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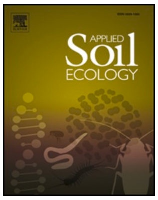

Research paper

Phylogenetically related fast-growing trees and their root traits affect soil microbial communities

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ABSTRACT

The soil microbiome plays a key role in tree growth and nutrient cycling. Although hybrid poplar clones are phylogenetically related, their influence on the soil microbiome may differ due to phenotypic differences, especially their root mean diameter or mass density (RMD). This influence remains relatively unexplored, limiting our understanding of how soil microbial communities contribute to the growth strategies of fast-growing trees. Our objective was to determine how phylogenetically related trees and their root traits influence soil microbial diversity and composition by studying five hybrid poplar clones growing in a plantation located in New Liskeard, Ontario, Canada. We collected soil cores at depths of 0–20 cm (topsoil) and 20–40 cm (subsoil) and analyzed fine root traits and soil bacterial and fungal communities. We found that phylogenetically related hybrid poplars influenced the soil microbiome by shaping the composition of microbial communities. Differences between clones were evident in the relative abundance of soil ectomycorrhizal fungi and Actinobacteriota in both topsoil and subsoil. The increase in relative abundance of ectomycorrhizal fungi was driven by high RMD and root length density (RLD), i.e., high fine root mass and length per unit soil volume. Fine roots with high RLD led to a higher relative abundance of Actinobacteriota in the topsoil, while recalcitrant fine roots (high lignin/nitrogen ratio) promoted their abundance in the subsoil. Thus, root traits are key factors in determining the effects of trees on soil microbiome. The soil microbiome, together with root traits, could be an important aspect to consider in tree selection.

1. Introduction

Soil microbial communities are responsible for a number of major soil functions, including soil carbon (C) and nutrient cycling, plant growth promotion, protection against soil-borne pathogens and host immune regulation (Berendsen et al., 2012; Gazitúa et al., 2021; Mendes et al., 2013). As primary decomposers of soil organic matter (OM), soil microorganisms are dependent on the quantity and quality of tree inputs (Bertin et al., 2003; Strickland et al., 2009). In this context, tree genetics can play a role in shaping the soil microbial diversity and composition (Karliński et al., 2020; Lottmann et al., 2010). For example, phylogenetically distant tree species can lead to significant differences in soil microbial composition, as they share a shorter common evolutionary history and are less likely to develop common traits than phylogenetically related species (Dukunde et al., 2019; Harvey and Pagel, 1991; Yang et al., 2019). However, little is known about the potential of phylogenetically related trees to shape distinct soil microbial communities, which limits our understanding of plant-soil interactions and tree growth.

Hybrid poplars (*Populus* spp.) are among the fastest-growing trees in Canada, helping to meet the growing global demand for wood products (Peichl et al., 2006). They are used as model organisms to study tree-

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microorganism interactions (Rubin, 2008; Sannigrahi et al., 2010), and isolates from poplar microbial communities have been shown to improve the health and growth of their host plants (Taghavi et al., 2009; Timm et al., 2016). Hybrid poplar clones are considered phylogenetically close, as they are derived from crosses between two or more species of the same genus *Populus*. Therefore, poplar genetic effects on the soil microbiome might be limited compared to environmental factors (Gehring et al., 2006; Karliński et al., 2020). However, in some studies, significant differences between hybrid poplar clones were detected for microbial groups such as saprotrophs and mycorrhizal fungi (Cregger et al., 2018; Khasa et al., 2002). Some clones may favor mycorrhizal interactions to improve their growth (Smith and Read, 2010), even in the subsoil through their deep roots (Dickmann et al., 1996). In a similar soil environment, it remains to be determined which soil microbial groups and functions show the most variation between phylogenetically related trees.

Studies have shown that hybrid poplar clones have different functional traits of fine roots (diameter < 2 mm) whether in topsoil or subsoil (Al Afas et al., 2008; Rabearison et al., 2024). These differences could mainly explain the variation in the soil microbiome of these phylogenetically related trees since fine roots provide energy sources (root secretions and exudates) for soil microorganisms (Babalola et al., 2021; Sun et al., 2021). For instance, fine roots with a smaller diameter and higher specific root length (SRL) had a higher relative abundance of rhizosphere Bacteroidota, a group identified as copiotrophic, compared to thicker fine roots due to the release of easily decomposable litter (Hu et al., 2023; Pérez-Jaramillo et al., 2017; Pervaiz et al., 2020). Higher root mass density (RMD), i.e. fine root biomass per unit soil volume, can increase mycorrhizal colonization, as it reflects the size of the root system and determines the niche available for mycorrhizal colonization (Demenois et al., 2018; Emery et al., 2018). Yet, a comprehensive understanding of the effects of root traits of hybrid poplars on the soil microbiome is still lacking. Since such traits are linked to the growth rate of poplar trees (Rabearison et al., 2024), assessing these effects would contribute to a better understanding of the growth strategy of fast-growing trees.

Soil microbial communities also differ according to root chemical traits of plant species, which determine the quality of organic compounds released into the soil (Wardle, 2004; Wilschut et al., 2019). Soil saprotrophic fungi are considered to be the main decomposers of recalcitrant compounds and may be more closely associated with root lignin concentration (Clemmensen et al., 2015; Lindahl and Tunlid, 2015). Oligotrophic bacteria (Acidobacteriota) could also be associated with this root chemical trait due to their ability to decompose recalcitrant matter (Fierer et al., 2007). On the other hand, the growth of soil copiotrophic bacteria, such as the Actinobacteriota and Firmicutes, is generally stimulated by increases in labile or easily decomposable OM and could then be associated with higher root N and soluble compounds concentrations (Fierer et al., 2007; Gschwendtner et al., 2016). However, some members of the Actinobacteriota have also been found to be oligotrophic (Hu et al., 2023; Li et al., 2021). Thus, the relationship between root chemical traits and the soil microbiome remains unclear.

Hybrid poplar clones and their roots could also influence soil microbial communities through indirect pathways. They can modify soil chemical properties, such as the soil organic carbon (SOC) and total nitrogen (N) (Rabearison et al., 2023), which in turn, will shape soil microbial communities (Gehring et al., 2006; Karliński et al., 2010). For instance, higher soil total C and N increased ectomycorrhizal colonization in a poplar plantation (Gehring et al., 2006). The relative abundance of soil Bacteroidota and Gamma-proteobacteriota, which are mainly copiotrophs, was positively related to soil total C and inorganic N, especially in the subsoil (Xia et al., 2020). This suggests that root traits might indirectly influence the soil microbial community through changes in soil chemical properties. Identifying these indirect effects could help select the optimal combination of soil types and tree genotypes for plantation management strategies.

