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Annual precipitation and soil moisture level strongly associate with the bacterial community structure in Interior Douglas-fir and Sub-Boreal Spruce ecozones in British Columbia

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SUMMARY Soil moisture and precipitation can affect forest soil bacterial community structure and regulate important interactions between key soil microbial processes and the environment. This study examined how mean annual precipitation and soil moisture content affect the alpha and beta diversity in the soil microbiome of the Interior Douglas Fir and Sub-Boreal Spruce ecozones in British Columbia. Since soil compaction and organic matter removal could impact moisture level, their association with soil microbial structure were also examined in this study. We used 16S rRNA gene libraries from the organic soil layer of 104 samples to test whether mean annual precipitation and soil moisture are associated with differences in soil bacterial community structure. Our results showed that sites with higher mean annual precipitation had higher soil moisture content (one-way ANOVA $p = 1.16 \times 10^{-7}$). We demonstrated that the relative abundance of *Mycobacterium*, *Patulibacteraceae*, and *Bradyrhizobium* were significantly impacted by higher mean annual precipitation and soil moisture content. Soil moisture content positively correlated (spearman $r = 0.58$, $p < 0.0001$) with alpha and beta diversity, and it significantly affected soil microbial community composition. Organic matter removal and soil compaction did not significantly impact soil moisture. Overall, our study highlighted that water availability is a key driver of forest soil microbial diversity in British Columbia. Thus, close monitoring of precipitation and soil moisture may provide meaningful information on forest health.

INTRODUCTION

Heterogeneity in forest soil microbial systems could be caused by environmental factors. Forest soil plays an important role in the productivity of forest biomes. Microorganisms such as bacteria, archaea, fungi, and protists are the most abundant residents of forest soils, even though the identities of only a fraction of these microbes have been revealed (1). These microbes serve as a prism through which the effects of environmental factors like moisture and pollution can be discerned and propagate to higher trophic members of the ecosystem (2).

The composition of soil microorganisms varies temporally, geographically, and spatially. Kivlin and Hawkes (2016) observed temporal heterogeneity in bacterial communities in neotropical rainforests, which they reasoned may be explained by temporal climate and soil resource fluctuations (3). Tree species and soil depth can associate with the amount of organic matter in the soil and thus leading to the diversity in the microorganism profile (4). The study by Wilhelm et al. (2017) presented a comprehensive forest soil microbiome dataset for North American soil in various ecozones from the Long-term Soil Productivity (LTSP) study (5, 6). The study examined environmental factors like latitude, precipitation, pH, along with the

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impacts of various intensities of timber harvesting on soil like compaction and organic matter (OM). Several subsequent investigations have used this dataset to study the effects of OM removal (7, 8) However, there has been limited investigation into whether the impact of OM removal is compounded by changes of water accessibilities in those soils. The first objective of our study was to examine the interactions between moisture and the effects of timber harvesting on soil compaction and OM removal.

Soil health refers to the ability of soil to maintain a wide variety of organisms (9). The ecological relationships between soil microbes and forests are important for the overall well-being of the forest ecosystem (10). Soil microorganisms sustain the supply of important inorganic elements such as carbon and nitrogen by decomposition (11). For instance, *Bradyrhizobium* and *Methylocystaceae* in the phylum of Proteobacteria are important players in carbon and nitrogen recycling. Species of *Mycobacterium* from the phylum of Actinobacteria can break down lignin and cellulose (4), while other *Mycobacterium* species in soil could be human pathogens (12). Moreover, species from Rhizobium can improve trees' resistance against plant pathogens (13). The destabilizing forces of climate change and increased demand for protecting forest natural resources are creating unparalleled need to better understand forest soil health. As moisture level is a vital factor for all organisms, better characterization on the impact of moisture region on the soil microbiome may guide approaches to mitigation and improved forest management practices targeting the soil microbiome (14). Prior studies have shown moisture can affect the diversity and functioning of microbes in soil (15), but few have investigated the interaction between soil microbial communities to varying degrees of moisture accessibility. Our second objective was to determine the effect of mean annual precipitation (MAP) and soil moisture on microbial composition in forests in the Interior Douglas-fir (IDF_{BC}) and Sub-Boreal Spruce (SBS_{BC}) ecozones of BC.

Precipitation. Rainfall is a primary source of water for soil ecosystems, and increased water access has been linked to higher productivity and biomass increase (16). Studies on forests have demonstrated an effect of rainfall reduction on the soil microbiome, but depending on the type of forest and soil, the response of microbial communities to water availability could be different. For instance, Felsmann et al (2015) simulated drought conditions in Germany and found reduced precipitation only affects active bacterial communities (17). Pereira et al (2019) report that rainfall reduction in Mediterranean forests does not affect bacteria but leads to an increase of fungi (18). These examples demonstrate that soil microbes in different regions respond to rainfall differently, and hence there is a need for designing region-specific studies to characterize soil microbial activities. As such, we sought to understand how BC soil microbial community is associated with different MAP patterns. Given water is essential for metabolic functions, we hypothesize that higher MAP is associated with greater soil microbial diversity.

