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Characterizing the influence of depth and glacial drift on microbial community diversity and potential carbon-cycling enzyme activity in permafrost soils

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ABSTRACT

Microbial communities in permafrost soils play an important role in breaking down organic matter and cycling nutrients. Despite difficult environmental conditions including below-freezing temperatures, yearly freeze-thaw cycles, and short growing seasons, these microbial communities remain active year-round. To better understand the microbial community diversity and potential activity across environmental gradients in northern Alaska, I sequenced the microbial community and measured the potential enzyme activity of three carbon-cycling enzymes at 5-centimeter increments in 60-centimeter soil cores spanning active layer and permafrost at three moist acidic tundra sites located on landscapes with different glacial histories in northern Alaska. I found that microbial community diversity was not driven by glacial drift, but decreased significantly with depth. There was higher hydrolytic enzyme activity per gram dry soil in soil horizons with higher carbon availability, regardless of microbial diversity, and oxidative enzyme activity per gram dry soil did not vary significantly with carbon availability, soil horizon, or glacial drift. This suggests that microbial communities are able to produce hydrolytic enzymes proportionally to the available soil carbon in active layer and permafrost soil horizons, regardless of the decline of microbial community diversity with depth.

Introduction

Arctic permafrost soils, while limited to around 20%-25% of the Northern Hemisphere, store approximately 50% of the estimated soil carbon pool (Hugelius et al., 2020; Mishra et al., 2021). Permafrost soils have been frozen for at least 2 consecutive years (Van Everdingen, 1998), though some permafrost soils in North America are up to 800,000 years old (Froese et al., 2008). Permafrost is overlain by an active layer, which experiences seasonal freeze-thaw cycles (Ollivier et al., 2014; Schnecker et al., 2014; Wagner et al., 2005). The frozen conditions in permafrost preserve intact organic matter (Coolen & Orsi, 2015; Hobbie et al., 2000), creating a unique habitat for soil microbial communities. While there are often large amounts of organic matter available for permafrost microbes to break down, the microbial communities must contend with freezing temperatures. To access organic matter stored in the soil, microbes produce different extracellular enzymes that target different nutrients or substrates (Sistla & Schimel, 2012). Despite the extreme cold and annual freeze-thaw cycles, microbial communities in active layer soils produce these enzymes on the same order of magnitude per gram soil as soil microbial communities from ecosystems in milder climates (Wallenstein et al., 2009). Improving the understanding of how composition and potential extracellular enzyme activity (EEA) of permafrost microbial communities respond to environmental variables will improve predictions of the impact of these communities on permafrost soil ecosystems in the context of Arctic warming and permafrost thaw. Two particularly important characteristics of the permafrost soil environment are depth and glacial history.

Soil microbial communities are well-known to respond to depth. In montane soils, depth differences had an equivalent magnitude to differences seen between surface soils from entirely different biomes (Eilers et al., 2012). This observation is not surprising, as abiotic factors known

to control microbial community structure such as temperature, pH (Deng et al., 2015; Eilers et al., 2012), carbon availability (Frank-Fahle et al., 2014; Rumpel & Kögel-Knabner, 2011; White et al., 2004), and nitrogen availability (Deng et al., 2015; Frank-Fahle et al., 2014; Rumpel & Kögel-Knabner, 2011; Schnecker et al., 2014) can all change with depth. Often, there is more labile carbon in organic active layer, and availability decreases with depth moving towards the permafrost (Frank-Fahle et al., 2014; White et al., 2004). This pattern is complicated by cryoturbation, where over time, freeze-thaw cycles lead to the subduction of more nutrient-rich active layer soils into deeper mineral layers (Bockheim, 2007; Kaiser et al., 2007). This vertical heterogeneity is a key difference between depth gradients in permafrost-affected soils and non-permafrost soils, which makes nutrient gradients more complicated than in other ecosystems. For example, the top layer of permafrost may be more chemically similar to organic active layer soils at the top of a soil profile than the mineral active layer directly above the permafrost (Ernakovich et al., 2015). While there is a large amount of carbon stored in permafrost (Mishra et al., 2021), it is possible that this organic matter may resist microbial decomposition because the carbon in permafrost is more difficult to decompose than in active layer soils (Wagner et al., 2005).

Despite the heterogeneity in nutrient availability that is common across depths in permafrost soils, microbial communities in these soils tend to follow similar patterns of microbial diversity and abundance as are found in lower latitudes. Namely, diversity and abundance decrease with depth. Active layer soils are found to have more diverse microbial communities than permafrost (Deng et al., 2015; Frank-Fahle et al., 2014; Jansson & Taş, 2014; Ollivier et al., 2014; Steven et al., 2008; Tripathi et al., 2018; Wang et al., 2022). Additionally, there is often a decrease in microbial abundance in deeper permafrost soils (Müller et al., 2018; Ollivier et al., 2014; Steven et al., 2008). EEA has also been observed to decrease in permafrost

soils compared to active layer soils (Varsadiya et al., 2022; Waldrop et al., 2010), but there are fewer studies of EEA in permafrost compared to other soil ecosystems. Additionally, in studies of EEA in both permafrost and temperate soils, many studies focus on samples taken from the top 15-20 centimeters of soil (Steinweg et al., 2013). However, carbon is stored in arctic soils below that depth in both active layer and permafrost, and microbial communities may be producing extracellular enzymes to access this carbon. EEA may be responsive to microbial community composition (Schnecker et al., 2014), and while it is known that microbial communities vary with depth in permafrost soils, the limited studies of EEA deep in permafrost soils prevents an understanding of whether the change in microbial communities correlates with a change in degradation potential. The potential EEA of microbial communities at different depths in Arctic soils is important because large amounts of organic matter can be stored deep in permafrost and is not restricted to the upper active layer.

Soil formation is another factor impacting soil biota, and as such the time since an Arctic landscape was deglaciated may also structure microbial communities. At these study sites in northern Alaska, the glacial drift indicates the amount of time the soil has been forming. Parent material in the northern foothills of Alaska has overall similar mineralogical properties regardless of glacial age (Bockheim et al., 1998; Munroe & Bockheim, 2001; Ping et al., 2005). Despite this, in older landscapes in this region, there are higher rates of microbial biomass and activity in the active layer (Whittinghill & Hobbie, 2011), and greater rates of litter decomposition, attributed to a lower pH at older sites (Hobbie & Gough, 2004). Soils that were deglaciated over 100,000 years ago in northern Alaska have been observed to be more consistently acidic than younger soils (Hobbie & Gough, 2002; Munroe & Bockheim, 2001; Whittinghill & Hobbie, 2011). On the North Slope of Alaska, more recently deglaciated

landscapes are dominated by moist non-acidic tundra, and older landscapes are dominated by moist acidic tundra (Hobbie et al., 2005; Hobbie & Gough, 2002; Munroe & Bockheim, 2001; Whittinghill & Hobbie, 2011). Because of this, some of the previously observed differences between microbial communities with regards to time since deglaciation may have been driven by differences in pH, because pH is an influential structuring factor across deglaciation gradients (Alfaro et al., 2017; Freedman & Zak, 2015) and soils in general (Fierer, 2017). Improving the understanding of whether previous findings about microbial response to landscape age were primarily due to the effect of pH, or if they reflect a distinction between microbial communities from soils located on different glacial drifts, will help determine if glacial drift is an important factor for predicting how microbial communities will respond to changing Arctic conditions.

