic gradients (Brassard et al., 2009; Ostonen et al., 2011). In higher latitude conditions, a greater fraction of plant biomass is allocated to fine roots as a result of adaptation to low nutrient supply driven by impaired nutrient cycling rates and limited soil solution movement, in comparison to lower latitudes (Reich et al., 2014). Still, the response of fine-root biology to climate variation remains poorly understood, and particularly little is known about how changes in baseline temperatures will alter: (1) fine-root absorptive capacity across different root orders; (2) their associations with mycorrhizal fungi; (3) biomass partitioning between absorptive vs transport roots; and (4) genotypic vs phenotypic controls in fine-root response to climate. Specifically, it is unclear whether in order to increase absorptive capacity, plants from cold climates favor production of functionally absorptive fine roots which may directly increase nutrient uptake and indirectly do so by maintaining associations with ectomycorrhizal fungi (Guo et al., 2008; Ouimette et al., 2013). Mycorrhizal fungi are important for nutrient acquisition and transfer to roots, soil aggregation and stabilization, and maintaining root hydraulic conductivity (by maintaining contact between soil and roots), and they play key roles in the adaptation of plants to the cold (Veen et al., 1992; Bogeat-Triboulot et al., 2004; Rillig & Mummey, 2006). Thus, a shift towards ‘more absorptive’ roots could have far-reaching consequences for fine-root turnover rates, decomposition, mycorrhizal associations, soil carbon sequestration (Gaudinski et al., 2010; Goebel et al., 2011; Ouimette et al., 2013), and resolving these belowground data gaps will improve understanding of plant adaptation to long-term climatic conditions.

Historically, all roots < 2 mm in diameter have been categorized as fine, short-lived absorptive roots and those > 2 mm in diameter as coarse, long-lived roots responsible for water and nutrient transport (Finér et al., 2007). However, this simplistic categorization does not account for large variations in root function among roots below a 2 mm or even 0.5 mm cutoff, where transport as well as absorptive roots can be found (Pregitzer et al., 2002; Guo et al., 2008). Generally, absorptive fine roots occupy the lowest branch orders, whereas transport fine roots displaying secondary xylem and suberized cork layers occur higher in the branching hierarchy. Transport fine roots play primarily structural and transport-based roles and have limited capacity for water and nutrient uptake (Pregitzer et al., 2002; Guo et al., 2008). Despite recognition of these functional differences, understanding how allocation to functionally divergent fine roots varies with climate is limited (McCormack et al., 2015).

It is unclear whether climate-related adjustments in fine-root anatomy and branching structure result from phenotypic
plasticity or from genotypic variation (i.e. among populations), or both. Importantly, if broad phenotypic plasticity occurs, species may be able to respond to rapid climatic change by adjusting allocation strategies for soil resource acquisition (Valladares et al., 2014). In contrast, if patterns of root development are relatively fixed within a genotype, this inflexibility may mean that trees in the face of warming environments may be at risk of failing to function well.

In order to address whether and how fine roots adjust to differences in climate, we characterized fine-root anatomy and mycorrhization of diverse populations of Scots pine along a gradient of environmental conditions found across different latitudes (Fig. 1). The very wide distribution of Scots pine (Pinus sylvestris) has led to adaptation of different populations to higher and lower temperatures (Palmroth et al., 1999; Martinez-Vilalta et al., 2009), which makes this both an important focal species and a useful model system. We specifically tested the hypotheses that: (1) at every relevant root branch order, populations from cold environments maintained a greater percentage of absorptive (rather than transport) fine roots than populations from warmer environments (branch orders where all fine roots had either an absorptive or transport potential function were irrelevant for this test); (2) compared to those from warmer environments, populations from cold locations have a greater fractional biomass allocation to the generally more absorptive lower order root classes; (3) because of hypothesis 1 or 2, or their combination, populations from cold climates maintain a greater potential absorptive capacity per gram of fine root across all root orders than those from warmer locations; and (4) cold-climate populations will also have greater colonization by mycorrhizal fungi. To test these hypotheses, we examined root anatomy, biomass partitioning among absorptive and transport fine roots, and mycorrhization in Scots pine populations along a latitudinal gradient from northern Scandinavia to Central Europe. Furthermore, we addressed the hypothesis (5), that cold-origin populations will show the 'high absorptive adaptation' fine root syndrome when grown in common gardens with warmer-origin populations. We addressed this question using a common garden experiment where high-latitude (northern) and mid-latitude (central) populations of Scots pine have grown together in Lithuania, a relatively warm, mid-latitude site for nearly 40 yr.

