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RESEARCH ARTICLE

ENVIRONMENTAL MICROBIOLOGY

The community of root fungi is associated with the growth rate of Norway spruce (*Picea abies*)

Leena Hamberg[1] | **Jarno Vanhatalo**[2,3] | **Sannakajsa Velmala**[1] | **Andy F. S. Taylor**[4] | **John MacKay**[5] | **Sébastien Caron**[6] | **Fred O. Asiegbu**[7] | **Risto Sievänen**[1] | **Pasi Raumonen**[8] | **Tuija Hytönen**[1] | **Taina Pennanen**[1]

[1]Natural Resources Institute Finland (Luke), Helsinki, Finland

[2]Department of Mathematics and Statistics, Faculty of Science, University of Helsinki, Helsinki, Finland

[3]Organismal and Evolutionary Biology Research Program, Faculty of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland

[4]The James Hutton Institute, Aberdeen, UK

[5]Department of Biology, University of Oxford, Oxford, UK

[6]Center for Forest Research and Institute for Integrative and Systems Biology, Université Laval, Québec, Canada

[7]Faculty of Agriculture and Forestry, University of Helsinki, Helsinki, Finland

[8]Computing Sciences, Tampere University, Tampere, Finland

Correspondence
Leena Hamberg, Natural Resources Institute Finland (Luke), P.O. Box 2, FI-00791 Helsinki, Finland.
Email: leena.hamberg@luke.fi

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Abstract

Our study delved into the relationship between root-associated fungi, gene expression and plant morphology in Norway spruce cuttings derived from both slow-and fast-growing trees. We found no clear link between the gene expression patterns of adventitious roots and the growth phenotype, suggesting no fundamental differences in the receptiveness to fungal symbionts between the phenotypes. Interestingly, saplings from slow-growing parental trees exhibited a higher richness of ectomycorrhizal species and larger roots. Some ectomycorrhizal species, typically found on mature spruces, were more prevalent on saplings from slow-growing spruces. The ericoid mycorrhizal fungus, *Hyaloscypha hepaticola*, showed a stronger association with saplings from fast-growing spruces. Moreover, saplings from slow-growing spruces had a greater number of Ascomycete taxa and free-living saprotrophic fungi. Aboveground sapling stems displayed some phenotypic variation; saplings from fast-growing phenotypes had longer branches but fewer whorls in their stems compared to those from the slow-growing group. In conclusion, the observed root-associated fungi and phenotypic characteristics in young Norway spruces may play a role in their long-term growth rate. This suggests that the early interactions between spruces and fungi could potentially influence their growth trajectory.

INTRODUCTION

The growth of trees depends largely on their ability to acquire nutrients (Näsholm et al., 2014). When resources are limited, trade-offs among growth, maintenance, storage, reproduction and defence are likely to occur (Franklin et al., 2014; Herms & Mattson, 1992). Beneficial root symbionts, such as mycorrhizal fungi, improve water and nutrient uptake (Chen et al., 2016), which can regulate the growth of trees and their root systems (Boukcim & Plassard, 2003). Yet, the development of roots also varies due to tree genetic diversity and varying environmental conditions, as well as their interaction (Salmela et al., 2020, 2024). Phenylpropanoid secondary metabolites, which play important roles in tree defence and stress tolerance consume a large fraction of the non-structural carbon (Franklin et al., 2014; Harding et al., 2014). Indeed, a direct competition between growth and defence has been proposed based on studies on poplars with contrasting growth types (Harding et al., 2014).

The microbiome of tree roots comprises mutualistic, endophytic and parasitic fungi (Kohout et al., 2021; Makarov, 2019). Among these, ectomycorrhizal (ECM) fungi are a crucial ecological group in boreal forest ecosystems (Smith & Read, 2010, p. 192). Norway spruce (*Picea abies* (L.) H. Karst.) naturally develops symbiotic ECM fungal associations with a diverse array of fungi, but as with other ECM hosts, only a few ECM species tend to dominate spruce ECM fungal communities (Gehring et al., 1998; Peter et al., 2001; Taylor et al., 2000; Tedersoo et al., 2008). The occurrence and abundance of dominant ECM fungal species in Norway spruce's fungal community are partly controlled by the host genotype (Velmala et al., 2013). This phenomenon has also been reported in pines *Pinus sylvestris* (Leski et al., 2010) and *Pinus elliottii* (Rosado et al., 1994), and poplar species *Populus deltoides* and *Populus trichocarpa* (Courty et al., 2011; Tagu et al., 2001, 2005). Host plants can have access to recalcitrant nitrogen (N) sources via ECM fungi and high ECM fungal richness has the potential to increase the functional complementarity of host nutrient acquisition by diversifying the production of fungal-derived exoenzymes (Velmala et al., 2014). However, the availability of soil N is a crucial factor that affects ECM community composition and functioning (Bogar et al., 2022; Lilleskov et al., 2002; Makarov, 2019).

In addition to ECM fungi, tree roots also host diverse communities of endophytic fungi (Jumpponen & Trappe, 1998; Kohout et al., 2021). The role of fungal endophytes on host plant physiology is less clear than ECM fungi and may range from beneficial to detrimental (Lukešová et al., 2015). In boreal regions, the endophytic communities in tree roots are mainly dominated by the so-called dark septate endophytes (DSE) (Grünig et al., 2008). Also, ericoid mycorrhizal fungi can colonise tree roots endophytically, although they mainly form mycorrhizal associations with ericoid dwarf shrubs (Vohník et al., 2013). Mycorrhizal fungi coexist with saprotrophic and pathogenic fungi (Johnson & Gehring, 2007; Piri & Hamberg, 2015). Saprotrophs decompose wood and litter, using dead organic matter for growth (Burke et al., 2011; Vasiliauskas et al., 2007), but some saprotrophic fungi can colonise fine roots of trees, thus exhibiting characteristics that are typical of mycorrhizal fungi (Vasiliauskas et al., 2007). Indeed, it is not always clear where the functional boundaries lie between fungal groups as some fungi exhibit features from several trophic modes (Kohout et al., 2021; Makarov, 2019).

The main aim of this study was to investigate differences between the morphology of young spruce saplings originating from fast- and slow-growing Norway spruces, and their root fungal communities. The results of our previous experiments focusing on the intraspecific diversity of Norway spruce showed that higher ECM fungal richness (Korkama et al., 2006, 2007) was associated with a faster growth rate of clonal 14-year-old spruces in the field. However, the observed positive relationship between the long-term growth rate and ECM fungal diversity in the field does not reveal the mechanistic role of ECM fungi for growth. Importantly, high ECM fungal diversity may also be a consequence of, rather than a cause of, faster growth. Thus, the present study aimed to address these gaps in mechanistic knowledge by investigating whether the superior height growth of trees with higher ECM fungal diversity and a higher proportion of athelioid fungi at 14 years of age (Korkama et al., 2006, 2007) is supported by a specific species or increased ECM fungal diversity in the early years of development.

We also aimed to compare the transcriptomes of adventitious roots of young saplings originating from fast- and slow-growing Norway spruces to identify genes that may play a role in the formation of diverging ECM fungal communities, particularly genes related to host defence, and to study the relationship between growth and the symbiotic ability of roots. Based on our previous findings there were no differences between spruce clones showing contrasting phenotypes in the ability to form ECM associations (Velmala et al., 2013, 2014).

