

This is an electronic reprint of the original article.

This reprint *may differ* from the original in pagination and typographic detail.

Author(s): Sannakajsa M. Velmala, Matti J. Salmela, Tommy Chan, Teemu Hölttä, Leena Hamberg, Risto Sievänen & Taina Pennanen

Title: Genotypes exhibit no variation in precision foraging in mycorrhizal Norway spruce seedlings

Year: 2022

Version: Published version

Copyright: The Author(s) 2022

Rights: CC BY 4.0

Rights url: <http://creativecommons.org/licenses/by/4.0/>

Please cite the original version:

Velmala, S.M., Salmela, M.J., Chan, T. et al. Genotypes exhibit no variation in precision foraging in mycorrhizal Norway spruce seedlings. *Plant Soil* (2022). <https://doi.org/10.1007/s11104-022-05672-2>

All material supplied via *Jukuri* is protected by copyright and other intellectual property rights. Duplication or sale, in electronic or print form, of any part of the repository collections is prohibited. Making electronic or print copies of the material is permitted only for your own personal use or for educational purposes. For other purposes, this article may be used in accordance with the publisher's terms. There may be differences between this version and the publisher's version. You are advised to cite the publisher's version.



Genotypes exhibit no variation in precision foraging in mycorrhizal Norway spruce seedlings

Sannakajsa M. Velmala · Matti J. Salmela · Tommy Chan · Teemu Hölttä · Leena Hamberg · Risto Sievänen · Taina Pennanen

Received: 9 May 2022 / Accepted: 23 August 2022
© The Author(s) 2022

Abstract

Aims Fine roots, that comprise the adjustable part of the root system, are important in spatially heterogeneous boreal forest soils. We investigated the soil exploring patterns of Norway spruce (*Picea abies*) seedlings of equal height belonging to families representing two contrasting growth phenotypes that have shown fast and slow growth rates in long-term experiments. We hypothesised that seedlings of the fast-growing phenotype would show a more explorative root growth strategy, intense branching, and root proliferation in response to the nutrient patch, and

that slow-growing phenotypes would be more tolerant to drought stress.

Methods Seedlings were grown in flat Perspex microcosms with a clod of humus placed in the mid-bottom part of each microcosm for eight months. The order-based and functional classification, branching topology, and size of seedling root systems were studied with WinRHIZO™ image-analysis software and root exploration patterns with LIGNUM-model simulations. In addition, transpiration, stomatal conductance, net assimilation rate responses were measured.

Results No differences were found in the early foraging of roots for the humus clod nor net assimilation rate and transpiration between the phenotype groups. Seedlings were favouring exploitation over exploration in the early phases of development regardless of growth phenotype group. However, in fast-growing phenotypes, the main roots were longer, and the lateral root pool favoured long and bifurcated laterals that formed larger absorptive root area.

Conclusions Our results indicate that in nutrient-poor conditions, better growth of lateral roots precedes future differences in the aboveground growth rate of Norway spruce.

Responsible Editor: Timothy J. Fahey.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11104-022-05672-2>.

S. M. Velmala (✉) · M. J. Salmela · L. Hamberg · R. Sievänen · T. Pennanen
Natural Resources Institute Finland (Luke),
Latokartanonkaari 9, 00790 Helsinki, Finland
e-mail: sannakajsa.velmala@luke.fi

T. Chan
Institute for Atmospheric and Earth System Research/
Physics, Faculty of Science, University of Helsinki, Gustaf
Hällströmin katu 2, 00014 Helsinki, Finland

T. Hölttä
Forest Sciences, Faculty of Agriculture and Forestry,
University of Helsinki, Latokartanonkaari 7,
00014 Helsinki, Finland

Keywords Norway spruce (*Picea abies*) · Root architecture · Lateral root · Convex hull · Intraspecific variation · Root exploitation

Introduction

Substantial spatial heterogeneity characterises boreal forest soils (Pennanen et al. 1999; Dehlin et al. 2006), indicating that plants will benefit from adjustable belowground strategies that allow roots to proliferate in patchy nutrient spots and to capture scarce resources (Hodge 2004). Fine roots comprise the dynamic part of the root system, explaining a significant share of terrestrial primary production (reviewed in McCormack et al. 2015) and exhibiting functional and physiological diversity (reviewed in Freschet et al. 2021): short-lived root tips are mainly responsible for water and nutrient uptake, whereas thicker fine roots and lateral roots act as transport routes and provide structural support (Keel et al. 2012). Belowground foraging strategies in boreal conditions comprise also an interspecific component as nutrient acquisition can be outsourced via collaboration for instance to mycorrhizal fungal partners (Bergmann et al. 2020).

Various functional traits manifest diversity in situ both among and within species (Siefert et al. 2015). While ecological belowground studies have largely focused on interspecific differences in fine roots (e.g., Freschet et al. 2021; Weigelt et al. 2021), substantial environment-related intraspecific variation also has been discovered in architectural, morphological and chemical root traits in varied tree species (Ostonen et al. 2011; Ostonen et al. 2017; Defrenne et al. 2019; Weemstra et al. 2021). Common-garden studies with uniform experimental conditions show that such variation arises not only from phenotypic plasticity but also from genetic variation, and in widely distributed forest trees genetic differences in functional traits among populations are generally closely related to major environmental gradients (Savolainen et al. 2007). This broad pool of natural genetic variation in economically valuable traits facilitates tree breeding programmes which have increased, for example, volume growth by up to 25% relative to unimproved material in the Nordic countries (Jansson et al. 2017).

Norway spruce (*P. abies* L. Karst) is among the most economically important tree species in northern Europe, and its range covers marked climatic and soil gradients that shift optimal functional trait values. Mature trees have a comparatively shallow root system that is highly plastic in response to the environment (Kalliokoski et al. 2008), and studies on

nutrient-foraging roots in situ have found intraspecific variation that may be related, e.g., to spatial heterogeneity in belowground resource availability (Ostonen et al. 2017; Weemstra et al. 2017; Weemstra et al. 2021). Common-garden studies have demonstrated a strong genetic basis of the species intraspecific variation above- and belowground, with its effects comprising interspecific interactions as well. Utilising long-term breeding trials in the field in Finland, Korkama et al. (2006) found that genotypes with faster growth hosted more diverse ectomycorrhizal species communities and had higher root tip and fine root density than those with slower growth. In accordance with Korkama et al. (2006), Hamberg et al. (2018) found that genotypes with faster growth in the field exhibited more explorative root growth, that is, they allocated root tips and biomass further away from the base of the seedling and had more root branches and tips. Senior et al. (2022) recently found that genetic variation in Norway spruce affected associated soil microbial communities and biochemical processes in a field trial and that some of these variables were related to growth rate (Senior et al. 2022). Velmala et al. (2013) sampled a wide range of genotypes included in Finnish breeding programmes and showed that seedling fine root traits were genetically determined also in greenhouse settings. Finally, in natural populations sampled between 60 and 66 °N in Finland, seedling root systems were smaller but accounted for a larger proportion of total biomass in more northern origins (Salmela 2021), with genetic variation in root and shoot functional traits occurring also locally within individual populations (Salmela et al. 2020; Salmela 2021).

Distinct growth strategies of plants are expected to be determined by covariation across multiple divergent functional traits (Coley et al. 1985; Grime 1977; Reich 2014), and one outstanding question in trait-based ecology is how such variation is coordinated across entire plants from root to shoot (Weigelt et al. 2021). For example, interspecific variation in leaf morphology, chemistry and metabolism is interrelated on a global scale, with photosynthetic capacity correlating positively with nitrogen concentration (Wright et al. 2004). Faster growth may be conditional on higher photosynthetic capacity, which in turn may be dependent on effective resource uptake from soil coordinated by different features of root systems (Coley et al. 1985; Reich 2014). Branching intensity

and specific root length are typical architectural and morphological fine root traits that are expected to be associated with aboveground growth (Weemstra et al. 2016), but their variation may be driven by different environmental factors (Defrenne et al. 2019; Weemstra et al. 2021). Furthermore, intraspecific analyses of seedling root systems suggest that both composite measures are independent from overall root system size (Salmela et al. 2020; Salmela 2021), a belowground feature that is challenging to estimate in situ and whose associations with aboveground growth consequently remain poorly known (Carmona et al. 2021; Weigelt et al. 2021). Overall, the multidimensionality of belowground traits among and within species is indicative of complex cross-plant trait correlations that may vary across environments. Tolerance mechanisms are crucial for forest trees which show high intraspecific variation in e.g. osmotic adjustments and photosynthetic rate in response to abiotic stresses such as drought that further reflects to plant productivity (Polle et al. 2019; Tan and Blake 1997). Drought is first sensed in roots, but the effects spread rapidly across the entire plant (Polle et al. 2019). Further, the drought responsive gene expression involves same gene families that are needed for plant growth and stress responses (Depardieu et al. 2021).