To investigate the impact of the environmental gradients of soil depth and glacial history on microbial community diversity and potential EEA, I sequenced the microbial community and measured the potential activity of three carbon-cycling enzymes in active layer and permafrost cores from sites at three different glacial drifts all located on moist acidic tundra in northern Alaska. The goal was to determine if glacial drift or depth and soil horizon more strongly predict microbial community diversity and potential EEA in acidic soils. Understanding how these variables impact the ability of microbial communities to degrade carbon will help clarify how organic matter is processed throughout a permafrost soil profile. My hypotheses were as follows:

1) Microbial communities will be more strongly influenced by depth than by time since deglaciation, because abiotic and biotic variables will likely differ more strongly by depth than with deglaciation given that all my study sites are located on moist acidic tundra.

2) Potential extracellular enzyme activity will be greatest in organic active layer soils, because there is a more diverse, abundant microbial community and more chemically

available carbon for microbes to decompose than in mineral active layer and permafrost soils.

Methods

Site selection

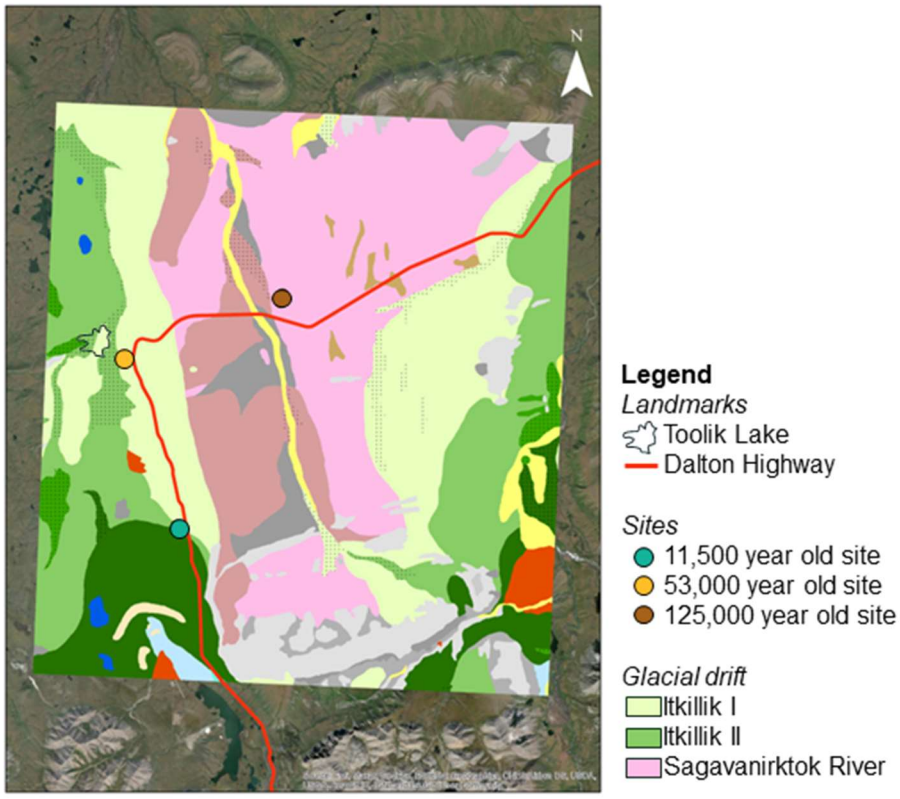

Figure 1. Study site locations on different glacial drifts near the Toolik Field Station in northern Alaska, USA. Glacial drift map is superimposed over satellite imagery of the study region. Map created by Fernando Montaño López.

Table 1. Site characteristics. All study sites were on Moist Acidic Tundra (MAT). Precise location was recorded in the field and used to determine the ages and glacial drifts (Hamilton, 2003). The mean pH and mean percent C is a mean of all active layer and permafrost samples taken at each site. More detailed pH data can be found in Appendix Figure A1.

	Time since deglaciation	Glacial drift	Location	Mean % C	Mean pH
Itkillik II	11,500 – 25,000 years	Itkillik II	68.54975, -149.49432	22.6% (14.3%)	5.06 (0.47)
Itkillik I	53,000 - 66,000 years	Itkillik I	68.624661, -149.5707	7.07% (9.52%)	4.63 (0.27)
Sagavanirktok River	125,000 – 728,000 years	Sagavanirktok River	68.653505, -149.381139	15.9% (15.8%)	4.35 (0.21)

Three sites located on glacial drifts of three different ages were selected for sampling. All three study sites were on Moist Acidic Tundra vegetation. Soil pH has a strong influence on microbial community composition in arctic soils (Wang et al., 2022), so sites with a similar pH were selected to allow me to discern with more detail whether time since deglaciation has a strong structuring effect on microbial communities (Appendix Figure A1).

Sample collection and processing

In August 2021, three sites were sampled. At each site, the sampling team selected four plots approximately 100 meters away from each other and took two permafrost cores and one active layer soil profile at each plot. For this project, active layer was defined as soil that was unfrozen when sampled, and permafrost was defined as soil that was frozen when sampled. Because sampling occurred in mid-August, it is possible that some of the samples defined as permafrost would have thawed later in the season. The permafrost cores were taken as deep as possible (ranging 30 cm – 60 cm below boundary of the unfrozen soil) and immediately placed in a cooler. At each site, active layer samples were collected with a trowel at 5-centimeter increments. Samples were maintained between -5°C and -15°C prior to downstream processing.

Permafrost cores from the three sites were brought back to the University of New Hampshire. These permafrost cores were aseptically scraped to remove any microbial contamination from instruments or between depths and sawed into pucks at roughly five-centimeter intervals in a cold room maintained between -5°C and -15°C. The frozen wet weight of each sample was measured, and samples were stored in individual Whirl-Pak bags in a -80° C freezer.

Each sample was freeze dried in a lyophilizer to remove all water from the sample while preserving the microbial community and extracellular enzyme pool present at the time of sampling. The dry weight of the sample was recorded after freeze-drying. The deeper of the two cores from each plot was selected for inclusion in this project, for a total number of 148 samples.

Bulk C for each sample was measured on an elemental analyzer (ThermoFisher Scientific, Bremen, Germany), which was verified with cocoa and peach standards every 10 samples and duplicates every 10 samples.

Enzyme assays

Enzyme assays were performed on freeze dried soils. EEA was assessed for two hydrolytic enzymes, beta-xylosidase (BX) and cellobiohydrolase (CBH), and one oxidative enzyme, phenol oxidase (POX). These enzymes were selected because they are carbon-cycling enzymes that target three different aspects of carbon decomposition which have been observed to vary in northern permafrost soils (Lynch et al., 2023; Schnecker et al., 2014, 2015; Varsadiya et al., 2022; Waldrop et al., 2010).

Table 2. Enzyme assay methods.

	Beta-xylosidase (BX)	Cellobiohydrolase (CBH)	Phenol oxidase (POX)
Type	Hydrolytic	Hydrolytic	Oxidative
Purpose	Break down hemicellulose	Convert cellulose into disaccharides	Assist in the degradation of polyphenols
Substrate	4-Methylumbelliferyl-β-D-xylopyranoside	4-Methylumbelliferyl-β-D-cellobioside	3-(3,4-dihydroxyphenyl)-L-alanine (L-DOPA)
Concentration of substrate used	500 μM	200 μM	25 mM
Incubation time	45 min	45 min	24 hours
Incubation temperature	4°C	4°C	4°C
Excitation wavelength	360 nm	360 nm	N/A
Emission wavelength	460 nm	460 nm	N/A
Absorbance	N/A	N/A	450 nm

The protocol for enzyme assays was adapted from DeForest (2009), Saiya-Cork et al. (2002), and Whalen (2016). V_{max} testing was conducted to determine the ideal incubation time and substrate concentration for hydrolytic enzyme assays. Incubation time and substrate concentration for the oxidative enzyme assays was determined based on prior enzyme assays performed on permafrost samples.

