Undergraduate Journal of Experimental Microbiology and Immunology (UJEMI) Vol. 26:1-13


# Differences in organic matter removal treatments do not influence soil microbial diversity in British Columbia managed forests

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**SUMMARY** Microbial diversity is essential to the wellbeing of a given soil ecosystem, correlating to higher primary productivity levels. Microbial composition between different layers of soil, or soil heterogeneity, has been linked to microbial diversity and a healthy soil ecosystem. Organic matter removal treatments are logging practices that involve removing organic matter such as trees, branches and leaves, from a certain harvested area. It is well known that organic matter removal treatments have an effect in soil physical properties and the soil microbiome. However, the effects of organic matter removal treatments on soil microbial diversity and soil heterogeneity have not been extensively explored. We hypothesized that harsher organic matter removal treatments would result in a reduction of soil heterogeneity, and by extension, microbial diversity. Using the QIIME2 pipeline, we analysed 16s rRNA gene sequencing data collected from British Columbia managed forest sites that had undergone various organic matter removal treatments. Alpha and beta diversity analyses were performed to evaluate microbial diversity between different organic matter removal treatment sites and compare the diversity between soil layers within each site, respectively. We found that changes in microbial diversity were not correlated to changes in organic matter removal treatments or soil heterogeneity, even when confounding variables were controlled for with a logistic regression model. Nevertheless, organic matter removal treatments seem to have an effect in the abundance of certain microbial taxa. These results show that organic matter removal treatments do not appear to play a role in microbial diversity. However, other abiotic factors potentially influence soil microbial diversity and more research should be conducted to further investigate this conclusion.

## INTRODUCTION

Primary productivity, the synthesis of organic compounds from inorganic carbon, is vitally important to nearly every ecosystem as it is the process that allows for the formation of a stable food web foundation (1). A key indicator of how productive a given ecosystem may be, especially a soil ecosystem, is how microbially diverse it is (2). Logging is one of the largest industries in British Columbia (3), with a key aspect of logging being organic matter removal (OM) treatments. There are three standard organic removal treatments; OM treatment 1 involves simply removing tree branches, OM treatment 2 involves removing branches and trunks, while OM treatment 3 involves removing branches, trunks and the upper organic layer of soil (4). Even though the general consensus is that OM treatments disrupt the physical properties of the soil, there have been conflicting reports in the literature regarding the impacts of organic matter removal methods on microbial diversity. Ponder et al. state that OM removal had few consistent effects and therefore has very little effect on soil diversity (5). On the other hand, Soto Cárdenas et al. state that the different degrees OM removal show consistent changes in bacterial populations within the soil (6).

As a result of the ambiguity found in the literature, we wish to examine how microbial properties among soil samples within British Columbia forests are impacted by various OM removal treatments. This decision to focus on forests in British Columbia was due to the unique soil properties and microbial profiles seen in each region (4). Through a thorough examination of the literature, we found that microbial heterogeneity amongst soil horizons is related to microbial diversity within a soil ecosystem (7). Soil horizons are layers of soil categorized by their biological, physical and chemical properties. Typically, OM removal

**Published Online:** September 2021

**Citation:** Juan Camilo Burckhardt, Adam Dorner, Chris Breden, David Liang. 2021. Differences in organic matter removal treatments do not influence soil microbial diversity in British Columbia managed forests. UJEMI 26:1-13

**Editor:** Daniela Morales, Stefanie Sternagel and Brianne Newman, University of British Columbia



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methods involve disruptions of the O horizon, which is near the surface and contains high levels of organic carbon, as well as the A horizon which is deeper and contains less organic carbon and other nutrients (8). As a result, we wish to not only examine the effects of OM removal treatments on the overall microbial diversity of forest sites, but also the microbial heterogeneity between A and O soil horizons; this is because, as mentioned before, microbial heterogeneity between layers is correlated to overall microbial diversity in a soil community, as well as overall primary productivity of a soil ecosystem (7). Finally, we wish to determine the nature of taxa present following different OM removal treatments. Based on these previous findings, we hypothesize that the higher intensity OM treatments, namely treatment 3, will reduce microbial diversity in soil ecosystems of British Columbia forests when compared to reference sites (REF) where no organic removal treatments have occurred. Furthermore, we predict that this reduction in overall microbial diversity will be linked to a reduction in microbial heterogeneity between A and O soil horizons due to the various OM treatments. Finally, we predict that higher intensity OM removal methods will select for certain taxonomies of microbes. To test these hypotheses, we examined 16s rRNA sequencing data collected from soil samples of BC forest sites that have undergone OM removal treatments, provided by the Mohn research group at the University of British Columbia (9) and analyzed how the data changes between various OM removal treatments as well as different soil horizons. In doing so, we learned that OM removal treatments do not seem to be linked to a reduction in soil heterogeneity or overall microbial diversity; our results suggest that sites that have undergone OM removal treatments may have unique microbial communities, but this may be due to extraneous variables such as temperature or precipitation as opposed to the OM removal treatments themselves. We also learned that specific OM removal treatments can influence the abundance of certain microbial classes, but the cause or implications of this finding remains to be further explored.

## METHODS AND MATERIALS

The following section contains summarized versions of the analyses done in this paper. All relevant code and scripts can be found in the supplementary material section. An overview flowchart of the processing and analysis pipeline can also be found in the supplementary materials section (Fig. S0).

**Data source: study sites, sample collection and 16S rRNA sequencing**. All data discussed in this paper proceeds from the paper "A metagenomic survey of forest soil microbial communities more than a decade after timber harvesting" by Wilhelm et al. (9). The following is a summarized version of their experimental design, sample collection and sample processing relevant to this paper. For specifics please refer to the original paper.