The aim of our study was to test the effect of phylogenetically related trees on soil microbial diversity and composition using different hybrid poplar clones, and to determine whether their root traits have direct or indirect (via some soil chemical parameters) effects on the soil microbiome. We hypothesized that soil bacterial and fungal communities, in topsoil and subsoil, would differ between hybrid poplar clones that have different traits. The difference between clones in soil fungal communities would occur more in mycorrhizal fungi than in other fungal groups since they might be more influenced by root traits that were found to differ significantly between clones (e.g., RMD and root length density (RLD), see Rabearison et al., 2024). For soil bacteria, there would be more differences between clones for copiotrophs than for oligotrophs as fast-growing trees mainly transfer labile compounds (Fierer et al., 2007). Root architectural traits (RMD and RLD) would have more direct than indirect effects on soil microbial communities as the increase in these traits could directly improve the niches and food sources available for soil microorganisms. Root chemical traits would have direct effects on the soil microbiome by modifying the availability of carbon sources for microbial growth, but also indirect effects through their impact on the soil C and N.

2. Materials and methods

2.1. Study site

The study was conducted in a hybrid poplar plantation established on agricultural land at the New Liskeard Agricultural Research Station, University of Guelph, Ontario, Canada (47°31′15″ N, 79°39′52″ W). The region has a humid continental climate with an average daily temperature of 2.6 °C and average annual precipitations of 786 mm (576 mm rain and 222 cm snow) based on climate data from 1981 to 2010 (Earlton station) (Environment Canada, 2023). The site features a soil with clay loam texture (Yan et al., 2019) and humic Gleysol type (IUSS Working Group WRB, 2015).

The experimental site was ploughed and cross-cultivated with agricultural disks in October 2006, followed by pre-emergent herbicide applications in spring 2007. One-year-old rooted cuttings were used to plant hybrid poplars in the Spring of 2007 with the spacing of 3.5 m × 3.5 m (816 stems ha^{-1}) and NPK 18–23-18 fertilization (110 g tree^{-1}) at a rate of 89.76 kg ha^{-1}. The presence of weeds was controlled by ploughing between rows with disks followed by herbicide applications between trees (RoundupTM) for the first two years after plantation establishment. The experimental design featured three complete blocks (replicates), each containing eight monoclonal plots of 100 trees (10 rows × 10 trees) randomly distributed within blocks. After 14 years of planting (2021), we selected, from the eight planted, five clones with different parentage and root traits (Rabearison et al., 2024): 1079 (*Populus* × *jackii* (*P. balsamifera* × *P. deltoides*)), 747210 (*P. balsamifera* × *P. trichocarpa*), 915005 (*P. balsamifera* × *P. maximowiczii*), 915319 (*P. maximowiczii* × *P. balsamifera*) and DN2 (*P. deltoides* × *P. nigra*).

2.2. Soil sampling and preparation

We systematically sampled soils between two trees in each monoclonal plot, excluding the two outer rows and columns to avoid edge effects. Soil cores were collected at two distances [87.5 cm (quarter) and 175.0 cm (center)] from a stem and at two soil depths (0–20 and 20–40 cm) using a PVC cylinder measuring 10 cm in diameter and 20 cm in length. We sharpened one edge of the PVC cylinder and used a wooden block and a hammer to help embed the cylinder into the soil. We collected soil samples at the 40–60 cm depth, but the DNA concentration in these samples was too low and out of range for further analysis. We obtained a total of 60 soil samples (2 distances × 2 soil depths × 5 clones × 3 replicate blocks), which were placed in sterile bags, stored on ice in a cooler in the field and at 4 °C upon arrival at the laboratory.

Within two weeks after the soil sampling, each soil core was broken into smaller pieces and carefully homogenized. We fresh-weighed each soil sample and took approximately 40 and 300 g for microbiome and soil analysis, respectively. Sub-samples for microbiome analysis were stored at −20 °C prior to DNA extraction, while those for soil chemical analysis were air-dried. We extracted and weighted the pebbles and found that they represented no more than 0.3 % of the soil mass in each sample. The remaining portion of each soil sample, accounting for around 85 % of the total mass, was fresh-weighed and stored in a refrigerator for root analysis. Fresh masses were required to calculate the adjusted soil volume to determine SOC stocks and root architectural traits.

2.3. Root and soil analyses

Complete details for root analysis can be found in Rabearison et al. (2024). For the current study, we selected root traits as in Rabearison et al. (2024): average root diameter (D), specific root length (SRL), root dry mass content (RMDC), root mass density (RMD), root length density (RLD), root carbon concentration (RCC), root nitrogen concentration (RNC) and root lignin concentration. Briefly, roots were washed and rinsed using a root washer (Gillison's Variety Fabrication Inc., USA) according to Smucker et al. (1982). Total root length and D (mm) were determined using a scanner at 400 dpi resolution (Epson Perfection V800; Epson, Canada) and WinRhizo Pro 2019 software (Regent Instruments, Canada). We calculated SRL (m g^{-1}) as the ratio of total root length to root dry mass, and root dry matter content (RDMC, mg g^{-1}) as the ratio of root dry mass to root fresh mass. We calculated RMD (g cm^{-3}) as the ratio of root dry mass to the soil volume from which roots were extracted, and RLD (cm cm^{-3}) as RMD multiplied by SRL. Finally, we finely ground dried root samples using a 2 mm sieve and an ultra-centrifugal mill (ZM200, Retsch GmbH., Germany), and analyzed RCC and RNC by dry combustion (Vario MAX cube; Elementar, Germany). Root lignin concentrations (mg g^{-1}) were analyzed by the method of Van Soest et al. (1991) using a fiber analyzer (Fibersac 24; Ankom, USA). We also included the root lignin/N ratio since it is a good predictor of root decomposability (Roumet et al., 2016).

