# Soil microbiomes reveal different strategies for nitrogen acquisition in aspen-dominated stands from Mexico and Canada

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# Abstract

Plant species shape soil microbiome composition through species-specific interactions. However, it is less clear how these host-microbe interactions vary across populations of genetically structured species, particularly those spanning diverse climatic and soil gradients. In this study, we explore the influence of host genetic composition and edaphic factors on the soil microbiome of *Populus tremuloides* Michx., one of North America's most widespread tree species. Using 16S, ITS, and 18S metabarcoding on soils from natural stands and soil, rhizosphere, and root samples from a greenhouse common garden setup, we examined microbiome diversity and community structure in two genetically distinct aspen genetic groups: one from Eastern Canada (Quebec), spanning boreal and cold temperate forests, and the other from Northwestern Mexico, representing warm temperate forests. While the soil microbial communities were distinguishable between the two genetic groups, we also observed significant differences among the three ecological regions. Variation in soil properties contributed to differences both between and within genetic groups, whereas under indoor and uniform soil conditions, genetic diversity within and between groups significantly impacted microbial communities. Despite taxonomic similarities, soil microbiomes associated with the Canadian and Mexican groups diverged functionally, reflecting distinct strategies for nitrogen acquisition. Boreal Canadian aspen stands and seedlings showed stronger associations with ectomycorrhizal fungi, while Mexican stands harbored a greater abundance and diversity of nitrogen-fixing bacteria. Our findings highlight how local adaptations to climate and soil conditions in aspen extend to their microbial partners, emphasizing the potential role of host-microbe interactions in shaping tree resilience and susceptibility to future climate changes.

# Introduction

Forests play a critical role in carbon cycling, particularly as carbon sinks, with their microbiota being a key component in this process (Baldrian 2017; Malhi, Baldocchi, and Jarvis 1999). Microorganisms act as carbon storage, they decompose organic material and provide vital services to forest species by supplying nitrogen and phosphorus, or promoting tree growth (Baldrian 2017; Uroz et al. 2016; Hawkins et al. 2023). At the same time, forest microbiomes also harbor pathogens that affect species population dynamics and forest services (Castello, Leopold, and Smallidge 1995; I. L. Boyd et al. 2013). The diversity and dynamics of microbial communities are key to understanding the functioning of forest ecosystems.

Globally, climate, growing season length, and edaphic factors shape forest species composition and their microbial communities (Baldrian, López-Mondéjar, and Kohout 2023). Along latitudinal gradients, forest ecosystems vary in species richness, macronutrient availability, and organic matter content (Liang et al. 2022; Gao et al. 2022; Reich et al. 2014; Malhi, Baldocchi, and Jarvis 1999). Boreal forests are generally low in species diversity and nutrients but have abundant organic matter (Pan et al. 2013; Liang et al. 2022). In contrast, tropical forests are rich in species and nutrients but contain less organic matter (Pan et al. 2013; Liang et al. 2022). Nutrient availability and tree species composition have a strong impact on the composition of microbial communities in the soil (Baldrian, López-Mondéjar, and Kohout 2023; Uroz et al. 2016). These include symbiotic components of the soil microbiome, such as mycorrhizal fungi, that are responsible for the transport of nitrogen and phosphorus to plants in exchange for carbon via root connections (Powell and Rillig 2018; Ward et al. 2022; Courty et al. 2010). Boreal forests are typically rich in ectomycorrhizal fungi, while arbuscular mycorrhizal fungi dominate in tropical forests (Steidinger et al. 2019). Mycorrhizal fungi exhibit diverse host preferences; they influence both forest growth and biodiversity, making them crucial to the overall health of the forest.

*P. tremuloides* Michx. is one of the few species in North America with a distribution range that spans multiple ecosystems and forms both ectomycorrhizal and arbuscular mycorrhizal associations (Teste, Jones, and Dickie 2020). *P. tremuloides* populations extend across diverse ecological regions: they dominate much of the boreal zone in the north, stretching into the eastern temperate zone in the middle-eastern part of North America, whereas in the west they spread through the high-elevation northwestern forested mountains, reaching as far south as the temperate Sierras of the United States of America and Mexico. Aspens are keystone forest species supporting wildlife habitats and contributing to ecosystem diversity (Rogers et al. 2020), but a decline in populations has been observed in recent years (Worrall et al. 2013), with drought identified as a key factor in the reduced growth of Western Canadian populations (Chen et al. 2017). *P. tremuloides* is naturally associated with riparian habitats but also a fire-adapted species (Shinneman et al. 2013) that thrives in post-disturbance environments. It is often among the first to appear after natural disturbances such as fire or pest outbreaks (Swift and Ran 2012) or in reclaimed soils following oil sand exploitation (Pinno and Errington 2015; Stefani et al. 2018). *P. tremuloides* exhibits both sexual reproduction, through wind-dispersed pollen and seeds during brief "windows of opportunity" (Jelinski and Cheliak 1992; Mock et al. 2008), and asexual reproduction, via root suckering. In the southwestern range (U.S.A. and Mexico), large clonal

stands are common (Kemperman and Barnes 1976; Mitton and Grant 1996), with the Pando clonal population in the Fishlake National Forest (U.S.A.) being an extreme example (DeWoody et al. 2008; Pineau et al. 2024). In areas like the Great Lakes and Central Canada, sexual reproduction occurs more frequently, contributing to smaller clone sizes (Namroud et al. 2005; Mock et al. 2008). This is consistent with experiments showing that the impact of drought on seed germination is higher for aspen originating from drier regions (Goessen et al. 2022).

Genetically, *P. tremuloides* is divided into four major genetic groups spread across distinct regions: Eastern Canada, Western Canada, the Midwestern and Southern U.S.A., and Mexico, each varying in levels of clonality and ploidy (Goessen et al. 2022), although more groups can be distinguished with more extensive sampling (Goessen, in prep.). Given their broad ecological distribution, the varying vegetation composition and density across regions, local climate adaptations within genetic groups are expected (Goessen et al. 2022). The range of naturally occurring microbial associations across these groups is still unknown. In a large-scale study comprising several aspen species, climate and geography have been shown to be the primary drivers of soil and foliar endophytic fungal communities, with arbuscular mycorrhizae more prevalent in warmer regions and ectomycorrhizal fungi more common in cooler areas (Van Nuland et al. 2023). However, no studies have yet compared the microbiomes within aspen species across different ecosystems.

Here we use amplicon sequencing of 16S, ITS, and 18S markers to investigate soil prokaryotic and fungal microbiomes in natural stands and greenhouse settings of *P. tremuloides* coming from two distinct genetic groups: Eastern Canada from boreal and cold temperate zones and Mexico from the warm temperate zone. Our research objectives were to: i) examine the differences in microbial communities between aspen-dominated stands in Canada and in Mexico and determine how soil and aspen genotypes influence them, ii) assess the abundance and diversity of ectomycorrhizal (EMF) and arbuscular mycorrhizal fungi (AMF) in these groups, and iii) evaluate the effect of aspen's genetics on microbial community structure, including EMF and AMF communities, in a greenhouse common garden setup.

# Materials and Methods

## Study sites

Twenty-six soil samples were collected at eight sites within a two-meter radius of *P. tremuloides* trees growing in patchy stands, generally within mixed forests (Fig. 1A-C, Supplementary Table 1). Sampling was conducted at five sites in Quebec, Canada, and three sites in Northwestern Mexico (Fig. 1B). In Canada, two sites (AMOS, STFE) are located well within the boreal zone, while one site (STET) falls within the Mixedwood Plains ecozone corresponding to the cold temperate region. The remaining two sites (ESSI, FORE) lie at the southern limit of the boreal zone, in the Saint Lawrence Estuary, where soil and climate characteristics resemble those of temperate region (Fig. 1E). Given these similarities, we grouped STET, ESSI, and FORE into a cold temperate region (Fig. 1E). Sampling in Northwestern Mexico was done at three sites within the warm temperate zone (Santiago, FP2, and FLOR1, Fig. 1, Supplementary Figure 1). One additional site, AB within the boreal zone in Canada, was sampled for the greenhouse experiment only (Fig. 1A-B,D). Sites were accessed in 2018 (ESSI, AMOS, STFE, FORE), and 2019 (the rest).

The sites AMOS, STFE, and AB are part of the Canadian clay belt and located within the boreal zone in Quebec, Canada. Apart from soils rich in clay and silt, these sites are characterized by the lowest mean annual temperatures (1.6 °C) among all sampled sites, and relatively high mean annual precipitation (869-872 mm). Sites AMOS (Guillemette and DesRochers 2008) and STFE are situated in aspen natural stands, with understory vegetation including plants such as ferns, *Rubus* spp., *Epilobium* spp., and seedlings of *Salix* spp., *Betula* spp., and *Abies balsamea*. The AB site is part of a natural post-fire mixedwood forest, featuring species such as *A. balsamea*, *Picea mariana*, *P. glauca*, and *B. papyrifera* (Bergeron 2000).

Three Canadian sites ESSI, FORE, and STET within the cold temperate zone are located along the Saint Lawrence Estuary. These sites are located on sandy soils, at low elevations, and experience high mean annual precipitation of up to 1211 mm. ESSI and FORE sites consist of natural mixed stands of *P. tremuloides* and conifers, situated near the roadside with varying levels of disturbance. The STET site is characterized by natural stands of *P. tremuloides* growing on abandoned farmland near a river and residential areas.

The three Mexican sites—Santiago, FP2, and FLOR1—are all located in Durango State within the warm temperate zone of the Sierra Madre Occidental range (temperate Sierras zone), within pine-oak forests. The sites are found at high elevations (2573-3050 m) with annual mean temperatures around 12.5-13.3°C and mean annual precipitation reaching 672 mm. In contrast to Canadian sites, these locations experience less temperature fluctuations throughout the year, whereas precipitation is seasonal. Rainfall occurs mostly in summer and autumn with long periods of dry weather in between. The FLOR1 and FP2 sites are dominated by *Arbutus bicolor*, *Arbutus* spp., *Pinus durangensis*, *Pinus arizonica*, and *Quercus rugosa*. The Santiago site is situated on

soils with sparse vegetation near a road, and a stream present only during the rainy season. Its natural stands are dominated by *Pinus arizonica*, *Quercus sideroxyla*, and *Arbutus* spp.

## Sampling protocol

Soil was taken in triplicates at 2 m distance from the tree. Samples were obtained using a 15 cm soil corer after removing the organic layer, and stored in plastic bags kept on ice in a cooler. Sampling equipment was cleaned to remove residual dirt and wiped with ethanol between each sample. In the lab, triplicates were mixed into a composite sample and further homogenized using a 6 mm mesh sieve. Root segments were removed from the soil samples. A 0.25 g subsample of the resulting soil was transferred to a PowerBead tube (DNeasy PowerSoil Kit, QIAGEN) for DNA extraction. The remaining soil was air-dried and used for physicochemical analyses.

## Greenhouse experiments

*P. tremuloides* saplings were grown in a greenhouse compartment at Laval University's Forestry faculty in Quebec City, from the roots collected at two locations in Quebec (AB, FORE) and two locations in Mexico (FP2 and Santiago). Samples from Quebec were received in September 2019 and were first propagated by putting them on trays with peat moss soil to stimulate root sprouting and then transferring cuttings of around 10 cm to a misting unit on perlite substrate for rooting. After developing roots, plants were transferred to the potting mix. Roots from Mexico were received 1-2 months after harvest (November 2019 and January 2020 for FP2 and Santiago, respectively). Due to the low propagation success rate of previous batches of Mexican roots, these roots were planted directly in a potting mix. The substrate for growing cuttings included 2 volumes of peat (Fafard), 1 volume of perlite, and 0.5 volume of vermiculite. Cuttings were repotted several times and transferred to 1.7 L pots between May and June 2020, and to 2.8 L pots between January and February 2021. The substrate for repotting was composed of 3 volumes of peat (Fafard), 1 volume of perlite, 1 volume of vermiculite, and 0.25 volume of compost (Biosol, de Fafard). Several pesticide treatments were used including Nova 40 WP (September 2020, August 2021), Bioceres (September 2020), and Avid (January 2021), and predator insects (Cucumeris, Dalotia, Orius, and Aphidus) were used to limit pests. Plants were fertilized weekly with nitrogen, phosphorus, and potassium (20-20-20, 1 g/L) and micronutrients (Plant-Prod Chelated Micronutrient Fertilizer Mix, 0.3 g/L). Overall, plants were grown under ambient temperature and long daylight in peat moss soil for 21-24 months.

Soil, root, and rhizosphere samples were collected from pots on 12 October 2021 and subsequently transferred to and processed in the research laboratory at the Laurentian Forestry Centre (Canadian Forest Service, Quebec City) to obtain DNA extracts. Soil was processed as above, whereas root and rhizosphere samples were obtained for DNA extraction using the following protocol. Roots with attached soil (rhizosphere soil) were transferred to a 25 mL solution containing 1X PBS (phosphate-buffered saline) and Tween 20 (0.1%) and shaken by inversion 10 times to detach soil from the roots. Tubes were then centrifuged at 4500 g for 10 minutes at 4°C. Roots were transferred to a petri dish half-filled with a fresh PBS solution and rinsed by pulling them gently through the solution with sterile tweezers. Clean roots were transferred to a

50 ml Falcon tube containing 25 ml PBS, submerged, vortexed for 15 seconds, and put twice in a sonication bath. Each sonication step was done at room temperature and at an operating frequency of about 40 kHz for 30 seconds 5 times with 30-second intervals between repetitions. Before the second sonication, roots were transferred to clean tubes containing 25 ml PBS. Roots were recovered from the tubes, pat-dried on absorbent paper and cut into 1 cm pieces with scissors pre-sterilized in 70% ethanol. Subsequently, 0.1 g of the root fragments were transferred to a 2 ml tube with a 0.5 mm steel bead and stored in the freezer (between -20 and -80°C). Frozen roots were homogenized twice for 45 seconds each on the TissueLyser II (QIAGEN) and collected for DNA extraction. The rhizosphere soil pellet was recovered from the tube after centrifugation of the roots with attached soil, after removing roots and the supernatant. The pellet was thoroughly mixed with a sterilized spatula and transferred to a small piece of Whatman filter paper to absorb the remaining PBS liquid. Finally, 0.25 g of rhizosphere soil was taken for DNA extraction.