Materials and Methods

Study sites

A latitudinal gradient was made from three transects (Swedish, Finnish and Polish) established across 32 Central and Northern European sites, ranging from 51 to 70°N. This included sites ranging from 56 to 68°N in Sweden (16 sites), 61 to 70°N in Finland (10 sites) and 51 to 54°N in Poland (six sites) (Fig. 1; Supporting Information Table S1). All stands were dominated by mature Scots pine (Pinus sylvestris L.) trees (over 80 yr old) and were selected without distinguishing whether they were...
naturally regenerated or planted. The stands of Scots pine in Scandinavia occupied predominantly podzolic soils, and in a few cases lithosols and brown forest soils. The stands of Scots pine in Poland were growing on sandy loam with a visible podzolic layer. Mean annual temperature (MAT) and other climatic variables for each site were obtained using the WorldClim database (http://www.worldclim.org) (Hijmans et al., 2005) and CGIAR-CSI (http://www.csi.cgiar.org) (Trabucco & Zomer, 2009). The common garden site was located in southern Lithuania (54.75°N, 23.58°E) in a temperate climate with MAT of 6.5°C and mean annual precipitation of 616 mm. This site was established in 1975 as a part the All-Union Forest Research Institute (VNIILM) initiative (Shutyaev & Giertych, 1997). The trees were 39 yr old at the time of sampling. To assess the effect of plant origin on the structure of different root orders, populations representing a broad range of climatic conditions across the geographical distribution of Scots pine were selected. Sample Scots pine trees planted at the common garden represent five populations ranging from the northern part of European Russia (63°N; 1°C) to western Ukraine (48°N; 5°C) (Fig. 1; Table S2). Samples in the latitudinal gradient were collected in 2012 (Sweden, at the end of July and beginning of August), 2013 (Finland, in July) and 2014 (Poland, in July). Samples from the common-garden experiment in Lithuania were collected in early September 2014. All roots were harvested in the summer or early fall. Sampling continued for 3 wk to sample roots in 16 sites in Sweden, 2 wk for 10 sites in Finland, 10 d for six sites in Poland and 6 d for five sites (i.e., populations) in the common garden experiment in Lithuania. Timing of root production phenology is generally related to seasons with peak root growth at the beginning of the growing season and in fall (McCormack et al., 2014; Abramoff & Finzi, 2015). Thus, the sampling period was selected to avoid intensive root growth in late fall (Repo et al., 2005; Withington et al., 2006). To examine climatic trends in studied traits, we used MAT because most other climatic variables showed significant correlation with it (Table S3). Preliminary analyses also tested an aridity index (AI) as an integrator of potential evapotranspiration and mean annual precipitation. However, as there was relatively low variation in AI (i.e., all sites classified as humid), and because MAT and AI were well correlated ($R^2 = 0.52, P \leq 0.05$), we again chose to primarily focus analyses on MAT as an important index of environmental and climate variation.

Root sampling

In the common garden experiment, we randomly selected three trees from each population. In the latitudinal transect, five trees were sampled randomly at each stand. From each tree we collected 30 samples of intact root branches. The roots were sampled from a depth of c. 0–10 cm. Samples were taken from individual trees located at least 20 m apart in the in situ transect, and at least 8 m apart in the common garden experiment. To sample root branches, we carefully removed plant litter to find distal root tips, and then we followed subsequent root branches to exposed lateral roots growing from the main lateral roots. The root branches, containing at least six intact orders, were removed from lateral roots using pruning scissors. We did not immediately separate roots from adhered organic and inorganic particles in the forests to preserve intact terminal orders. Roots were transported back to the lab where soil and adhering materials were carefully removed with gentle washing in a water bath. All root branches contained at least the first through sixth root orders. The first six root orders generally represented all roots < 1 mm in diameter. Roots were kept in plastic bags and stored in the refrigerator (at c. 5°C) until further analysis. During storage, the bags were opened and fresh moistened towels were changed daily. This prevented the deterioration of roots during storage, which lasted up to 1 wk.