Air-dried subsamples for soil chemical analysis were ground to 2 mm. Complete details of the soil organic C (SOC) stock and soil total N concentration analyses can be found in Rabearison et al. (2023). Briefly, soil C and N concentrations were analyzed by dry combustion (Vario MAX cube; Elementar, Germany). Soil C/N ratio was calculated by dividing soil organic C concentration by total N concentration. Our previous soil pH analysis revealed average values of 6.9 (±0.2) and 7.4 (±0.2) at the 0–20 and 20–40 cm depths respectively, after analyzing one subsample from each clone in each block and at each depth (Rabearison et al., 2023).

2.4. DNA extraction, library preparation and sequencing, and bioinformatic processing

Soil DNA was extracted from 0.25 g of bulk soil using the DNeasy Powersoil Pro kit with the QIAcube system following the manufacturer's instructions (QIAGEN, Valencia, CA, USA). The concentration of DNA extracts was measured with the Qubit™ dsDNA HS Assay Kit and the Qubit™ 3.0 fluorometer device (Thermo Fisher). DNA extracts were diluted to 10 ng/μL with 10 mM Tris pH 8.0 solution using the QIAgility automated system (Qiagen) (no dilution if DNA concentration was lower than 10 ng/μL) and shipped on dry ice to the Centre d'expertise et de services, Génome Québec (Montréal, Canada) for library preparation and sequencing. Selected primers were 515 F-Y (5′-GTGYCAGCMGCCGCGGTAA-3′) and 926R (5′-CCGYCAATTYMTTTRAGTTT-3′) targeting the V4-V5 regions of the 16S rRNA gene of bacteria and archaea (Parada et al., 2016) and ITS9F (5′-GAACGCAGCRAAIIGYGA-3′) and ITS4R (5′-TCCTCCGCTTATTGATATGC-3′) targeting the ITS2 region of fungi (Menkis et al., 2012). Amplicon libraries were sequenced on an Illumina MiSeq platform using a PE300 v3 kit.

Bioinformatics analyses were performed using QIIME2 (Bolyen et al., 2019), version 2023.02 within the Q2Pipe pipeline (Nagati et al., 2024). The overall procedure was similar for each taxonomic marker. Briefly, fastq files were imported and sequence reads were truncated at their 5′ and 3′ ends based on primer length (16S rRNA genes) or specific primer sequence (ITS2 region) and per base sequence quality score, respectively. The QIIME2 plugin DADA2 (Callahan et al., 2016) was selected as the denoising method, which allowed for filtering, dereplication, merging of paired-end reads, and chimera identification. The SILVA (Quast et al., 2012) and UNITE (Abarenkov et al., 2010) databases were used for the taxonomic assignment of the resulting 16S rRNA gene ASVs and fungal ITS2 ASVs, respectively, using the QIIME2 plugin feature-classifier classify-sklearn. For the 16S rRNA gene, ASVs assigned to Eukaryota, mitochondria, chloroplast and unassigned ASVs at the kingdom level were filtered out of the ASV table. For the fungal ITS2 regions, only ASVs assigned to the kingdom Fungi were retained in the ASV table. The fungal ASV table was further analyzed using FUNGuild (Nguyen et al., 2016) to infer the ecological guilds of fungi based on their taxonomy. Metabarcoding raw sequence data and metadata were deposited in the Sequence Read Archive of the NCBI (BioProject ID: 1240279, accession number: PRJNA1240279).

As our objective was to determine the effect of clones and their root traits on soil fungal and bacterial composition, and since variations of root traits and soil chemical parameters were different at each soil depth (Rabearison et al., 2023, 2024), further analyses were conducted separately for each soil depth. We chose thresholds of 68,231 and 5060 sequences per sample for soil fungi to randomly rarefy data from the 0–20 and 20–40 cm depths, respectively, as these rarefaction thresholds were sufficient to capture the vast majority of ASVs in the samples. Thresholds of 65,983 (0–20 cm depth) and 5770 (20–40 cm depth) sequences were selected for bacteria. ASV tables generated by QIIME2 were imported into R as phyloseq objects (phyloseq package version 1.46.0) (McMurdie and Holmes, 2013).

2.5. Statistical analyses

All statistical tests were performed using the R software version 4.2.2 (R Development Core Team, 2013) and all plots were created using the ggplot2 package (Wickham, 2016). The effects of distance from trees on soil fungal and bacterial diversity and composition were not significant and hence were dropped, but data from both distances were retained to determine clone and root trait effects.

2.5.1. Effects of clones on soil microbial diversity and composition

Alpha diversity indices (i.e., observed richness (number of ASVs), Shannon index) of soil fungal and bacterial communities were calculated using the “estimate_richness” function of the phyloseq package. We compared observed richness and Shannon index between clones at each depth by using linear mixed models where block (replicate) was considered as a random effect (lme4 and lmerTest packages, Bates et al., 2015; Kuznetsova et al., 2017). The predictor effect was significant when the probability level (*P*) was below the theoretical probability level $\alpha = 0.05$. Model assumptions (independence of residuals, equality of variance (homoscedasticity), and normality of residuals) were tested by diagnostic graphs and Shapiro-Wilk tests. When the model assumptions were not met, square root or Ordered Quantile normalization (orderNorm() function in bestNormalize package) transformations were performed. Tukey's Honest Significant Difference (HSD) test was applied as a *post-hoc* method using “emmeans” function (emmeans package) to make pairwise comparisons where appropriate (Lenth et al., 2018).

Beta diversity was assessed on rarefied ASV tables using Bray-Curtis dissimilarity matrices (“vegdist” function) and permutational multivariate analysis of variance (PERMANOVA, “adonis” function) with 999 permutations using the vegan package. PERMANOVA tests were done between clones on soil fungal and bacterial community composition (ASV matrices), considering block (replicate) as random factors (strata).

Post-hoc pairwise comparison was tested where appropriate using the “pairwise.adonis” function in the “pairwiseAdonis” package.

To test our hypotheses, we calculated and compared the relative abundance of soil fungal guilds and bacterial phyla between clones at each depth. For soil fungi, we used FUNGuild to determine the functions of fungal ASVs (Nguyen et al., 2016). We only considered assigned functional categories with highly probable and probable confidence to avoid over-interpretations. We then simplified functional groups into saprotroph, ectomycorrhiza, pathogen, and other mycorrhizae to facilitate data interpretation. The fungal ASVs identified as ‘Unassigned’ or with multiple guilds were classified into ‘Other’. We compared the relative abundance of soil fungal guilds between clones by using linear mixed models where block (replicate) was considered as a random effect. To meet model assumptions, square root was applied to the ‘ectomycorrhiza’ variable. We also produced a taxonomic profile of the top 20 soil fungal families for each clone to complement the results of soil fungal guilds (Fig. S1). For soil bacteria, phyla with a relative abundance higher than 2 % were compared between clones. As relative abundances of bacterial phyla are generally zero-inflated (Ho et al., 2019), we used zero-inflated mixed models using “glmmTMB” function in glmmTMB package to compare clones (Brooks et al., 2017). We performed *post-hoc* pairwise comparisons using “emmeans” function where appropriate.