Data comes from the Long-term Soil Productivity Study (LTSP), a project designed to look at soil quality and productivity of managed forests in North America. The overall goal of the LTSP project is to look at how soil compaction and organic matter (OM) removal treatments impact managed forests. Soil samples were collected from eighteen LTSP sites between 2008 and 2014 in four main regions: California, Texas, British Columbia and Ontario. For each LTSP site, samples were collected from experimental plots that were unharvested or harvested with three different intensity levels of OM removal treatments. OM removal treatments are characterized by the debranching of trees *in-situ* (OM1), removal of branches and trunks (OM2), or removal of branches, trunks and top soil layer (OM3) of forests (Fig. S1A). The reference site (REF) was an unharvested neighboring plot. For each experimental plot, samples were collected at two depths representing the organic layer (O horizon; 0.1 meters) and mineral layer (A horizon; 0.3 meters) of the soil (Fig. S1A). Samples were processed and their physical properties (carbon, nitrogen, pH, moisture, and bulk density) were determined. Other geographical properties such as ecozone and mean annual temperature were also reported in the metadata. For each soil sample, amplicon sequences from bacteria were collected using sequencing of the 16S rRNA gene. The 16S rRNA sequencing libraries can be found in the European Sequencing Archive under the study accessions identifiers PRJEB8599 and PRJEB12501.

**Microbial 16S rRNA processing.** 16S microbial data was processed using QIIME2 version 2020.8.0 (10). Demultiplexed sequences were truncated at 150 base pairs (bp) and combined into identical amplicon sequence variants (ASVs) using the DADA2 plugin version 2020.8.0 (11). Low frequency reads, chloroplast and mitochondria sequences, and sequences preceding from regions other than British Columbia were filtered using QIIME2. A phylogenetic tree relating representative ASVs was created with the QIIME2 phylogeny tool. Lastly, QIIME2 classifier tool and the 97% version of the GREENGENES sequence database (12) was used to train a naive Bayes classifier to assign taxonomy to ASVs. The QIIME2 script can be found in Soil_QIIME2_script.txt

**Microbial 16S rRNA alpha and beta diversity analyses**. A rarefaction plot generated with QIIME2 (Fig. S2) was used to determine the sequencing depth at which the alpha and beta diversity analyses would be conducted. A sequencing depth of 6041 was chosen and used to run the QIIME pipeline to generate the beta-diversity Emperor principal coordinate analysis (PCoA) plots and following diversity metrics: Shannon, Pielou's Evenness, observed features, Faith's, Jaccard, Bray-Curtis and Weighted and Unweighted UniFrac. QIIME2 "alpha-diversity significance" and "beta-diversity significance" commands were used respectively to visualize alpha-diversity boxplots and distance metric beta-diversity boxplots. For formatting purposes, Weighted UniFrac PCoA plots were also done in R using the packages Phyloseq (13), DESeq2 (14) and ggplot2 (15). Phyloseq was used to store and filter the data generated with QIIME2 into a functional R object. DESeq2 was then used to rarefy the data and ordinate it into Weighted UniFrac PCoA plots, and ggplot2 was used to format the resulting plots.

Alpha and beta diversity analyses were done to compare microbial diversity and composition between the different OM removal treatments (REF, OM1, OM2, OM3). Furthermore, within each OM removal treatment, changes in the differences in microbial communities between the A and O soil horizons were also explored using beta-diversity metrics (Fig. S1B). Plots based on other variables such as collection site or ecozone were also produced (Fig. S3), but were not explored in depth in this study.

**Alpha diversity metric logistic regression.** Data for all alpha diversity metrics (Shannon, Pielou's Evenness, observed features and Faith's Phylogenetic Diversity) was downloaded from outputs generated with the "QIIME2 view" online software (https://view.qiime2.org/). Alpha diversity data was loaded into R and merged with metadata variables through the "join()" function. An analysis of the Weighted UniFrac PCoA plot was conducted to determine variables that showed strong clustering patterns (Fig. S3). These variables were then inputted into a generalized linear model to determine the weighted effect each of them had in explaining changes in alpha diversity. If two variables were strongly correlated with each other, only one of them was retained in the model. The final regression model consisted of the following explanatory variables: Ecozone, Soil horizon, OM removal treatment, collection site, soil compaction and moisture content. Lastly, ggplot2 was then used to plot the model predicted alpha diversity values grouped by their respective OM removal treatment.

**Indicator taxa analysis and logistic regression**. Indicator taxa analysis was conducted in R using the phyloseq and Indicspecies (16) libraries. QIIME2 data was loaded into a phyloseq object, filtered based on region (British Columbia) and ASV counts were grouped based on class taxonomy rank using an in-house developed function (group_by_taxonomy). Then, the "multipatt" function was used to conduct an indicator taxa analysis to determine differentially abundant microbial classes specific to an OM removal treatment. Microbial classes whose indicator value had a false discovery rate of less than 5% ($p$-value $< 0.05$) were considered significant. Afterwards, taxonomic ASV counts were transformed into relative abundance using an in-house developed function (RelativeTaxa). A logistic regression model was used to weight the impact that OM removal treatments have in explaining relative abundance changes of significant classes when controlling for confounding variables (ecozone, soil horizon, collection site, soil compaction, and moisture content). The classes that were significantly explained by OM removal treatment (corrected $p$-value $< 0.05$) were retained.

The model-predicted relative abundance values were grouped by OM removal treatment and plotted using ggplot2 to visualize the differences in abundance.

**Statistical analyses.** Significance of alpha diversity differences between OM removal treatments was tested using the Kruskal-Wallis test (for all groups and in a pairwise manner) with QIIME2; p-values < 0.05 were considered significant. Beta diversity significance between OM removal treatments was tested using a pairwise PERMANOVA done by QIIME2 software; p-values < 0.05 were considered significant. Logistic regression model coefficient significance was calculated using the "summary()" and "glm()" R functions, which implement Student t-tests to determine statistical significance (p-value < 0.05). Finally, indicator taxa analysis with multipatt function of the Indicspecies library implements permutations tests to determine the statistical significance (p-value < 0.05) of the indicator value of a taxon.