Once removed from the bags, the roots were divided into individual orders according to the method described by Pregitzer et al. (2002), taking into account that all tip-ended roots are first (lowest) order, second-order roots start at the junction of first-order roots, and so on. Dissections of roots from first through sixth orders were made using a steel scalpel; during the dissections the roots were kept moist. Furthermore, only roots that appeared to be alive determined based on texture and visual appearance were collected for analyses. In case of first-order root tips, we studied only fibrous roots (see Zadworny & Eissenstat, 2011), and avoided pioneer roots which differ morphologically and functionally from fibrous roots (Bagniewska-Zadworna et al., 2012). Pioneer roots were identified based on their morphology (easily distinguished from fibrous root tips by larger diameter and greater length). Moreover, first-order root tips were almost completely colonized by mycorrhizal fungi and we collected them irrespective of the specific mycorrhizal morphotypes that fine roots formed.

Anatomical analyses

For anatomical analyses, 15 segments were chosen randomly from each root order from each analyzed tree of each site. The number of root order segments analyzed from the common garden experiment in Lithuania totaled 1350; there were 7050 from sites along the transect in Sweden; 3960 from Finland; and 2430 from Poland. Anatomical root observations were made on a total of $\approx 14,790$ segments of root orders. Because of insufficient sample material for complete analysis, we did not use fifth- and sixth-order roots from site S13, and were able to use only two trees from sites F7, F8 and P4.

Following order dissections, roots were immediately fixed in a mixture of 2% formaldehyde (Polysciences, Warrington, FL, USA) and 2% glutaraldehyde (Polysciences) in 0.1 M cacodylate buffer (Polysciences) according to Bagniewska-Zadworna et al. (2012). For lower root orders, the entire root was fixed. In higher order roots, when their lengths frequently exceeded 15 mm, the root samples were limited to 10 mm. After 24 h in fixing buffer, the roots were washed once in 0.05 M cacodylic buffer, washed twice in deionized water, and then dehydrated in gradually increasing series of ethyl alcohol (Polish Chemical Reagents, Gliwice, Poland) for 1 h at each concentration (10%, 30%, 50%, 70%, 90%, 96%, 100%). After these dehydration steps roots were infiltrated and embedded using Technovit 7100 (Heraeus.
Kulzer, Wehrheim, Germany) directly after the final dehydration in 100% ethyl alcohol.

Cross-sections 5 μm thick (embedded in Technovit 7100) were obtained using a rotary microtome (Leica RM2265) and stained with 0.5% toluidine blue (Sigma, St Louis, MO, USA) dissolved in 1% sodium tetraborate (Sigma). Each root was observed under an Axioskop 20 microscope (Carl Zeiss, Oberkochen, Germany) at 10, 20 and 40× objective magnification and then classified as having either absorptive or transport potential based on the presence or absence of phellem, the outer layer of cork periderm (and loss of root absorptive function because expanded suberized phellem inhibits ion and water uptake; Fig. 2). In addition, roots were observed under UV light to ensure that there were no suberized cork layers. Roots with potential for nutrient uptake were called absorptive whereas those with expanded phellem (i.e. outer layer of cork periderm, observed only in roots with secondary growth), were assumed to have lost ability to efficiently take up water and ions (Peterson et al., 1999; Enstone et al., 2001; Meyer & Peterson, 2011) and were identified as transport roots. For each site, based on the anatomical observations, we calculated percentage of absorptive and transport roots within Scots pine roots across the branching hierarchy.

