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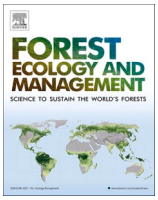

Soil factors and genetic variation regulate intraspecific growth in Norway spruce (*Picea abies*)

Matti J. Salmela [a,*,1], Sannakajsa M. Velmala [b], Katri Himanen [c], Tiina Ylioja [b], Taina Pennanen [b]

[a] *Natural Resources Institute Finland, Paavo Havaksen tie 3, Oulu 90570, Finland*
[b] *Natural Resources Institute Finland, Latokartanonkaari 9, Helsinki 00790, Finland*
[c] *Natural Resources Institute Finland, Juntintie 154, Suonenjoki 77600, Finland*

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ABSTRACT

Genotypic tradeoffs along environmental gradients help maintain diversity in functional traits in the wild and limit the range of suitable environments for each genotype in tree breeding programmes. Little is still known of the capacity of abiotic and biotic soil variation to generate marked shifts in genotypic performance ranks. We examined the potential of belowground soil factors to bring about genotype × environment interactions in a tree species by combining data from extant field trials and a new seedling-based progeny experiment. We first analysed genotypic growth patterns of Norway spruce (*Picea abies*) at two field trial locations, a native forest site and a former agricultural field, that exhibited biotic and abiotic soil variation. While we found significant genotype × location interactions in growth, we also observed positive between-location genotypic correlations, indicating similar genotypic rank orders across divergent soil types. Contributing to the genotype × environment interactions, differences in age at the time of growth measurements may explain why genetic variances nevertheless differed between the locations. The subsequent progeny experiment with soil and seeds collected at the trial locations enabled controlled treatments in a growth chamber that tested the capacity of between-location soil variation to induce genotype × environment interactions in seedling traits. The progeny experiment revealed that the soil treatment had major effects on averages in all 14 shoot and root functional traits, with four groups of correlated traits (e.g., estimates of shoot and root system size) identified by a principal component analysis. Seed collection location affected only few traits, and the more southern agricultural field trial yielded slightly larger seedlings with delayed phenology. Yet, despite significant genetic variation, no seedling trait manifested genotype × soil treatment interactions, which may be due to the soil treatments not mirroring spatial heterogeneity of the soils at the trial locations, or to our limited subsample of ten genotypes in the progeny experiment. Taken together, our results on adult trees and seedlings indicate that overall tree growth is impacted by variation in belowground environmental factors, but further research with more comprehensive sampling is needed to determine whether they have potential to generate location-specific patterns of genotypic performance in economically valuable tree species.

1. Introduction

Various types of tradeoffs preserve inter- and intraspecific biodiversity in the wild. According to a classical hypothesis, a competitive strategy of plants that maximises fitness in a specific environmental setting is determined by correlated variation of traits across different organs and functions (Grime, 1977; Coley et al., 1985), and interspecific surveys on functional trait variation on various geographic scales *in situ* provide evidence for such covariation among traits (Wright et al., 2004; Annighöfer et al., 2022; Tumber-Dávila *et al.*, 2022). However, large-scale patterns of variation that have developed among species may not describe those that have evolved within specific environments or individual species (Laughlin & Messier, 2015; Siefert et al., 2015; Messier et al., 2017; Anderegg et al., 2018).

Two key components of intraspecific variation, genetic factors and phenotypic plasticity, are separable in a common-garden experimental set-up that in widely distributed forest trees has exposed the ubiquity of local environment-driven genetic differentiation in varied functional

* Corresponding author.
E-mail address: matti.salmela@luke.fi (M.J. Salmela).
[1] ORCID: 0000-0002-4892-761X

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traits related to phenology, growth and growth allocation (Howe et al., 2003; Savolainen et al., 2007). Variation within traits may be introduced also, e.g., by the parental reproductive environment whose carryover effects may persist in progeny over many years: in Norway spruce (*Picea abies*), higher temperature during seed maturation is associated with delayed cessation of leader shoot growth and development of cold hardiness in autumn in seedlings (e.g., Johnsen & Skrøppa, 1996; Johnsen et al., 2005). In addition, seed weight and cone production are increased by higher temperature and a better parental nutritional status (Karlsson & Örlander, 2002).

The interplay between the environment and intraspecific variation in plants is not entirely unidirectional: tree genotypes host divergent microbial communities in the rhizosphere and modify local soil chemistry (Korkama et al., 2006; Pérez-Izquierdo et al., 2019; Senior et al., 2022), and compatible pairings between tree genotypes and species of ectomycorrhizal fungi that cooperate with their hosts in resource acquisition may yield beneficial consequences on plant fitness (Gehring et al., 2017). Consequently, intraspecific variation in trees contributes to complex interspecific feedback loops in natural settings whose effects extend from alterations of biotic and abiotic belowground growth conditions back to the expression of plant functional traits (Revillini et al., 2016).

According to the common hypothesis of correlated trait variation within plants, a high aboveground growth rate is expected to be paired with more acquisitive and explorative root growth patterns that enhance resource foraging capacity (Chapin, 1980; Reich, 2014; Weemstra et al., 2016). Like shoot traits, fine root traits often exhibit environment-related intraspecific variation *in situ* (Helmisaari et al., 2009; Ostonen et al., 2011; Zadworny et al., 2016; Ostonen et al., 2017), the genetic basis of which has been reported in a small number of common-garden studies (Zadworny et al., 2016; Zadworny et al., 2021). Yet, detailed analyses of plant-wide correlations between roots and shoots are still rare. Intraspecific covariation of traits within trees can be investigated, for instance, with a seedling-based common-garden approach that distinguishes genetic and plastic effects, that facilitates the thorough phenotyping of many functional traits in the same seedling, and that yields large sample sizes (Salmela et al., 2020; Salmela, 2021). Here, our model system is Norway spruce, a widely distributed and economically valuable Eurasian conifer whose abundant naturally occurring intraspecific genetic diversity in functional traits (e.g., Ekberg et al., 1979; Salmela, 2021) has benefitted, for instance, Nordic tree breeding programmes (Jansson et al., 2017). Genetic variation in root traits has been documented in breeding material used in Finland (Velmala et al., 2013), with evidence for more explorative seedling root growth in genotypes with faster long-term growth in field settings (Korkama et al., 2006; Hamberg et al., 2018; Velmala et al., 2023).

Environment-dependent genotypic ranks are expected when genotypes exhibit variation in functional traits and when their performance is monitored across divergent environmental settings (cf. Grime, 1977), determining the environmental range suitable for a given genotype in breeding programmes (Falconer & Mackay, 1996). In trees, such genotype × environment interactions have been associated with among-site differences in soil chemistry and temperature conditions (Li et al., 2017). In Finnish forestry practices, both boreal forests and former agricultural fields have been used as planting sites, potentially resulting in genotypic rank order shifts due to a contrast in biotic and abiotic belowground conditions. We test the capacity of soil variation to generate genotype × environment interactions in Norway spruce by comparing growth between a natural forest stand where nutrients and microbiota are heterogeneously distributed (e.g., Pennanen et al., 1999; Saetre & Bååth, 2000), and a former agricultural field that initially lacked tree-associated microbes and the small-scale heterogeneity of forest soils (Wall & Hytönen, 2005). A progeny trial established with seeds collected at the two trial locations will reveal seed origin effects on various seedling functional traits, with larger size and delayed autumn phenology expected in seedlings originating from the former agricultural field found in a warmer, more southern location (Karlsson & Örlander, 2002; Wennström et al., 2002; Johnsen et al., 2005). We anticipate that variation in soil will affect various seedling phenotypes and that if soil factors are key determinants of genotype × environment interactions in growth in the field, we will observe comparable interactions also in a diverse selection of root and shoot functional traits. Finally, we expect that if root system variation giving rise to varied longer-term genotypic growth trends in the field arises already at an early developmental stage, characteristics improving resource acquisition (e.g., larger root systems, more intensive branching or higher specific root length) will be found in genotypes that exhibit more rapid growth in the field (Reich, 2014; Weemstra et al., 2016).