For each soil sample, a soil slurry was created by blending either 0.5 g (for active layer or organic soil) or 1.0 g (for mineral soil) with 125 mL of 50 mM sodium acetate buffer with a pH of 5.0. The soil slurry was mixed on a stir plate, to ensure consistency during pipetting into well plates. The samples were plated according to the plate layouts in Appendix Table B1 and Appendix Table B2 and measured in a BioTek Synergy H1 Microplate Reader according to methods outlined in Table 2. The calculations of the EEA were done according to the methods in Appendix B.

DNA extractions, PCR, and sequencing

DNA extractions were performed by adapting the MagMAX Microbiome Ultra Nucleic Acid Isolation Kit for extracting soils using 96-well bead plates on the KingFisher Flex. A previous comparison of this kit's results to those from 96-well bead plate extractions with the Qiagen PowerSoil DNA isolation kit found that, for the purpose of characterizing microbial community composition, there was little bias introduced by the extraction protocol (Shaffer et al., 2021).

Samples were randomly ordered before being weighed out into one of two 96-well MagMAX Microbiome Bead Plates. After being freeze dried, these samples carried a strong static charge. To reduce issues with the statically charged samples "jumping" into other wells while loading the extraction plates, all wells except for the well being loaded were covered with labelling tape, and the samples were treated with a Milty Zerostat 3 Anti-Static Gun before being loaded into the sample well. Because of the different volume and density of organic and mineral samples, I used 200-250 mg of mineral soil samples and 40-60 mg of samples with a large amount of organic plant matter for the DNA extraction, as suggested in protocols for the extraction of DNA from plant matter using the KingFisher Flex (Anderson et al., 2018).

After being weighed out, I added lysis buffer, sealed the plate, and agitated it for 2-5 minutes. After agitation, sample plates were spun down at 2,500 rpm for 5-10 minutes and moved to the KingFisher Flex immediately for extraction. 44 extraction blanks were randomly distributed across the two 96-well plates. Plate extractions were then performed on the KingFisher Flex following the standard MagMAX_Microbiome_Soil_Flex program. This extraction protocol requires 53 minutes on the KingFisher, all of which is automated except for one step where the sample plate is temporarily removed to add binding bead mix to each well of the plate. At the end of the extraction, the elution plate with extracted DNA was stored at -80°C.

To describe the bacterial and archaeal communities, I used PCR to amplify the V4-V5 region of the 16S rRNA gene using the primers 515F and 926R (Parada et al., 2016). For each 12 µl reaction, I added 0.7 µl of 5 µM forward primer, 0.7 µl of 5 µM reverse primer, 2 µl of template DNA, 6 µl of DreamTaq hot start (green) (Thermo Fisher Scientific, Waltham, MA), and 2.6 µl of H_2O solution containing 0.5 µg/µl of bovine serum albumin (BSA) to reduce potential PCR inhibitors (Kreader, 1996). I maintained a no template control blank set up the same as the DNA sample wells, but without any template DNA, as well as a negative control blank containing only nuclease free water. The thermal cycling protocol was a 180 second HotStart at 95˚C, followed by 35 cycles of 30 seconds at 95˚C (denaturation), 30 seconds at 55˚C (annealing), and 60 seconds at 72˚C (extension), followed by a final step for 12 minutes at 72˚C. PCR products were sent to the UNH Hubbard Center for Genome Studies for barcoding with Illumina's Nextera barcode system, and sequenced with 2x250 bp paired-end sequencing using the Illumina Novaseq 6000 (Illumina, San Diego, CA, USA).

Bioinformatic analyses

Bioinformatic analyses were done with R Version 4.3.1 (R Core Team, 2023) and use of UNH's PREMISE computing cluster. Amplicon sequencing data was received from the UNH Hubbard Center as demultiplexed FastQ files. Remaining adapters were removed using cutadapt before ASV assignment using the dada2 pipeline. I used a version of the dada2 pipeline (Callahan et al., 2016) adapted for use with NovaSeq error rates (Holland-Moritz et al., 2023) to process samples. Primers were removed from sequences using cutadapt, and any reads lower than 20 bp were removed. Quality plots were used to determine the location to trim the base pairs. Forward and reverse reads were both trimmed to 225 base pairs. The error rates were binned, and the results of different error rate learning functions were compared to determine the

best function for this data. The best function was option one, which was used to approximate observed error rates and assign the sequence variants to the data. The samples were pseudo-pooled. Then, the dada2 pipeline was used to dereplicate samples, remove chimeras, and assign taxonomy using Silva db v138. For these samples, the original sequencing depth ranged from 0 to 560,900 reads, with an average sequencing depth of 65,800 reads.

Using the R package phyloseq (McMurdie & Holmes, 2013), I filtered out any taxa categorized as mitochondria at the family level and chloroplast at the order level, as well as all taxa that were unassigned at the phylum level. Additionally, I removed all samples with a sequencing depth less than 10,000 reads. After samples with fewer than 10,000 reads were dropped, the average sequencing depth was 125,300 reads. These samples were then normalized to the median sequencing depth before statistical analysis.

Contamination of blanks

Ultimately, after being filtered through the dada2 pipeline and removing all samples with fewer than 10,000 reads, there were 54 soil samples, 14 extraction blanks, and 0 PCR blanks remaining of the 148 samples, 42 extraction blanks, and 2 PCR blanks that were sequenced. When the 14 blanks were plotted in a Principal Coordinate Analysis (PCoA) ordination with the sample data, the blanks were spread across a similar distribution as the sample data, suggesting contamination in the DNA extraction (Appendix Figure C1). There were three general clusters of blanks in the PCoA, which correlated with three different areas where those blanks were clustered on the two 96-well extraction plates.

The suspected source of contamination in these blanks is that the freeze dried soil samples carried a strong static electric charge, which may have led to small amounts of some samples contaminating other wells across the plate. However, there were still 28 other extraction

blanks across the two plates that were not contaminated, so I plotted a PCoA ordination of the community data to see if there seemed to be patterns based on age, soil depth, and soil horizon (Figure 6). The samples do not group by age, but they do group by both soil horizon and soil depth. It is highly unlikely that if the entirety of both extraction plates were contaminated randomly, I would see the soil horizon and soil depth groupings displayed in Figure 6.

I determined that the soil depth signal in the PCoA of the microbial community data was not correlated with the sequencing depth of the samples. Additionally, to check if the entire dataset was strongly contaminated, I compared the microbial community data in the active layer and permafrost to rhizosphere microbial community data provided by a collaborator on this project who had already sequenced rhizosphere samples from these same sites. When plotted on an NMDS, the active layer and permafrost community data had the same spread on the first axis of the NMDS as the rhizosphere community data and were primarily separated on the second axis of the NMDS (Appendix Figure C2). Additionally, the rhizosphere community data clustered more closely to the active layer and permafrost community data than about half of the contaminated blanks from this project. Based on this process, I chose to continue with a preliminary analysis of the microbial community data, with the knowledge that there is some contamination represented in the sample data.