2.5.2. *Direct and indirect effects of root traits on soil microbial community*

We conducted piecewise structural equation modeling (SEM) to assess and compare the direct and indirect (via some soil chemical parameters) effects of root traits of clones on the soil microbiome using the ‘psem” function (‘piecewiseSEM’ R package) (Lefcheck, 2016). The piecewise SEM approach combines a series of separate linear models with local parameter estimation into a single directed acyclic graph, and is suitable for incorporating the categorical variable “clone” in the path model (Lefcheck, 2016). A conceptual model of hypothetical relationships was first established, assuming that root traits influenced by phylogenetically related hybrid poplars (1) would have direct effects on the soil microbiome (2) and/or modify first the soil chemistry (3) which in turn would impact soil microbiome (4) (Fig. 1). Our experimental site offers the opportunity to test the direct or indirect (via some soil chemical parameters) effects of root traits, as all plots presented similar soil physical and chemical characteristics (same fertilization treatment) before hybrid poplar planting within each of the soil layer.

We selected the relative abundance of soil ectomycorrhizal fungi and the Actinobacteriota phylum (bacteria) to determine root trait effects on the soil microbiome as these were the only groups differing significantly between clones at both soil depths. Ectomycorrhizal fungi play an important role in helping trees acquire soil nutrients (Smith and Read, 2010), while Actinobacteriota was among the most abundant bacterial groups in our soils and could contribute largely to litter decomposition (Buresova et al., 2019, 2021). Therefore, we had four different path models corresponding to these two microbial groups and the two soil depths. Bivariate correlations between root traits were first assessed using Pearson's correlation coefficients (Tables S1 and S2). We then performed linear mixed models between each root trait and each microbial group (Tables S3 and S4) and selected only root traits that were non-collinear and had a significant *P*-value and the lowest AICc (Akaike Information Criterion, *aictab* function and AICcmodavg package) for subsequent path analyses. We selected one of the three soil chemical parameters (SOC, soil total N or C/N ratio) that resulted in the adequate and best overall model fit. Overall model fit is indicated by the χ^2 test, with a *P* value that should be greater than 0.05 (Lefcheck, 2016). To be able to compare the direct and indirect effects of root traits, we estimated them using the *bootEff* and *semEff* functions in the semEff package (Murphy, 2022). We used 1000 bootstrapped estimates for each response to calculate the 95 % confidence interval for effects (Ren et al., 2023). Effects from the emEff package are standardized effects, adjusted for multicollinearity among predictors (a.k.a. semi-partial correlations) and thus slightly lower than the unadjusted standardized coefficients (Murphy, 2022).

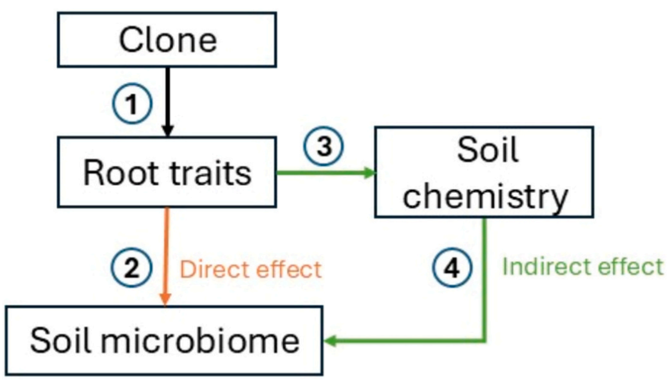

Fig. 1. Conceptual model showing the direct and indirect effects of root traits on soil microbial communities.

3. Results

3.1. *Clone effect on soil microbial diversity and composition*

Soil fungal observed richness and Shannon index were significantly different between clones at the 0–20 cm depth, but no differences were found at the 20–40 cm depth (Fig. 2a, c). At the 0–20 cm depth, clone 915,319 had higher soil fungal observed richness than clones 747,210 and DN2 and higher soil fungal Shannon index than clones 1079 and DN2 (Fig. 2a, c). There were no significant differences in soil bacterial observed richness and Shannon index between clones at any soil depth, although a slight variation ($P = 0.06$) in bacterial Shannon index was noted at the 20–40 cm depth (Fig. 2b, d).

Clones showed significant differences in soil fungal community composition at both 0–20 and 20–40 cm depths, as indicated by PERMANOVA (Table 1). Soil fungal composition of clone 915,319 differed from that of clones 1079 and DN2 at the 0–20 cm depth, while it differed from clone 1079 at the 20–40 cm depth (Tables S5 and S6). Soil bacterial composition varied slightly between clones at the 0–20 cm depth ($P =$

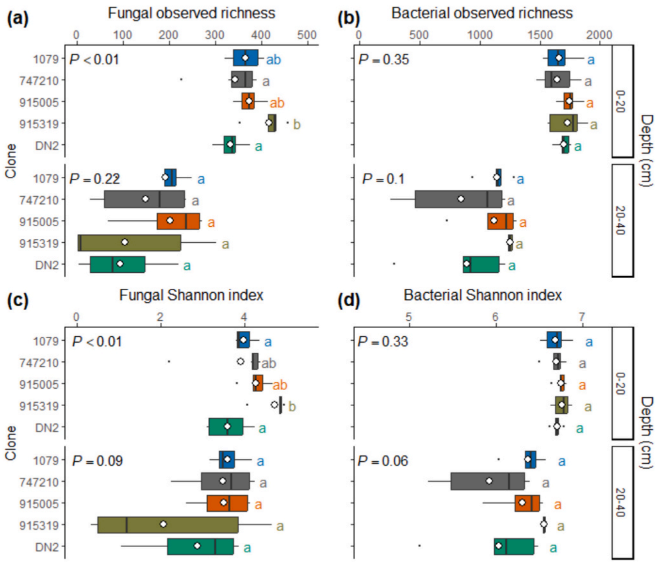

Fig. 2. Differences in alpha diversity of soil fungal (a,c) and bacterial (b,d) communities between clones at the 0–20 and 20–40 cm depths. Boxplots include the median (black vertical lines) and the mean (white circles) of observed richness (a,b) and Shannon index (c,d) for each clone. Different lowercase letters indicate significant differences between clones.