2. Materials and methods

2.1. Genotypic sampling in field trials

We studied 34 genotypes of Norway spruce that were planted as clonal cuttings into the soil in two field trials in southern Finland in the late 1970 s and that represent controlled crosses between parental genotypes originating from Finland, the Baltic countries, Russia and Central Europe. The trials (Table 1), separated by approximately 190 km, are situated in a former agricultural field (Nurmijärvi, 60.50° N, 24.70° E, L60A hereafter) and a moist forest heathland (Kangasniemi, 61.95° N, 26.68° E, L61F hereafter). Both trials consisted of five spatial blocks, each with one plot per genotype and four genotypic replicates planted per plot. Due to the spatial proximity of each genotype's replicates within blocks in the height data collected in 1983 and 1987, we first calculated block-specific genotypic averages and used these values as replicate measurements in statistical analyses, yielding four or five replicates per genotype and one per block. We calculated block-specific genotypic averages for further use also for growth data collected in 2010 and 2019 in cases where multiple genotypic replicates remained in a single plot. We obtained location-specific estimates of long-term average temperature and precipitation conditions (1970−2000) using the WorldClim Version 2.1. dataset (30-sec resolution; Fick & Hijmans, 2017).

Ten out of the 34 genotypes provided open-pollinated seeds at both locations for a new progeny experiment (cone collection at L60A on 2 December 2019 and 9 January 2020, at L61F on 12 December 2019). Cones were placed in a heating cabinet at 30 °C until cone scales opened, full seeds were extracted and seed wings removed manually, and radiography was applied to confirm seed quality. Seeds were then stored at

Table 1
Information on two field trials of Norway spruce genotypes in Finland that were included in the current study. The species has six regions of provenance in Finland that mark areas environmental similarity and guide the use of seeds collected in natural stands. The Measurements column indicates in which years height (H) and diameter at breast height (D) were recorded.

	Location	
	Nurmijärvi	**Kangasniemi**
Code	L60A	L61F
Site type	Fmr. agricultural field	Native forest
Latitude (° N)	60.50	61.95
Longitude (° E)	24.70	26.68
Elevation (m a.s.l.)	100	103
Region of provenance	1	2
Established	1977	1979
Genotypes	62	77
Shared genotypes	34	
Number of plots	310 (2 m × 2 m)	385 (2 m × 8 m)
Genotypic replicates/ plot	4	4
Number of blocks	5	
Planting distance (m)	1 × 1	2 × 2
Area (ha)	0.124	0.616
Measurements	H: 1987, 2019; D: 2010, 2019	H: 1983, 2019; D: 1983, 2019

−18 °C until sowing, and pooling yielded 20 location-specific genotypic samples. Hereafter, we will refer to progeny sharing the maternal parent as families. Average seed weight per family was estimated based on a single 60-seed sample.

Height and diameter at breast height were assessed in over 40-year-old trees at the time of cone sampling. However, thinning in 1988 resulted in a poor replication of genotypes at L60A where some treetops were noted to be damaged at the time of cone sampling, preventing robust statistical comparisons to corresponding recent data from L6IF. To determine whether growth by age ten at L60A was indicative of growth patterns over a longer period, we used data on diameter at breast height instead which was estimated in November 1987 and June 2010. In 1987, genotypic averages of diameter correlated positively with those for height ($r = 0.734$, $P < 0.0001$), indicating that both are informative traits for describing growth rate. In 2010 two replicates per block remained for 18 genotypes; for these we used the average value such that there was no replication of genotype within blocks.

2.2. Progeny experiment with controlled treatments

We established a progeny experiment with the cone-producing genotypes in a controlled growth chamber (FitoClima 1200, Aralab, Rio de Mouro, Portugal) to separate the effects of soil variation, seed collection location and family on seedling functional traits. We sampled soil at the field trial locations in late May 2020. The samples from L60A, soil within the breeding trial (L60 afforestation treatment) and soil at a nearby open field located ~ 300 m from the breeding trial (L60 field treatment), represent a local-scale contrast between fallow field with no recent history of growing trees and former arable/fallow soil in which trees have been growing for approximately 40 years. At L61F we collected soil within the breeding trial (L61 forest treatment). The larger-scale L60 *vs.* L61 contrast represents a comparison of natural forest soil with a long history of tree growth to former or present-day agricultural soil. We sieved (Ø < 2 mm) soil from ten separate soil cores (Ø 9 cm) and mixed these samples per treatment for a nutrient analysis and soil DNA extraction (Supplementary material, Materials and methods).

Setting up the treatments, we mixed sieved soil samples with natural Sphagnum peat (pH 4.2) and vermiculite (11:4:1), and homogenised physical and nutritional conditions. We analysed three samples per treatment for nutrient content and pH (Table 2a). After cold stratification overnight, we sowed seeds on moist soil in trays (35.2 cm × 21.6 cm × 8.7 cm, 50 cm^3 per cell; 67 cells per tray), with 13 replicates per location-specific family (i.e., three treatments × two field trial locations × ten families × 13 replicates per family) and four trays of 67 cells per treatment. We randomised the order of families within each treatment and kept the trays in dark for three weeks (16 h at 20 °C/8 h at 15 °C).

After germination, seedlings grew in simulated natural conditions (Table 2b) that were based on long-term monthly data (1959−2019) on maximum and minimum temperatures (Klein Tank et al. 2002) from a weather station (Heinola, 61.20° N, 26.05° E, elevation 92 m a.s.l.) located between the field trials. We watered seedlings manually and rotated trays within the chamber each week, maintaining relative humidity at 50−60%. Photosynthetic photon flux density (PPFD) at the seedling level was approximately 200 μmol m^{-2} s^{-1}.

2.3. Seedling-wide phenotyping of shoot and root functional traits

When a visible apical bud appeared between needles, we recorded timing of bud set as the number of days since June 20, the first day of cycling conditions. We measured seedling height and sampled intact seedling root systems from moist soil. We prepared complete root systems for imaging as in Salmela (2021), and analyses with WinRHIZO Pro Version 2020 (Regent Instruments Inc., Quebec City, Canada) provided estimates of total root length, average diameter and the number of root tips. These measures yielded additional estimates of branching intensity (number of root tips/total root length, root system architecture), and specific root length (total root length/belowground biomass, root morphology). We measured needle biomass, stem biomass and belowground biomass after samples had been in an oven at 50 °C for four days, generating estimates of root-to-shoot ratio (belowground biomass/aboveground biomass), root tip-to-shoot ratio (number of root tips/aboveground biomass), leaf mass fraction (needle biomass/total biomass) and stem mass fraction (stem biomass/total biomass).

Table 2
Experimental conditions for the Norway spruce progeny experiment with three soil treatments. **a)** Abiotic characteristics of the soil treatments, with two separate soil samples collected at L60A. Values shown are averages of three replicates. **b)** Monthly adjusted light and temperature cycles that resembled natural conditions at 61° N in Finland.

a)

Variable	L60 afforestation	L60 field	L61F forest
pH	5.07	5.54	4.54
C %	5.91	2.62	6.80
N %	0.352	0.199	0.283
Al mg/kg	26567	17967	10867
B mg/kg	5.55	3.79	1.29
Ca mg/kg	4200	4497	2980
Cd mg/kg	0.166	0.135	0.103
Cr mg/kg	38.0	29.0	23.9
Cu mg/kg	18.10	19.87	9.92
Fe mg/kg	24600	22067	12500
K mg/kg	4697	3057	832
Mg mg/kg	5567	4987	1797
Mn mg/kg	451	529	220
Na mg/kg	270	345	266
Ni mg/kg	16.47	11.97	6.80
P mg/kg	991	996	713
Pb mg/kg	11.5	7.5	11.1
S mg/kg	379	241	277
Zn mg/kg	77.5	70.0	39.0
Humidity %	3.59	1.69	3.08
OM %	12.3	5.8	13.3
Ash %	87.7	94.2	86.7

b)

Month of treatment	Light:dark cycle (h)	Light/dark temperature (°C)
1 (June)	19:5	20.0/9.6
2 (July)	18:6	22.1/12.4
3 (August)	16:8	19.9/11.1
4 (September)	13:11	13.8/6.6
5 (October)	10:14	7.3/2.0
6 (November)	7:17	5.0/2.0

We estimated ectomycorrhizal morphotypes and colonisation rate in 303 root systems (80 or 81 per treatment). We determined the number of colonised tips in complete root systems under a dissecting microscope, rounding up estimates of colonisation rate to the nearest 5%. We subjected a subsample of morphotyped mycorrhizas to Sanger sequencing of the ITS region to verify the visual identification (NCBI Genbank accension numbers ON454507−ON454529). We obtained missing estimates of belowground biomass for these 303 samples by fitting a non-linear regression to all data on total root length and belowground biomass (n = 372), yielding the equation

$$belowground \quad biomass = 0.00110 \times total \quad root \quad length^{0.7715}.$$

To estimate carbon and nitrogen concentrations in needles, we ground dried needles after biomass measurements using steel beads and TissueLyser II (Qiagen). To obtain the required amount of 100 mg for an analysis with CHN628 (LECO Corporation), we typically combined four to five individual samples that had similar biomass measurements, resulting in three to four replicates per family and treatment (114 samples in total). We did pooling per family over the two trial locations.