Statistical analysis of enzyme activities

The statistical analysis of the EEA for hydrolytic and oxidative enzymes was done for the EEA per gram dry soil and the EEA per gram C of each sample. First, I defined a full core as 0 cm – 60 cm below the surface of the soil for analysis, because not all sites had samples taken from depths greater than 60 cm. Two outliers were removed from the analysis after performing 2-way ANOVAs for the activity of all three enzymes, first excluding the outliers (Appendix

Table D3) and then including the outliers (Appendix Table D4). I used the aov function from the vegan package (Oksanen et al., 2022) to perform 1-way ANOVAs and compare the means of each enzyme across the different sites. For each enzyme, the model structure was EEA_perc ~ age * depth_bin. Age and depth were both treated as categorical variables. Because the significance of the results was the same regardless of whether the outliers were included or excluded, an outlier from the Itkillik I site and an outlier from the Sagavanirktok River site were removed from the rest of the analysis.

To investigate the patterns in EEA more closely, each sample was categorized into the following soil horizons: organic active layer, mineral active layer, organic permafrost, or mineral permafrost. The organic/mineral distinction in the active layer was based on the average depth of the organic horizon recorded at time of sampling in the field. The organic/mineral distinction in the permafrost was based on a threshold of 12% C; samples below 12% C were classified as mineral and samples above 12% C were classified as organic. This cutoff was chosen because a histogram of the % C values of permafrost samples showed that there was a group of samples with less than 12% C, and then a group of samples with greater than 16% C. These two methods of determining organic vs. mineral soil horizons aligned; the % C values in the organic active layer and organic permafrost were not statistically different from each other, but they were statistically different from the percent C values in mineral active layer and mineral permafrost (Appendix Figure A2a). There were significant differences in the mean percent C across different glacial drifts: Itkillik I had the lowest percent C, and Itkillik II had the highest percent C (Appendix Figure A2b).

Because these are cryoturbated permafrost soils, these soil horizons are not all found at the same depth across sites, or even at the same depth for cores at the same site. Additionally,

some cores have more than one organic permafrost horizon or more than one mineral permafrost horizon. There were only organic permafrost horizons at the 11,500 year old and 125,000 year old sites, so the organic permafrost horizon was omitted from statistical analysis when using ANOVAs to understand how soil horizon impacted EEA. However, mean EEA data in organic permafrost is included in figures showing means across soil horizons.

I used these soil horizon distinctions to categorize the samples from all 12 soil cores (4 per glacial drift). Then, for each horizon in each core, I calculated the mean activity of each enzyme per gram C and summed up the total activity of each enzyme per gram C within the horizon. Because the horizons were not all the same thickness, I corrected the enzyme sums on a per centimeter basis. Then, I used the aov function from the vegan package (Oksanen et al., 2022) to conduct 2-way ANOVAs to assess the effect of soil horizon and age of site on the activity of each of the three enzymes, as well as the effect of these variables on the percent C of each sample. For each enzyme, the model structure was EEA_mean ~ age * horizon or EEA_sum ~ age * horizon. I also conducted these analyses on the percent C data to see how carbon in each sample responded to age and soil horizon.

Statistical analysis of microbial communities

Using the R package phyloseq, I split the microbial community data based on domain into bacterial and archaeal communities. For each domain, I then calculated the relative abundance of the community with depth and calculated three metrics of alpha diversity for each sample: observed richness, Shannon index, and CHAO1 index (estimate_richness from the phyloseq package). I took the means and standard deviations of the Shannon index of diversity by age and by soil horizon, and conducted 2-way ANOVAs to assess the effect of soil horizon and age of site on the archaeal and bacterial alpha diversity. The organic permafrost horizon was

removed from the analysis based on soil horizon, because only one sample categorized as organic permafrost was successfully sequenced.

To assess beta diversity, I used ordinate from the R package phyloseq to run a Principal Coordinate Analysis (PCoA) using a Bray-Curtis dissimilarity matrix. Additionally, I ran several PERMANOVAs using the function adonis2 from the R package vegan (Oksanen et al., 2022) to assess the response of the microbial community data to the variables of age, depth, and soil horizon. I also used the function betadisper from the vegan package to analyze the multivariate homogeneity of group dispersions. Finally, I used the vegdist function vegan package to create Euclidean distance matrices for the continuous depth variable and the continuous age variable so I could use the function mantel to run two separate Mantel tests. The first compared the Euclidean distance matrix of depth to the Bray-Curtis dissimilarity matrix of the microbial community, and the second compared the Euclidean distance matrix of age to the microbial community matrix.

Results

Carbon-cycling enzyme activity

EEA varied with depth and across sites (Appendix Figure D1, Figure 2). When calculated on a per gram dry soil basis (Figure 2), hydrolytic EEA tended to decrease with depth, with some variation. The mean EEA in organic active layer and organic permafrost was significantly different than in mineral active layer and mineral permafrost; the mean BX activity in organic horizons was about 9 times higher than in mineral horizons and the mean CBH activity in organic horizons was about 14 times higher than in mineral horizons (Table 4). In soils with more organic matter, such as at the 11,500 year old site, the hydrolytic enzyme activity remained higher at greater depths before the transition into mineral soils. However, the mean hydrolytic

EEA per gram soil was not significantly different at sites of different ages (Table 3). When calculated on a per gram C basis, hydrolytic EEA was not significantly different at sites of different ages (Appendix Table D1), though for both BX and CBH, the average activity was highest at the 53,000 year old site, second highest at the 125,000 year old site, and lowest at the 11,500 year old site. Across all ages, hydrolytic EEA (BX & CBH) was higher in organic active layer and, where present, organic permafrost than in mineral active layer or mineral permafrost, but these differences were not statistically significant (Appendix Table D2, Figure 2b and 3c).

The oxidative enzyme POX per gram soil did not differ with depth or at different sites (Appendix Figure C1c). The mean POX activity was between 6.7 – 7.3 μmol g $soil^{-1}$ hr^{-1} across the different sites (Table 3), and between 6.8 – 7.2 μmol g $soil^{-1}$ hr^{-1} across the different soil horizons (Table 4). When expressed per gram C instead of per gram soil, POX responded significantly to depth, age, and soil horizon in different statistical tests. At the 53,000 year old site, POX activity per gram C was 1.9 times higher than at the 125,000 year old site, and 3.6 times higher than at the 11,500 year old site (Appendix Table D1). Across all sites, POX activity was higher for mineral active layer and mineral permafrost than organic active layer and organic permafrost (Appendix Table D2, Figure 2). It seems, however, that this is due to the relationship between these variables and percent carbon. The carbon data follows the same trends as POX activity per gram C: it responds to the soil horizon and age across the full core (Appendix Figure A2), and the mean percent carbon responds to age and horizon in a 2-way ANOVA (Appendix Table D5). Because the mean bulk POX activity per gram dry soil is similar at each glacial drift when the POX EEA is considered per gram carbon, the statistically significant patterns are likely a product of the patterns in the carbon data by glacial drift (Appendix Figure D3c).

Two-way ANOVAs were performed to assess the effect of soil horizon (organic active layer, mineral active layer, or mineral permafrost – organic permafrost was excluded from this 2-way ANOVA because it was only present at 2 of 3 glacial drifts) and glacial drift on the activity of each enzyme per gram C (Appendix Table D5). Neither BX activity nor CBH activity responded significantly to age or soil horizon. The mean POX activity per gram C responded to both age (P = <0.001) and horizon (P = <0.001), and the sum of POX activity also responded to age (P = <0.001) and soil horizon (P = <0.001). This analysis was repeated for the EEA per gram dry soil (Appendix Table D7); for both hydrolytic enzymes, the mean EEA per gram dry soil responded significantly to soil horizon but did not respond to age. The mean POX activity per gram dry soil did not respond significantly to either age or horizon.