The aboveground phenology and morphological traits of young Norway spruces were also investigated as they could provide indications of the initiation of an active growing period and features that may explain differences in growth rate (Skrøppa & Steffenrem, 2019). In Norway spruce, bud burst is known to show both phenotypic plasticity and genetic diversity (Salmela et al., 2020, 2024). Previous research on spruces originating from seeds has shown morphological differences in roots between the slow- and fast-growing Norway spruce phenotypes; slow-growing phenotypes had higher fine-root density, resulting in more condensed root systems (Velmala et al., 2014). Moreover, fast-growing phenotypes exhibited larger root extensions and allocated biomass further away from the seedling base (Hamberg et al., 2018).

First, we hypothesised that ECM fungal community composition contributes to the divergent growth rate of the Norway spruce phenotypes, and thereby differences in fungal communities can be detected before visible differences in the growth rate of the clones emerge. Second, we hypothesised that the slow- and fast-growing phenotypes do not show differences in their roots' gene expressions. Third, we hypothesised that bud burst happens earlier in saplings originating from fast- than slow-growing spruces and that these spruce groups have different growth habits in the early stage of development.

TABLE 1 The number of slow- and fast-growing Norway spruce (*Picea abies*) saplings included in this work. Saplings belonging to fast-growing clone 15 were available only for some investigations. Bud burst was observed in spruce saplings that grew for five growing seasons in a forest.

Phase	Growth rate								
	Slow				Fast				
	52	61	70	76	15	18	20	35	Total
Glasshouse study									
• 5 months in a glasshouse	3	-	3	-	-	3	1	3	13
• 18 months in a glasshouse	3	3	3	3	3	3	3	3	24
Forest study									
• 5 months in a forest	12	12	12	12	7	12	12	12	91
• 5 growing seasons in a forest	10	10	10	5	-	10	10	10	65

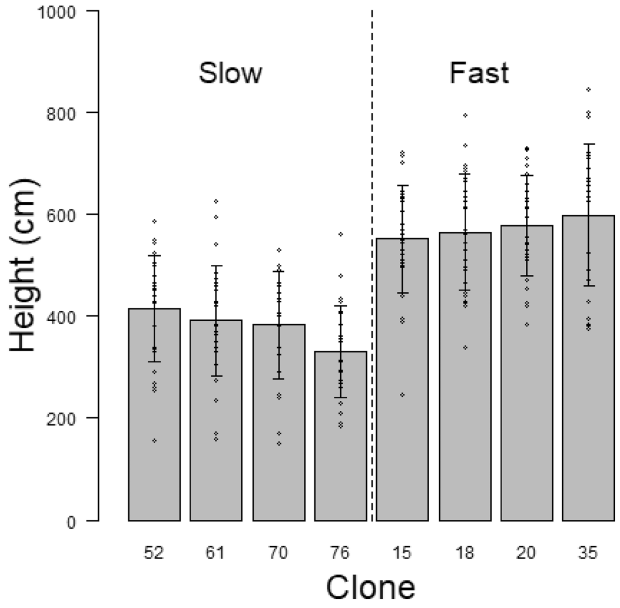

FIGURE 1 The mean height of 20-year-old parental Norway spruce trees (cm) used for propagation in the present study. 'Slow' refers to slow-growing trees (slow trunk height development) and 'Fast' to fast-growing trees (fast trunk height development). Means ± standard deviations and original values have been presented ($n = 213$, i.e., 24–27 per spruce clone).

EXPERIMENTAL PROCEDURES

Data

Rooting of the clonal cuttings in the nursery

Norway spruce (*P. abies*) saplings included (Table 1) were vegetatively propagated from branch cuttings of trees reported as slow- and fast-growing in several long-term field experiments (evaluations based on Korkama et al., 2006; Venäläinen, 1993). The cuttings were taken in 2009 from 20-year-old parental trees in the city of Pieksämäki (Pieksänmaa, 62°17′12″ N, 27°04′41″ E), Eastern Finland (Figure 1). Saplings cultivated from these cuttings were divided into two categories based on the trunk height development of the parental trees: slow- and fast-growing saplings.

One set of cuttings was grown for five- and 18-months for root RNA analyses in a glasshouse (glasshouse study, Table 1, Supporting Information 1.1). To study the growth and natural mycorrhisation of spruce saplings a larger set of cuttings was grown in a glasshouse and after that in a nursery field (altogether for 2 years) using common sapling production practices, that is, they were irrigated and fertilised regularly (forest study, Table 1).

RNA extraction, sequencing and bioinformatics of clonal cuttings

After five- and 18-month growth in a glasshouse, adventitious roots from a subset (glasshouse study, Table 1) were washed carefully under tap water and immediately detached from the plant, wrapped in aluminium foil and frozen in liquid nitrogen. Samples were stored at −70°C degrees until RNA extraction.

The total root RNA was extracted in a random order according to Chang et al. (1993) with modifications described in Pavy et al. (2008) (Supporting Information 1.2). RNA processing for hybridisation to the oligonucleotide microarray (GEO, platform no GPL15033, The Prostate Centre, Vancouver General Hospital, Canada) and initial array data processing and reading of the files, transformations, normalisations, and correction of redundant probes followed the steps described in Raherison et al. (2012) (Supporting Information 1.3). The array results were verified with RT-qPCR.

Outplanting of rooted cuttings into forest stand and sampling after 5 months and 5 years

On 18 May 2011, cuttings grown for the forest study (Table 1) were planted randomly in 12 rows (0.5 m between the rows), 0.4 m apart, on a *Vaccinium*

myrtillus type clear-cut site in southern Finland, Tuusula (60°21′42″ N, 25°02′55″ E). At that time, there was no difference in height between the slow- and the fast-growing saplings (the mean height for both groups was 12.4 cm). The planting area was 12 × 8 m^2 and was fenced to protect saplings from interference caused by humans and animals.

The total plant height was measured after 5 months in the field on 18 October 2011. On 28 October 2011, 91 saplings were excavated with their roots from the site (Table 1). The excavation was done carefully, and if the roots of different saplings were mixed, we separated them carefully to be sure that all roots belonged to the correct host sapling. Thus, only those roots surely attached to a host sapling were included in subsequent analyses. Roots were washed gently under tap water, and their fresh weight (g) was measured. Root tip density (the number of root tips divided by the root length, cm) was calculated for 48 spruce saplings (23 slow- and 25 fast-growing spruces, evenly distributed across clones) as follows: The total number of root tips from the most distant parts of roots from 12 root segments per sapling was divided by the total length of roots used to count root tips. Random samples from root tips, that is, from fine roots (<2 mm in diameter), ca. 4 × 50 mg per sapling, were taken from different parts of roots (from the roots in the proximity of the base of the saplings and from the outermost parts of roots) for subsequent laboratory analyses to investigate fungal communities of roots. The total dry weight of roots (including those parts used in root tip density investigations) and shoots were measured after they were dried for 2 days at 60°C.

After five growing seasons (20 October until 1 November 2015), 65 saplings were measured for height, they were excavated from the forest site and their roots were washed (Table 1). Altogether 2 × 200 mg of fine roots were collected per sapling. An additional sample from fine roots was collected in a 2 mL Eppendorf tube per sapling for spruces taller than 90 cm in height having root fresh weight of more than 65 g to obtain a representative sample from larger saplings. These samples represented 20% of both slow- and fast-growing spruces. Saplings and root samples were frozen at −20°C for further investigations. Frozen roots were defrosted, the length of the longest lateral root was measured and the number of root branches at least 2 mm in diameter was counted at discrete distances from the base of each sapling starting at a distance of 20 cm and then at 10 cm intervals up to 180 cm. Similarly, the number of root branches less than 2 mm in diameter, that is, fine roots, was counted at the same measuring points. Two representative root branches were photographed alongside a measuring tape. Photographs of 29 sapling roots (15 slow- and 14 fast-growing spruces, evenly distributed across clones) were converted to black-and-white in the GIMP image processing program and moved to WinRHIZO (Pro) Version 2017 for root tip density determination (10–12 root segments per sapling from the most distant parts of roots). Root tip density was counted similarly to the 2011 data. Shoot and root weight (g) were measured after they were dried at room temperature for a month and after that at 55°C for 24 h.