Mycorrhiza parameter analysis
We measured mantle cross-section area, parenchyma cortex cross-section area, root cross-section absorptive area (parenchyma cortex cross-section + mantle cross-section area; i.e. the total cross-section area of root tissue whose function is primarily associated with resource uptake), as well as the ratio of mantle cross-section area to root cross-section absorptive tissue area (mantle/cortex) and the percentage of mantle area to total root area across the first – third root orders, using cross-sections of roots collected from the latitudinal transect. Root anatomical and mycorrhizal characteristics were measured within transverse sections in two crossing radial directions (Ostonen & Lõhmus, 2003) using an Axioskop 20 microscope equipped with AxioVision software (Carl Zeiss). Root traits (i.e. root diameter, the thickness of the mantle and thickness of parenchyma cortex) were expressed in μm and then converted into area (μm²). The ratio of mantle cross-section area to root cross-section absorptive tissue area was calculated as the mantle area/absorptive area ratio and the percentage of mantle area to total root area was calculated as mantle area/root × 100. Mantle area was calculated for roots that had apparent colonization as they possessed mantle and Hartig net. Since all roots above third order in the low latitudes lacked any apparent fungal mantle, we analyzed only the first three root orders to have a complete representation of the mantle characteristics along the transect.

Patterns of biomass partitioning among root orders
In order to estimate the pattern of biomass partitioning (i.e. the partitioning of total fine-root biomass to different root orders or to different classes of fine roots) along the latitudinal gradient and in the common garden experiment, we determined the contribution of each root order to the combined biomass of the first six root orders in 13 sites across Finland and Poland (sites F7, F8 and P4 were not included due to insufficient sample material) and in all five populations in the common garden experiment in Lithuania. Intact root branches were dissected into individual orders, up to sixth order, as described above. We used two root samples per tree, and five trees per site along the latitudinal gradient, and in the common garden experiment three trees were selected from each population. After dissection, the roots of each individual order and individual branch were dried at 65°C for 3 d and weighed; and contribution of each root order to the total branch mass was calculated. Based on anatomical results, we divided the mass of individual root orders into groups of absorptive and transport roots and calculated percentage of their mass.

Statistical analysis
The effect of MAT, root order and seed origin were analyzed by GLM models using factorial ANOVA. We used a mixed model where root traits were dependent variables, root order and MAT independent variables, and individual trees were considered as a random factor. Using variance components, we found that individual trees had a very low contribution to the total variance of the proportion of roots that were absorptive (1.1%, P = 0.426; 3.7%, P = 0.269; for transect and common garden study, respectively). Therefore, the effects of individual trees are not shown. We used regression analysis to determine the impact of MAT on proportion of absorptive root within root orders. The Bliss angular transformation (Snedecor & Cochran, 1967) was used for statistical analyses of traits expressed as percentages of absorptive roots. For anatomical variables related to the biomass allocation and mantle traits we used log10 transformation to meet the assumption of normality. However, figures show the nontransformed data. Statistical relationships were considered significant at P ≤ 0.05. All analyses were conducted using STATISTICA v.8.0 (StatSoft Tulsa, OK, USA).

Results
In support of Hypothesis 1 we observed that trees growing in cold-climate sites maintained a greater percentage of absorptive roots within each relevant branching order than those from warmer locations (Fig. 3). The average percentage of fine roots that are absorptive decreased significantly with MAT (P < 0.001) and orders (P < 0.001), and we observed a strong interaction between MAT and order (P < 0.001) (Table S4). MAT explained a large portion of the variation in root functional types (absorptive vs transport) among each of the second through fourth root branching orders (Fig. 3). All first-order roots were absorptive and the percentage of roots that were absorptive decreased with increasing branch order; moreover, the magnitude of these differences depended on temperature. To illustrate the difference between the coldest and warmest sites, compared on an absolute basis, there were 15%, 22%, 11% and 1% more absorptive roots in the second, third, fourth and fifth orders, respectively, in the
Table 1: Examples of individual root branches. Light field photomicrographs of Scots pine (*Pinus sylvestris*) first, second, third, fourth, fifth and sixth order roots classified as absorptive (left column) and transport root orders (right column). Among all sites, no absorptive roots were observed among the sixth root order and no transport roots were observed within the distal, first root order. Asterisks, parenchyma cortex cells; arrowheads, cork/phellem layer; arrows, vascular cambium cells; T, secondary tracheids. Bars, 50 μm.