## RESULTS

**North American regions have distinct soil and microbial profiles**. The data source by Wilhelm et al. contained data from highly productive forests in 4 different North American regions (British Columbia, Ontario, Texas and California) (9). We wanted to determine the differences in regards to soil and microbial properties of the samples collected from these regions. Thus, we analyzed the reported carbon and nitrogen content in the soil of these regions by plotting their unique carbon/nitrogen values against each other. It appears that soil from each region contains its own distinct range of carbon and nitrogen (Fig. 1A). British Columbia has the highest range of carbon and nitrogen in soil, Texas has the lowest, and Ontario and California span from low to high. Interestingly, carbon and nitrogen content seem to have a strong, linear, positive relationship. We followed this analysis by looking at microbial composition of the data from all regions to see if there were unique clustering patterns in relationship to the region the samples were collected from. We used Weighted UniFrac to account for bacterial abundance and phylogenetic relatedness in our analysis. It appears that microbial composition is driven by geographical region and that British Columbia has a unique microbial community in relation to the other regions (Fig. 1B). Because of the specific soil properties and microbial profiles seen in each region, we decided to focus our analysis only in British Columbia. We made this decision to help us eliminate confounding variables in our study and help us zoom in into variables that we are interested in -- OM treatment and soil horizons.

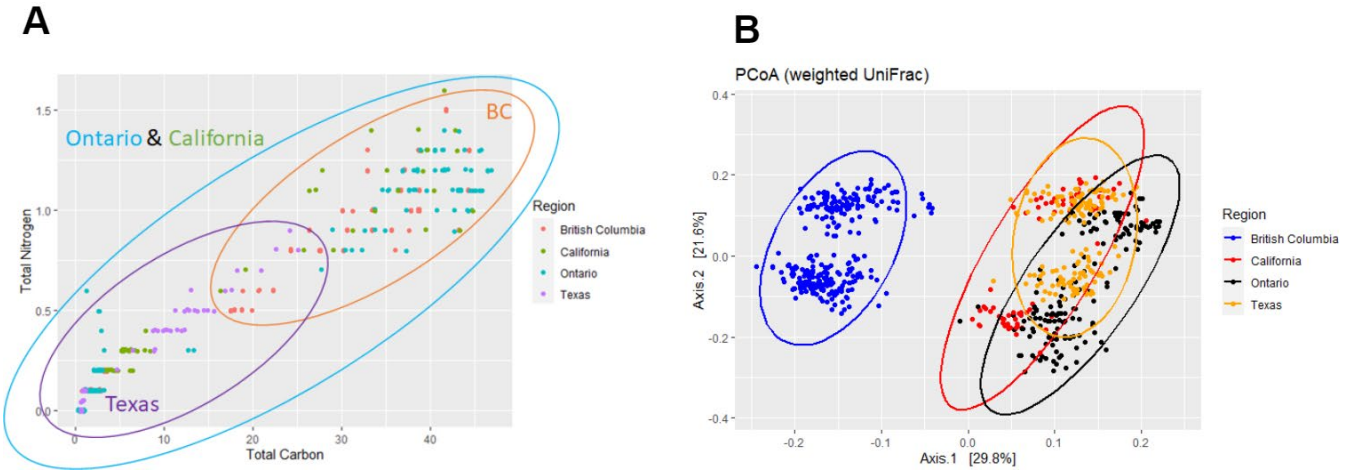



**FIG. 1 Different carbon, nitrogen and microbial profiles between studied regions.** Data from 4 North American regions were contained within the data source. (A) Data points (samples) were plotted based on soil total carbon and total nitrogen and colored in reference to the North American region collected. (B) Weighted UniFrac principal coordinate analysis (PCoA) of bacterial communities from the different North American regions. Variance explained of the PCoA plot is given in brackets.

**Soil horizons showed differences in microbial composition but no changes based on OM treatments.** We wanted to investigate if there were any changes in microbial composition between the A horizon and the O horizon depending on the OM removal treatment. We filtered to maintain only the ecosites located in British Columbia (BC), followed by analysis of the filtered ASV data using beta diversity metrics (Weighted UniFrac, Table; Unweighted UniFrac, Jaccard, Bray-Curtis, not shown) through QIIME2 and RStudio. PCoA ordination showed distinct clustering of samples based on soil horizon in every OM removal treatment and the REF (Fig. 2). As expected, the O and A horizons showed distinctive clustering patterns and therefore had significantly different microbial compositions (Fig. 2). Differences between each OM treatment showed no observable differences in clustering patterns (Fig. S4); however, a pairwise PERMANOVA analysis of OM removal treatment groups demonstrated OM3 being significantly different ($p<0.05$) from every other OM group (Table 1). Microbial composition differed with soil horizon, as in the OM3 treatment (Fig. 2D), there was a complete removal of the O horizon layer. This is the likely explanation behind why OM3 microbial composition significantly differs from the other treatments as the O horizon consists of a distinct clustering pattern compared to the A horizon (Fig. 2). All other OM treatments showed no significant changes in microbial composition based on the PERMANOVA. Thus, these results show that there are no significant changes in microbial diversity between the O and the A horizon based on OM treatment, with the exception of OM3, which completely removed the O horizon layer.