The above-ground parts of five-growing-season saplings were scanned from three directions in the laboratory using 3D laser Scanner Model Focus 3D S 120 (FARO, US) with resolution ¼ or ½ (4×, TLS Colour). Scanning data were moved to Scene 5.4 (FARO, US) to clean and combine point cloud data from different directions. Scanning data was processed into quantitative structure models (QSMs) using the TreeQSM program in Matlab (Calders et al., 2015; Raumonen et al., 2013). To get optimal QSMs, the TreeQSM reconstruction parameters were optimised for the best shoot structure of each spruce based on a metric considering shoot height, the number of branches and the first-order branches (originated from a trunk; second-order branches originated from the first-order branches, etc.), total length of branches and the first order branches. To consider stochasticity in TreeQSM reconstruction, five QSMs with the optimal parameters were reconstructed for each of the 28 spruce shoots (15 slow- and 13 fast-growing spruces, evenly distributed across clones) and the computed tree attributes were averages from the five QSMs. The data included measurements of stem diameter at 0.5 m (cm), stem and branch volume (dm^3), maximum distance from the main stem to branch tip (m), number of whorls (locations of branch junctions) and branches. Laser scanning for root systems of the spruces was not used since the resolution of laser scanning was not adequate for fine roots.

DNA extraction, sequencing and bioinformatics for root fungal communities

To investigate fungal communities in roots excavated after 5 months (forest study, Table 1), fungal DNA was extracted from four replicates per sapling following the instructions of NucleoSpin96 Plant II kit (Macherey Nagel) with small modifications (Supporting Information 1.4). Diluted, combined (three DNA samples per sapling 1:1:1) and purified PCR samples per sapling were sent to 454 sequencing to the Norwegian Sequencing Centre, NSC. A forward primer ITS1F was used as a forward primer (Gardes & Bruns, 1993) and ITS2 (White et al., 1990) as a reverse primer to investigate the ITS1 region of fungi.

Four root DNA samples were extracted per five-growing-season sapling (Table 1, NucleoSpin96 Plant II kit), and the combined samples per sapling were sent to Illumina sequencing (MiSeq, Institute of Biotechnology, University of Helsinki) to investigate fungal

communities of roots based on ITS2 regions of genes. Illumina MiSeq with ITS2 region was used since 454 sequencing was not technically supported anymore. A forward primer gITS7 (Ihrmark et al., 2012) and a reverse primer ITS4 (White et al., 1990) were used in sequencing.

Bioinformatic analyses of the root sequence data from five-month and five-growing-season saplings were performed using PipeCraft v.1.0 (Anslan et al., 2017, Supporting Information 1.4). Taxonomy assignment was performed using the UNITE's Reference Database (UNITE Community, 2017). Non-fungal operational taxonomic units (OTUs) were removed from the data. Also, OTUs with an e-value higher than e^{-25} were excluded. OTUs included in the data had at least the following percent identity (sequence similarity): for OTUs at the phylum level 70%, at the class level 75%, at the order level 80%, at the family level 85%, at the genus level 90% and the species level 95%. Query coverage was at least 85% for each OTU included in the data. OTUs with the same taxon specification were combined.

The total number of read counts after bioinformatic analysis was 126,590 in the data collected from five-month saplings and 826,166 in the five-growing-season saplings. There were 61 and 450 different OTUs in the roots of five-month and five-growing season saplings, respectively. Reduced data sets were used in analyses of differences in single OTUs between the spruce groups. These data sets included five and 86 analysable OTUs found from the roots of five-month and five-growing-season saplings, respectively. These OTUs occurred at least in 20% of samples and/or included at least 0.2% of read counts. The program FUNGuild was used to assign each OTU found in this study to one non-overlapping functional group based on their trophic mode (Nguyen et al., 2016).

Budburst

Apical bud burst was observed in 35 slow- and 30 fast-growing spruce saplings four times from the 18th of May until the 28th of May 2012 when all buds burst (Table 1). The occurrence of bud burst was recorded when the apical bud of a sapling was open, and green needles were visible.

Statistical analyses

Gene expression

Data analyses for gene expressions for 37 arrays of all saplings (glasshouse study, Table 1) were carried out using R version 3.2.3 (R Development Core Team, 2015), and Bioconductor 3.2 (Gentleman et al., 2004, Supporting Information 2.1). The identification of differently expressed genes from 10,403 positive probes between sampling timepoints and slow- and fast-growing saplings was done with two approaches with Bioconductor R packages: (1) significance analysis of microarrays estimating the false discovery rate by using *siggenes* (Schwender, 2012), and (2) the linear model approach to investigate differences between the gene expressions of the slow- and fast-growing spruces at two time points (after five and 18 months) by using *limma* (Ritchie et al., 2015). Weighted correlation network analysis with package WGCNA (Langfelder & Horvath, 2008) was used to identify modules of genes (from the 18-month-old spruces) that are associated with the stem height of 20-year-old parental trees (Figure 1).

Differences in the root and shoot morphology

All analyses were performed separately for morphology data collected after five months and five growing seasons (forest study, Table 1). Differences in shoot height (cm) and dry weight (g), main stem and branch volume (dm^3), main stem diameter (cm), main stem: branch ratio, maximum distance from trunk to branch tip (m), root maximum length (cm) and dry weight (g), shoot: root ratio for corresponding dry weights, and root tip density between the slow- and fast-growing groups were investigated with a Gaussian model (Supporting Information 2.2). We used Bayesian inference and the *rjags* package in R (Plummer, 2019; R Core Team, 2020). Differences in count variables (number of whorls and branches) between the shoots of spruce groups were investigated using a negative binomial model, which is an overdispersed version of the Poisson count distribution (Supporting Information 2.3). Three chains, each with 500 samples' burn-in and 2000 samples, were computed. Model convergence was evaluated using trace plots and a potential scale reduction factor (PSRF, Gelman et al., 2014, p. 285).

The number of lateral and fine roots of saplings as a function of the distance from the base of saplings was investigated using the function-valued quantitative trait model GPQTL mapping (Vanhatalo et al., 2019, Supporting Information 2.4).

Fungal associations with slow- and fast-growing saplings

Hierarchical modelling of species communities (HMSC) was used to analyse fungal data collected from roots excavated after 5 months and five growing seasons (forest study, Table 1) using the *Hmsc* package in the statistical program R (R Core Team, 2019; Tikhonov, Opedal et al., 2020; Tikhonov, Ovaskainen et al., 2020). HMSC is a multivariate hierarchical generalised linear mixed model fitted with Bayesian inference (Ovaskainen & Abrego, 2020, p. 39).