- **Traits**
  - profuse mantle presence of Hartig net
  - vascular cambium cells retained parenchyma cells
  - well preserved cortex vascular cambium lack of continuous cork
  - parenchyma cells alteration vascular cambium lack of continuous cork

- **Root order**
  - **absorptive**
    - 1 no absorptive roots
    - 2 vascular cambium cork layer crushed parenchyma cells
    - 3 vascular cambium layers of phellem cells crushed parenchyma cells
    - 4 vascular cambium secondary tracheids phellem layers
    - 5 vascular cambium secondary tracheids numerous phellem layers
    - 6 vascular cambium phellem layers

- **Traits**
  - no transport roots
  - vascular cambium cork layer crushed parenchyma cells
  - vascular cambium layers of phellem cells crushed parenchyma cells
  - vascular cambium secondary tracheids phellem layers
  - vascular cambium secondary tracheids numerous phellem layers
  - vascular cambium phellem layers

Fig. 2 Examples of individual root branches. Light field photomicrographs of Scots pine (*Pinus sylvestris*) first, second, third, fourth, fifth and sixth order roots classified as absorptive (left column) and transport root orders (right column). Among all sites, no absorptive roots were observed among the sixth root order and no transport roots were observed within the distal, first root order. Asterisks, parenchyma cortex cells; arrowheads, cork/phellem layer; arrows, vascular cambium cells; T, secondary tracheids. Bars, 50 μm.
coldest sites (<0°C MAT), compared to the warmest sites (>8°C, Fig. 3).

Similar patterns were observed for the populations in the common garden (Table S4; Fig. 3). The percentage of roots that were absorptive was higher in lower root orders, and in populations with cold origins, and the variation in relation to climate of origin was strongest in the lowest order roots (Fig. 3). The generally similar results from the common garden and from the spatial transects suggested that these patterns were likely (and perhaps largely) related to genetic control of tree growth, and resulted from the adaptation of northern tree genotypes to short growing seasons and harsh climate (Oleksyn et al., 1992). Moreover, the relationship between AI and percentage of absorptive roots was generally similar to that found for MAT. AI had slightly more explanatory power along the transect, whereas temperature of seed origin better explained differences between Scots pine trees along the transect, whereas temperature of seed origin better explained differences between Scots pine trees provenances growing in the common garden (Fig. 3; Table S5). Additional environmental variables that correlated closely with the percentage of fine roots with absorptive capacity are shown in the Table S6.

Since there were no (or very weak) differences in proportional biomass by root orders (R² = 0.04, P = 0.07; estimated by regression analysis), all changes in biomass partitioning to absorptive vs transport roots were due to variation within order between absorptive vs transport roots. Overall, as we hypothesized at colder sites, a greater percentage of total fine-root biomass (first through sixth orders) was allocated to absorptive roots (R² = 0.39, P < 0.05, Fig. 4), and a similar relationship, but statistically insignificant, was found for AI (R² = 0.22, P = 0.1). On average, the biomass percentage of absorptive root constituted 34% of fine-root biomass in the warmest sites (MAT > 8°C) and increased to 61% at the coldest, most northern sites (MAT < 0°C). Likewise, a greater percentage of fine-root biomass was allocated to absorptive roots in northern populations growing in a common garden (R² = 0.81, P < 0.05, Fig. 4). For AI this relationship was statistically insignificant (R² = 0.51, P = 0.17).

Parenchyma cortex is a transition zone for the exchange of pho-toassimilates, soil water and nutrients between the plant and fungi, whereas the mycorrhizal mantle extends the root surface area. Hence, both play a key role in plant nutrient and water acquisition from soil. In agreement with our hypothesis, we found that across the three most distal root orders these traits differed along the temperature gradient. Total cross-section of absorptive root area was greater in colder, compared to warmer sites (Fig. 5a; Tables S7, S8). Mantle cross-section area and ratio of mantle cross-section area to total cross-section absorptive area (i.e. mantle + parenchyma) increased as MAT decreased (Fig. 5b,c; Tables S7, S8). Likewise, the percentage of total root cross-section area occupied by the mantle was highest in the low MAT sites in all analyzed root orders (Fig. 5d; Tables S7, S8). AI relationships to mycorrhizal root trait characteristics are shown in Table S9. Thus, not only do cold-climate Scots pines maintain a greater percentage (relative to transport roots) of acquisitive roots than those from warmer locations, but those acquisitive roots also

