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RESEARCH ARTICLE

Plant–soil feedbacks among boreal forest species

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Abstract

1. Plant–microbial interactions in soils are considered to play a central role in regulating biodiversity in many global ecosystems. However, studies on plant–soil feedbacks (PSFs) and how these affect forest stand patterns in boreal regions are rare.
2. We conducted a fully reciprocal PSF glasshouse experiment using four boreal tree species. *Alnus glutinosa*, *Betula pendula*, *Picea abies* and *Pinus sylvestris* seedlings were grown under controlled conditions in sterilised soil with or without soil inoculum collected under mature trees of each of the four species. Bacterial, fungal and oomycete communities in the rhizosphere were investigated using metabarcoding and correlated with differences in plant biomass.
3. Alder grew best in conspecific soil, whereas birch grew equally well in all soil types. Pine and spruce grew best in heterospecific soil, particularly in soil from their successional predecessor. Ectomycorrhizal fungi (EMF) enhanced the growth of most seedlings, and Actinomycetota supported alder and birch growth and fungal plant pathogens hampered pine growth. Increased growth was linked to the ability of trees to recruit specific EMF and root-associated fungi in heterospecific soils.
4. *Synthesis*. This study experimentally examines the influence of root-associated microbiota on the growth of boreal tree species. The observed plant–soil feedbacks mirror the successional patterns found in boreal forests, suggesting a possible contribution of soil microbiota to the successional progression. Species-specific ectomycorrhizal fungi and a few bacteria rather than fungal plant pathogens or oomycetes seem to drive the feedbacks by promoting seedling growth in heterospecific soils.

KEYWORDS
Alnus glutinosa (alder), bacterial community, *Betula pendula* (silver birch), fungal community, *Picea abies* (Norway spruce), *Pinus sylvestris* (Scots pine), plant–soil feedbacks, succession

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1 | INTRODUCTION

Feedback mechanisms between plants and soil microbiota are major drivers of global biodiversity, including diversity in tropical and temperate forests (Bennett et al., 2017; Domínguez-Begines et al., 2020; Mangan et al., 2010), meadows and grasslands (Bauer et al., 2015; Klironomos, 2002), and Mediterranean-climate shrublands (Teste et al., 2017). A plant–soil feedback (PSF) occurs when plants alter abiotic and biotic soil characteristics, which, in turn, promote or hamper their own performance, as well as the performance of surrounding plant populations. The type of feedback mechanism determines inter- and intraspecific dynamics in forests. Positive feedbacks, where tree-mediated changes in soil biota aid conspecific seedling establishment, often result in the dominance of one tree species over the others (Lance et al., 2020). Conversely, negative PSFs, which promote the establishment of heterospecific seedlings, can result in either the coexistence of two or more species or compositional shifts. For example, two tree species can have reciprocal negative PSFs, meaning that the growth of each species is poorer in conspecific soil and better in the soil of the other species. This would lead to the stable coexistence of the two species, resulting in mixed stands (Mangan et al., 2010). By contrast, nonreciprocal negative feedback of multiple plant species could promote unidirectional shifts in species composition (van der Putten et al., 2013), in which different species only coexist during the transition between successional stages (Bauer et al., 2015; Kardol et al., 2006). Even though PSFs may affect the directional dynamics of plant communities, we lack an understanding of the mechanisms that underpin these feedbacks, particularly those driven by soil microbial communities.

The mechanisms underlying biotic PSFs may depend on the type and strength of interactions different species establish with soil microorganisms and the successional stage of the forest. It is generally accepted that life traits determine which plants will dominate early successional stages (Petrokas et al., 2020). However, successional patterns in forests may also be affected by biotic PSFs modifying soil conditions, making the area more favourable for the establishment of certain tree species (Bennett et al., 2017; Domínguez-Begines et al., 2020; Mangan et al., 2010). In boreal forests, ectomycorrhizal fungi (EMF) and nitrogen-fixing bacteria facilitate tree establishment in disturbed areas by providing nutrients to the host in exchange for sugars from photosynthesis (Policelli et al., 2020). EMF also create a sheath around vulnerable feeder roots, offering protection from soil-borne root pathogens (Bennett et al., 2017; Colinas et al., 1994). Conversely, root pathogens, such as many oomycetes, which concentrate near adult host trees can inhibit conspecific seedling establishment by increasing mortality rates, thus favouring the establishment of other nonhost species (Domínguez-Begines et al., 2020). In tropical forests, the driving force for negative PSFs is widely considered to be soil-borne pathogens accumulating near adult plants, which decrease the regeneration capabilities of the most abundant species, thereby promoting the formation of mixed-species stands (Bell et al., 2006; Mangan et al., 2010). In temperate forests, EMF offer a competitive advantage to their hosts by allowing these trees to access soil nutrients that would otherwise be less accessible or by offering protection from pathogens (Bennett et al., 2017; Bennett & Klironomos, 2019), resulting in positive PSFs. However, even though PSFs can result in changes in soil characteristics (e.g. via plants and microorganisms changing the nutrient profile or water availability) and microbial communities (by promoting or hampering the growth and establishment of host species) (Bennett & Klironomos, 2019), little is known about how microorganisms involved in PSFs might contribute to forest stand composition in boreal forests. In boreal forests, pathogen-driven negative feedbacks may be less important than the positive feedbacks provided by mutualistic soil organisms that facilitate seedling establishment (as is the case in many temperate forests) (Bennett et al., 2017), although the relative importance of soil-borne pathogens, EMF and beneficial microorganisms in tree regeneration in northern forests is mostly unknown.

In European boreal regions, young forest stands are dominated by light-demanding species such as silver birch (*Betula pendula*) or alder (*Alnus glutinosa*). Birch is a typical early coloniser that inhabits areas that were cleared of tree cover as a result of a disturbance (Angelstam & Kuuluvainen, 2004; Beck et al., 2016), whereas alder often forms stands along rivers and lakes but can also be found away from water sources, particularly in the relatively humid boreal forests (Condé et al., 2003; Sundseth et al., 2009). Early-colonising deciduous trees are less common overall in boreal forests than Norway spruce (*Picea abies*) or Scots pine (*Pinus sylvestris*), which dominate in mature forests (Angelstam & Kuuluvainen, 2004; Caudullo et al., 2016), either in mixed stands with other species or, especially in the case of spruce, in pure stands covering large areas of the boreal forest (Caudullo et al., 2016; Houston Durrant et al., 2016b). Nonreciprocal negative feedbacks promoting the establishment of heterospecific species could potentially facilitate this progression from an early successional forest to a mature spruce or pine forest. For example, later-successional species might be less susceptible to the pathogens accumulated around early successional species which could promote their establishment. However, despite the well-established successional dynamics in boreal forests, little is known about how soil microbial communities and PSFs contribute to their establishment.

