Roots constitute a major segment of plant biomass, and variation in belowground traits in situ correlates with environmental gradients at large spatial scales. Local adaptation of populations maintains intraspecific genetic variation in various shoot traits, but the contribution of genetic factors to adaptation to soil heterogeneity remains poorly known. I established a common-garden experiment with three Norway spruce *Picea abies* populations sampled between 60° and 67° N in Finland, each represented by 13 or 15 maternal families, to determine whether belowground traits are as genetically differentiated among populations as those in the shoot along a collective latitudinal gradient of temperature and soil heterogeneity. Two growing season simulations enabled testing for among-population differences in phenotypic plasticity. I phenotyped 777 first-year seedlings from shoot to root to capture functional traits that may influence survival in the wild: autumn phenology, shoot growth, root system size, root architecture, root morphology and growth allocation. All traits exhibited within-population genetic diversity, but among-population differentiation ranged from strong in shoot traits to nonexistent in root system architecture and morphology that are scaled to root system size. However, latitudinal trends characterised root-to-shoot ratio and root tip-to-shoot ratio that account for among-population differences in aboveground growth. Overall trait variability was multidimensional with variable among- versus within-population trends: for example, phenology and shoot growth covaried across populations, but their association within individual populations was variable. Shoot growth correlated positively with root system size, but not with root architecture or morphology. Finally, the two higher-latitude populations exhibited greater phenotypic plasticity in shoot traits and growth allocation. The results demonstrate varying patterns of genetic variation in functional traits of Norway spruce in the boreal zone, suggesting simultaneous adaptation to multiple environmental factors. Functional traits that exhibit phenotypic plasticity, genetic diversity and little covariation will promote long-term survival of populations in fluctuating environments.

Keywords: adaptation, environmental heterogeneity, phenotypic plasticity, plant economic spectrum, roots, trait-based ecology
Introduction

Biodiversity in the wild is layered at numerous distinct levels, with variation at each tier possessing related ecological and evolutionary implications: variation among species can promote their coexistence in a community (Bolnick et al. 2011), while intraspecific diversity increases the survival probability of populations in a changing environment (Aitken et al. 2008). Environmental heterogeneity along varying geographic distances regulates diversity simultaneously across such hierarchy. For example, global gradients in climate and soil conditions are associated with broadscale above- and belowground phenotypic diversity in leaves and fine roots across species and continents (Wright et al. 2004, Freschet et al. 2017), although environment–trait associations may turn out weak in such wide-spectrum in situ data due to phenotypic differences among species within communities (Reich 2014) or intraspecific variation in functional traits (Siefert et al. 2015).

Intraspecific variation in functional traits in situ emerges from the collective effects of phenotypic plasticity and genetic diversity (Siefert et al. 2015), the disclosure of which demands a homogeneous common-garden setting. Genetic differentiation among populations and genetic diversity within populations characterise many shoot traits of widely distributed forest trees, conferring home-site fitness advantage in reciprocal transplants of populations (Savolainen et al. 2007, Aitken et al. 2008, Alberto et al. 2013). For example, in a common-garden study with 19 populations of Scots pine Pinus sylvestris in Finland, seedlings from higher-latitude origins formed apical buds earlier at the end of the growing season such that latitude explained 95% of variation among population averages of timing of bud set (Mikola 1982). The trait’s within-population genetic variability is likewise plentiful, and in the two-population greenhouse study by Savolainen et al. (2004) standard deviations of total phenotypic variation ranged between six and eight days.

Similar to shoot traits, variation in root form and function derives from plasticity and genetic factors. Genetic variation in root characteristics has been described for instance in Nordic tree breeding material (Velmala et al. 2013, Senior et al. 2019) and within a natural population of Norway spruce Picea abies in Finland (Salmela et al. 2020). Genetic variation among natural populations has been found for instance in root biomass in whitebark pine Pinus albicaulis across its range in western North America (Bower and Aitken 2008), and larger averages of root-to-shoot ratio are associated with warmer population origins in the model system Arabidopsis thaliana on the Iberian Peninsula (Montesinos-Navarro et al. 2011, Wolfe and Tonsor 2014) and with cooler climates in coastal Douglas fir Pseudotsuga menziesii var. menziesii in the Pacific Northwest (St. Clair et al. 2005). In a set of six eastern European origins of Scots pine, detailed order-based phenotyping of roots indicated a positive association between latitude and allocation to absorptive fine roots, which is consistent with in situ data across a larger spatial range in Europe (Zadworny et al. 2016). Still, in studies on intraspecific adaptation, an appreciable imbalance persists in trait representation across the entire plant phenotypic spectrum that stems largely from laborious phenotyping required for fine root traits expected to regulate resource acquisition in heterogeneous soils and overall plant performance, including architecture, morphology and physiology of different size-based classes of fine roots and belowground associations with microbes (Freschet et al. 2021). Meanwhile, ecological research on the adaptive benefit of root variation has accentuated interspecific differences in fine roots of adult trees sampled in situ and their environmental determinants (Weemstra et al. 2016, Laliberté 2017, Freschet et al. 2021), and in cases where attention has been drawn to significant intraspecific variation (Ostonen et al. 2007, 2011, 2017, Defrenne et al. 2019, Weemstra et al. 2021), the genetic and plastic elements of trait variability are inseparable. Consequently, a common-garden technique combined with comprehensive phenotyping is required to detect the heritable component in root functional trait variation and to determine whether comparable levels of among-population differentiation have emerged along the entire plant phenotypic spectrum from shoot to root.