These models were estimated to investigate OTU richness in functional groups found in slow- and fast-growing spruces. The whole data set gathered after bioinformatic analyses included 61 OTUs after 5 months, and 450 OTUs after five growing seasons. However, in the five-month data, the number of OTUs per functional group was too low for HMSC analyses. In the five-growing-season data, the number of OTUs per functional group was treated as a response and the explanatory variables were (1) a spruce category (a factor with two levels: fast- or slow-growing saplings), and (2) the log-transformed total read count per spruce root to accounting for sequencing depth, that is, variation in the total count of sequences. The number of sampling units ($n = 65$), spruce clones ($n = 7$), and spatial components ($n = 65$), that is, the location of each spruce sapling on a site expressed as x- and y-coordinates, were included as random factors to the models. Two Markov Chain Monte Carlo (MCMC) chains with 150,000 iterations were used to estimate the models. The first 50,000 iterations were discarded as burn-in, and the rest were thinned by 100 yielding altogether 2000 posterior samples. Potential scale reduction factor (PSRF) of parameters and posterior trace plots were examined to verify the convergence of MCMC chains (Ovaskainen & Abrego, 2020, pp. 75–76). Associations between functional groups were investigated using a library *corrplot* in R (Wei & Simko, 2017).

HMSC was also used to investigate the abundance of fungal species and higher taxonomic units (OTUs) in the roots of slow- and fast-growing spruces. Here we used reduced analysable data sets (five OTUs in the five-month data, and 86 OTUs in the five-growing-season data) since OTUs with low frequency and number of read counts could not be analysed using HMSC. Models were estimated similarly as for functional groups, except that the read count of each OTU was used as responses in the analysis and the phylogeny of fungal species was included in the models to account for variation relating to taxonomy associations (Ovaskainen & Abrego, 2020, pp. 114–125, Supporting Information 2.5). The phylogenic structure of fungal species was obtained using the *ape* package in R, version 5.3 (Paradis & Schliep, 2018). For the five-growing-season data, two Markov Chain Monte Carlo (MCMC) chains with 2,750,000 iterations were used to estimate the models. The first 250,000 iterations were discarded as burn-in, and the rest were thinned by 500 yielding altogether 10,000 posterior samples.

Budburst

Differences in the bud burst timing in the slow- and fast-growing spruce clones were investigated using a Bayesian time-to-event model (Table 1, Supporting Information 2.6). The model was estimated in R using the *INLA* package (R Core Team, 2022; Rue et al., 2017).

All figures were drawn in R (R Core Team, 2022). Library *ggplot2* was used in figure drawing (Wickham, 2016).

RESULTS

Comparison of spruce clones after five- and 18-months growth in the glasshouse

Gene-expressions of roots in nursery-grown saplings

The gene expression as determined by RNA levels increased when roots developed: the overall expression of sequences encoding catalases and transferases, kinases (involved in the regulation of protein and macromolecule modification), cellular development and multicellular organismal processes in intracellular organelles and membranes was higher 18 than 5 months after root initiation. However, less than 0.5% of genes were differentially expressed between the timepoints.

Six differentially expressed genes were found between slow- and fast-growing clones 18 months after root initiation but with marginal statistical significance ($p = 0.051–0.075$) in both the significance analysis of microarrays and the linear model analysis (Supporting Information 3.1). These six genes were less expressed in the slow-growing clones, and are involved in metabolic, cellular or biological processes, transport, stress response and abiotic or biotic stimulus. The correlation network analysis grouped the gene expression profiles into seven modules, of which three had a significant positive relationship with the stem height of 20-year-old parental trees ($p < 0.001$). Genes associated with tree height were four unknown proteins, seven genes associated are induced by abiotic and biotic stimuli or stress (e.g., low temperature, desiccation and salt stress), one was transmembrane transporter, an enzyme involved in secondary metabolite synthesis, and two were structural constituents of a ribosome (Supporting Information 3.2). Moreover, three of the six genes that were marginally significantly differentially expressed between the slow- and fast-growing saplings in the data—collected 18 months after planting—were associated with tree height: an unknown protein, and proteins with transporter and transferase activity.

Morphology, phenology and fungal communities in forest-grown trees

Morphology

Five months after planting at a forest site, the means (θ parameter in the models) of height of spruce saplings originating from clones showing slow and fast shoot

TABLE 2 Differences in the root and shoot morphology of slow- and fast-growing Norway spruce (*Picea abies*) saplings 5 months ($n = 91$) and five growing seasons ($n = 65$) after the saplings were planted at a forest site. Main stem: branch ratio is main stem volume divided by branch volume and shoot: root ratio is shoot dry weight divided by the root dry weight. We present the posterior means ± standard deviations (SD) for the group-wise expected morphologies; that is, the estimated mean parameters (θ for Gaussian models and λ for Negative-Binomial models) for both spruce groups. We present also posterior probabilities that the mean, and in the case of Gaussian models the SD ($1/\sqrt{\tau}$), of slow-growing spruces is higher than that of the fast-growing spruces p (slow > fast). Note: the probability of the fast-growing spruces having higher values than the slow-growing ones is 1- p (slow > fast). Variables not measured in a specific year have been indicated with a line (-).

	5 months after planting				Five growing seasons after planting			
	Slow, θ or λ	Fast, θ or λ	Comparison		Slow, θ or λ	Fast, θ or λ	Comparison	
Variable	Mean ± SD	Mean ± SD	$P(\theta_{slow} > \theta_{fast})$	P $(SD_{slow} > SD_{fast})$	Mean ± SD	Mean ± SD	$P(\theta_{slow} > \theta_{fast})$	P $(SD_{slow} > SD_{fast})$
Shoot height (cm)	21.6 ± 3.6	20.6 ± 4.2	0.779	0.153	81.7 ± 22.0	86.1 ± 15.0	0.234	0.976
Shoot dry weight (g)	5.0 ± 1.5	3.6 ± 1.6	0.994	0.384	76.7 ± 64.1	70.3 ± 36.1	0.695	0.998
Main stem volume (dm^3)	-	-	-	-	0.10 ± 0.12	0.06 ± 0.06	0.814	0.995
Main stem diameter (cm)	-	-	-	-	0.79 ± 0.51	0.63 ± 0.17	0.843	1.000
Branch volume (dm^3)	-	-	-	-	0.09 ± 0.11	0.07 ± 0.05	0.728	0.994
Main stem: branch ratio	-	-	-	-	1.38 ± 1.00	1.03 ± 0.85	0.802	0.726
Number of whorls	-	-	-	-	3	2	0.901	-
Number of 1st order branches	-	-	-	-	14	11	0.790	-
Number of 2nd-5th order branches	-	-	-	-	13	11	0.791	-
Max. distance to branch tip (m)	-	-	-	-	0.26 ± 0.10	0.30 ± 0.10	0.177	0.540
Shoot: root ratio	3.9 ± 0.6	4.4 ± 2.1	0.161	<0.001	4.6 ± 1.2	5.3 ± 1.2	0.032	0.555
Root maximum length (cm)	-	-	-	-	95.5 ± 41.7	74.3 ± 31.9	0.908	0.921
Root dry weight (g)	1.3 ± 0.5	0.9 ± 0.5	0.992	0.357	18.7 ± 16.7	14.4 ± 8.9	0.839	1.000
Root tip density (tips per cm)	21.4 ± 5.2	18.4 ± 4.5	1.000	1.000	5.2 ± 1.6	5.7 ± 1.8	0.213	0.354

height growth did not differ significantly ($p\ [\theta_{slow} > \theta_{fast}] < 0.90$, Table 2). However, shoots and roots were heavier and the root tip density was higher in the slow-growing than in the fast-growing spruce saplings with high posterior probability ($p\ [\theta_{slow} > \theta_{fast}] \geq 0.95$). There was more variation (measured using SD) in the shoot: root ratio among the fast- than slow-growing spruces after 5 months ($1-[p\ (SD_{slow} > SD_{fast})] > 0.99$, Table 2).