2.4. Statistical analyses

2.4.1. Field trials

For sapling height, 34 genotypes had four or five replicates per

genotype at both locations. For an analysis on diameter estimated in mature trees, we included genotypes for which at least two replicates from different blocks remained at both locations, resulting in a final sample of 32 genotypes. We analysed variation in growth using an analysis of variance (ANOVA) model with the following factors: location (fixed factor), genotype (random factor), genotype × location interaction (random factor), and block within location (random factor). We repeated the same test also for the ten genotypes included in the progeny experiment.

Because height measurements at the two locations were taken at different ages, we were mainly interested in genotype × location interactions; this includes also interactions due to environment alone. When genotypic growth is positively correlated across environments, a significant main effect of genotype will be found in ANOVA, and a significant interaction term may reflect between-environment differences in the genotypic variance. When marked genotypic rank order changes occur, a significant interaction term will be observed without a significant main effect of genotype. To calculate genotypic correlations (r_{GE}) between L60A and L61F, we first estimated variance components for the factors using the restricted maximum likelihood (REML) method and assuming all factors were random. We then used the equation

$$r_{GE} = \frac{\sigma^2_G}{\sqrt{(\sigma^2_{G(L60A)} \times \sigma^2_{G(L61F)}}},$$

where σ^2_G = the genotypic variance component estimated across locations, $\sigma^2_{G(L60A)}$ = the genotypic variance component at L60A, and $\sigma^2_{G(L61F)}$ = the genotypic variance component at L61F (Windig, 1997). Due to between-location differences in average growth, we estimated coefficients of variation (*CV*) for the effects of genotype and residual (within-family) variation using the equation

$$CV = \frac{\sqrt{\sigma^2_{G/R}}}{\mu},$$

where $\sigma^2_{G/R}$ = genotypic ($_G$) or residual ($_R$) variance component at the location and μ = location average for the trait.

To test whether early growth in saplings was predictive of longer-term growth patterns within the two locations, we used Pearsoń correlation and genotypic averages of sapling growth and growth by age 33 (height, L60A, 32 genotypes) or age 40 (diameter, L61F, 34 genotypes).

2.4.2. Progeny experiment

Following Salmela et al. (2020), we used a principal component analysis (PCA) to identify groups of seedling functional traits varying in a correlated manner. For each trait, we used ANOVA to test for the effects of treatment (fixed factor), location (fixed factor), location × treatment interaction (fixed factor), family (random factor) and family × treatment (genotype × environment) interaction (random factor). Note that in the progeny experiment the location factor refers to seed origin.

We analysed variation in ectomycorrhizal colonisation rate with a generalised linear model with a normal probability distribution and identity link function. We included treatment, location, family and family × treatment interaction as factors.

We performed separate ANOVAs on carbon and nitrogen concentrations of needles, including the following factors: treatment (fixed), family (random) and family × treatment interaction (random). We excluded location due to the pooling of samples over L60A and L61F. Only carbon concentration exhibited a significant main effect of family (see Results); to test whether genetic variation in carbon concentration was associated with variation in other functional traits, we used Pearsoń correlation to associate family averages estimated for the concentration with those for the individual traits estimated in all 675 replicates.

To explore whether variation in seedling functional traits was associated with genotypic growth in the field, we used Pearson's correlation to associate family averages of functional traits with the corresponding genotypic averages in the field. Due to statistically significant genotype × location interactions in the field (see Results), we carried out separate analyses for L60A and L61F; in the progeny data, we used overall marginal averages for families estimated across treatments owing to non-significant family × treatment interactions. Because of significant correlations among traits, variation in 14 functional traits of seedlings could be outlined by four PCs (see Results), and when testing for correlations between seedlings and growth in the field, we used family averages for PC1 (shoot and root growth), PC2 (phenology and growth allocation), PC3 (root architecture) and PC4 (root morphology).

We analysed all tree data with IBM SPSS Statistics Version 28. We did bacterial and fungal community permutational analyses of variance (PERMANOVA) with the adonis function and Non-Metric Multidimensional scaling NMDS with metaMDS function from the vegan package (Oksanen et al., 2020) in R (R Core Team, 2020), with relative abundance of OTUs and soil type (L60 afforestation, L60 field or L61 forest) as explanatory factors.

3. Results

3.1. Soil biotic and abiotic variation

Location and soil type affected bacterial and fungal communities in contemporary soil samples (PERMANOVA, Supplementary Table S1). The L60 field soil had the most pathotrophic fungi (Ascomycota being the largest phylum) while Basidiomycota dominated in forest soils (Supplementary Table S2). In bacteria, the dominant groups Proteobacteria, Actinobacteria or Acidobacteria were rather similar in nearby L60 afforestation and L60 field soils compared to the L61 forest soil. Ectomycorrhizal diversity was higher in forest soils (data not shown). Both bacterial and fungal communities varied in NMDS ordination according to location, and within L60 there was significant variation between the afforested forest and field soil (data not shown). Chemical profiles varied among soil samples, with higher carbon content and lower pH in the L60 afforestation soil compared to the nearby L60 field soil (Table 2). Soil pH was lowest and carbon content highest in the L61F forest soil. However, concentrations of most mineral nutrients in the L60 afforestation soil still resembled those in the nearby L60 field soil. Total nitrogen content was lowest in the L60 field soil and highest in the nearby L60 afforestation soil.

The WorldClim climate data suggested that L60A has experienced higher average temperatures, less precipitation and less intra-annual fluctuations in temperature and precipitation than L61F (Supplementary Table S3 and S2).

3.2. Genotypic variation in the field

Genotypic averages of sapling height varied from 3.27 m to 5.52 m at age ten at L60A, and from 0.380 m to 1.13 m at age five at L61F (Fig. 1a, b). ANOVA revealed highly significant effects of location, genotype × location interaction, and block within location (Table 3a). Due to growth measurements in different years, the main effect of location is confounded with time; at age five, genotypes at L61F had reached 11.2−33.3% of the average genotypic height at age ten at L60A (Fig. 1a). The main effect of genotype was not strong ($P = 0.067$), but the genotypic correlation between the locations was moderate and positive ($r_{GE} = 0.532$) (Table 3a, Fig. 1b). Variance components revealed that the genotype × location interaction explained a larger proportion of total variation than the main effect of genotype, indicative of location-specific patterns of genotypic variation. The genotypic variance component was about eight times larger at L60A than at L61F, but mean-standardised *CV*s indicated that there was more among- and within-genotype variation in sapling height at L6IF than at L60A (Table 3c).