A further perk of in-depth shoot–root phenotyping is that it provides the means for defining trait dimensionality, i.e. whether functional traits that can influence performance vary in a correlated manner or independently. Synchronisation in trait variations on a global scope has been considered a prerequisite for optimal adaptation for instance such that faster growth rates are coupled with more acquisitive root characteristics (Reich 2014, Weemstra et al. 2016), but accumulating inter- and intraspecific evidence points to more complex patterns in the multi-trait phenotypic space where probable regulators of plant fitness can vary independently and correlate with different environmental factors (Kramer-Walter et al. 2016, Defrenne et al. 2019, Senior et al. 2019, Salmela et al. 2020). These patterns may echo simultaneous adaptation to various co-occurring yet unrelated selective agents (Laughlin 2014).

The large size and longevity of forest trees amplify their exposure to spatial and temporal heterogeneity, challenges that could be overcome by adaptive phenotypic plasticity. For instance, spring phenology in natural stands is advanced by warmer spring temperatures (Rousi and Heinonen 2007). Plasticity can evolve when genotypes in a population vary in how they respond to a change in the environment (Via and Lande 1985), and theory predicts increased plasticity for instance in margins of distribution ranges when there is maldaptive gene flow from the environmentally contrasting range centre (Chevin and Lande 2011) and in temporally fluctuating environments where constant plastic adjustments are required (Lande 2014). Among-population variation in plasticity indicates that populations with greater capacity for phenotypic adjustments may be more tolerant of rapid environmental changes, but empirical support for the theoretical predictions remains insufficient. Overall, differences in plasticity among populations have been studied less than genetic differentiation in trait averages in a single
common garden because their estimation by population × environment interactions calls for a minimum of two test environments (Matesanz and Ramírez-Valiente 2019).

Environmental variation within Finland caters a convenient framework for probing genetic variation in natural plant populations from a whole-plant viewpoint: average temperatures decrease linearly with increasing latitude (Salmela 2014) while nutrient limitation, estimated for instance as inorganic nitrogen deposition, builds up towards the north (Mustajärvi et al. 2008, Ostonen et al. 2011). Previously, Salmela et al. (2020) showed that various shoot and root functional traits exhibit within-population genetic variation in Norway spruce in southern Finland, but due sampling within a small area, potential environmental factors contributing to such diversity could not be identified. Here, I hypothesise that genetic differentiation among discrete tree populations has developed across shoot and root functional traits such that earlier shoot phenology and smaller size, two typical features of higher-latitude populations (Savolainen et al. 2007), are associated with architectural or morphological features of roots that may enhance the uptake capacity of nutrients: more intensive branching, higher specific root length and/or increased relative allocation of biomass to roots (St. Clair et al. 2005, Freschet et al. 2017). In the event of simultaneous adaptation to varied climate and soil factors along this latitudinal transect, I expect the degree of among-population differentiation to vary across traits, leading to a multidimensional organisation of intraspecific shoot–root functional trait diversity (cf. Laughlin 2014, Kramer-Walter et al. 2016). Further, trait covariation could be inconsistent among versus within populations (cf. Laughlin and Messier 2015) for instance due to the range of environmental heterogeneity varying across these two sampling scales (Messier et al. 2017b). Lastly, on top of genetic differentiation in trait averages among populations, different origins could be genetically diverged in phenotypic plasticity of climate-related traits due to greater among-year fluctuations in temperature conditions in the northern part of the country (Salmela 2014), an expectation that is testable in a common-garden layout with replicated treatments (Via 1993).

Material and methods

Sampling design and treatments

Expanding the spatial sampling area of Salmela et al. (2020) from about 1 km to 6° of latitude and over 600 km, I sampled three populations of Norway spruce in Finland: Lapinjärvi (60°39’N, 26°8’E, 35–65 m a.s.l.; Lapinjärvi(60°) hereafter) and Puolanka (64°40’N, 28°4’E, 290–370 m a.s.l.; Puolanka(64°) hereafter) are national gene reserve forests where seeds were collected in 2006 and 2012, and Ro vaisiemi near the Arctic Circle (66°24’N, 26°53’E, 175 m a.s.l.; Ro vaisiemi(66°) hereafter) is a natural reference stand for breeding purposes where seeds were collected in 2003. Each population was represented by 15 open-pollinated and randomly selected maternal seed families. This design is standard across a variety of species in forest genetics because it permits the joint estimation of naturally occurring quantitative genetic diversity among and within populations, i.e. among maternal families from the same population, in a common garden setting (Fig. 1a; Howe et al. 2003).