Soil moisture. Productivity of soil is directly tied to moisture, as a water potential below -36 MPa in the organic horizon inhibits the cycling process required for microbial decomposition (19). Once moisture falls below this critical point, microbial activity ceases greatly and mass mortality occurs, thereby selecting for soil microbes with greater stress tolerance (20, 21). The general relationship between soil moisture and microbial diversity follows a parabolic trend with maximal diversity occurring in the middle range of moisture concentration (22). Studies on Western Canada have noted soil moisture as a primary factor in influencing the composition and enzyme activity of forest soils (15). However, it has not been identified what levels of moisture facilitate or suppress the diversity of soil microbiome in BC. As higher MAP may serve as a proxy for soil moisture, we hypothesize that higher soil moisture will correlate with greater soil microbial diversity.

METHODS AND MATERIALS

Forest Soil Microbiome Data. The dataset contained two ecozones in BC: IDF_{BC} and SBS_{BC}. The MAP, mean annual temperature, longitude, latitude, sampling depth, elevation, soil type,

climate, and herbicide use at each ecozone were recorded. For each soil sample, the moisture content at sampling time, pH, soil bulk density, soil compaction, total nitrogen content, total carbon content, and carbon/nitrogen ratio were measured and recorded. Only organic soil samples were used in the study. A total of 104 16S rRNA amplicon libraries (V1-V3) for IDF_{BC} and SBS_{BC} were downloaded from the European Nucleotide Archive repository (PRJEB8599). ROCHE 454 Titanium platform was used to generate the amplicon libraries as described in the original study (5).

Preliminary data filtering in Python. The steps for preliminary data filtering in Python are outlined in Script 0 (https://github.com/EmiliaCXY/soil_microbiome_diversity) To restrict the manifest table to samples from the IDF_{BC} and SBS_{BC} ecozones, samples from the other ecozones were removed. Samples from the organic soil layer with pH > 0 were retained. This removed all samples for OM3 (severe OM removal), since only mineral soil samples could be obtained from this treatment. The moisture content metadata category was also organized into 5 bins: 40-50, 50-60, 60-70, 70-80, and 90-100%. The filtered manifest table was imported into QIIME 2 for downstream analysis (23).

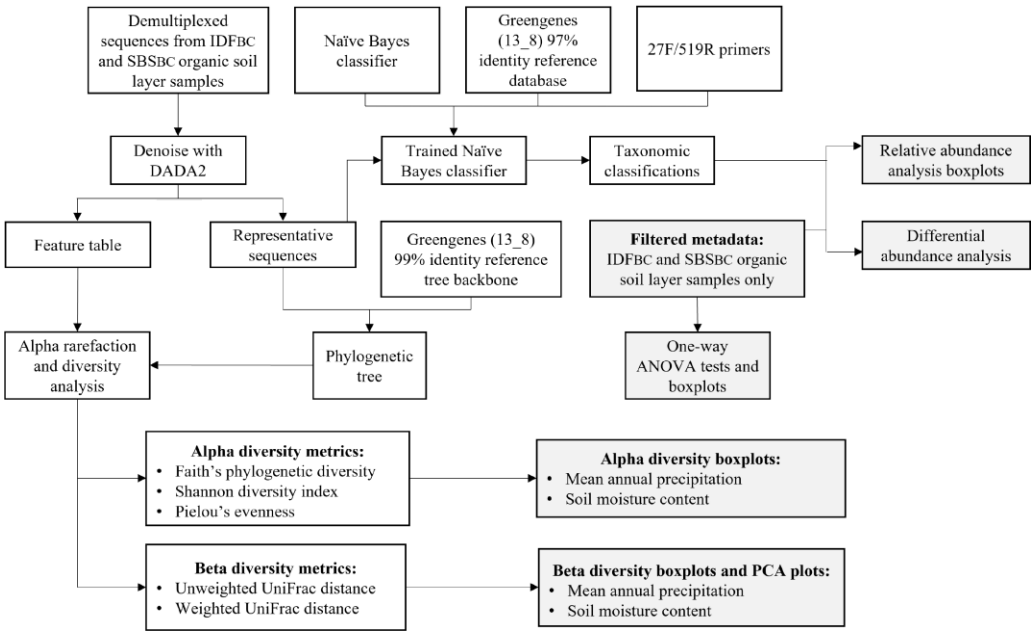

FIG. 1 QIIME 2 and R workflow. The dataset was filtered in Python to retain organic soil layer samples with pH > 0 from the IDF_{BC} and SBS_{BC} ecozones. The filtered dataset was imported into QIIME 2 and denoised with DADA2 to generate a feature table and identify the representative sequences. A phylogenetic tree was generated by inserting the representative sequences into a reference tree backbone. The feature table and phylogenetic tree were used to generate alpha and beta diversity metrics, which were later visualized as boxplots and PCA plots in R. A trained Naive Bayes classifier was used to taxonomically classify the representative sequences. The taxonomic classifications were imported into R for differential and relative abundance analyses. One-way ANOVA tests were performed in R as well. The white and grey boxes refer to steps performed in QIIME 2 and R, respectively.

Data processing and analysis in QIIME 2. QIIME 2 DADA2 software package was used to denoise the demultiplexed single-end sequences (23, 24). The sequences were truncated to 340 base pairs (bp) and a feature table containing the representative amplicon sequence variants (ASVs) for the BC ecozones was generated. Next, alpha rarefaction was performed (24). The minimum and maximum sequencing depths were set to 10 and 8500 sequence reads, respectively. A rarefaction depth of 3038 sequence reads per sample was selected in order to retain 48.03% of the ASVs and 88.82% of the samples. These steps are outlined in Script 1.