Five growing seasons after planting, the height and weight of shoots did not differ significantly between the slow- and fast-growing spruce saplings ($p\ [\theta_{slow} > \theta_{fast}] < 0.90$). The number of whorls (locations where branches start to grow around a trunk in different directions) was marginally significantly higher for slow-growing than fast-growing spruces ($p\ [\lambda_{slow} > \lambda_{fast}] = 0.90$, λ parameter in the negative binomial models denotes to means). The maximum distance from a trunk to the branch tip was longer among the fast- than the slow-growing spruces with moderate posterior probability (0.82).

After five growing seasons, the longest lateral roots of slow-growing spruces extended ca. 21 cm further from the base of the saplings than in the fast-growing ones ($p\ [\theta_{slow} > \theta_{fast}] = 0.91$, Table 2). The slow-growing spruces had more lateral (≥2 mm in diameter) and fine roots (<2 mm in diameter) than the fast-growing saplings along the root length, especially at distances 30–120 cm from the base of saplings (Figure 2), but root tip densities did not differ any more five growing seasons after planting to a forest site (Table 2). The shoot:root ratio was higher in the fast-than in the slow-growing saplings: in the fast-growing spruces, the relative difference between shoot and root weight was clearly larger

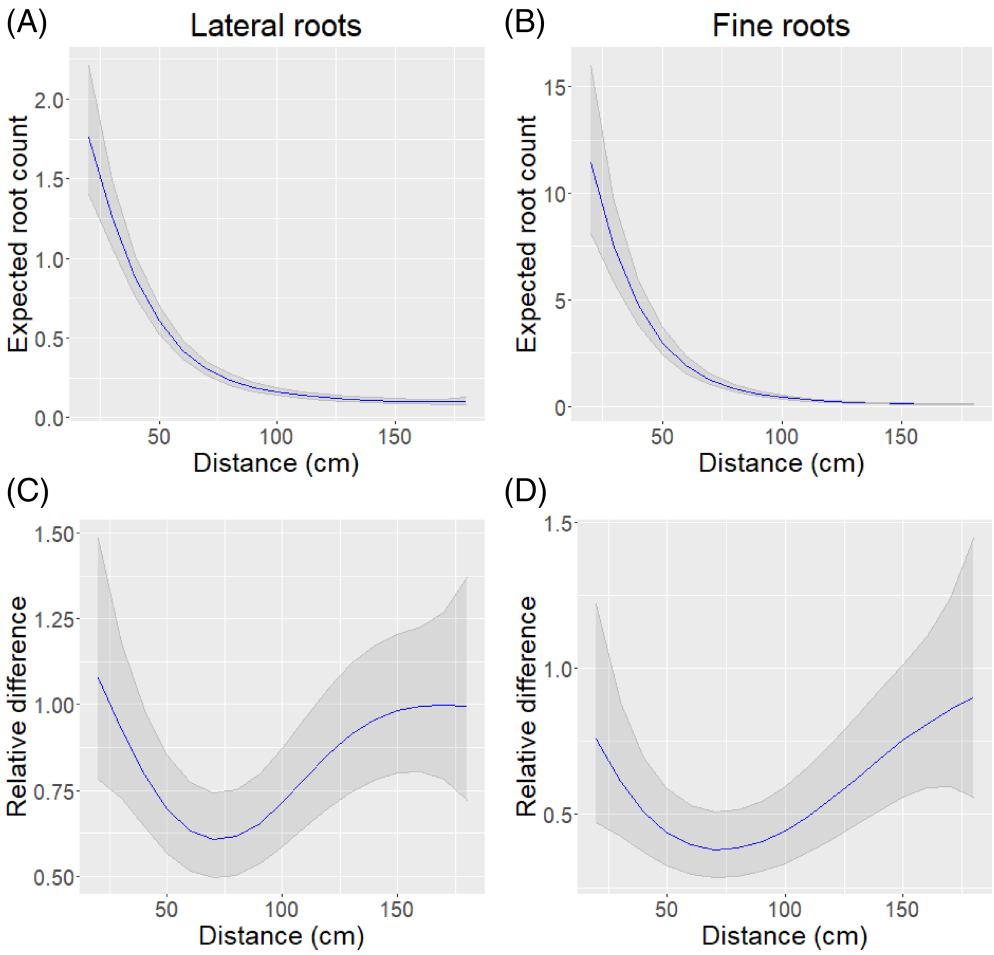

FIGURE 2 The posterior mean (solid line) and 95% credible interval (shaded area) of the expected number of lateral (A) and fine roots (B) of the slow-growing Norway spruce saplings as a function of the distance from the base of saplings five growing seasons after the spruces were planted to a forest site. Relative differences in lateral (C) and fine roots (D) of fast- and slow-growing spruces (E[fast]/E[slow]) are shown to reveal differences between the spruce groups. A relative difference of less than one indicates lower numbers among fast- than slow-growing spruces. Lateral roots are ≥2 mm and fine roots <2 mm in diameter.

(shoot weight higher compared to root weight) than in the slow-growing group ($1-p\,[\theta_{slow} > \theta_{fast}] = 0.97$).

After five growing seasons, variation in shoot height, shoot weight, trunk volume and diameter, branch volume, and root weight and maximum length was higher among the slow-growing spruces ($p\,[SD_{slow} > SD_{fast}] \geq 0.90$).

Budburst

Budburst did not occur at different times in the fast-growing and the slow-growing spruces (the posterior mean of β_{slow} was 0.939 and 95% credible interval [−2.488, 4.385]).

Fungal community of roots

Five months after the spruce saplings were planted at a forest site, Thelephoraceae (OTU IDs 27, 30–33 and 36–37) was the most abundant taxon on both the roots of slow- and fast-growing spruces accounting for ca. 90% of all DNA sequences in both groups (Figure 3, Supporting Information 3.3). The second most abundant OTU in terms of DNA sequence representation was the ECM fungus *Inocybe jacobi*, but it was clearly lower in abundance (6%). Four growing seasons later, the majority of the DNA sequence reads derived again from ECM fungi (Supporting Information 3.4). The most abundant OTUs both among the slow- and fast-growing spruces were the ECM fungi *Amphinema* spp. (44 and 29%, respectively), and *Lactarius rufus* (11 and 18%) (Figure 3, Supporting Information 3.3). Interestingly, some ECM species occurred more frequently on the roots of slow- than the fast-growing spruces, such as *Amanita fulva* (occurred on 31% of the slow- but only on 10% of fast-growing spruces), *Lactarius necator* (34 vs. 10%), *Russula decolorans* (20 vs. 3%), *Russula vesca* (23 vs. 13%) and *Tomentella terrestris* (23 vs. 10%).