Table 1
Effects of clones on soil microbial community composition at the 0–20 and 20–40 cm depths using the permutational multivariate analysis of variance (PERMANOVA) based on the Bray-Curtis dissimilarity matrices.

Communities	Soil depth	Variable	Df	Sum of squares	R^2	F	*P*
Fungi	0–20	Clone	4	1.52	0.23	1.83	**<0.001**
		Residual	24	4.97			
	20–40	Clone	4	1.74	0.18	1.27	**<0.01**
		Residual	23	7.86			
Bacteria	0–20	Clone	4	0.43	0.18	1.27	0.06
		Residual	24	2.02			
	20–40	Clone	4	0.87	0.23	1.33	**0.04**
		Residual	18	2.94			

Significant effects ($P < 0.05$) are highlighted in bold.

0.06), but it differed significantly between clones at the 20–40 cm depth (Table 1), where soil bacteria composition under clone 915,319 was different from that of clone DN2 (Table S7).

The relative abundance of soil ectomycorrhizal fungi was significantly different between clones at both the 0–20 and 20–40 cm depths (Fig. 3a, b). The taxonomic profile of the top 20 soil fungal families also confirmed this difference between clones, with an observed variation in the family Hymenogastraceae comprising several ectomycorrhizal fungi (Fig. S1). Clone 915,319 showed a lower relative abundance of soil ectomycorrhizal fungi than the other clones, with values significantly lower than those of clones 1079, 915005, and DN2 at the 0–20 cm depth, and lower than that of clone DN2 at the 20–40 cm depth (Fig. 3a, b). The relative abundance of soil pathogenic fungi differed between clones only at the 0–20 cm depth, while the relative abundance of soil saprotrophic fungi was similar between clones at both soil depth (Fig. 3a, b). Among the main bacterial phyla, only the relative abundance of soil Actinobacteriota varied between clones at both 0–20 and 20–40 cm depths (Fig. 3c, d). The relative abundance of soil Actinobacteriota was greater in clone 1079 than in clone 747,210 at the 0–20 cm depth, while clone DN2 had a greater relative abundance of this phylum compared to clone 915,319 at the 20–40 cm depth (Fig. 3c, d). Clone effects were also observed for the relative abundances of soil Acidobacteriota, Planctomycetota, Gemmatimonadota, Methylomirabilota and Myxococcota at the 20–40 cm depth only (Fig. 3d).

3.2. Direct and indirect effects of root traits on the relative abundance of soil ectomycorrhizal fungi and Actinobacteriota

Root mass density and RDMC were the root traits having direct and indirect effects (via SOC) on the relative abundance of soil ectomycorrhizal fungi at the 0–20 cm depth, according to the best piecewise SEM model (χ^2 test $P = 0.15$ and $R^2 = 0.41$, Fig. 4a). Root mass density had significant and mostly direct positive effects on the relative abundance of soil ectomycorrhizal fungi at this soil depth (direct effects = 0.50 vs.

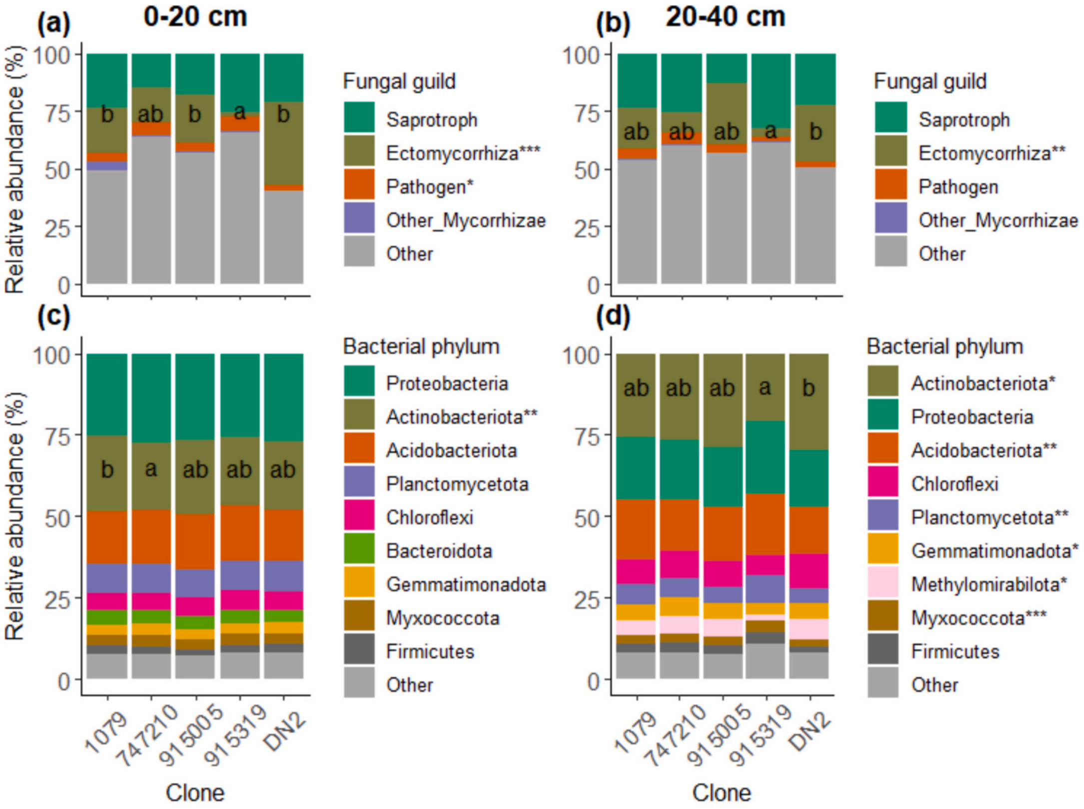

Fig. 3. Differences in relative abundance of soil fungal guilds (a,b) and main bacterial phyla (c,d) between clones at the 0–20 (a,c) and 20–40 cm (b,d) depths. $P < 0.001$***; $P < 0.01$**; $P < 0.05$*. The fungal communities identified as 'Unassigned' or with multiple nutrition modes were classified into 'Other'. Soil bacterial phyla whose relative abundance was less than 2 % were assigned to "Other". Different lowercase letters indicate significant differences in the relative abundance of soil Actinobacteriota or ectomycorrhizal fungi between clones. Complete *post-hoc* pairwise comparisons for the other soil bacterial phyla or fungal guilds are in Table S8.