After subsetting to variants present in BC soil samples, we used the QIIME 2 fragment insertion approach to construct a rooted phylogenetic tree (25). Input files for this step include representative sequences and a reference tree backbone from Greengenes (sepp-refs-gg-13-8.qza) downloaded from QIIME 2 (23). This approach inserts variants in the input file into the provided tree backbone based on sequence similarity, enabling faster generation of a relatively accurate phylogenetic tree (24).

We performed alpha and beta diversity analyses with QIIME 2 using the phylogenetic tree produced in the previous step. Assessed metrics included Shannon diversity, Faith's phylogenetic diversity index, Pielou's evenness index, weighted UniFrac distance, and

unweighted UniFrac distance. Data visualization was performed with R programming language (R 4.0.5) and RStudio (v1.4.1106) using tidyverse, vegan, phyloseq, ggplot2, and ggpubr packages (26-32). Kruskal-Wallis, PERMANOVA, and Spearman correlation tests were performed to determine statistical significance of the results. The steps in QIIME 2 are outlined in Script 2, and steps in R are outlined in Script 9.

We trained a Naïve Bayes classifier using the q2-feature-classifier plugin (33). To improve the specificity of the classifier, we extracted V1-V3 regions from the Greengenes (release 13_8) 97% database using the same primers as the ones used in generating the sequencing data (i.e. universal primers 27F and 519R) as our training sequences (5). The training data were then trimmed to 340 bp to match the length of variants generated from DADA2. After training, we applied the classifier to assign taxonomy to our quality-controlled variants. The results were outputted with the *qiime tools export* command for downstream analysis. These steps are outlined in Script 4.

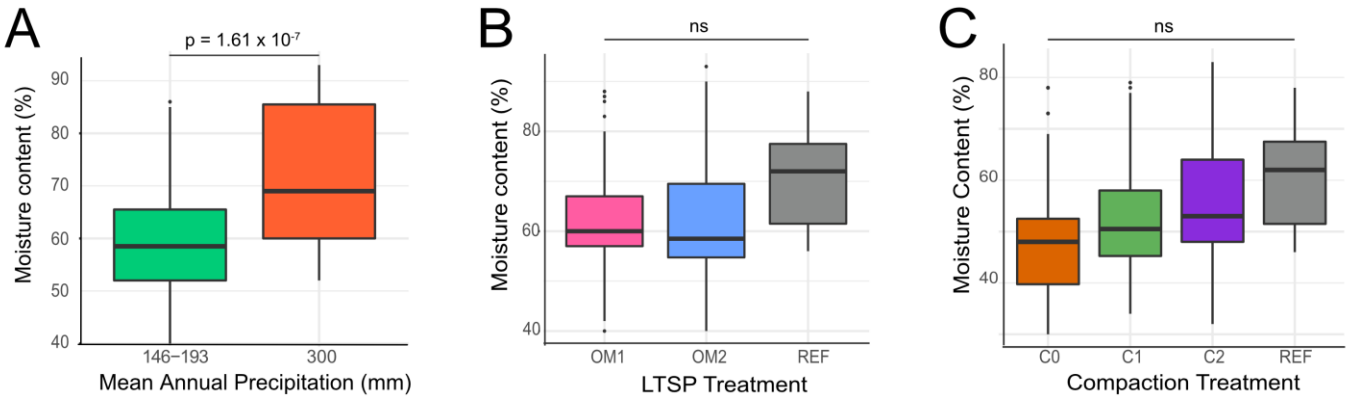

FIG. 2 Soil moisture content was significantly impacted by mean annual precipitation but not OM removal and soil compaction. Boxplots showing the correlation between (A) soil moisture and mean annual precipitation, (B) soil moisture and OM removal (LTSP treatment), and (C) soil moisture and compaction treatment. For the mean annual precipitation groups: N146-193 mm = 52 and N300 mm = 42. For the OM removal treatments: NOM1 = 43 (OM1), NOM2 =38, NREF =and 13. For the soil compaction treatments: NC0 = 27, NC1 = 27, NC2 = 27, and NREF = 13. ns = not significant ($p > 0.05$, $q > 0.05$). (*) = statistical significance ($p < 0.05$, $q < 0.05$).

Differential abundance analysis based on annual precipitation and soil moisture. We imported variants with taxa classification, the metadata file, and the phylogenetic tree into R as a phyloseq object for differential abundance analysis. Variants with low abundance (<0.005% of total sequencing reads in samples of interests) were excluded. The DESEQ2 package was used to perform the differential abundance analysis (34, 35). For MAP, differential abundance analysis was performed on the two MAP groups (146-193mm and 300mm). For soil moisture, pairwise comparisons between low, medium, and high moisture groups were carried out. These steps are outlined in Script 5-8.

Determining the Effects of MAP, Soil Compaction, and OM Removal on Soil Moisture. The soil metadata was imported into R and filtered to retain organic layer soil samples that had a soil pH > 0 and were from the two BC ecozones. R was used to generate boxplots and perform one-way ANOVA tests to determine if MAP, soil compaction, or OM removal significantly affected soil moisture content. These steps are outlined in Script 3.