The experimental layout resembled a classical reciprocal transplant of populations that allows testing for home-site fitness advantage and genetic variation in phenotypic plasticity. The two treatments simulated ordinary (annual growing degree days close to the local 60-year average) growing seasons for light and temperature cycles at the extremes of the sampled latitudinal transect based on longstanding weather station records in European Climate Assessment and Dataset (Klein Tánk et al. 2002). The South treatment (average light cycle temperature 18.6°C, average dark cycle temperature 10.6°C, average photoperiod ca 16.1 h) was based on the summer of 1980 at Kotka (60°27’N, 26°57’E, 23 m a.s.l., ca 50 km from Lapinjärvi(60°)) average growing season length 179 days, CV = 10.0%; average June–August growing degree day sum 1089 day degrees, CV = 10.2%), and the North treatment (average light cycle temperature 15.4°C, average dark cycle temperature 7.7°C, average photoperiod ca 18.5 h) simulated the summer of 1989 at Ro vaisiemi (66°34’N, 25°50’E, 195 m a.s.l., ca 50 km from Ro vaisiemi(66°); average growing season length 137 days, CV = 10.9%; average June–August growing degree day sum 864 day degrees, CV = 12.6%). Distinct temperatures of light and dark cycles changed weekly, while photoperiod tracked its daily variation at the sampled latitudes (Fig. 1b). Consequently, seedlings experienced a four-week polar day with constant light at the beginning of the North treatment, during which time temperature dropped to the lower nighttime value daily between 11 p.m. and 01 a.m. In the South treatment maximum photoperiod was approximately 19 h. I used two growth chambers for the experiment.

I stratified seeds in cold water at +10°C overnight which then germinated on moist paper in petri dishes kept in an 18-h photoperiod (+16°C in light, +12°C in dark). After six days I transferred germinating seedlings to trays (cell size 30 × 30 × 60 mm, 33 cm³). All seedlings grew in a mixture of Sphagnum peat (NPK 16-4-17) and vermiculite (3:1, based on volume). I randomised the order of families and populations within each treatment and planted ten replicates per family and treatment (900 seedlings in total). The two treatments commenced 16 days after planting and finished after about 19 weeks when all seedlings had developed apical buds. To minimise effects of unaccounted chamber factors, I rotated the treatments between the two chambers once a week. Within chambers I rotated trays twice a week. I watered seedlings by spraying three times each week. Photosynthetically active radiation in the chambers, measured with the a quantum meter, was at maximum ~120 μmol m⁻² s⁻¹ which approximates growing in shade in a forest.
Phenotypic measurements

The use of first-year seedlings made possible a larger sample size given the space available in controllable growth chambers and phenotyping of entire plants from the top of the shoot to root tips at a developmental stage when selection on functional traits in natural stands is strong (Petit and Hampe 2006). This meant that an estimate of total root system size was obtainable in addition to size-scaled composite traits related to root architecture and morphology that can be measured also for smaller sub-sections of complete root systems. Table 1 lists the measured traits, estimation methods for the composite traits and expected patterns of among-population variation. I monitored timing of bud set, i.e. the appearance of a visible apical bud, three times a week and recorded it as the number of days since the first day of treatment. I measured shoot growth after buds had appeared in all seedlings, which was followed by the careful sampling of complete root systems from moist peat. I washed the seedling root systems cautiously under a running tap, spread them in a small amount of water on a transparent plastic tray and scanned them using a photo scanner and a resolution of 400 dpi following the manual of WinRHIZO Pro Ver. 13a. The minimum length of external links for the software was 2 mm, and I inspected all analyses visually and performed manual edits when necessary to improve the accuracy of the data. Based on Salmela et al. (2020), I prioritised two measures of intact root system size (total root length and the number of root tips) that could be used together with belowground biomass to estimate branching intensity (root system architecture) and specific root length (root system morphology) at the whole root system level (Table 1). In addition to scaling the number of root tips to total root length for branching intensity, I estimated root tip-to-shoot ratio by scaling the number of

Figure 1. (a) The experimental common-garden layout that enables the estimation of three types of genetic variation: (1) genetic differentiation in functional traits among three Norway spruce populations along the sampled latitudinal gradient, (2) genetic variation among 13 or 15 maternal families within each population and (3) genetic variation in phenotypic plasticity among populations. (b) Experimental temperature and photoperiod settings for the two growing season simulations, the period of which is shown on the x-axis. Temperatures for the light and dark cycles changed weekly, but photoperiod shifted daily according to local sunrise and sunset times.
Earlier bud set in higher-latitude populations

Estimation method for composite traits

Expected spatial trend

...
equivalent results. I performed all statistical analyses with IBM SPSS Statistics Ver. 26.

Results

Structuring of genetic variation in functional traits among and within populations

Eight out of the ten traits examined with analysis of variance exhibited a significant population effect, indicating genetic differentiation among populations (Table 2). In timing of bud set, shoot growth, root system size, root-to-shoot ratio and root tip-to-shoot ratio the contribution of population to total variation ranged from 14.4% to 62.8% and was largest for shoot traits. Patterns of among-population differentiation were branching intensity and specific root length estimated for intact root systems (Table 2, Fig. 2g–h). The number of lateral shoot branches varied among populations (df = 2, Wald $\chi^2 = 115$, $p < 0.0001$), with a larger average in Lapinjärvi($60^\circ$) (0.682 branches) than in Puolanka($64^\circ$) (0.0561 branches) or Rovaniemi($66^\circ$) (0.0185 branches).