The aim of this study was to understand how PSFs, particularly those driven by microorganisms, influence stand dynamics in boreal forests. To investigate this, we examined how species-specific soil communities influence growth patterns of boreal tree species. Specifically, our aim was to address the following research questions: (I) Do tree seedlings grow better in soils inoculated with microbial communities from heterospecific trees? (II) Are negative feedbacks between the studied species driven by the recruitment of beneficial or harmful microorganisms? To address the first question, we conducted a glasshouse experiment using seedlings of four species that are common in boreal forests. In a fully reciprocal design, we grew 336 seedlings of alder (*Alnus glutinosa*), silver birch (*Betula pendula*), Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies*) in pots with sterilised soil inoculated with a small amount of forest soil inoculum (as in Mangan et al., 2010) or without forest soil inoculum, which simulated

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soil not colonised by microorganisms. We measured seedling biomass after 5 months and analysed the composition of fungal, bacterial and oomycete communities in the rhizosphere to determine the effect of specific microbial guilds on seedling growth (question II). Finally, to further address question II and to test whether negative feedbacks were caused by oomycete pathogens, we applied oomycete-specific biocide to half the pots throughout the experiment to suppress the oomycete population. Overall, this study focusses on exploring the PSFs among boreal tree species which have received comparatively less attention than PSFs in other ecosystems, such as grasslands or tropical forests.

2 | MATERIALS AND METHODS

2.1 | Soil collection

The soil used as bulk was collected near the campus of the Swedish University of Agricultural Sciences in Uppsala (59°48′33.0″ N 17°39′49.1″ E). The upper horizon of the soil was excavated from an old pastureland that had not been recently colonised by any vegetation. The soil was a mineral soil that was poor in organic matter with a nitrogen content of *c*. 0.1%. We chose this soil to simulate the initial successional stage in disturbed soils. The soil was sterilised at Ionisos Baltics by gamma irradiation (35–50 kGy) and then stored in sealed bags for 7 days at room temperature until the seedlings were planted.

Soil used as inoculum was collected from Fiby urskog (59°52′55.1″ N 17°21′13.1″ E), an old-growth mixed forest in central Sweden, with permission from Uppsala Länsstyrelsen (permit number 521-3573-2018). Soil was collected from below dominant silver birch (*Betula pendula* Roth), Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* (L.) H. Karst.) and alder (*Alnus glutinosa* (L.) Gaertn.) trees growing in the same stand. The litter layer was removed and 0.5 kg of soil in contact with the root system of the adult tree was collected from three points around each tree, that is a total of 1.5 kg of soil per tree (fresh weight). Soil from six mature trees of each species was sampled and kept separated, considering the soil from each tree as an independent soil sample (Reinhart & Rinella, 2016; Gundale et al., 2019). Although the sampled tree species coexist in the same forest, individual trees were several metres apart, allowing for the collection of soil conditioned by an adult of a specific species. The inoculum soils were sieved through a 2-mm mesh and then stored at room temperature for 12–24 h until the seedlings were planted.

2.2 | Seedling production

We obtained pine, spruce and birch seeds from Swedish seed orchards and used alder seeds that had been collected from healthy-looking trees in the vicinity of Uppsala. All seeds were surface sterilised before sowing. Pine and spruce seeds were immersed in a mixture of 4.7% NaOCl and one drop of TWEEN® 20 for 15 min and then thoroughly rinsed in sterile water and left to soak for 24 h in sterile water. After 24 h, floating seeds were discarded, and the remaining seeds were sown in vermiculite. Alder and birch seeds were sterilised in a mixture of 2.5% NaOCl and one drop of TWEEN® 20 for 10 min, rinsed and then immediately sown in vermiculite. All seeds were grown in a clean growing facility at 20°C with a 16 h:8 h, light:dark photoperiod for 1 month. During this period, they were watered weekly with distilled water and once with a nutrient solution 2 weeks after sowing. After that, the seedlings were not fertilised again.

2.3 | Experimental design

We established a fully reciprocal pot experiment in July 2018 using 336 pots in total with one seedling per pot. Seedlings of each of the four studied species were planted in: 24 pots with conspecific soil inoculum; 12 pots with each of the three heterospecific soil inocula (36 pots in total); and 24 pots without inoculum, to simulate soil which has not been colonised by microbiota. One-month-old seedlings were removed from the vermiculite and planted in 2-L pots containing 94% V/V gamma-sterilised bulk soil and 6% V/V inoculum soil. We used a small portion of inoculum soil together with the same bulk soil across all pots to control for abiotic differences between inoculum soils (Mangan et al., 2010). Hereafter, we will use the term 'inoculated soil' to refer to this mixture of sterile and inoculum soil. We will use the terms 'alder soil', 'birch soil', 'pine soil' and 'spruce soil' to refer to each of the four inoculated soils used in the experiment. We use the term 'sterile soil', to refer to pots containing only sterile bulk soil.

The total experimental period was 5 months. During July and August, the seedlings were grown in a glasshouse without artificial light or temperature control. After that, they were grown at 20°C and under a 16 h:8 h, light:dark photoperiod for 3 months until harvest. There was no experimental blocking, and the distribution of the pots in the glasshouse was completely random in order to avoid micro-environmental biases due to light, temperature or moisture that could affect seedling growth. The pots were elevated above the glasshouse tables to prevent cross-contamination via root contact with run-off water. Throughout the experiment, the pots were watered twice a week. Every 2 weeks, half of the pots were treated with 150 mL of Subdue® MAXX® (metalaxyl) at a concentration of 0.05 mL L^{-1} to prevent oomycete growth in order to determine the contribution of oomycete pathogens to the feedbacks. The remaining pots were watered with the same amount of distilled water.

2.4 | Measurements and sample preparation

At harvest, the above-ground part of the plant was separated from the root system. To collect the rhizosphere soil for microbial profiling, three random subsamples of fine roots were sampled at harvest, placed in 50 mL centrifuge tubes containing 30 mL of

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phosphate-buffered saline (PBS) and vortexed to detach the soil. The roots were removed and the tubes were centrifuged the same day at 10,000×*g* for 10 min. The PBS was decanted, and the soil pellet was kept at −20°C until DNA extraction.

To evaluate whether our experimental design introduced abiotic differences between inoculated soils, at the end of the experiment, a sample of the soil was collected at random from the pot, avoiding the rhizosphere, and sent to the Department of Soil and Environment at the Swedish University of Agricultural Sciences (Uppsala) for chemical analysis (i.e. pH, phosphorus and potassium via ammonium lactate extraction, total carbon and total nitrogen). The rest of the soil was discarded and the root system was washed with water to remove all the remaining soil. In addition, clusters of *Frankia* nodules on the roots of alder seedlings were counted. Shoots and roots were then dried separately at 60°C for a week and dry matter was determined.

Two to three dried leaves were chosen randomly from each seedling and ground in a ball mill until a fine powder was obtained. Subsamples were then weighed in tin capsules and sent to the UC Davis Stable Isotope Facility (Davis, CA, USA) for ^{15}N isotope analysis to estimate the fraction of nitrogen in the plants that may have been provided by EMF (Hobbie & Hobbie, 2008).

2.5 | DNA extraction, library preparation and sequencing

DNA was extracted from each of the 360 soil pellets (336 rhizosphere samples from the pot experiment + 24 soil samples used as inoculum that were collected from mature trees) using the NucleoSpin® Soil kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions.