All scripts and data visualization output are available at https://github.com/EmiliaCXY/soil_microbiome_diversity

RESULTS

MAP significantly affected soil moisture content whereas OM removal and soil compaction had no significant impacts. We performed one-way ANOVA to test if sites with different MAP, OM removal, or soil compaction treatments had different soil moisture levels. Our results revealed that the samples in the ecozone with 300 mm MAP had significantly higher soil moisture content than the ones with 146-193 mm MAP ($p = 1.16 \times 10^{-7}$) (Fig 2A). Soil moisture content did not significantly differ between the OM1 (minimal OM removal), OM2 (moderate OM removal), and REF (reference/control) ($p = 0.116$, $\alpha = 0.05$) treatments (Fig 2B). Similarly, the soil moisture content of the C0 (minimal compaction), C1 (moderate compaction), C2 (severe compaction), and REF (reference/control) treatments were not significantly different from each other ($p = 0.053$) (Fig 2C).

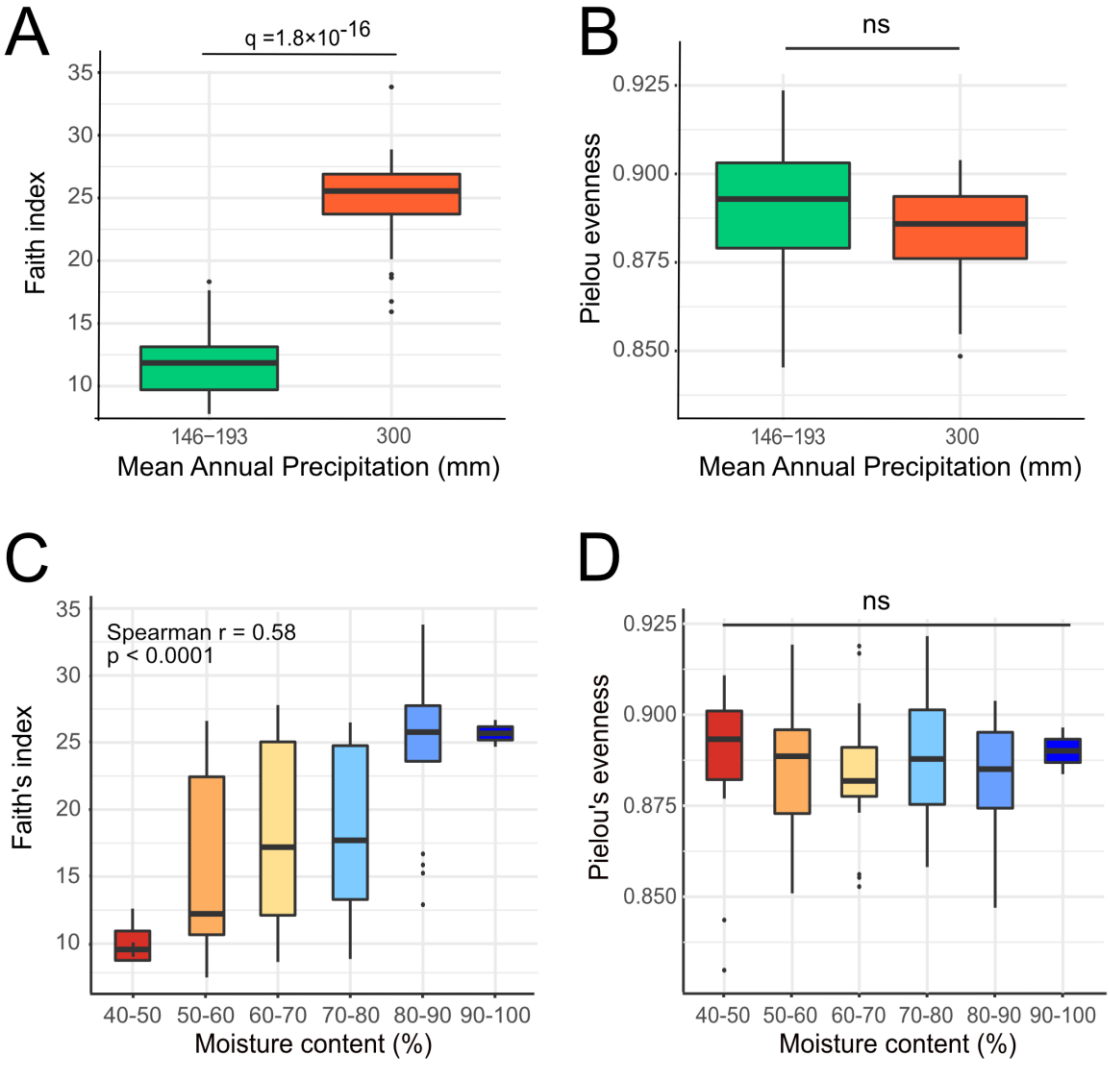

FIG. 3 Sites with higher MAP and soil moisture content had greater alpha diversity. (A) Faith's phylogenetic diversity index boxplot for low (146-193 mm) and high (300 mm) mean annual precipitation levels ($N_{146\text{-}193\ mm} = 52$, $N_{300\ mm} = 42$). (B) Pielou's evenness index box plot for both mean annual precipitation levels. (C) Faith's phylogenetic index boxplot for each soil moisture content group ($N_{40\text{-}50\%} = 13$, $N_{50\text{-}60\%} = 30$, $N_{60\text{-}70\%} = 32$, $N_{70\text{-}80\%} = 10$, $N_{80\text{-}90\%} = 16$, $N_{90\text{-}100\%} = 3$). (D) Pielou's evenness index box plot for each moisture content group. $q < 0.05$ and $p < 0.0001$ = statistical significance. ns = not significant.