In the current study, we employ explicit genetically determined growth strategies within Norway spruce and a microcosm experiment to test whether genotypes exhibit functional trait variation in response to soil patchiness and whether such variation is related to their long-term growth performance in field trials. Velmala et al. (2014a) and Hamberg et al. (2018) surveyed lateral root branches in six genotypes and reported that fast-growing genotypes in field trials had spread-out root systems and more root tips and allocated more biomass away from the base than those that grew slowly. We add four more genotypes to the dataset, consider the heterogenous character of the growth substrate and apply a comprehensive root phenotyping technique and modelling of the root–soil contact area such that accurate estimates of complete root system size are obtainable on top of more commonly investigated architectural and morphological root traits. Genotypes exhibiting the fast-growing phenotype have shown stronger pathogen-triggered shoot responses than those representing the slow-growing phenotype (Velmala et al. 2014b). Moreover slow-growing white spruce genotypes have been

observed to be better adapted to drought conditions than fast- and intermediate genotypes (Bigras 2005). These observations suggest genetic trade-off in performance between standard and stressful growth conditions (cf. Coley et al. 1985). Thus, we impose mild drought on seedlings to test whether similar intraspecific trade-offs are detectable in photosynthetic traits that are considered key elements of growth in plants (Reich 2014; Wright et al. 2004). More specifically, we hypothesise that genotypes of the fast-growing phenotype will exhibit a more explorative root growth strategy manifested as **1**) a larger number of root branches and tips, **2**) longer fibrous lateral roots with larger surface area, and/or **3**) more intensive root branching in association with a nutrient hotspot than genotypes expressing a slow-growing phenotype. Finally, based on the detected patterns of functional trait covariation (e.g., Wright et al. 2004) and classical expectations of trade-offs between growth potential and stress tolerance (Coley et al. 1985; Grime 1977), we anticipate that **4**) genotypes of the fast-growing phenotype will have higher photosynthetic rates under benign well-watered conditions, while in drier conditions genotypes of the slow-growing phenotype will be able to maintain photosynthesis at a higher level.

Material and methods

Selection of genotypic material

Our sampling scheme consists of genotypes of Norway spruce that are of natural origin and that have been tested in replicated tree breeding trials in Finland. The genotypes were either crossed with a specific paternal pollen donor or open-pollinated in the field (Supplementary Table S1). Their genetic variation can be assessed as differences in functional traits among maternal seed families in a common-garden setting. Five maternal families were classified as fast- and the other five as slow-growing based on stem height at the age of 14-yr from over five replicated field experiments per maternal family conducted in Finland (Napola 1997, Supplementary Table S1). The fast-growing maternal families (F) F612, F1162, F1064, F298, and F902 have had excellent success rates 89–102 (reference level 50) and the slow-growing maternal families (S) S427, S503, S394, S1183,

and S1278 have had much lower long term success rates 31–35 (Supplementary Table S1, maternal families ordered according to descending success rate). Hereafter these two distinct groups of maternal families will be referred to as the fast- and slow- growing phenotypes. However, no among-growth phenotype differences in the aboveground stem growth of young seedlings are expected because this differentiation appears only after several growing seasons in the field, as reported by Velmala et al. (2014a) and Hamberg et al. (2018).

Growth conditions

Seedlings were grown in 200×300 mm size flat (7 mm thickness) Perspex microcosms with soil volume of ~240 ml. A 25 ml clod (Ø 60 mm) of fresh sieved forest humus layer from a spruce dominated forest (60° 21'18.576" N, 24° 59'17.808" E) (mesh size 2 mm) was placed in the mid-bottom part of each microcosm (Supplementary Fig. S1). The transparent side was covered with black plastic and the top corners with aluminium foil to prevent light on roots. Ten microcosms, each representing one maternal family, were randomly placed in a shallow Orthex Smart Store™ plastic box and nine of these boxed were placed into two growth chambers (Binder KBW 720/400, WTB Binder Labortechnik GmbH, Tuttlingen, Germany) with 18 hours of light at 20 °C and a six-hour dark period at 15 °C under 40% ventilation. The 18-hour day length resembles a crude average day length between May and August in southern Finland. Full-day light tubes were used and the photosynthetically active radiation in the chambers, measured with an Apogee MQ-200 quantum meter (Apogee Instruments, Inc., Logan, Utah, USA) at the seedling root neck height was 100–120 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Seedlings were rotated inside each box and between chamber shelves once a week. Boxes were rotated between chambers every second week.

Growth experiment

Prior to the study's eight-month experiment, a four-month pilot study was conducted to follow the early direction of root growth in relation to the humus clod and to test the growth substrate (Supplementary Material).

Seeds that were soaked overnight germinated on water agar plates in darkness and were transplanted into microcosms containing growth substrate mixture 1:1:3 of sand ($\text{Ø} < 2 \text{ mm}$), vermiculite (Vermipu Oy), and light Sphagnum-peat (White 420 F6), pH 4.6–4.8, conductivity 1.4 mS/cm fertilized with Kekkila starter 6 NPK 16–4–17 with slow-release N (Kekkila Group, Vantaa, Finland) within ten days of the primary root becoming visible (nine replicate seedlings per maternal family). Weekly irrigation of 10 ml of tap water was applied with a syringe and additional moist (2 ml) was sprayed once a week onto the whole vertical soil surface area. Seedlings were given additional fertilization twice during the third month (weeks 10 and 12, 5 ml of 0.1% water soluble Turve Superex NPK 11–5–26, Kekkila Group, Vantaa, Finland).

After one month each seedling was inoculated with 2 ml of vegetative hypha of ectomycorrhizal fungus *Thelephora terrestris* (40% vol/vol in half strength MNN nutrient solution, Marx 1969). *Thelephora terrestris* is a common ectomycorrhizal fungus in Finnish seedling nurseries and it tolerates well various experimental conditions. The purpose of the inoculation was to equalize the colonization status of seedlings as mycorrhizal fungal colonization affects root development (Velmala et al. 2014a). After two months the seedlings were re-inoculated with 2 ml of fresh sieved forest humus on the clod in the bottom part of the microcosm. After 18 weeks four 30×6×2 mm rubber spacers were installed in margins to ensure ventilation for growing roots, and weekly irrigation was increased to 20 ml of tap water in addition to spraying. At the age of six months 5 ml of soil mixture of sieved forest humus layer (stored 8 weeks in +4 °C) and Nutricote® 70-pellets (NPK 16–4–8) were added on the humus clod. The mixture was made according to the suggested application rate of 3 g of slow-release fertilizer into 1 l of soil substrate.

During the seventh month of the experiment, half of the seedlings were subjected to drying soil. Seedlings in the well-watered treatment were continuously watered with 20 ml of water weekly. During the first week of the dry treatment seedlings received 5 ml of watering and none after that. Microcosms were weighted weekly, and the target dryness was ensured by opening the microcosms for 10 to 30 min to speed up the drying. At the end of the experiment the dry

microcosms had only 20% of the moisture left when compared to the well-watered treatment.

Leaf gas exchange and water potential measurements

Measurements of transpiration rate ($\text{mmol m}^{-2} \text{s}^{-1}$), stomatal conductance (GH_2O that is total water vapor conductance [$\text{mmol m}^{-2} \text{s}^{-1}$]) and net CO_2 assimilation rate [$\mu\text{mol m}^{-2} \text{s}^{-1}$] (ambient CO_2 400 ppm, PAR 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, leaf temperature 20 °C, vapour pressure deficit 1.40 kPa) were conducted with a Portable Gas Exchange Fluorescence System GFS-3000 (Heinz Walz GmbH, Effeltrich, Germany) from four to six replicate seedlings of each maternal family (altogether 57 seedlings) at seven months of age. Seedlings were measured in a random order between 10 am and 3 pm over three days, and measurements were taken within 5–10 min after the seedling was placed in the cuvette. During the last two weeks of the experiment half of the seedlings were not watered. To determine the timepoint when drying started to affect net assimilation and transpiration rates, dark respiration rate and leaf water potential of two fast and two slow maternal families were followed until the seedlings started to close stomata (data not shown). The experiment was terminated after eight months, at which point gas exchange measurements were taken again with the same settings as described above. Water potential was measured with a Scholander type pressure chamber.

Sampling

Seedling stem height and collar diameter (10 mm above root neck) were measured with a ruler and a vernier calliper. Shoots were separated from the roots and air dried for two weeks (overnight 40 °C) before the determination of dry weight. Soil samples from the growth substrate mixture and humus clod from each microcosm were collected into 5 ml Eppendorf-tubes which were stored 1–3 weeks in +4 °C and then kept frozen –20 °C until the determination of soil nutrient content. The soil was washed away only from the humus clod and the revealed roots were coloured for 30 min at room temperature by pipetting 1 ml of 1% aqueous safranin-O red on the root surface to let it attach to lignin in cell walls. The remaining surrounding soil was washed away with tap water after the stain had adhered on the humus-colonizing roots.

Whole root systems were photographed and scanned with Epson Perfection V700 Photo scanner (Seiko Epson Corporation, Suwa, Japan) as tagged image file format (TIFF) files with a 400-dpi resolution. Roots were investigated under a dissecting microscope before determination of dry weight (air dried for two weeks and overnight at 40 °C).

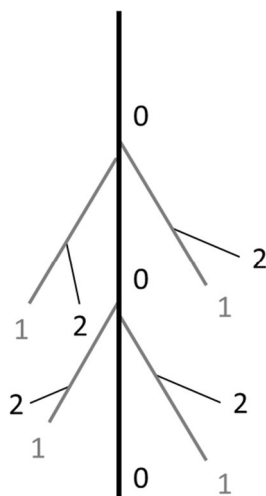
Soil nutrient capacity

Soil moisture, ash and total nutrient content were analysed with LECO TGA-701 (Leco Corporation, USA) according to ISO 11465 and ICP-emission spectrometer iCAP 6500 DUO (Thermo Scientific, United Kingdom) according to ISO 11466. Soil samples for IPC were digested by the closed wet $\text{HNO}_3\text{-HCl}$ digestion method in a microwave (CEM Mars 6 XPress, USA). Carbon and nitrogen content were determined with a LecoCN TruMac®-analyzer (LECO Nordic Ltd.; analysis based on ISO 10694 and ISO 13878 standards). Soil pH was determined from a water suspension according to Soil Quality-Determination of pH (ISO 10390) with a SympHony SB79P pH meter (VWR International, Finland).