A significant family(population) factor for all traits explored with analysis of variance indicated that genetic diversity is present locally in shoot and root functional traits, with some evidence of larger within-population genetic variances towards the north in timing of bud set, shoot growth, branching intensity, specific root length, root-to-shoot ratio and root tip-to-shoot ratio (Table 2). In all, the statistical models explained 60–81% of total variation in shoot-related traits and 11–42% of variation in root traits.

Correlated variation across the shoot–root trait spectrum

PCA identified four trait dimensions with eigenvalues above one in the complete dataset, the largest of which (PC1) summarised variation in timing of bud set, shoot growth, root-to-shoot ratio and root tip-to-shoot ratio (Table 3a). Root system size constituted the second component (PC2), correlating with total root length, belowground biomass and the number of root tips. Specific root length (PC3) and branching intensity (PC4) were not strongly correlated with shoot growth (Table 3a–b). Variation among populations visualised with PCs was in agreement with patterns in individual traits, with greater among-family variation in PC1 in Puolanka($64^\circ$) and Rovaniemi($66^\circ$) than in Lapinjärvi($60^\circ$) (Fig. 3a–b).

Trait associations differed depending on the population (Table 3a). Timing of bud set, root-to-shoot ratio and root tip-to-shoot ratio covaried with shoot growth at the among-population level, but the correlations broke down within populations. As evidenced by a significant timing of bud set × population interaction, the slope between phenology and shoot height differed among populations (df = 2, df = 771, F = 51.3, p < 0.0001), with steeper slopes in Rovaniemi($66^\circ$) and Puolanka($64^\circ$) than in Lapinjärvi($60^\circ$) (Fig. 4). With family means the overall trend remained similar, but the population × timing of bud set interaction was not statistically significant (df = 2, df = 37, F = 2.69, p = 0.081). Shoot growth and root system size represented two distinct PCs across populations, but within individual populations measures of root system size and shoot growth often loaded on the same component (Table 3a).

Genetic variation in phenotypic plasticity among and within populations

A replicated common-garden layout revealed significant population × treatment interactions in six out of the ten functional traits examined with analysis of variance, signalling genetic differentiation in phenotypic plasticity in timing of bud set, shoot growth, total root length, root-to-shoot ratio and root tip-to-shoot ratio (Table 2). Puolanka($64^\circ$) and Rovaniemi($66^\circ$) exhibited greater plasticity than Lapinjärvi($60^\circ$) in all traits except for total root length (Fig. 2a–c, i–j). The population × treatment interaction was marginally significant for the number of lateral shoot branches (df = 2, Wald $\chi^2 = 5.48$, p = 0.065), with population responses mirroring those for shoot height and aboveground biomass: Lapinjärvi($60^\circ$) had very similar means in both treatments (0.671 branches in the South treatment and 0.692 branches in the North treatment), but treatment averages increased in Rovaniemi($66^\circ$) (from 0.0148 to 0.0231 branches) and Puolanka($64^\circ$) (from 0.0259 to 0.121 branches) in response to the North treatment. Total root length was the only belowground trait with a significant interaction term, with greater plasticity in Lapinjärvi($60^\circ$) and Puolanka($64^\circ$) than in Rovaniemi($66^\circ$). As indicated by a significant family(population) × treatment interaction, root-to-shoot ratio was the only trait with within-population genetic variation in phenotypic plasticity (Table 2).

Discussion

The central goal of this study was to compare patterns of genetic variation in above- and belowground traits that may facilitate adaptation to climate and soil heterogeneity in boreal tree populations. The occurrence of genetic diversity on a local, within-population scope was a shared characteristic of all traits, but major differences among traits were evident in the magnitude of among-population differentiation: population accounted for the largest proportion of total variation in timing of bud set, whereas two commonly investigated aspects of root growth, architecture and morphology, exhibited no evidence of among-population differences. Further, the setup revealed population-specific trait correlations and evidence of latitudinal variation in phenotypic plasticity.
Table 2. Analyses of variance with mixed-effect models for ten seedling functional traits. The table shows significant factor effects underlined and in bold.