Alpha diversity in the soil microbiome was higher in samples exposed to a higher level of MAP. Analysis of Shannon, Faith's phylogenetic diversity, and Pielou's evenness indices showed a significant difference in microbiome species richness between the two levels of precipitation (Fig 3A, B, Supplementary Fig 1A). Taken individually, Faith's phylogenetic diversity index was significantly greater in higher rainfall, indicating that higher precipitation increases phylogenetic diversity (Kruskal-Wallis $q = 1.8\times10^{-16}$, $\alpha = 0.05$) (Fig 3A). On the other hand, Pielou's evenness index was lower in the 300 mm MAP samples (Kruskal-Wallis

q = 0.049), suggesting that sites with high MAP had less even bacterial communities (Fig. 3B).

We divided soil moisture level into intervals of 10% and obtained six groups in total covering from 40% to 100% moisture content. Faith's phylogenetic diversity index revealed an increasing trend between soil moisture and species diversity which saturated as soil moisture content reached 80% (Fig 3C). The positive correlation was statistically significant but moderate (spearman r = 0.58, $p < 0.001$, $\alpha = 0.05$). Similarly, the Shannon diversity index also demonstrated a positive correlation between soil moisture and diversity (Supplementary Fig 1B, spearman r = 0.55, $p < 0.001$, $\alpha = 0.05$). The Pielou's evenness index did not show significant differences across samples from different moisture levels (Fig 3D).

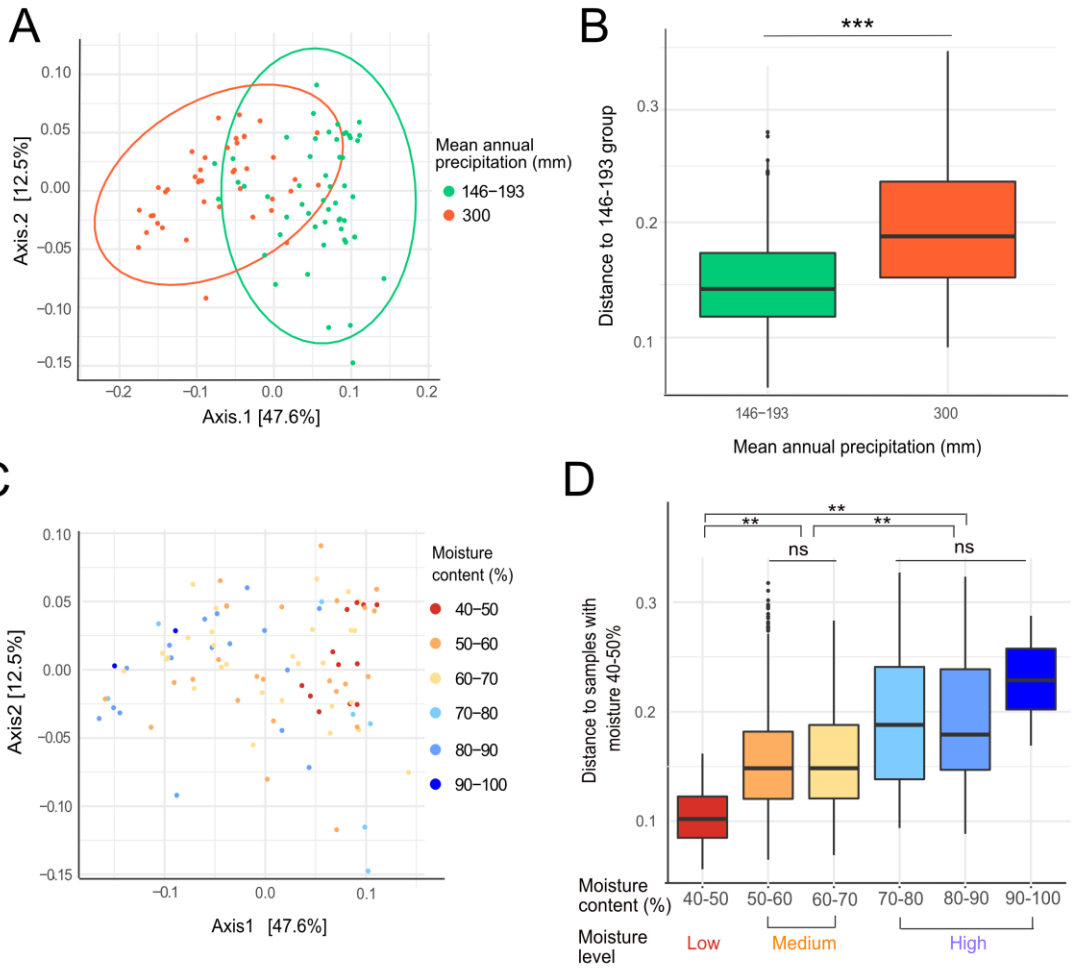

FIG. 4 Sites with higher mean annual precipitation and soil moisture content had higher beta diversity. (A) PCA analysis of Weighted UniFrac distance for mean annual precipitation. (B) Boxplot for a pairwise comparison of Weighted UniFrac distance for both low (146-193 mm) and high (300 mm) levels of mean annual precipitation. (C) PCA analysis of Weighted UniFrac distance for soil moisture content. (D) Pairwise comparisons of Weighted UniFrac distance against samples with 40-50% (low) moisture content. (**) = statistical significance ($p < 0.01$). (***) = statistical significance ($p < 0.001$). ns = not significant.