2.5.1 | Fungi

The fungal ITS2 was amplified using the forward gITS7 (Ihrmark et al., 2012) and reverse ITS4/ITS4arch (Kyaschenko et al., 2017; White et al., 1990) primers, which were extended by a linker base (T), a unique eight-base identification tag (differing in at least three positions) and a terminal base (C) (Clemmensen et al., 2016). Each reaction consisted of 25 ng template, 2.75 mM $MgCl_2$, 0.5 μM, 0.3 μM and 0.15 μM of the ITS7, ITS4 and ITS4arch primers, respectively, and 0.025 U μL^{-1} polymerase in buffer (DreamTaq Green, Thermo Scientific, Waltham, MA, USA). Cycling conditions involved an initial denaturation at 95°C for 5 min, followed by 24–31 cycles (optimised for each sample) of 30 s at 95°C, 30 s at 56°C and 30 s at 72°C, with a final elongation for 10 min at 72°C. PCR products were purified using the AMPure kit (Beckman Coulter, Brea, CA, USA) and quantified fluorometrically with the Qubit DNA quantification kit (Thermo Fisher Scientific, Waltham, MA, USA). They were pooled in equal amounts, and each pool was cleaned again with the EZNA Cycle Pure kit (Omega Bio-Tek, Norcross, GA, USA). Four pools were sequenced using two Pacific Biosciences Sequel SMRT cells per pool after the addition of sequencing adapters by ligation (SciLifeLab, Uppsala, Sweden).

2.5.2 | Bacteria

Bacterial and archaeal communities were studied by amplifying the V3–V4 region of the 16S rRNA gene with primers pro341F and pro805R (Takahashi et al., 2014) following a two-step PCR protocol (Berry et al., 2011). In the first step, amplification was performed using primers with Nextera adaptor sequences in duplicate 15-μL reactions containing 10 ng DNA, 0.25 μM of each primer, 0.5 mg mL^{-1} BSA and Phusion High-Fidelity PCR Master Mix (Thermo Fisher Scientific). Sample DNA was amplified under the following reaction conditions: 3 min at 98°C, followed by 25 cycles of 30 s at 98°C, 30 s at 55°C and 30 s at 72°C, and a final elongation of 10 min at 72°C. PCR products were pooled and purified using the AMPure PCR purification kit (Agencourt Bioscience Corporation, Beverly, MA, USA). The second amplification step was performed in duplicate 30 μL reactions with Phusion High-Fidelity PCR Master Mix, 10% of the final concentration of purified product from the first PCR, and 0.2 μM of both forward and reverse primers with Nextera barcoding regions. The reaction conditions were the same as those used in the first step, except that the number of cycles was decreased to eight and the elongation step of each cycle was increased to 45 s. PCR products were purified as in the first step and pooled in equimolar ratios. The three pools were sequenced on one Illumina® MiSeq lane per library at SciLifeLab using 2 × 250 bp paired-end sequencing chemistry.

2.5.3 | Oomycetes

For oomycetes, ITS1 was amplified using the oomycete-specific primers ITS6 and ITS7 (Vannini et al., 2013). One tag was used for each soil sample. Template DNA was amplified in a 25-μL reaction with DreamTaq polymerase in its buffer (Thermo Fisher Scientific, Waltham, MA), 2 μL of template, and each primer at a final concentration of 0.5 μM. The reaction conditions were as specified in Vannini et al. (2013) apart from the following modifications: 32–35 cycles, an initial denaturation step at 95°C for 30 s and a final elongation of 6 min. Amplicons were purified with NucleoMag® NGS Clean-up and Size Select (Macherey-Nagel) following the manufacturer's protocol. The concentration of the purified PCR product was measured using the Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific). The four pools were sequenced using one lane of Illumina® MiSeq at SciLifeLab.

2.6 | Analysis of metabarcoding data

Quality control, screening and clustering of fungal and oomycete sequences into operational taxonomic units (OTUs) were performed using the bioinformatics SCATA pipeline (scata.mykopat.slu.se).

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2.6.1 | Fungi

Sequences with less than 200 base pairs, an average base quality of <20 or a score of <10 at any position, were discarded from the dataset, as were any sequences with less than 90% primer match or 100% tag match, or a mismatched tag. Homopolymers were collapsed into three bases, and the dataset was further processed as in Castaño et al. (2020). After the removal of plant OTUs, 594,134 reads remained, which were clustered into 2513 OTUs with clustering conditions as specified by Castaño et al. (2020). The 456 most abundant fungal OTUs (each with at least 12 sequences), representing 94% of total fungal reads were taxonomically and functionally classified. Each OTU was compared against the UNITE and INSD databases using massBLASTer in PlutoF (Abarenkov et al., 2010). We used PROTAX (Somervuo et al., 2016) in PlutoF to obtain the first taxonomical profile of each OTU, using a 50% classification probability. We assigned taxonomic identities and functional classification to the OTUs using FungalTraits (Põlme et al., 2020), which were further curated using the UNITE database, DEEMY (Agerer & Rambold, 2017) and published literature (Clemmensen et al., 2015; Sterkenburg et al., 2015).

2.6.2 | Bacteria

The 16S rRNA gene sequences were trimmed using the FASTX Toolkit (http://hannonlab.cshl.edu/fastx_toolkit), and paired-end sequences were merged using PEAR (Zhang et al., 2014). Quality filtering of the assembled sequences (maximum expected error value of 1), OTU clustering and chimaera detection were performed with VSEARCH (Rognes et al., 2016). The resulting OTU sequences were aligned with SINA (Pruesse et al., 2012) and taxonomy was assigned using the SILVA database as a reference (release 138.1), removing any OTUs classified as 'Chloroplast' or 'Mitochondria'. The resulting dataset, containing 7300 OTUs, was then partitioned into core taxa, which are generally abundant and widely distributed, and satellite communities, which mostly occur in lower abundance and at fewer sites, following Jeanbille et al. (2016). Previous research has shown that the core community is predominantly shaped by environmental factors, whereas satellite communities are mostly dispersed randomly and do not reflect treatment effects (Jeanbille et al., 2016; Magurran & Henderson, 2003; Ulrich & Zalewski, 2006). Therefore, only the 4041 core OTUs, representing 99% of reads, were used in subsequent analyses.

2.6.3 | Oomycetes

Sequences shorter than 150 base pairs were removed and the remaining sequences were screened for primers (90% match), sample tags (100% match), average base quality (>10) and individual base quality (>2). Homopolymers were collapsed into three bases, and the remaining sequences were clustered into OTUs with a clustering distance of 1%. Clusters were matched to reference sets from *Phytophthora*-ID and a list of sequences obtained from NCBI. The resulting dataset was further cleaned to remove tag jumps and OTUs with less than two reads and that were present in less than two samples. To identify clusters, OTUs from the cleaned dataset were identified using BLAST and the resulting file was analysed in MEGAN (Huson et al., 2007) using a least common ancestor analysis (minimum score of 50 and minimum identity of 70%). Sequences placed in the SAR supergroup (a group of phyla that includes Stramenopiles, Alveolates and Rhizarians) by MEGAN were then manually identified with BLAST, keeping only those with more than 80% coverage and a similarity of >95%. Sequences with ≥99% similarity were identified to species level, those with 97%–98.9% similarity to genus level, and the remaining OTUs were identified only as oomycetes. The final dataset contained 61 OTUs.

