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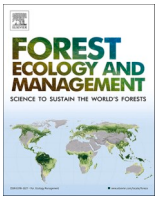

Tree species traits and mycorrhizal association shape soil microbial communities via litter quality and species mediated soil properties

Petr Heděnec [a,h,*], Haifeng Zheng [a], David Pessanha Siqueira [a,b], Qiang Lin [c], Yan Peng [a,d], Inger Kappel Schmidt [a], Tobias Guldberg Frøslev [e,f], Rasmus Kjøller [e,f], Johannes Rousk [g], Lars Vesterdal [a]

[a] Department of Geosciences and Natural Resource Management, Faculty of Science, University of Copenhagen, Rolighedsvej 23, 1958 Frederiksberg C, Denmark
[b] Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, Rio de Janeiro, Brazil
[c] Laboratory of Medical Microbiology, Vaccine and Infectious Disease Institute, University of Antwerp, Antwerp, Belgium
[d] Key Laboratory for Humid Subtropical Eco-Geographical Processes of the Ministry of Education, School of Geographical Sciences, Fujian Normal University, Fuzhou 350007, China
[e] Department of Biology, University of Copenhagen, Universitetsparken, 15, Copenhagen 2100, Denmark
[f] Section for GeoGenetics, GLOBE Institute, University of Copenhagen, Øster Voldgade 5-7, Copenhagen 1350, Denmark
[g] Department of Biology, Microbial Ecology - MEMEG, Lund University, Ecology Building, Lund SE-223 62, Sweden
[h] Institute for Tropical Biodiversity and Sustainable Development, University Malaysia Terengganu, 21030, Kuala Nerus, Terengganu, Malaysia

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ABSTRACT

Soils harbor a vast diversity of soil microbiota, which play a crucial role in key ecosystem processes such as litter transformation and mineralization, but how complex plant-soil interactions shape the diversity and composition of soil microbiota remains elusive. We performed amplicon sequencing of DNA isolated from mineral topsoil of six common European trees planted in multi-site common garden monoculture stands of broadleaved maple and ash associated with arbuscular mycorrhiza (AM), broadleaved beech, lime and oak associated with ectomycorrhizal fungi (ECM) and coniferous spruce associated with ECM. The main aim of this study was to evaluate the effects of tree species identity, traits and mycorrhizal associations on diversity, community structure, cohesion, and shift in the relative abundance of taxonomic and functional groups of soil bacteria, fungi and nematodes. Our results revealed that soils beneath broadleaved trees hosted higher OTU richness of bacteria, fungi, and nematodes than under Norway spruce. Broadleaved tree species associated with AM fungi showed higher cohesion of bacterial and fungal communities than broadleaved trees associated with ECM fungi, but the cohesion of nematode communities was higher under trees associated with ECM fungi than under trees associated with AM fungi. Copiotrophic bacteria, fungal saprotrophs and bacterivorous nematodes were associated with ash, maple and lime having high soil pH, and high litter decomposition indices, while oligotrophic bacteria, ectomycorrhizal fungi and fungivorous nematodes were associated with beech, oak and Norway spruce that had low soil pH and low litter decomposition indices. Tree species associated with AM fungi had a high proportion of copiotrophic bacteria and saprotrophic fungi while trees associated with ECM fungi showed a high relative abundance of oligotrophic bacteria, ECM fungi and fungivorous nematodes. The different abundances of these functional groups support the more inorganic nutrient economy of AM tree species vs the more organic dominated nutrient economy of ECM tree species. The bacterial community was indirectly affected by litter quality via soil properties, while the fungal community was directly affected by litter quality and tree species. The functional groups of nematodes mirrored the communities of bacteria and fungi, thereby indicating the main and active groups of the tree species-specific microbial communities. Our study suggested that tree species identity, traits, and mycorrhizal association substantially shape microbial communities via a direct effect of litter chemistry as well as via litter-mediated soil properties.

* Corresponding author at: Department of Geosciences and Natural Resource Management, Faculty of Science, University of Copenhagen, Rolighedsvej 23, 1958 Frederiksberg C, Denmark.
E-mail addresses: petr.hedenec@umt.edu.my, peh@ign.ku.dk (P. Heděnec).

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1. Introduction

Soil microorganisms play an important role in key ecosystem processes such as nitrogen fixation, carbon sequestration or mineralization of dead organic matter (Ribbons et al., 2016; Trivedi et al., 2013), therefore, the investigation of factors shaping diversity and composition of soil microbial communities has become the central point of recent microbial ecology (Fierer, 2017). Despite an increased number of studies focused on soil microbial communities in past decades (Delgado-Baquerizo et al., 2020; Fierer, 2017; Fierer and Jackson, 2006; Griffiths et al., 2000; Rousk et al., 2010), the mechanisms shaping diversity and composition of soil microbial communities still require further study. Dominant vegetation shapes diversity and composition of soil microbiota either directly via their litter chemistry or indirectly via their effect on soil properties (Prada-Salcedo et al., 2022). For example, the diversity and composition of soil bacterial community are strongly associated with soil properties such as pH (Rousk et al., 2010) and soil moisture (Evans et al., 2014), while diversity and composition of fungal communities were reported to be mostly associated with aboveground plant vegetation (Prada-Salcedo et al., 2022) and litter quality (Urbanová et al., 2015). However, the role of tree species identity (e.g. ash, maple or spruce) and traits (e.g. litter qualitty) in shaping bacterial and fungal diversity in soils, as well as the mechanisms behind, deserve to be studied in tree species experiments across multiples sites with due control of possible confounding effects of site factors.

Tree species influence soil physical and chemical properties through different quality leaf litter (De Schrijver et al., 2012; Vesterdal et al., 2008) yet our understanding of litter mediated effects on the composition and diversity of soil biota still entails several challenges. The quality of leaf litter varies strongly between tree species and the quality of leaf litter as a substrate likely affects soil microbiota directly (Pietsch et al., 2014; Wardle et al., 2004), while indirect effects on soil microbiota occur via tree species effects on soil chemistry (Dawud et al., 2017; Hedĕnec et al., 2020). Tree species producing litter with high C:N ratio and low base cation content are reported to support the fungal-dominated energy channel with slow nutrient turnover while plant species producing litter with low C:N ratio and high base cation content were reported to support the bacterial-controlled channel with fast decomposition and nutrient release (Pietsch et al., 2014; Wardle et al., 2004). Soil bacteria are mostly associated with decomposition of easily degradable compounds such as starch or glucose (Fierer et al., 2007), while soil fungi are important for decomposition of more recalcitrant compounds such as lignin (Algora Gallardo et al., 2021; Urbanová et al., 2015).

Mycorrhizal fungi form associations with plant roots. For example, arbuscular mycorrhizal fungi (AM) and ectomycorrhizal fungi (ECM) form symbiosis with the roots of most of the trees on Earth (van der Heijden et al., 2015). The AM fungi primarily scavenge for inorganic forms of N and P released by saprotrophic microbes, while ECM fungi rely on organic N and P sources which require extracellular enzymes that allow degrading complex organic compounds such as proteins, chitin and inositol phosphates (van der Heijden et al., 2015). Mycorrhizal associations shape activity and biomass of soil bacterial and fungal communities. For instance, tree species associated with AM fungi support bacterial growth while tree species with ECM fungi support fungal growth (Hedĕnec et al., 2020). In addition, the community composition of ECM fungi differs among tree species (Ferlian et al., 2021), however the effect of mycorrhizal associations on diversity, community cohesion and relative abundance of various groups of soil microbiota is not yet fully understood.

Amplicon sequencing methods revealed a vast microbial diversity, but this method also includes relic extracellular DNA, which is abundant in soil and obscures estimates of soil microbial diversity (Carini et al., 2016). Investigating trophic interaction in soils can highlight the active fraction of the soil microbial community, because microbial feeders such as nematodes are expected to be consumers of active microbes (Nasri et al., 2020). Soil nematodes are highly abundant top-down regulators of microbial communities and thus comprise a wide range of functional groups including bacterivores and fungivores (Geisen, 2016). In addition, soil nematodes include herbivores, predators, parasites and omnivores, which play important roles in regulation of primary consumers and thus indirectly affect decomposition and transformation processes in soils (Cesarz et al., 2013).

The high functional and taxonomic diversity of the organisms recently found in soils presumably reflects the complexity of soil microbial communities in terms of the number and strength of interconnections between various taxa (Herren and McMahon, 2017; Wong et al., 2018), but the factors shaping interconnectivity of soil microbial communities require further studies. Analyses of microbial community interconnectivity often involves construction of complex neural networks based on many parameters that are difficult to calculate and interpret. To solve this problem, a new metric called community cohesion has been suggested (Herren and McMahon, 2017), but it remains to be tested at wider scale. Positive and negative community cohesion indices quantifies the degree of connectivity of a microbial community based on positive and negative correlations of relative abundances between different taxa within a community matrix. Positive cohesion index refers to high connectivity as reflected by positively correlated taxa indicating mutualism or facilitation (Herren and McMahon, 2017). In contrast, negative cohesion index refers to high negative connectivity among taxa within a community indicating competitive exclusion or preference for different niches (Herren and McMahon, 2017).

In this study, we evaluated the influence of six common European tree species with different leaf litter quality and mycorrhizal association on diversity, structure, cohesion, and relative abundance of taxonomic and functional groups of soil microbial communities using Illumina MiSeq sequencing of the DNA. Our study platform was a unique 47-year-old common garden experiment replicated at six sites that controlled for possible confounding site-related effects (Vesterdal et al., 2013). The same common garden experiment has previously revealed tree species effects on soil C and N stocks (Steffens et al., 2022; Vesterdal et al., 2008), soil respiration and C and N turnover (Vesterdal et al., 2012), pH and base saturation (Schelfhout et al., 2017), ^{15}N abundance and N cycling traits (Callesen et al., 2003), N and water balances (Christiansen et al., 2010), and most recently microbial biomass, growth and composition based on PLFA analysis (Hedĕnec et al., 2020).