## DNA extraction and library preparation and sequencing

DNA extraction on soil, rhizosphere, and root samples was performed using the DNeasy PowerSoil Kit (QIAGEN) following the manufacturer's instructions. DNA concentration of the extracts was assessed with the Qubit 3.0 fluorometer (Thermo Fisher Scientific, MA, USA) using the dsDNA BR (broad range) or HS (high sensitivity) assay kit (Life Technologies, CA, USA). Amplicon library preparation was performed as described by Illumina (Illumina 2013) using user-defined primers. Selected primer pairs 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, Needham, and Fuhrman 2016), and ITS9F (5′-GAACGCAGCRAAIIGYGA-3′) and ITS4R (5′-TCCTCCGCTTATTGATATGC-3′) targeting the ITS2 region of fungi (Menkis et al. 2012; White et al. 1990). PCR reactions were set up by mixing 25.0 µL of 2X HotStarTaq Plus Master Mix (QIAGEN, Germany), 0.5 µL of each 10 µM HPLC-purified (or desalted) primer (Invitrogen, USA), 19 µL of RNase-free water (QIAGEN) and 5.0 µL of DNA diluted at 5 ng/µL, for a total volume of 50 µL. Thermal cycling conditions for the 16S rRNA gene and ITS2 region were as follows: initial denaturation at 95 °C for 5 min; 35 cycles or 40 cycles for ITS2 at 94 °C for 45 s, 50 °C for 45 s, 72 °C for 1 min; and a final elongation step at 72 °C for 10 min. For the 18S rRNA gene of AMF, a nested PCR approach was used, as previously described (Stefani et al. 2020). In the first step, samples were amplified using primers AML1 (5′-ATCAACTTTCGATGGTAGGATAGA-3′) and AML2 (5′-GAACCCAAACACTTTGGTTTCC-3′) targeting the V3-V4-V5 region of the 18S rRNA gene with the following thermal cycling conditions: 95 °C for 5 min; 35 cycles of 94 °C for 1 min, 57 °C for 1 min, 72 °C for 1 min; and a final elongation step at 72 °C for 10 min. PCR products were used as template in a second PCR reactions with primers nu-SSU-0595-5' (5′-CGGTAATTCCAGCTCCAATAG-3′) and nu-SSU-0948-3′ (5′-TTGATTAATGAAAACATCCTTGGC-3′) targeting the V4 region of the 18S rRNA gene with the following thermal cycling conditions: 95 °C for 5 min; 35 cycles of 94 °C for 40 sec, 57 °C for 40 sec, 72 °C for 40 sec. Amplicon libraries were sequenced on an Illumina MiSeq platform using a PE300 v3 kit at the Centre de recherche du CHU de Québec-Université Laval.

## Data acquisition and processing

### Climate and soil physicochemical properties

For each sampling site, we used ClimateNA v7.50 (Wang et al. 2016) to obtain annual mean temperature, annual mean precipitation, continentality (the difference between the mean of the warmest month and mean of the coldest month), and mean precipitation for winter and spring seasons, for years between 1991 and 2020. Elevation was extracted for each site using Python package *pyhigh* v0.0.6.

Soil obtained through sieving with 6 mm mesh was air-dried and subjected to physciochemical analyses. Soil was sieved with 2 mm mesh size, and a soil subsample was additionally ground to obtain a 0.5 mm fraction using a disk mill for the determination of percent total carbon (C) and nitrogen (N). N and C were quantified with TruMac CNS analyzer (LECO Corporation, MI, United States). Using a 1:2 soil:water ratio, the soil pH was determined in the aqueous phase with a Thermo Scientific Orion 2-Star pH meter following (Carter and Gregorich 2007). Phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), aluminum (Al), and sodium (Na) were extracted with a Mehlich III extraction buffer (Carter and Gregorich 2007) and quantified using an ICP-OES (Optima 7300 DV, Perkin Elmer, Waltham, MA, United States). Cation exchange capacity (CEC) was calculated using quantified positively charged ions. The soil texture (sand, silt, and clay) was determined following the hydrometer method (Carter and Gregorich 2007).

### Bioinformatic analysis

Reads (16S, ITS, and 18S) were processed using the Q2Pipe pipeline (https://github.com/NRCan/Q2Pipe, versions 10-2022 (natural stands), 01-2024 (greenhouse samples), and 05-2024 (18S samples)), which leverages the functionality of the Qiime2 platform (Bolyen et al. 2019). In short, read quality was initially evaluated using Figaro (Weinstein et al. 2019) and Falco (https://zenodo.org/badge/latestdoi/214499063) to identify trimming positions. Subsequently, the reads were trimmed, filtered, and merged into Amplicon Sequence Variants (ASVs) with DADA2 (Callahan et al. 2016). Before the filtering step, ITS reads were trimmed using CutAdapt (Martin 2011) to remove variable primer lengths. ASVs with frequencies below 0.05% were discarded, and the remaining ASVs were assigned to taxonomic levels. For taxonomic classification, we used databases Unite v9 for the ITS2 region in fungi (Nilsson et al. 2019), Silva v138 for the 16S and 18S rRNA genes (Yilmaz et al. 2014), and FUNguild (December 2022) for the assignment of fungal guilds (Nguyen et al. 2016). For the 16S rRNA genes, only sequences assigned to bacteria and Archaea were retained, excluding sequences classified as Eukaryota, mitochondria, or chloroplast. For the ITS2 region, we retained sequences classified as fungi, and for the 18S rRNA genes those classified as Eukaryota. In natural stands we retained a total of 1,423,526 reads (16S), 1,555,740 reads (ITS), and 1,677,810 reads (18S) for further analyses. For the greenhouse dataset, we retained 739,825 reads (16S), 2,082,460 reads (ITS), and 1,671,470 reads (18S). For estimating alpha diversity, read counts were rarefied using rarefaction curves as a guide to control for uneven read numbers across samples.

## Statistical analyses

All downstream analyses were performed with R v4.4.1 and Python v3.8 software.

In the natural stands dataset, four samples were excluded from the ITS analysis due to low read counts (1x STET and 3x Santiago), resulting in 46 samples with 16S rRNA gene sequences, 42 samples with ITS2 region sequences, and 46 with 18S rRNA gene sequences. The greenhouse dataset included 12 soil, 12 rhizosphere, and 12 root samples, all of which were sequenced for 16S, 18S rRNA genes and ITS2 regions. A subset of nitrogen fixing bacteria were selected based on taxonomic classification, and included all ASVs for which the genus name contained the word “rhizobium” or “Rhizbium”, genus *Frankia*, and phylum Cyanobacteria.

Differences in each soil property between genetic groups, ecological regions and sampling sites were tested with one-way ANOVA with nested groups (genetic group/region/site) using R function lm(). Outliers were removed and data was transformed when necessary, assuring normal distribution of residuals and homogeneity of variance. If conditions were not met, a generalized least square (GLS) model was used with gls() function from the R package *nlme* to account for unequal variance between the groups and correlated errors within the nested structure. GLS models were fitted separately for the site, region, and genetic group level. Benjamini-Hochberg multiple testing correction was applied to *P*-values at a false discovery rate of 0.05. Principal component analysis (PCA) on micro and macronutrients was done with R function prcomp().

Abundance tables for each taxonomic marker (16S, 18S, ITS) were imported into a phyloseq R format. To visualize community composition by ordination, we retained ASVs present in 5% or more of the samples, to remove potential sequencing artifacts and reduce the noise (Cao et al. 2020). Read counts were then transformed using centered log-ratio (CLR) transformation with the R package *microbiome*. PCA was performed on CLR-transformed abundances using the prcomp() function, with no scaling. To project soil nutrient variables onto the ordination space, we conducted an envfit analysis with the R package *vegan* v2.6, testing significance with 999 permutations. In the natural stands dataset, we tested the effects of hierarchical grouping (genetic groups/regions/sites) on community structure using PERMANOVA via the adonis() function in the *vegan* package, with significance tested using 999 permutations. In the case of the greenhouse datasets, we tested the effects of hierarchical grouping for the whole dataset (sample type/genetic group/site) and within each sample type (genetic group/site) using the same method.

Alpha diversity metrics were estimated from the rarefied data using the alpha() function from the *microbiome* v1.26 package. Metrics included Gini-Simpson index (the probability that two randomly selected samples are different) and the total species richness (Chao1). Differences between regions were tested with one-way nested ANOVA or GLS models as described above. Pairwise differences were tested with the Tukey test. To assess the relationship between soil properties and alpha diversity, we fitted beta regression model using the betareg() function from the R package *betareg* v3.2. Gini-Simpson index was used as the response variable, and subsequent explanatory variables were tested: pH, C:N, MAT (mean annual temperature), K and P. The best model was selected using stepwise model selection with R package *StepBeta* v2.1.0.

Pseudo $R^2$ of each predictor was calculated by subtracting pseudo $R^2$ of the model without the predictor from the pseudo $R^2$ of the full model.

We measured the effect of the genetic distance among aspen trees on the microbiome community structure using single nucleotide polymorphism (SNP) data from (Goessen et al. 2022). Vcftools v0.1.16 (Danecek et al. 2011) was used to extract SNPs with mean depth across all individuals of more than 2, less than 15% of missing data, and a frequency of minor alleles above 5%, giving a total of 53,714 SNPs. Euclidean distances between genotypes were estimated with dist() function, whereas the distances between microbial communities were the same as in the envfit analysis (euclidean distances between CLR-transformed abundances). To evaluate the association between genetic and microbial distance matrices we applied Mantel's test with 10,000 permutations using the mantel() function from the R package *vegan* v2.6. PCA on SNPs was performed with snpgdsPCA() function from R package *SNPRelate* v1.40.

Differential abundance between taxa from the three sampled ecological regions (boreal, cold and warm temperate) was estimated with ANCOM-BC v2.6 (Lin and Peddada 2020), which models an unsampled fraction of taxa in compositional data. Taxa present in at least 35% of the samples were selected to remove those that occur infrequently. Differences between regions were estimated independently at the genus and family level, by first combining read counts of ASVs from the same genus or family. Taxa that were not defined or uncultured were grouped at the lowest possible taxonomic level. The sampling site was included as a random effect in the model. To assess differential abundance between regions and sites in fungal guilds, combined abundances for the selected guilds were CLR-transformed. Differences between regions were tested with one-way nested ANOVA or GLS models as described above. Pairwise differences were tested with the Tukey test. Benjamini-Hochberg multiple testing correction was applied to *P*-values at a false discovery rate of 0.05.

To show how often ASVs and taxa that are shared among or are unique to different groups, we used non-rarefied read counts with a relative abundance threshold of 0.001 to remove only the least abundant ASVs or taxa. We also checked the frequency of taxa without filtering for relative abundance and obtained very similar results. Taxa were classified as present within a specific group (genetic group, region, site), if at least one sample in that group had the given taxon.

# Results

## Soil properties contribute to the diversity of soil microbial communities in aspen-dominated stands

We compared soil and microbiome characteristics between two genetic groups: Canada and Mexico (NENA and MX in (Goessen et al. 2022)), and across three ecological regions—boreal, cold temperate, and warm temperate—referred to as regions. Soils from the two genetic groups were distinguished based on soil physicochemical properties (Fig. 1D-E, Supplementary Table 2). Nested ANOVA analyses showed that the strongest differences between genetic groups included higher Fe in Canadian soils and higher K and Mn in Mexican soils (Supplementary Fig. 2). Other properties varied primarily between sites and/or regions. For example, Mexican (warm temperate) soils had on average higher pH, but the levels between sites varied considerably. Boreal sites were characterized by the highest levels of Mg (along with Fe), whereas cold temperate sites showed the lowest levels of Mn, Mg, K, and Ca. Macronutrients (N, C, and P) did not vary between genetic groups, but mostly between sites and regions. Boreal sites scored the highest levels of N and C, but had the lowest C:N ratio. Boreal and warm temperate sites also showed high levels of cation exchange capacity (CEC) (Supplementary Fig. 2).

Bacterial, archaeal, and fungal communities were distinct between the genetic groups, regions, and between sites (PERMANOVA, $P < 0.001$, Fig. 2A-B). We investigated the impact of soil properties and genetic relatedness of trees on microbial community structure. Community structure was correlated with many soil nutrients (envfit analysis, $P < 0.05$, Fig. 2A-B, Supplementary Table 3), suggesting that soil properties were driving part of the variation of bacterial, archaeal, and fungal communities. In bacteria and Archaea, the most correlated properties included clay and sand texture, Fe, Ca, and Mn ($R^2 = 0.45$-$0.67$, Supplementary Table 3), whereas in fungal communities it was mainly K, Ca, and Mn ($R^2 = 0.46$-$0.54$, Supplementary Table 3). Because at the global scale, soil differences can be correlated with other factors specific to genetic groups, we also investigated the impact of soil at the smaller, genetic group scale. In Canada, bacterial/archaeal community structure was mostly correlated with Ca, Mg and clay ($R^2 = 0.74$-$0.86$, Supplementary Table 4), and fungal communities with sand, clay and silt ($R^2 = 0.71$-$0.76$, Supplementary Table 4), reflecting differences between the three ecological regions. In Mexico, bacterial/archaeal communities were best correlated with pH, CEC, and clay ($R^2 = 0.51$-$0.66$, Supplementary Table 5), whereas for fungal communities silt was the dominating factor ($R^2 = 0.90$), followed by C:N ratio ($R^2 = 0.57$, Supplementary Table 5).

To measure the impact of aspen genetic variation on the microbial community structure, we compared genetic distances between individual trees sequenced in a previous study (Supplementary Fig. 3, Goessen et al. 2022) with the distance between soil microbiome communities collected under those trees. As the Mexican and Canadian aspens belong to two distinct genetic groups, we measured the distances within each genetic group, to avoid confusion of genetic differences with other factors. We found that the aspen genetic distance did not explain

the microbial community distances, except for a small effect found for bacterial and archaeal communities in Mexico (Mantel's test, $P = 0.05$, Fig. 2C,G).