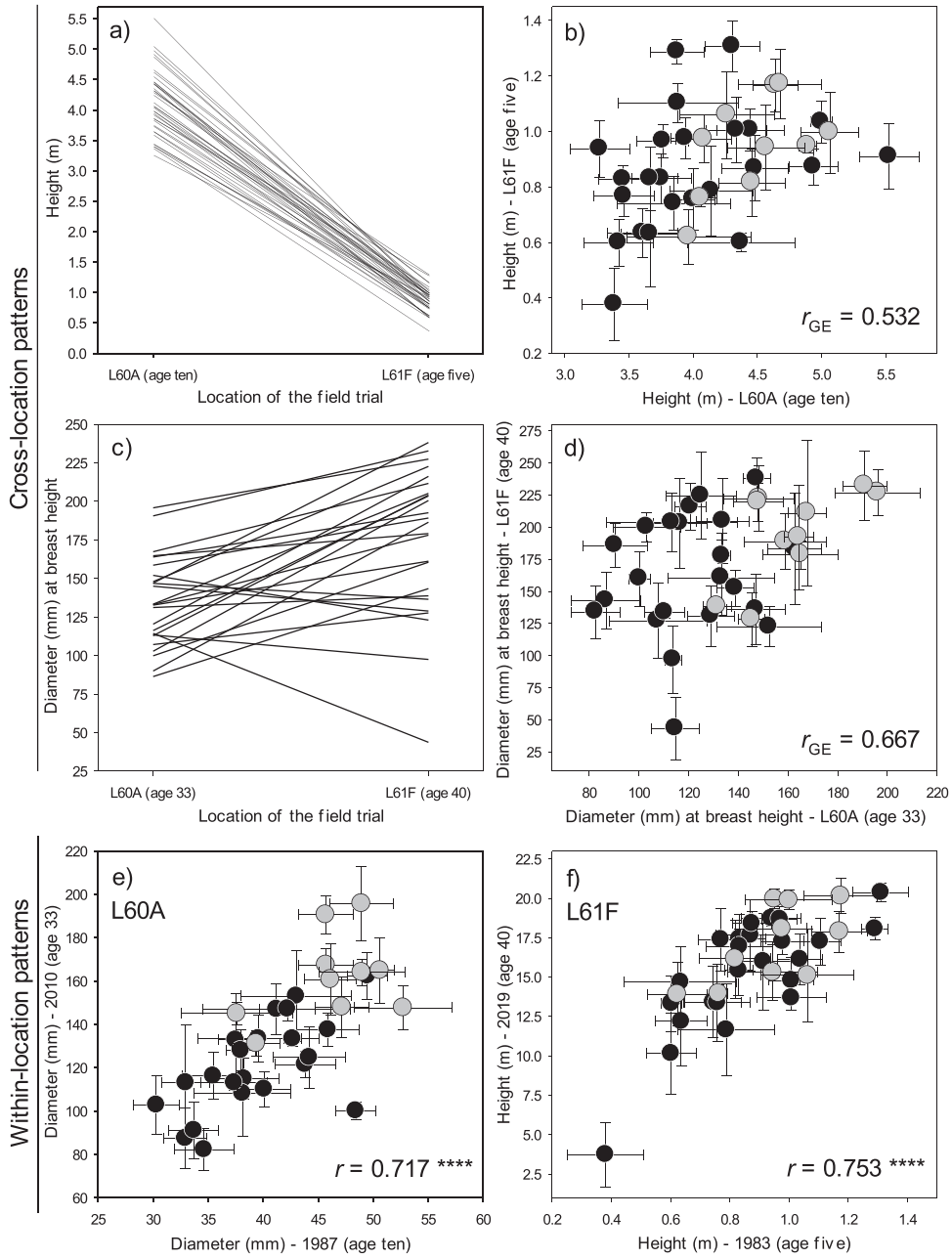

Fig. 1. **a)** Reaction norms for 34 genotypes of Norway spruce that were measured for height growth at two different locations in Finland at age ten (L60A) or age five (L61F). **b)** Scatterplot of genotypic averages (± SE) of sapling height at the two locations. **c)** Reactions norms for 32 genotypes measured for diameter at breast height at age 33 (L60A) or age 40 (L61F). **d)** Scatterplot of genotypic averages (± SE) of diameter at the two locations. **e)** Scatterplot of genotypic averages (± SE) of diameter at breast height measured in 1987 (age ten) and in 2010 (age 33) at the L60A. Data from both years were available for 32 genotypes. **f)** Scatterplot of genotypic averages (± SE) of height measured in 1983 (age five) and in 2019 (age 40) at L61F. Data from both years were available for 34 genotypes. In **b), d), e)** and **f)** the ten cone-producing genotypes sampled for the progeny experiment are marked in grey. **** = $P < 0.0001$.

Patterns of variation in diameter at breast height in mature trees (age 33 *vs.* age 40) within and across the locations resembled those found in sapling height (Table 3b, Fig. 1c). Variance components showed that the main effect of genotype was larger than that of the genotype × location interaction, signalling a stronger positive genotypic correlation across the locations ($r_{GE} = 0.667$) than for sapling height (Table 3b, Fig. 1d). The genotypic variance component in diameter was greater at L61F than at L60A, but *CVs* were similar at both locations (Table 3c). On the other hand, the within-genotype *CV* was again greater at L61F than at L60A.

There were no significant genotype × location interactions in the ten seed-producing genotypes sampled in the progeny experiment (Table 3a, b), a subset capturing 48.8% and 59.2% of the genotypic variation in sapling height at L60A and L61F, respectively (Fig. 1b). For diameter, the corresponding proportions were 53.4% at L60A and 56.9% at L61F (Fig. 1d). Average seed weight was higher at L60A (7.57 mg) than at L61F (7.01 mg).

Early growth by age five or age ten was a strong predictor of longer-term genotypic growth over 33—40 years, with positive correlations of similar strength for genotypic averages of diameter at breast height at L60A (1987 *vs.* 2010, Fig. 1e), and for genotypic averages of height at L61F (1983 *vs.* 2019, Fig. 1f).

Table 3
Analyses of variance (ANOVA) on **a)** height in saplings, and **b)** diameter at breast height in mature trees across two field trial locations, L60A and L61F, in Norway spruce in Finland. The subsample of ten genotypes represents those included in the progeny experiment. **c)** Variance components and coefficients of variation for height and diameter at L60A and L61F.

a) Height (m)		**34 genotypes**				**10 genotypes**		
Factor	df	MS	*F*	VC (%)	df	MS	*F*	VC (%)
Location	1	894	644 ****	5.29 (93.9%)	1	308	2140 ****	6.16 (95.9%)
Genotype	33	1.07	1.70 ns	0.0447 (0.794%)	9	0.597	2.51 ns	0.0359 (0.559%)
Genotype × location interaction	33	0.632	3.44 ****	0.0901 (1.60%)	9	0.238	1.01 ns	0.00219 (0.0341%)
Block(location)	8	0.940	5.12 ****	0.0222 (0.394%)	8	0.143	0.603 ns	0
Residual	262	0.183		0.184 (3.27%)	72	0.236		0.227 (3.533%)
b) Diameter (mm)		**32 genotypes**				**10 genotypes**		
Factor	df	MS	*F*	VC (%)	df	MS	*F*	VC (%)
Location	1	102415	21.2 ****	820 (23.1%)	1	25033	16.8 *	573 (19.5%)
Genotype	31	7831	2.30 *	574 (16.2%)	9	6253	3.42 *	450 (15.3%)
Genotype × location interaction	31	3400	1.94 **	325 (9.16%)	9	1828	0.923 ns	0 (0%)
Block(location)	8	3300	1.88 ns	48.0 (1.35%)	8	1632	0.824 ns	0 (0%)
Residual	208	1754		1782 (50.2%)	66	1980		1922 (65.3%)
c) VCs and CVs								
Height:								
L60A (age ten), genotype: 0.240, 11.8%								
L61F (age five), genotype: 0.0295, 19.3%								
L60A (age ten), residual: 0.319, 13.6%								
L61F (age five), residual: 0.0486, 24.8%								
Diameter:								
L60A (age ten), genotype: 651, 19.1%								
L61F (age five), genotype: 1136, 19.5%								
L60A (age ten), residual: 672, 19.4%								
L61F (age five), residual: 2899, 31.1%								

df = degrees of freedom, MS = mean square, *F* = *F*-ratio, ns = non-significant ($P > 0.05$), * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$, VC = variance component, CV = coefficient of variation. In **a)** and **b)**, percentages for VCs show the proportion of total variation in the functional trait explained by each factor. In **c)**, CVs are shown as percentages.

3.3. Variation in seedling functional traits

Overall, PCA identified four PCs and groups of correlated traits with eigenvalues above one that together explained 83.1% of total variation among seedling functional traits: size-related traits (PC1), growth allocation and phenology (PC2), stem mass fraction and root system morphology (PC3), and root system architecture (PC4) (Table 4). All traits exhibited a highly significant main effect of treatment in ANOVA (Table 5, Fig. 2).