The main aim was to answer the following questions: (1) how do tree species identity (tree species), traits (litter quality) and mycorrhizal associations (AM. ECM) shape diversity, community structure and cohesion of soil microbiota via litter quality and tree species mediated soil properties? (2) do litter chemistry and tree species mediated soil properties differ in their relative importance for diversity and cohesion between communities of soil bacteria, fungi and nematodes? (3) do tree species identity, traits and mycorrhizal associations shift the relative abundance of soil bacteria, fungi and nematodes groups via litter quality and tree species mediated soil properties? and (4) is the diversity, community structure and functional groups of soil nematodes mirroring the diversity and community structure of bacteria and fungi?

We expect that the different litter quality of the tree species will be reflected in higher alpha diversity of soil bacteria, fungi and nematodes in broadleaves than in Norway spruce and also in AM broadleaves compared to ECM broadleaves. We also expect that broadleaved trees associated with AM fungi will differ in diversity and cohesion of bacterial, fungal and nematodes communities from the broadleaved tree species associated with ECM fungi. We hypothesize that fungal communities will be controlled by litter quality since fungi are the decomposers of particulate organic matter and are tightly linked with plants, while the bacterial community will be more controlled by soil properties such as pH and nutrient concentrations. Finally, we hypothesize that the diversity and community structure of soil nematodes will be strongly linked with diversity and community structure of soil

bacteria and fungi.

2. Materials and methods

2.1. Site description and sampling design

The six common European tree species: broadleaves beech (*Fagus sylvatica* L.), pedunculate oak (*Quercus robur* L.), lime (*Tilia cordata* L.), sycamore maple (*Acer pseudoplatanus* L.), ash (*Fraxinus excelsior* L.) and coniferous Norway spruce (*Picea abies* (L.) Karst.) were planted in single species plots of approximately 0.25 ha in 1961 (Kragelung) and 1973 (Mattrup, Odsherred, Vallø, Viemose and Wedellsborg) (Table S1). Ash and maple have AM association whereas beech, oak, lime and Norway spruce all have ECM association (Hedĕnec et al., 2020). The selected experimental sites were planted on old forest land (Odsherred, Vallø, Viemose, Wedellsborg) and former cropland (Kragelund, Mattrup) (Fig. S1; Table S1). The minimum and maximum distance between experimental sites is 30 and 200 km respectively. The sites are distributed throughout Denmark mainly situated on relatively nutrient-rich soils developed from till deposits and classified as Luvisols (Mattrup, Odsherred, Vallø, Viemose), Phaeozem (Wedellsborg) and Alisol (Kragelund) (Callesen et al., 2003). The soil texture of the parent material has clay content increasing from 8 % (Kragelund) to 30 % (Wedellsborg) (Vesterdal et al., 2008). The sites were mainly located in, or close to, rural and intensively managed agricultural areas. Climatic conditions regarding mean annual precipitation (580–890 mm), mean annual temperature (7.5–8.4 °C) and the length of the growing season are relatively similar (Vesterdal et al., 2008). Litter turnover rates were high and there was only little forest floor accumulation in most of the plots with deciduous stands (Vesterdal et al., 2012). Further site information can be found in Vesterdal et al. (2008) and Hedĕnec et al. (2020). Freshly fallen litter for analyses of lignocellulosic content from each tree species was collected in autumn 2018 from Viemose site. Understory vegetation in plots with broadleaved species were dominated by *Anemone nemorosa* L. in early spring in the four old forest sites. After the leaf flushing, only maple, ash and oak plots had some scattered understory vegetation. Other tree species were absent of understory vegetation due low light availability (Hoffmann, 2007). Forest floor and understory vegetation was removed from soil surface before sampling using small shovel. Soil samples were collected in the May and October 2019 from 0 to 5 cm of the mineral topsoil using a soil corer (4 cm diameter). Six soil cores were taken on each plot to make one composite sample per plot. The distance between six soil cores was approximately 10 m (Fig. S1). The distance between soil cores and tree species was approximately 1.5 m from the nearest tree higher than 2 m (Fig. S1). The soil corer was disinfected using 70 % ethanol to avoid cross-contamination. In total, 68 composite samples (6 sites with 6 monoculture stands) were taken during two sampling campaigns in the spring and autumn 2019 whereas one ash and one spruce plots were missing due to windstorm. The soil samples were sieved through a 2 mm mesh and stored at 4 °C for soil a physico-chemical analysis and at −20 °C for the DNA extraction.

2.2. Soil physico-chemical properties and litter chemistry analyses

The soil moisture was measured gravimetrically after drying at 105 °C. The pH was measured at the soil:water ($CaCl_2$) ratio of 1:5 and analyzed with a Radiometer combination-electrode GK2401 (Radiometer, Copenhagen, Denmark). The TOC, TN and C/N ratio was measured by dry combustion (Dumas method) in a Leco CSN 2000 Analyzer (Matejovic, 1993). Information on indices for litter C and N turnover based on litter decomposition constants (Olson, 1963) for C (kf C) and N (kf N) as well as topsoil C and N net potential mineralization rates were available from previous studies in the common garden plots (Christiansen et al., 2012; Vesterdal et al., 2012). Total concentrations of Ca in soils and litter were determined after microwave-assisted digestion in concentrated HNO_3, and the digests were subsequently analyzed for total element contents by ICP-OES (Perkin Elmer Optima 3000XL). Lignocellulosic material was measured according to National Renewable Energy Laboratory (NREL) procedures (Sluiter et al., 2010). Water and ethanol extraction were performed on a Soxhlet apparatus. Concentrations of cellulose, hemicellulose was performed in an Ultimate HPLC (Thermo Fisher Scientific Inc., Waltham, MA USA). Lignin was determined as the dry weight of the samples (after acid hydrolysis) taking the ash content into account (Sluiter et al., 2010).

2.3. DNA extraction, PCR amplification and Illumina MiSeq sequencing

Ten grams of sieved and homogenized soil were used for DNA extraction using DNeasy PowerMax Soil Kit (Qiagen, Hilden, Germany) according to manufacturer's instruction. Extracted eDNA concentration was checked using a Qubit fluorometer (Thermo Fisher Scientific Inc., USA), diluted to 10 ng/μl and stored at − 20 °C. Different combinations of tagged primers with unique MID/barcodes tags of 6 bp at the 5′ end, preceded by 1,2 or 3N's (Frøslev et al., 2021) were used for amplifying of the 16S rRNA gene for bacteria (341F 5́-CCTAYGGGRBGCASCAG-3‘ and 806R 5́-GGACTACHVHHHTWTCTAAT-3́ (Caporaso et al., 2011)), the ITS2 region for fungi (gITS7 5‘-GTGARTCATCGARTCTTTG-3‘and ITS4 5́-TCCTCCGCTTATTGATATGC-3‘ (Bruns et al., 1990; Ihrmark et al., 2012)) and the 18S rRNA gene for eukaryotes (TAReuk454FWD1 5́-CCAGCASCYGCGGTAATTCC-3‘ and TAReukREV3 5́-ACTTTCGTTCTTGATYRA-3‘ (Stoeck et al., 2010)), respectively. Altogether 1 μl DNA template was used for the 25 μl PCR reaction containing 14.60 μl MQ water, 2.50 μl 10x buffer, 2.50 μl MgCl2, 0.20 μl dNTP's (25 mM), 1.50 μl reverse and forward primers (10 μM), 1 μl BSA (20 μg/μl) and 0.20 μl AmpliTaq Gold® DNA Polymerase (5U/μl). The PCR conditions used for different microbial groups were as follows: bacteria – initial denaturation 95 °C (5 min), then 35 cycles of 95 °C (30 sec), 54 °C (30 sec), and 72 °C (90 sec), elongation at 72 °C for 10 min; fungi – initial denaturation 95 °C (5 min), then 32 cycles of 95 °C (30 sec), 55 °C (30 sec), and 72 °C (1 min), elongation 72 °C for 7 min; and eukaryotes – initial denaturation 98 °C (7 min), then 15 cycles of 98 °C (30 sec), 53 °C (30 sec), and 72 °C (45 sec), again 15 cycles of 98 °C (30 sec), 48 °C (30 sec), and 72 °C (45 sec) and elongation 72 °C for a 10 min cycle. Agencourt AMPure beads XP (Beckman Coulter, CA, USA) were used to purify the PCR products, and DNA concentration of PCR products was measured using Qubit fluorometer to make an equimolar mix for each of the microbial groups. The PCR products were pooled into two equal pools per marker. The six pools in total were built into six separated sequencing libraries using the TruSeq DNA PCR-Free Library Preparation Kit (Illumina). Sequencing of the hypervariable V3-V4 regions of DNA was performed at the Danish National High Throughput DNA Sequencing Centre using Illumina Miseq v.3 platform (Illumina) with samples divided on six libraries mixed in equal proportions with 300 bp paired end runs (Frøslev et al., 2021). The raw sequence data are available in the European Nucleotide archive (https://www.ebi.ac.uk/ena/data/view/PRJEB52275).

2.4. Bioinformatics analyses

The SEED pipeline version 2.1.2 was used for filtering and trimming of sequence reads obtained from Illumina MiSeq sequencer (Větrovský et al., 2018). The reads were merged into paired end sequences with at least 30 bp overlap (Větrovský et al., 2018). All sequences with ambiguous bases and average base quality scores lower than 30 were removed from the dataset. Sequences without primers and identifiers as well as sequences with mismatched identifiers were also removed. Remaining sequences were sorted into samples according to the MID sequences. Chimeric sequences were detected using algorithm UCHIME included in USEARCH 7.0.1090 (Edgar et al., 2011) and deleted. Chimera free sequences were clustered using UPARSE implemented within USEARCH 7.0.1090 (Edgar, 2013) at a 97 % similarity level.