Alpha diversity indices varied regionally for bacterial and archaeal communities (Gini-Simpson index, $F = 9.7$, $P = 4.13E-04$, Fig. 2B), and for fungal communities (richness, $F = 7.7$, $P = 0.002$, Fig. 2F). Boreal sites had a higher bacterial/archaeal diversity than warm temperate sites (Gini-Simpson index, Tukey test, $P = 3.14E-04$), whereas warm temperate sites showed higher fungal species richness than both boreal and cold temperate sites (Tukey test, $P < 0.02$). However, alpha diversity metrics varied even more between sites (Fig. 2B,F). We tested if soil nutrients were driving variation in alpha diversity. As many soil and climate factors influence diversity of bacterial and fungal groups (Labouyrie et al. 2023), we modeled Gini-Simpson index using beta regression, with the following uncorrelated variables: pH, C:N, MAT (mean annual temperature), P, and K. The selected models explained a small portion of variance (pseudo $R^2 = 0.16$ for bacteria/Archaea, and pseudo $R^2 = 0.04$ for fungi, Supplementary Table 6). In bacteria/Archaea, the strongest predictor was the interaction between pH, MAT, and K (pseudo $R^2 = 0.05$, $P = 0.06$), whereas in fungi, no factor or interaction was a good predictor of diversity, likely because alpha diversity did not vary a lot across samples. Alpha diversity of bacteria/Archaea and fungi were positively correlated with each other (Spearman's Rank correlation, rho = 0.38, $P = 0.01$).

We observed up to 3 times log-fold changes in the taxonomic composition of microbial taxa among boreal and temperate regions (genus level, Fig. 3, family level, Supplementary Fig. 4). However, none of the highly abundant genera showed significant differences in relative abundance between the pairs of regions, likely due to high variability across sites (Fig. 3A, C). At the genus level, a large proportion of bacterial and archaeal microbiome was shared between Canada and Mexico (69.4%, Fig. 3B) or between the three regions (56.7%, Fig. 3B), whereas much fewer genera were unique to a genetic group or a region (30.6% and 27.9%, respectively, Fig. 3B). In fungi, the number of genera unique to genetic group or region (46.8% and 39% respectively, Fig. 3D), was similar to the number of genera shared by the groups (53.2% and 40.5%, respectively, Fig. 3D), indicating higher regional specificity of fungal genera compared to bacteria. Conversely, at the ASV level, most variants were unique to the genetic group, region, or site (Fig. 3B, D). Bacterial genera present across all sampled sites primarily belonged to the Phyla Proteobacteria (n = 52), Acidobacteriota (n = 21), Chloroflexi (n = 18), Actinobacteriota (n = 17), Planctomycetota (n = 16), and Bacteroidota (n = 14), among others. Shared fungal genera were mostly from Ascomycota (n = 66) and Basidiomycota (n = 44), with smaller contributions from Mucoromycota (n = 3), Rozellomycota (n = 2), Chytridomycota (n = 1), and Mortierellomycota (n = 1).

Boreal and warm temperate regions were taxonomically most differentiated (Fig. 3, Supplementary Fig. 4). Bacterial taxa most enriched in the boreal region included *Ktedonobacteracea*, A21b, and *Subgroup 7*, whereas the warm temperate region of Mexico had highly abundant families such as *Oxaobacteraceae* (*Masillia* spp.), *Mycobacteriaceae* (*Mycobacterium* spp.), or *Burkholderiaceae*. Cold temperate sites had only a few taxa that were more abundant than in both boreal and warm temperate sites (WD260), with most taxa having a relative abundance intermediate between the two regions (*Ktenobacteracea*, AD3, *Mycobacteracea*). Fungal taxa enriched in boreal sites included families *Elaphomycetacea*

(*Elaphomyces* sp.), *Inocybaceae* (*Inocybe* spp.), and *Russulaceae* (*Lactarius* spp., *Russula* spp.), in cold temperate region *Tricholomatacea* (*Tricholoma* sp.), and in the warm temperate region *Pseudeurotiaceae* (*Pseudogymnoascus* spp.), *Aspergillaceae* (*Penicillium* spp.), and *Umbelopsidaceae* (*Umbelopsis* spp.). Notably, fungal genera with higher abundances in boreal and cold temperate sites were often ectomycorrhizal (Fig. 3B).

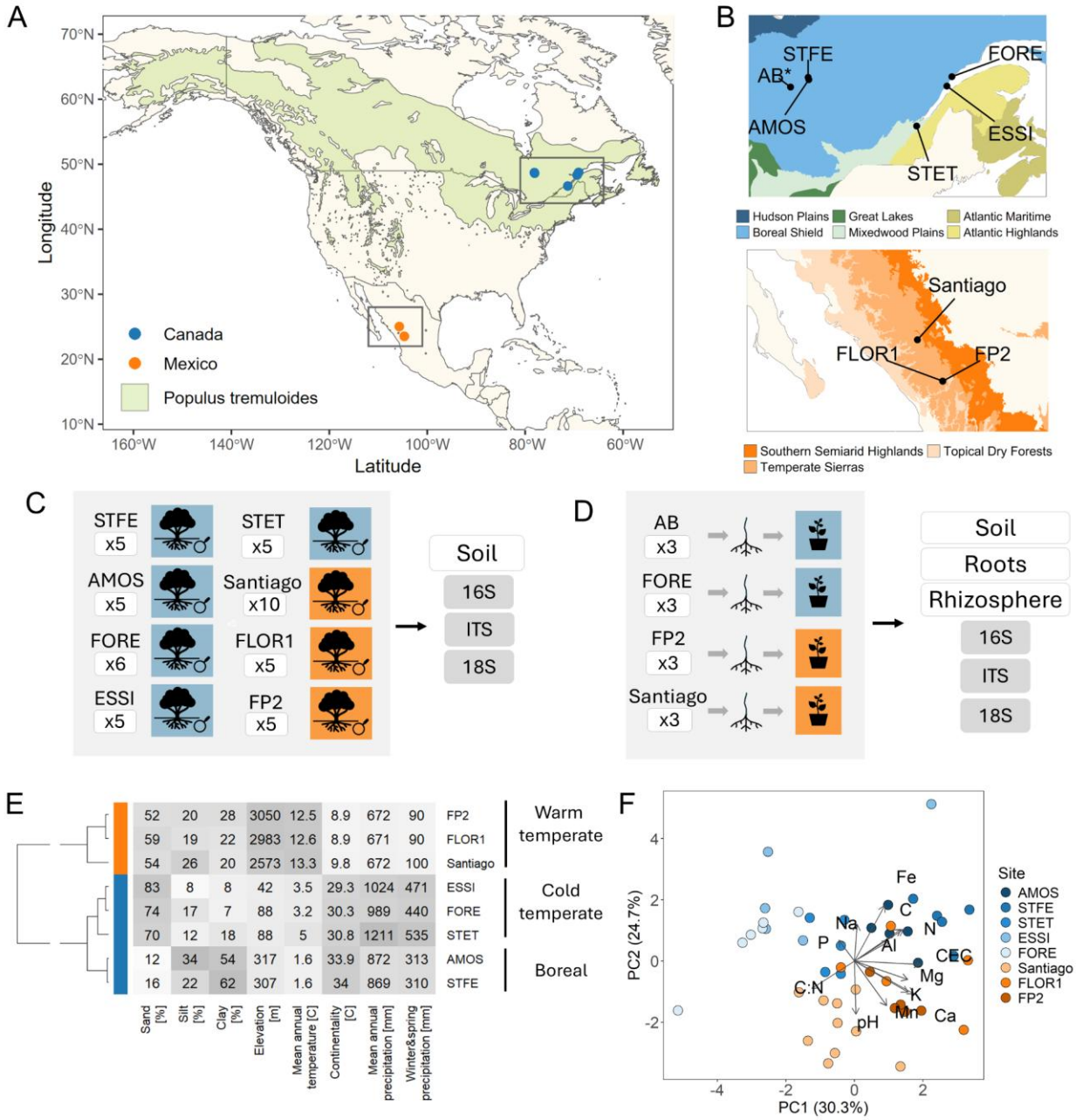

Fig 1. Study design and characteristics of the sampling sites. A) Distribution of aspen with the sampling locations. B) Sampling locations in Quebec, Canada and Northwestern Mexico. Note that site AB was used for the greenhouse study only. C) Datasets collected from the natural stands. D) Design of the greenhouse setup and analyzed datasets. E) Soil texture and geoclimate characteristics in seven natural stand locations. Climate variables represent 1990–2020 climate normals. F) Principal component analysis of soil physicochemical properties. Ecoregions displayed in panel (B) were obtained from the Government of Canada website (https://natural-resources.canada.ca/forest-forestry/sustainable-forest-management/forest-classification) and the United States Environmental Protection Agency website (https://www.epa.gov/eco-research/ecoregions-north-america). Distributions and tests of all soil properties are provided in Supplementary Figure S2.

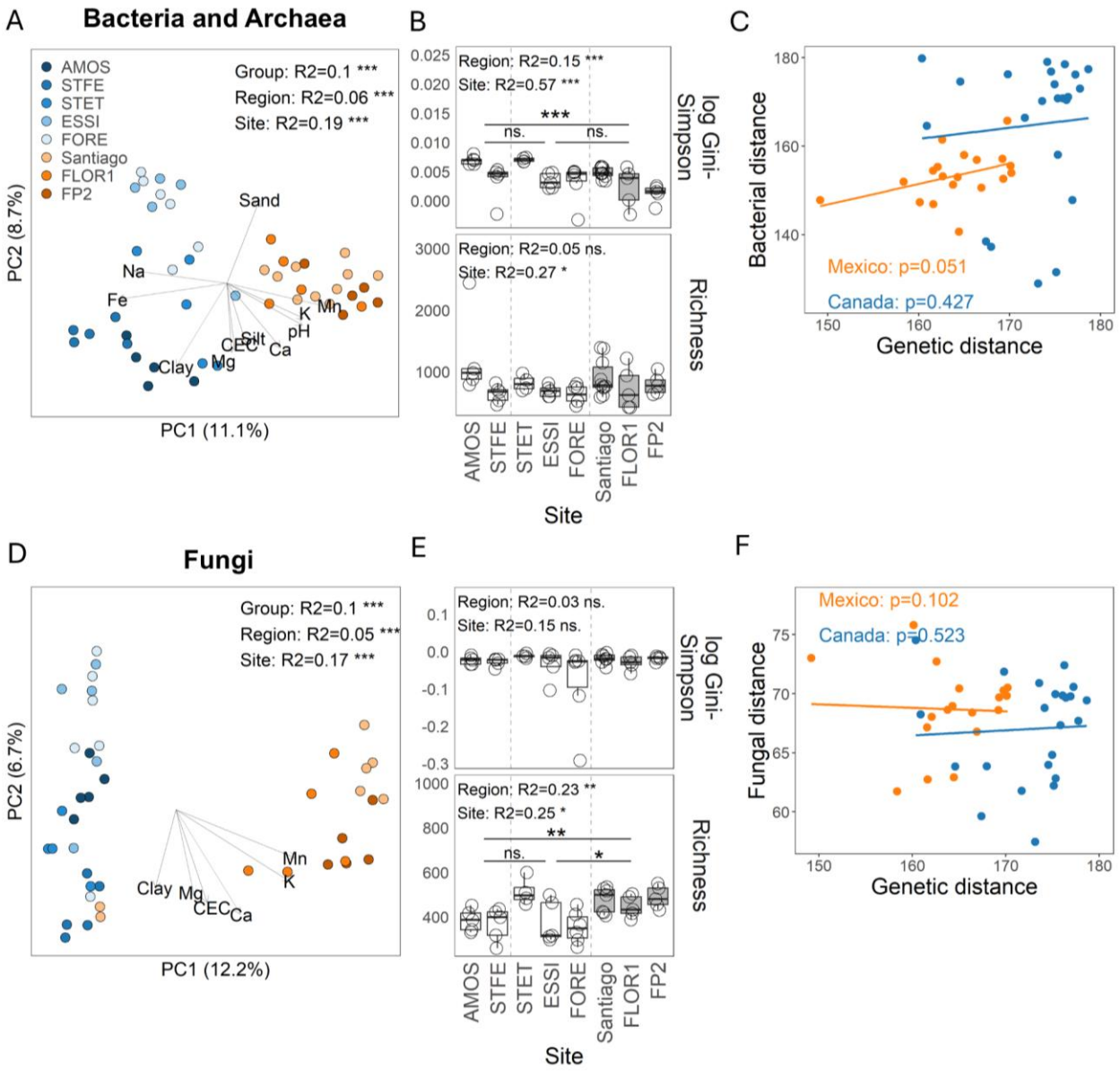

Fig 2. Factors driving soil bacterial, archaeal, and fungal community structure. Ordination of A) bacterial/archaeal, and D) fungal communities. Vectors show significant soil properties fitted onto the ordination of microbial structure with the envfit analysis. Only soil properties with significant vectors at $P < 0.01$ are shown. $R^2$ values refer to PERMANOVA analysis with nested groupings: genetic group/region/site. B, E) Alpha diversity metrics in bacterial and archaeal (B) and fungal (E) communities. Differences between regions and sites were tested with nested ANOVA, and pairwise differences with the Tukey test. C, F) Mantel's test comparing euclidean distances of bacterial and archaeal (C) and fungal (F) communities with euclidean distances of individual tree genotypes. Principal component analysis on aspen variants is shown in Supplementary Figure S3.