Image analysis, order-based and functional classification and root system topology

WinRHIZO™ Pro-image analysis software Version 2013a (Regent Instruments Inc., Quebec, Canada) was used to measure the size, architecture, and morphology of complete root systems. Data is created as centrifugal, i.e., developmental segments (Berntson 1997) in the software. That is, the branching order of the imaged roots was recorded using the concept of Gravelius ordering (Fabrika et al. 2019). This means that the sequence of root segments starting from the base of the seedling and classified by WinRHIZO™ as being in the same root gets the lowest order (zero), and roots (sequences of root segments) forking off the main root are of order one and so on (Fig. 1). The first two segments with the lowest orders, i.e., the innermost root segments were treated separately in the data and are hereafter called the main root (order zero) and the lateral roots (order one). The higher order branches, i.e., the outer root segments were pooled together to form the tertiary level of segments, hereafter called fine roots (orders two to four).

Fig. 1 Schematic diagram of root topology according to Gravelius ordering (Fabrika et al. 2019) and as it is produced in WinRHIZO™: the main root of the seedling gets the lowest order (zero), the first order lateral roots are attached to the main root, and the outermost tertiary level of segments (2nd, 3rd order) form the fine roots



The dichotomous branching index (DBI) was calculated from the segment data containing the external path length and number of root tips according to Šmilauerová and Šmilauer (2002): $DBI = [Pe - \min(Pe)] / [\max(Pe) - \min(Pe)]$ where Pe , the external path length, is the sum of the number of segments in all paths from each external segment to the base segment, $\min(Pe) = \mu[\min(a) + 1] - 2^{\min(a)-1}$, is the theoretical external path length for a fully dichotomous topology where μ is the number of root tips, i.e., exterior segments and $\min(a) = \lceil \log_2(\mu - 1) \rceil + 2$ the number of segment in the longest single path from an external segment to the base segment, and $\max(Pe) = 0.5(\mu^2 + 3\mu - 2)$ is the theoretical external path length in a fully herringbone system (Fitter 1987; Beidler et al. 2015). This estimates the degree to which a root system is fully herringbone (1) or fully dichotomous (0). DBI values less than 0.5 indicate a dichotomous topology.

The count of thin fibrous (absorptive roots) and thicker branchless pioneer roots (transport roots) was estimated by sorting all lateral root branches attached to the main root (zero order) into five different functional categories based on the lateral root branching topology (Supplementary Fig. S2): *i*) short fibrous roots (≤ 15 mm) and longer branches that have *ii*) no further branches or root tips (max one tip), *iii*) two or more than two root tips but no longer branches, *iv*) 1–2 longer branches, and *v*) more than two longer branches (Supplementary Fig. S2). Root tips were assumed to be ≤ 5 mm in length. Roots in categories *ii*

and *iii* are typical pioneer roots and in categories *i*, *iv* and *v* fibrous absorptive roots.

Also, specific root length $SRL \text{ cm g}^{-1}$ (root length/root dry weight) and branching intensity BI (number of root tips/total root length, also called root tip density) were calculated. It is possible to generate based on WinRHIZO™ data a two-dimensional replica of the measured root system that is statistically like the original. Such replicas were made using LIGNUM software (Sievänen et al. 2010). They were used to calculate the convex hull areas of the root systems by drawing the tightest convex polygon around the root branches. The convex hull area was used as an estimate of the interactive rhizosphere area. The values are averages of ten replicate simulations of each lateral root branch class above short fibrous roots (*ii–v*). Convex hull area is not simulated to *i*-class short roots as their convex hull area is the same as their root surface area. The count and convex hull area of lateral root branches of each class mentioned above (*i–v*) that grew into the humus clod were calculated.

Data and statistical analyses

Finally, 76 seedlings out of 90 were included in the analyses since 14 seedlings ceased of growing in microcosm in the early phase of the experiment. Thus, 37 replicates from the fast and 39 replicates from the slow growth phenotype were included (5–10 per maternal family).

Principal component analysis PCA with varimax rotation was used to summarize and support the interpretation of information in root data with the function *principal* from the **psych** library (Revelle 2021). Varimax rotation strengthens the loadings of trait with one component only. Components with eigenvalue larger than one were retained. Correlation of (> 10.71) was considered strong.

To test our working hypothesis that seedlings from the two growth phenotypes show differences in the measured traits, we fitted linear mixed models *lme* with growth phenotype as explanatory fixed variable and maternal family as random factor from packages **nlme** (Pinheiro et al. 2018). For ecophysiological measurements following the onset of dry conditions both treatment and phenotype were used as explanatory variables. To avoid repeated analysis of highly correlated root parameters mixed models were

estimated to shoot height and only to one representative root trait having the strongest correlation with each PC1–5: total root length, BI, SRL, length of main root, and percentage of tips in the humus clod. Also two other parameters (number of lateral roots and fibrous:pioneer-ratio) that did not correlate strongly with any of the PC were studied separately. Percentages were logit-transformed in the model $y = \log(y/100/(1-y/100))$.

Permutational multivariate analyses of variances for the number, humus contact, and simulated convex hull areas of lateral roots in different topology classes were calculated with *adonis* from package **vegan** with maternal family nested within growth phenotype. Further, to visualize the branching topology of first-order laterals (branching, precision, and convex hull), a 2-dimensional non-metric multidimensional scaling was conducted with *metaMDS* from package **vegan** (Oksanen et al. 2020) with Bray-Curtis dissimilarity indices.

Pearson's correlation coefficients for family means for transpiration, stomatal conductance, net assimilation rate, and water potential and the representative root and shoot parameters were computed with function *cor.test* from **Stats** library.

Statistical testing and figures were done with the R software (R version 4.0.2) environment for statistical computing and graphics (R Core Team 2020). We considered $p \leq 0.05$ as statistically significant and $0.05 < p \leq 0.10$ as indicative.

Results

Seedling aboveground growth

Shoot height varied among maternal families but it was not associated with the growth phenotype (Table 1). These results were in accordance with the pilot experiment (Supplementary Table S2). Shoot height had a positive correlation with root weight, length, and collar diameter ($r = 0.63, 0.54,$ and 0.59 , respectively, $p < 0.001$). Shoot weight is not reported as an approximately 10–12 mm piece of stem was cut above the collar for ecophysiological measurements and assessment of wood formation and structure.

Root data exploration with rotated principal component analysis

Most root variables were highly correlated as they are generated from the same original data and many traits are interrelated. The rotated principal components simplified the root data including 19 parameters into five principal components (PC) that explained almost 90% of the variation (Table 1): PC1 alone accounted for half of the variation. Root collar diameter, root dry weight, root projection and surface area, root volume, total root length, length of lateral and tertiary roots, root tip number, number of tertiary root branches, and the sum of convex hull areas were strongly positively correlated with PC1. Moreover, dichotomous branching index (DBI) had strong negative correlation with PC1. Branching intensity (BI) i.e., root tip density correlated strongly with PC2. Number of tips in the humus clod and the percentage of all root tips in the humus clod correlated strongly with PC3. Both PC2 and PC3 explained around 10% of the variation. Specific root length (SRL) correlated with PC4, and the main root length with PC5. In the further analyses PC1 is represented by total root length, PC2 by BI, PC3 by percentage of all root tips in the humus clod, PC4 by SRL, and PC5 by main root length. However, results can be generalized to other highly correlated parameters.

Root system size and architecture, described by total root length (PC1) and BI (PC2), respectively, varied significantly between families but not systematically between the growth phenotypes (Table 1). Roots of both growth phenotypes were dichotomously branching and had a very low DBI (< 0.2). Root branching patterns and responses to nutrient patches, represented by the proportion of root tips in the humus clod (PC3) and SRL (PC4), did not differ between the growth phenotypes. The main root (PC5) was longer in the fast growth phenotype (Table 1).