From each cluster, the most abundant sequence was selected as a representative sequence for subsequent analysis. All singletons and chimeric sequences were removed. Bacterial sequences were clustered using BLAST against local SILVA database (Yilmaz et al., 2014). The extraction of the ITS2 region was processed by ITSx (Bengtsson-Palme et al., 2013). The non-fungal ITS2 sequences were removed from the dataset and fungal ITS2 sequences were clustered using BLAST search against local UNITE database (Nilsson et al., 2019). Eukaryotic sequences were clustered using BLAST against local PR^2 database (Guillou et al., 2013). In total, approximately 1,000,000 bacterial (16S rRNA gene), 1,500,000 fungal (ITS2) and 950,000 eukaryotic (18S rRNA gene) sequence reads were clustered into 9200, 3900 and 6600 singleton-free OTUs respectively. The bacterial, fungal and eukaryotic sequence reads were resampled to equal depth of 3000, 2200 and 3600 sequences per sample, respectively. After removing rare OTUs with relative abundance lower than 0.1 %, 175 bacterial, 146 fungal, and 82 nematode OTUs were maintained. Categorization of bacteria into functional groups was based on the available publications (Bastian et al., 2009; Fierer et al., 2007). Bacteroidetes and the classes alpha and gamma Proteobacteria were identified as copiotrophs while delta Proteobacteria, Actinobacteriota and Acidobacteriota were identified as oligotrophs (Bastian et al., 2009; Fierer et al., 2007). Categorization of fungi into functional groups was based on the FungalTraits database (Põlme et al., 2020). Categorization o functional groups of nematodes was based on NEMAguild database (Nguyen et al., 2016). The obtained raw OTUs tables including assigned taxonomy are available in Multimedia component. Relative abundances of functional groups of soil bacteria, fungi and nematodes were calculated as a summary of OTUs affiliated to specific taxa identified and assigned to specific functional groups.

2.5. Statistical analyses

Statistical analyses were performed using R (www.r-project.org). All plots were performed using the "ggplot2" package (Wickham, 2016). Multi-way analyses of variance (ANOVA) followed by Tukey HSD test was used to test effects of tree species, mycorrhizal associations and random sampling sites on soil properties, parameters of litter quality, diversity, cohesion and relative abundance of soil biota and their functional groups. The effect of sampling time in the ANOVA model was assigned as an error variable to account for pseudo-replication of repeated measurements. Alpha diversity indices (OTUs richness, Shannon index Pielou index) were calculated using the vegan package (Oksanen et al., 2012). Positive and negative cohesion indices representing positive and negative connectivity among OTUs within a community were calculated according to Herren and McMahon, (2017). Relative importance of soil properties and litter chemistry for the microbial community was estimated using random forest regression with the "rfPermute" package (Liu et al., 2020). To visually interpret community dissimilarity, non-metric multidimensional scaling (NMDS) ordination was conducted. The "envfit" function was used to test correlation ($p < 0.01$) of soil properties and litter traits with community structure of soil bacteria, fungi and nematodes (Oksanen et al., 2012). The analysis of dissimilarity was used to test effect of tree species, random site effects, mycorrhizal associations, sampling time, litter quality and soil properties on soil microbial communities based on Hellinger-transformed Bray-Curtis distance of individual OTUs in community matrix using "adonis" function (PerMANOVA) in "vegan" package (Oksanen et al., 2012). Pearsońs correlation coefficient was used to test correlation of soil properties and litter traits with the relative abundance of various taxonomic and functional groups of soil bacteria, fungi and nematodes. Partial least squares path modelling (PLS PM) was carried out to test the effects of tree species, litter chemistry and soil properties on relative abundance of most abundant taxonomic groups of bacteria, fungi and nematodes using the "plspm" package (Sanchez, 2013). The categorial variables used in PLS PM were binary coded whereas "1" indicated the broadleaved or AM species, and "0" the coniferous or ECM species respectively. Linear regression was used to test significant relationship between bacterial and fungal communities with communities of soil nematodes.

3. Results

3.1. Litter quality and tree species mediated soil properties

Tree species differed in litter quality variables such as concentrations of Ca, extractives, lignin, cellulose, hemicellulose and lignin:cellulose: hemicellulose ratio (L:C:H ratio) in leaf litter (Table 1). For example, lime, beech, oak and Norway spruce had higher concentration of lignin than ash and maple. Litter from Norway spruce showed higher concentration of cellulose and hemicellulose than broadleaf trees. Broadleaf trees associated with AM had higher Ca and extractives in litter while broadleaves associated with ECM showed higher lignin, cellulose and hemicellulose concentration. In contrast, litter from Norway spruce was lower in Ca concentration.

Litter decomposition indices of C and N in forest floor (Table 1) as well as soil net mineralization rates of C and N differed significantly among the studied tree species (Table 2). Soils below Norway spruce had lower litter decomposition indices as well as soil net mineralization rates than in soils planted by broad-leaf tree species. Among broadleaf trees, ash and maple, which are associated with AM fungi, showed higher litter decomposition indices and soil net mineralization rates than lime, beech and oak, which are associated with ECM fungi.

Soil physico-chemical properties differed among the studied tree species (Table 2). Soils under ash, maple and lime showed higher pH and higher concentration of Ca than soils beneath beech, oak and Norway spruce. Soils with broadleaved trees had higher soil moisture than soils below Norway spruce. Soils in Norway spruce were higher in TOC, TN and C/N ratio than soils planted by broadleaved trees. Among broadleaf trees, soil beneath trees associated with ECM fungi were higher in C/N ratio than soils planted by trees associated with AM fungi (Table 2).

3.2. Tree species effects on diversity, structure and cohesion of soil microbial communities

The alpha diversity indices of bacteria, fungi and nematodes differed significantly among tree species (Table 3). Soils in Norway spruce hosted lower alpha diversity of bacteria, fungi, and nematodes than soils planted to broadleaf trees. Soils under broadleaves associated with AM fungi had higher OTU richness of nematodes than broadleaves associated with ECM (Table 3).

The analyses of dissimilarity based on Hellinger-transformed Bray-Curtis distance matrix of OTUs revealed that tree species was the most important driver of community structures of bacteria, fungi and nematodes followed by the effect of site and mycorrhizal association (Table S2). Community structures of fungi and nematodes were clustered within sampling periods but contributed little to explain community structures (Table S2). The ordering of samples by non-metric multidimensional scaling (NMDS) based on the Hellinger-transformed Bray-Curtis distance matrix of OTU showed a clear divergence in structure of bacterial and fungal communities among broadleaves and Norway spruce (Fig. 1A-B). In contrast, community structure of nematodes showed only small differences between broadleaved trees and Norway spruce (Fig. 1C). The microbial community structure in soils under broadleaved trees associated with AM fungi was separated from that in soils under broadleaved trees associated with ECM fungi (Fig. 1D-F). Analyses of dissimilarity indicated an effect of soil physico-chemical properties and decomposition traits on the microbial community structure in addition to the variation explained by tree species identity (Table S2).

The community structure based on the Hellinger-transformed Bray-Curtis distance matrix in Norway spruce was associated with high TOC and C/N ratio, while the community structure in soils beneath

Table 1
Litter quality and decomposition indices of six common European tree species.

Tree	Ca (mg kg^{-1})	Lignin (mg kg^{-1})	Cellulose (mg kg^{-1})	Hemicellulose (mg kg^{-1})	L:C:H ratio	Extractives (mg kg^{-1})	kf C	kf N
Ash (AM)	21.8 ± 4.8a	18.7 ± 3.4b	10.5 ± 0.5c	8.5 ± 1.1b	0.22 ± 0.06b	30.8 ± 1.8a	0.83 ± 0.2a	0.83 ± 0.2a
Maple (AM)	11.3 ± 1.8b	21 ± 2.9b	13.3 ± 0.9b	8.2 ± 0.6b	0.19 ± 0.03c	23.9 ± 3a	0.74 ± 0.2a	0.70 ± 0.2a
Lime (ECM)	16.6 ± 0.2b	32.6 ± 2.4a	12.7 ± 1.6b	11.2 ± 0.7a	0.23 ± 0.04b	9.6 ± 0.8c	0.68 ± 0.2a	0.66 ± 0.2a
Oak (ECM)	19.4 ± 4ab	30.3 ± 3.6a	12.1 ± 1.7b	9.5 ± 0.9b	0.27 ± 0.05a	19.7 ± 4.8b	0.40 ± 0.2b	0.33 ± 0.2b
Beech (ECM)	10.4 ± 1.7b	37.6 ± 2.5a	13.5 ± 1.2b	10.3 ± 0.7a	0.28 ± 0.05a	9.4 ± 2.4c	0.29 ± 0.1c	0.21 ± 0.1c
Spruce (ECM)	9.9 ± 0.3c	29.4 ± 1.1a	16.9 ± 0.0.4a	11.4 ± 0.1a	0.15 ± 0.01d	17.3 ± 1.1b	0.18 ± 0.1d	0.13 ± 0.1d
ANOVA	*	***	***	***	***	***	***	***
Mycorhizal association	AM > ECM*	AM < ECM***	AM < ECM*	AM < ECM***	AM < ECM**	AM > ECM***	AM > ECM*	AM > ECM*

Information on indices for litter C and N turnover based on litter decomposition constants (Olson, 1963) for C (kf C) and N (kf N) were available from previous studies in the common garden plots (Christiansen et al., 2012; Vesterdal et al., 2012). The kf C and kf N reffer rates of C and N released from forrest floor per year. Significance levels: 0.05 (*), 0.01 (**) and 0.001 (***). Mean values ± SEM with different lettering differ significantly in Tukeys HSD pairwise comparison. Differences between broadleave trees with AM (Ash, Maple) and ECM (lime, beech oak) symbiosis were tested by Tukey test. Asterisks *, ** and *** indicates significance levels of $P < 0.05$, 0.01 and 0.001 respectively.

Table 2
Soil properties and mineralization indices of six common European tree species.