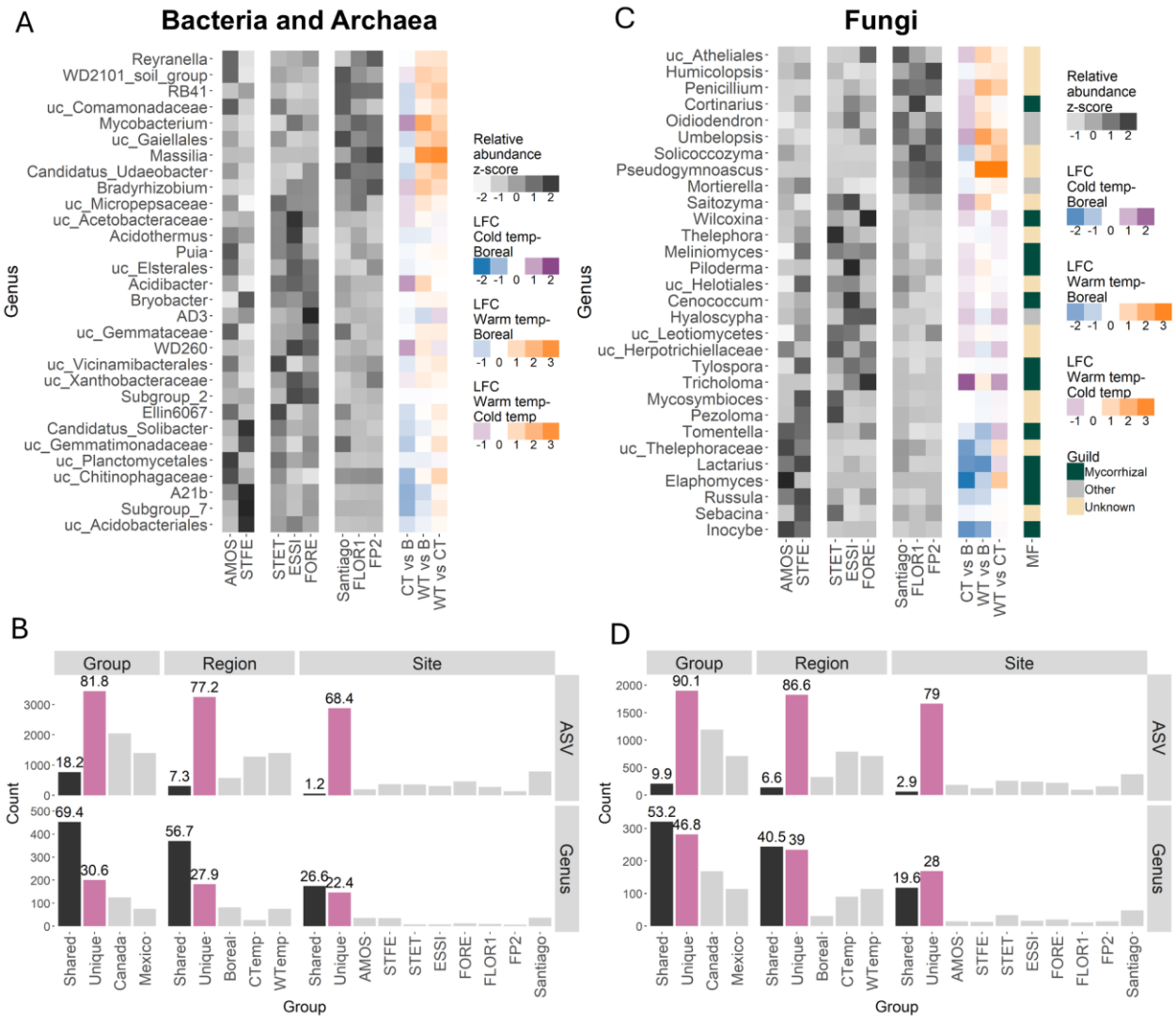


Fig 3. Taxonomic composition of the most highly abundant soil taxa. A, C) Grey heatmaps show standardized relative abundances and colored heatmaps show differential abundance (natural log fold change, LFC) between the pairs of regions in bacterial and archaeal (A), and fungal (C) taxa clustered at the genus level. Taxa most abundant in boreal region (B) are in blue, in cold temperate region (CT) in purple, and in warm temperate region (WT) in orange. The second color heatmap in (C) named “MF” indicates taxa classified as mycorrhizal fungi (green), other guild (grey), or unknown (beige) using FunGuild. Prefix “uc” in taxonomic names indicates all unidentified or uncultured genera clustered at the higher level. Genera are ordered such that the topmost genera have the highest mean relative abundance in the warm temperate region, the middle ones - in the cold temperate region, and the bottom ones - in the boreal region. B, D) Counts of ASVs and genera shared across (black), or unique to genetic group, region, or site (pink), for bacterial and Archaea (B), and fungi (D). Grey bars indicate the number of taxa specific to the given class. Note that categories are not mutually exclusive. The numbers above bars indicate percentages of all taxa. Differential abundance between bacterial, archaeal, and fungal families is shown in Supplementary Figure S4.

## Communities of ectomycorrhizal (EMF) and arbuscular mycorrhizal fungi (AMF) vary between ecozones

We compared the relative abundance of eight fungal guilds between boreal, cold, and warm temperate regions. Consistent with the prevalence of mycorrhizal fungi among the most enriched genera in Canada (Fig. 3C), we observed significant regional differentiation in ectomycorrhizal fungi (EMF, one-way nested ANOVA, $F = 8.7$, $P = 0.002$, Fig. 4A), the most abundant among eight analyzed fungal guilds (Supplementary Fig. 5). The boreal region had a higher relative abundance of EMF compared to both cold and warm temperate regions (Tukey test, $P < 0.05$), whereas cold and warm temperate regions had similar levels of EMF (Tukey test, $P = 0.42$). In contrast, alpha diversity remained constant across the three regions ($F = 0.12$, and $F = 2.33$, respectively for the Gini-Simpson index and species richness, $P > 0.1$, Fig. 4A).

Because the ITS2 marker is not reliable in detecting highly variable rDNA regions of arbuscular mycorrhizal fungi (AMF), we sequenced all samples for the 18S marker designed specifically to amplify AMF sequences. Since not only AMF but also other taxonomic groups were identified in our data set with 18S primers, we used the proportion of AMF reads relative to all reads captured by 18S as a proxy for their relative abundance. We found no difference in AMF relative abundance between the three regions ($F = 1.24$, $P = 0.34$, Fig. 4B). However, the Gini-Simpson index and richness differed significantly between the regions ($F = 10.67$, and $F = 9.68$, respectively, $P = 0.001$, Fig. 4B), with the highest levels found in the cold temperate region (Tukey test, $P < 0.04$, Fig. 4D).

To examine the relative abundance and alpha diversity of nitrogen-fixing bacteria, we identified taxa belonging to rhizobia and *Cyanobacteria* in aspen stands (Sepp et al. 2023). We found significant differences between the regions in both relative abundance ($F = 60.11$, $P = 3.06E-13$, Fig. 4C) and alpha diversity ($F = 11.95$, $F = 20.21$, for Gini-Simpson index and richness, respectively, $P < 5.0E-4$, Fig. 4C). While samples from Mexico showed reduced relative abundance in EMF and lower diversity of AMF, both of which facilitate nitrogen acquisition, they also showed higher relative abundance of selected nitrogen-fixing bacteria than in the cold temperate region (Tukey test, $P = 0.002$) and boreal region (Tukey test, $P = 6.48E-13$). Similarly, alpha diversity indices were higher in Mexico than in cold temperate (Tukey test, $P < 0.02$, Fig. 4C) and boreal regions (Tukey test, $P < 5.0E-4$, Fig. 4C).

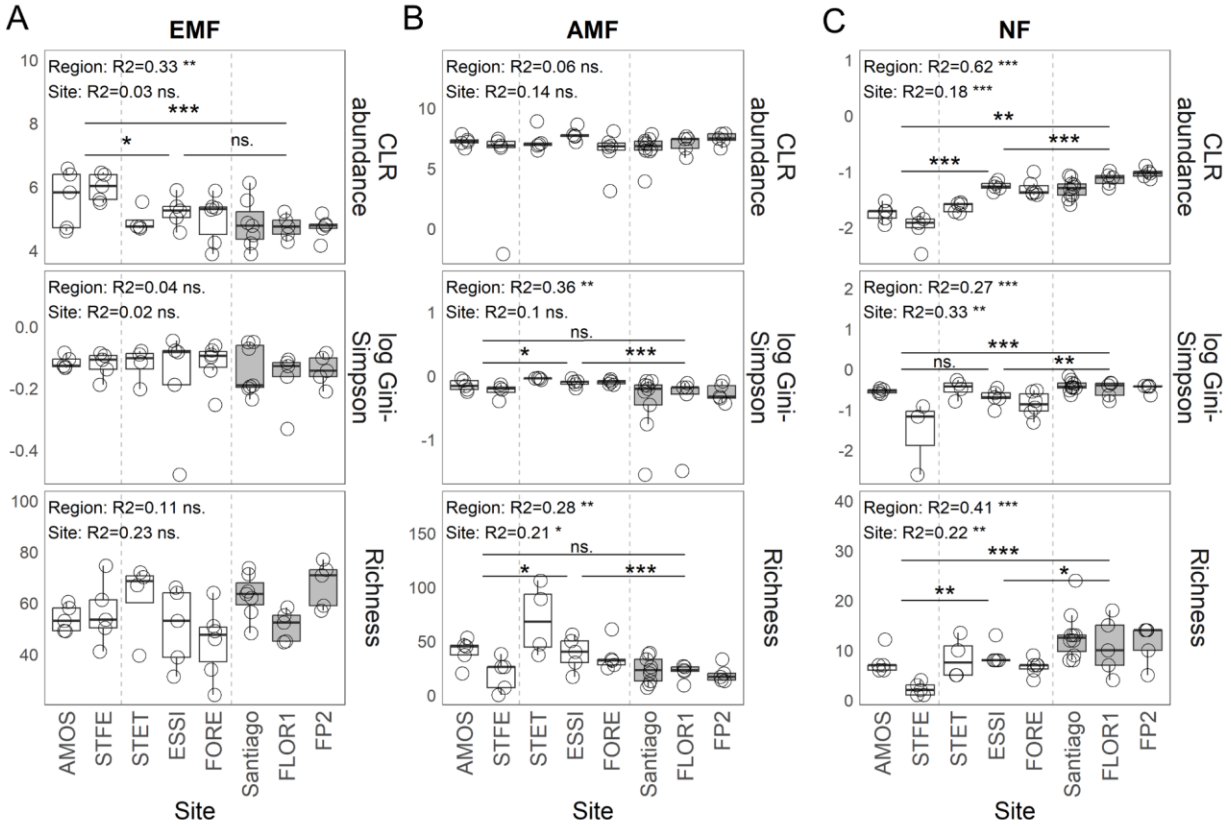


Fig 4. Abundance and alpha diversity of EMF (A), AMF (B), and selected nitrogen-fixing bacteria, NF (C) between the three regions of aspen natural stands. Sites are divided with dashed lines into boreal, cold temperate, and warm temperate regions. CLR stands for center log-ratio transformed abundance. Grey color indicates genetic groups or regions from Mexico. Differences between regions were tested using nested ANOVA. Pairwise differences between regions were performed with the Tukey test. ns. stands for not significant after multiple test correction with the Benjamini-Hochberg procedure at 0.05; * $P < 0.05$, ** $P < 0.01$,*** $P < 0.001$. Three Gini-Simpson outlier values were removed from the plot for better visibility.

## Greenhouse common garden confirms the influence of the aspen genetic makeup on bacterial, fungal, and EMF communities

To disentangle the effects of soil and host genotype on the community structure and EMF/AMF abundance, we characterized the soil, rhizosphere, and root microbiome of aspen seedlings from 4 sites (2 from Mexico, 2 from Canada) grown in the same potting soil and under the same ambient conditions (Fig. 1C). We found that community structures differed between the sample types of bacterial/archaeal (PERMANOVA, $R^2 = 0.11$, $P < 0.001$, Fig. 5A) and fungal communities ($R^2 = 0.09$, $P < 0.001$, Fig. 5B). Similar to results in the natural stands, we found significant differences between the two genetic groups in the soil (bacteria: $R^2 = 0.12$, $P = 0.008$; fungi: $R^2 = 0.13$, $P = 0.002$, Fig. 5A-B), and rhizosphere communities (bacteria: $R^2 = 0.12$, $P = 0.034$; fungi $R^2 = 0.12$, $P = 0.008$, Supplementary Fig. 6). The signal from roots was a little weaker in bacteria

and Archaea ($R^2$ = 0.1, $P$ = 0.2; fungi $R^2$ = 0.12, $P$ = 0.035, Supplementary Fig. 7). Site explained more variation than genetic group within soil and rhizosphere communities ($R^2$ = 0.2-0.22, Fig. 5A-B, Supplementary Fig. 6), but the effect was smaller and not significant in the roots ($R^2$ = 0.16-0.18, Supplementary Fig. 7). This result confirmed that community differences were influenced by the host genotype in the soil, and also in the rhizosphere. No particular genus dominated in either of the genetic groups (Fig. 5C-D, Supplementary Figs. S6-S7). Alpha diversity indices in the soil, rhizosphere, and root samples were similar between the genetic groups and sites (Fig. 5E-F, Supplementary Figs. S6-S7).

We examined whether aspen from Canada forms more associations with mycorrhizal fungi than those from Mexico and whether Mexican aspen promotes more nitrogen-fixing bacteria than their Canadian counterparts when grown under the same conditions. We did not find AMF in any of the samples, likely because they were absent or not able to develop in the potting mix. Regarding EMF, we found in total six genera in the soil, rhizosphere, and roots (Fig. 6B-D), which together had higher relative abundance in aspen plants from Canada in all three sources (generalized least squares, $P$ = 3.86E-07, and $P$ = 1.26E-12; nested ANOVA, $P$ = 0.07, for the soil, rhizosphere and roots respectively, Fig. 6A). This is in contrast to other fungal guilds which had similar abundances between the two genetic groups (Supplementary Fig. S8). No significant differences in alpha diversity were found between sites from the two genetic groups (Fig. 6A). We also found no differences in the relative abundance or alpha diversity of nitrogen-fixing bacteria between the two genetic groups (Supplementary Fig. 9), which could be a consequence of frequent fertilization of the potting mix. In general, we confirmed that EMF fungi were more commonly found in association with aspen from Canadian sites, than from Mexico.