In the data collected after five growing seasons, the number of saprotroph, pathotroph-saprotroph-symbiotroph (taxa with no clear life strategy) and ECM species (OTUs)

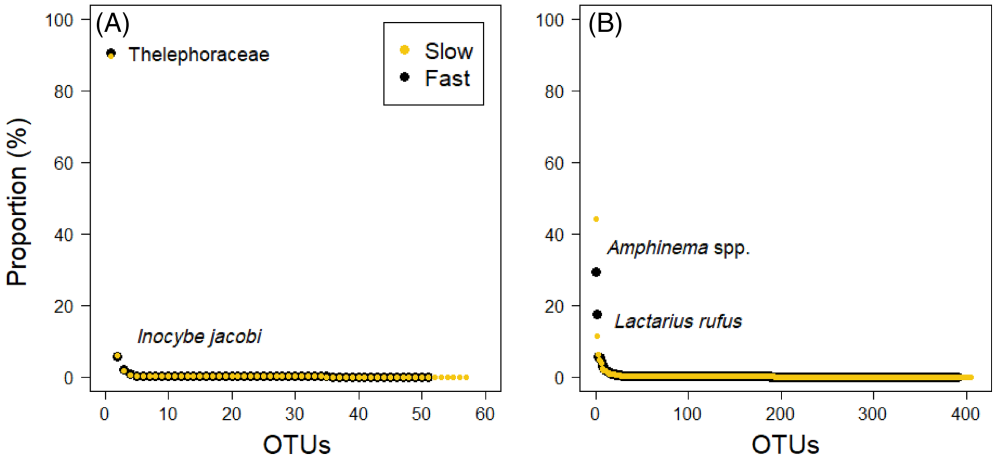

FIGURE 3 The most abundant operational taxonomic units (OTUs) among the slow- and fast-growing spruce saplings: (A) 5 months and (B) five growing seasons after the saplings were planted at a forest site. The proportion is the raw DNA read counts out of the total number of raw read counts (%) separately for slow- and fast-growing spruces. Note that the symbols for OTUs in slow- and fast-growing spruces are partly overlapping. See Supporting Information 3.3.

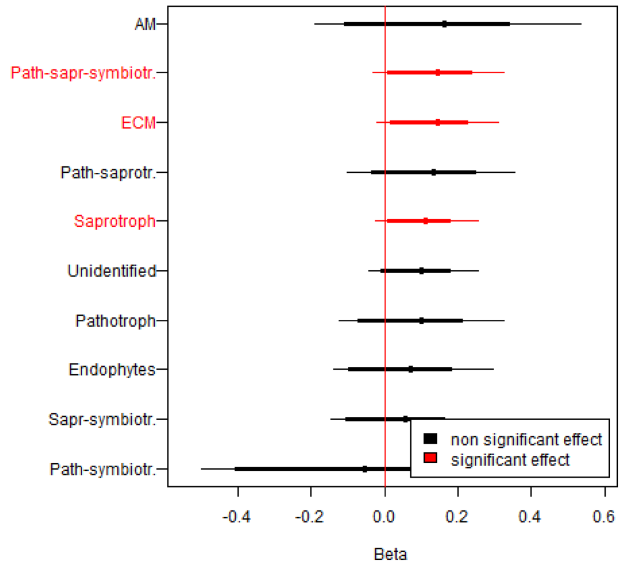

FIGURE 4 Posterior densities for the slow-growing spruce effect, $ß_{slow}$, on the number of operational taxonomic units (OTUs) in functional groups based on the hierarchical modelling of species communities (HMSC). Dots show the posterior means, thick lines the 80% central posterior range and thin line the 90% central posterior range. Posterior mean values more than zero indicate higher values in the OTU numbers of the slow- than in the fast-growing Norway spruce saplings five growing seasons after the saplings were planted at a forest site. Results relating to the whole data set (450 OTUs, 10 functional groups) are presented. Marginally significant posterior probabilities for effect ($0.90 \le p\ (ß_{slow} > 0) < 0.95$) have been presented in red in the figure.

was indicatively higher among the slow- than fast-growing spruces ($0.90 \le p\ [ß_{slow} \ge 0] < 0.95$, Figure 4). Based on the HMSC analysis, spatial association in OTU numbers (transition in OTU richness in space) was found to extend up to 9 m within a field site. Furthermore, a positive association (occurrence at the same time) with at least 95% posterior probability was found among many functional groups (Supporting Information 3.5). In the data collected after 5 months, the number of OTUs in different functional groups could not be analysed (values were too low for HMSC).

No phylogenetic signal nor spatial effect was found in the species community analysis (HMSC) regarding the single OTU data collected after 5 months. Furthermore, no differences between the slow- and fast-growing spruces in terms of single OTU abundances were found. However, there was a negative association (found together less often than expected by chance) between Thelephoraceae (OTU IDs 27, 30–33 and 36–37) and *Laccaria* sp. ($p \ge 0.95$, Supporting Information 3.6).

After five growing seasons, spatial association in OTUs (transition in OTU abundance) extended up to 0.72 m within the field site but no phylogenetic signal between the samples was found. The read count of 16 OTUs was significantly or marginally significantly higher ($p\ [ß_{slow} > 0] \ge 0.90$) in the roots of slow- than fast-growing spruces (Figure 5, Supporting Information 3.7). Most of the OTUs were saprotrophic fungi (Auricularia spp., *Cladophialophora chaetospira*, Hyaloscyphaceae, *Mortierella macrocystis*, *Penicillium armarii*, *Peterozyma toletana* and *Umbelopsis dimorpha*), and rest were pathotroph-saprotroph (Herpotrichiellaceae), saprotroph-symbiotroph (*Chaetosphaeria* sp.), symbiotroph (*Lactarius necator*, *Leptodontidium* spp., *Wilcoxina rehmii*) or unidentified OTUs belonging to Ascomycota, Chaetothyriales, Helotiales and Thelephoraceae. The ericoid mycorrhizal fungus, *Hyaloscypha hepaticola* was the only OTU that was clearly higher in the roots of fast-growing saplings. Many positive associations were found between OTUs (Supporting Information 3.8).