![Fig. 3 Cold-climate enhances root acquisitive capacity. Effect of mean annual temperature (MAT) of sites along the transect (blue symbols), and of location of seed origin of populations in the common garden (red symbols), on the percentage of fine roots with absorptive capacity, shown for different branching orders. Percentage of absorptive roots (i.e. absorptive roots as a percent of total (absorptive + transport) root number) shown for Scots pine (Pinus sylvestris) populations growing in situ along a transect latitudinal gradient (i.e. sites in Finland, Poland, Sweden) (blue color) and in a common garden in central Lithuania (red color) within second-order (a), third-order (b), fourth-order (c) and fifth-order (d) roots. Linear regression was used to estimate patterns of second-order (R² = 0.224, P = 0.006; R² = 0.629, P = 0.109), third-order (R² = 0.138, P = 0.035; R² = 0.834, P = 0.03), fourth-order (R² = 0.106, P = 0.068; R² = 0.709, P = 0.073) and fifth-order (R² = 0.049, P = 0.221; R² = 0.771, P = 0.049) roots in relation to MAT within the transect and common garden studies, respectively. Values are means of individual trees data pooled together per stand. First-order roots not shown as all first-order roots were absorptive, and sixth-order roots not shown as all sixth-order roots were classified as transport roots.](image-url)
deploy a greater network of fungal hyphae which acquire soil resources that are traded to the plant in exchange for carbon substrates.

Discussion

Plants of populations from cold conditions are driven to put greater investment towards uptake of water and nutrients as lower temperatures and generally acidic soils suppress the mineralization rates and accessibility of some nutrients (McMichael & Burke, 2002; Lambers et al., 2008; Sorensen et al., 2008). In response to harsh growing conditions in high latitudes, plants have evolved mechanisms that can compensate for lower nutrient availability in colder soils (Chapin, 1974; Schimel et al., 1996; Persson, 2002). Our results corroborate these findings and suggest that the Scots pine growing at lower temperatures may partially compensate for reduced availability of nutrients by maintaining greater uptake capacity higher up in the root branching hierarchy than populations growing in warmer climates (Fig. 3).

In addition to increasing absorptive surface area, maintaining a higher proportion of potentially absorptive roots may lead to greater root exudation and increased nutrient availability through stimulation of soil microbial extracellular enzymes and mineralized organic matter (Bengtson et al., 2012; Dijkstra et al., 2013; Bardgett et al., 2014). Plants may also increase nutrient uptake through physiological adjustment of root-uptake kinetics of transporters or active nutrient transport at the cell membrane (BassiriRad, 2000; Lee et al., 2007). However, in cold environmental conditions, limited mobility and slow decomposition may constrain uptake kinetics at the individual root level. Therefore, increased production of active transporters may reduce the energy available for plant growth as discussed by Fernández-Martínez et al. (2014). Overall, these observations suggest that in cold environments, maintaining a high proportion of absorptive root mass (Fig. 4) may be an efficient way to compensate for limited nutrient availability not only along latitudinal gradients, but also along altitudinal temperature-related gradients, as shown in studies from temperate and tropical mountain gradients with decreasing temperature leading to marked allocation shifts to the fine-root system and root morphological changes (Girardin et al., 2010, 2013; Hertel & Schöling, 2011; Moser et al., 2011; Mao et al., 2015). Nevertheless, slower growth rates in high-latitude conditions suggest that in addition to adaptive adjustments to maximize nutrient uptake, plants may also reduce nutrient constraints via slow plant growth (Marschner, 1995). Indeed, the

Fig. 4 Scots pine (Pinus sylvestris) trees have a greater percentage of fine roots that are absorptive at low mean annual temperature (MAT).

Percentage of roots that are absorptive (as a percentage of total root mass across six root orders) in relation to mean annual temperature (MAT) for Scots pine populations growing in situ in Finland and Poland (no data for mass percentage of absorptive roots were collected in Sweden) (blue symbols) and in a common garden in central Lithuania (red symbols). Simple regression was used to estimate $R^2 = 0.39$ and $P = 0.021$ (transect study) and $R^2 = 0.81$ and $P = 0.036$ (common garden experiment). Values are site means representing multiple individual trees from each stand. Total number of analyzed trees was 67. Estimates included most, but not all root orders $< 2$ mm in diameter as some higher order roots whose diameter may still have been $< 2$ mm were not measured (seventh, eighth and ninth order). Therefore, estimates of the proportion allocated to transport roots should be viewed as a minimum.