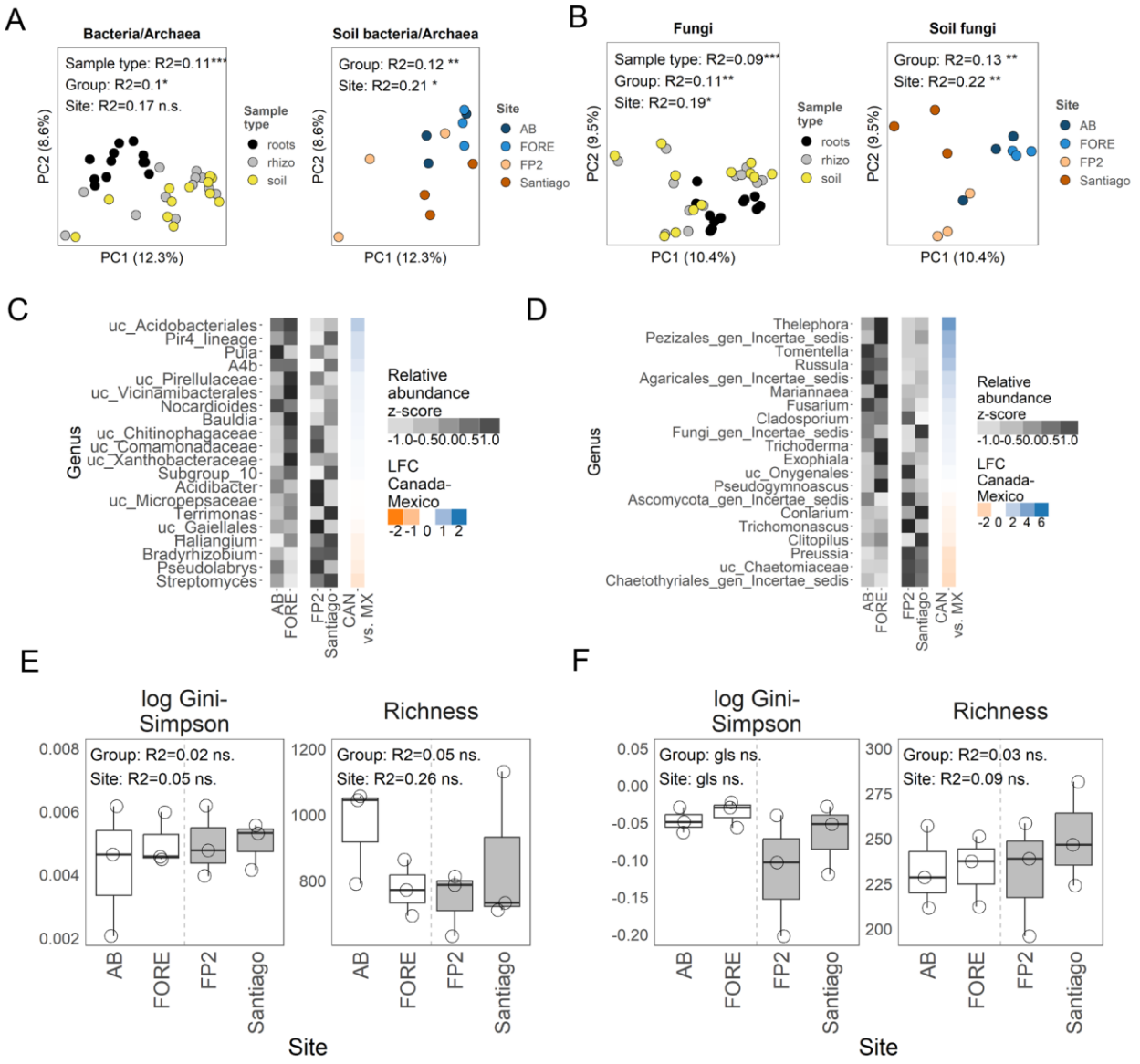


Fig 5. Soil communities of aspen seedlings from Canada and Mexico growing in greenhouse conditions. A-B) Ordination and PERMANOVA analysis of bacterial/archaeal (A) and fungal (B) communities visualized for samples from the three sample types (soil, rhizosphere, roots) and for soil samples only. C-D) Grey heatmaps with standardized relative abundances and colored heatmaps with differential abundance (natural log fold change, LFC) between the genetic groups in bacterial/archaeal (C), and fungal (D) taxa clustered at the genus level. Taxa differentially abundant in Mexico are in orange, and differentially abundant in Canada are in blue. E-F) Comparison of alpha diversity for bacterial/archaeal (E), and fungal (F) communities. Grey boxes indicate samples from Mexico. Differences between genetic groups were tested using nested ANOVA, or generalized least squares (gls). ns. stands for not significant after multiple test correction with Benjamini-Hochberg procedure at 0.05; * $P < 0.05$, ** $P < 0.01$,*** $P < 0.001$. Results for roots and rhizosphere communities are shown in Supplementary Figures S6-S7.

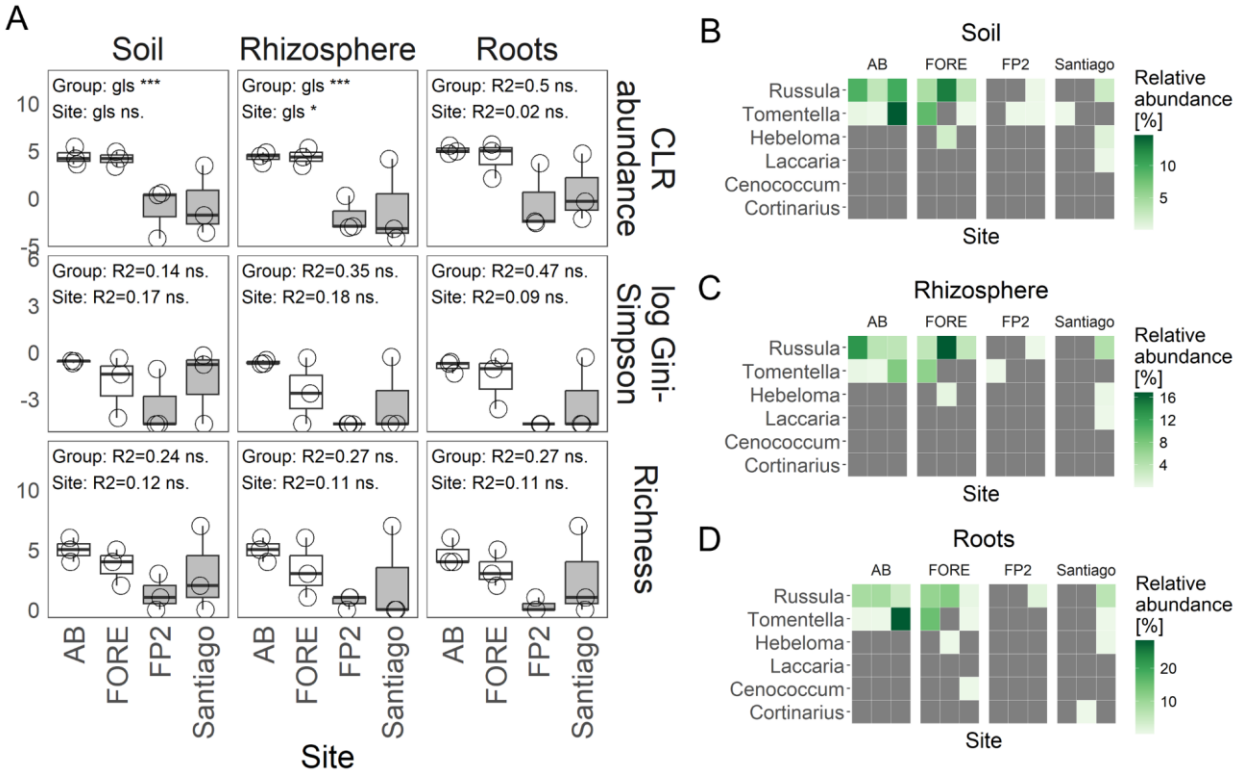


Fig 6. Relative abundance and alpha diversity of EMF communities of aspen seedlings from Canada and Mexico growing in greenhouse conditions. A) Comparison of center log-ratio transformed (CLR) abundance, log of Gini-Simpson index, and species richness in EMF communities between two genetic groups. Grey boxes indicate samples originating from Mexico. Differences between genetic groups were tested using nested ANOVA, or generalized least squares (gls). B-D) Heatmaps with a relative abundance of EMF genera measured in soil (B), rhizosphere (C), and roots (D).

# Discussion

We investigated the soil communities associated with two genetically and geo-climatically distinct genetic groups of *P. tremuloides*: one sourced from Northwestern Mexico and the other from Eastern Canada. Our study examined how soil properties and host tree genotype influence the composition, structure and diversity of these communities and tested the impact of tree origin using a common garden setup. We found that both host genotype and soil properties contribute to the divergence of the aspen soil microbiome. Additionally, mirroring the geo-climatic variation across sampled sites, we observed differences in community structure and composition among three forest regions—boreal, cold temperate, and warm temperate. These regions were primarily differentiated by the relative abundance and diversity of ectomycorrhizal fungi (EMF), arbuscular mycorrhizal fungi (AMF), and nitrogen-fixing bacteria.

## Factors driving differences in diversity and community structure of aspen

The two studied aspen genetic groups diverged around 800,000 ya (Goessen 2023) and occupy somewhat distinct habitats at the southern and northern extremes of the species' range: Northwestern Mexico and Quebec, Canada, respectively. Due to the high elevation of the Mexican stands, this temperate genetic group experiences much cooler weather than the adjacent dry tropical forests and obtains nearly as much annual rainfall as temperate parts of Canada. Nevertheless, aspen stands from Mexico differ markedly from Canadian stands in lower temperature seasonality and a different precipitation regime which is constrained to the rainy season. Other factors also vary between the two genetic groups including abiotic (soil, altitude, photoperiod) and biotic (host genetic differentiation, forest taxonomic composition, and surrounding understory vegetation) – all of which can contribute to divergence in their associated microbiomes (Guo et al. 2023; Chamard et al. 2024; Adair and Douglas 2017; Baldrian 2017). We found that microbial communities indeed varied between the two genetic groups, however most variation was distributed among samples and among sites. This is consistent with findings of strong spatial variability and a smaller impact of seasonal changes on *P. tremuloides* bacterial and fungal root communities in the U.S.A. (Nash et al. 2025). In our study, we focused on analyzing the impact of soil properties and host genotype on the overall microbial community.

Soil properties are one of the key factors shaping microbiota, influencing microbial growth rates, diversity and composition primarily through pH and nutrient availability, and their interaction with climate (Ding, Travers, and Eldridge 2022; Rousk et al. 2010; Osburn et al. 2024; Sridhar et al. 2022). For example, interaction between pH and temperature was shown to predict continental and global extent of forest soil bacterial richness much better than pH alone (Luan et al. 2023). We observed that pH varied only slightly between Mexico and Canada and levels of macronutrients such as N, C, P and remained similar, but they all varied significantly among the sites. However, the overall combination of soil parameters clearly differentiated the two genetic groups. At the continental scale, Fe, K, Ca, Mn correlated best with microbial structure, but they also strongly differed between groups, making it difficult to disentangle the effect of those nutrients from other group-specific factors. Nevertheless, several nutrients were strongly correlated with community structures ($R^2$ = 0.5-0.9) also on the local (genetic group) levels, suggesting at least partial contribution of soil to aspen microbiome differences between sites and regions. We also found that a part of bacterial diversity was explained by the interaction between mean annual temperature, soil macronutrients and pH. This effect was weak (pseudo $R^2$ = 0.16), which could result from nonlinear relationships between predictor and soil variables or from other unmeasured spatial effects that differentiate samples and sites in our study.

Host plant genotypes can influence soil microbiomes through root exudates that attract and inhibit specific microbes (Morgan, Bending, and White 2005). Metabolic profiles of root exudates show natural variation between (Rathore et al. 2023) and within species (Mönchgesang et al. 2016) that eventually impact the composition of the rhizosphere microbiome (Seitz et al. 2022). Aspen is genetically structured into four major genetic groups, with those from Mexico and Eastern Canada being the most divergent ($F_{ST}$ = 0.174; Goessen et al. 2022). Within genetic groups, genetic differentiation between sites is low, accounting for just 0.3–4.4% of the total variation (Goessen et al. 2022). Consistent with this, we found no strong relationship between aspen genotypes and

their microbial communities within genetic groups of natural stands. The only weak trend was observed in bacteria and Archaea from Mexico. Aspen trees from Mexico are more differentiated, likely due to clonal reproduction in isolated mountainous areas, which could explain a stronger trend in this genetic group. This suggests that at the observed level of genetic differentiation, host genotype effects are overshadowed by other factors. In contrast, greenhouse common garden experiments clearly demonstrated the influence of genotype at both local (between study sites) and global (between genetic groups) scales. While host genotype effects are present locally, they appear weaker than the effects of external factors.

Because plant roots influence microbial community assembly by attracting and promoting growth of certain microbes (Badri et al. 2009), the surrounding vegetation can significantly shape the soil microbiome of aspen stands. In Canada and in the U.S.A., *P. tremuloides* is not typically associated with high-diversity environments. As a pioneer species, it is more commonly found in early-successional habitats or areas recovering from disturbances, such as forest fires, logging, or agricultural abandonment (Shinneman et al. 2013; Swift and Ran 2012; Stefani et al. 2018), where competition for light, nutrients, and space is minimal. Consequently, its presence is often restricted to specific niches or transitional phases in forest succession. On the other hand, in Mexico it can be associated with diverse habitats (Simental-Rodríguez et al. 2014). To assess the relative impact of vegetation, as well as soil, and host genotype on soil microbiome communities in natural stands, a detailed plant inventory, and a broader sampling would be necessary.

## A gradient in taxonomic composition of aspen soil microbiome

Most (~70%) of the bacterial microbiome could be found in both Canada and Mexico. Bacterial taxa commonly found across all sampling sites in our study belong to phyla that were previously consistently found as common components of poplar soil (Proteobacteria, Acidobacteriota, Chloroflexi, Actinobacteriota, Planctomycetota) or poplar roots (Bacteroidota, (Zanin et al. 2024)), and were also in general the most commonly found phyla in soil and rhizosphere (Ling, Wang, and Kuzyakov 2022). Two genera we noted as highly abundant, *Bradyrhizobium* and *Mycobacterium,* were also reported previously (Zanin et al. 2024). One commonly found genus, enriched in Mexico, was *Massilia*, which was previously found to be a core component of the *Populus* phyllosphere (Dove et al. 2021; Liu et al. 2023). Although taxonomic enrichments between regions were not significant, likely due to large heterogeneity across sites, some trends in the distribution of taxa were evident. For example, *Massilia*, *Bradyrhizobium* and *Mycobacterium* dominated in warm temperate sites of Mexico, and boreal sites had higher abundance of Subgroup 7 (Acidobacteriota), and A21b (*Burkholderiales,* Proteobacteria). *Bradyrhizobium* is a genus containing many nitrogen-fixing bacteria, and *Mycobacterium* is a widely distributed decomposer in soils across forest ecosystems (Walsh et al. 2019). *Massilia* is a common rhizosphere component (Ofek, Hadar, and Minz 2012), and interestingly, it was shown to exhibit high phosphatase activity in forest soil when growth of fungal hyphae was suppressed (Brooks et al. 2011). Subgroup 7 is not commonly reported, but was previously found, e.g., in agriculturally modified soils in Brazil (de Chaves et al. 2019; Navarrete et al. 2015), while A21b in soils from Antarctica (Krauze et al. 2021). It is not clear however if these species have any association with aspen, and may simply be common components of the soil in that region.