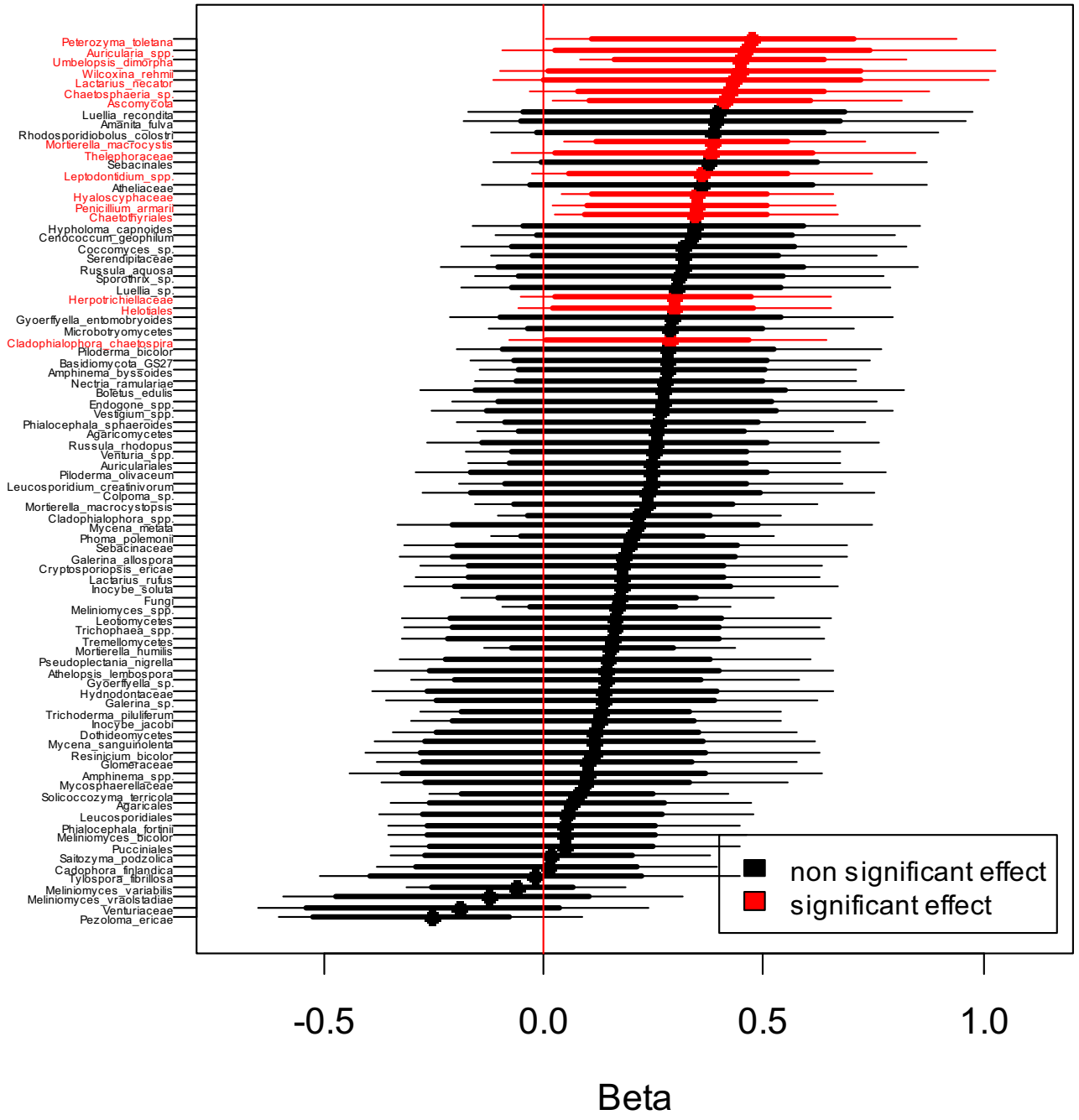

FIGURE 5 Posterior densities for the slow-growing tree effect, $ß_{slow}$ (based on hierarchical modelling of species communities [HMSC]), on the read counts of single operational taxonomic units (OTUs) in spruce roots, excavated five growing seasons after the spruces were planted at a forest site. Dots show the posterior means, thick lines the 80% central posterior range and thin line the 90% central posterior range. Posterior mean values more than zero indicate higher values in the read counts of the slow- than in the fast-growing Norway spruce saplings. Significant and marginally significant posterior probabilities $P(ß_{slow} > 0) \geq 0.90$ have been presented in red in the figure. See Supporting Information 3.7 where the OTU names from the bottom to the upper part of the figure are presented. *Pezoloma ericae* is currently called *Hyaloscypha hepaticola*.

DISCUSSION

We investigated differences between the morphology of young spruce saplings originating from fast- and slow-growing Norway spruces, and their root fungal communities. The fast-growing spruce saplings supported slightly different fungal associates on their roots compared to the slow-growing ones, but contrary to Korkama et al. (2006, 2007), the number of ECM species was higher among the slow-growing spruces than the fast-growing ones. Nevertheless, some species-specific similarities in ECM fungi were found in the studied clonal rootlets compared to our earlier study by Korkama et al. (2007); higher relative abundance of

Ascomycetes in slow-growing clones and a tendency showing more athelioid *Tylospora* sp. in the fast-growing ones. As we hypothesised, no clear differences in spruce root gene expressions were found soon after root formation. Gene expression patterns in roots did not show any differences in defence-related transcripts, but a small number of genes related to metabolic, cellular and biological processes and in response to abiotic or biotic stimulus were indicatively more active in the fast-growing clones. Against our hypothesis, the timing of apical bud opening in spring did not differ between the spruce phenotypes, but fast-growing Norway spruce trees allocated fewer resources to roots than slow-growing ones in their first years in the field, indicating that they acquire needed nutrients with less extensive root systems.

Unlike our expectation based on the previous observations (Korkama et al., 2006, 2007), the number of ECM fungal OTUs was lower on the fast-growing spruces, possibly because of the rather fertile outplanting site which may reduce the need for fast-growing clones to invest C to fungi. A decrease in ECM fungal abundance in response to increased N availability has been found in many studies and has been associated with a reduction in the allocation of photosynthate C to the below-ground organs and their ECM fungi (e.g., Choma et al., 2017; Högberg et al., 2011). As the allocation of C to the belowground part of a plant decreases, ECM fungi are less competitive against other soil microorganisms (Högberg et al., 2003; Lilleskov et al., 2011). For example, in beech (*Fagus sylvatica*), low carbon productivity has resulted in low ectomycorrhizal diversity (Druebert et al., 2009). Furthermore, ECM species producing big fruiting bodies and occurring typically in older trees, such as *Lactarius necator* and *Amanita fulva* (Rudawska et al., 2018), were more common in the slow-growing spruce saplings. Symbiosis with these ECM fungal species may be costly for young spruce trees and the lower frequency of these species in the fast-growing spruces may thus partly support their better growth. On the contrary, inconspicuous fruiting bodies forming athelioid ECM fungi *Tylospora* sp. were found to be more common in the fast-growing saplings (Supporting Information 3.3), similarly as in their 14-year-old parental clones in the study by Korkama et al. (2006).

Furthermore, the occurrence of saprotrophic fungal species or taxa with no clear life strategy (pathotroph-saprotroph-symbiotroph species) was lower among the fast-growing spruces. Saprotrophs are decomposers of organic material, and therefore important in decomposing and nutrient cycling (Crowther et al., 2012) but their functional role in spruce roots is not well known. Higher saprotroph species abundance on the slow-growing roots may indicate that the slow-growing spruces show higher root turnover rates or are more prone to disturbances such as root herbivory. In the early stages of mycelial decomposition, fungal communities are almost exclusively composed of saprotrophs (Brabcová et al., 2016). Saprotroph species *Penicillium armarii* and *Mortierella macrocystis* were more abundant on the roots of slow- than fast-growing spruces (Supporting Information 3.7). Interestingly, these species belong to genera that are found to be important primary colonisers of decaying fungal mycelia (Brabcová et al., 2016)—also supporting the idea of more disturbed roots of slow-growing clones.

Both ECM fungi and saprotrophs were more abundant in the roots of slow- than fast-growing spruces; however, their establishment following a random experimental design suggests that differences were unlikely due to microsite variations. Possibly, the more extensive lateral roots and more numerous fine roots of the slow-growing spruces (see Table 2, Figure 2) can host more ECM and saprotrophic species or taxa with no clear life strategy (pathotroph-saprotroph-symbiotrophic species) than the more confined roots of fast-growing saplings.