Fig. 5 Fungal association of cold-grown Scots pine (Pinus sylvestris) increases active surface area for nutrient and water absorption. Mycorrhizal per root trait characteristics: (a) root cross-section absorptive area (parenchyma cortex cross-section + mantle cross-section area) ($\mu$m$^2$), (b) mantle cross-section area ($\mu$m$^2$), (c) ratio of mantle cross-section area to root absorptive tissues cross-section area (mantle/cortex) and (d) percentage of mantle cross-section area in root cross-section area (%) in relation to mean annual temperature (MAT) along transect temperature gradient (i.e. Finland, Poland, Sweden) within first-order (black), second-order (red) and third-order roots (blue). Linear regression was used to estimate $R^2$ values reported in Supporting Information Table S8. Values are site means representing multiple individual trees from each stand.
relatively slow growth of northern (vs central) populations of Scots pine in common garden conditions, not differentially limited by nutrient availability, indicates that their slow growth is not simply a consequence of nutrient restriction, but possibly an evolved adaptive response to cold climate (Stephan & Liesebach, 1996; Oleksyn et al., 1999).

We determined that the relationship between percentage of absorptive roots and aridity index (AI) was generally similar to that found for mean annual temperature (MAT), but we focused on the effect of temperature variation on the expression of root traits because the gradient in temperature was more consistent than AI, likely due to the fact that all sites included in this study were broadly classified as 'humid'. However, the correlations between different environmental variables along the latitudinal gradient (Table S3) suggest that tree-root resource acquisition strategies also may be reasonably related to multiple environmental factors (Tables S5, S6). Indeed, temperature can have both direct effects on plant-root processes and indirect ones involving broad changes in soil nutrient conditions because organic matter decomposition, water viscosity, membrane permeability, nutrient movement and availability are all generally lower at lower temperatures, which then has further effects on metabolic processes and microbial activity (McMichael & Burke, 2002; Sorensen et al., 2008; Reich et al., 2014). For example, soil fertility has been shown previously to vary consistently along the comparable climate gradient in Northern Europe considered in the present study (Salesmaa et al., 2008; Ostonen et al., 2011; Leppälammi-Kujansuu et al., 2014), with a trend of increasing soil carbon to nitrogen ratio (C:N) of the organic layer from lower to higher latitudes and from warmer to colder temperatures. Despite these broad patterns in soil fertility, we focused on MAT as an important driver of fine-root structure, because MAT influences nutrient conditions by regulating the rate of organic matter decomposition and MAT is generally correlated with soil fertility along the latitudinal gradient.

Absorptive roots, in addition to increasing the ability of plants to acquire nutrients directly, also offer a tissue base for colonizing by mycorrhizal fungi that secrete enzymes to mobilize organically bound nutrients, even in cold soils (Tibbett et al., 1998, 1999; Brundrett, 2002). We measured two key traits (parenchyma cortex and mantle cross-section area) related to both the host capacity to support mycorrhizal fungi and subsequent fugal growth on the root. We observed that roots of northern populations maintained greater parenchyma cortex cross-section areas available for fungal colonization, effectively increasing potential exchange between plant roots and mycorrhizal fungi. Strong host plant dependence on mycorrhizal fungi was evident from the increased mantle area and higher percentage of mantle area in root absorptive tissues with decreasing temperatures (Fig. 5b–d). Thicker mantles and higher percentage of mantle in root cross-section absorptive area, together with associated extramatrical hyphae, are instrumental in maintaining efficient nutrient uptake by roots at low temperatures (Tibbett & Cairney, 2007).

The shifts of fine-root biomass allocation to absorptive roots that favor higher mycorrhizal colonization (Figs 4, 5) represent important adaptations of Scots pine to cold climate conditions that likely facilitate nutrient mobilization and uptake by Scots pine populations growing in colder climates with short growing seasons (Hansen et al., 1997; Iversen et al., 2015). Indeed, Ostonen et al. (2011) observed changes in fine-root strategies in Picea abies forests across a European climatic gradient from southern Germany to northern Finland. Root acclimation was manifested by the increase in the proportion of biomass of ectomycorrhizal roots relative to total fine-root biomass. Thus, high branching intensity might also be a driver of nutrient acquisition in nutrient-poor habitats, as suggested by Kong et al. (2014). This is partially supported by our data that showed a consistent trend of increasing first-order root biomass along a temperature gradient ($R^2 = 0.29, P = 0.057$; data not shown).