Fungal taxa proved to have a more constrained distribution, with around half of the genera (~53%) common across and half (~47%) specific to only one of the two genetic groups. Also, 39% of fungal genera were found uniquely within one of three climate regions. This observation is consistent with higher global estimates of environmental specificity for fungi than bacteria (Loos et al. 2024). Regional endemism was notably found in poplar mycobiome and was explained by combination of soil, climate effects and geographical distance (Van Nuland et al. 2023). Several most abundant fungal genera in our dataset were reported previously as associated with poplar soil (*Cenococcum*, *Cortinarius*, *Inocybe*, *Lactarius*, *Mortierella*, *Tomentella, Tricholoma*) (Zanin et al. 2024; Cripps and Miller 1993). Similar to bacteria, fungal taxa were not significantly enriched in any of the three regions, but few of them showed some regional preferences. Boreal sites were preferentially occupied by *Elaphomyces*, *Inocybe* and *Lactarius* and cold temperate sites by *Tricholoma*. The only common mycorrhizal genus preferentially found in Mexico was *Cortinarius*, and three non-mycorrhizal highly enriched taxa included *Pseudogymnoascus*, *Penicillium* and *Umbelopsis*. All three genera are known to be associated with the rhizosphere (Summerbell 2005; Xia, Rufty, and Shi 2021).

## Shifts in mycorrhizal communities along a latitudinal gradient

Ectomycorrhizal fungi (EMF) and arbuscular mycorrhizal fungi (AMF) differ in their capabilities to acquire nutrients. AMF, which are associated with nearly 80% of all land plants, rely on phosphorus (P) and nitrogen (N) released by saprotrophic microbes in the surrounding soil (Phillips, Brzostek, and Midgley 2013). In contrast, EMF are primarily associated with trees and possess soil-degrading enzymes that allow them to directly access N and P from organic matter (Phillips, Brzostek, and Midgley 2013). These decomposing capabilities give EMF a competitive advantage in boreal forests, where organic material tends to accumulate, and where EMF indeed dominate (Steidinger et al. 2019). In addition, the diversity of plants tends to be correlated with the diversity of mycorrhizal fungi (Fei et al. 2022), predicting a higher MF diversity at sites with rich surrounding vegetation, including other tree and understory species.

In aspen, an expected latitudinal shift in EMF is evident: EMF are significantly more abundant in boreal sites compared to temperate stands in Canada and in Mexico. Greenhouse common garden confirmed that these EMF patterns are driven by specific aspen genotypes rather than soil or vegetation differences. The importance of AMF communities partially aligned with our expectations. While AMF were anticipated to be more prevalent in the temperate and southernmost sites of the aspen range (Steidinger et al. 2019), we observed a uniform relative abundance across all sampling locations, however we noted the highest diversity in temperate stands in Canada. As we were not able to measure AMF associations in the greenhouse study, it is less clear whether this uniformity in abundance reflects consistent AMF associations across aspen genetic groups, if it is a consequence of differences in understory vegetation among sites (Fei et al. 2022), in particular the sparse vegetation in high-altitude aspen habitats in Mexico, or other climate or soil factors (Van Aarle, Olsson, and Söderström 2002).

Trees can also obtain nitrogen through symbiotic or free-living nitrogen-fixing bacteria and Archaea (E. S. Boyd and Peters 2013). Globally, rhizobial nitrogen fixers are more diverse in hot,

stable, and seasonally humid regions (Sepp et al. 2023) and are more abundant in arid biomes (Steidinger et al. 2019). This aligns with our findings, where the highest relative abundance and richness of selected nitrogen-fixing bacteria were observed in Mexico. This pattern may also explain the lack of exceptionally high AMF diversity in this genetic group, suggesting that nitrogen-fixing bacteria either complement or dominate nitrogen acquisition in aspen.

## Aspen stand persistence and dynamics in the face of climate change

*P. tremuloides* likely originated in southern North America (Goessen 2023), and had to adapt to novel temperature regimes while it expanded northward, particularly to boreal regions. Notably, three genes that were identified to diverge across climate gradients are responsible for auxin biosynthesis and distribution in roots (Goessen 2023). Auxins are plant hormones that regulate plant growth and development (Gomes and Scortecci 2021), but are also known to influence arbuscular mycorrhiza colonization and promote their development (Gutjahr 2014). Auxin signalling has been also proposed to play a role in the formation of poplar-*Laccaria* ectomycorrhizal roots (Vayssières et al. 2015). These genes may therefore be key candidates for plant-fungal interactions that emerged during adaptation to novel climate conditions. It is however unclear how these plant-microbe interactions will change in response to rapid climate change, including an increasing drought frequency.

Global change can affect microbiomes directly (Knight et al. 2024), but it also has indirect effects on ecosystems through shifts in host distribution, changes in phenological timing, and increasing mismatches between symbiotic partners (Classen et al. 2015). Understanding the role of key plant-soil interactions in forest productivity and resilience is essential, as is determining the consequences of their potential degradation—whether they can be replaced by new interactions and at what rate. For example, in the context of assisted migration, an important question arises: Can we relocate trees adapted to drier climates without considering their microbial associations? A resurgent idea is that organisms can influence their environments in a way to promote ecosystem resilience (Hunter 2018). Some studies have demonstrated that plant genotypes modify their rhizosphere microbiome differently due to variation in nitrogen cycling processes, and in that way they influence the biomass of subsequent generations growing in conditioned soil (Przybylska et al. 2024). Others have shown that transplantation of plant soil microbiomes from different geographic origins can increase tree survival faced with various climate stresses (Allsup, George, and Lankau 2023) or can affect plant phenology (Ware et al. 2021). Therefore, understanding microbiome dynamics is key to understanding plant resilience to global changes, and such beneficial microbial communities should be conserved and harnessed to support the desired tree responses in forest management (Heuertz et al. 2023).

## Conclusions

We demonstrated that soil physicochemical properties and host genotype shape soil microbiomes of two genetic groups of *P. tremuloides* from Mexico and Canada. Our findings highlight the regional importance of nutrient-exchange symbioses in aspen stands: EMF dominate in the boreal forest, AMF are most diverse in temperate forests, and nitrogen-fixers abound in seasonally dry, warm regions, aligning closely with the expected global patterns of symbiosis distribution. We also demonstrated that aspen seedling genotypes from Canadian sites grown in the greenhouse associate more commonly with EMF, showing that plant genetics determines host-microbial interactions that mirror global ecosystem functional divergence. Further research is needed to identify microbial species that associate with aspen genotypes from different ecological regions and to uncover the heritability and molecular mechanisms driving these interactions. These insights will lay the groundwork for future management strategies in sustainable agriculture and forestry incorporating and utilizing microbiomes.

# Data availability

All raw sequence data generated in this study have been deposited in Sequence Read Archive (SRA) under project accession number PRJNA1230202. Processed data and code will be available at [zenodo link].

# Acknowledgments

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# Supplementary Tables

Supplementary Table 1. Description of sampling sites.
Supplementary Table 2. Soil physicochemical properties.
Supplementary Table 3. Envfit analysis measuring correlation between soil properties and ordination of bacterial/archaeal and fungal microbial structure in the complete dataset.
Supplementary Table 4. Envfit analysis measuring correlation between soil properties and ordination of bacterial/archaeal and fungal microbial structure in Canadian genetic group.
Supplementary Table 5. Envfit analysis measuring correlation between soil properties and ordination of bacterial/archaeal and fungal microbial structure in Mexican genetic group.
Supplementary Table 6. Coefficients, 95% confidence intervals, *P*-values and pseudo $R^2$ in the selected beta regression model explaining Gini-Simpson in bacteria/Archaea and fungi.

# Supplementary Figures

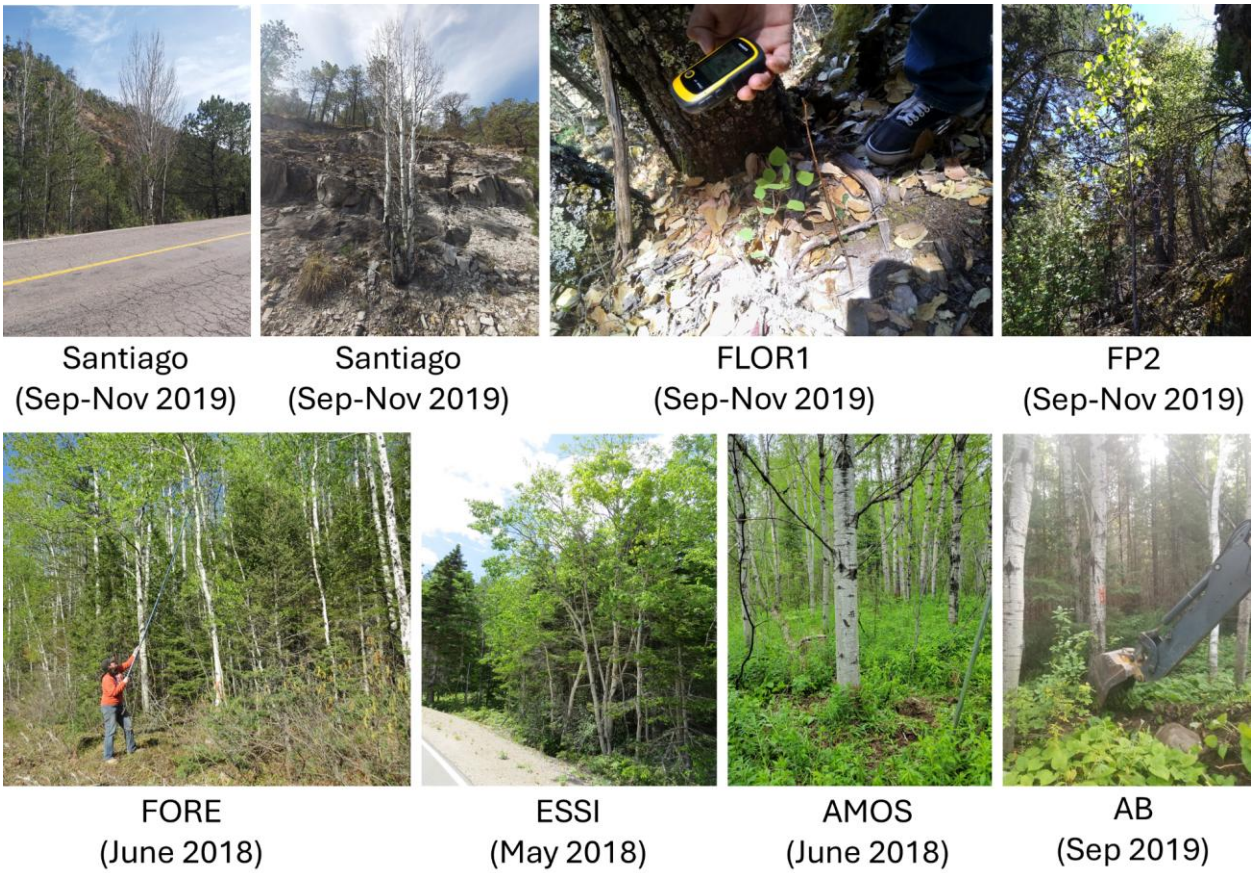


Fig S1. Diversity of sampled aspen habitats in Mexico (Santiago, FLOR1, FP2) and Canada (FORE, ESSI, AMOS, AB).