Branching topology

A clear majority of lateral roots directly attached to the main root (visual explanation of branching topology classes in Supplementary Fig. S2) were short fibrous roots that formed the first class (*i*) of the lateral root branching topology (Fig. 2a). The number of laterals that branch from the main root (Fig. 2a, Table 1) and the ratio between absorptive fibrous

Table 1 Shoot height and root parameters analysed after eight months growing period for two growth phenotypes, fast and slow. On right a summary of the principal component (PC) analysis on 19 root traits showing eigenvalues, proportion variance (%) and loadings (Pearson's correlation coefficients), with

strong correlations (>|0.7|) emphasised in bold. Representative traits chosen for linear mixed model analyses are underlined with their fixed effects reported. Sample number in brackets. $P < 0.05$ are marked with an asterisk (*) and $P < 0.1$ with an apostrophe (')

Parameter	Fast (37)	(Min-max)	Slow (40)	(Min-max)	$F_{1,8}$	p	PC1	PC2	PC3	PC4	PC5
Shoot height cm	9.98	5.5–15.1	9.24	5.4–17.1	0.5	0.48					
<i>Eigenvalue</i>							9.44	2.62	1.95	1.36	1.02
<i>Proportion variance</i>							49.7%	13.8%	10.2%	7.2%	5.4%
Collar Ø mm	1.66	1.1–2.4	1.52	0.9–2.6			0.74	–0.13	–0.08	–0.45	–0.04
Root dry weight g	0.41	0.1–0.7	0.35	0.1–0.7			0.9	–0.19	–0.01	–0.31	0.07
Root Projection Area cm ²	74.1	0.2–214.6	51.4	0.2–147.9			0.93	–0.25	–0.05	0.17	0.03
Root Surface Area cm ²	233	41–674	162	30–465			0.93	–0.25	–0.05	0.17	0.03
Root vol cm ³	6.18	0.4–20.9	4.17	0.4–13.9			0.9	–0.27	–0.08	0.16	0
Root tot length cm	726	270–1733	532	176–1247	1.8	0.213	0.96	–0.13	0.02	0.21	0.07
<u>Main root length cm</u>	36	28.0–48.9	32.9	20.7–44.6	5	0.06'	0.07	0.04	–0.08	–0.01	0.98
Lateral root length cm	287	151–451	223	119–397			0.82	–0.17	–0.05	0.14	0.07
Tertiary root length cm (2nd, 3rd, 4th order)	445	51–1339	307	31–904			0.93	–0.1	0.03	0.21	0.04
Number of root tips	947	267–1700	750	369–1450			0.8	0.55	0	0.09	0.04
<u>Number of lateral roots, 1 st order branches</u>	116	49–383	111	35–420	0.6	0.7	0.03	0.44	–0.51	0	0.12
Number of 2nd, 3rd, 4th order branches	866	184–1610	669	258–1371			0.82	0.53	–0.04	0.11	0.03
<u>Specific root length SRL cm mg⁻¹</u>	1.74	1.0–3.3	1.55	1– 2.1	1.3	0.29	0.39	–0.01	0.06	0.88	–0.02
<u>Branching intensity BI tips cm⁻¹</u>	1.42	0.8–2.8	1.51	0.6–2.7	0.5	0.51	–0.32	0.89	–0.03	–0.09	–0.05
Dichotomous Branching Index DBI	0.11	0.05–0.2	0.13	0.05–0.3			–0.82	–0.08	–0.02	0.03	0.08
<u>Fibrous:Pioneer-ratio</u>	0.17	0.03–0.5	0.14	0.01–0.5	2.9	0.12	–0.33	0.69	0.13	0.08	0.06
Number of tips in humus	67.9	3–202	63.4	7–209			0.22	0.32	0.89	0.02	–0.03
<u>Percentage % of tips in humus</u>	8.14	0.4–25.5	8.42	0.7–22.2	0.4	0.56	–0.23	–0.07	0.92	0.05	–0.01
Convex hull area cm ²	1290	435–2684	914	209–1911			0.78	–0.39	0.03	0.23	0.05

roots (i, iv–v) and pioneer roots (ii–iii) did not vary between the growth phenotypes (Table 1).

The branching topology class pattern was independent of the growth phenotype (Table 2), and the 2-D visualization (Fig. 3a) showed that the growth phenotype centroids overlapped with each other.

The humus clod did not direct root growth. This observation was supported by the pilot experiment where only 5% of seedlings grew directly towards the humus clod (data not shown). After eight months, roots that were in contact with the humus clod showed a bimodal distribution with short roots (i) and roots with long branches (v)

being equally abundant (Fig. 2b). However, only 5.5% of all roots in microcosms belonging to class (i) grew to the humus clod compared with 58% of roots in class (v). The pattern of lateral root branching topology classes that were in contact with the humus clod showed indicative differences between the growth phenotypes: there were more class (i) and (v) laterals in fast-growing phenotypes (Table 2, Fig. 2b). The pattern of lateral root branching topology classes that were in contact with the humus clod showed indicative differences between the growth phenotypes (Table 2, Fig. 2b). However, growth phenotype explained less than 2% of the variation in number

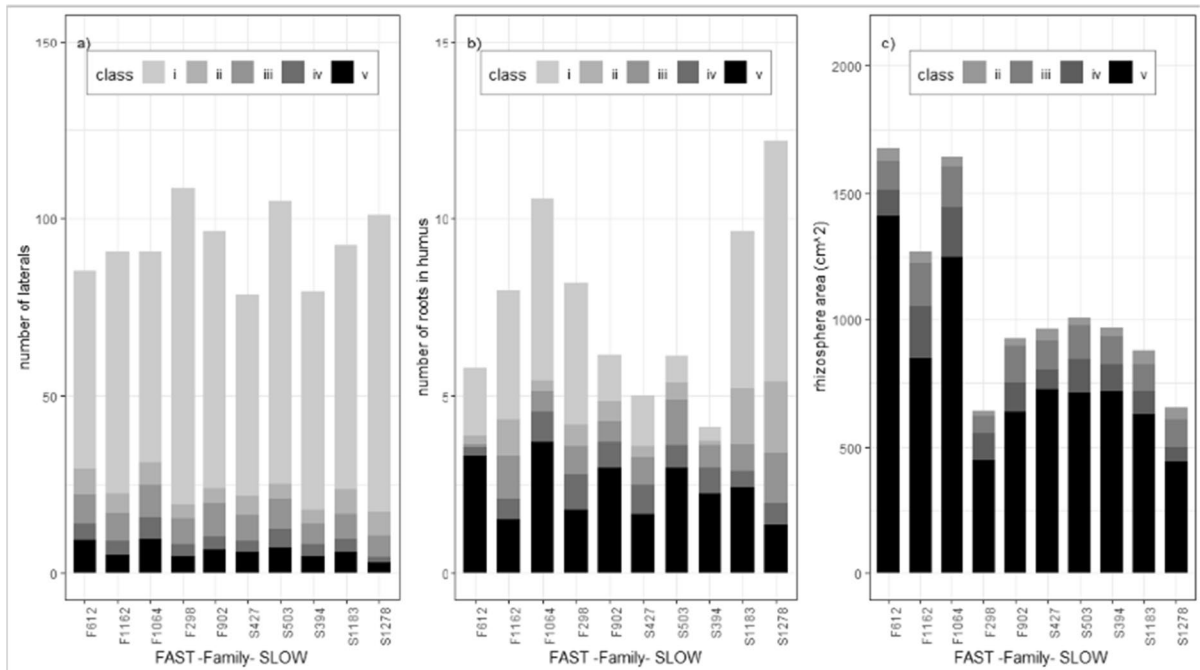


Fig. 2 Branching topology of first order laterals: i) short fibrous roots (≤ 15 mm) and longer branches that have ii) no further branches and max one tip, iii) two or more than two root tips but no longer branches, iv) 1–2 longer branches, and v) more than two longer branches (Illustration of root segments in Supplementary Fig. S2). **a)** the mean number of root classes attached to the main root, **b)** the number of root classes

attached to the main root that have grown into the humus clod at the end of the experiment, **c)** the simulated convex hull area of the root classes attached to the main root. Note that surface area is not simulated to i-class short roots. Fast- (F612, F1162, F1064, F298, F902) and slow- (S427, S503, S394, S1183 and S1127) growing families are ordered according to descending future success rate (Supplementary Table 1)

Table 2 Multivariate branching topology data including the number of lateral roots in different classes, number of roots that grew into humus and the surface area of roots in different classes. Permutational analysis of variance for Branching topology of first order laterals. $P < 0.05$ are marked with an asterisk (*) and $P < 0.1$ with an apostrophe (')

Topology classes i-v	Fast	Min-max	Slow	Min-max	F	R2	P
Number of laterals in							
i	67.16	27–128	68.30	21–136	1.00	0.013	0.22
Ii	5.59	1–13	5.43	0–19			
Iii	8.19	3–13	6.95	1–16			
Iv	4.49	0–14	3.45	0–10			
V	7.54	1–16	5.98	1–18			
Number in humus							
i	3.11	0–27	2.43	0–19	1.06	0.014	0.09'
Ii	0.54	0–3	0.80	0–7			
Iii	0.65	0–4	0.93	0–7			
Iv	0.62	0–3	0.65	0–3			
V	2.7	0–7	2.20	1–6			
Surface area in class (cm ²)							
ii	39	3–105	41	0–118	5.54	0.062	<0.01**
Iii	136	24–357	115	6–286			
Iv	147	0–635	93	0–287			
V	968	169–2122	665	13–1661			