2.7 | Statistical analyses

Statistical analyses were performed either with R version 4.1.2 (R Core Team, 2021) or with JMP® Pro 16.0.0 (SAS Institute Inc., Cary, NC, 1989-2023). One-way ANOVA was used to determine the effect of soil inoculum, soil type (sterile or inoculated) and metalaxyl treatment on seedling growth. The responses of the four seedling species to different inoculum soils varied, so each species was analysed separately. Seven plant specimens that died during the experiment were excluded. Post hoc comparisons were made using Fisher's LSD test ('LSD.test' function from the package agricolae (de Mendiburu, 2021)) with a false discovery rate adjustment at $\alpha = 0.05$. The relative abundance of each fungal guild or bacterial phylum was correlated with seedling biomass, as was the effect of soil and seedling species on the soil microbial community composition. Seedling biomass was square-root-transformed to fit normality assumptions.

The effect of seedling species, soil inoculum and metalaxyl treatment on community composition was assessed separately for fungi, bacteria or oomycetes by performing a permutational multivariate analysis of variance (PERMANOVA) using the 'adonis2' function from the vegan package (Oksanen et al., 2020), which was set to test the marginal effects of the variables. For this, OTU abundances were standardised using the Hellinger transformation. Differences in community composition were visualised with a principal coordinate analysis based on the Bray–Curtis dissimilarity index. The association between seedling root and shoot biomass and the composition of the microbial communities was analysed using the 'envfit' function of the vegan package. To calculate α-diversity indices (species richness and Shannon diversity index), corresponding functions from the vegan package were used. To analyse frequency shifts of microbial taxa between different seedling-inoculum combinations, we performed an indicator species analysis using the 'multipatt' function from the indicspecies package (De Caceres & Legendre, 2009).

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3 | RESULTS

3.1 | Effect of soil on seedling biomass

We observed that soil inoculum had an effect on seedling growth, with each tree species showing a unique pattern of growth when exposed to soil inoculum collected from adult trees of each of the studied tree species (Figure 1a; Table 1). Spruce, pine and alder grew significantly better in inoculated soil than in sterile soil, reaching average biomass values that were 3.5, 5 and 2.5 times greater than in sterile soil, respectively. By contrast, there was no significant difference in birch growth in sterile or inoculated soil. Alder was the only species that grew better in conspecific soil than in heterospecific soils, exhibiting a positive PSF (Table 1). Alder seedlings achieved the greatest biomass when grown in soil with inoculum from alder or birch, and their growth was associated with a depletion of foliar δ^{15}N and a lower foliar carbon to nitrogen (C:N) ratio. Birch grew equally well in almost all soils, including sterile soil, and showed higher foliar C:N ratios than any other species. Birch showed the poorest growth in alder soils (Figure 1), and foliar δ^{15}N values were also lowest in alder soils. Pine seedlings grew best in birch soil. Average pine biomass in birch soil was 64% greater than in conspecific pine soil. Although birch soil contained slightly higher levels of nitrogen and potassium than some of the other soils, there were no significant differences in nitrogen or potassium content between soils planted with pine seedlings (Figure S1). Spruce seedlings grew best in pine soil, where their average biomass was 55% greater than in conspecific soil. Foliar δ^{15}N values of pine and spruce seedlings were higher in seedlings grown in soils with inoculum than in sterile soils, showing the opposite pattern to that found in alder. Phosphorus content did not differ significantly between inoculated soils. Metalaxyl treatment did not affect the seedling growth of any of the studied species ($R^2 < 0.05$, $p > 0.05$ for each species), possibly because it did not significantly alter oomycete community composition in most seedlings (Tables 2 and 3).