Beta-diversity analysis demonstrated distinct clusters of samples based on MAP levels but not based on soil moisture levels. Principal Component Analysis (PCA) for both weighted and unweighted UniFrac distances showed similar clustering patterns for both the 146-193 mm and 300 mm groups, indicating greater similarity among samples of the same precipitation level than samples from differing levels (Fig 4A, Supplementary Fig 2). PERMANOVA analysis of weighted and unweighted UniFrac distances found that sample diversity significantly differed between precipitation levels ($p = 0.001$) (Supplementary Tables 1, 2).

To further explore inter-sample dissimilarity, we focused on weighted UniFrac distances as this metric accounts for both phylogenetic similarity and richness. PCA did not reveal distinct sample clusters by moisture content (Fig 4C). However, the weighted UniFrac distances between samples in the 40-50% moisture content group with samples in the other moisture content groups were associated with moisture level differences (Fig 4D). This trend

occurred in a stepwise manner (Fig 4D). Pairwise PERMANOVA showed that samples with 40-50% soil moisture were significantly different from other groups ($q < 0.050$ for all pairs) (Supplementary Table 3), Samples from the 50-60% moisture level group and 60-70% group did not differ significantly. Likewise, no statistically significant difference was found among samples with 70-80%, 80-90%, and 90-100% moisture content. As such, we re-stratified the data into three categories: Low represents 40-50%, Medium represents 50-70%, and High represents 70-100% moisture content. All group pairs were found to be significantly different from one another (Fig 4D). Taken together, we observed that the difference in soil microbiome composition was associated with moisture content, and the impact of moisture may be discrete.