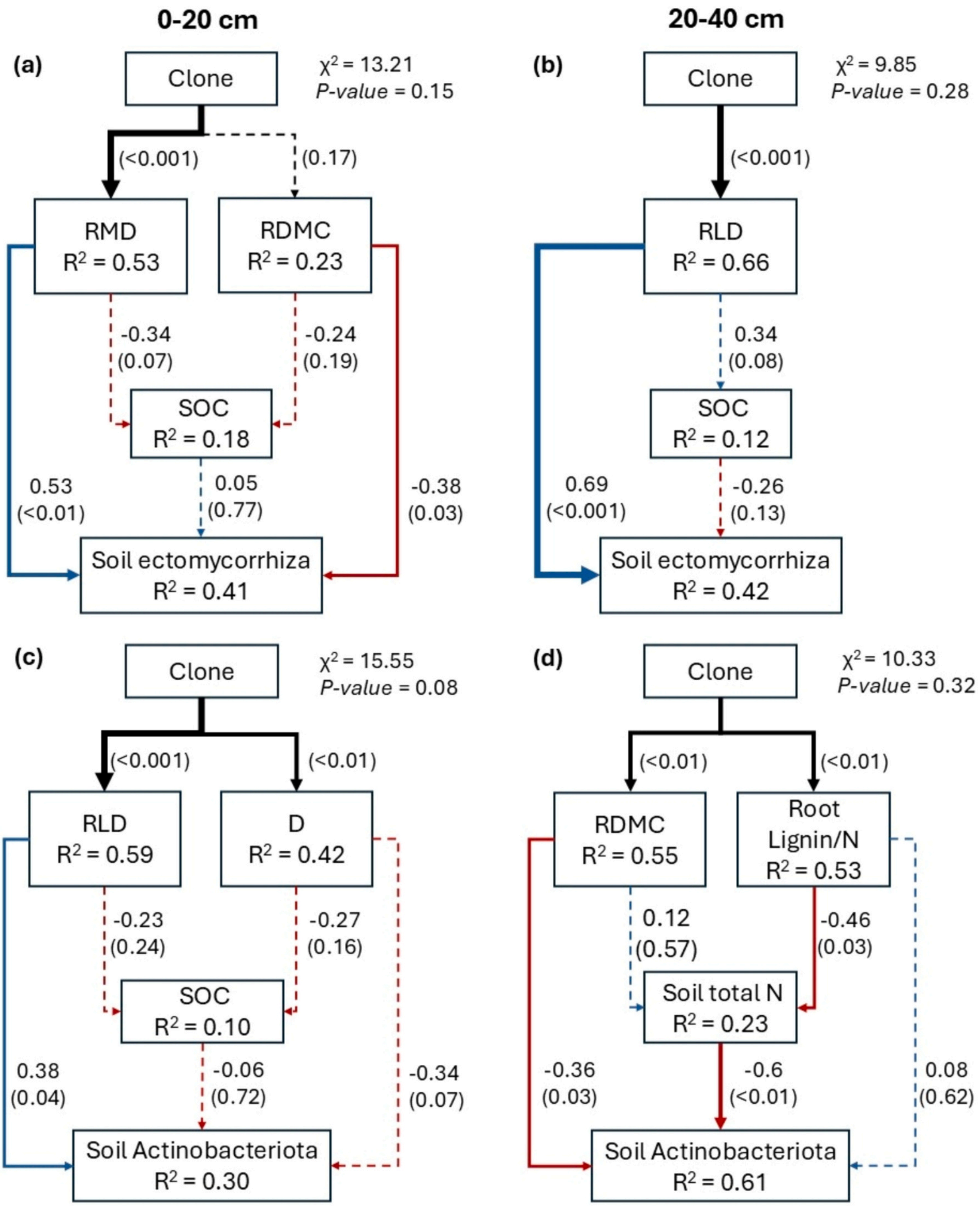

Fig. 4. Path analysis showing the direct or indirect effects of root traits on the relative abundance of soil ectomycorrhizal fungi (a,b) and Actinobacteriota (c,d) at the 0–20 (a,c) and 20–40 cm (b,d) depths. D: average root diameter; RDMC: root dry matter content; N: nitrogen; RLD° root length density; RMD: root mass density; SOC: soil organic carbon. Solid and dashed arrows indicate significant and non-significant relationships respectively. The thickness of the arrows increases with the significance of the relationship. Blue arrows: positive relationship; red arrows: negative relationship; black arrows: effect of the categorical variable 'clone'. The standard estimate and *P* value (in brackets) of each relationship are shown to the right of each arrow.

indirect effects = −0.02), whereas RDMC mainly had direct negative effects (direct effects = −0.37 vs. indirect effects = −0.01, Table 2). At the 20–40 cm depth, RLD affected the relative abundance of soil ectomycorrhizal fungi directly and indirectly (via SOC) according to the best path model (χ^2 test $P = 0.28$ and $R^2 = 0.42$, Fig. 4b). The direct positive effects (0.65) of RLD on the relative abundance of this fungal guild was significant and more pronounced than the indirect negative effects (−0.24) (Table 2).

The relative abundance of soil Actinobacteriota was influenced directly and indirectly (via SOC) by RLD and root mean diameter (D) at the 0–20 cm depth, according to the best piecewise SEM model (χ^2 test $P = 0.08$ and $R^2 = 0.30$, Fig. 4c). Root length density had more direct positive effects (0.38) on the relative abundance of soil Actinobacteriota at this soil depth compared to its indirect effects (0.02); while root D had more direct negative effects (−0.34) than indirect positive effects (0.02) on the relative abundance of this phylum (Table 2). At the 20–40 cm, RDMC and root lignin/N ratio of clones directly or indirectly influenced the relative abundance of soil Actinobacteriota via total soil N (χ^2 test P

Table 2
Summary of direct or indirect effects of root traits on the relative abundance of soil Actinobacteriota and ectomycorrhizal fungi at the 0–20 cm and 20–40 cm depth.