Tree	Moisture (%)	pH	Ca (mg kg^{-1})	TOC (mg kg^{-1})	TN (mg kg^{-1})	C:N ratio	Soil C mineralization	Soil net N mineralization
Ash (AM)	23.7 ± 8.5ab	4.2 ± 0.3a	1521 ± 825a	38.3 ± 12.6ab	2.9 ± 1b	13.5 ± 1.9c	0.56 ± 0.23a	11.2 ± 2.3ab
Maple (AM)	23.6 ± 7.4ab	4.4 ± 0.3a	1049 ± 629a	35.6 ± 12.1b	2.6 ± 0.9b	13.7 ± 1.6c	0.53 ± 0.15a	15.5 ± 1.1a
Lime (ECM)	23.6 ± 7.5ab	4.3 ± 0.4a	796 ± 422b	34.2 ± 12.8b	2.4 ± 0.9b	14.4 ± 2.1b	0.43 ± 0.24b	10.1 ± 2.6b
Oak (ECM)	24.6 ± 7.3a	3.7 ± 0.2b	480. ± 328c	45.8 ± 21.6a	3.1 ± 1.3a	14.8 ± 1.9b	0.58 ± 0.29a	12.7 ± 5.7a
Beech (ECM)	24.4 ± 7.6a	3.82.3b	445 ± 257c	36.9 ± 9.4ab	2.6 ± 0.8b	14.5 ± 1.7b	0.49 ± 0.25b	10.9 ± 3.5b
Spruce (ECM)	17.3 ± 11.7b	3.3 ± 0.2b	561 ± 227b	65.5 ± 28.4a	3.5 ± 1.4a	18.8 ± 1.1a	0.28 ± 0.16c	6.3 ± 3.6c
ANOVA	**	***	***	***	**	***	***	**
Mycorhizal association	ns	AM > ECM***	AM > ECM**	ns	AM < ECM*	AM < ECM***	ns	AM > ECM*

Information on indices for the topsoil C and N net potential mineralization rates were available from previous studies in the common garden plots (Christiansen et al., 2012; Vesterdal et al., 2012). Significance levels: 0.05 (*), 0.01 (**) and 0.001 (***). Mean values ± SEM with different lettering differ significantly in Tukeys HSD pairwise comparison. Differences between broadleave trees with AM (Ash, Maple) and ECM (lime, beech oak) symbiosis were tested by Tukey test. Asterisks *, ** and *** indicates significance levels of $P < 0.05$, 0.01 and 0.001 respectively.

Table 3
Alpha diversity indices for bacteria, fungi and nematodes in soils from six common European tree species.

	Bacteria			Fungi			Nematodes		
Tree	OTUs richness	Shannon index	Pielou indecx	OTUs richness	Shannon index	Pielou indecx	OTUs richness	Shannon index	Pielou indecx
Ash (AM)	161 ± 15a	4.3 ± 0.2a	0.85 ± 0.03	75 ± 12a	3.1 ± 0.2	0.72 ± 0.05	20 ± 7a	2.5 ± 0.6a	0.84 ± 0.12ab
Maple (AM)	153 ± 12a	4.2 ± 0.1a	0.83 ± 0.03	73 ± 14ab	2.9 ± 0.2	0.68 ± 0.04	21 ± 6a	2.6 ± 0.5a	0.88 ± 0.08a
Lime (ECM)	157 ± 13a	4.2 ± 0.2a	0.85 ± 0.03	72 ± 10b	3 ± 0.3	0.70 ± 0.05	20 ± 4a	2.5 ± 0.3a	0.85 ± 0.04a
Oak (ECM)	160 ± 6a	4.4 ± 0.1a	0.83 ± 0.03	73 ± 10ab	2.9 ± 0.	0.68 ± 0.04	16 ± 4b	2.2 ± 0.4ab	0.79 ± 0.09b
Beech (ECM)	162 ± 6a	4.3 ± 0.2a	0.84 ± 0.03	73 ± 9ab	2.9 ± 0.3	0.69 ± 0.04	18 ± 5b	2.5 ± 0.4a	0.85 ± 0.05a
Spruce (ECM)	135 ± 16b	4.1 ± 0.2b	0.85 ± 0.03	61 ± 13c	2.7 ± 0.2	0.67 ± 0.04	12 ± 4c	1.7 ± 0.54b	0.70 ± 0.13c
ANOVA	***	***	ns	*	ns	ns	**	***	**
Mycorhizal association	ns	ns	ns	ns	ns	ns	AM > ECM***	ns	ns

Significance levels: 0.05 (*), 0.01 (**) and 0.001 (***). Mean values ± SEM with different lettering differ significantly in Tukeys HSD pairwise comparisons. Differences between broadleave trees with AM (Ash, Maple) and ECM (lime, beech oak) symbiosis were tested by Tukey test. Asterisks *, ** and *** indicates significance levels of $P < 0.05$, 0.01 and 0.001 respectively.

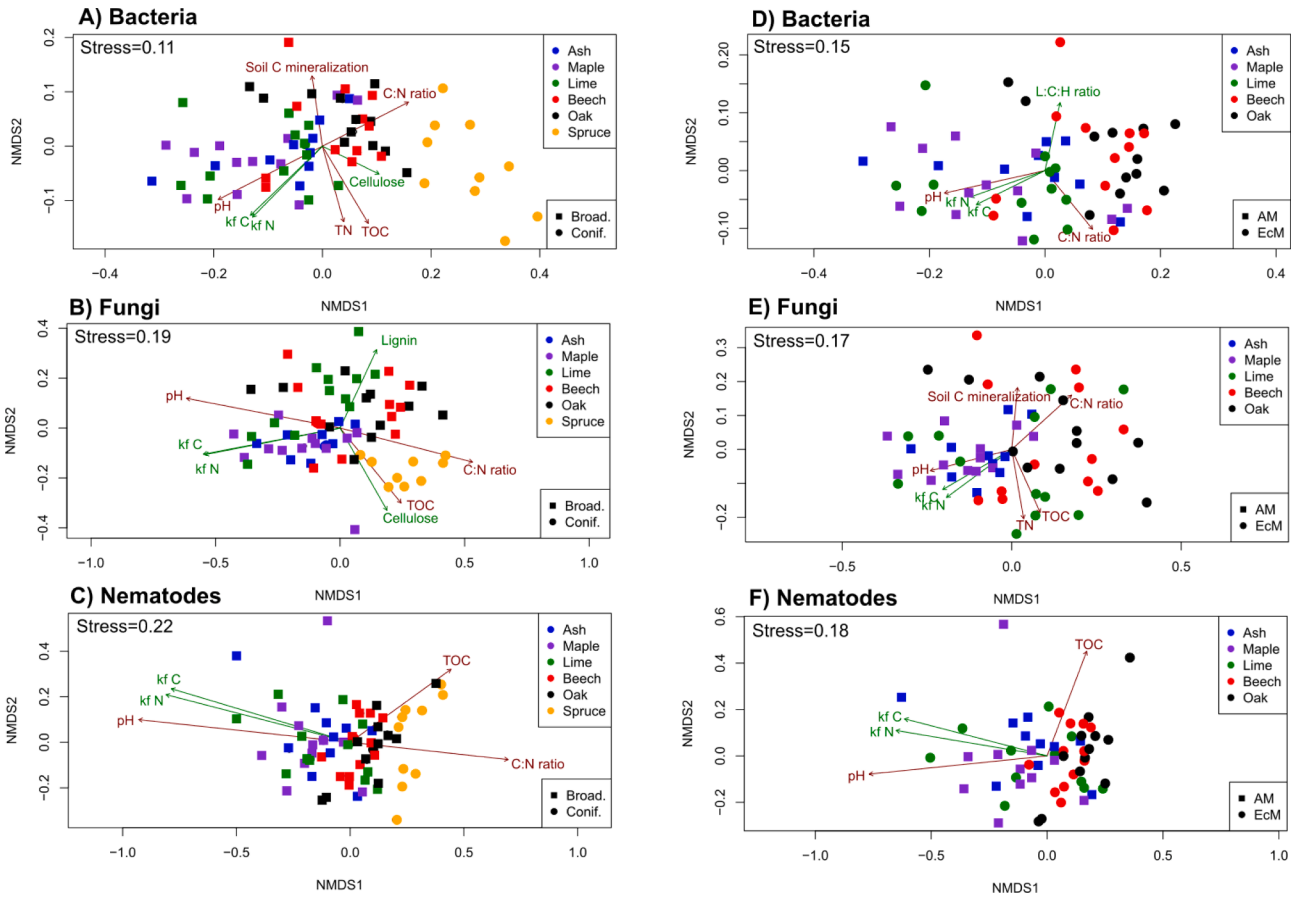

Fig. 1. Non-metric multidimensional scaling of community structure of soil bacteria (A,D), fungi (B,E) and nematodes (C,F) in soils between six common European tree species (A-C) and mycorhizal associations of broadleaved trees (D-F).

broadleaved trees was associated with high soil pH and fast litter decomposition or soil mineralization rates (Fig. 1A-C). Similarly, the community structure in soils beneath broadleaved trees associated with AM fungi were associated with high pH and fast litter decomposition while the community structure under broadleaves associated with ECM fungi was associated with high C:N ratio, TOC, TN and slow decomposition rates (Fig. 1D-F).

Positive and negative cohesion indices of soil bacterial, fungal and nematode communities differed among tree species (Table 4). Cohesion indices (positive as well as negative) of bacterial communities were high in soils beneath Norway spruce, ash, maple and lime while soils under beech and oak showed low cohesion of bacterial communities. Cohesion indices of fungal communities were highest in soils under ash and maple, while soils in Norway spruce and oak showed lowest cohesion of fungal communities. A contrasting pattern in community cohesion was found for nematodes with lowest cohesion indices in soils under ash and maple and highest in soils beneath Norway spruce and oak. The overall cohesion indices (representing overall cohesion among taxa) was highest in spruce and maple and lowest under beech and oak because of their low cohesion of the bacterial community. Broadleaved tree species associated with AM fungi showed higher overall cohesion as well as cohesion of bacterial, fungal communities than broadleaved trees associated with ECM fungi. In contrast, cohesion of nematode communities was higher under trees associated with ECM fungi than under trees associated with AM fungi within broadleaves.

Table 4
Cohesion of bacterial, fungal and nematode communities between various common European tree species.