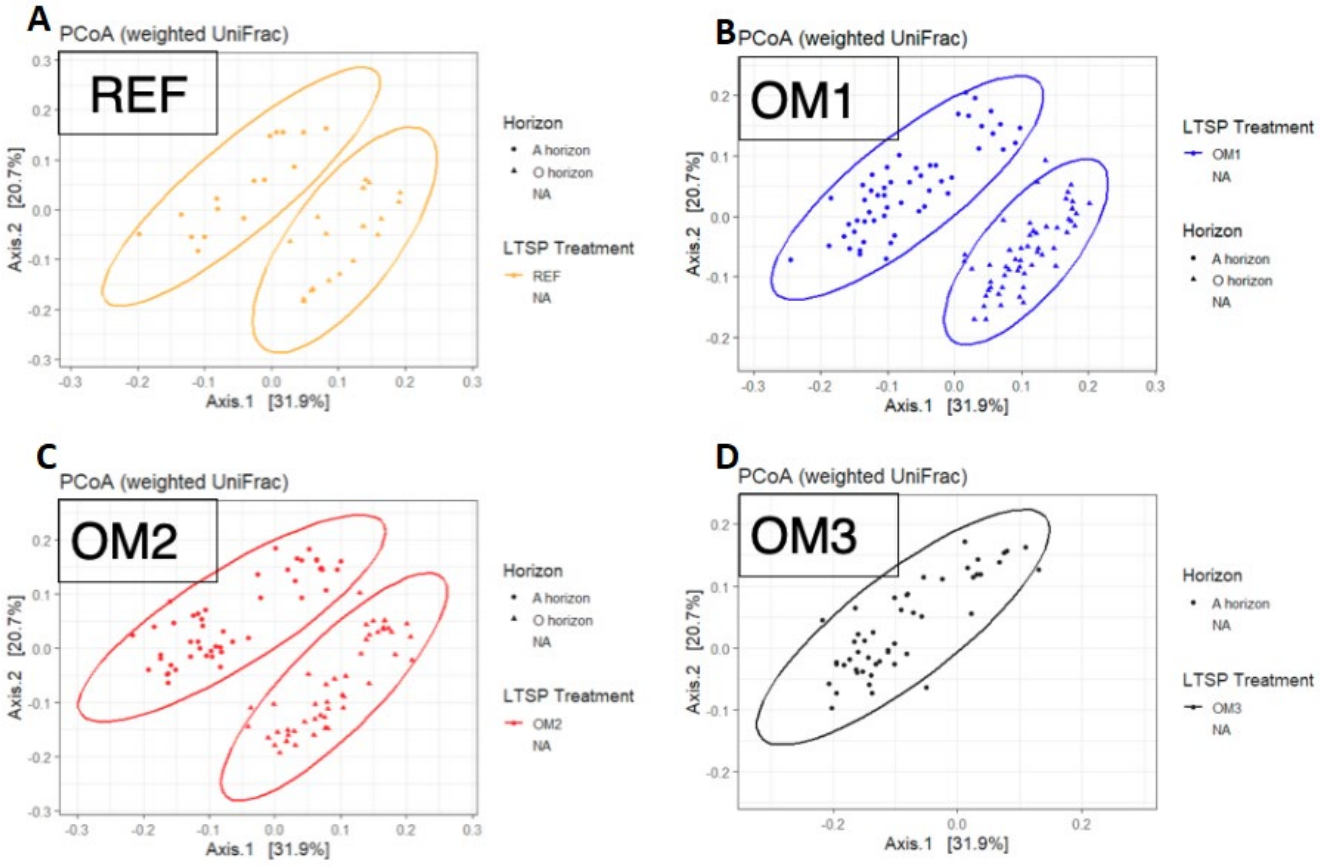


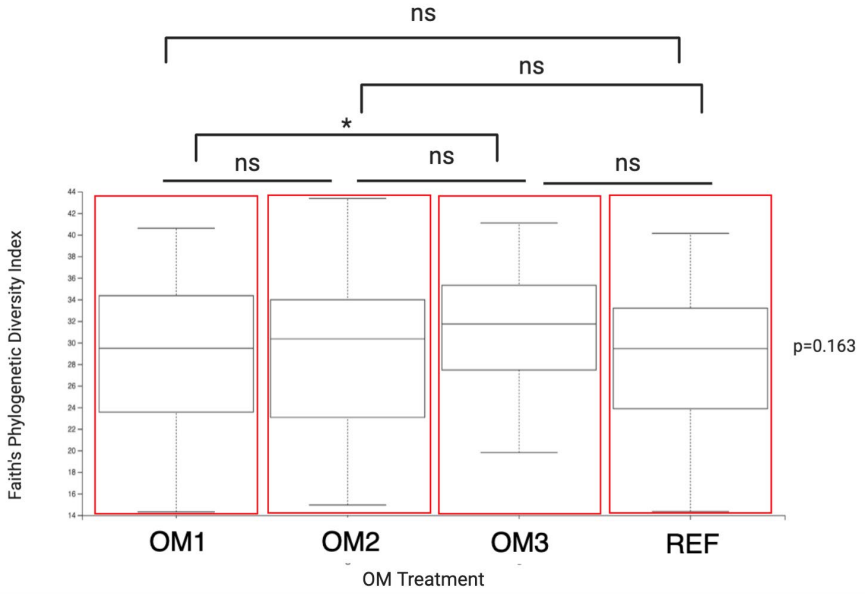

**FIG. 2 No changes in microbial diversity between soil horizons within each OM treatment group.** Weighted UniFrac PCoA ordinations of ASV data visualizing microbial diversity between soil horizons depending on OM removal. (A-D). Levels of OM removal: Reference plots (REF), yellow ellipses; OM1, blue ellipses; OM2, red ellipses; OM3, black ellipses. Type of Soil Horizon: O Horizon, circles; A horizon, triangles. (D) OM3 plot showed a disappearance of the O horizon. Variance identified by each PCoA axis is given in brackets. NA: North America.