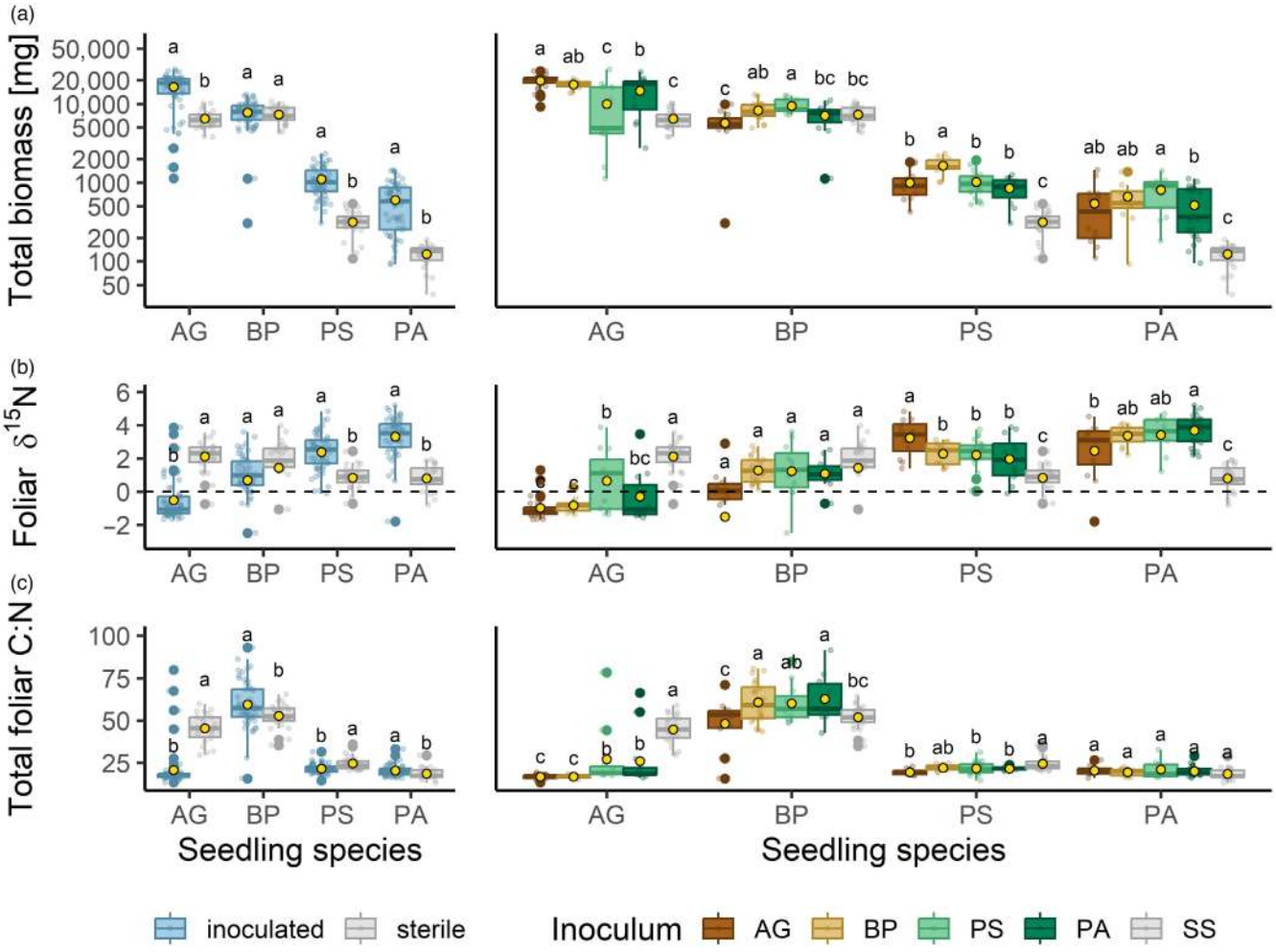


FIGURE 1 Performance of tree seedlings of *Alnus glutinosa* (AG), *Betula pendula* (BP), *Pinus sylvestris* (PS) or *Picea abies* (PA) in sterile soil inoculated with soil from older trees of the same four species and in sterile soil without inoculum (sterile soil; SS). The total biomass (mg at harvest; a), foliar δ^{15}N (b) and total foliar C:N ratio (c) of each seedling species (*x*-axis) in different soil types (fill colour). The yellow dots show mean values, while the dots below and above the boxplots show outliers. The letters above the plots were obtained through a Fisher's LSD test with a false discovery rate adjustment performed separately for each seedling species; different letters indicate significant differences between treatments within each seedling species ($p \leq 0.05$).

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TABLE 1 Effect of soil on seedling biomass shown as R^2 values from one-way ANOVA.

Seedling	Sterile vs. inoculated	Conspecific vs. heterospecific	Inoculated soil
Alder	0.39***	0.15**	0.29***
Birch	0.01 ns	0.03 ns	0.26***
Pine	0.45***	0.02 ns	0.36***
Spruce	0.27***	0.04 ns	0.08 ns

Note: Sterile soil is only included in the analysis of sterile versus inoculated soil. Inoculated soil is a factor with four levels (i.e. alder, *Alnus glutinosa*; birch, *Betula pendula*; pine, *Pinus sylvestris*; and spruce, *Picea abies*).

Abbreviation: ns, not significant.

**$p<0.01$, ***$p<0.001$.

TABLE 2 Effect of experimental factors on fungal, bacterial and oomycete community composition shown as R^2 values from PERMANOVA.

	Fungi	Bacteria	Oomycetes
Inoculated vs. sterile bulk soil	0.13***	0.09***	0.04***
Seedling species	0.18***	0.18***	0.05***
Conspecific vs. heterospecific soil	0.01**	0.01 ns	0.00 ns
Inoculum species	0.04***	0.08***	0.02 ns
Metalaxyl	0.00 ns	0.00 ns	0.01 ns

Note: Sterile soil is only included in the analysis of inoculated versus sterile bulk soil.

Abbreviation: ns, not significant.

**$p<0.01$, ***$p<0.001$.

3.2 | Soil microbial communities

The root-associated microbial community of the seedlings differed between inoculated and sterile soils (Table 2; Figure 2). Although the bulk soil was sterile at planting, it did not remain sterile throughout the experiment, likely due to the microorganisms present in the glasshouse environment and on the seedlings. However, the differences in soil community composition between inoculated and sterile soils suggest that the community recruited by the seedlings in inoculated soils originated from the forest inoculum soils and not from the nursery or the greenhouse.

The PCoA analysis showed compositional differences between the soil communities in different seedlings in the experiment and the communities under adult trees in the field. Fungal and bacterial communities in the pots differed more between tree species than in the original soil collected from the field (Figures S2 and S3). Seedling species was the most significant factor affecting the microbial community structure, accounting for 18% of the fungal and bacterial variation (Table 2). Seedlings tended to recruit similar taxonomic/functional groups across all inoculated soils (Figure 3; Tables S1 and S2). Nevertheless, variation in the composition of fungal and bacterial communities was observed within seedling species depending on the type of inoculated soil, with inoculum explaining 4% and 8% of the fungal and bacterial variation, respectively. This effect was not observed for oomycete communities (Tables 2 and 3), whose composition was similar across inoculated soils for all seedlings (Figure 2; Table S3). However, despite these similarities, differences between tree types (Table 2) are possible due to multivariate dispersion differences, as both conifer trees had lower average distances to the median than broadleaved species (0.4173, 0.4985 vs. 0.6013, 0.6076 for spruce, pine, alder and birch, respectively).

Within alder seedlings, inoculum species explained 19% of the variation in fungal community composition (Table 3). The composition of fungal guilds in birch and alder soils was similar, with EMF the dominant guild (Figure 3). Alder growth was mainly associated with the relative abundance of EMF ($p=0.003$, $R=0.38$; Table S4) and Actinomycetota ($p<0.001$, $R=0.59$; Table S5) and was also correlated with nodule formation ($p<0.001$, $R=0.69$). The highest levels of nodule formation occurred in alder soils. Nodule formation was also correlated with Actinomycetota abundance ($p<0.001$, $R=0.58$), which was correlated with a higher soil nitrogen content ($R=0.26$, $p=0.04$).

The microbial community of birch was predominantly shaped by the soil inoculum, which explained 10% of fungal and 16% of bacterial variation (Table 3). However, the composition in terms of fungal guilds and bacterial phyla was relatively similar across all inoculated soils (Figures 2 and 3; Tables S1 and S2). The relative abundance of Actinomycetota correlated with birch growth ($p=0.037$, $R=0.27$; Table S5); however, the relative abundance of EMF or fungal pathogens did not ($p=0.418$, $R=0.11$; $p=0.846$, $R=0.03$, respectively; Table S4).

For both conifers, the largest differences in terms of fungal composition were observed between inoculated and sterile soils (Table 3). The composition of fungal guilds in inoculated soils was relatively similar within and between the two coniferous species (Figure 3; Table S1) but differed greatly from that of sterile soil, which contained primarily saprotrophs and pathogens. EMF were positively correlated with the growth of both species (pine, $p=0.027$, $R=0.29$; spruce, $p=0.003$, $R=0.39$; Table S4), whereas fungal pathogens, which were relatively more abundant in pine seedlings inoculated with spruce soil than with pine or birch soil, correlated negatively with pine growth ($p=0.048$, $R=-0.26$; Table S4).

Indicator species analysis showed that alder seedlings were able to recruit EMF from most soils; however, only *Tomentella testaceogliva*, an EMF associated with alder inoculum, was significantly correlated with alder growth (Figure 4; Table S6). Birch recruited EMF from all inoculated soils (Table S6). However, *Hyaloscypha finlandica*, an indicator species in pine soil, was the only EMF that was positively correlated with birch biomass (Figure 4). Pine seedlings were able to recruit some EMF and root-associated fungi in birch soil (Table S6), whereas indicator species in conspecific soil were moulds and saprotrophs. An EMF from the genus *Trichophaea* and a root-associated fungus from the genus *Oidiodendron* were indicator species for pine growing in birch soil and were positively correlated with seedling

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TABLE 3 Effect of soil and treatment on the fungal, bacterial and oomycete community composition in the rhizosphere of each seedling shown as R^2 values from PERMANOVA.