	Positive cohesion				Negative cohesion			
Tree	**Bacteria**	**Fungi**	**Nematodes**	**Total**	**Bacteria**	**Fungi**	**Nematodes**	**Total**
Ash (AM)	0.27 ± 0.02b	0.17 ± 0.02a	0.14 ± 0.02c	0.21 ± 0.02a	−0.23 ± 0.01a	−0.09 ± 0a	−0.08 ± 0.02b	−0.15 ± 0.01a
Maple (AM)	0.27 ± 0.02b	0.17 ± 0.02a	0.13 ± 0.03c	0.21 ± 0.02a	−0.23 ± 0.01a	−0.09 ± 0.01a	−0.08 ± 0.04b	−0.16 ± 0.01a
Lime (ECM)	0.27 ± 0.02b	0.15 ± 0.03b	0.14 ± 0.03c	0.21 ± 0.02a	−0.23 ± 0.01a	−0.07 ± 0.01b	−0.09 ± 0.04b	−0.15 ± 0.01a
Oak (ECM)	0.26 ± 0.01c	0.14 ± 0.01c	0.16 ± 0.01b	0.19 ± 0.01b	−0.22 ± 0.01b	−0.07 ± 0.01b	−0.11 ± 0.02a	−0.14 ± 0.01b
Beech (ECM)	0.26 ± 0.01c	0.14 ± 0.01b	0.15 ± 0.02b	0.19 ± 0.01b	−0.22 ± 0.01b	−0.07 ± 0.01b	−0.11 ± 0.02a	−0.14 ± 0.01b
Spruce (ECM)	0.31 ± 0.02a	0.14 ± 0.01c	0.18 ± 0.02a	0.21 ± 0.02a	−0.23 ± 0.01a	−0.07 ± 0.01b	−0.12 ± 0.03a	−0.15 ± 0.01a
ANOVA	***	***	***	***	***	***	**	***
Mycorhizal association	AM > ECM*	AM > ECM*	AM < ECM**	AM > ECM**	AM > ECM*	AM > ECM***	AM < ECM**	AM > ECM*

Positive and negative cohesion indexes representing positive and negative connective levels among OTUs within a community matrix. Significance levels: 0.05 (*), 0.01 (**) and 0.001 (***). Mean values ± SEM with different lettering differ significantly in Tukeys HSD pairwise comparisons. Differences between broadleave trees with AM (Ash, Maple) and ECM (lime, beech oak) symbiosis were tested by Tukey test. Asterisks *, ** and *** indicates significance levels of $P < 0.05$, 0.01 and 0.001 respectively.

3.3. Relative importance of tree species mediated soil properties and litter quality for diversity, structure and cohesion of microbial communities

Random forest analyses revealed that the most important factors (based on increase of mean square error) affecting bacterial OTU richness was soil pH, soil TN and litter cellulose concentration (Fig. 2A). Fungal OTU richness was most affected by litter decomposition indices (Fig. 2B). The OTU richness of nematodes was primarily affected by soil pH, followed by litter cellulose concentration and L:C:H ratio (Fig. 2C). Soil pH was the most important factor affecting positive cohesion index of bacterial (Fig. 3A), fungal (Fig. 3B) and soil nematode communities (Fig. 3C). Soil pH and litter decomposition indices were the most important factors affecting negative cohesion index of the bacterial community (Fig. 3E), while the concentration of lignin and litter decomposition indices were the most important factors associated with negative cohesion index of fungal communities (Fig. 3F) and communities of nematodes (Fig. 3G) respectively. Overall positive cohesion (OTU of bacteria, fungi and nematodes altogether) was most affected by pH followed by soil C:N ratio and concentration of Ca in litter (Fig. 3D). Negative total community cohesion was most affected by pH followed by concentration of lignin and decomposition index of N (Fig. 3H).

3.4. Tree species effect on taxonomic and functional groups of soil biota

The relative abundance of bacterial phyla (based on sequence reads) differed significantly among tree species (Fig. 4A; Table S3). Norway spruce soils had higher relative abundances of Acidobacteriota, Actinobacteriota and Firmicutes than soils in broadleaf tree species (Fig. 4A). In contrast, soils below broadleaved tree species showed higher relative abundance of Proteobacteria than Norway spruce soils (Fig. 4A). Broadleaves associated with AM fungi showed higher abundance of Proteobacteria and Bacteroidetes while broadleaves associated with ECM showed higher relative abundance of Acidobacteriota (Fig. 5A). Soils beneath ash, maple and lime showed high relative abundance of copiotrophs while beech, oak and Norway spruce soils showed high relative abundance of oligotrophs (Fig. 4B). Broadleaves associated with AM fungi had the higher share of copiotrophs, while ECM broadleaves were had a higher share of oligotrophs (Fig. 5B).

The dominant fungal phyla were Basidiomycota, Ascomycota and Mortierellomycota respectively. The relative abundance of Basidiomycota was higher in soils in Norway spruce, oak, beech and lime than in ash and maple soils (Fig. 4C; Table S3). In contrast, the relative abundance of Ascomycota was higher in soils in ash, maple and lime than in oak and beech soils. The fungal community was dominated by soil saprotrophs and ectomycorrhizal fungi. Broadleaves associated with AM fungi showed higher relative abundance of Ascomycota and Mortierellomycota while broadleaves associated with ECM fungi had higher relative abundance of Basidiomycota (Fig. 5C). Soils beneath lime, beech oak and Norway spruce had higher relative abundances of ectomycorrhizal fungi than soils in ash and maple (Fig. 4D). In contrast, soils under ash, maple and Norway spruce had higher relative abundances of soil saprotrophs than lime, beech and oak. Soils under broadleaves associated with AM fungi showed high relative abundance of saprotrophs while soil under broadleaves associated with EcM fungi showed high relative abundance of Ectomycorrhizal fungi (Fig. 5D; Table S3).

Two dominant classes of nematodes Enoplea and Chromadorea were identified. The relative abundance of taxonomic groups of nematodes did not differ significantly among tree species (Fig. 4E; Table S3). In contrast, soils under broadleaves associated with AM fungi were dominated by Chromadorea while soils beneath broadleaves associated with EcM were dominated by Enoplea (Fig. 5E). The community of soil nematodes was equally dominated by the functional groups: bacterivores, omnivores and fungivores respectively. The relative abundance of omnivores did not differ significantly among tree species (Fig. 4F). In addition, soils under broadleaves differed in relative abundance of fungivores being higher in soils beneath broadleaves associated with ECM (Fig. 5F). Soils in Norway spruce, beech and oak had higher abundance of fungivores than soils beneath ash, maple and lime. Bacterivorous nematodes were more abundant in soils beneath ash, maple, lime and beech than under Norway spruce and oak.

Bacterial phyla, fungal classes and the two identified classes of soil nematodes based on sequence reads were associated with soil physicochemical properties and litter traits (Fig. S2A). For example, Acidobacteriota decreased and Proteobacteria increased with pH and litter decomposition rates. Basidiomycota decreased while Mortierellomycota increased with pH and litter decomposition indices. In case of functional groups of soil bacteria, fungi and nematodes, copiotrophs, plant pathogens, litter saprotrophs, herbivores and omnivores were associated with high pH and high litter decomposition rates, while oligotrophs, ectomycorrhizal fungi, and fungivores were associated with low pH and low litter decomposition rates (Fig. S2B).

The PLS-PM revealed that tree species (tree traits and mycorrhizal associations) had significantly positive direct effects on litter quality (litter decomposition indices of C and N, Ca in litter and extractives in litter) and soil properties (moisture, pH, Ca in soil and mineralization indices of C and net N), however tree species only had a significant direct effect on relative abundance of fungal classes (Fig. 6A-C). Litter quality had a direct effect on soil properties and also an indirect effect via litter-mediated soil properties on relative abundance of bacterial phyla and the two classes of nematodes (Fig. 6A, C). In contrast, relative abundance of fungal classes was significantly directly affected by litter quality and tree species traits (Fig. 6B)). In conclusion, tree species affected composition of bacteria and nematodes indirectly via a direct effect on litter chemistry and fungal composition via a direct effect on litter chemistry and tree traits.

3.5. Interactions of communities of nematodes with bacterial and fungal communities

The linear regression model indicated positive correlation between OTU richness of nematodes and bacteria ($R^2 = 0.14$; $p = 0.01$) and a strong positive correlation between fungal and bacterial OTU richness ($R^2 = 0.48$; $p < 0.001$), while OTU richness of nematodes and fungi were unrelated (Fig. S3A-C). Linear regressions based on Hellinger-transformed Bray-Curtis distance revealed a positive correlation ($R^2 = 0.45$; $p < 0.001$) of bacterial community with fungal community. Furthermore, the community of nematodes was positively related with bacterial ($R^2 = 0.24$; $p < 0.001$) and fungal ($R^2 = 0.12$; $p = 0.01$) communities respectively (Fig. S3D-F).

3.6. Site-related effects on diversity, community structure, cohesion and relative abundance of soil bacteria, fungi and nematodes

Site locations differed significantly in edaphic factors such as soil texture and site related climatic factors (Table S1). Site effects were partly related to land-use history. Litter quality and soil properties differed among sampling sites (Table S4-S5)). For example, Kragelund showed highest soil mineralization of C and N than other sites while Wedellsborg showed highest litter decomposition rates of C and N. There was a divergence in community structure between former cropland (Mattrup and Kragelund) and sites with a long-term forest legacy (Fig. S4A-C). In contrast, the alpha diversity indices of bacteria, fungi and nematodes differed among sampling sites but there was no clear pattern related to land use history (Table S5). Similar differences among sampling sites but no clear effects of former land use were also found for positive and negative cohesion indices (Table S6) and for relative abundance of taxonomic and functional groups of soil bacteria (Fig. S5A-F, Table S7).