| Shoot:             | df | Trt | Pop | Pop × Trt | Fam(Pop) | Fam(Pop) × Trt | Res | Range of maternal family means within populations |
|--------------------|----|-----|-----|-----------|----------|----------------|-----|--------------------------------------------------|
| Timing of bud set  |    |     |     |           |          |                |     | Lapinjärvi(60°): 84–99 days (VC: 14.6)          |
|                    |    |     |     |           |          |                |     | Puolanka(64°): 56–74 days (VC: 14.4)            |
|                    |    |     |     |           |          |                |     | Rovaniemi(66°): 48–66 days (VC: 16.3)           |
| Shoot height       |    |     |     |           |          |                |     | Lapinjärvi(60°): 4.43–5.70 cm (VC: 0.0798)       |
|                    |    |     |     |           |          |                |     | Puolanka(64°): 2.88–3.95 cm (VC: 0.0716)         |
|                    |    |     |     |           |          |                |     | Rovaniemi(66°): 2.79–4.21 cm (VC: 0.137)        |
| Aboveground biomass|    |     |     |           |          |                |     | Lapinjärvi(60°): 85.4–119 mg (VC: 52.3)         |
|                    |    |     |     |           |          |                |     | Puolanka(64°): 42.9–66.4 mg (VC: 51.8)          |
|                    |    |     |     |           |          |                |     | Rovaniemi(66°): 38.1–66.4 mg (VC: 64.7)        |
| Root:              |    |     |     |           |          |                |     |                                                |
| Total root length  |    |     |     |           |          |                |     |                                                |
|                    |    |     |     |           |          |                |     | Lapinjärvi(60°): 162–246 cm (VC: 411)          |
|                    |    |     |     |           |          |                |     | Puolanka(64°): 141–183 cm (VC: 70.7)           |
|                    |    |     |     |           |          |                |     | Rovaniemi(66°): 127–195 cm (VC: 368)           |
| Belowground biomass|    |     |     |           |          |                |     |                                                |
| Number of root tips|    |     |     |           |          |                |     |                                                |

(Continued)
Functional shoot and root traits differ in their latitudinal differentiation

Thorough seedling phenotyping demonstrated that among-population differentiation was of smaller magnitude belowground than in shoot traits in boreal Norway spruce along a shared gradient of temperature and soil heterogeneity. The observation that shoot phenology and growth varied markedly among populations from contrasting temperature and light environments agrees with countless common-garden studies on European and North American tree species (Savolainen et al. 2007, Aitken et al. 2008, Alberto et al. 2013), but analogous assessments of belowground genetic diversity are still comparatively rare (Freschet et al. 2021).

Here, the effect of population on total variation in root traits was variable: branching intensity and specific root length, two common metrics that depict root architecture and morphology and whose broad-scale variation in situ suggests they are involved in adaptation to soil conditions (Freschet et al. 2017), were the only traits that did not vary...
among populations despite all their components – total root length, belowground biomass and the number of root tips – expressing a significant population effect. The finding of no latitudinal trend in branching intensity and specific root length within this geographic region is in agreement with the results of Ostonen et al. (2017) who measured the same traits in fine roots of the same species at five sites between 60° and 68°N in Finland. More recently, Weemstra et al. (2021) noted that variation in branching intensity and specific root length of fine roots in Norway spruce in situ was higher within elevations than across a 600-m elevational range in the French Alps. However, Ostonen et al. (2011) found that root tip length and fine root biomass were larger in higher-latitude Norway spruce stands in Finland, and a common-garden study with eastern European origins of Scots pine described a parallel positive correlation between latitude and the percentage of absorptive fine roots (Zadworny et al. 2016). These studies with more precise phenotypic assessments of fine roots suggest the number of root tips should be supplemented by additional size-related measurements in further common-garden studies with small seedlings.

While latitudinal variation in branching intensity and specific root length was not significant in this experiment, signals of environmental adaptation were explicit in root-to-shoot ratio and root tip-to-shoot ratio, two measures of growth allocation that scale root measurements to shoot growth. Population averages of both traits increased towards higher latitudes, which is in agreement with in situ observations across species (Mokany et al. 2006, Poorter et al. 2012) and with a common-garden experiment on coastal Douglas fir in which origins from higher elevations and cooler climates in the Pacific Northwest allocated more biomass to roots (St. Clair et al. 2005). The observed among-population variation in root tip-to-shoot ratio resembles the in situ

Figure 2. Treatment(Trt)-specific population means (± SE) for ten shoot and root functional traits. The figure shows among-population variation in phenotypic plasticity for the traits that exhibited a significant population × treatment interaction (Pop × Trt: p < 0.05), with the smaller treatment average compared to the larger one. ns = non-significant.
Table 3. (a) Principal component (PC) analyses with ten functional traits for the complete dataset and for each population–treatment combination. The table shows eigenvalues for each PC, and the percentage indicates the proportion of total variation explained. (b) Bivariate trait correlations in the complete dataset. Values below the diagonal are based on all measurements and those above the diagonal are based on maternal family means. Both tables present Pearson’s correlation coefficients, with strong correlations ($r < -0.7$ or $r > 0.7$) shown underlined and in bold.