	Fungi			Bacteria			Oomycetes		
Seedling	Live vs. sterile	Inoculum species	Metalaxyl	Live vs. sterile	Inoculum species	Metalaxyl	Live vs. sterile	Inoculum species	Metalaxyl
Alder	0.23***	0.19***	0.01 ns	0.12***	0.19***	0.01 ns	0.03 ns	0.08 ns	0.06*
Birch	0.10***	0.10***	0.01 ns	0.10***	0.16***	0.01 ns	0.02 ns	0.07 ns	0.02 ns
Pine	0.29***	0.13***	0.01 ns	0.19***	0.14***	0.01 ns	0.08***	0.07 ns	0.03 ns
Spruce	0.29***	0.10**	0.02 ns	0.20***	0.12**	0.02 ns	0.10***	0.08 ns	0.02 ns

Note: Sterile soil is only included in the analysis of live (i.e. inoculated soil) versus sterile soil.

Abbreviation: ns, not significant.

*$p \leq 0.05$, **$p < 0.01$, ***$p < 0.001$.

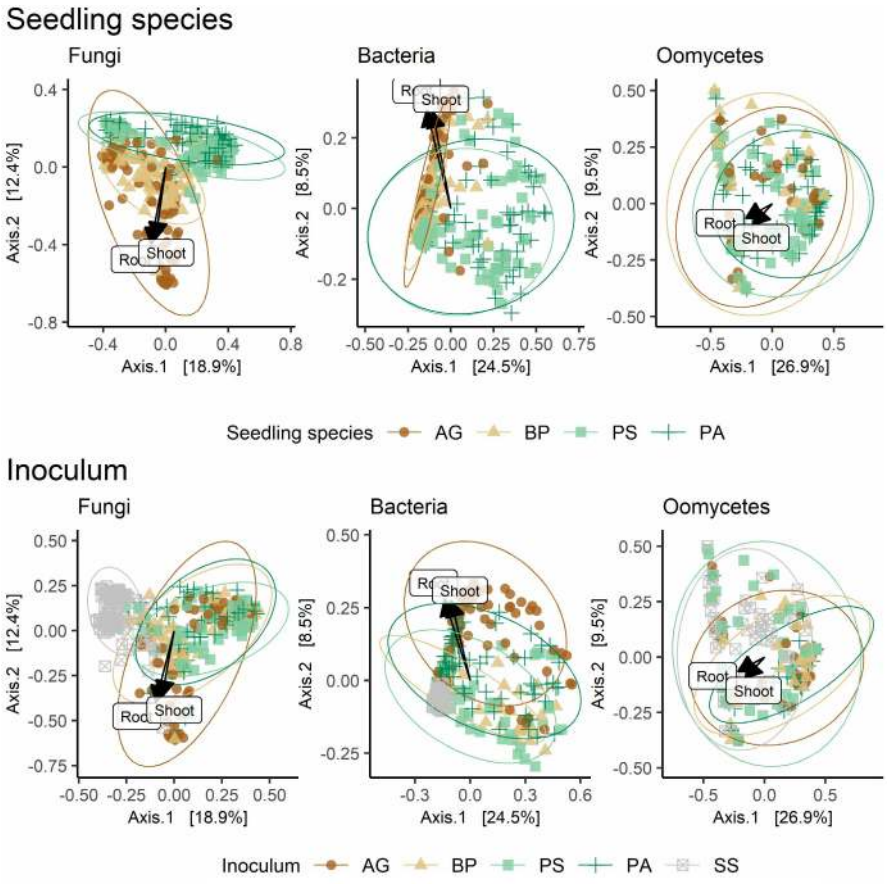


FIGURE 2 Principal coordinate analysis plots of fungal, bacterial and oomycete communities based on Bray–Curtis distances. Differences in the length and direction of vectors indicate the relative strength of the association between ordination axes and the root or shoot biomass (i.e. 'Root' and 'Shoot', respectively). For fungi and bacteria, vector axes were multiplied by 0.5 to fit better within the plot. Ellipses correspond to the seedling species (above) or inoculum species (below) indicated by the symbol shape (AG, *Alnus glutinosa*; BP, *Betula pendula*; PS, *Pinus sylvestris*; PA, *Picea abies*). Inoculum refers to the soil from adult trees of the same four species as the seedlings and sterile soil (SS) is sterile bulk without inoculum. The percentage of the total variance explained by each PC is indicated in parentheses.

growth (Figure 4; Table S6). By contrast, spruce seedlings were able to recruit many EMF and root-associated fungi in pine soil (nine out of 19 indicator taxa) (Table S6), but none of these fungi was significantly correlated with seedling growth. In conspecific soils, spruce seedlings mainly recruited moulds, and only five out of 14 indicator taxa were EMF or root-associated fungi.

Within each seedling species, bacterial community composition differed between inoculum types (Table 3), even though the main phyla were conserved across all soils (Figure 3). Several individual phyla were strongly correlated with growth (Table S5). *Isoachlya*, the only oomycete genus that showed a significant correlation with seedling growth, was negatively correlated with spruce seedling growth ($p = 0.002$, $R = -0.42$; Table S7). *Isoachlya* was associated with alder soils, where it showed a higher relative abundance than in any other soil ($p < 0.001$).

4 | DISCUSSION

In this study, we provide experimental evidence that soil microbiota may contribute to boreal forest stand dynamics. In a glasshouse experiment, we observed that seedlings of birch, alder, pine and spruce had species-specific responses to different soil microbiota and recruited unique root-associated microbial communities depending

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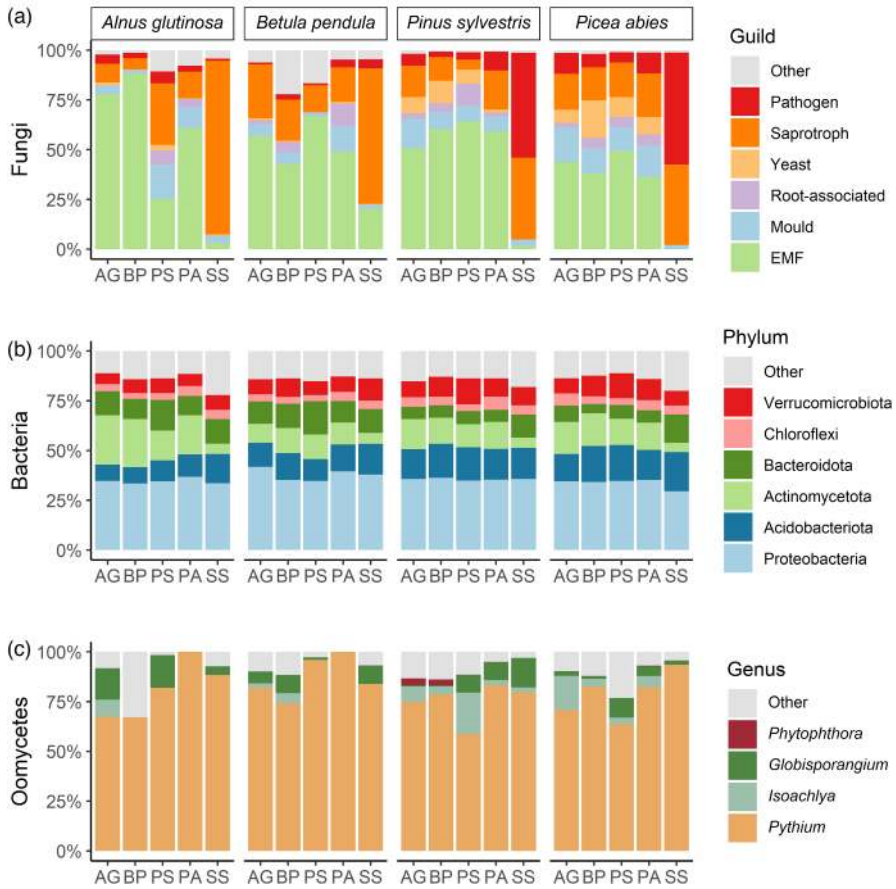