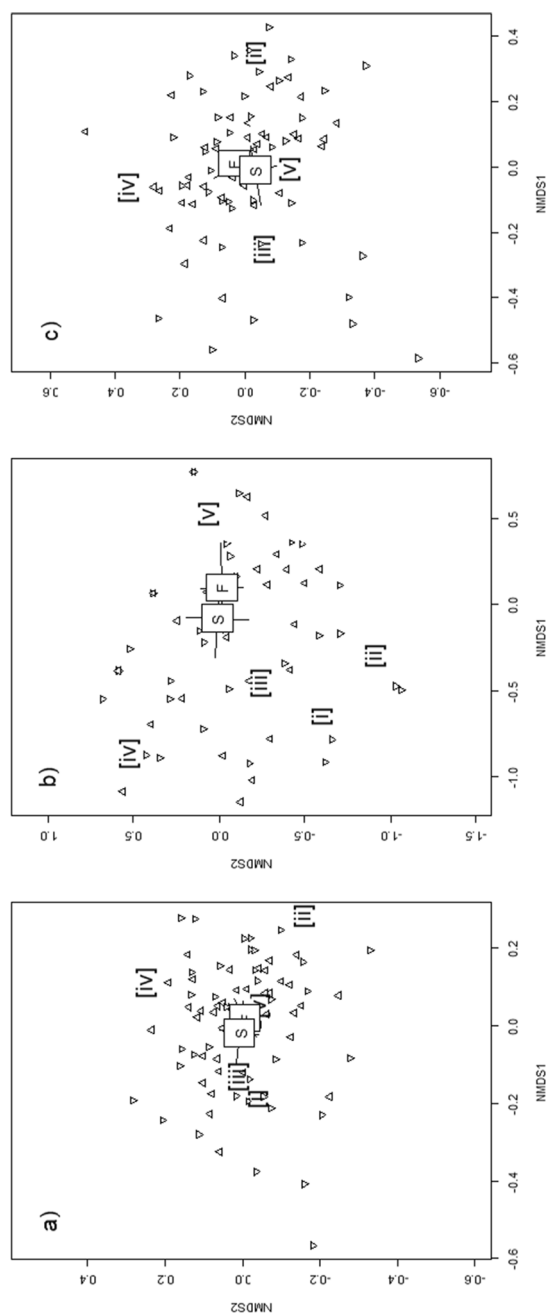


Fig. 3 2-dimensional non-metric multidimensional scaling NMDS plots of branching topology of first order laterals of seedlings representing fast ▲ (erect triangle) and slow ▼ (inverted triangle) phenotypes. Square brackets with roman numerals show the location of branching classes: i) short fibrous roots (≤ 15 mm) and longer branches that have ii) no further branches and max one tip, iii) two or more than two root tips but no longer branches, iv) 1–2 longer branches, and v) more than two longer branches (Illustration of root segments in Supplementary Fig. S2). F and S stand for the standard error of the 95% confidence interval of the mean. **a** number of roots in each topology class, **b** number of roots of each class in humus, **c** simulated convex hull area in each topology class

of roots of each class in humus, which is reflected also in the visual presentation (Fig. 3b). The total convex hull area had a positive correlation with PC1 and total root length, which did not differ between the growth phenotypes. However, the convex hull area was highest in the fibrous roots with more than two long branches (v): over 70% of the absorptive root area came from these laterals and around 14% of the area both from long laterals with many root tips and few long branches (classes iii and iv) (Fig. 2c). The root convex hull pattern differed significantly between the growth phenotypes (Table 2), which explained 6% of the variation in the multivariate data. Thus, in the fast-growing phenotypes root area is especially expanded by branched fibrous roots (v) (Table 2, Figs. 2c and 3c).

Ecophysiological measurements

Transpiration, stomatal conductance, and net assimilation rate did not differ significantly between the growth phenotypes based on the point measurements at seven months (data not shown). Neither were there any correlations between root traits and transpiration, stomatal conductance, or net assimilation rate at seven months.

None of the seedlings showed any visual signs of damage after exposure to dry conditions. The net assimilation rate decreased during the eighth month but did not differ significantly due to dry conditions, or growth phenotype (Table 3). After one month in dry conditions,

transpiration ($F_{1,62}=10.34$, $p<0.01$) and stomatal conductance ($F_{1,62}=9.21$, $p<0.01$) were significantly lowered in comparison to the well-watered condition, but there were no differences between the growth phenotypes (Table 3). The reduction in transpiration rate and stomatal conductance were 54% and 54% in the fast and 45% and 33% in slow- growing phenotype, respectively. Water potential did not vary significantly due to dry conditions, or growth phenotype (Table 3). There was a two-fold increase in water use efficiency under drought ($F_{1,60}=12.28$, $p<0.001$), but again no differences were found between phenotypes.

At eight months family means of root total length, the number of lateral roots and percentage of tips in humus clod did not have any relationship with family means of assimilation rate, transpiration, stomatal conductance nor water potential in neither condition. Main root length had strong negative correlation with water potential family means ($r=-0.75$, $p=0.02$), and indicative negative correlations with assimilation rate, transpiration, and stomatal conductance (all $r<-0.59$, $p=0.07$) but only in the well-watered condition. Under dry conditions specific root length had strong correlation with assimilation rate ($r=0.72$, $p=0.02$), and indicative moderate correlation with transpiration and stomatal conductance (both $r>0.58$, $p<0.08$). Shoot height had indicative moderate negative correlation with transpiration and stomatal conductance (both $r<-0.60$, $p=0.07$), and branching index indicative moderate correlation with transpiration and

Table 3 The phenotype mean, min and max of point measurements of transpiration, stomatal conductance (total water vapor conductance), net assimilation rate, water use efficacy and

water potential at 7 and 8 months after subjecting half of the seedlings were subjected to well-watered and half to dry conditions for the last month. Sample number in brackets

	8 months, Well-watered		8 months, Dry	
	Fast (16)	Slow (20)	Fast (18)	Slow (19)
Transpiration E [mmol m ⁻² s ⁻¹]	0.64	0.70	0.29	0.46
Min-max	0.2–1.4	0.3–1.7	–0.1– 1.1	0–1.3
Stomatal conductance GH ₂ O [mmol m ⁻² s ⁻¹]	46.1	50.4	21.3	34
Min-max	13–98	18–123	–4–77	–2–103
Assimilation rate A [μmol m ⁻² s ⁻¹]	2.71	3.21	2.51	3.37
Min-max	1.3–4.9	1.6–6.3	0.1–5.7	–0.3– 7.7
Water use efficiency A/E	4.8	5.1	12.5	10.6
Min-max	3.3–8.6	2.7–8.6	–1.9–36.5	–14.3–49.1
Water potential Ψ (MPa)	–0.55	–0.48	–0.52	–0.58
Min-max	–0.36– –0.88	–0.30– 0.70	–0.30– –0.70	–0.24– –0.90

stomatal conductance (both $r > 0.57$, $p < 0.09$). The family mean ratio between fibrous to pioneer roots had moderate indicative correlations with assimilation rate, transpiration and stomatal conductance (all $r = 0.61$, $p = 0.06$).

Soil nutrients and mycorrhizas

The nutritional content of the soil substrate and humus clods was measured at the start and at the end of the experiment. At the beginning of the experiment humus clods had lower pH and higher concentrations of C, macronutrients N, P, S and Ca, and heavy metals such as Cd and Pb than the surrounding soil (Table 4). The C/N ratio of the surrounding scarce soil increased from 59 to 64, and of the humus clod from 23 to 25, which resembles C/N ratio levels typical of relatively nutrient-rich Norway spruce boreal forest site types (Pennanen et al. 1999). The content of P was at the start five and in the end 15

times higher in the humus clod than in the surrounding soil. Due to the long timespan of the experiment and because sieved forest humus was twice applied to the nutrient patch together with additional slow-release fertilizer, we could see some accumulation of micronutrients such as Fe, Mn, Zn, B, and Na, possibly also due to diffusion from the outer soil layers (Table 4). Seedlings were inoculated with a pure culture of *Thelephora terrestris* and ectomycorrhizal fungi originating from humus that resulted in a high $> 80\%$ colonization rate of root tips (data not shown).

Discussion

In this microcosm experiment we studied seedling root exploration and tolerance to drought in Norway spruce families from contrasting growing phenotypes. We found that the fast- and slow-growing phenotype groups, each represented by five maternal families,

Table 4 Nutritional content of the soil substrate and humus clods per dry weight at the start of the experiment and at the end after 8 months presented with two significant digits. Highest values are strengthened and lowest underlined. Light Sphagnum peat pH 4.7 (Kekkilä Oy)

Sample and month	Soil start	Soil end	Humus start	Humus end
pH	5.3	5.6	<u>4.1</u>	4.6
C % dw	<u>4.7</u>	4.5	46	24
N % dw	0.08	<u>0.07</u>	2.00	0.97
P µg/g	190	<u>58</u>	930	890
K mg/g	5.9	2.8	<u>0.64</u>	2.0
S µg/g	140	<u>70</u>	2300	1500
Ca mg/g	1.2	<u>0.8</u>	5.4	5.2
Mg mg/g	15	7.6	<u>0.8</u>	3.6 ^{Nut}
Fe mg/g	6.3	2.9	<u>2.8</u>	7.3^{Nut}
Mn µg/g	40	<u>20</u>	59	180^{Nut}
Zn µg/g	11	<u>6.6</u>	45	58^{Nut}
Cu µg/g	5.1	<u>3.2</u>	14	21^{Nut}
B µg/g	0.75	<u>< 0.40^{LOD}</u>	4.1	4.9^{Nut}
Na µg/g	120	<u>100</u>	250	430^{Nut}
Al mg/g	5.70	2.90	<u>2.60</u>	5.60 ^{Nut}
Cd µg/g	<u>< 0.07^{LOD}</u>	<u>< 0.07^{LOD}</u>	0.46	0.27
Cr µg/g	34.00	13.00	<u>6.50</u>	15.00 ^{Nut}
Ni µg/g	21.00	9.30	<u>7.00</u>	9.50 ^{Nut}
Pb µg/g	<u>< 1.00^{LOD}</u>	<u>< 1.00^{LOD}</u>	80.00^a	66.00

LOD value below the limit of detection. Nut = The higher content of these nutrients in the humus clod at the end of the experiment are most likely due to the addition of Nutricote® 70-grains. In some cases, nutrients may have diffused down the microcosms from the outer soil layers. a: soil collected close to a highway, average of Pb in Finnish soil is 20 mg/kg, the limit for contaminated soil is 2500 mg/kg

showed only minor differences in root growth and architecture. All families adopted a dichotomous rooting habit. However, we found indications that fast-growing families had larger rhizosphere interaction surface between branched lateral roots and soil. Still, the physiological state of families was uniform.