(a) Complete dataset

| Trait | shoot height | above-ground biomass | root tip-to-shoot ratio | shoot width | above-ground biomass | root tip-to-shoot ratio | shoot width | above-ground biomass | root tip-to-shoot ratio |
|-------|---------------|----------------------|-------------------------|-------------|----------------------|-------------------------|-------------|----------------------|-------------------------|
| Total root length | 0.720 | 0.00234 | 0.287 | 0.633 | 0.259 | 0.0920 | 0.633 | 0.259 | 0.0920 |
| Biomass | 0.106 | 0.127 | 0.266 | 0.176 | 0.202 | 0.0935 | 0.633 | 0.259 | 0.0920 |
| Number of root tips | 0.0610 | 0.0935 | 0.0691 | 0.124 | 0.0935 | 0.0691 | 0.0935 | 0.124 | 0.0935 |
| Specific root length | 0.387 | 0.155 | 0.176 | 0.176 | 0.155 | 0.176 | 0.176 | 0.176 | 0.155 |
| Aboveground biomass | 0.035 | 0.571 | 0.241 | 0.387 | 0.241 | 0.387 | 0.241 | 0.387 | 0.241 |
| Number of root tips | 0.299 | 0.480 | 0.299 | 0.480 | 0.299 | 0.480 | 0.299 | 0.480 | 0.299 |
| Specific root length | 0.0118 | 0.0229 | 0.0118 | 0.0229 | 0.0118 | 0.0229 | 0.0118 | 0.0229 | 0.0118 |
| Root to shoot ratio | 0.625 | 0.371 | 0.625 | 0.371 | 0.625 | 0.371 | 0.625 | 0.371 | 0.625 |
| Biomass | 0.0529 | 0.899 | 0.0529 | 0.899 | 0.0529 | 0.899 | 0.0529 | 0.899 | 0.0529 |
| Specific root length | -0.0118 | 0.0229 | -0.0118 | 0.0229 | -0.0118 | 0.0229 | -0.0118 | 0.0229 | -0.0118 |
| Root to shoot ratio | 0.725 | 0.371 | 0.725 | 0.371 | 0.725 | 0.371 | 0.725 | 0.371 | 0.725 |

(b) Principal component analyses with ten functional traits for each population–treatment combination.

| Trait | shoot height | above-ground biomass | root tip-to-shoot ratio | shoot width | above-ground biomass | root tip-to-shoot ratio | shoot width | above-ground biomass | root tip-to-shoot ratio |
|-------|---------------|----------------------|-------------------------|-------------|----------------------|-------------------------|-------------|----------------------|-------------------------|
| Total root length | 0.720 | 0.00234 | 0.287 | 0.633 | 0.259 | 0.0920 | 0.633 | 0.259 | 0.0920 |
| Biomass | 0.106 | 0.127 | 0.266 | 0.176 | 0.202 | 0.0935 | 0.633 | 0.259 | 0.0920 |
| Number of root tips | 0.0610 | 0.0935 | 0.0691 | 0.124 | 0.0935 | 0.0691 | 0.0935 | 0.124 | 0.0935 |
| Specific root length | 0.387 | 0.155 | 0.176 | 0.176 | 0.155 | 0.176 | 0.176 | 0.176 | 0.155 |
| Aboveground biomass | 0.035 | 0.571 | 0.241 | 0.387 | 0.241 | 0.387 | 0.241 | 0.387 | 0.241 |
| Number of root tips | 0.299 | 0.480 | 0.299 | 0.480 | 0.299 | 0.480 | 0.299 | 0.480 | 0.299 |
| Specific root length | 0.0118 | 0.0229 | 0.0118 | 0.0229 | 0.0118 | 0.0229 | 0.0118 | 0.0229 | 0.0118 |
| Root to shoot ratio | 0.625 | 0.371 | 0.625 | 0.371 | 0.625 | 0.371 | 0.625 | 0.371 | 0.625 |
| Biomass | 0.0529 | 0.899 | 0.0529 | 0.899 | 0.0529 | 0.899 | 0.0529 | 0.899 | 0.0529 |
| Specific root length | -0.0118 | 0.0229 | -0.0118 | 0.0229 | -0.0118 | 0.0229 | -0.0118 | 0.0229 | -0.0118 |
| Root to shoot ratio | 0.725 | 0.371 | 0.725 | 0.371 | 0.725 | 0.371 | 0.725 | 0.371 | 0.725 |
patterns described by Ostonen et al. (2011) when the number root tips colonised by mutualistic ectomycorrhizal fungi was scaled to the basal area of the sampled stand. Thus, these composite allocation characteristics that incorporate accompanying variation in genetically highly variable aboveground growth may be more informative reflectors of adaptation to boreal soils with limited resources in Norway spruce than branching intensity or specific root length. On the other hand, Prescott et al. (2020) recently postulated that greater allocation to roots in growth-limiting conditions might in fact be caused by the transfer of surplus carbon belowground and not by more intensive resource foraging. I did not score ectomycorrhizal colonisation in this experiment, and it remains to be established in common garden how large a role these fungal partnerships play in soil adaptation among populations of Norway spruce, a species in which genetic diversity within breeding trials has been shown to regulate the community composition of associated fungi (Korkama et al. 2006, Velmala et al. 2013). It has been hypothesised that microbial alliances would be the most beneficial when soil resources are in short supply (Revillini et al. 2016).