FIGURE 3 Relative abundance of each fungal guild (a), bacterial phylum (b) or oomycete genus (c) in the rhizosphere of *Alnus glutinosa*, *Betula pendula*, *Pinus sylvestris* and *Picea abies* seedlings growing in sterile soil inoculated with soil from below mature trees of *Alnus glutinosa* (AG), *Betula pendula* (BP), *Pinus sylvestris* (PS) or *Picea abies* (PA) or with sterile soil (SS). The statistics of the comparisons are shown in the corresponding Tables S1–S3.

on the tree species that conditioned the soil. Seedling biomass was positively correlated with the proportion of EMF and specific bacterial phyla. Our results suggest that the ability of pine and spruce to recruit beneficial root-associated microbial communities from heterospecific soils may be one of the mechanisms underlying mixed-stand formation in boreal forests. Similarly, the tendency of alder to form pure stands may be partly driven by beneficial soil microbiota recruited from conspecific soil.

The observed plant growth differences across soils and tree species followed growth patterns seen in nature. Alder, which tends to form highly shaded single-species stands near water bodies or in soils with a high water-table (Houston Durrant et al., 2016a), was the only species examined in this study that grew best in conspecific soil. Furthermore, the growth of the other seedling species was poorest when grown in alder soil. Alder growth also correlated strongly with Actinomycetota and the presence of root nodules. Alder is known to associate with nitrogen-fixing Actinomycetota belonging to the genus *Frankia* (Benson & Silvester, 1993), which aid its establishment by providing nitrogen to the tree. This association with nitrogen-fixing soil microbiota may facilitate alder establishment in areas with waterlogged soils which often experience high rates of denitrification by providing alder access to nitrogen (Hamonts et al., 2013). However, although there were significantly more Actinomycetota in the rhizosphere of alder seedlings than in the rhizosphere of the other seedlings, the relative abundance of *Frankia* was among the lowest and did not affect biomass. *Frankia* is an endophyte (Benson & Silvester, 1993), which could explain why it was not detected abundantly in the rhizosphere. In addition, alder seedlings exhibited depletion of foliar $\delta^{15}N$ that mirrored growth patterns in different soils, indicating that some of the nitrogen had been provided to the plant via microbial assimilation of primarily inorganic nitrogen with low ^{15}N values (Craine et al., 2015; Hobbie & Hobbie, 2008). Alder seedlings were also associated with nitrophilic EMF such as *Tuber* and *Tomentella*, with the former linked to higher seedling biomass. The dominance of these or other EMF species that are short-distance exploration types is expected under conditions of high nitrogen availability (Lilleskov et al., 2002; Sterkenburg et al., 2015).

Silver birch grew equally well in inoculated and in sterile soil, which suggests that birch may rely more on abiotic conditions, such as mineral nutrients, light or temperature, than on recruiting growth-enhancing microorganisms to assist in its establishment. This is in line with a study by Ibáñez et al. (2022) which found that birch regeneration did not respond to changes in soil biota but was mostly driven by changes in abiotic soil properties. Birch is an early-colonising, light-demanding species which tends to establish on relatively bare ground soon after a disturbance (Condé et al., 2003; Špulák & Kacálek, 2020). The capacity of birch seedlings to grow on soils without or with just a few generalist microbial symbionts may give them an advantage over other species when colonising areas that have been cleared by a disturbance, such as a forest fire.

Scots pine can also be considered an early successional species due to its high-light requirements and can often be found forming

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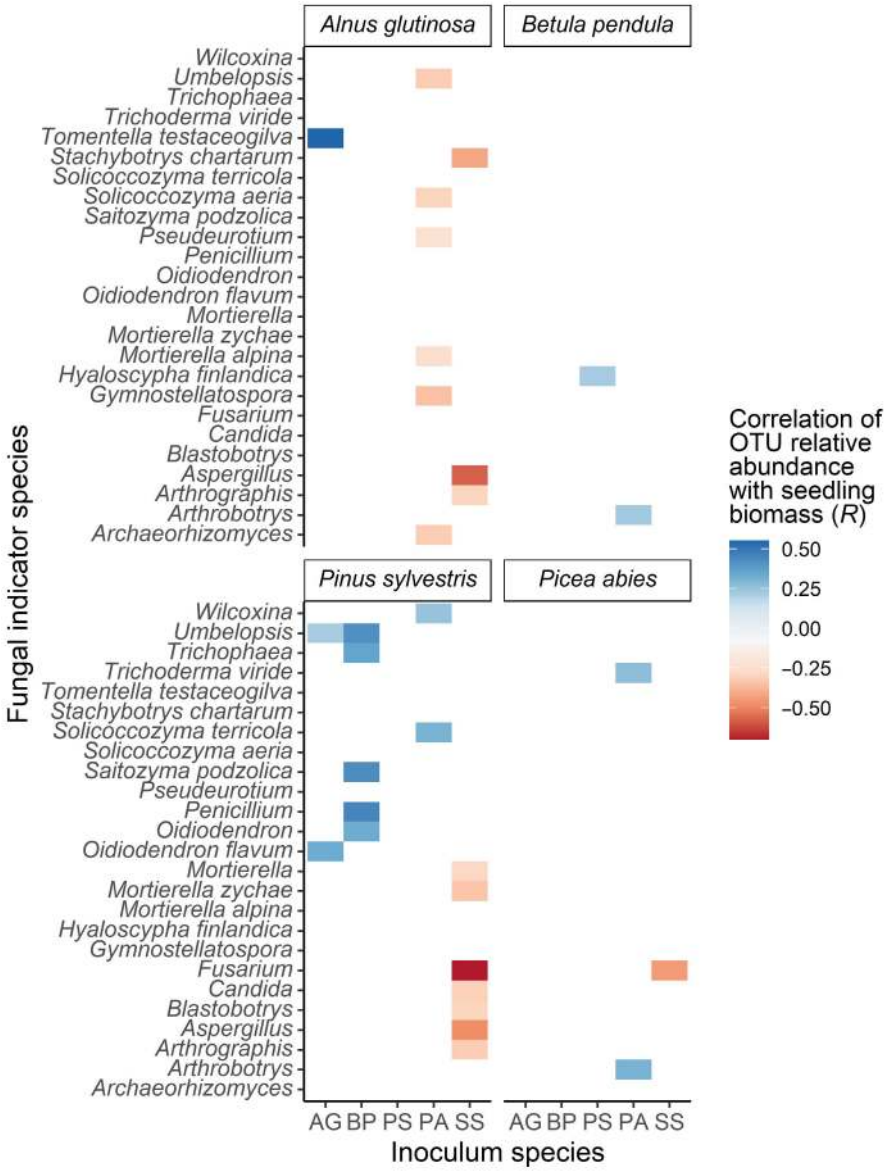