Intraspecific variation in root morphology and architecture is independent of growth phenotype

Intraspecific trait variation is important for performance. In many aspects the families of the fast- and slow-growing phenotypes grew alike. As expected, the phenotypes did not differ in their aboveground growth. Neither did the data support our first hypothesis regarding the number of root branches and root tips. The total root length, number of root tips, root tip density (BI), specific root length (SRL), the number of lateral branches, or the ratio of fibrous and pioneer roots did not vary between the phenotypes. Only the main root was on average slightly longer in families of fast-growing phenotypes.

Our data does not suggest stable indirect root measures for intraspecific comparisons that lead to good growth performance from temporally and spatially highly adjustable root characteristics. Moreover, based on our data, the often measured BI and SRL seem not to be good predictors for spruce growth. Liese et al. (2017) concluded that BI is a key belowground trait that relates the mycorrhizal association type to precision foraging and thus influences resource uptake rates and function. SRL has earlier been observed to be independent from the whole plant economic spectrum (Kramer-Walter et al. 2016, Salmela et al. 2020; Salmela 2021). In accordance with our results, many studies have found intraspecific variation in SRL of Norway spruce but no connection to latitudinal gradient (Ostonen et al. 2013), nor growth phenotype (Hamberg et al. 2018), or populations (Salmela et al. 2020) have been reported. Furthermore, recently Freschet et al. (2021) conclude that these easily measurable traits are often vaguely related to functions, and that emphasis should be made to measure traits related to a precise function. Likewise, to understand root trait variation in relation to the environment, and whether general intraspecific patterns exist across species a broader set of root traits and environmental variables should be measured (Weemstra et al. 2021). Weemstra et al. (2021)

noted that variation in BI and SRL of fine roots in Norway spruce in situ was higher within elevations than across a 600-m elevational range in the French Alps. They suggested that intraspecific root trait variation may depend more on small-scale heterogeneity than large-scale environmental variation. However, Ostonen et al. (2011) found in nutrient poor subarctic soil that root tip length and fine root biomass were larger in higher-latitude Norway spruce stands and emphasized the importance of a collaborative continuum of tree nutrient acquisition.

Branching topology affects the root-soil interaction area that favours fast-growing phenotypes

The second hypothesis is partly supported by the data: The total length and convex hull area of lateral roots were on average higher in the fast-growing phenotype, but the differences were not statistically significant. However, the patterns of the contact area between lateral roots and soil varied between the phenotypes indicating that fast-growing families gain more interaction surface from the branched and bifurcated fibrous lateral roots, whose convex hull is the largest.

The observed longer main roots and the lateral root branching topology patterns of fast-growing families correspond well with previous findings of Hamberg et al. (2018), irrespective of homogenous growing substrate in their study. The root systems of their seedlings were considerably bigger than in the present study, and fast-growing phenotypes had larger number of side branches and root tips especially further away from the base of seedlings. Hamberg et al. (2018) speculated that this characteristic could be one that differentiates fast- from slow-growing phenotypes before the divergence in their aboveground growth rate.

Fine roots have a pivotal role in both absorption and transport of nutrients and water. The formation of branches enables plants to extend roots to a larger area and to increase their contact area with soil (Beidler et al. 2015). Interestingly, more than half of the most ramified lateral roots with long branches grew into the humus. Furthermore, the phenotypes indicatively differed in the type of lateral roots that were in contact with the humus clod. Considering the slight differences in the overall branching topology, fast-growing phenotypes seem to have larger interaction surface with the nutrient spot.

Nutrient spots do not direct early root growth

We found no support for our third hypothesis that fast-growing families would proliferate more fine roots in response to a nutrient patch compared to slow-growing seedlings when growing in a substrate of very low nutrient content. Seedlings from both phenotypes were mining the soil with equal intensity as they did adopt a dichotomous rooting habit instead of a herringbone type. Dichotomous branching may be favoured in uneven soil as it intensifies patchy soil exploitation and is better in acquiring diffusion-limited resources even though it is less efficient in exploring soil space and capturing mobile resources (Fitter 1987; Fitter et al. 1991). For example, it has been suggested that nitrate uptake efficiency of dichotomously branching root system from a heterogeneous and static soil supply is higher than for herringbone type roots (Dunbabin et al. 2004).

The stability of root architecture has been suggested to be an adaptation of conifers to deal with the recalcitrant litter of boreal forests (Chen et al. 2016; Cheng et al. 2016). The proposition that thick root species, such as conifers, forage without consideration of nutrient patches and thus avoid opportunistic proliferation in nutrient hotspots (Chen et al. 2018) was supported by our study as the nutrient patch did not direct the early root growth. Neither did the amount of root tips in humus early on have any relationship with the number of roots in humus in the end of the experiment. In plants, the most successful competitors exploit nutrient patches more completely because of higher growth rates and larger root systems (Campbell et al. 1991).

Spruce seedlings benefit from outsourcing the nutrient acquisition to a mycorrhizal partner (Bergmann et al. 2020) which extends the nutrient acquisition area multifold, as mycelia may be suggested more important than root proliferation in nutrient patch exploitation of trees (Tibbett 2000, Chen et al. 2016, Cheng et al. 2016, Defrenne et al. 2019, McCormack and Iversen 2019). Moreover, under variable soil resource conditions, shifts in fine root and mycorrhizal fungal biomass rather than in fine-root morphology have been reported (Weemstra et al. 2017; Ostonen et al. 2011). Thus, this interspecific interaction may compensate for the tree's inability to proliferate roots in organic patches in scarce conditions (Cheng et al. 2016). Boukcim and Plassard

(2003) found clear differences in non-mycorrhizal fine root architecture between one fast- and one slow-growing Norway spruce family, but the difference disappeared when roots were mycorrhizal. In this study, seedlings seemed to utilise the high-scale, but low-precisions foraging (Campbell et al. 1991) regardless of their phenotype. This implies that root foraging precision to nutrient hot spots may not be the driving factor behind long-term differences in growth rate of spruce. Most spruce families used in the experiment are known to equally form ectomycorrhizal symbiosis (Velmala et al. 2014a) and our seedlings were inoculated with *Thelephora terrestris* to minimize the effects of varying colonization on spruce fine root traits (Velmala et al. 2014a). Thus, even if we did not measure the nutrient uptake by hypha, it is likely that the nutrients from the humus clod were partly taken up by the fungal hypha.

Studies focusing on between- or within species root traits as discussed above, often utilize homogeneous growing media or common garden-layout where the effects of soil physical, chemical, and biological environment are minimized. This is due to increasing complexity of the picture if wider variation in environmental or tree genetic properties is included in the system. Indeed, Ostonen et al. (2017) showed a trilateral relation between the morphological traits of absorptive spruce fine roots, exploration types of root colonizing fungi, and soil bacterial community structure. Further, Salmela et al. (2020) demonstrated that in addition to soils of different nutritional status and accompanying microbiota, varying temperature affected Norway spruce root traits, and the variation was often environment-specific, implying that a great genetic diversity in spruce is enabling the adaptation of roots. Changes in root architecture but not biomass in response to soil nutrient status and intraspecific competition in dragon spruce *Picea asperata* has been reported (Nan et al. 2013). A moderate genetic component determining mycorrhizal associations of Norway spruce has also been shown (Velmala et al. 2013). Nevertheless, by studying mature stands Senior et al. (2019) found no evidence that breeding for increased growth in Norway spruce would impact root tissue density or chemical properties of fine roots, but that growth rate alters the soil microbial communities and soil biological properties and thus the ecosystem services they provide (Senior et al. 2022).

Rapid responses to drought do not differ between growth phenotypes

The indicators of the physiological state of families including assimilation rate, stomatal conductance, and transpiration were uniform at seven months and did not vary according to phenotype. The short and relatively mild drought period limited transpiration through a decrease in stomatal conductance, which increased water use efficiency. However, there was no significant difference between the phenotypes, and we did not find support for our fourth hypothesis: seedlings from both growth phenotypes showed rapid response to drought that was reflected in the net assimilation rate, stomatal conductance, transpiration, and water uptake efficiency. The seedlings of the fast-growing phenotype responded slightly faster by closure of stomata, but the difference was not statistically significant. Thus, our data does not give support for the assumption that the future fast-growing phenotypes would be more sensitive to stress, contrasting to the previous findings in Velmala et al. (2014b). As Tron et al. (2015) claimed, there is no unique root ideotype for dry ecosystems, but exploitative roots are adaptive to intermittent water supply. Main root length had a positive relationship with water potential under conventional condition, and after drought SRL had a positive correlation with net assimilation rate. Interestingly, high fibrous to pioneer root ratio indicatively suggested efficient photosynthetic machinery.

Conclusion

For a comprehensive understanding of the long-term growth performance of coniferous trees the research focus on roots is justified. The congruence between current and previous observations on the early root growth of genetically independent spruce origins that have contrasting long-term growth, and the observed physiological state of seedlings that varied regardless of the phenotype lays the focus on roots. The efficiency of the photosynthetic machinery or the water transport capacity from soil to leaf at the early seedling stage are probably not major factors behind the varying long-term growth rates. Our results suggest that despite a uniform mode of early root growth and

root exploitation in nutritionally heterogeneous soil, Norway spruce seem to possess early topological differences that enable families of the fast-growing phenotype to monopolise heterogeneously distributed nutrients by means of their more extensive root surface area. These differences in roots topology might be later reflected also in the photosynthetic machinery.