**TABLE 1 Significance in changes in microbial composition of soil layers between OM Treatments assessed by permutational multivariate analysis of variance (pairwise PERMANOVA).** Abbreviations: OM, Organic Matter; OM1, tree stem removal; OM2, whole tree removal; OM3, whole tree and topsoil layer removal; REF, reference plot. *$P < 0.05$. Test statistic used was pseudo-F.

| Group 1 | Group 2 | Sample size | Permutations | Pseudo-F | p-value | q-value |
|---|---|---|---|---|---|---|
| **OM1** | OM2 | 200 | 999 | 1.057057284110850 | 0.337 | 0.337 |
| **OM1** | OM3* | 155 | 999 | 14.45847001227430 | 0.001 | 0.002 |
| **OM1** | REF | 140 | 999 | 2.1588570110122000 | 0.05 | 0.075 |
| **OM2** | OM3* | 143 | 999 | 11.392393105433200 | 0.001 | 0.002 |
| **OM2** | REF | 128 | 999 | 1.9210528856885700 | 0.065 | 0.078 |
| **OM3** | REF | 83 | 999 | 11.468848994407700 | 0.001 | 0.002 |

**No significant differences in soil microbial diversity with respect to OM treatment.** Next, we wanted to determine whether there were any significant differences in microbial diversity between each OM removal treatment. We analyzed microbial diversity differences between the different levels of OM removal treatments through various alpha Diversity metrics, statistical analysis methods used to study microbial diversity within a single environment. Faith's Phylogenetic Diversity (Fig. 3) showed no significant differences between any of the treatments with the exception of OM1 with OM3 ($p < 0.05$). Analysis via Shannon Diversity and Pielou's Evenness (Fig. S5) showed similar non-significant differences between each treatment (with some exception). Overall, these results show no significant differences in microbial genetic relatedness, a measure of microbial diversity, based on different OM

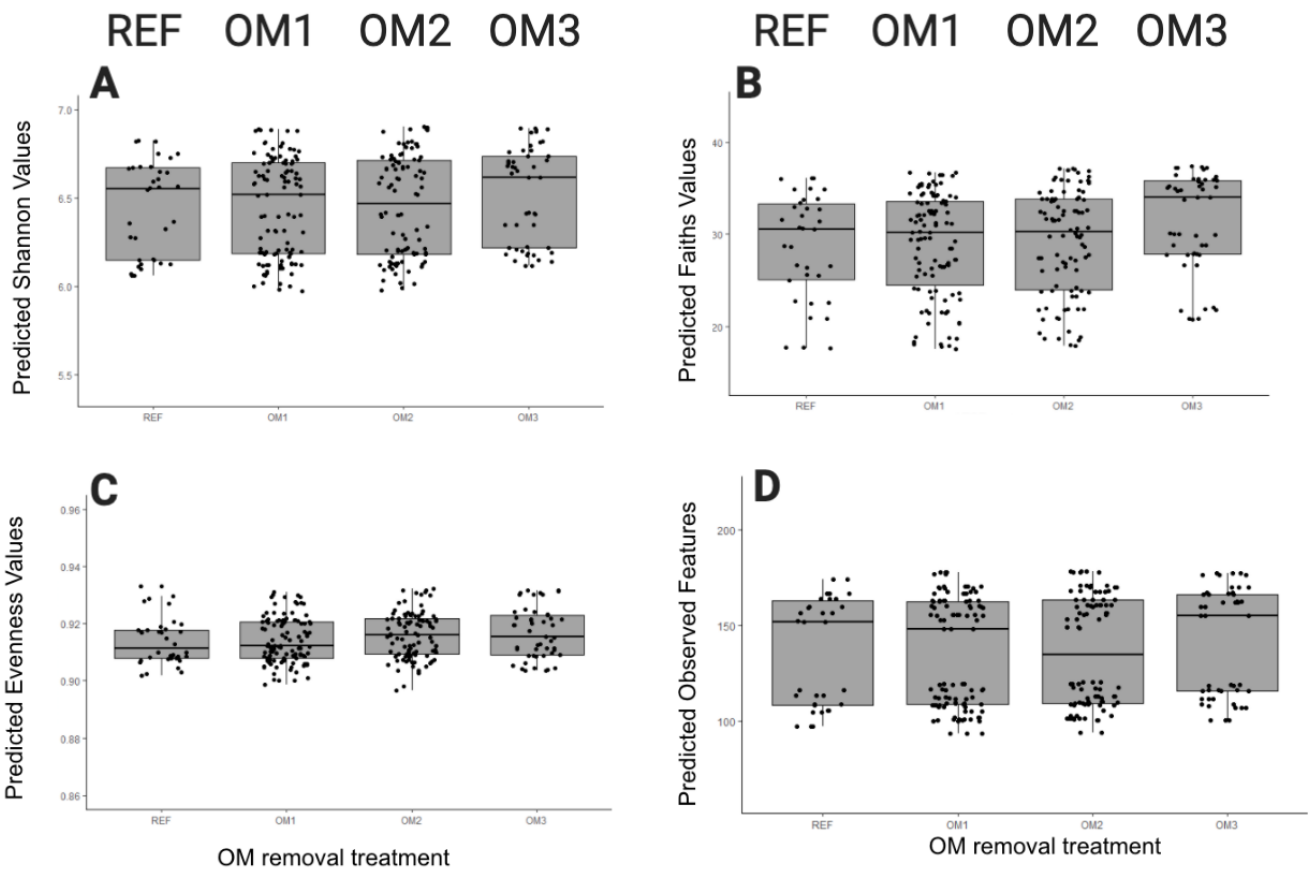
treatments.




**Logistic regression analysis showed no significant differences in alpha diversity metrics between OM treatments when controlling for confounding variables.** Visualization of the Weighted UniFrac PCoA plot based on ecozone, soil horizon, collection site, soil compaction, moisture content, annual temperature and annual precipitation, showed observable clustering patterns (Fig. S3). We hypothesized that these variables could act as confounding factors masking the subtler effects that OM removal treatment had in alpha diversity changes. Thus, we conducted a logistic regression analysis of different alpha diversity metrics to determine if OM removal treatments had a significant impact in microbial diversity when controlling for confounding variables (Fig. 4, Table S1). A logistic regression is a statistical technique that analyzes the relationship between multiple independent variables and a single dependent variable, in our case alpha diversity (17).

Fig. 4 shows the model-predicted alpha diversity box plots for Shannon Diversity, Faith's Phylogenetic Diversity, Pielou's Evenness, and Observed Features, when grouping by OM removal treatment. Overall, no significant differences in microbial richness between the distinct OM treatment groups was observed. One exception appears to be OM3 in the Faith's Phylogenetic Diversity plot (Fig. 4B). Given that Faith's Phylogenetic Diversity is a measure of genetic relatedness, OM3 seems to contain a different taxonomic composition compared to the rest of the OM treatments. A one-way ANOVA comparison of the predicted Faith's alpha diversity values in relationship to OM removal grouping showed a trend towards statistical significance (p-value = 0.0634). However, the results show that in general, even controlling for several potential confounding variables, there were no observable differences in microbial diversity between the different OM treatments.