In brief, the L60 field treatment was associated with early timing of bud set, high root-to-shoot and root tip-to shoot ratios, and low needle and stem mass fractions, with root systems characterised by high total root length, belowground biomass, number of root tips, and specific root length, and low average root diameter and branching intensity (L60fi in Fig. 2). The L60 afforestation treatment exhibited later timing of bud set and the most aboveground growth in terms of shoot height, needle and stem biomass, which were also reflected in a low root-to-shoot ratio and high needle mass fraction (L60af in Fig. 2). Belowground, root systems in the L60 afforestation treatment manifested the highest branching intensity and low specific root length. The L61 forest treatment exhibited later timing of bud set, the least aboveground growth and high stem mass fraction, with the smallest root systems in terms of total root length, belowground biomass, and the number of root tips (L61fo in Fig. 2). In addition, the L61 forest treatment exhibited the largest root average diameter and low specific root length.

A significant main effect of location on timing of bud set, needle biomass, total root length, belowground biomass and the number of root tips (Table 5) indicated that seeds collected at L60A yielded seedlings that on average set their buds slightly later and that grew slightly larger than those originating from seeds sampled at L61F (Fig. 2). The location × treatment interaction was significant only in needle biomass.

Table 4
Principal component analysis (PCA) with 14 seedling functional traits estimated in the Norway spruce progeny experiment. Eigenvalues are shown for each PC, with the percentage indicating the proportion of total variation explained. Values in the table are Pearson's correlation coefficients, and each trait's strongest correlation with a PC is shown underlined and in bold.

	Principal component			
	PC1	PC2	PC3	PC4
	3.97	3.46	2.97	1.24
Trait	28.3%	24.7%	21.2%	8.87%
Shoot traits:				
Timing of bud set (days)	0.150	***0.621***	-0.172	0.0200
Shoot height (cm)	***0.851***	0.233	-0.091	0.0529
Needle biomass (mg)	***0.786***	0.520	0.173	0.0544
Stem biomass (mg)	***0.931***	0.219	-0.162	0.0283
Root traits:				
Total root length (cm)	***0.676***	-0.305	0.602	-0.212
Belowground biomass (mg)	***0.795***	-0.264	0.420	-0.0950
Root average diameter (mm)	-0.216	0.299	***-0.687***	-0.123
Number of root tips	***0.715***	-0.256	0.577	0.183
Root branching intensity (tips/cm)	0.0473	0.105	-0.0895	***0.983***
Specific root length (cm/mg)	0.203	-0.210	***0.704***	-0.300
Growth allocation:				
Root-to-shoot ratio (%)	-0.104	***-0.901***	0.259	-0.138
Needle mass fraction (%)	-0.00285	***0.967***	0.0113	0.136
Stem mass fraction (%)	0.174	0.00465	***-0.818***	0.0649
Root tip-to-shoot ratio (tips/mg)	0.0335	***-0.765***	0.541	0.189

3.4. Phenotypic plasticity in other seedling traits

Ectomycorrhizal colonisation in roots: Estimates of ectomycorrhizal colonisation rate in seedling root systems ranged from 10% to 100%, with a grand average 87.9%. Colonisation rate was influenced by treatment (Wald $X^2 = 138$, df = 2, $P < 0.0001$), with a lower average rate in the L60 field treatment (70.2%) than in the L61 forest treatment (96.2%) or the L60 afforestation treatment (97.4%). The effects of location (Wald $X^2 = 1.88$, df = 1, $P = 0.171$) or location × treatment interaction (Wald $X^2 = 0.244$, df = 2, $P = 0.885$) were not significant.

Carbon and nitrogen concentrations in needles: Carbon and nitrogen concentrations in needles were affected by treatment (carbon: $F_{2,\ 18.081} = 74.8$, $P < 0.0001$, nitrogen: $F_{2,\ 18.260} = 635$, $P < 0.0001$). For carbon

Table 5
Analyses of variance (ANOVA) on 14 functional traits measured in the Norway spruce progeny experiment. Note that in the progeny experiment the location factor refers to seed origin.

		Timing of bud set (days)		**Shoot height (cm)**		**Needle biomass (mg)**	
Factor	**df**	MS	*F*	MS	*F*	MS	*F*
Treatment (Trt)	2	7716	42.2 **** / 9.83%	39	63.4 **** / 19.7%	12270	51.4 **** / 17.2%
Location (Loc)	1	1036	4.02 * / 0.578%	1.64	2.77 ns / 0.348%	1456	6.81 ** / 0.800%
Loc × Trt interaction	2	342	1.32 ns / 0.170%	0.375	0.634 ns / 0%	667	3.12 * / 1.22%
Family (Fam)	9	2878	15.8 **** / 11.5%	6.93	11.3 **** / 11.3%	2060	8.63 **** / 9.38%
Fam × Trt interaction	18	182	0.707 ns / 0%	0.615	1.04 ns / 0.159%	239	1.12 ns / 0.384%
Residual	642	258	77.9%	0.591	68.6%	214	71.0%
		Stem biomass (mg)		**Total root length (cm)**		**Belowground biomass (mg)**	
Treatment (Trt)	2	594	31.4 **** / 10.3%	293591	144 **** / 39.3%	14019	109 **** / 25.7%
Location (Loc)	1	56.5	3.11 ns / 0.324%	7906	4.33 * / 0.529%	1054	6.57 ** / 0.886%
Loc × Trt interaction	2	27.4	1.52 ns / 0.250%	2348	1.29 ns / 0.122%	337	2.10 ns / 0.622%
Family (Fam)	9	225	11.9 **** / 13.5%	11492	5.65 *** / 4.41%	1280	9.91 **** / 7.16%
Fam × Trt interaction	18	19.0	1.04 ns / 0.0333%	2033	1.11 ns / 0.373%	129	0.804 ns / 0%
Residual	642	18.2	75.6%	1827	55.2%	161	65.7%
		Root average diameter (mm)		**Number of root tips**		**Specific root length (cm/mg)**	
Treatment (Trt)	2	0.301	261 **** / 48.3%	505955	129 **** / 28.1%	29.7	121 **** / 39.6%
Location (Loc)	1	0.00133	1.37 ns / 0.0740%	23682	4.37 * / 0.641%	0.00945	0.0497 ns / 0%
Loc × Trt interaction	2	0.000606	0.621 ns / 0%	6566	1.21 ns / 0.172%	0.0693	0.365 ns / 0%
Family (Fam)	9	0.00545	4.73 ** / 2.99%	32246	8.22 **** / 4.99%	0.527	2.14 ns / 1.33%
Fam × Trt interaction	18	0.00115	1.18 ns / 0.398%	3917	0.723 ns / 0%	0.247	1.30 ns / 0.808%
Residual	642	0.000977	48.3%	5414	66.1%	0.190	58.3%
		Root branching intensity (tips/cm)		**Root-to-shoot ratio (%)**		**Needle mass fraction (%)**	
Treatment (Trt)	2	8.66	52.6 **** / 18.0%	39481	138 **** / 28.3%	2605	104 **** / 20.0%
Location (Loc)	1	0.0119	0.0697 ns / 0%	102	0.249 ns / 0%	55.3	1.32 ns / 0%
Loc × Trt interaction	2	0.0853	0.500 ns / 0%	603	1.48 ns / 0.00321%	98.0	2.34 ns / 0.646%
Family (Fam)	9	0.166	1.01 ns / 0.0195%	1716	6.00 *** / 3.20%	153	6.10 *** / 2.94%
Fam × Trt interaction	18	0.165	0.966 ns / 0%	285	0.699 ns / 0%	25.1	0.599 ns / 0%
Residual	642	0.170	81.9%	409	68.5%	41.9	76.4%
		Stem mass fraction (%)		**Root tip-to-shoot ratio (tips/mg)**			
Treatment (Trt)	2	279	48.7 **** / 15.2%	119	76.4 **** / 26.4%		
Location (Loc)	1	19.1	2.94 ns / 0.522%	0.236	0.182 ns / 0%		
Loc × Trt interaction	2	2.32	0.355 ns / 0%	0.570	0.440 ns / 0%		
Family (Fam)	9	27.3	4.76 ** / 3.84%	9.30	5.98 *** / 5.94%		
Fam × Trt interaction	18	5.73	0.880 ns / 0%	1.56	1.20 ns / 0.625%		
Residual	642	6.51	80.4%	1.30	67.0%		

df = degrees of freedom, MS = mean square, *F* = *F*-ratio, ns = non-significant ($P > 0.05$), * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$. Percentages after significance levels show the proportion of total variation in the functional trait explained by each factor (based on variance components).