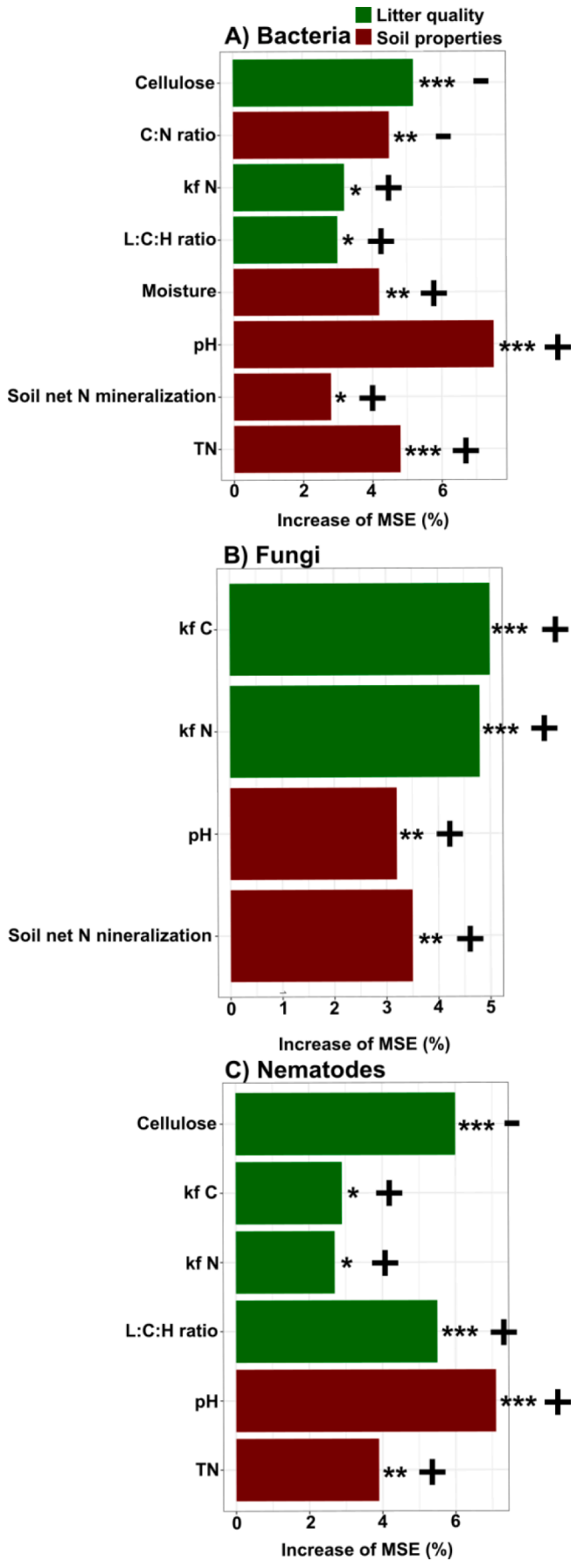

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Fig. 2. The relative importance of tree species mediated soil properties and litter quality affecting OTUs richness of bacteria (A), fungi (B) and nematodes (C). Only factors with p value < 0.05 are present. Estimated permutation p-values for random forest importance metrics: 0.05 (*), 0.01 (**) and 0.001 (***). MSE – Mean square error. The + and – signs refer positive and negative effect of soil properties or litter traits on OTU richness based on Pearson correlation coefficients.

4. Discussion

4.1. Factors affecting diversity, structure and cohesion of soil microbial communities

Soils beneath broadleaf trees showed on average higher OTU richness than soils under Norway spruce. Also, community structures of bacteria, fungi and nematodes differed between tree species with distinct separations of broadleaves and Norway spruce. Only nematodes showed differences in OTU richness between and of AM and ECM hosts. This indicate that tree traits based on phylogenetic differences among broadleaved and coniferous tree species are more important for bacterial and fungal diversity than mycorrhizal associations. We suggest that this can be attributed to broadleaved trees producing high quality leaf litter with low C:N ratio and low concentrations of recalcitrant compounds, which promotes more available nutrients than in Norway spruce (Meier and Bowman, 2008).

Our results revealed that diversities of fungi, bacteria and nematodes were controlled by both litter quality and tree species mediated soil properties. Soil pH showed the largest effect on bacterial diversity, while C and N litter decomposition indices had the largest effect on fungal diversity. We therefore suggest that tree species mainly shape bacterial diversity by modification of the soil pH and C:N ratio (Heděnec et al., 2020; Liu et al., 2018), while soil fungal communities are mostly governed by leaf litter quality such as lignin and cellulose content (Algora Gallardo et al., 2021). Nematode diversity was mainly related to soil pH and litter cellulose content, and we suggest that soil environment and substrate quality shape diversity of nematodes indirectly via a bottom-up effect of bacterial and fungal communities (Cesarz et al., 2013).

Community structures of bacteria, fungi and nematodes diverged between tree species and mycorrhizal association suggesting adaptation of microbial communities to easily degradable substrate under trees associated with AM and to recalcitrant substrate under trees associated with ECM (Eagar et al., 2022; Heděnec et al., 2020). Apart from litter-mediated effects, we also suggest an effect of tree species on microbial diversity via rhizodeposition (Paterson et al., 2007) including root exudates (Doornbos et al., 2012). For example, a study by Brant et al. (2006) showed that root C inputs exert a large control on microbial communities in various forest ecosystems. We also found fungal and nematode communities clustered along sampling periods in spring and summer, respectively, indicating a possible seasonal effect (Wu et al., 2016).

Our results revealed that soil from ash, maple and Norway spruce showed higher community cohesion of microbial communities than in lime, beech and oak. Communities with greater (positive or negative) cohesion indices refer higher connectivity between taxa or functional groups that share similar niche or compete for similar resource (Herren and McMahon, 2017). For example, Norway spruce has high concentration of lignin in litter, low pH and high C:N ratio in soils, therefore we expect that soil under Norway spruce will host taxa (e.g. Actinobacteria) and functional groups (Wood saprotrophs) associated with lignin decomposition and tolerating low N and more acid conditions (Urbanová et al., 2015). In contrast, ash and maple have higher pH and low C:N ratio in soils and thus supporting taxa and functional groups associated in decomposition of easily decomposable substrate.

Soil pH was the most important factor explaining positive cohesion index of fungal and nematode communities, while concentration of lignin and the litter decomposition index for N were the most important

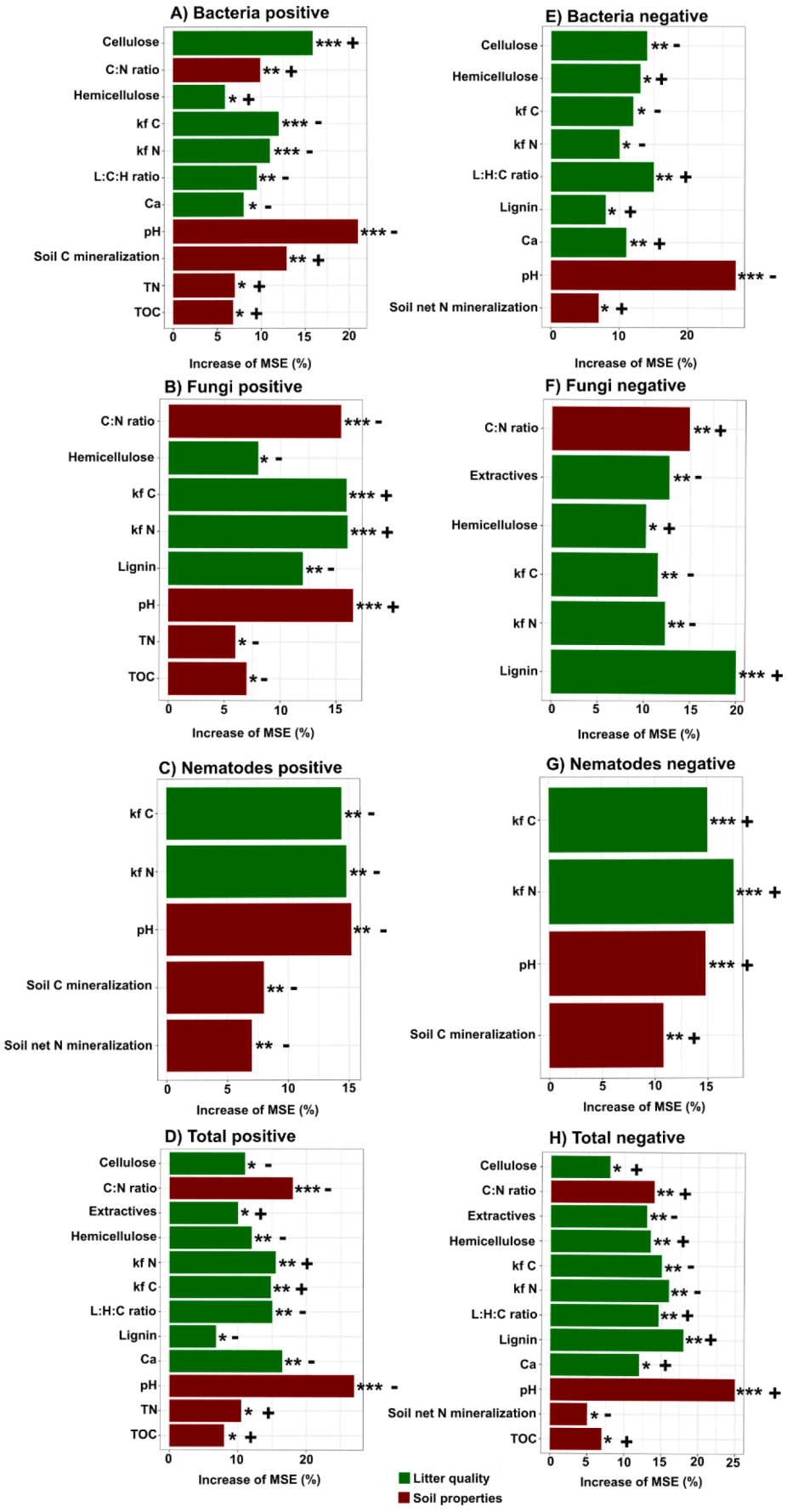

Fig. 3. The relative importance of litter quality and litter mediated soil properties affecting positive (A-D) and negative (E-H) cohesion of bacterial (A, E), fungal (B, F) nematodes (C, G) and total (D, H) microbial community. Only factors with p value < 0.05 are present. Estimated permutation p-values for random forest importance metrics: 0.05 (*), 0.01 (**) and 0.001 (***). MSE – Mean square error. The + and – signs refer positive and negative effect of soil properties or litter traits on OTU richness based on Pearson correlation coefficient.