**FIG. 3 No microbial diversity in BC forests according to OM removal treatments.** Box plot visualization of Faith's Phylogenetic Diversity for different OM treatments in BC forest sites. Upper and lower box boundaries represent the interquartile range (25%-75%); the middle line represents the median; upper and lower range of whiskers represent maximum and minimum values, respectively. The Kruskal-Wallis (all groups) *p* value is indicated. * indicates statistical significance ($p < 0.05$).

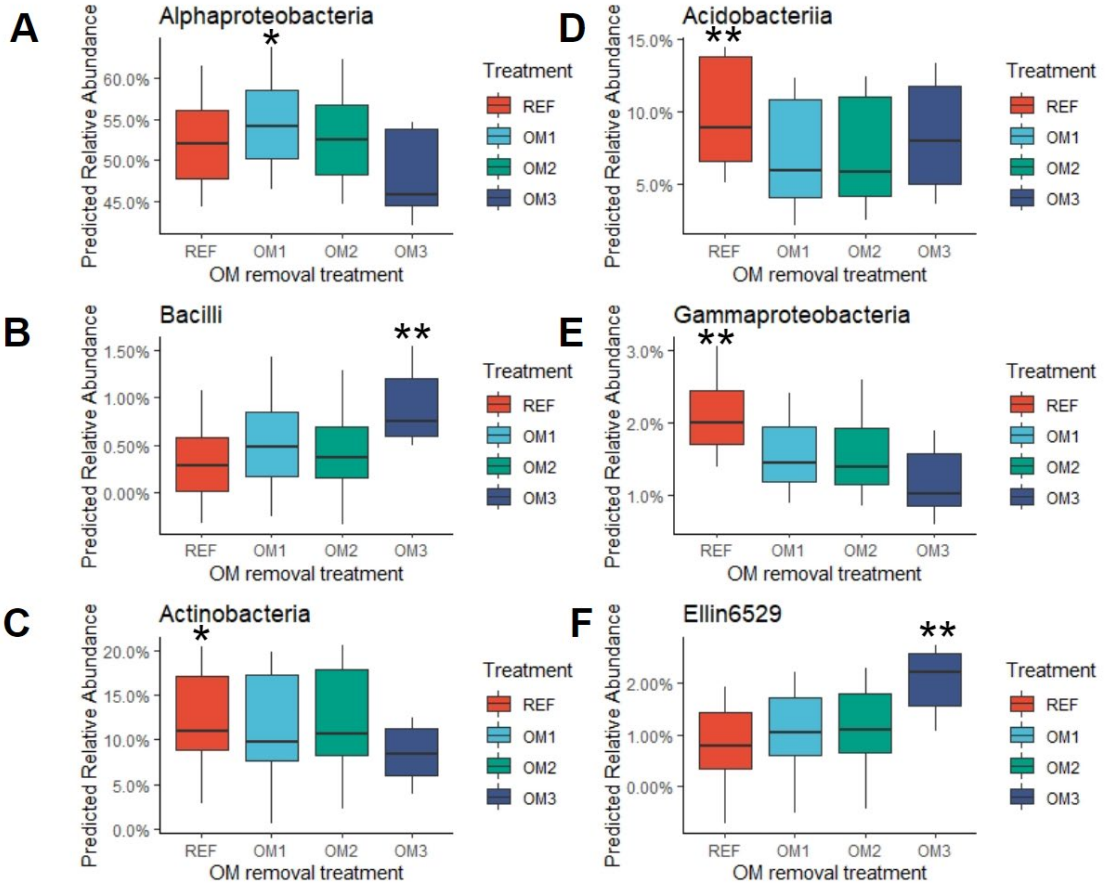



**Indicator taxa analysis identified microbial classes that were significantly more abundant at specific OM removal treated groups, even when confounding variables were controlled for**. We wanted to see if there was an enrichment of certain taxa depending on OM removal treatment. We created a taxonomic composition bar plot at the microbial class level to see if there were any visible differences between OM removal treatments when separated by soil horizons (Fig. S6). However, looking at the composition bar plot gave us inconclusive results. Thus, we decided to perform an indicator taxa analysis to determine which microbial classes were more abundant relative to OM removal treatment type. The analysis returned 25 microbial classes with a significant indicator value ($p < 0.05$) determined by a permutation statistical test. To further control for other previously described confounding variables (Fig. S3), we inputted the relative abundance of each of the 25 significant classes through a logistic regression model. We only retained the classes that had their relative abundance significantly explained by OM removal treatment. Of these remaining microbial classes, the relative abundance values predicted by our model were then grouped by OM treatment and visualized with boxplots (Fig. 5, Fig. S7, Table S2). Fig. 5 shows a representative subset of the microbial classes which have a relative abundance that is

**FIG. 4 Controlling for confounding factors showed no significant differences between OM treatments.** A logistic regression analysis controlling for ecozone, soil horizon, LTSP treatment, collection site, soil compaction and moisture content was done on several Alpha Diversity metrics; (A) Shannon Diversity; (B) Faith's Phylogenetic Diversity; (C) Pielou's Evenness; (D) Observed Features. Upper and lower box boundaries represent the interquartile range (25%-75%); the middle line represents the median; upper and lower range of whiskers represent maximum and minimum values, respectively. REF: reference plot.

generally over 1%. Overall, taxonomic classes were enriched either in the control (REF) or in OM removal treatment 3 (OM3) group - opposite extremes in the range of intensities of OM removal treatment. These results support the idea that OM removal treatments have an effect in microbial composition by affecting the abundance of certain taxa. However, the

specific mechanistic relationship between taxonomic abundance, soil properties and harvesting practices is beyond the scope of this paper.