Table 3. Means and standard deviations of EEA per gram soil by age. Letters in a column denote statistically different groups for the mean of that EEA across the 0 – 60 cm core, as per Tukey HSD.

	Mean BX activity (nmol g soil^{-1} hr^{-1})	Mean CBH activity (nmol g soil^{-1} hr^{-1})	Mean POX activity (μmol g soil^{-1} hr^{-1})
11500	88.7 (171.0) *a*	68.3 (122.5) *a*	6.7 (2.3) *a*
53000	48.3 (110.2) *a*	29.8 (72.9) *a*	7.3 (1.4) *a*
125000	106.0 (244.2) *a*	96.7 (374.7) *a*	6.9 (2.3) *a*

Table 4. Means and standard deviations of EEA per gram dry soil by soil horizon. Letters in a column denote statistically different groups for that EEA across the 0 – 60 cm core, as per Tukey HSD.

	Mean BX activity (nmol g soil^{-1} hr^{-1})	Mean CBH activity (nmol g soil^{-1} hr^{-1})	Mean POX activity (μmol g soil^{-1} hr^{-1})
Organic active layer	180.0 (262.7) *a*	143.2 (390.7) *a*	6.9 (2.5) *a*
Mineral active layer	20.5 (31.5) *b*	8.5 (12.4) *ab*	6.8 (1.9) *a*
Organic permafrost	175.0 (286.2) *a*	164.3 (189.6) *a*	7.2 (2.4) *a*
Mineral permafrost	19.2 (24.9) *b*	14.1 (33.3) *b*	7.2 (1.7) *a*

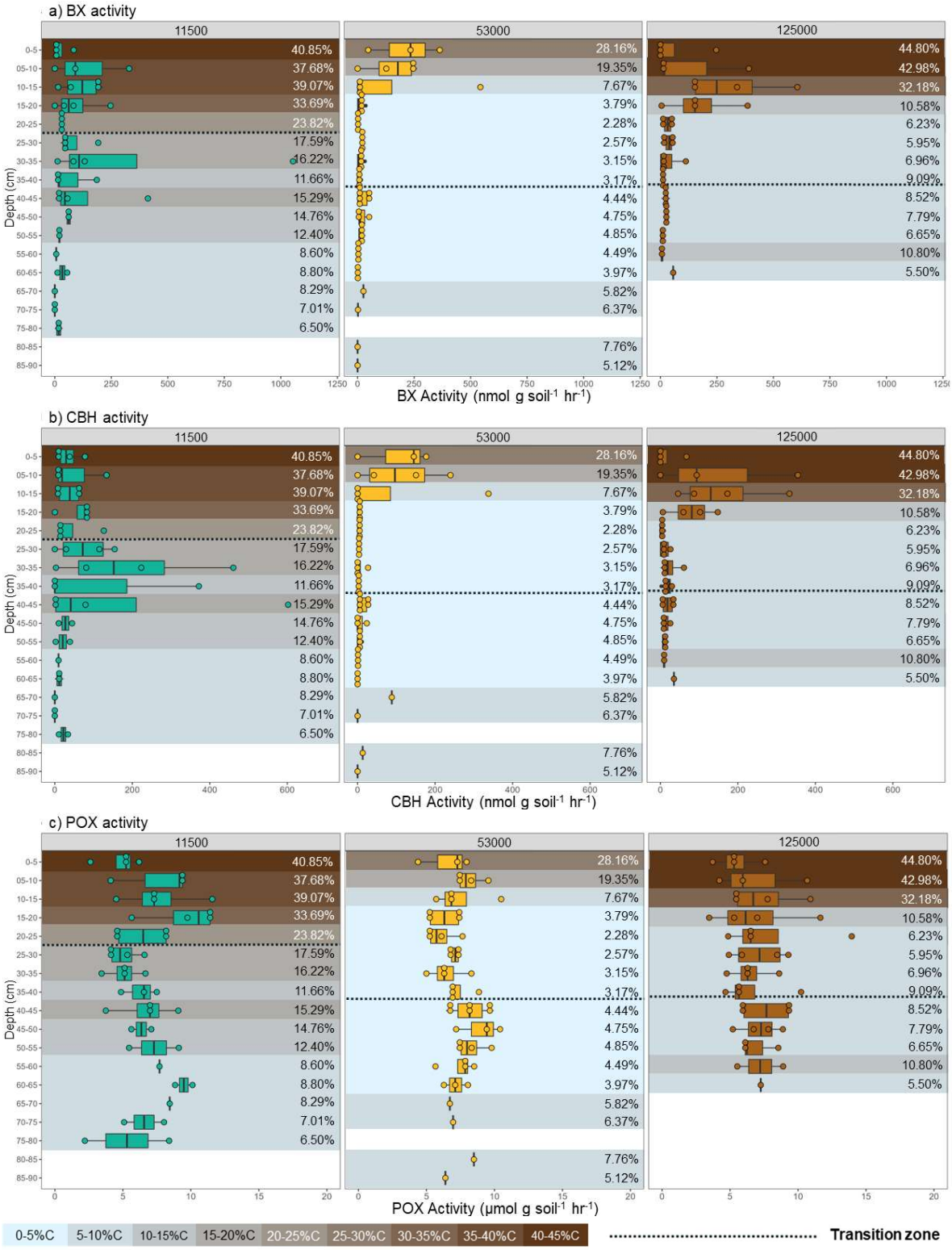

Figure 2. EEA in nmol (BX, CBH) or µmol (POX) per gram dry soil per hour at each site. Each point represents a sample. The box displays the median between the 25th and 75th percentiles. The whiskers extend to display a 95% confidence interval. The dashed line represents the transition zone between active layer and permafrost. The percent C values on the right of each panel represent the mean percent C of all samples taken in each 5-cm increment at each site. The color scale represents the gradient from low percent C in light blue to high percent C in dark brown.