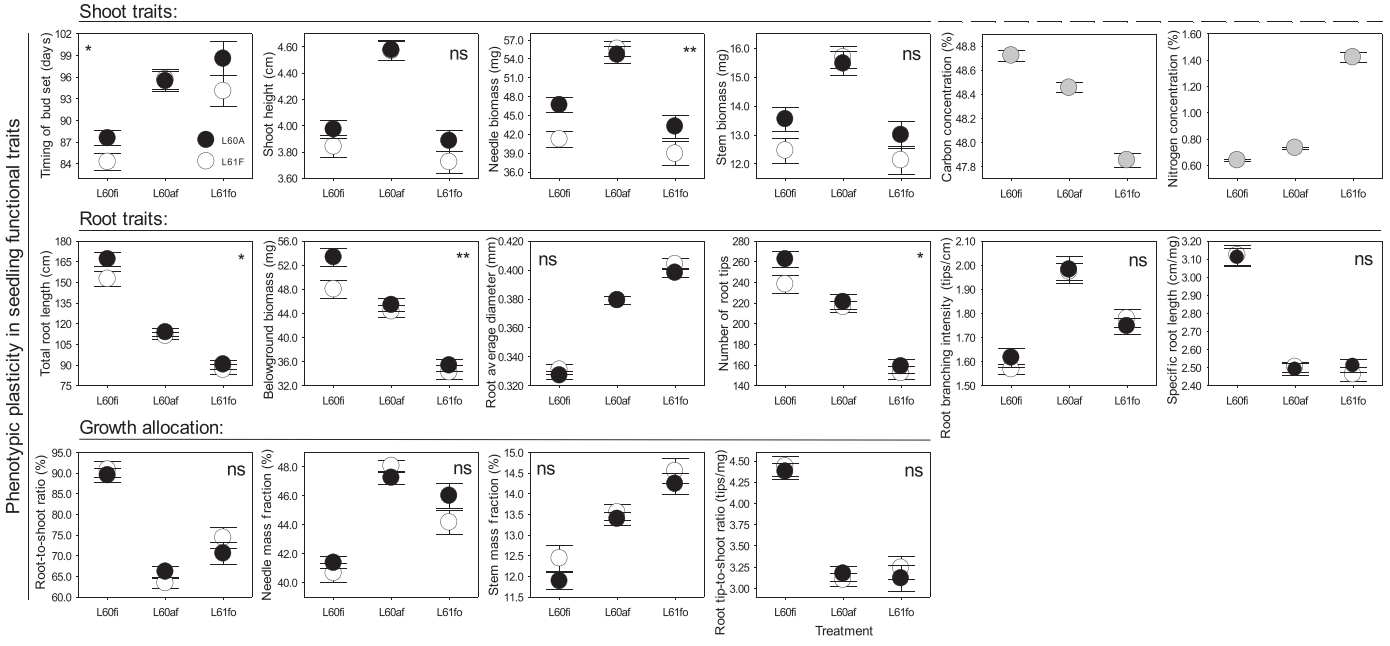

Fig. 2. Marginal treatment averages (± SE) for six shoot traits, six root traits and four measures of growth allocation measured in the Norway spruce progeny experiment with three soil treatments: L60af(afforestation), L60fi(field) and L61fo(forest). Averages are shown by the location of seed collection (L60A or L61F) for all other traits except for carbon and nitrogen concentrations in needles, with symbols within the figures marking whether the main effect of location was statistically significant: ns = non-significant ($P > 0.05$), * = $P < 0.05$, ** = $P < 0.01$. Averages per location were not obtainable for carbon and nitrogen concentrations which were estimated in pooled needle samples.

concentration, the L61 forest treatment exhibited the lowest average (47.9%) and the L60 field treatment had the highest average (48.7%), with treatment explaining 68.3% of total variation (Fig. 2). For nitrogen concentration the L60 field treatment exhibited the lowest average (0.638%) and the L61 forest treatment had the highest average (1.42%), with treatment explaining 91.8% of total variation (Fig. 2).

3.5. Genetic variation in seedling functional traits

A significant main effect of family revealed genetic variation in all other functional traits estimated in all seedlings except for root branching intensity and specific root length (Table 5, Fig. 3).

Carbon concentration in needles exhibited a significant main effect of family ($F_{9,\ 18.112} = 4.13$, $P < 0.01$), with family averages ranging from 48.0% to 48.5% (Fig. 3). Family explained 9.15% of total variation in carbon concentration. Family did not have a significant effect on nitrogen concentration ($F_{9,\ 18.361} = 1.08$, $P = 0.423$; Fig. 3) or ectomycorrhizal colonisation rate (Wald $X^2 = 3.26$, df = 9, $P = 0.953$).

We found no significant family × treatment interactions in functional traits estimated in all replicates, i.e., family responses to soil variation were uniform (Table 5). Further, ectomycorrhizal colonisation rate (Wald $X^2 = 13.7$, df = 18, $P = 0.746$) and nitrogen concentration in needles ($F_{18,\ 84} = 0.559$, $P = 0.919$) did not exhibit significant family × treatment interactions. We detected a significant and modest family × treatment interaction only in carbon concentration in needles ($F_{18,84} = 1.79$, $P < 0.05$), accounting for 3.87% of total variation.

3.6. Associations between seedling functional traits and growth in field trials

We did not observe statistically significant correlations between family averages of seedling functional traits described by PC1−PC4 and corresponding genotypic averages of sapling height at age ten at L60A ($r = -0.385$–0.0331, $P > 0.269$) (Fig. 4). At age five at L61F, all other correlations were statistically non-significant ($r = -0.427$–0.228, $P > 0.216$) except for the one with PC3 ($r = -0.653$, $P < 0.05$). Of the individual traits comprising PC3, a significant seedling—sapling correlation occurred in stem mass fraction ($r = 0.736$, $P < 0.05$) but not in specific root length ($r = -0.213$) or root average diameter ($r = 0.268$) ($P > 0.453$). We found no significant associations when using diameter at breast height at ages 33 or 40 instead of sapling height (Supplementary Figure S1).

4. Discussion

Examining genotypic variation and its dependence on the environment in tree growth, we found significant genotype × location interactions in growth in Norway spruce in the field, with positive genetic correlations between two sites differing, e.g., in biotic and abiotic soil environments. In a new progeny experiment, families manifested phenotypic plasticity in a selection of shoot and root functional traits in response to soil treatment, with a minor effect of the seed collection location on growth and phenology. In contrast to the field observations, no family × treatment interactions arose in seedling functional traits despite significant main effects of treatment and family. Finally, genetic differences found in seedlings did not correlate with genotypic variation in growth in the field.