## DISCUSSION

In an effort to determine how the various OM treatments affected soil microbial diversity in managed British Columbia forests the 16S rRNA gene amplicon metadata supplied from Wilhelm et al. was filtered and used for various diversity and taxonomic analyses (9). After baseline filtering, alpha diversity plots between each of the OM treatments were created. In addition, beta diversity plots comparing each of the OM treatments and the O and A horizons were also produced. No significant differences between the OM treatments were noticed with the alpha diversity plots, and no obvious clustering between the different OM treatments were observed in the Weighted UniFrac PCoA plot, either. However, through the Weighted UniFrac PCoA plot we realized that the O horizon was significantly different compared to the A horizon. When visualizing other abiotic variables compared to only OM treatments, we noticed that there were distinct clustering patterns on the Weighted UniFrac plot (Fig. S3). This suggested that they could be potential confounding variables that are accounting for

**FIG. 5 Representative indicator taxa classes showcase significant differences based on OM removal treatment.** Significant bacterial classes from the Indicator Taxa analysis were run through a logistic regression model to control for confounding variables. The model-predicted relative abundance of the representative classes was plotted based on OM treatment. Four taxonomic classes are represented: Alphaproteobacteria (A), Bacilli (B), Actinobacteria (C), Acidobacteria (D), Gammaproteobacteria (D) and Ellin6529 (F). Upper and lower box boundaries represent the interquartile range (25%-75%); the middle line represents the median; upper and lower range of whiskers represent maximum and minimum values, respectively. Indicator value significance was calculated based on the permutation. Indicator value with **p-value* < 0.05; **p-value < 0.001.

microbial diversity, and a logistic regression analysis was employed to control for them.

**Limited Differences in microbial diversity between the OM treatments using alpha diversity.** After filtering the ASV data, various alpha and beta diversity plots were generated to determine differences in microbial diversity between the different OM treatments and soil horizons. When all of the ASV data was considered there were no significant differences in diversity noticed. The Faith's Phylogenetic Diversity plots (Fig. 3) showed similar values for the REF and each OM treatment indicating that the diversity within each treatment was similar. These results were found to be similar to a previous study that used Shannon Diversity to look at various ecozones in British Columbia and other locations throughout North America (18). It was hypothesized that a difference would be noticed due to the increasing disturbance from each OM treatment, the factor that would drive differences in microbial diversity. After our initial analysis it seemed quite possible that the filtering was not specific enough, resulting in data with many uncontrolled variables that went against the hypothesis by suggesting that there were no significant differences in soil microbial diversity between OM treatments. The Faith's Phylogenetic Diversity boxplots (Fig. 3) were not specific enough and more analyses were required for a conclusion.

**No microbial diversity between the OM treatments and limited differences between soil horizons using beta diversity.** The next step was to analyze the results from the Weighted UniFrac PCoA plot (Fig. 2), and when the data was visualized by OM treatment, no significant clustering was noticed. Another group had previously done a similar study where they used a different analysis related to a PCoA plot known as Canonical analysis of principal coordinates (CAP) and noticed a significant difference in soil microbial diversity between the REF and all three OM treatments, as well as between the varying levels of soil compaction, supporting our hypothesis (19). Again, our observed result went against the hypothesis, as we expected to notice a trend where an increasing OM removal treatment intensity would reduce heterogeneity between the O and A soil horizons. Looking at the Weighted UniFrac PCoA plots for each of the REF, OM1 and OM2 treatments (Fig. 2) there were distinct differences between the O and A horizons. However, no discernable trend was seen showing the reduction of heterogeneity between the two soil horizon layers based on differing OM treatments, and rather remained constant throughout. It was noticed that the entire O horizon was removed during the OM3 treatment, which is a reasonable conclusion since the upper organic layer of the forest is scraped away in this treatment (9). Since the O horizon is the upper layer where the organic matter and decomposing leaves are located, under a treatment that removes the upper organic layer we would expect to notice the removal of the O horizon when analyzing with the Weighted UniFrac PCoA plot (Fig. 2). The O horizon being removed from OM3 has significant consequences for the soil ecosystem, especially regarding the overall inter-layer microbial diversity. The microbial composition between O and A horizons would likely vary due to the differences in environment, with the O horizon being an organic layer with decomposing leaves and the A horizon having a larger number of minerals from parent material. It has been shown previously that the O horizon is a rich source of labile nutrients, and disturbing this layer can have serious effects on site fertility and nutrient cycles (20). Based on the Weighted UniFrac plot alone (Fig. 2), we show that the level of OM treatment did not drive these expected differences in composition, likely because the treatment did not alter the soil environment as much as expected, especially in the OM1 and OM2 treatments. The treatment did impact the environment in OM3, and this was likely to be a result of the removal of the upper layer of the soil.

**Confounding variables affecting the findings of limited differences in microbial diversity.** While analyzing the Weighted UniFrac PCoA plot, when other variables were selected rather than OM removal treatment, significant clustering was noticed (Fig. S3). These results indicate that there are a variety of factors that need to be considered when determining any significant differences in soil microbial diversity. In order to see meaningful trends of the OM treatments, these parameters need to be considered, potentially by filtering for these abiotic factors, to correct for the influence they may have on the results. However, further filtering could result in a significant decrease in sample size, which presents other issues, as it could potentially decrease the significance of our results and less meaningful

relationships. This trade-off between increased control of our data and sample size must be considered and would require an increased number of samples if it were to be done effectively. The clustering of samples located within similar conditions based on these chosen parameters was intriguing as it showed that there may be significant differences in soil microbial diversity. The clustering within similar conditions is likely a direct result of certain soil microbes being selected for due to the environment they are within. The diversity of microbes changes as the environment changes, and particular microbes can increase or decrease in abundance if they have a particular biological function that benefits or is disadvantaged from the change (21). For example, the Alphaproteobacteria class of bacteria has been shown to vary significantly with precipitation levels (22). Having microbial composition change based on these factors to begin with makes the issue a more complex one than initially anticipated. Proposing such a broad question without considering this unique set of parameters proved to be too comprehensive, leaving further required analysis to answer our research question.