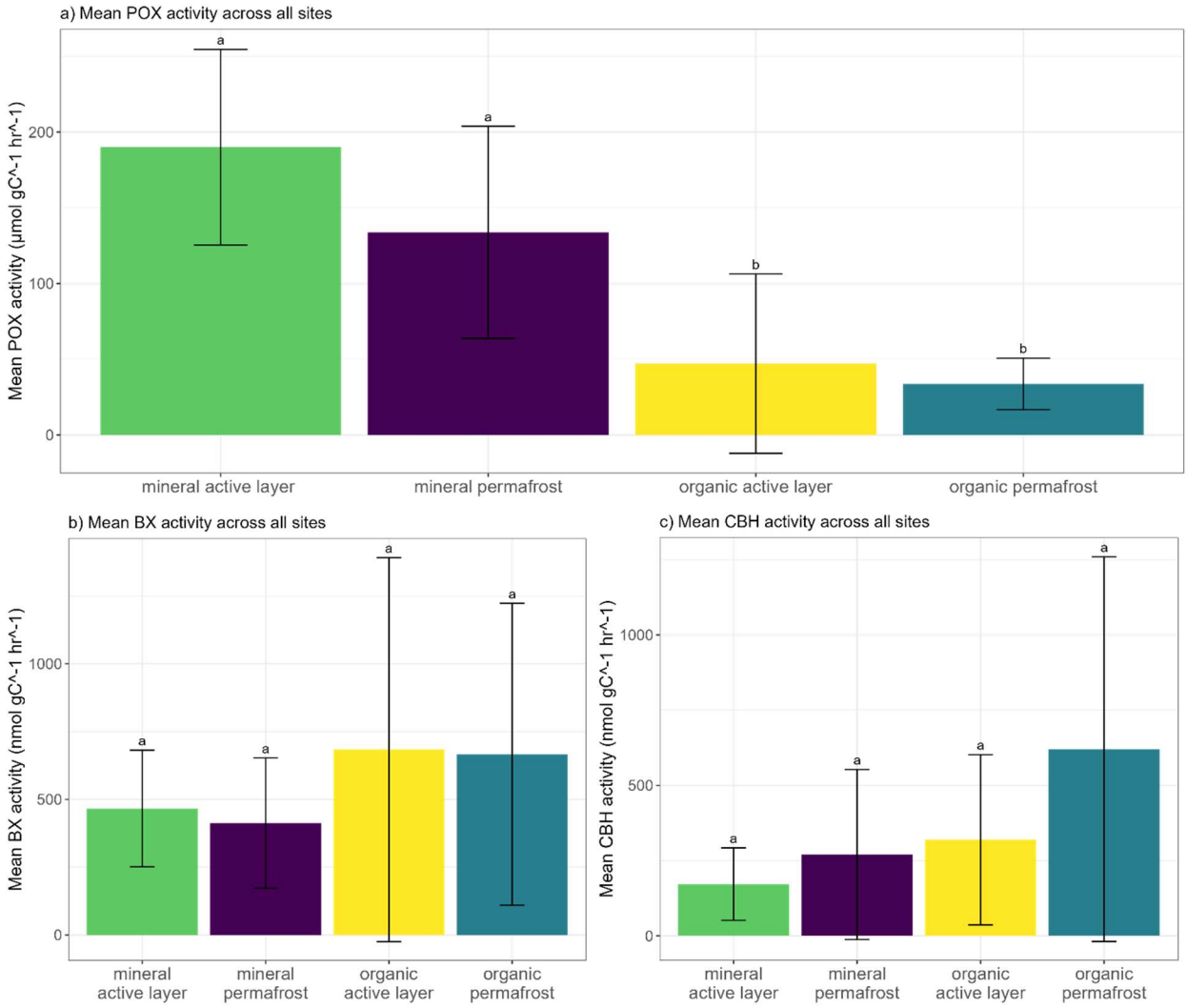

Figure 2. Mean activity per gram C of each enzyme in each soil horizon. Error bars represent one standard deviation. The oxidative enzyme POX was significantly higher in mineral active layer and permafrost than in organic active layer and permafrost. There were no significant differences in activity per gram C for either hydrolytic enzyme, though the organic active layer and permafrost activity was higher for both BX and CBH than in the mineral active layer and permafrost.

Microbial community diversity

Alpha diversity

The alpha diversity of the bacterial community decreased with depth at all ages (Figure 4b). The alpha diversity of the archaeal community was lower than that of the bacterial community and did not show a strong trend with depth (Figure 4a). The relative abundance of different archaeal and bacterial phyla also changed with depth (Appendix Figure E1).

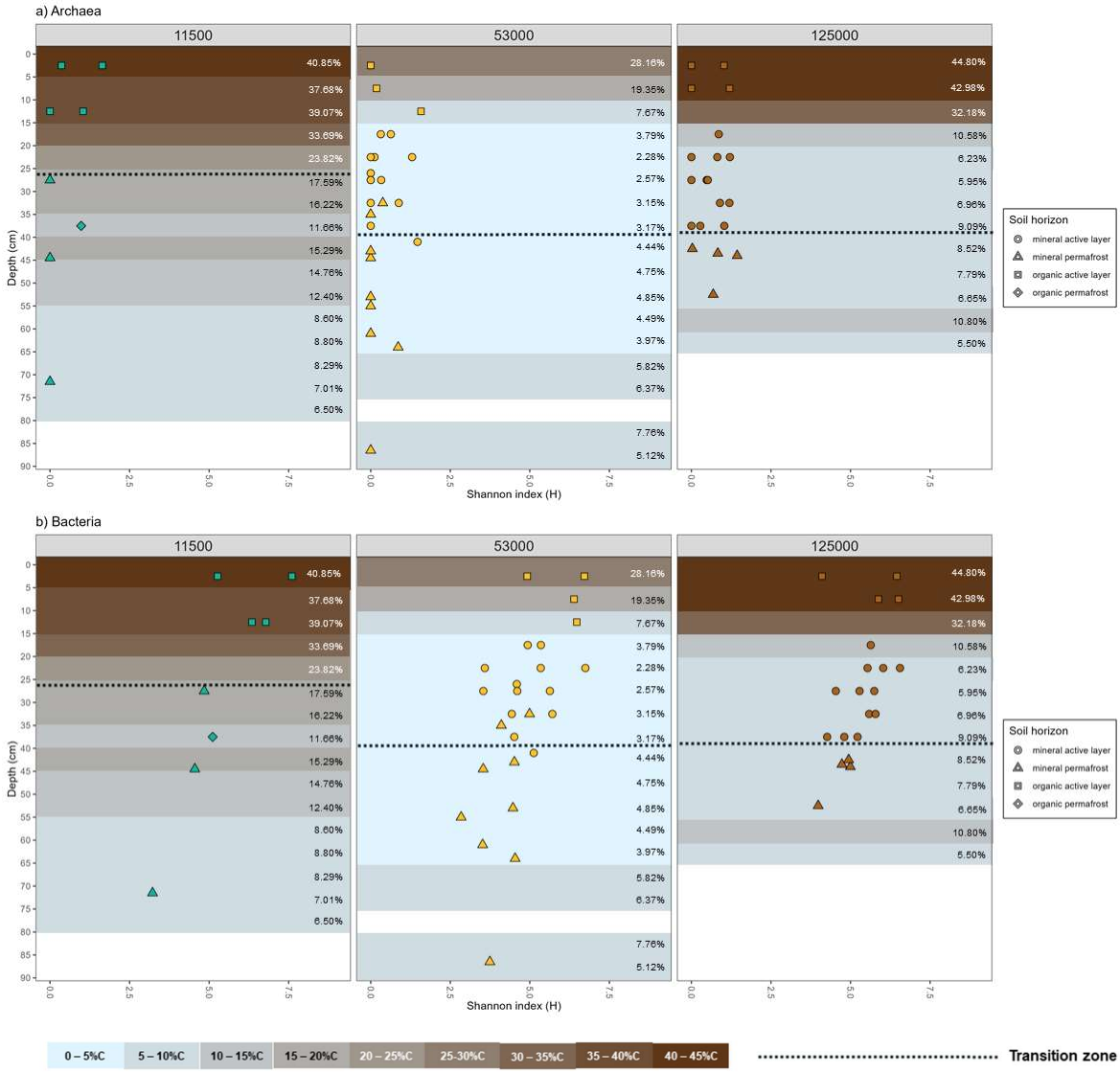

Figure 4. Shannon index of alpha diversity of microbial communities with depth at all three sites. The observed richness (Appendix Figure E2) and CHAO1 index (Appendix Figure E3) of both the archaeal and bacterial communities are available in Appendix E. The percent C values on the right of each panel represent the mean percent C of all samples taken in each 5-cm increment at each site. The alpha diversity of each microbial community is represented as an individual point instead of aggregated into a mean per 5-cm increment because the depth below the surface was treated as a continuous variable.