4.1. Genotypic variation in growth was dependent on the field trial location

Our finding that the field trial location generated significant genotype × location interactions agreed with our hypothesis, but moderate and positive genotypic cross-environment correlations suggested that genotypes that grew more rapidly in a former agricultural field at L60A tended to do so also in native forest conditions at L61F. Magnitudes of genotypic correlations found here are typical in growth in multi-site comparisons of Nordic breeding material in Norway spruce (Berlin et al., 2015; Chen et al., 2017) and in Scots pine in Finland (Haapanen, 1996), and some of these trials have been established on agricultural land. The observation that significant genotype × location interactions occurred together with positive genetic correlations indicates that while some genotypes may have shifted their ranks due to location, the interactions typify also between-location differences in the magnitude of genotypic variance. Indeed, the genotypic variance for height at age ten at L60A was greater than at age five at L60F, but in diameter in mature trees the difference was smaller. Hence, it is possible that the genotype × location interactions are partially due to measurements taken at

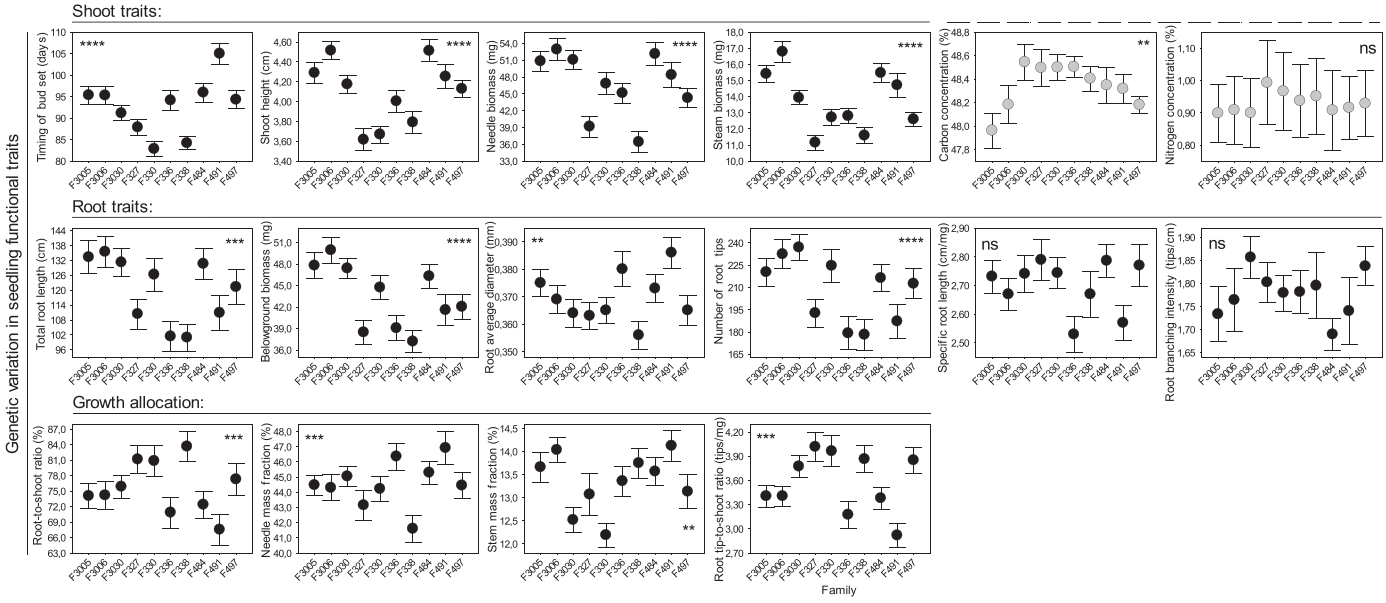

Fig. 3. Marginal family averages (± SE) for six shoot traits, six root traits and four measures of growth allocation measured in the Norway spruce progeny experiment across three soil treatments. Symbols within figures mark whether the main effect of family was statistically significant in ANOVA: ns = non-significant ($P > 0.05$), *** $P < 0.001$, **** = $P < 0.0001$. In contrast to all other traits, carbon and nitrogen concentrations in needles were estimated in pooled needle samples (n = 114) instead of all individual replicates (n = 675).

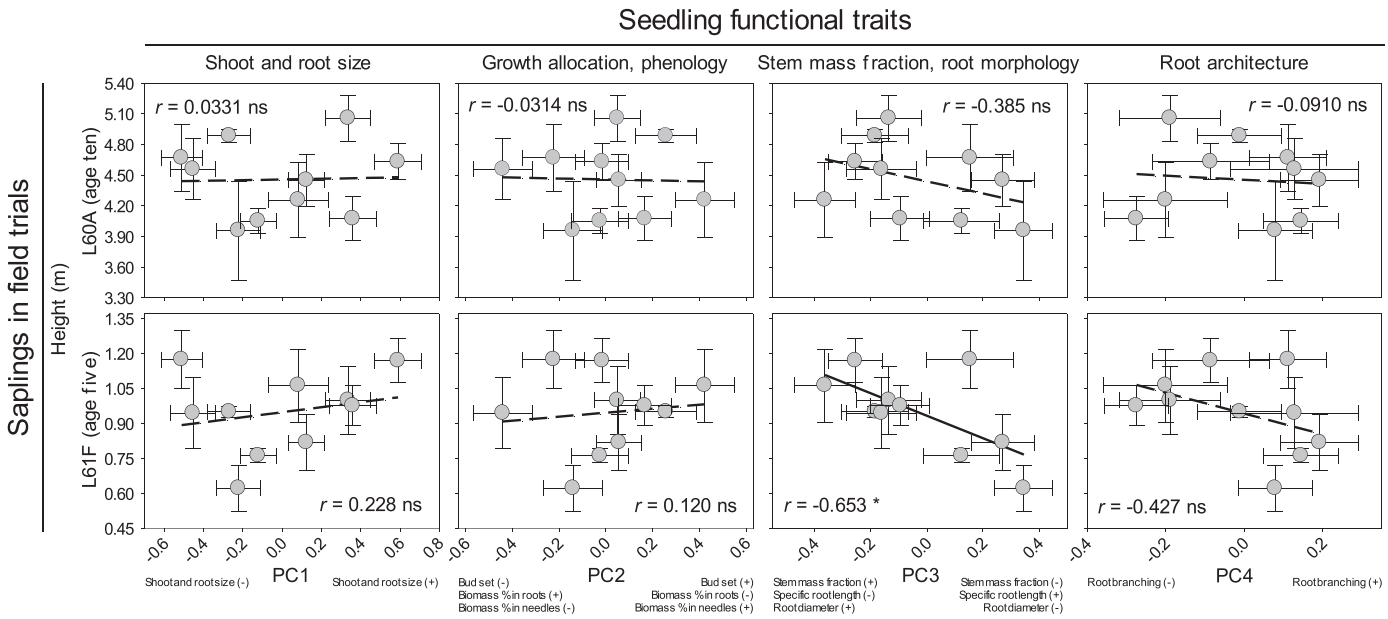

Fig. 4. Associations between marginal family averages (± SE) of the four principal components (PC; Table 4) identified among 14 shoot and root functional traits in the Norway spruce progeny experiment across three soil treatments, and average height (± SE) of the corresponding genotypes at age ten (L60A) or age five (L61F) at the two field trial locations. Pearson's correlation coefficients are shown in each scatterplot: ns = statistically non-significant ($P > 0.05$), * = $P < 0.05$. Non-significant associations are illustrated with dashed lines. Trait correlations are summarised for each PC, with (+) indicating a positive association and (−) marking a negative association with the component.

different ages and sizes.

Another probable source of the interactions in growth is between-location environmental variation. Berlin et al. (2015) did not find strong links between genotype × environment interactions in growth and environmental variables among southern Swedish breeding trials of Norway spruce, while Chen et al. (2017) found that low spring and autumn temperatures explained a proportion of genotype × environment interactions via frost damage (Chen et al., 2017). The specific contribution of soil factors to interactions is often not known (e.g., Haapanen, 1996), but findings by Mikola et al. (2014) suggest that small-scale (i.e., within-site) heterogeneity in soil conditions was not sufficient to generate strong genotype × environment interactions in growth in *Betula pendula*. Our study sites spanned a greater environmental distance, and initial within-location belowground spatial heterogeneity was likely lower at the ploughed and afforested agricultural field of L60A than at the native forest site of L61F. When measurements of within-location variation were adjusted with trait averages using *CV*s, there was more among- and within-genotypic variation in sapling height at L61F, possibly due to more heterogenous forest soil conditions. However, this between-location difference in the *CV*s for among-genotype variation did not appear in diameter measured in mature trees, the large root systems of which may exceed the diameter of spatially autocorrelating patches in forest soil (Lilleskov et al., 2004).