Soil microbe	Root trait	Std. Effects	Root trait	Std. Effects
	0–20 cm depth		20–40 cm depth	
Ectomycorrhiza	Direct RMD	**0.50**	Direct RLD	**0.65**
	Indirect RMD (via SOC)	−0.02	Indirect RLD (via SOC)	−0.24
	Direct RDMC	**−0.37**		
	Indirect RDMC (via SOC)	−0.01		
Actinobacteriota	Direct RLD	0.38	Direct RDMC	**−0.36**
	Indirect RLD (via SOC)	0.02	Indirect RDMC (via soil N)	−0.06
	Direct D	−0.34	Direct lignin/N	0.07
	Indirect D (via SOC)	0.02	Indirect lignin/N (via soil N)	**0.24**

Numbers in bold indicate a significant effect at $P < 0.05$. D: average root diameter; RDMC: root dry matter content; N: nitrogen; RLD° root length density; RMD: root mass density; SOC: soil organic carbon. Effects from the emEff package are standardized effects, adjusted for multicollinearity among predictors (a.k.a. semi-partial correlations) and thus slightly lower than the unadjusted standardized coefficients (Murphy, 2022).

$= 0.32$ and $R^2 = 0.61$, Fig. 4d). Root dry mass content had negative effects on the relative abundance of soil Actinobacteriota at this soil depth, with significant direct effects (−0.36) greater than indirect effects (−0.06) (Table 2). However, root lignin/N ratio showed positive effects on the relative abundance of soil Actinobacteriota at this soil depth, and its significant indirect effects (0.24) via total soil N were greater than direct effects (0.07) (Table 2).

4. Discussion

4.1. Soil microbial composition and diversity

After 14 growing seasons, hybrid poplar genetics influenced soil fungal and bacterial community composition and fungal diversity, but not bacterial diversity, partially supporting our hypothesis. This influence was particularly observed in the topsoil, but also occurred in the subsoil. Thus, even between phylogenetically related trees, tree genetics shaped the soil microbiome, modulating microbial community composition and microbial group abundance (Cregger et al., 2018; Tagu et al., 2005; Ulrich et al., 2008), and not solely in the upper part of the soil profile but also deeper in the soil. These effects could be explained by the fact that clones originate from different parentages and have different growth strategies and plant traits (Elferjani et al., 2016; Rabearison et al., 2024).

Hybrid poplar genetics only affected soil fungal diversity at the 0–20 cm depth. The greater soil fungal diversity observed in 915,319 compared to DN2 may be due to the low abundance of ectomycorrhizal fungi under clone 915,139. A reduced abundance of ectomycorrhizal fungi in the soil could lessen competition with other fungal groups, thereby promoting overall fungal diversity (Fitter and Garbaye, 1994). The lower OM input from clones in the subsoil (Rabearison et al., 2023) likely reduced the effects of clones on soil fungal diversity at this soil depth compared to the topsoil. In addition, the similarity of some factors intrinsically linked to soils at each depth, such as pH and texture between our clones (Rabearison et al., 2023) could have led to the very low variation in soil bacterial diversity. Indeed, several studies demonstrated that soil bacterial diversity is more influenced by variations in soil pH and texture than by variations in plant-related variables (Fierer and Jackson, 2006; Xia et al., 2020).

4.2. Effects of hybrid poplars and their root traits on soil ectomycorrhiza

Variation in soil fungal communities between clones was mainly reflected in the relative abundance of ectomycorrhizal fungi at both depths, supporting our hypothesis. We found that the relative abundance of soil ectomycorrhizal fungi was the highest in clone DN2 and the lowest in clone 915,319, whereas these two clones were the most productive compared to the other clones according to our previous study (Rabearison et al., 2023). This indicates that promoting associations with ectomycorrhizal fungi might be a growth strategy for clone DN2, but not for clone 915,319. Ectomycorrhizal fungi help trees acquire nutrients such as N or phosphorus (Cairney, 2011; Smith and Read, 2010). Interestingly, the genus *Naucoria* was the most observed ectomycorrhizal fungus in our hybrid poplar plantation, deserving further investigation as it was less common in poplars (Szuba, 2015), but more observed in alders (Sumorok et al., 2008).

Our additional metabarcoding analyses using 18S rRNA gene primers also revealed that clone DN2 had higher alpha diversity and relative abundance of soil arbuscular mycorrhizal fungi (Glomeromycetes) compared to clone 915,319 in the topsoil (Figs. S2 and S3). Therefore, the most productive clone DN2 had stronger associations with these two mycorrhizal fungi than the second most productive clone 915,319. These results reinforce our previous findings in Rabearison et al. (2024), suggesting that these two clones have distinct growth strategies. Specifically, clone DN2 may optimize its growth by maximizing soil exploration through a greater RMD and enhanced mycorrhizal associations, whereas the growth strategy of clone 915,319 may rely more on its fine roots, which are richer in N and likely have a high capacity for nutrient assimilation (Jia et al., 2013; Rabearison et al., 2024). These divergent growth strategies could help identify tree genotypes adapted to future climate conditions. In environments where climate change is expected to limit mycorrhizal development (Bennett and Classen, 2020), genotypes with nutrient-efficient fine roots (e.g., clone 915,319) may be favored. However, in scenarios of declining soil fertility (Mondal, 2021), clones capable of extensive soil exploration and benefiting from mycorrhizal associations (e.g., clone DN2) may show greater resilience.

Root architectural traits had the most direct effects on soil ectomycorrhizal fungi. The relative abundance of soil ectomycorrhizal fungi directly increased with RMD and RLD, as these traits indicate the niches available for ectomycorrhizal colonization (Demenois et al., 2018; Emery et al., 2018). This result echoes the fungal collaboration gradient in the root economics space of plants, where concentrating high density of root mass or root length in a given soil volume allows for the development of collaboration with mycorrhizal fungi as a strategic way for trees to explore soils (Bergmann et al., 2020; Wen et al., 2021). Extramatrical fungal hyphae from ectomycorrhizal fungi expand the soil volume available for nutrient acquisition by trees, hence increasing root depletion zone (Cairney, 2011). Therefore, RMD and RLD are beneficial for tree growth and soil resource acquisition, not only through increased root surface, but also via ectomycorrhizal colonization. However, RLD also had a slight indirect negative effect on this fungal guild via an increase in SOC at the subsoil. Organic matter-rich soils probably cover nutrient demand of trees, leading trees to form fewer ectomycorrhizal associations (Cairney, 2011).