The alpha diversity of the archaeal community was low relative to the bacterial community and did not differ significantly when grouped by age (Figure 5a) or by soil horizon (Figure 5b). The alpha diversity of the bacterial community was not significantly different when grouped by age (Figure 5c), but there were significant differences when grouped by soil horizon

(Figure 5d). The diversity of the bacterial community was highest in the organic active layer, second highest in the mineral active layer, and lowest in the mineral permafrost (Table 5). In a 2-way ANOVA, the mean Shannon index *H* of archaeal communities did not respond to either age or soil horizon (Appendix Table E1). In contrast, the mean Shannon Index *H* of bacterial communities responded to both age ($P = 0.034$) and soil horizon ($P = <0.001$) in a 2-way ANOVA (Appendix Table E1).

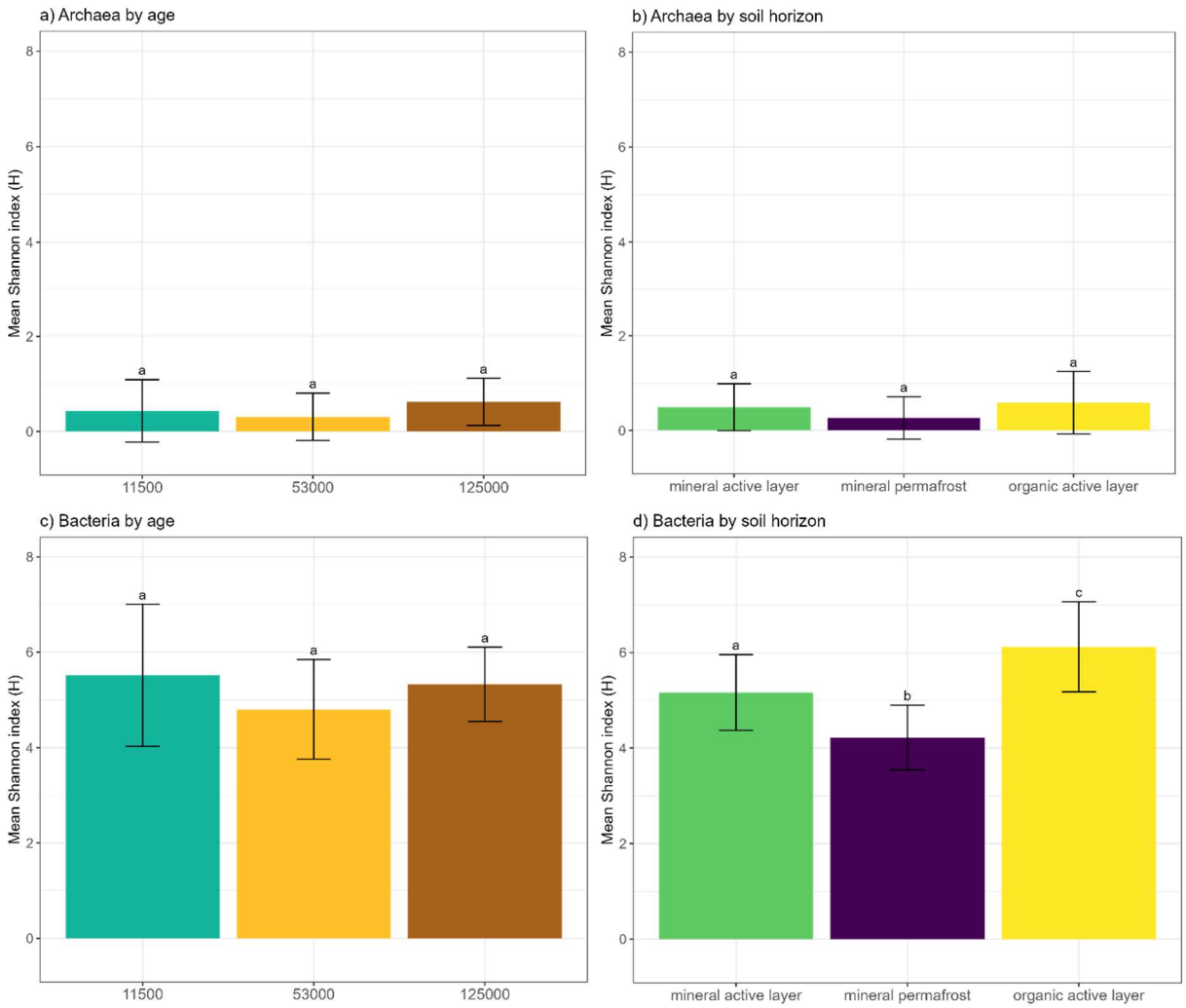

Figure 5. Mean Shannon diversity for bacteria & archaea by soil horizon and glacial drift. Error bars represent one standard deviation. The only significant differences between groups were for bacteria by soil horizon.

Table 3. Means and standard deviations of Shannon index of diversity by soil horizon.

	Bacterial community	Archaeal community
	Shannon index (*H*)	Shannon index (*H*)
Organic active layer	6.12 (0.94) *c*	0.59 (0.66) *a*
Mineral active layer	5.16 (0.79) *a*	0.49 (0.50) *a*
Mineral permafrost	4.22 (0.68) *b*	0.26 (0.45) *a*

Beta diversity

A principal coordinates analysis using the Bray-Curtis dissimilarity measure was done to visualize the spread of the microbial communities. The microbial communities from each sample did not clearly cluster by age of site (Figure 6a), but they grouped together by both soil horizon (Figure 6b) and soil depth (Figure 6c). Soil depth, however, explained more variation in the microbial community diversity data. In a PERMANOVA considering the effect of age and depth expressed as a continuous variable, age and depth were both significant; age explained 4.904% of variation in the community between samples and depth explained 29.334% of variation in the community between samples (Table 6). In a separate PERMANOVA considering the effect of age and soil horizon, age again explained 4.904% of variation in the community between samples and soil horizon explained 13.038% of variation in the community between samples (Table 7). When examining soil horizon in the principal coordinates analysis, organic active layer samples separated from the mineral active layer and permafrost samples on the first axis, and the mineral active layer and permafrost samples then separated on the second axis (Figure 6b). A homogeneity of dispersion test for the microbial data and age was not significant, suggesting the PERMANOVA significance isn't due to differences in group dispersion. However, a homogeneity of dispersion test for the microbial data and depth was significant, as was the case for the microbial data and soil horizon, so PERMANOVA significance for these variables could be due to differences in group dispersion.