Patterns in root anatomical development observed for the different latitudinal populations growing in the common garden were similar to those observed along the latitudinal transect (Fig. 3). Differences in patterns of root development among the different populations grown in the common garden may be explained, at least in part, by differential, and genetically fixed, photoperiodic control over plant growth. Populations from colder climates are controlled by photoperiod and end seasonal aboveground growth before the onset of harsh winter conditions (Körner & Basler, 2010). This photoperiodic adaptation has been shown to relate closely to earlier cessation of annual stem growth in northern populations (Oleksyn et al., 1992, 2001). However, root growth does not appear to be limited in a similar way and so long as thermal conditions are favorable, roots may continue to be produced and develop while they also benefit from elevated belowground carbohydrate supplies following the cessation of shoot growth (Smit-Spins et al., 1985; Hansen et al., 1996; Oleksyn et al., 2000; Abramoff & Finzi, 2015; Iversen et al., 2015; Blume-Werry et al., 2016). Continued growth of roots in northern populations after the cessation of shoot and stem growth (Oleksyn et al., 1992, 2001) may further enhance proportional biomass of absorptive roots (Fig. 4). This phenomenon helps to explain increased CO$_2$ exchange (photosynthetic capacity) and higher N concentrations observed in leaves of populations originating from cold sites grown in common garden experiments (Reich et al., 1996; Oleksyn et al., 1998), as the higher proportion of absorptive roots enhances acquisition and transfer of nutrients to leaves. Still, the ultimate consequences of increased leaf nitrogen are unclear because needles of the more northern populations growing in the common garden may also have increased risk of mortality due to higher microbial and herbivore pressure (Moles et al., 2011; Sturrock et al., 2011).

Our results enhance understanding of intraspecific variation of functional fine-root adjustments along a large-scale geographical transect and shed light on anatomical adaptations of roots that have the potential to enhance their absorptive capacity. These findings highlight different mechanisms of woody plant adaptations to cold environments and their potential adjustments in response to shifts in climate. Given the striking differences in fine-root structural development observed in the different populations of Scots pine along both the latitudinal transect and in the common garden, it is clear that quantitative appreciation of
intraspecific variation in fine-root traits is needed to understand plant adjustments to varying climatic conditions and to predict responses of forested areas to global changes. Furthermore, as one of the most important tree species in Eurasia, it is particularly important to understand potential responses of Scots pine to environmental change. The overall patterns of root structure and development along the in situ climate gradients (Figs 3, 4) studied here indicated strong adaptations to local climate. At the same time, the observation that these patterns together with whole-plant strategies for aboveground growth appear to be at least partly fixed within genotype as demonstrated by results in the common garden experiments, indicate limited plasticity of Scots pine to adjust to rapid climate warming (but see Reich & Oleksyn, 2008). Therefore, despite strong ecotopic variation within Scots pine, its populations may have limited capacity to adjust root function in the face of a changing climate and soil milieu.

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Author contributions
M.Z. and J.O. designed the experiments; M.Z. oversaw the study and drafted the manuscript with contributions from M.L.M., J.M., P.B.R. and J.O.; M.Z. and J.M. performed analyses; all authors discussed the results; M.Z. and J.O. established the collaborations necessary for this paper and J.O. sought funding for it.

References


Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

**Table S1** Site locations of transect study

**Table S2** Origin of Scots pine populations in common garden experiment in Lithuania

**Table S3** Correlation coefficient of different climatic variables based on study sites

**Table S4** Factors contributing to root development variance

**Table S5** AI contribution to percent of absorptive roots

**Table S6** Equations describing the relationship between percentage of absorptive roots and latitude (Lat.), growing season days (GLS) and mean temperature during the growing season ~May to August (MGT)

**Table S7** Factors contributing to mycorrhizal traits variance

**Table S8** Equations describing relationship between ectomycorrhizal traits and MAT

**Table S9** AI contribution to mycorrhizal root trait characteristics

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