The direct effects of the root architectural traits on soil ectomycorrhizal fungi could also be linked to root chemical traits. At the 0–20 cm soil depth, high RMD was associated with a high root lignin/N ratio and low RNC, both of which correlated with a high relative abundance of soil ectomycorrhizal fungi (Tables S1 and S3). Consequently, the increase in recalcitrant compounds from trees would have benefited soil ectomycorrhizal fungi in our fast-growing plantation, as these chemical traits suggest low root decomposition rates (Jimoh et al., 2024; Roumet et al., 2016). Ectomycorrhizal fungi are efficient in breaking down lignocellulosic materials through oxidative degradation (Lindahl and Tunlid, 2015; Rineau et al., 2012). In addition, higher root dry mass content (RDMC) had negative effects on the relative abundance of ectomycorrhizal fungi at both soil depths (Fig. 4 and Table S3), particularly in soils of clone 915,319, where RDMC was higher (Rabearison et al., 2024). Fine roots with higher RDMC are usually linked to higher root tissue density and a conservative strategy (Lin et al., 2024; Roumet et al., 2016), which could limit C exchange between fine roots and ectomycorrhizal fungi.

4.3. Effects of hybrid poplars and their root traits on soil Actinobacteriota

The Actinobacteriota, one of the most abundant bacterial phyla in soils, differed between our phylogenetically related clones at both soil depths. Our hypothesis on the importance of copiotrophs over oligotrophs in our fast-growing plantation cannot be validated based solely on the phylum level, as the life strategy of Actinobacteriota as a whole can be either copiotrophic or oligotrophic in the literature (Stone et al., 2023). Soil Actinobacteriota are ecologically important for litter decomposition, especially for enhancing the decomposition of recalcitrant materials by producing extracellular enzymes (Buresova et al., 2021; Větrovský et al., 2014). This suggests that there were probably variations in the quality of organic material input between hybrid poplar clones as a result of differences in aboveground or root traits (Rabearison et al., 2024). Many bacteria in this phylum also show great potential as plant growth promoting rhizobacteria through various mechanisms, including phytohormone production, phosphate solubilization and biological nitrogen fixation, according to a review of studies by Boukhatem et al. (2022).

Root length density had direct positive effects on soil Actinobacteriota at the 0–20 cm depth, possibly due to the increased availability of food sources (root exudates and dead fine roots) through the increase in root surface area (Babalola et al., 2021; Birt et al., 2022). At this soil depth, a higher relative abundance of soil Actinobacteriota was also slightly associated with thinner fine roots, where turnover rates and inputs would be greater compared to thicker fine roots (McCormack et al., 2012; Weemstra et al., 2016). Growth of this bacterial phylum would therefore be dependent on the quantity of organic compounds from fine roots. Moreover, the weak indirect effects of these traits via SOC at this depth suggest that topsoil Actinobacteriota accessed their nutrients directly from roots rather than through soils. Root chemical traits had no effect on soil Actinobacteriota at this depth, leaving their life strategy unclear (Hu et al., 2023; Stone et al., 2023). However, within this phylum, we found more bacteria from the class MB-A2–108 and the genus *Gaiella* (Fig. S4), which are frequently associated with low-nutrient environments and recalcitrant OM and could be considered oligotrophic bacteria (Lan et al., 2022; Wei et al., 2024; Zhang et al., 2019).

We found that root lignin/N ratio had indirect positive effects on soil Actinobacteriota via a decrease in total soil N in the subsoil. A higher lignin/N ratio is an indicator of lower decomposition rates of organic compounds (Roumet et al., 2016). Therefore, more recalcitrant fine roots (higher lignin/N ratio) would be more conducive to soil Actinobacteriota at this depth, probably through the transfer of recalcitrant and N-poor compounds into the soil. Soil Actinobacteriota at this soil depth would clearly have an oligotrophic life strategy (Hu et al., 2023; Li et al., 2021). Several studies argued that bacteria belonging to Actinobacteriota, such as those of the MB-A2–108, were involved in the decomposition of recalcitrant compounds by producing lignolytic enzymes (Buresova et al., 2021; Lan et al., 2022; Větrovský et al., 2014). They were also among the most abundant bacteria decomposing OM in the subsoil (Sagova-Mareckova et al., 2016; Steger et al., 2019). Thus, contrary to our hypothesis, poplar hybrid clones likely had a greater effect on oligotrophs than on copiotrophs at both soil depths, based on detailed analyses of the results. Furthermore, the negative relationship between soil Actinobacteriota and RDMC could indicate that lower root inputs would reduce the abundance of this phylum (Roumet et al., 2016).

5. Conclusion

Hybrid poplar clones, despite being phylogenetically related trees, had a significant impact on the soil microbiome by shaping its community composition through their root traits. Our research observed differences among clones in terms of the relative abundance of soil ectomycorrhizal fungi, which may play a crucial role in the growth of certain clones. Growth strategies of clones could then either depend on, or function independently from soil ectomycorrhizal associations. The increase in their relative abundance was primarily driven by high RMD and RLD, reflecting the role of roots as niches for mycorrhizal colonization. Hybrid poplar genetics also influenced soil bacteria, where the relative abundance of Actinobacteriota was promoted by high RLD in the topsoil and by more recalcitrant fine roots in the deeper soil layer. Our results may help optimize the management of fast-growing plantations, particularly in the selection of clones and soil types. This study provides a framework for identifying trees that are potentially adapted and resilient to climate change, either by selecting genotypes that rely more on their intrinsic traits if future climate is expected to limit mycorrhizal development or those enhancing soil mycorrhizal interactions in scenarios of decreased soil fertility.

CRediT authorship contribution statement

Toky Jeriniaina Rabearison: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Vincent Maire:** Writing – review & editing, Validation, Supervision, Software, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Annie DesRochers:** Writing – review & editing, Resources, Methodology, Investigation, Conceptualization. **Vincent Poirier:** Writing – review & editing, Resources, Methodology, Investigation. **Dennis Alejandro Escolástico-Ortiz:** Writing – review & editing, Software, Formal analysis. **Marie-Josée Morency:** Writing – review & editing, Resources. **Christine Martineau:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

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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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2025.106389.

Data availability

Metabarcoding raw sequence analyzed in this study were deposited in the Sequence Read Archive of the NCBI, accession number PRJNA1240279; and root and soil data are available in Supplementary data.

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