Acknowledgements Funded by Academy Finland Projects 292967 and 325995. We thank M. Oksanen, T. Hytönen, and S. Tiikkainen for assistance in establishing the experiment and for taking care of the seedlings weekly, U. Jauhiainen and H. Paavola-Koskinen for assistance during the drought treatment, sampling, and in digitizing and photographing roots. Special thanks for Dr. Jussi Heinonsalo for lending the flat transparent Perspex microcosms and M-L Napola for selecting Norway spruce families.

Authors' contributions SV, MS, TH, RS, and TP conceived the ideas and designed methodology; SV, MS, and TC collected the data; SV, MS, TC, TH, LH, and RS analysed and interpret the data; SV, MS, TP drafted and led writing of the publication; TC, TH, LH, RS critically revised the manuscript. All authors contributed to the publication and have approved the final version. We agree that the data is appropriately investigated.

Funding Open access funding provided by Natural Resources Institute Finland (Luke). This work was funded by Academy of Finland Projects 292967 and 325995.

Data availability The data that support the findings of this study is available in spreadsheets from <https://zenodo.org/https://doi.org/10.5281/zenodo.7034181> under CC BY 4.0 licence.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose. The founding sponsors had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, and in the decision to publish the results.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Beidler K, Taylor B, Strand A, Cooper E, Schönholz M, Pritchard S (2015) Changes in root architecture under elevated concentrations of CO₂ and nitrogen reflect alternate soil exploration strategies. *New Phytol* 205:1153–1163. <https://doi.org/10.1111/nph.13123>
- Bergmann J, Weigelt A, van der Plas F, Laughlin DC, Kuyper TW, Guerrero-Ramirez N, Valverde-Barrantes OJ, Bruehlheide H, Freschet GT, Iversen CM, Kattge J, McCormack ML, Meier IC, Rillig MC, Roumet C, Semchenko M, Sweeney CJ, van Ruijven J, York LM, Mommer L (2020) The fungal collaboration gradient dominates the root economics space in plants. *Sci Adv* eaba3756, 6:27, eaba3756 <https://doi.org/10.1126/sciadv.aba3756>
- Berntson GM (1997) Topological scaling and plant root system architecture: developmental and functional hierarchies. *New Phytol* 135:621–634. <https://doi.org/10.1046/J.1469-8137.1997.00687.X>
- Bigras FJ (2005) Photosynthetic response of white spruce families to drought stress. *New Forest* 29:135–148. <https://doi.org/10.1007/s11056-005-0245-9>
- Boukcim H, Plassard C (2003) Juvenile nitrogen uptake capacities and root architecture of two open-pollinated families of *Picea abies*. Effects of nitrogen source and ectomycorrhizal symbiosis. *J Plant Physiol* 160:1211–1218. <https://doi.org/10.1078/0176-1617-00973>
- Campbell BD, Grime JP, Mackey JML (1991) A trade-off between scale and precision in resource foraging. *Oecologia* 87:532–538. <https://doi.org/10.1007/BF00320417>
- Carmona CP, Bueno GC, Toussaint A, Träger S, Díaz S, Moora M, Munson AD, Pärtel M, Zobel M, Tamme R (2021) Fine-root traits in the global spectrum of plant form and function. *Nature* 597(7878):683–687. <https://doi.org/10.1038/s41586-021-03871-y>
- Chen W, Koide R, Adams T, DeForest J, Cheng L, Eissenstat D (2016) Root morphology and mycorrhizal symbioses together shape nutrient foraging strategies of temperate trees. *PNAS* 113:8741–8746. <https://doi.org/10.1073/pnas.1601006113>
- Chen W, Koide RT, Eissenstat DM (2018) Nutrient foraging by mycorrhizas: from species functional traits to ecosystem processes. *Funct Ecol* 1–12. <https://doi.org/10.1111/1365-2435.13041>
- Cheng L, Chen W, Adams T, Wei X, Li L, McCormack ML, DeForest JL, Koide RT, Eissenstat DE (2016) Mycorrhizal fungi and roots are complementary in foraging within nutrient patches. *Ecology* 97:2815–2823. <https://doi.org/10.1002/ecy.1514>
- Coley PD, Bryant JP, Chapin FS 3rd. (1985) Resource availability and plant antiherbivore defense. *Science* 230(4728):895–899. <https://doi.org/10.1126/science.230.4728.895>
- Defrenne CE, McCormack ML, Roach WJ, Addo-Danso SD, Simard SW (2019) Intraspecific fine-root trait-environment relationships across interior Douglas-Fir forests of Western Canada. *Plants* 8:199. <https://doi.org/10.3390/plants8070199>
- Dehlin H, Nilsson MC, Wardle DA (2006) Aboveground and belowground responses to quality and heterogeneity of organic inputs to the boreal forest. *Oecologia* 150:108–118. <https://doi.org/10.1007/s00442-006-0501-5>
- Depardieu C, Gérardi S, Nadeau S, Parent GJ, Mackay J, Lenz P, Lamothe M, Girardin MP, Bousquet J, Isabel N (2021) Connecting tree-ring phenotypes, genetic associations and transcriptomics to decipher the genomic architecture of drought adaptation in a widespread conifer. *Mol Ecol* 30:3898–3917. <https://doi.org/10.1111/mec.15846>
- Dunbabin V, Rengel Z, Diggle AJ (2004) Simulating form and function of root systems: efficiency of nitrate uptake is dependent on root system architecture and the spatial and temporal variability of nitrate supply. *Funct Ecol* 18:204–211. <https://doi.org/10.1111/j.0269-8463.2004.00827.x>
- Fabrika M, Scheer L, Sedmák R, Kurth W, Schön M (2019) Crown architecture and structural development of young Norway spruce trees (*Picea Abies Karst.*): a basis for more realistic growth modelling. *BioResources* 14:908–921
- Fitter AH (1987) An architectural approach to the comparative ecology of plant root systems. *New Phytol* 106:61–77. <https://doi.org/10.1111/j.1469-8137.1987.tb04683.x>
- Fitter AH, Stickland TR, Harvey ML, Wilson GW (1991) Architectural analysis of plant root systems I. Architectural correlates of exploitation efficiency. *New Phytol* 118:375–382. <https://doi.org/10.1111/j.1469-8137.1991.tb00018.x>
- Freschet GT, Roumet C, Comas LH, Weemstra M, Bengough AG, Rewald B, Bardgett RD, De Deyn GB, Johnson D, Klimešová J, Lukac M, McCormack ML, Meier IC, Pagès L, Poorter H, Prieto I, Wurzbürger N, Zadworny M, Bag-niewska-Zadworna A et al (2021) Root traits as drivers of plant and ecosystem functioning: current understanding, pitfalls and future research needs. *New Phytol* 232:1123–1158. <https://doi.org/10.1111/nph.17072>
- Grime JP (1977) Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *Am Nat* 111:1169–1194 <https://www.jstor.org/stable/2460262>
- Hambergen L, Velmala SM, Sievänen R, Kalliokoski T, Pen-nanen T (2018) Early root growth and architecture of fast- and slow-growing Norway spruce (*Picea abies*) families differ—potential for functional adaptation. *Tree phys* 38:853–864. <https://doi.org/10.1093/treephys/tpx159>
- Hodge A (2004) The plastic plant: root responses to heterogeneous supplies of nutrients. *New Phytol* 162(1):9–24. <https://doi.org/10.1111/j.1469-8137.2004.01015.x>
- Jansson G, Hansen JK, Haapanen M, Kvaalen H, Steffenrem A (2017) The genetic and economic gains from forest tree breeding programmes in Scandinavia and Finland. *Scan J For Res* 32(4):273–286. <https://doi.org/10.1080/02827581.2016.1242770>
- Kalliokoski T, Nygren P, Sievänen R (2008) Coarse root architecture of three boreal tree species growing in mixed stands. *Silva Fenn* 42:189–210. <https://doi.org/10.14214/sf.252>
- Keel SG, Campbell CD, Högberg MN, Richter A, Wild B, Zhou X, Hurry V, Linder S, Näsholm T, Högberg P (2012) Allocation of carbon to fine root compounds and their residence times in boreal forest depend on root size class and season. *New Phytol* 194:972–981. <https://doi.org/10.1111/j.1469-8137.2012.04120.x>