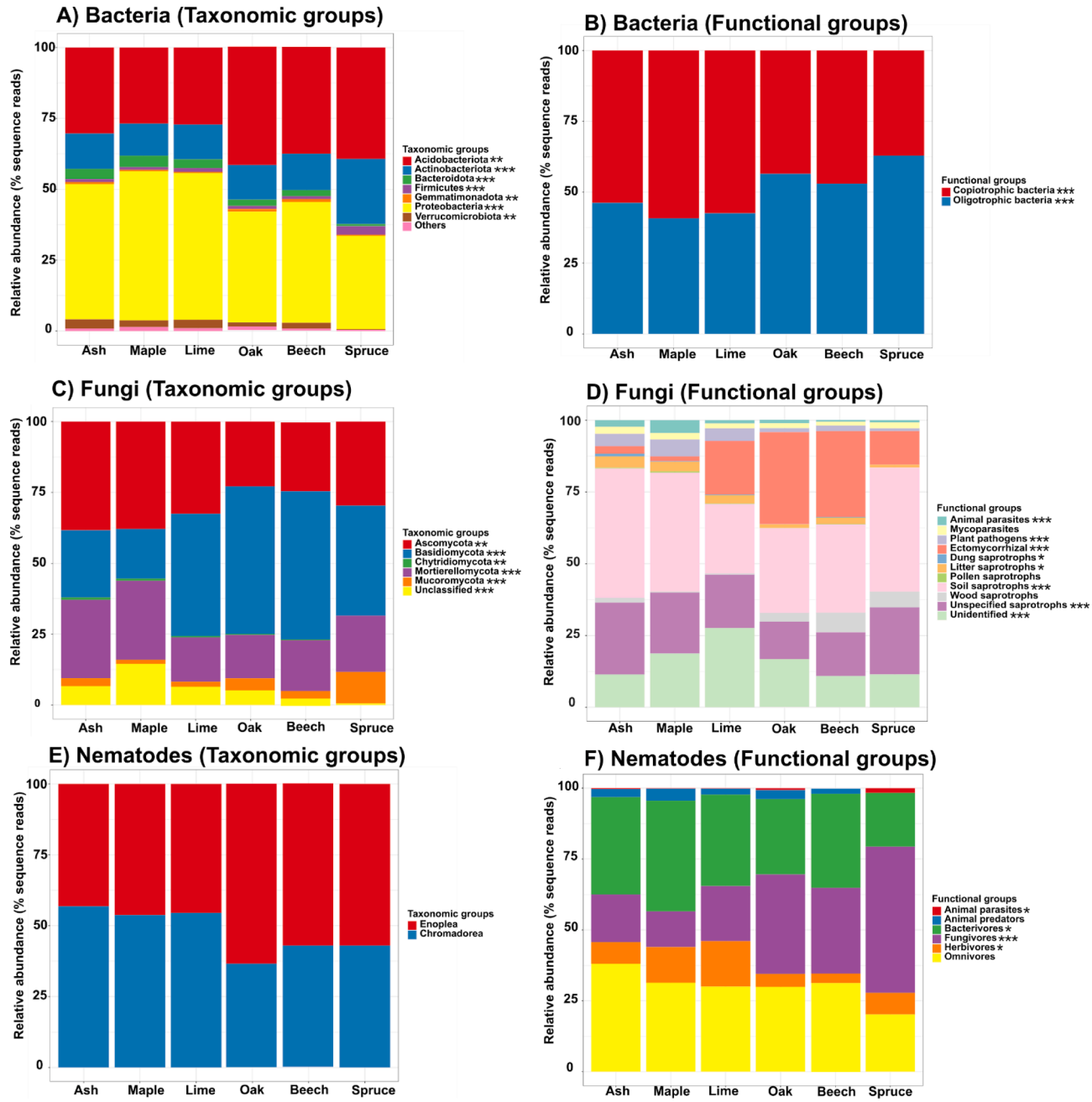

Fig. 4. Relative abundance of taxonomic and functional groups of bacteria (A-B), fungi (C-D), nematodes (E-F) in soils under six common European tree species.

factors associated with negative cohesion index of fungal communities and communities of nematodes respectively. Therefore, we hypothesize that extremes like ash and spruce along the pH gradient shaped the degree of cohesion in the soil microbial community (Cruz-Paredes et al., 2017).

4.2. Tree species mediated shifts in relative abundance of fungal and bacterial groups in soil

We found high relative abundance of Proteobacteria in soils beneath ash, maple and lime, while soils under oak, beech and Norway spruce showed high relative abundance of Acidobacteriota, Actinobacteriota and Firmicutes. There was a high relative abundance of oligotrophic bacteria in soils under oak, beech and Norway spruce while copiotrophs were dominant in soils under ash, maple and lime. Oligotrophic (K-strategists) bacteria scavenge for the nutrients from recalcitrant soil organic matter while copiotrophs (r-strategists) scavenge for nutrients from easily available soil organic matter (Bastian et al., 2009; Fierer et al., 2007; Ho et al., 2017). Same is true for fungi and nematodes. Our results therefore suggest a strong litter mediated effect of tree species on soil properties such as pH and C:N ratio (Dawud et al., 2016; Vesterdal and Raulund-Rasmussen, 2011), which in turn shapes diversity and composition of functional groups of soil bacteria (Hedènec et al., 2020). Schelfhout et al. (2017) and Steffens et al. (2022) reported such substantial effects of tree species on soil C and N stocks, pH and base saturation in the same common garden experiments. Moreover, our results also indicate a strong positive association of copiotrophs with litter decomposition indices and soil C and N mineralization rates. The tree species-mediated quality of the soil environment is consequently an important driver of functional responses of soil bacterial community within the studied range of common European tree species. Our findings in European tree species are supported by Fierer et al. (2007), who

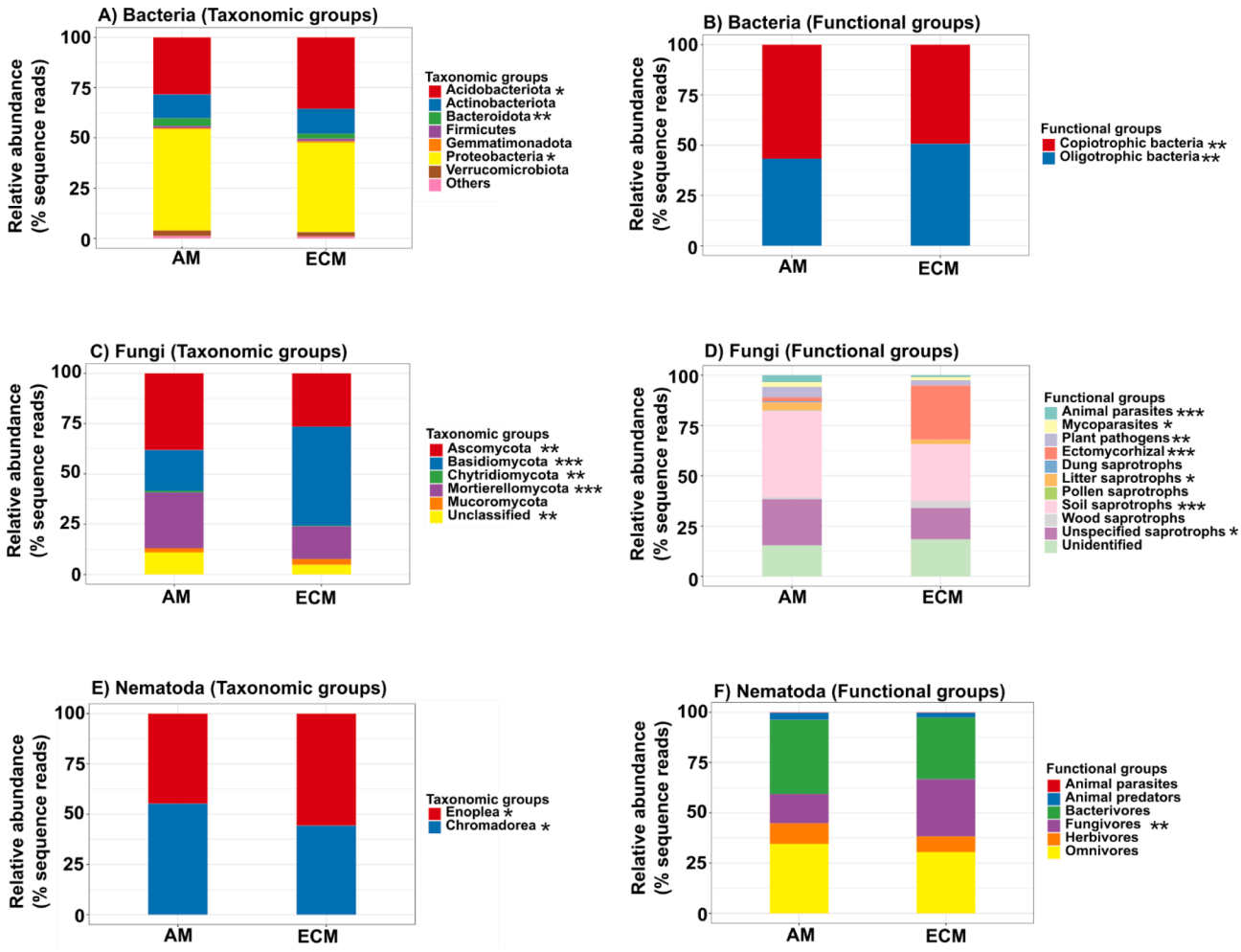

Fig. 5. Relative abundance of bacteria (A), fungi (B), nematodes (C), bacterial functional groups (D), fungal functional groups (E) and functional groups of nematodes (F) in soils under broadleaved trees associated with AM and ECM fungi respectively.

showed increased relative abundance of copiotrophic bacteria (Bacteroidetes, Proteobacteria) and decreased abundance of oligotrophic bacteria (Acidobacteria, Actinobacteria) with increasing C mineralization rate. However, we suggest that overal pattern of various functional groups of soil microbiota would change if more OTU could be identified at species level and assigned to specific life strategy.