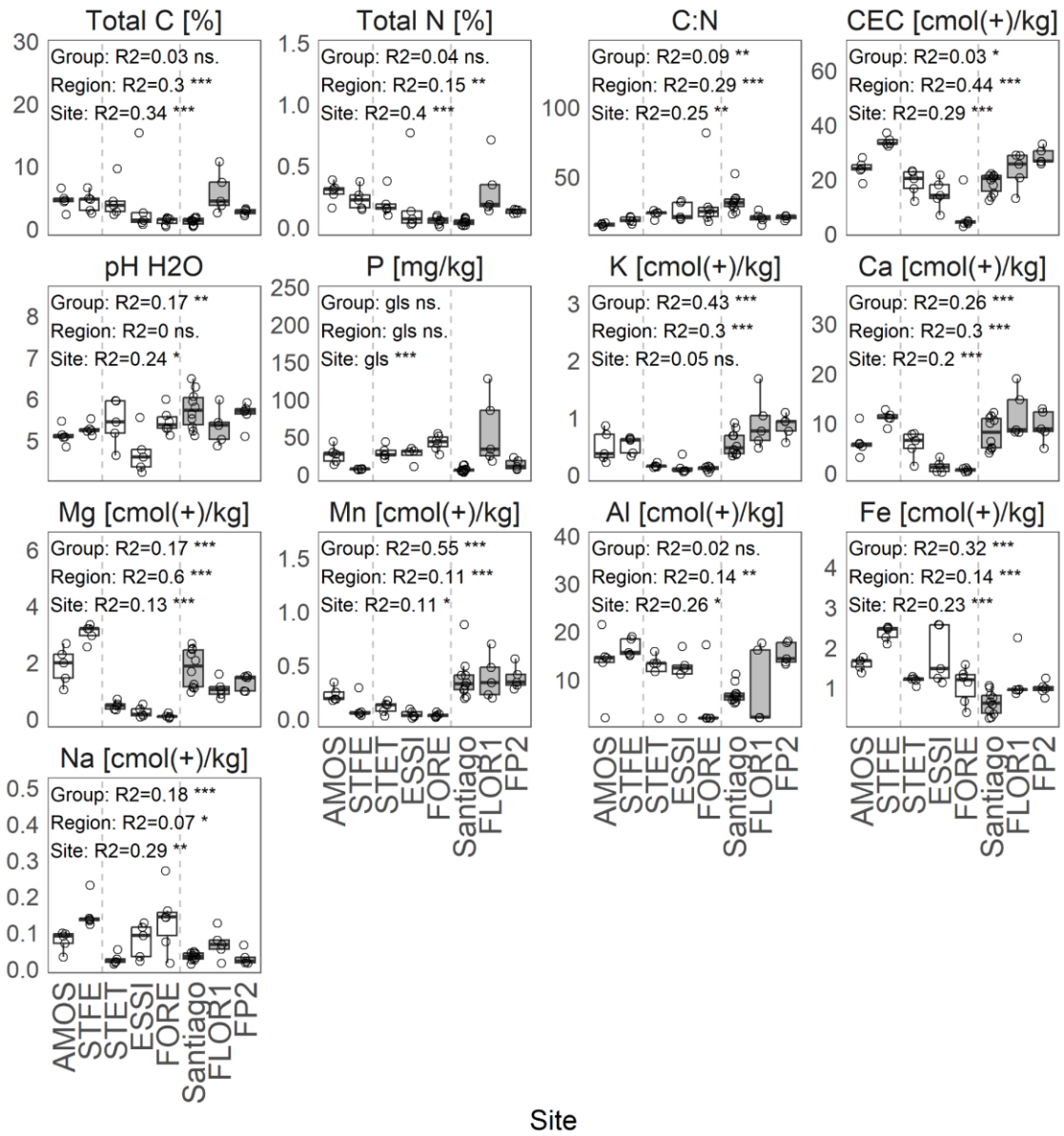


Fig S2. Comparison of soil physicochemical properties between genetic groups, regions and sites. Differences between genetic groups, regions and sites were tested using nested ANOVA, or generalized least squares (gls) in case of non-homogeneity of variance. Grey boxes indicate samples originating from Mexico. Dashed lines separate three regions: boreal, cold temperate, and warm temperate. ns. stands for not significant after multiple test correction with Benjamini-Hochberg procedure at 0.05; * $P < 0.05$, ** $P < 0.01$,*** $P < 0.001$. A single phosphorus (P) outlier (P>400), was removed for better visibility.

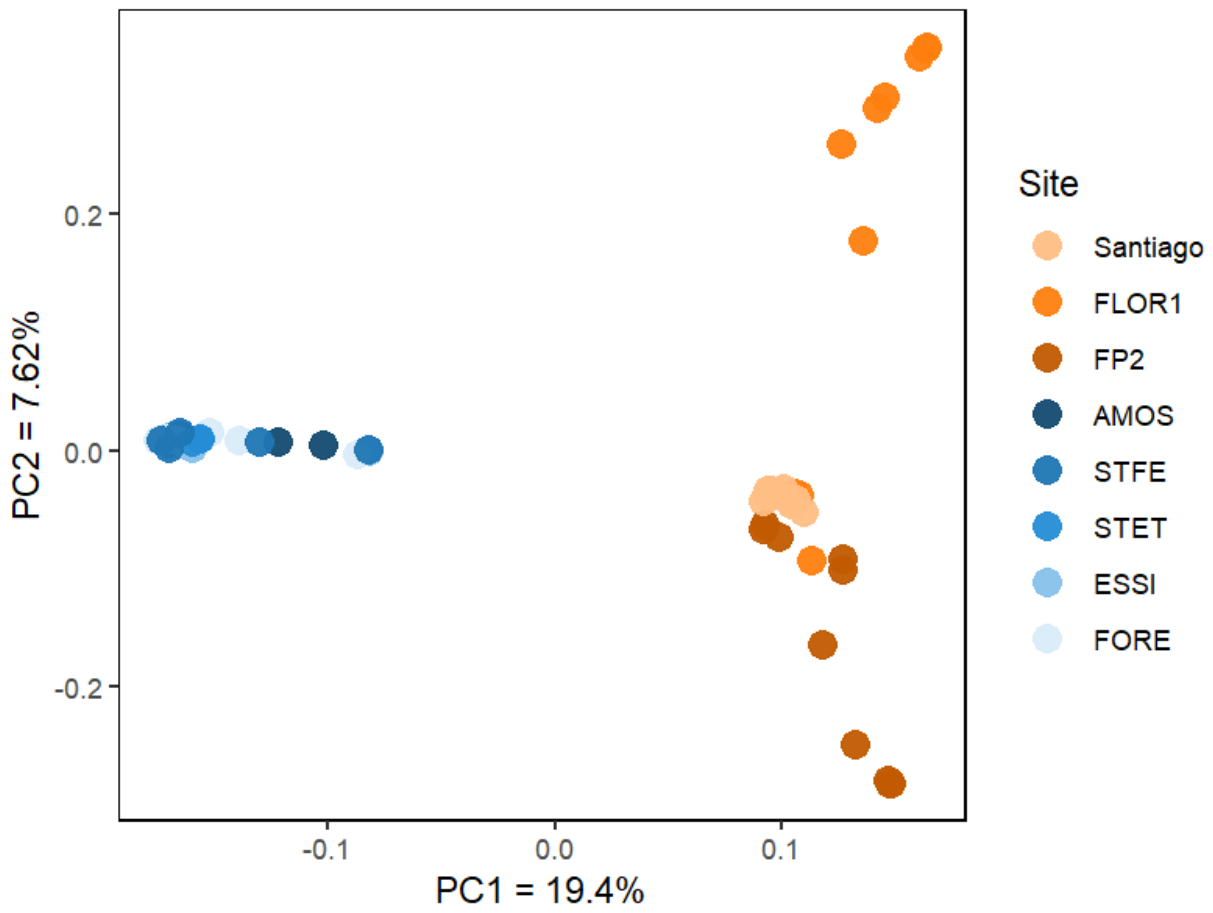


Fig S3. Principal component analysis using 53,714 genome-wide SNPs sequenced from aspen trees.

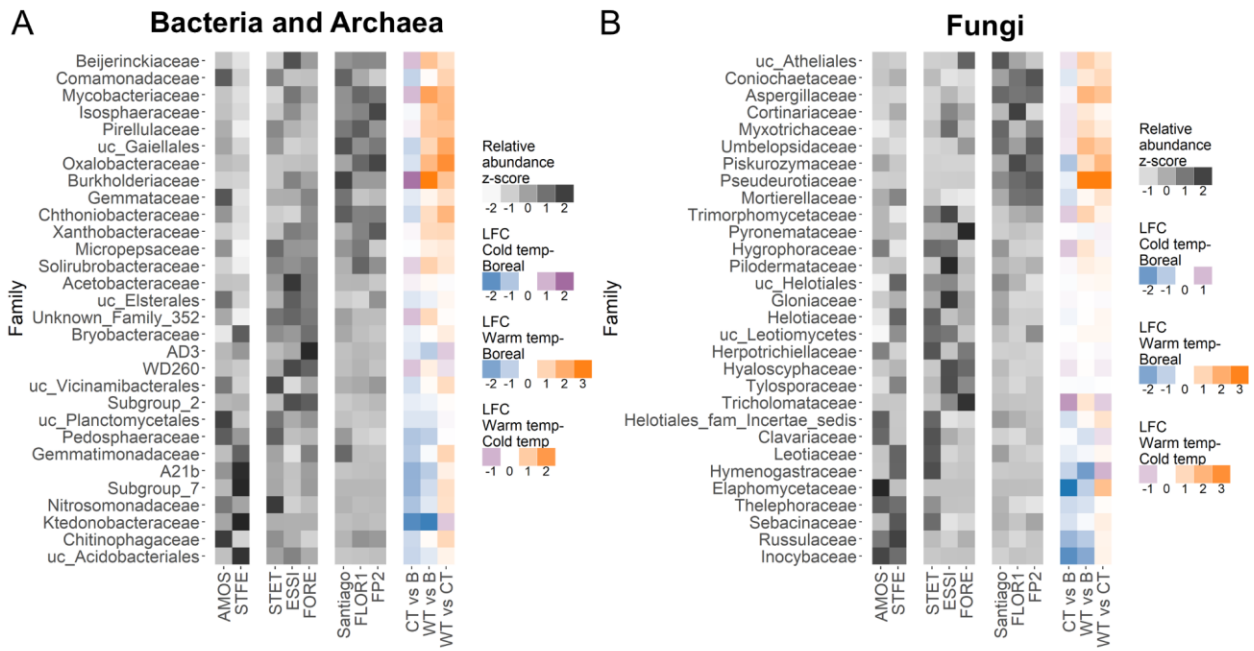


Fig S4. Differential abundance across three regions (boreal B, cold temperate CT and warm temperate WT) in top 30 most abundant soil families. A, B) Standardized relative abundances and differential abundance between the pairs of regions in bacterial and archaeal (A), and fungal (B) taxa clustered at the family level. Color heatmaps show estimates of natural log fold change (LFC) obtained with ANCOM-BC between pairs of regions. Taxa most abundant in boreal region (B) are in blue, in cold temperate region (CT) in purple, and in warm temperate region (WT) in orange. Prefix “uc” in taxonomic names indicates all unidentified or uncultured families clustered at the higher level. Genera are ordered such that topmost families have the highest mean relative abundance in warm temperate region, middle ones - in the cold temperate region, and bottom ones - in the boreal region.

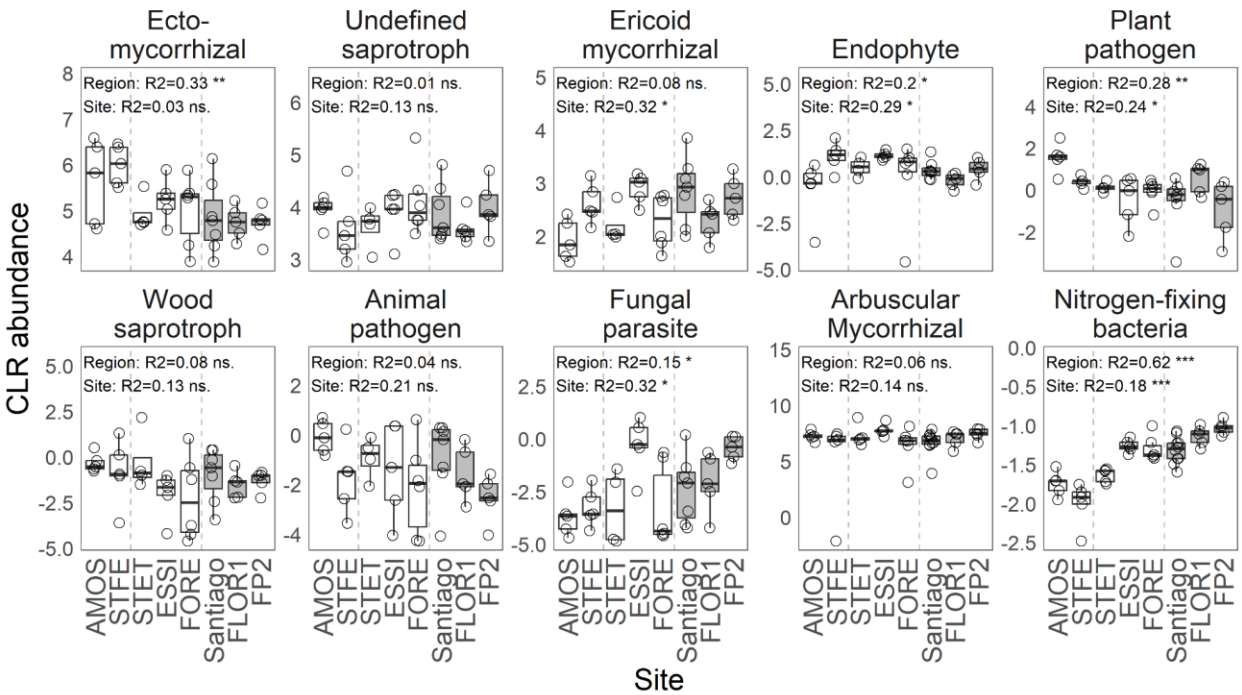


Fig S5. Comparison of log-ratio transformed (CLR) abundance of eight selected fungal guilds including EMF (ITS dataset), along arbuscular mycorrhiza, AMF (18S dataset), and selected nitrogen-fixing bacteria (16S dataset) between regions. Grey boxes indicate samples originating from Mexico. Dashed lines separate three regions: boreal, cold temperate, and warm temperate. Differences between genetic groups were tested using nested ANOVA. Ns. stands for not significant after multiple test correction with Benjamini-Hochberg procedure at 0.05; * $P < 0.05$, ** $P < 0.01$,*** $P < 0.001$.