**Controlling for confounding variables affecting microbial diversity between OM treatments.** Performing a logistic regression analysis on the alpha diversity metrics allows the results to be interpreted while considering the variances caused by confounding variables that were not controlled for in the Faith's Phylogenetic Diversity plot. When considering all of Shannon Diversity, Pielou's Evenness, observed features, and Faith's Phylogenetic Diversity results were calculated to determine which differences were significant. Parameters that had a significant influence on changes in soil microbial diversity based on the logistic regression were determined to be ecozone, sample collection site, compaction level, soil horizon and moisture content (Table S1). Observing that ecozone had an influence on soil microbial diversity was not a surprise, as this has been shown before when using soil samples that were collected from ecozones throughout North America (18). This result is consistent with both alpha and beta diversity analysis, which showed that there are many factors that influence soil microbial diversity besides just OM treatment. Collecting samples from two different ecozones will impact the data since the microbial composition was seen to vary between both, and the same goes for the other parameters shown to be significant. One intriguing parameter was the compaction level, since this metric is likely affected by the OM treatment, but it was not studied in detail. When analyzing the logistic regression based on OM treatment there were no significant differences observed in soil microbial diversity (Fig. 4). This result is consistent with the results from both Faith's Phylogenetic Diversity and the Weighted UniFrac PCoA plot (Fig. 2, Fig. 3). This shows that even after controlling for certain confounding variables that there were no significant differences between each of the treatment conditions in soil microbial diversity using the various alpha diversity metrics. Once again, this result was similar to one concluded in previous studies looking at soil samples from various managed forests in North America (18). Unexpectedly, there were still no significant differences in microbial diversity between each of the OM treatment levels, even after controlling for various variables, indicating results against our hypothesis. It is possible that there are a number of variables to be considered in addition, and there are further analyses to explore this relationship between microbial diversity and OM treatments.

**Taxonomic analysis within the OM treatments and correcting for confounding variables.** Most taxa showed similar abundance between the OM treatment groups, with a few exceptions. It has been previously documented that abundance of Acidobacteria remains relatively constant throughout increasing compaction levels due to logging, and this was also observed with our analysis (Fig. 5) (23). This constant abundance was attributed to the fact that the Acidobacteria class was resilient towards the effects caused by logging. Alphaproteobacteria and Gammaproteobacteria had been shown to have only a slight resilience towards compaction effects from logging, and had differed slightly as compaction level increased; this was also observed in the Hartman et al. study in 2013 (23). Our results (Fig. 5) disagreed with the literature in regards to Chloroflexi and Ktedonobacteria specifically, as they have shown resilience towards logging effects, while we observed that there were noticeable differences in abundance for the OM3 treatments compared to the REF, OM1 and OM2 (23). This could be accredited to differences in definition of soil compaction and what the OM3 treatment consists of. The OM3 treatment is much more invasive and

results in the scraping of the upper layer of soil, compared to simply compacting the soil. Certain taxa abundance that had varied in ways that were similar to previously shown in literature, while others had disagreed. Overall, most of the taxa had been shown to not vary significantly in abundance between the different OM treatments (Fig. 5).

**Conclusions** In conclusion, while there were intriguing differences between soil composition separating managed forests in British Columbia compared to other sites in North America, we did not find the anticipated changes in soil microbial diversity between OM treatments. Our hypothesis that OM treatment would affect microbial diversity was not shown through our analysis. Even after correcting for certain abiotic factors such as mean annual precipitation, the same result was seen, showing no significant differences in diversity based on OM treatment. There are more abiotic factors to consider (such as elevation and temperature), as well as more types of analysis available to determine differences in soil microbial diversity, and it may be of interest to focus on the specific taxa that showed changes in abundance in the OM3 treatment from the taxonomic analysis.

**Future Directions** We were unable to find a correlational relationship between OM removal treatments and soil heterogeneity reduction/microbial diversity reduction; this was shown by a lack of clustering patterns based on OM treatment (Fig. 2, Fig. S4). However, we noticed that some clustering was present when sorting samples based on other abiotic factors, such as mean annual precipitation (Fig. S3). As such, we recommend future studies that attempt to control for extraneous variables and emphasize finding a causal relationship between OM removal treatments and microbial diversity. Previously it has been shown that the CAP analysis can help control for these extraneous variables, as such this could be a potential next step in analyzing this data (19) While our logistic regression model did not show any significant microbial diversity differences based on OM treatment, this model can be further improved upon by considering more variables and other types of distributions that may increase its accuracy. Furthermore, other regression models could also be explored. Additionally, controlled experiments to test the impacts of other variables on microbial diversity in these managed forest regions may be worthwhile as well. Furthermore, filtering of existing data may be done in order to explore said relationships; however, future researchers must be conscientious of maintaining enough data points to land at statistically significant results, as losing statistical power is a danger of over-filtering. Finally, an exploration of managed forests in other regions of the initial study may be meaningful to analyze consistency with the results observed in BC (4).

## ACKNOWLEDGEMENTS

We thank Mihai Cirstea for his invaluable guidance, discussion, and suggestions for the steps of our project as well as technical support for RStudio and QIIME2. We thank Dr. D. Oliver and Reynold Farrera for their comments and critical reading of the manuscript. We also thank Dr. W. Mohn for providing the soil data used for our project. All acknowledged members are a part of the University of British Columbia Department of Microbiology and Immunology. This work was supported by MICB 447.

## CONTRIBUTIONS

J.C.B. did the methods sections, part of the results and part of figures/supplementary figures and abstract. A.D. did the introduction and future directions sections, and part of the abstract. C.B. did the discussion section, the references, and part of the abstract. D.L. did the results section, figures/supplementary figures and tables, and part of the abstract. Everyone helped edit and make the entire manuscript cohesive and flow well. Several rounds of revisions were conducted as a group.

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