The fungal community data revealed a large proportion of saprotrophic fungi in soils under the AM associated species ash and maple while soils under lime, beech and oak had higher relative abundance of ECM fungi consistent with their mycorrhizal association. It is likely that ECM fungi in the latter tree species take over some of the decomposition activity from saprotrophic fungi, especially of recalcitrant material, in line with the hypothesis put forward by Lindahl and Tunlid (2015). Furthermore, soils under lime, beech, oak and Norway spruce were dominated by Basidiomycota, of which many engage in ECM associations (Põlme et al., 2020; Tedersoo et al., 2014). We detected a small proportion of ECM fungi in soils under ash and maple, which are typically associated with AM fungi, but this can be an artefact of beech seedlings, which were also present here and there in plots with sufficient light, particularly under ash. In contrast, soils under ash and maple were higher in abundance of Mortierellomycota, which are dominantly saprotrophic (Põlme et al., 2020; Tedersoo et al., 2014). Surprisingly we found lower relative abundance of ECM fungi and higher share of saprotrophs in soils under Norway spruce than in the other ECM associated species. This may be due accumulation of distinctly larger forest floor mass on top of the mineral soil in Norway spruce in comparison with all broadleaf species (Vesterdal et al., 2008). Surprisingly we recorded only very small numbers of sequence reads assigned to AM fungi from group Glomeromycota in soils under AM trees. This is likely because Glomeromycota are rather present in rhizosphere than in bulk soil, which was sampled in our study (Öpik et al., 2006). We sampled the top 5 cm of the mineral soil where saprotrophs can penetrate from the forest floor, while the relative abundance of mycorrhizal fungi increase with soil depth (Mundra et al., 2021; Šnajdr et al., 2008).

We identified litter decomposition indices as key factors shaping diversity and composition of functional groups of soil fungi. The relative abundance of saprotrophic fungi and plant pathogens increased with high C and N litter decomposition indices while ectomycorrhizal fungi showed the opposite trend, even with spruce being slightly lower in relative abundance of ECM fungi (Lindahl et al., 2007). We suggest that high decay rates can also shape diversity of soil saprotrophs via higher nutrient availability (Cruz-Paredes et al., 2021; Uroz et al., 2016). Decaying leaf litter can be considered as reactive interfaces where nutrient cycles are intensified and thus promoting available niches for variety of saprotrophic fungi (Uroz et al., 2016). In contrast, ECM fungi often thrive on more recalcitrant substrates with high C:N and high lignin:cellulose content, which require extracellular enzymes for decomposition (Lindahl and Tunlid, 2015; Phillips et al., 2013). We hypothesize that AM associated tree species with a high proportion of copiotrophic bacteria and saprotrophic fungi supporting more inorganic nutrient economy of AM tree species while trees associated with ECM fungi with high proportion oligotrophic bacteria, ECM fungi promoting

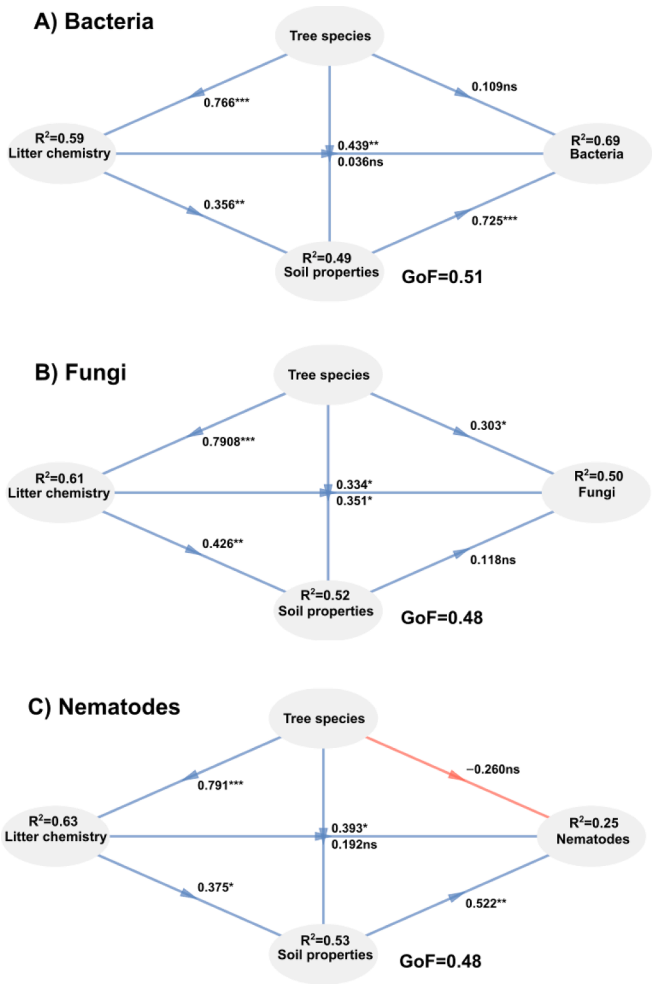

Fig. 6. Directed graph of the partial least squares path model (PLS-PM) of the tree species (tree traits and mycorrhizal associations), litter quality (litter decomposition indices of C and N, Ca in litter and extractives in litter) and litter mediated soil properties (moisture, pH, Ca in soil and mineralization indices of C and net N) on relative abundance of bacterial phyla (A), fungal classes (B) and classes of nematodes (C. Path coefficients and explained variability (R^2) were calculated after 999 bootstraps. Blue and red arrows represent positive and negative effects, respectively. The categorial variables used in PLS PM were binary coded whereas "1″ indicated the broadleaved or AM species, and "0" the coniferous or ECM species. The model was assessed using the goodness of fit (GoF). Significance levels are indicated by * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ respective. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

more organic dominated nutrient economy of ECM tree species (Phillips et al., 2013).

4.3. Links between nematodes and communities of bacteria and fungi

The community composition of nematodes was characterized by higher relative abundance of bacterivorous nematodes in ash and maple, while beech, oak and Norway spruce had higher relative abundance of fungivorous nematodes. Our eDNA sequence-based results are in line with results reported by Cesarz et al. (2013) based on micro-morphological characteristics. We hypothesize that litter traits shift the channeling of energy through the decomposer food web. For instance, tree species producing high quality leaf litter support bacterivores, which rely on the bacterial energy channel, while tree species producing low quality leaf litter will support fungivores relying on the fungal energy channel. In addition, we found higher diversity in nematodes in soils under AM species suggesting a possible link with higher bacterial growth reported in a previous study by Hedĕnec et al. (2020) from the same sites. In contrast, we reported higher cohesion in the community of nematodes and also high relative abundance of fungivorous nematodes under trees associated with ECM suggesting that the community of nematodes is linked with high biomass of ECM fungi as reported by Kitagami and Matsuda (2022).

Bacterial and fungal communities were closely correlated with the community of nematodes, which indicates a strong positive bottom-up effect of soil bacterial and fungal communities on the community of nematodes. Similarly, Singer et al. (2020) reported a positive correlation of sequence reads and OTU richness of Apicomplexa with sequence reads and OTU abundance of their invertebrate hosts indicating a strong positive feedback mechanism between hosts and parasites. In addition, we suggest that correlations between bacterial and fungal community structures and the community structure of nematodes provide information about the active part of the microbial community since the turnover of nematodes is linked with presence of their food resources (Cesarz et al., 2013; Matlack, 2001; Nasri et al., 2020). For example, Matlack (2001) suggest that aboveground disturbance affects the nematode community only to the extent that it influences the availability of potential hosts or prey in the soil.

We welcome future studies using RNA-based sequencing to shed further light on the active microbial communities (Bang-Andreasen et al., 2019). Furthermore, Nasri et al. (2020) showed decreased density of bacterial feeding nematodes in marine sediments after addition of antibiotics ciprofloxacin. Our study indicates that the bottom-up effect of fungi and bacteria on nematodes have potential to reveal the active part of soil microbiome. This suggests that the combined amplicon sequencing of bacterial, fungal and nematode communities is relevant to unravel the active fraction of microbial community, which may be differently linked to environmental conditions.

5. Conclusions

The relative abundances of copiotrophic bacteria, fungal saprotrophs and bacterivorous nematodes were associated with ash maple and lime having high soil pH, and high C and N litter decomposition indices. Relative abundance of oligotrophic bacteria, ectomycorrhizal fungi and fungivorous nematodes were associated with beech, oak and Norway spruce having low soil pH and low C and N decomposition indices. Broadleaved tree species associated with AM fungi had higher overall microbial community cohesion as well as cohesion of bacterial and fungal communities than broadleaved trees associated with ECM fungi. In contrast, cohesion of nematode communities were higher under trees associated with ECM fungi than under trees associated with AM fungi. Soils beneath Norway spruce hosted a lower diversity of bacteria, fungi, and nematodes than soils planted to broadleaf trees. Diversity, structure and cohesion of the bacterial community were mainly explained by soil pH, in turn driven by leaf litter quality, while fungal diversity and cohesion were directly associated with litter lignin concentration and decomposition indices for C and N, and by soil pH. The communities of bacterial and fungal feeding nematodes were closely positively correlated with communities of bacteria and fungi, respectively. Our study suggested tree species identity, traits, and mycorrhizal association substantially shape microbial communities via a direct effect of litter chemistry as well as via litter-mediated soil properties.

CRediT authorship contribution statement

Petr Hedĕnec: Writing – original draft, Conceptualization, Methodology. **Haifeng Zheng:** . **David Pessanha Siqueira:** . **Qiang Lin:** . **Yan Peng:** . **Inger Kappel Schmidt:** . **Tobias Guldberg Frøslev:** . **Rasmus Kjøller:** Conceptualization, Methodology. **Johannes Rousk:** Conceptualization, Methodology. **Lars Vesterdal:** Conceptualization,

Methodology.

Declaration of Competing Interest

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

Data availability

Data are in supplementary file.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2022.120608.

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