The ericoid mycorrhizal fungus *Hyaloscypha hepaticola* (Mrnka et al., 2020) and *Meliniomyces* species were more associated with the fast- than slow-growing spruces (Supporting Information 3.7). Recent research has indicated that *H. hepaticola*, asexual *Meliniomyces* species and *Cadophora finlandica* are congeneric, and all of them belong to the genus *Hyaloscypha* (Fehrer et al., 2019, Mrnka et al., 2020, see also Grelet et al., 2010). These fungal species are also known as DSE which can improve plant nutrient uptake and growth, increase tolerance against root pathogens and herbivores, and adverse environmental conditions (Alberton et al., 2010; Fadaei et al., 2020; Mandyam & Jumpponen, 2005; Mrnka et al., 2009; Schulz & Boyle, 2005; Sharples et al., 2000; Yang & Goulart, 2000). DSE fungi have been isolated from apparently healthy roots where they often co-occur with ectomycorrhizas, but they are also common on non-mycorrhizal tree roots (Ahlich & Sieber, 1996; Summerbell, 1989, 2005). In different understory vegetation species, *H. hepaticola* has significantly improved the nitrogen and phosphorous content of leaves, and plant—especially shoot—growth (i.e., shoot:root ratio, Myers & Leake, 1996, Kosola et al., 2007, Kowal et al., 2018, Fadaei et al., 2021). This fungal symbiont has been reported to be cost-efficient for host plants since it provides more nutrients for the host's growth than it requires C (Kowal et al., 2018). Therefore, the costs of the *H. hepaticola* in terms of C for the fast-growing hosts are low—probably an important feature in the rather poor boreal forest soils.

The lack of differences in root fungal communities after 5 months may be due to the nursery growth substrate as suggested by strong dominance by nursery-derived *Thelephoraceae* (OTU IDs 27, 30–33 and 36–37). Thus, at this stage, no spatial associations in

fungal communities were found. On the contrary, after five growing seasons in a forest site, the fungal communities of the roots were more divergent as indicated by a spatial effect extending over 0.72 m within the field site (HMSC: single OTUs data). Roots extended ca. 75–96 cm from the base of the saplings (see Table 2), that is, roughly the same distance as the spatial effect observed in the fungal data.

Studying how coniferous trees grow is long-lasting, and as the sequencing methods have evolved much faster, the root fungi in our experiments have been analysed by single root-tip based Sanger sequencing (Korkama et al., 2006), and in the present study, by using two different sequencing platforms: 454 sequencing and Illumina Miseq for data collected 5 months and five growing seasons after saplings were planted to a forest site, respectively. Although it has been found that both sequencing platforms used in the present study produce rather similar results, the Illumina generates shorter reads but provides sequencing at greater depth than 454 metabarcoding (Luo et al., 2012). Thus, results based on OTU data gathered from different time points are not fully comparable. Still, we believe that the most pronounced differences between the data sets are due to differences in growing conditions and age of saplings (younger saplings grown for a short time vs. older saplings grown for a longer time in the field).

The microarray analysis enabled comparisons of transcript levels for roughly one-third of the expressed genes in the Norway spruce genome, which comprises almost 30 thousand genes (Nystedt et al., 2013). Only a few marginally differently expressed genes could be identified between the roots of slow- and fast-growing saplings at the age of 18 months. The differentially expressed genes with sequences linked to metabolic, cellular and biological processes, transport and responses to abiotic or biotic stimulus were consistently higher in the fast-growing clones and some of them were positively associated with the stem height of 20-year-old parental trees of these Norway spruce saplings. Nevertheless, the genes annotated and known so far to be involved in the regulation of susceptibility to fungal infection and defence showed no systematic pattern related to the phenotypic growth groups of Norway spruce clones, and thus major differences in susceptibility of the clones to ECM fungi are not expected. Molecular analysis of ECM formation has revealed that it is the fungal partner that secretes molecules manipulating host immunity and metabolism, thereby promoting symbiosis establishment (Plett & Martin, 2015). However, more research is needed to reveal what is happening between ECM fungi and the roots of a host plant during the formation of ECM symbiosis.

Soon after planting in the field, the fast-growing spruce saplings showed lower root biomass and fewer root tips per cm than the slow-growing saplings. In an earlier study, where spruces were grown from seeds and cultivated in fertilised nursery peat, the roots of the fast-growing group extended further away from the base of the seedlings and had higher numbers of branches and tips than the slow-growing group (Hamberg et al., 2018). The differences in observations may be explained in part by differences in root formation between cut branches and seed-derived saplings; cuttings have no primary root (radicle) and all roots are adventitious. The contradictory results may indicate the phenotypic plasticity of spruces in their growth patterns in response to environmental variation and we cannot rule out genetic variation (Salmela et al., 2020).

Five growing seasons after the saplings were planted in a forest site, the shoot biomass and root tip density did not differ anymore between the spruce groups. The roots of slow-growing saplings were still heavier and extended ca. 20 cm further from the base of saplings and had more lateral and fine roots along the root length than those of the fast-growing spruces. Yet, the fast-growing spruces tended to be taller than the slow-growing ones, indicating differences in the root-to-shoot allocation of resources between the two groups. Efficient water and nutrient uptake could be possible without an extensive root system if a suitable root symbiont is available (see Boukcim & Plassard, 2003), and therefore, a plant may allocate resources more toward shoot growth.

The laser scanning-based morphotyping of the stems indicated that the slow- and fast-growing groups have different growth habits already in the sapling stage. The typical growth habit in the fast-growing spruces comprised better stem height development including thinner but taller main stems with fewer whorls, and branches that extend further away from the trunk than in the slow-growing spruces. Whether ca. 4 cm longer branches in the fast-growing spruces can explain the higher growth due to increased light uptake remains to be investigated in the future.

CONCLUSIONS

At the initiation of root formation, there does not seem to be any clear functional separation or major differences in gene expression between the fast- and slow-growing spruce saplings. Thus, we conclude that susceptibility to ECM symbiosis formation is not a major factor in shaping the root fungal communities. However, differences in root symbionts were observed in the young Norway spruce saplings along with early differences in growth rates, and the observed differences in fungal communities were partly consistent with our previous results on the same clonal origins, suggesting a key role of ECM fungi in the early development of Norway spruce. The roots of fast-growing spruces were shorter and included less resource-demanding ECM fungi, saprotrophic fungal species, and taxa with no

clear life strategy (pathotroph-saprotroph-symbiotroph species) than the roots of slow-growing spruces having more Ascomycetous fungi.

AUTHOR CONTRIBUTIONS

Leena Hamberg: Data curation (lead); methodology (lead); formal analysis (lead); visualisation (lead); writing—original draft (lead). **Jarno Vanhatalo**: Formal analysis (supporting); supervision (lead); writing—review and editing (equal). **Sannakajsa Velmala**: Conceptualisation (equal), investigation (equal); methodology (equal); data curation (equal); formal analysis (equal); writing—original draft, review, and editing (equal). **Andy FS Taylor**: Conceptualisation (equal), writing—review and editing (supporting). **John MacKay:** Conceptualisation (equal), methodology (equal); writing—review & editing (equal). **Sébastien Caron:** Investigation (supporting). **Fred O. Asiegbu:** Conceptualisation (equal), writing—review & editing (supporting). **Risto Sievänen:** Methodology (supporting); writing—review & editing (supporting). **Pasi Raumonen:** Methodology (supporting); software (supporting); writing—review & editing (equal). **Tuija Hytönen:** Investigation (supporting). **Taina Pennanen**: Funding acquisition (lead); conceptualisation (lead); project administration (lead). methodology (equal); writing—review & editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

Microarray experiment data have been submitted to the Gene Expression Omnibus (GEO) under accession numbers GSE35624 and GSE246281. The sequence data have been submitted to the NCBI database under BioProject PRJNA1033583 (BioSample accessions SAMN38035401–SAMN38035556): https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1033583. OTU tables including sequence read counts with sample metadata are openly available in the Dryad database: https://doi.org/10.5061/dryad.7pvmcvf13.

ORCID

Leena Hamberg https://orcid.org/0000-0002-0009-7768
Jarno Vanhatalo https://orcid.org/0000-0002-6831-0211

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SUPPORTING INFORMATION

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