Overall, differences among traits in the degree of among-population differentiation probably mark coinciding adaptation to multiple different climatic and soil factors in the boreal zone (St. Clair et al. 2005, Bower and Aitken 2008, Montesinos-Navarro et al. 2011). Average growing season length varies by over a month between 60° and 67°N in Finland (Salmela 2014), and according to Ostonen et al. (2011), inorganic nitrogen deposition at 67°N is approximately 20% of what it is at 60°N. No quantitative information is available on exact soil characteristics at the selected localities. Another phenomenon that could cause differences in above- versus belowground growth is lagging timing of root growth relative to shoot in boreal ecosystems as a result of air warming and cooling more rapidly than soil (Abramoff and Finzi 2015). Here, I sampled seedlings after shoot growth had ceased, and it is possible that belowground biomass would have continued to accumulate even longer.

Although the patterns of among-population differentiation varied greatly depending on the functional trait, a finding...
common to all traits in this study was the occurrence of significant within-population genetic diversity. This localised variation is often considered to be maintained by effective long-distance gene flow in wind-pollinated tree species (Savolainen et al. 2007, Aitken et al. 2008, Alberto et al. 2013). Other contributing factors might be fine-scale spatial heterogeneity at the home sites of populations or temporally fluctuating selection on functional traits. When spatial heterogeneity is high for instance due to topography, genetic differentiation can develop among different microhabitats separated by short distances, as was found by Campbell (1979) who assessed genetic variation in growth, phenology and survival in coastal Douglas fir on a 6100-ha watershed in central Oregon. Evidence of temporal changes in the intensity or direction of selection has been found in reproductive phenology of annual and perennial herbaceous species (Exposito-Alonso et al. 2018, Ehrén and Valdés 2020) and in a common-garden experiment with ponderosa pine (Pinus ponderosa) in western Montana in which selection favoured fast-growing origins before a beetle outbreak and origins with slow growth afterwards (de la Mata et al. 2017). Within-population genetic diversity in the current experiment exhibited an increasing trend with latitude particularly in branching intensity, specific root length, root-to-shoot ratio and root tip-to-shoot ratio, which might indicate greater fine-grained soil heterogeneity in more northern habitats with striking seasonal variation in environmental conditions.

**Trait dimensionality is variable among versus within populations**

The observation that functional traits differed in the degree of genetic diversity among versus within populations was manifested also in the outcome of a principal component analysis which identified four trait dimensions across populations (cf. Messier et al. 2017b with a matching interspecific approach). Comparable among-population differentiation in shoot traits, root-to-shoot ratio and root tip-to-shoot ratio resulted in robust covariation of these traits across the latitudinal gradient, but many associations were unstable within populations despite significant local-level genetic variation in all traits. On the other hand, shoot growth was positively correlated with total root length, belowground biomass and the number of root tips within populations, but because of differing patterns of among-population genetic diversity above- versus belowground, these measures of root growth comprised their own trait dimension across populations. Likewise, ecological data show that global trait integration may not accurately depict equivalent covariation at lower ranks of biodiversity, e.g. within individual species or in specific environments (Laughlin and Messier 2015, Messier et al. 2017a, b, Anderegg et al. 2018). For example, Grady et al. (2013) examined populations of *Populus fremontii* in a common garden and found that in warm conditions conservative features of leaf traits (e.g. low photosynthesis and nitrogen content) were associated with faster growth rates although global in situ data express an opposite general trend (Wright et al. 2004). In the current data, the population-specificity of the phenology–growth association suggests selective pressure on timing of bud set is lighter in southern Finland, with another common-garden experiment on a southern Finnish spruce population providing corroborative evidence (Salmela et al. 2020). Different populations in species with wide distributions may be under dissimilar selective pressures: for instance, water availability may drive local adaptation in southern populations of whitebark pine in western North America, but further north and at high elevations growing season length may have greater influence on genetic differentiation in functional traits (Bower and Aitken 2008).

Acquisition of nutrients and water from soil may be enhanced by higher branching intensity or specific root length (Weemstra et al. 2016, Freschet et al. 2017), which could also result in their covariation with shoot growth. Variation in these two root functional traits was within a limited range in comparison to all other traits in the current experiment, and they were not aligned with each other or the two major trait dimensions. The separation of specific root length from branching intensity and growth rate was described also by Kramer-Walter et al. (2016) in a common-garden experiment with seedlings of 66 tree species from New Zealand, and Defrenne et al. (2019) found that variation in specific root length in interior Douglas fir (Pseudotsuga menziesii var. glauca) in British Columbia was not associated with environmental factors, while branching intensity correlated with phosphorus availability. Collectively, these results suggest that variation in branching intensity and specific root length is driven by different belowground factors, but due to the high within-population and low among-population genetic variation, their role as growth regulators in Norway spruce populations remains unidentified. It may be that the necessary resource uptake for faster shoot growth is facilitated by longer roots and a greater number of root tips rather than by high root tip frequency, or that variation in branching intensity and specific root length becomes important for instance at later developmental stages in the wild as root systems of seedlings proliferate in forest soils with patchily distributed nutrients. Indeed, a basic survival mechanism of populations in natural environments with fluctuating selective pressures might stem from an interaction of genetic variation and many independently varying functional traits: the former would increase the adaptive capacity of individual traits (Aitken et al. 2008) while the latter would extend the range of potential multi-trait combinations (Laughlin 2014, Valverde-Barrantes and Blackwood 2016).