4.2. Shoot and root functional traits in seedlings were affected by soil conditions

The additional progeny experiment with treatments in a controlled experimental setting provided the means for testing how variation in various soil factors between the field trials affects different above- and belowground functional traits in Norway spruce and whether such heterogeneity is sufficient to give rise to noticeable genotype × environment interactions that might change genotypic rank orders depending on the environment. Matching our hypothesis, various seedling traits were responsive to contemporary soil variation between the field trial locations which may derive, e.g., from the effects of intraspecific genetic diversity in a tree species on the associated soil microbiota. Four distinct classes of correlated seedling traits identified by PCA manifested varying plastic responses among functional traits involved in environmental adaptation, agreeing with previous seedling studies with natural populations of Norway spruce (Salmela et al., 2020; Salmela, 2021) and expressing the capacity of plants to respond to multiple distinct environmental factors at the same time (Laughlin, 2014). Meanwhile, the seed collection location influenced only few traits, explaining a minor proportion of variation in timing of bud set and shoot and root growth. The result that heavier seeds from the afforested former field at L60A yielded slightly larger first-year seedlings with delayed phenology is in line with studies showing a positive correlation between seed size and seedling growth (Mikola, 1980) and those on the epigenetic memory of Norway spruce, a phenomenon where photoperiod and temperature during seed maturation confer long-lasting effects on functional traits for instance such that lower temperatures advance timing of bud set and cold acclimation in seedlings (e.g., Johnsen et al., 2005). Due to open-pollinated seeds at both field trial locations, the location effect in seedlings may also derive from genetic differences between the local pollen donors.

Variation observed in the progeny experiment suggests that averages in many above- and belowground functional traits differ due to soil conditions and that overall site productivity in general is influenced by local soils. Resembling field observations on Scots pine in Finland where increased overall growth has been noted on agricultural land (Haapanen, 1996), seedlings grew larger shoots in afforested field soil (the L60 afforestation treatment) than in native forest soil (the L61 forest treatment). Whether comparable variation within and among various shoot and root traits is expressed also in mature trees at the field trial locations is not known, and the more specific trait combinations contributing to long-term growth patterns in the field remain unidentified. For instance, nitrogen concentration in needles of seedlings suggested better nutritional status and higher capacity for photosynthesis in afforested field and native forest soils (e.g., Oleksyn et al., 1998; Wright et al., 2004) — two treatments that did not induce the largest shoot biomass. Pooled needle samples prevented us from including carbon and nitrogen concentrations in the PCA with other functional traits, but in global *in situ* data tree height was recently found to be detached from the leaf

economics spectrum (Maynard et al., 2022). Higher nitrogen concentrations in afforested field and native forest soils may be related to their greater ectomycorrhizal species diversity compared to agricultural soil, or to another belowground trait that was not measured but that would capture the absorptive capacity of roots better than the selected traits.

As in previous studies on Norway spruce (Velmala et al., 2013; Salmela et al., 2020; Salmela, 2021; Velmala et al., 2023), an analysis of shoot—root covariation in seedlings showed that shoot growth correlated positively with estimates of intact root system size. Although challenges in obtaining field measurements have limited data on the same belowground traits in natural ecosystems (e.g., Weigelt et al., 2021; Weemstra et al., 2023), recent *in situ* examples by Tumber-Dávila *et al.* (2022) and Annighöfer et al. (2022) provide evidence for similar positive correlations on wider geographic scales. The result that two frequently used measures of root architecture and morphology, branching intensity and specific root length, did not correlate with shoot growth agrees with prior intra- and interspecific studies on Norway spruce and other species (Kramer-Walter *et al.*, 2016; Hamberg et al., 2018; Salmela et al., 2020; Salmela, 2021; Velmala et al., 2023); this may be caused by the traits' poor linkage with specific physiological functions (Freschet et al., 2021). Further, large levels of unexplained variation in ratio variables like branching intensity and specific root length may be related to high sampling variation in their individual components, lowering the precision of their ratio (Jasieński & Bazzaz, 1999). Assuming the observed shoot—root correlations persist throughout ontogeny, it is possible that the enhanced resource foraging capacity that drives high grow rates in natural settings (Reich, 2014; Weemstra et al., 2016) is better described by estimates of root system size and closely associated metrics than the common descriptors of root architecture and morphology.

4.3. Patterns of genetic variation in seedling functional traits were constant across soil environments

Most shoot and root functional traits expressed genetic variation, but our sampling protocols may have been too coarse to reveal genetic differences in nitrogen concentration in needles and ectomycorrhizal colonisation rate in roots, two traits that have expressed genetic variation in previous studies with broader genotypic sampling (Oleksyn et al., 1998; Oleksyn et al., 2003; Velmala et al., 2013). In the case of branching intensity and specific root length, the lack of genetic variation in these 'soft' traits (i.e., traits that are easy to measure but whose association with specific functions is unclear; Freschet et al., 2021) may be explained by statistical properties of ratios that are based on variables estimated with varying precision (Jasieński & Fakhri, 1999). Genetic variation in branching patterns might also be dependent on the ectomycorrhizal colonisation (Velmala et al., 2014), complicating the comparison of studies executed in differing conditions.

Despite all seedling traits exhibiting plasticity across the soil treatments, we did not observe significant family × treatment (analogous to genotype × environment) interactions in the traits estimated in all 675 seedlings. The result that patterns of genetic variation in functional traits did not vary depending on the soil treatment may signal that the between-location variation in belowground factors is not sufficient to generate genotypic interactions with the environment, or that our treatments with contemporary soil samples did not reproduce the specific features of belowground conditions (e.g., patchiness of available resources in the early stages of the field trials) with more potential to cause interactions. Mikola et al. (2014) also found that local spatial heterogeneity can have large effects on average growth in *B. pendula* without impacting genotypic rank orders or variances. However, in our study significant interactions were not evident in field growth either when analysing only the seed-producing genotypes included in the progeny experiment, suggesting that a more diverse genotypic sample in terms of long-term growth patterns might have yielded different results (Saltz et al., 2018). Previously, Salmela et al. (2020) found family × soil treatment interactions in total root length and branching intensity within a southern Finnish Norway spruce population, but the current phenotyping approach was not as detailed due to the complexity of intact root systems. The difference between the two studies may also be due to the current sampling of genetic material that consists of artificial crosses between parents from different countries, or to the specific soil types screened in the experiment.

A limited genotypic sample in the progeny experiment, sampling only up to 60% of genotypic variation in growth in the field, may explain also why we did not find robust support for significant associations between genetic variation in seedling functional traits and longer-term growth in the field trials. It is also possible that cross-environment decoupling of trait variation occurs between closely controlled experimental conditions and more complex natural environments, particularly without large datasets (Poorter et al., 2016; Laughlin et al., 2017). Based on previous inter- and intraspecific findings (e.g., Hamberg et al., 2018; Salmela et al., 2020; Annighöfer et al., 2022), we expect high growth rates to be coupled with various features of root systems that describe their size and capacity to acquire resources, but it remains to be determined at which developmental stage such detectable trait covariation emerges in long-lived species.

5. Conclusions

We analysed trait variation in Norway spruce at different developmental stages, aiming to determine effects of soil variation on patterns of genotypic variation in growth in field trials. While we found that seedlings grew larger in soil from a former agricultural field and that all shoot and root functional traits were responsive to between-field trial location variation in soil factors, our treatments in a controlled experimental set-up did not generate genotype × environment interactions in seedlings. This may be due to the treatments not picking up the explicit belowground soil contrasts that are striking enough to prompt variable genotypic responses, or to a limited genotypic sample. In further analyses of existing field trials, potentially interfering effects of other sources of variation (e.g., trial designs, climate, management practices) need to be controlled for in order to single out explicit effects of soil factors. In conclusion, functional trait variation in trees is affected by belowground environmental variation and genetic factors for instance such that average growth is improved on agricultural land, but more research is needed for the identification of specific environmental agents that have capacity to give rise to genotypic rank order changes. Because the drivers of interactions can be difficult to identify even with multi-site datasets (e.g., Haapanen, 1996; Berlin et al., 2015; but see Chen et al., 2017), comprehensive genotypic sampling with treatments simulating natural conditions as accurately as possible will provide the most informative accompaniments to field observations. Although the underlying differences in root and shoot functional trait expression are not known, available evidence indicates that genetic correlations tend to be moderate and positive also between agricultural land and forest sites (Haapanen, 1996).

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CRediT authorship contribution statement

Tiina Ylioja: Writing – original draft, Resources, Methodology, Investigation. **Taina Pennanen:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Sannakajsa M. Velmala:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Katri Himanen:** Writing – original draft, Resources, Methodology, Investigation. **Matti J**

Salmela: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

The dataset is available at Zenodo (DOI: 10.5281/zenodo.10730284) and from the corresponding author.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.foreco.2024.121799.

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