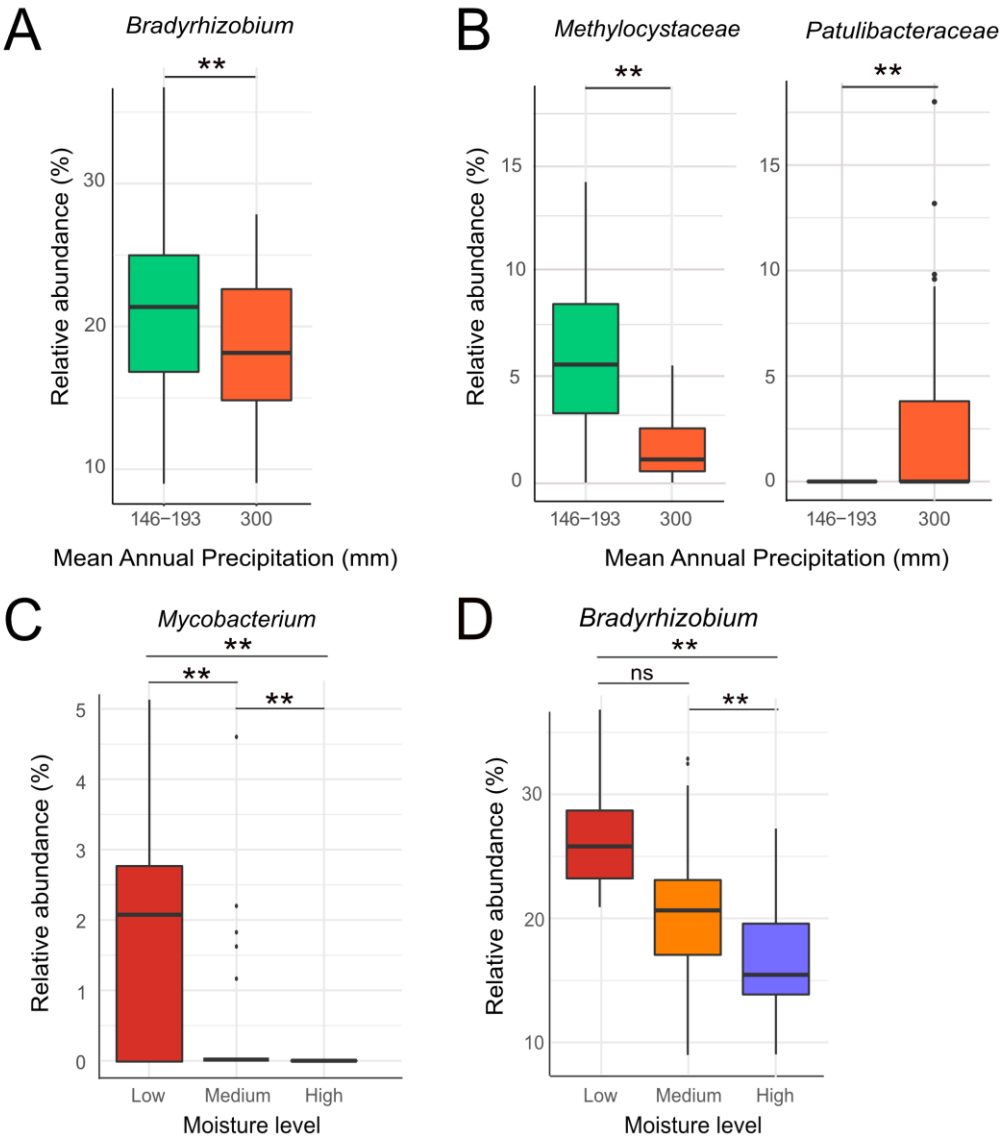

FIG. 5 *Bradyrhizobium* and *Methylocystaceae* were significantly more abundant in sites with low MAP and low soil moisture, whereas *Patulibacteraceae* was most abundant in sites with high MAP. Relative abundance of (A) *Bradyhizobium* and (B) *Methylocystaceae* and *Patulibacteraceae* in sites with low (146-193 mm) and high (300 mm) mean annual precipitation. Relative abundance of (C) *Mycobacterium* and (D) *Bradyrhizobium* in sites with low (40-50%), medium (50-70%), and high (70-100%) soil moisture content. $p < 10\text{-}3$ and (**) = statistical significance. ns = not significant.

MAP and soil moisture associated taxa. Taxonomic analysis revealed that *Bradyrhizobium*, *Rhodoplanes*, and *Methylocystaceae w*ere the three most abundant microbes in both SBS_{BC} and IDF_{BC} ecozones (Supplementary Fig 3). Relative abundance analysis of samples at the family and genus level revealed multiple instances of differentially abundant taxa between the 146-193 mm and 300 mm groups. At the family level, *Mycobacteriaceae*, *Methylocystacea*e, and *Bradyrhizobiaceae* were significantly enriched in low MAP samples,

while the opposite was true for *Patulibacteraceae* (Fig 5B). Next we investigated the genera that contributed to these significant differences.We found that *Bradyrhizobium* from *Bradyrhizibiaceae* and *Mycobacterium* from *Mycobacteriaceae* showed preferential abundance in sites with low MAP (Fig 5A, $p = 1.7\times10^{-5}$; Supplementary Fig 4A, $p = 1.52\times10^{-46}$). As such, while rainfall certainly affected the relative abundances of various bacteria, it was not unilateral in its impact, with varying degrees of correlations between abundance and MAP.

Taxa associated with the three moisture content groups (low, medium, high) were determined using differential abundance analysis. All moisture level pairs (low vs medium, medium vs high, low vs high) had at least one genus that showed statistically significant difference in abundance. Nine genera (*Candidatus Koribacter*, *Mycobacterium*, *Bradyrhizobium*, *Pedomicrobium*, *Streptomyces*, *Pseudonocardia*, *Sphingomonas*, *Afifella*, and *Devosia*) were significantly different between the low and high groups (Supplementary Fig 4B). We found that one (*Mycobacterium*) and two genera (*Bradyrhizobium* and *Mycobacterium*) had significant abundance differences between low and medium moisture groups and medium and high groups, respectively (Fig 5C, D). These genera overlapped with the nine genera discovered in the comparison between low and high moisture groups (Fig 5C, D; Supplementary Fig 4B). *Mycobacterium* showed up in all three pairwise comparisons (low vs medium, medium vs high, low vs high), and its abundance decreased as moisture level increased, which mirrored the lower abundance of *Mycobacterium* in sites with higher MAP (Fig 5C, Supplementary Fig 4A). Moreover, *Bradyrhizobium* was significantly more abundant in soils with low and medium moisture than in soils with high moisture, consistent with their associations with lower MAP (Fig 5A, D). The preferential abundance of six genera (*Pedomicrobium*, *Streptomyces*, *Pseudonocardia*, *Sphingomonas*, *Afifella*, and *Devosia)* for high moisture levels were driven by four samples in our data, hence, this observation may not be extrapolatable and is not addressed in the current study (data not shown).

DISCUSSION

Differences in MAP and soil moisture had consistent effects on the phylogenetic diversity and alpha diversity of soil bacteria. Soil bacterial composition in sites with lower MAP and lower phylogenetic diversity resembled more resolved trends observed in soil moisture (Fig 3, Fig 4). Faith's diversity and phylogenetic dissimilarity increased stepwise alongside soil moisture (Fig 3C, Fig 4D). The correlation between precipitation, moisture, and phylogenetic diversity may be caused by only a small variety of soil microbial taxa that are adapted to thrive in more arid conditions (36). These taxa may be adapted to water stress more effectively. This filtering effect has been discovered in the rhizosphere of crop soil in both bacteria and fungi, where water stress reduced the levels of soil phylogenetic diversity and enzyme activity, to the detriment of the crop (37). Although the surface of forest soil has greater porosity, biotic activity, and organic matter levels, deeper soil horizons of crop soil often display the characteristics of past forests (38). The corresponding low evenness across the different MAP and moisture content groups (Fig 3B, D) suggests that drought-tolerant taxa may occupy niches with less competitive overlap. Moisture content in this dataset was quantified at the moment of soil sample collection, allowing representation of short-term events like temperature change and precipitation, but not changes in long-term moisture regime. It is therefore noteworthy that corresponding trends in alpha-diversity, MAP, and soil moisture indicate that these results illustrate changes in the soil microbiome associated with water availability. Likewise, significant trends in taxa abundance, such as that of *Bradyrhizobium* (Fig 5, Supplementary Fig 4), give additional support to the link between MAP and soil moisture. Taken together, the diversity of forest soil bacterial communities may have implications on soil quality, as the presence of different microbes is associated with more variable functional diversity (39).