**Phenotypic plasticity manifests genetic variation among populations**

The simulation of a reciprocal population transplant revealed evidence of local adaptation to home-site light and temperature cycles in shoot growth: Puolanka (64°) and Rovaniemi (66°) grew larger in the North treatment with longer photoperiods and cooler temperatures, while for
Lapinjärvi (60°) the South treatment with shorter photoperiods and warmer temperatures was more optimal. Further, the approach exposed greater phenotypic plasticity in timing of bud set, shoot growth, root-to-shoot ratio and root tip-to-shoot ratio in the higher-latitude populations, which could signal adaptation to larger among-year fluctuations in temperature conditions in northern Finland (Salmela 2014). Increased plasticity in higher-latitude origins was recently described also in *Populus trichocarpa* in the Pacific Northwest based on temporally replicated measurements of phenology and growth in a field trial (Liu and El-Kassaby 2019). On the other hand, Cooper et al. (2019) found in *P. fremontii* in Arizona that increased plasticity in spring and autumn phenology characterised populations from lower elevations with hot and dry environments, suggesting local-level environmental heterogeneity is related to differing spatial gradients in different regions.

The only belowground trait with a significant population × treatment interaction was total root length in which plasticity was the lowest in Rovaniemi (66°). More detailed information on fine-grained soil heterogeneity at the sampled sites is required to explore potential causes of reduced belowground plasticity in the northernmost part of the sampled gradient observed in response to a light cycle and temperature treatment. Further, adding soil as another experimental factor in a common garden design would allow testing whether Norway spruce populations exhibit local adaptation to native soils with abiotic and biotic elements, whether there are differences in the relative importance of climate versus soil adaptation in the boreal zone, and whether among-population variation in the plasticity of root functional traits emerges when seedlings are exposed to natural soil heterogeneity. Currently the evidence for local adaptation to soil, i.e. positive consequences of growing in native soils on populations, is mixed. Soil fungi appear to play an important role in belowground adaptation in interior Douglas fir (Pickles et al. 2015), and in coastal Douglas fir in British Columbia, population transfers across environmental gradients showed that height growth of trees declined as the difference between the ectomycorrhizal communities of the non-native populations and those of the native hosts grew larger (Kranabetter et al. 2015). Contrastingly, Downie et al. (2020) found no indications of local adaptation to belowground fungal communities in a common-garden trial with Scottish populations of Scots pine.

The detected among-population variation in phenotypic plasticity under experimental environments does not necessarily demonstrate adaptive responses that are beneficial for fitness in the wild. Of more direct ecological relevance would be to explore plastic responses to heterogeneity that populations experience in their native environments, and to directly test associations between plastic responses and for instance overwinter survival. In long-lived trees with age-dependent mortality and overlapping generations (Petit and Hampe 2006), systematic long-term monitoring of genotypes representing different populations over a range of environments relevant to each origin would be needed to elucidate the mechanism by which natural selection acts on plasticity (Via 1993). Alternatively, a replicated common-garden design with many populations would allow for statistically associating various reaction norm parameters (Via et al. 1995, Arnold et al. 2019) with environmental gradients, as was done for instance by Cooper et al. (2019).

**Conclusions**

Common-garden designs have already been used to investigate interspecific variation in root traits of tree seedlings and its associations with climate and soil variables (Kramer-Walter et al. 2016, McCormack et al. 2020), but their application has been significantly more frequent in intraspecific tree studies that explore local adaptation of populations by correlating broad patterns of genetic diversity with climatic gradients (Savolainen et al. 2007, Aitken et al. 2008, Alberto et al. 2013). These forest genetic studies have predominantly focused on shoot traits related to phenology, growth and stress responses, and as a result, genetic adaptations to abiotic and biotic soil factors remain poorly known. Here, seedlings from different populations of Norway spruce grew in controlled chamber environments that mimicked natural light and temperature cycles within a latitudinal gradient, revealing trait-dependent patterns of among-population variation that could signal adaptation to varied environmental factors. Based on the current results and related common-garden and in situ studies on conifers (Ostonen et al. 2011, 2017, Zadworny et al. 2016, Weemstra et al. 2021), traits that express root system size, the proportion of fine roots within root systems and biomass allocation to roots versus shoots may be more useful belowground complements to climate-related shoot traits than branching intensity and specific root length. Together, the coexisting intraspecific phenomena of genetic diversity, multidimensionality of functional traits and phenotypic plasticity may echo robust survival strategies of long-lived species in habitats that are characterised by consequential spatial and temporal heterogeneity and changing selective pressures (Aitken et al. 2008, Laughlin 2014). Accordingly, the accuracy of predictive models on distribution range shifts under climate change can be improved by accounting for these explicit levels of intraspecific variability, the effects of which tend to be less dramatic expected changes than in cases where no evolutionary capacity is assumed (Benito Garzón et al. 2019).

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