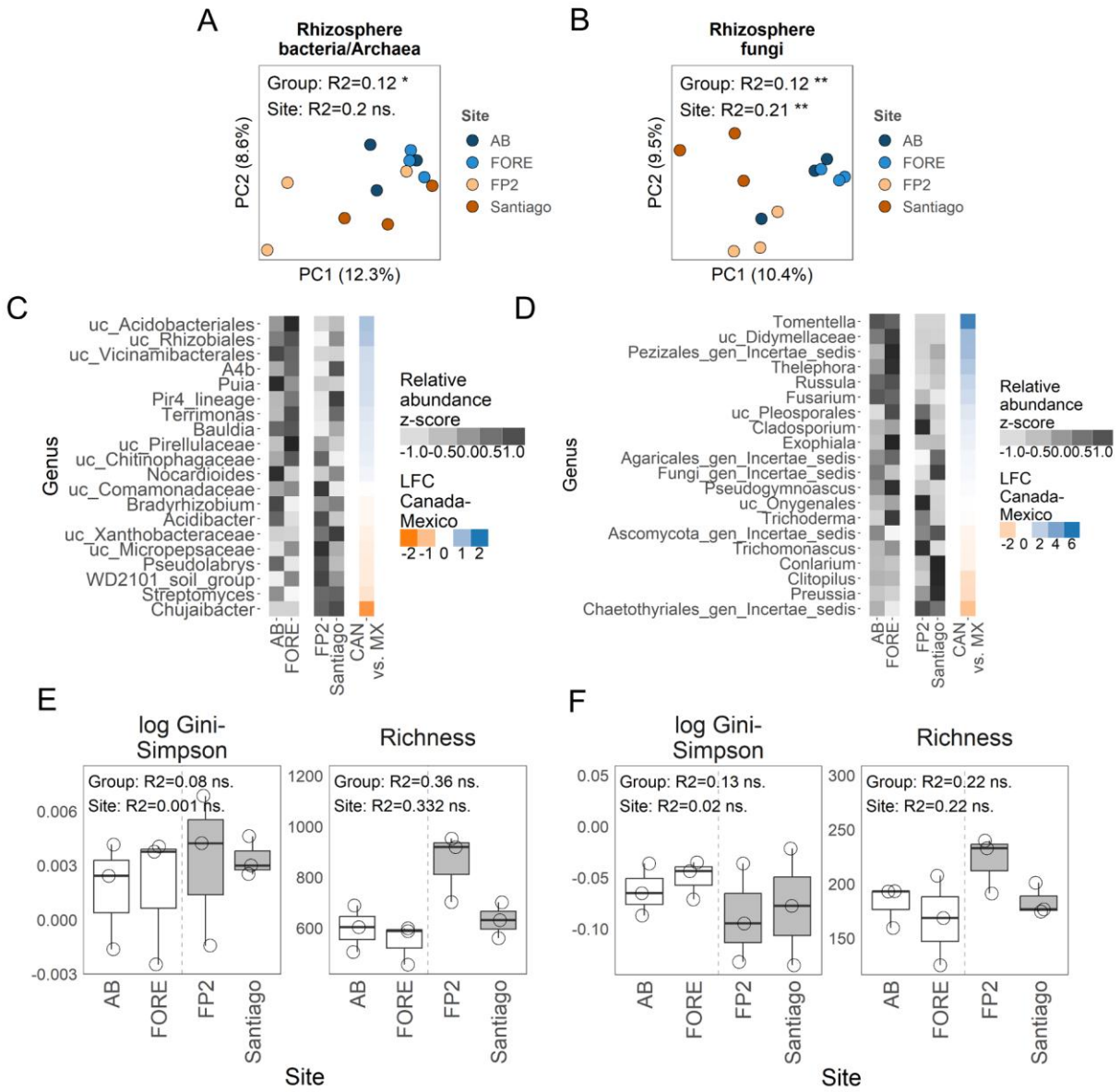


Fig S6. Rhizosphere communities of aspen seedlings from Canada and Mexico growing in greenhouse conditions. A-B) Ordination and PERMANOVA analysis of bacterial/archaeal (A) and fungal (B) communities. C-D) Grey heatmaps with standardized relative abundances and colored heatmaps with differential abundance (natural log fold change, LFC) between the genetic groups in bacterial/archaeal (C), and fungal (D) taxa clustered at the genus level. Taxa differentially abundant in Mexico are in orange, and differentially abundant in Canada are in blue. E-F) Comparison of alpha diversity for bacterial/archaeal (E), and fungal (F) communities. Grey boxes indicate samples from Mexico. Differences between genetic groups were tested using nested ANOVA. ns. stands for not significant after multiple test correction with Benjamini-Hochberg procedure at 0.05; * $P < 0.05$, ** $P < 0.01$,*** $P < 0.001$.

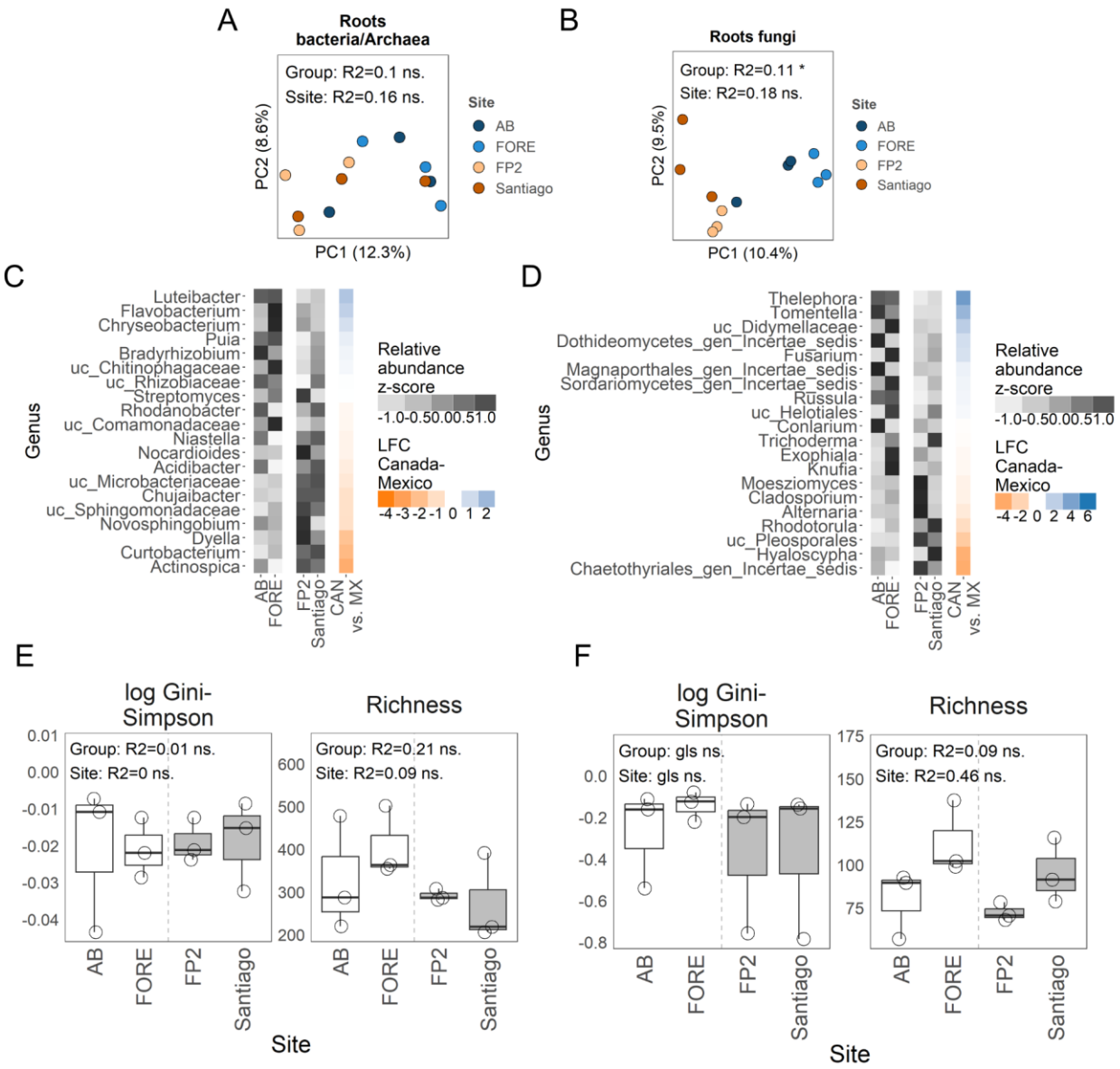


Fig S7. Roots communities of aspen seedlings from Canada and Mexico growing in greenhouse conditions. A-B) Ordination and PERMANOVA analysis of bacterial/archaeal (A) and fungal (B) communities. C-D) Grey heatmaps with standardized relative abundances and colored heatmaps with differential abundance (natural log fold change, LFC) between the genetic groups in bacterial/archaeal (C), and fungal (D) taxa clustered at the genus level. Taxa differentially abundant in Mexico are in orange, and differentially abundant in Canada are in blue. E-F) Comparison of alpha diversity for bacterial/archaeal (E), and fungal (F) communities. Grey boxes indicate samples from Mexico. Differences between genetic groups were tested using nested ANOVA, or generalized least squares (gls). ns. stands for not significant after multiple test correction with Benjamini-Hochberg procedure at 0.05; * $P < 0.05$, ** $P < 0.01$,*** $P < 0.001$.

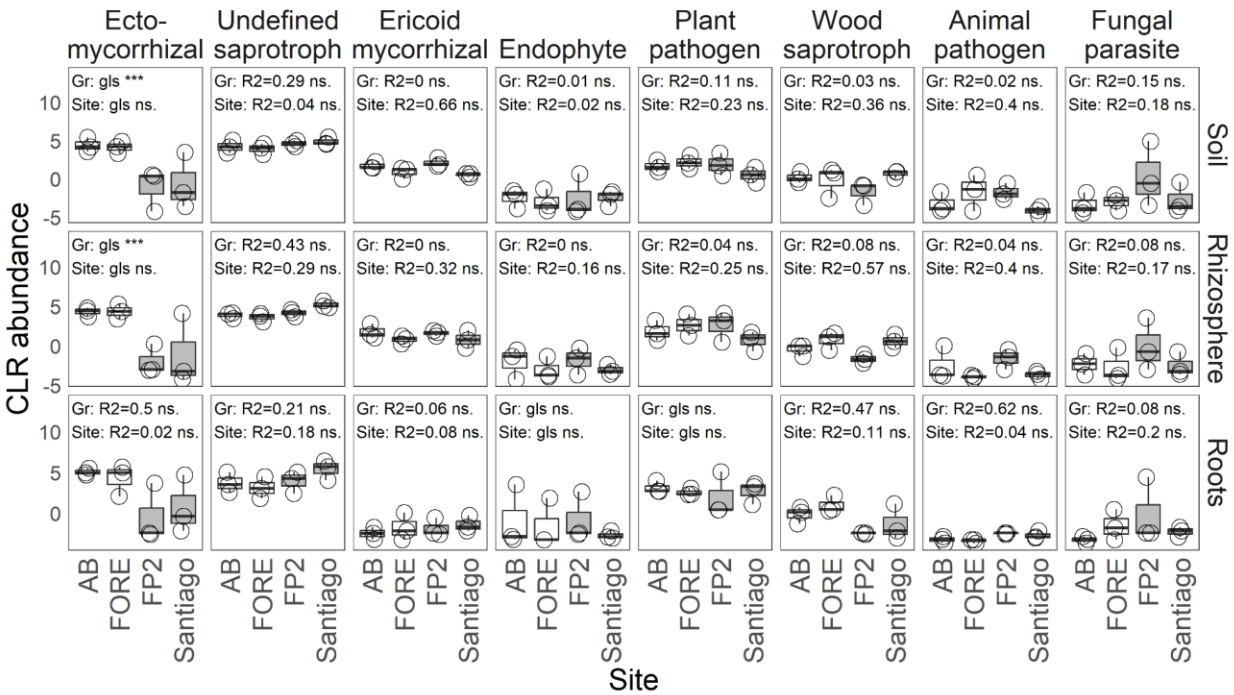


Fig S8. Comparison of log-ratio transformed (CLR) abundance of eight selected fungal guilds including EMF between genetic groups (Gr) in soil, rhizosphere, and root samples from the greenhouse common garden. Grey boxes indicate samples originating from Mexico. Differences between genetic groups were tested using nested ANOVA, or generalized least squares (gls). ns. stands for not significant after multiple test correction with Benjamini-Hochberg procedure at 0.05; * $P < 0.05$, ** $P < 0.01$,*** $P < 0.001$.

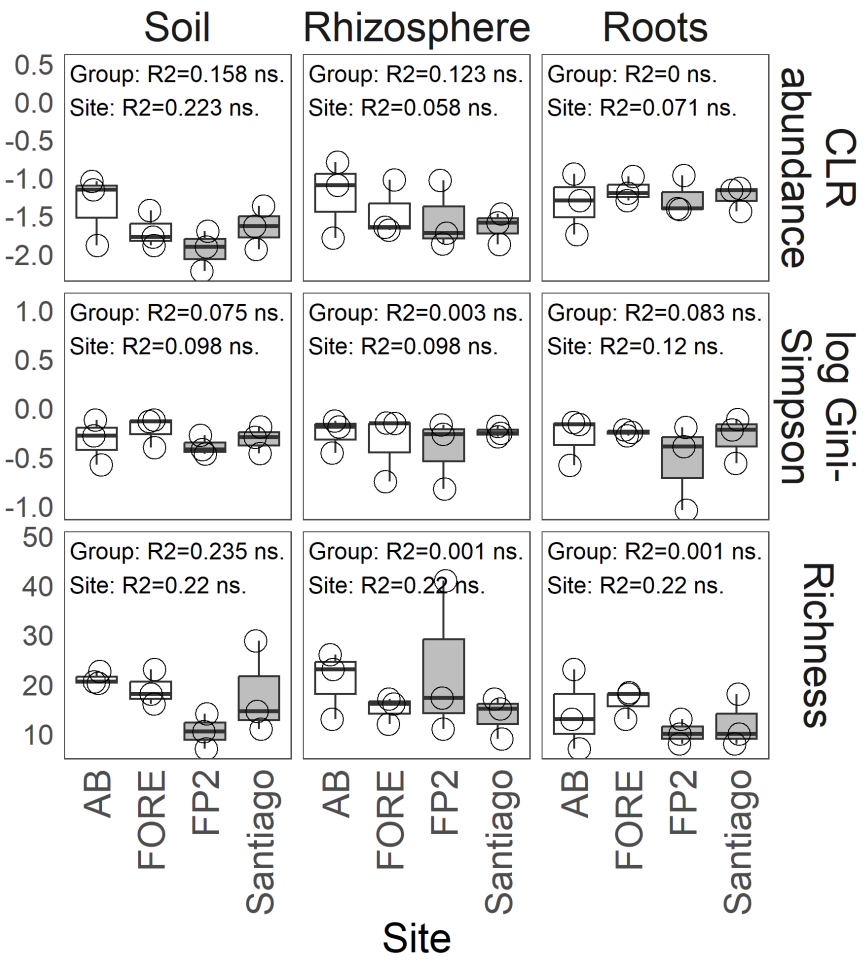


Fig S9. Comparison of log-ratio transformed (CLR) abundance, and alpha diversity of selected nitrogen-fixing bacteria between genetic groups in soil, rhizosphere, and root samples from the greenhouse experiment. Grey boxes indicate samples originating from Mexico. Differences between genetic groups were tested using nested ANOVA. ns. stands for not significant after multiple test correction with Benjamini-Hochberg procedure at 0.05.

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