- Korkkama T, Pakkanen A, Pennanen T (2006) Ectomycorrhizal community structure varies among Norway spruce (*Picea abies*) clones. *New Phytol* 171:815–824. <https://doi.org/10.1111/j.1469-8137.2006.01786.x>
- Kramer-Walter KR, Bellingham P, Millar TR, Smissen RD, Richardson SJ, Laughlin DC (2016) Root traits are multi-dimensional: specific root length is independent from root tissue density and the plant economic spectrum. *J Ecol* 104:1299–1310. <https://doi.org/10.1111/1365-2745.1256>
- Liese R, Alings K, Meier IC (2017) Root branching is a leading root trait of the plant economics Spectrum in temperate trees. *Front Plant Sci* 8:315 <https://www.frontiersin.org/articles/10.3389/fpls.2017.00315/full>
- Marx DH (1969) The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology* 59:153–163
- McCormack ML, Iversen CM (2019) Physical and functional constraints on viable belowground acquisition strategies. *Front Plant Sci* 10:1215 <https://www.frontiersin.org/article/10.3389/fpls.2019.01215>
- McCormack ML, Dickie IA, Eissenstat DM, Fahey TJ, Fernandez CW, Guo D, Helmissaari HS, Hobbie EA, Iversen CM, Jackson RB, Leppälampi-Kujansuu J, Norby RJ, Phillips RP, Pregitzer KS, Pritchard SG, Rewald B, Zadworny M (2015) Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. *New Phytol* 207:505–518. <https://doi.org/10.1111/nph.13363>
- Nan H, Liu Q, Chen J, Cheng X, Yin H, Yin C, Zhao C (2013) Effects of nutrient heterogeneity and competition on root architecture of spruce seedlings: implications for an essential feature of root foraging. *PLoS One* 8:e65650. <https://doi.org/10.1371/journal.pone.0065650>
- Napola ML (1997) Kuusen risteytysjälkeläistöjen valinta kasvullista joukkomonistusta varten. *Metsänjalostussäätiön työraportteja* 39. Helsinki 1997. 24 s
- Oksanen J, Blanchet FFG, Friendly M, Kindt R, Legendre P, McGlenn D, Minchin PR, O'Hara RB, Simpson GL, Solyomos P, Stevens MHH, Szoecs E, Wagner H (2020) *Vegan*: community ecology package. R package version 2.5–7. <https://CRAN.R-project.org/package=vegan>
- Ostonen I, Helmissaari HS, Borken W, Tedersoo L, Kukumägi M, Bahram M, Lindroos AJ, Nöjd P, Uri V, Merilä P, Asi E, Löhmus K (2011) Fine root foraging strategies in Norway spruce forests across a European climate gradient. *Glob Change Biol* 17:3620–3632. <https://doi.org/10.1111/j.1365-2486.2011.02501.x>
- Ostonen I, Rosenvald K, Helmissaari HS, Godbold D, Parts K, Uri V, Löhmus K (2013) Morphological plasticity of ectomycorrhizal short roots in *Betula* sp and *Picea abies* forests across climate and forest succession gradients: its role in changing environments *Front Plant Sci* 4. <https://doi.org/10.3389/fpls.2013.00335>. <https://www.frontiersin.org/articles/10.3389/fpls.2013.00335>
- Ostonen I, Truu M, Helmissaari HS, Lukac M, Borken W, Vanguelova E, Godbold DL, Löhmus K, Zang U, Tedersoo L, Preem JK, Rosenvald K, Aosaar J, Armolaitis K, Frey J, Kabral N, Kukumägi M, Leppälampi-Kujansuu J, Lindroos AJ et al (2017) Adaptive root foraging strategies along a boreal-temperate forest gradient. *New Phytol* 215:977–991. <https://doi.org/10.1111/nph.14643>
- Pennanen T, Liski J, Bååth E, Kitunen V, Uotila J, Westman CJ, Fritze H (1999) Structure of the microbial communities in coniferous Forest soils in relation to site fertility and stand development stage. *Microb Ecol* 38:168–179. <https://doi.org/10.1007/s002489900161>
- Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2018) *nlme*: Linear and Nonlinear Mixed Effects Models. R package version 3.1–137. URL: <https://CRAN.R-project.org/package=nlme>
- Polle A, Chen SL, Eckert C, Harfouche A (2019) Engineering drought resistance in Forest trees. *Front Plant Sci* 9:1875. <https://doi.org/10.3389/fpls.2018.01875>
- R Core Team (2020) *R*: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>
- Reich PB (2014) The world-wide 'fast-slow' plant economics spectrum: a traits manifesto. *J Ecol* 102:275–301. <https://doi.org/10.1111/1365-2745.12211>
- Revelle W (2021) *Psych*: procedures for psychological, psychometric, and personality research. Northwestern University, Evanston, Illinois. R package version 2.1.6, <https://CRAN.R-project.org/package=psych>
- Salmela MJ (2021) Patterns of genetic diversity vary among shoot and root functional traits in Norway spruce *Picea abies* along a latitudinal gradient. *Oikos* 130:1143–1157. <https://doi.org/10.1111/oik.08203>
- Salmela MJ, Velmala SM, Pennanen T (2020) Seedling traits from root to shoot exhibit genetic diversity and distinct responses to environmental heterogeneity within a tree population. *Oikos* 129:544–558. <https://doi.org/10.1111/oik.06797>
- Savolainen O, Pyhäjärvi T, Knürr T (2007) Gene flow and local adaptation in trees. *Annu Rev Ecol Evol Syst* 38:595–619. <https://doi.org/10.1146/annurev.ecolsys.38.091206.095646>
- Senior JK, Iason GR, Gundale M, Whitham TG, Axelsson EP (2019) Genetic increases in growth do not lead to trade-offs with ecologically important litter and fine root traits in Norway spruce. *For Ecol Manag* 446:54–62. <https://doi.org/10.1016/j.foreco.2019.05.021>
- Senior JK, Gundale MJ, Iason GR, Whitham TG, Axelsson EP (2022) Progeny selection for enhanced Forest growth alters soil communities and processes. *Ecosphere* 13:e3943. <https://doi.org/10.1002/ecs2.3943>
- Siefert A, Violle C, Chalmandrier L, Albert CH, Taudiere A, Fajardo A, Aarssen LW, Baraloto C, Carlucci MB, Cianciaruso MV, de L Dantas V, de Bello F, Duarte LDS, Fonseca CR, Freschet GT, Gaucherand S, Gross N, Hikosaka K, Jackson B et al (2015) A global meta-analysis of the relative extent of intraspecific trait variation in plant communities. *Ecol Lett* 18:1406–1419. <https://doi.org/10.1111/ele.12508>
- Sievänen R, Perttunen J, Nikinmaa E, Posada JM (2010) Functional structural plant models - case LIGNUM, in *Plant Growth Modeling, Simulation, Visualization and Applications*, eds. B. Li, M. Jaeger, and Y. Guo, Proceedings - PMA09, IEEE Computer Society, Los Alamitos, California, ISBN 978-0-7695-3988-1, 3–9

- Šmilauerová M, Šmilauer P (2002) Morphological responses of plant roots to heterogeneity of soil resources. *New Phytol* 154:703–715. <https://doi.org/10.1046/j.1469-8137.2002.00416.x>
- Tan W, Blake TJ (1997) Gas exchange and water relations responses to drought of fast- and slow-growing black spruce families. *Can J Bot* 75:1700–1706. <https://doi.org/10.1139/b97-884>
- Tibbett M (2000) Forum. Roots, foraging and the exploitation of soil nutrient patches: the role of mycorrhizal symbiosis. *Funct Ecol* 14:397–399. <https://doi.org/10.1046/j.1365-2435.2000.00417.x>
- Tron S, Bodner G, Lai F RL, Leitner D (2015) Can diversity in root architecture explain plant water use efficiency? A modeling study. *Ecol Model* 312:200–210. <https://doi.org/10.1016/j.ecolmodel.2015.05.028>
- Velmala SM, Rajala T, Haapanen M, Taylor AFS, Pennanen T (2013) Genetic host-tree effects on the ectomycorrhizal community and root characteristics of Norway spruce. *Mycorrhiza* 23:21–33. <https://doi.org/10.1007/s00572-012-0446-y>
- Velmala SM, Rajala T, Heinonsalo J, Taylor AFS, Pennanen T (2014a) Profiling functions of ectomycorrhizal diversity and root structuring in seedlings of Norway spruce (*Picea abies*) with fast- and slow-growing families. *New Phytol* 201:610–622. <https://doi.org/10.1111/nph.12542>
- Velmala SM, Rajala T, Smolander A, Petäistö RL, Lilja A, Pennanen T (2014b) Infection with foliar pathogenic fungi does not alter the receptivity of Norway spruce seedlings to ectomycorrhizal fungi. *Plant Soil* 385:329–342. <https://doi.org/10.1007/s11104-014-2238-y>
- Weemstra M, Mommer L, Visser EJW, van Ruijven J, Kuiper TW, Mohren GMJ, Sterck FJ (2016) Towards a multidimensional root trait framework: a tree root review. *New Phytol* 211:1159–1169. <https://doi.org/10.1111/nph.14003>
- Weemstra M, Sterck FJ, Visser EJW, Kuiper TW, Goudzwaard L, Mommer L (2017) Fine-root trait plasticity of beech (*Fagus sylvatica*) and spruce (*Picea abies*) forests on two contrasting soils. *Plant Soil* 415:175–188. <https://doi.org/10.1007/s11104-016-3148-y>
- Weemstra M, Freschet GT, Stokes A, Roumet C (2021) Patterns in intraspecific variation in root traits are species-specific along an elevation gradient. *Funct Ecol* 35:342–356. <https://doi.org/10.1111/1365-2435.13723>
- Weigelt A, Mommer L, Andraczek K, Iversen CM, Bergmann J, Bruehlheide H, Fan Y, Freschet GT, Guerrero-Ramírez NR, Kattge J, Kuiper TW, Laughlin DC, Meier IC, van der Plas F, Poorter H, Roumet C, van Ruijven J, Sabatini FM, Semchenko M et al (2021) An integrated framework of plant form and function: the belowground perspective. *New Phytol* 232:42–59. <https://doi.org/10.1111/nph.17590>
- Wright I, Reich P, Westoby M et al (2004) The worldwide leaf economics spectrum. *Nature* 428:821–827. <https://doi.org/10.1038/nature02403>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.