MAP and soil moisture content impact beta diversity by selecting for specific bacterial groups. Beta diversity analysis revealed significant differences in soil bacteria community composition between sites with different MAP and soil moisture content (Fig 5,

Supplementary Fig 4). Sites with lower MAP and soil moisture content had a significantly higher abundance of *Bradyrhizobium* (Fig 5A, D). The members of this genus are found in high abundance in North American forest soils, but while generally known for forming nodules on legume roots, forest soil *Bradyrhizobium* generally lacks the capacity for nodulation (40). We hypothesize that trends in the relative abundance of soilborne *Bradyrhizobium* may reflect direct differences in activity according to adaptations to water stress, in which ability to degrade aromatic compounds may be inhibited at higher MAP and moisture conditions (40). These trends in *Bradyrhizobium* may also be indirectly caused by interactions with plant species, such as *Fagus sylvatica* (European beech), that are sensitive to differences in MAP (41).

Likewise, *Methylocystaceae* had greater relative abundance at lower MAP (Fig 5B). Members of the genus *Methylocystaceae* are often methylotrophs or methanotrophs, suggesting that their affinity for low MAP may reflect a dependence on a level of soil aeration afforded only by drier soils (42). The limited diffusion of gases through the wetter soil may therefore decrease *Methylocystaceae* abundance. Conversely, the low rainfall samples were entirely devoid of *Patulibacteraceae* (Fig 5B), suggesting a need for conditions associated with higher levels of precipitation. Given that *Patulibacteraceae* can commonly be found in anaerobic waste digesters (43, 44), it seems that many members of this family prefer similarly wet and anaerobic conditions.

In particular, our results demonstrated a significant decrease of two genera, *Mycobacterium* and *Bradyrhizobium*, in high moisture level sites. *Mycobacterium* showed a strong preference for soil with less than 50% moisture content, and *Bradyrhizobium* appeared to prefer 40-70% moisture levels (Fig 5C, D). Both genera appear to be main contributors to the soil microbiome, hence, changes in their abundance are easier to detect. Walsh et al (2010) suggest *Mycobacterium* prefers a wet soil environment, which contradicts with our finding (13). Given soil microorganisms are interacting, changes in the major players could propagate to other members in the ecosystem, leading to differences observed in diversity (45). *Mycobacterium* and *Bradyrhizobium* belong to Actinobacteria and Proteobacteria respectively, which show high connectedness in co-occurrence networks (45). Similar mechanisms may apply to other genera as well, but our sample size may be too small to detect the interactions (n = 104). However, it is possible that some genera may have been misclassified as *Mycobacterium,* a subset of which has recently been reclassified as *Mycolicibacterium* (46). This new genus is predominantly composed of environmental species whereas *Mycobacterium* consists of major human pathogens (46, 47). Nonetheless, our results suggest soil moisture level can impact the composition of soil microbial systems, and this impact is most evident on organisms that dominate the soil microbiome. The differential abundance of certain microbes demonstrated that microbial abundance could be used to indicate changes in MAP and soil moisture which are two important factors in evaluating soil quality.

Limitations This study had several limitations both generated by the dataset and the analysis techniques used. Likewise, our focus on two ecozones in BC greatly limits extrapolation. These soil trends can only be applied to regions in BC of similar ecozone type. They cannot be applied to sites across North America, such as ecozones in California and Texas included in the original dataset. Additionally, differences in moisture between the two BC ecozones may be confounded by other variables not included in our analyses, such as pH, carbon/nitrogen ratio, and tree and soil types. As well, it is possible that some taxa were misclassified.

Conclusions This study analyzed the effects of MAP and soil moisture content on the soil microbiome in two BC forest ecozones. Our results revealed that higher MAP and soil moisture were associated with increased phylogenetic diversity and shifts in the relative abundances of major members of the soil microbiome, specifically *Bradyrhizobium*, *Methylocystaceae* and *Patulibacteraceae*. Typical consequences of timber harvesting, like OM removal and compaction, did not significantly affect soil moisture at the time of sampling at our sites. Determining the relationship between soil moisture and MAP not only provides the framework for analyzing these factors in new datasets, but also provides foundational

information to monitor the impacts of climate change and forest management practices on bacterial soil biodiversity and function.

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Future Directions In the future, studies that focus distinctly on the relationship between the identified taxa and soil moisture could determine the generalizability of the observed effects. Modelling these conditions in a controlled experiment to test our expectations about these taxa may yield further insights on both the intricacies of this relationship and the auxiliary environmental factors that affect soil taxa like *Bradyrhizobium*. This study only examined the organic layer, due to its proximity to rainfall and the harvested surface. Investigating the effects of MAP on deeper soil horizons, subsoil, and populations in different rooting zones would provide a more comprehensive understanding of biogeochemistry and plant microbe interactions in forests of different rainfall levels.

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CONTRIBUTIONS

All authors contributed to data processing and downstream analysis. X.E.C. processed the data for phylogenetic and diversity analysis in QIIME 2 for all metadata categories of interest and trained the Naïve Bayes classifier for taxonomic analysis. X.E.C. also performed differential abundance analysis for the soil moisture metadata category. J.N. investigated the relationship between MAP, OM removal (LTSP treatment) and soil moisture, and interpreted the results of alpha and beta diversity and taxonomic analyses. R. C. generated alpha and beta diversity analyses and performed relative abundance analysis for the mean annual precipitation metadata category. Each author contributed to writing and editing the draft manuscript.

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