FIGURE 4 Heatmap showing the correlation of the relative abundance of individual fungal indicator species with seedling biomass for each seedling species with each type of inoculum (AG, *Alnus glutinosa*; BP, *Betula pendula*; PS, *Pinus sylvestris*; PA, *Picea abies*). Only the statistically significant correlations are presented ($p \leq 0.05$), the rest can be seen in Table S6.

stands with birch after a disturbance (Houston Durrant et al., 2016b). In this study, Scots pine grew best in soil with birch inoculum. Pine seedling growth correlated with the abundance of EMF, which associate with plant roots to form symbioses that provide nitrogen and phosphorus to the plant, thereby facilitating growth (Anderson & Cairney, 2007; Boukhatem et al., 2022). Pine seedlings growing on birch soil were able to recruit specific EMF and root-associated fungi that correlated positively with growth, which suggests that soil microorganisms might be aiding pine establishment in birch-conditioned areas. In boreal forest, Scots pine is often out-competed by Norway spruce (Houston Durrant et al., 2016b). In this study, fungal pathogens, which were more abundant in spruce soil than in birch or pine soil, potentially inhibited pine growth in spruce soil, suggesting that soil microbiota may also play a role in the transition of pine stands to spruce-dominated forest.

Norway spruce is generally considered to be a late-stage coloniser because of its shade tolerance (Angelstam & Kuuluvainen, 2004) and a preference for soils originating from older stands (Stuiver et al., 2016). In our experiment, Norway spruce grew best in pine soil, which further suggests a possible microbial mechanism for the successional transition from pine to spruce dominance over time. Interestingly, unlike the indicator species for the other tree species, the indicator species for spruce growing in pine soil were fungal species belonging to the genera *Amphinema*, *Piloderma*, *Russula* and *Hydnellum*. All these fungal species (except *Russula* spp.) form extensive mycelia with a high capacity to capture nitrogen from the soil and retain it (Agerer, 2001). *Hydnellum* spp. (Arnolds, 1991; Hobbie & Högberg, 2012) and *Piloderma* spp. (Cox et al., 2010; Jörgensen et al., 2022; Lilleskov et al., 2011) have high $\delta^{15}N$ signatures and sensitivity to nitrogen deposition, indicating that these species may be particularly beneficial for nitrogen uptake by plants under low nitrogen conditions. Interestingly, these species have also been associated with late stages of forest development (Varenius et al., 2017), where organic nitrogen may be the dominant source of nitrogen (Lilleskov et al., 2011). The high foliar $\delta^{15}N$ signatures of pine and spruce seedlings grown in inoculated soils (relative to sterile soils) suggest a shift

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in the nitrogen source potentially towards ^{15}N-enriched organic matter (Hobbie & Högberg, 2012). Although we did not find individual EMF taxa associated with spruce growth, the number of EMF indicator species for Norway spruce growing in pine soil was greater than in conspecific soils, which may allow spruce seedlings to proliferate in mature pine stands, potentially aiding in a forest transition towards spruce-dominated stands.

The patterns of stand formation in boreal forests have been attributed to the varying light or nutrient demands of the different tree species (Angelstam & Kuuluvainen, 2004; Petrokas et al., 2020), as well as the interactions between trees and other plants, animals and soil biotic and abiotic properties (Aponte et al., 2011; Silvertown, 2004; Soong et al., 2020; Stuiver et al., 2016). Our experiment showed a possible effect of microbiota on seedling growth, suggesting that the interaction of specific soil microorganisms with trees could play a role in shaping tree species composition in boreal forest stands. Even though seedlings may not reflect adult tree responses, seedling growth is nevertheless likely to have an important influence on succession because it determines the number of trees that become established and that will potentially develop into adults. In future, research should further explore the contribution of the soil microbiome to boreal forest stand dynamics. Combining experimental and field studies, as well as surveys of forest soils from stands at varying successional stages, could provide deeper insight into the role of PSFs in succession and forest regeneration.

We show that PSFs may potentially drive forest stand composition in boreal forests. Major soil-borne plant pathogens such as oomycetes can play an important role in the regulation of forest stand composition by preventing seedling establishment (Domínguez-Begines et al., 2020), but this was not observed in our study. Instead, we found that EMF were associated with better growth of all seedlings apart from birch, whereas Actinomycetota were correlated with greater alder and birch seedling biomass. Actinomycetota abundance was correlated with a higher soil nitrogen content, which may be the mechanism through which Actinomycetota in the rhizosphere stimulate growth. The absence of strong fully reciprocal negative feedbacks suggests that growth-promoting microbes rather than pathogens might play a role in shaping tree species composition in boreal regions.

AUTHOR CONTRIBUTIONS

Jonàs Oliva, Karina E. Clemmensen, Miguel Ángel Redondo and Sara Hallin designed the study; Carles Castaño, Jaanis Juhanson and Miguel Ángel Redondo carried out the experiment; Dora Štraus and Jonàs Oliva analysed the data. Dora Štraus wrote the first draft; all authors contributed to the subsequent versions of the manuscript. All authors have read and agreed to the published version of the manuscript. Dora Štraus and Miguel Ángel Redondo contributed equally.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

DATA AVAILABILITY STATEMENT

The data obtained in this study are publicly available in Figshare at https://doi.org/10.6084/m9.figshare.21688049 (Štraus et al., 2022).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1: Nitrogen (N) and potassium (K) content in inoculated and sterile soils across all seedlings (left) and in pots with Scots pine seedlings (*Pinus sylvestris*, right).

Figure S2: Principal coordinate analysis plots of fungal, bacterial and oomycete communities in field-collected inoculum soil ('Field soil')

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and in pot soil inoculated with the field soil ('Pot soil') based on Bray–Curtis distances.

Figure S3: Relative abundance of each fungal guild, bacterial phylum or oomycete genus in soil collected in the forest under mature *Alnus glutinosa* (AG), *Betula pendula* (BP), *Pinus sylvestris* (PS) and *Picea abies* (PA) trees and used as inoculum ('Field soil') and in the rhizosphere of AG, BP, PS and PA seedlings growing in pots with sterile soil inoculated with inoculum soil ('Pot soil').

Table S1: Differences in fungal guild relative abundance between seedlings of the same species inoculated with different soils.

Table S2: Differences in the relative abundance of the dominant bacterial phyla between seedlings of the same species inoculated with different soils.

Table S3: Differences in the relative abundance of oomycete genera between seedlings of the same species inoculated with different soils.

Table S4: Correlation between fungal guild relative abundance across all inoculated soils and final seedling biomass shown as *R* values.

Table S5: Correlation between relative abundance of dominant bacterial phyla across all inoculated soils and final seedling biomass shown as *R* values.

Table S6: Fungal indicator species associated with each seedling in each soil and the correlation of their relative abundance with seedling growth; statistically significant correlations ($p \leq 0.05$) are in bold.

Table S7: Correlation between relative abundance of the three most abundant oomycete genera (>1% total reads) and *Phytophthora* across all inoculated soils and final seedling biomass shown as *R* values.

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