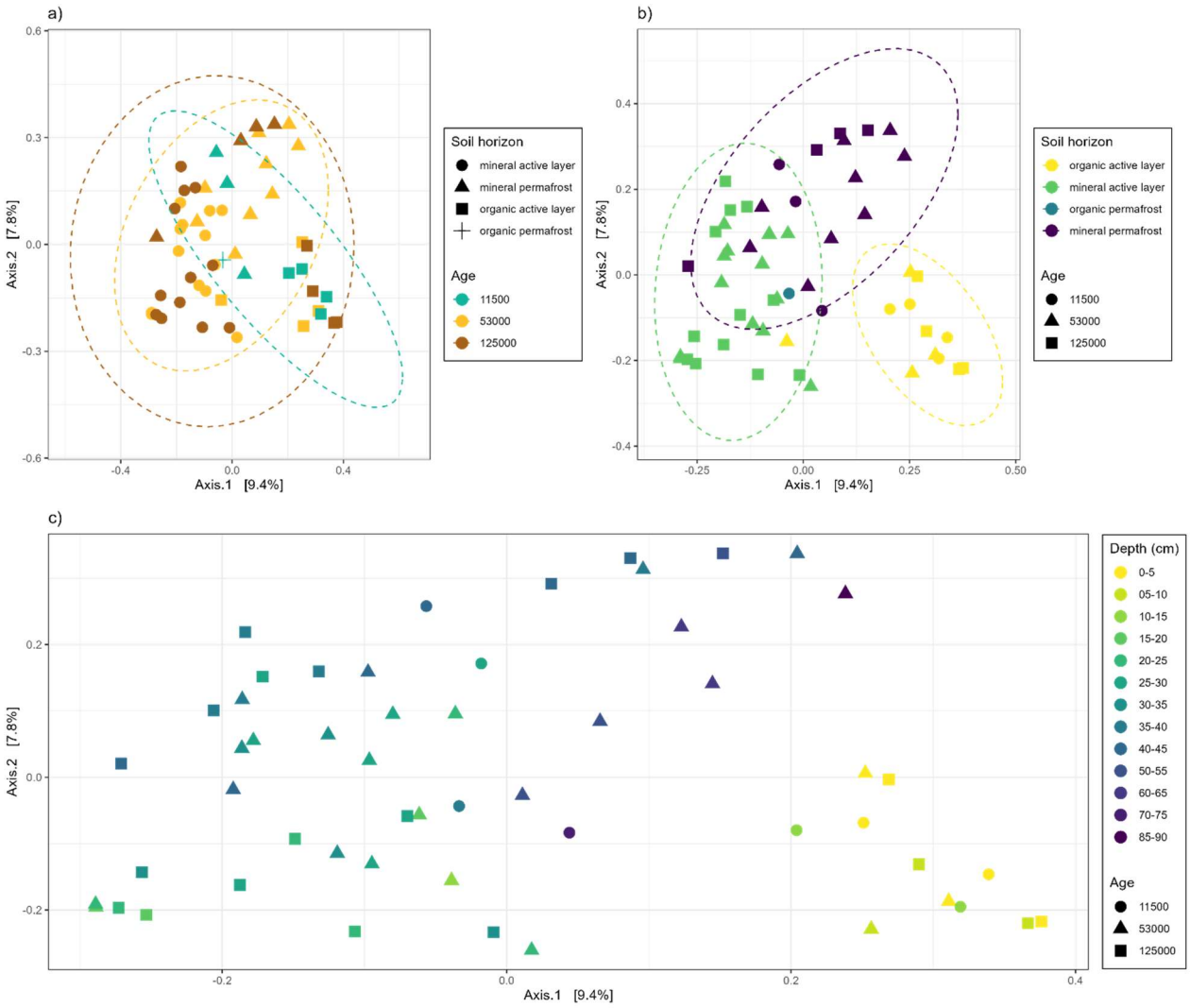

Figure 6. Principal coordinates analysis (PCoA) ordination of the microbial communities across all three sites using the Bray-Curtis dissimilarity measure. Ellipses represent the 95% confidence interval of a multivariate t-distribution of each group. The same PCoA is shown in each panel; 6a shows the microbial communities grouped by age, 6b shows the communities grouped by soil horizon, and 6c shows the communities categorized by 5-centimeter depth increments.

Table 4. Summary of results of PERMANOVA analyzing the response of microbial community beta diversity to age and depth. The structure of this model was physeq1_bray ~ (age+depth_bin). When percent C data was added to this model, it only explained an additional 1.854% of variation and was not statistically significant (Appendix Table E3). When percent C and pH data was added to this model, percent C only explained an additional 1.893% of variation and was not significant, and pH only explained an additional 2.161% of variation and was not significant (Appendix Table E5).

	Df	SumOfSqs	R2	F	Pr(>F)	Significance
age	2	1.0498	0.04904	1.4543	0.004	**
depth	12	6.2788	0.29334	1.4497	0.001	***
Residual	39	14.0763	0.65762			
Total	53	21.405	1			

Table 5. Summary of results of PERMANOVA analyzing the response of microbial community data to age and soil horizon. The structure of this model was physeq1_bray ~ (age+horizon). When percent C data was added to this model, it only explained an additional 2.014% of variation and was not statistically significant (Appendix Table E4).

	Df	SumOfSqs	R2	F	Pr(>F)	Significance
age	2	1.0498	0.04904	1.4344	0.003	**
horizon	3	2.7907	0.13038	2.5421	0.001	***
Residual	48	17.5645	0.82058			
Total	53	21.405	1			

A Mantel test was used to calculate the correlations between differences in the microbial community composition and differences in the variables of age and depth in the soil (Table 8). There was not a significant relationship between the microbial community and age, but there was a significant relationship between the microbial community and soil depth ($P = 0.001$), showing that communities that are more dissimilar in terms of depth (i.e. communities that are further apart in depth) are more dissimilar in the composition of the microbial community.

Table 6. Mantel test results from two separate Mantel tests: one analyzing the correlation between the microbial community dissimilarity matrix and the Euclidean distance matrix for age, and one analyzing the correlation between the microbial community dissimilarity matrix and the Euclidean distance matrix for depth.

	Mantel statistic r	Significance
Age	0.0448	0.163
Depth	0.4965	0.001 **

Discussion

Permafrost microbial communities have the remarkable ability to remain active throughout the soil profile despite the unique challenges posed by these frozen soils. Understanding the variables that structure these communities and their behavior will help me understand how microbes process carbon throughout the permafrost soil profile, and how microbial activity might change as the Arctic changes. My goal was to understand the relative contribution of depth or glacial history in shaping microbial community diversity and their potential EEA in permafrost. Because all my study sites were located under moist acidic tundra with similar parent material, I predicted that depth would be a stronger driver of microbial dynamics than glacial history, as pH and other environmental variables would be more likely to change on the environmental gradient created by depth. I expected that potential EEA would be greatest in organic active layer soils where there was a more diverse and abundance microbial community, and that microbial communities would also be most diverse in surface soils and decrease with depth. I found that potential hydrolytic EEA was driven by carbon availability, and therefore significant along a depth gradient, but potential oxidative EEA did not vary along soil profiles regardless of carbon availability. EEA only sometimes responded to glacial drift. The alpha diversity of microbial communities decreased significantly with depth but was similar across glacial drifts. The beta diversity of microbial communities responded significantly to both soil horizon and depth, and glacial drift explained <5% of variation in the microbial communities.

Hydrolytic and oxidative enzymes have diverging responses to depth, soil horizon, and carbon.

In the soil profile, there were slightly different gradients with depth for hydrolytic EEA at each site. BX and CBH activity were highest in the upper organic active layer for the 53,000 and

125,000 year old sites and peaked below the transition zone in the 11,500 year old site (Figure 2a and 2b). The mean BX and CBH activity per gram dry soil in organic active layer and organic permafrost (Table 4) was very similar to the mean BX and CBH activity reported for organic soil samples taken >5 cm below the surface in summer in another study near the Toolik Field Station, Alaska, USA (Sistla & Schimel, 2013); the mean BX and CBH activity per gram dry soil in mineral permafrost (Table 4) was also on the same order of magnitude as mean BX and CBH activity reported for frozen mineral soil samples in the same study (Sistla & Schimel, 2013). The mean BX and CBH activities recorded in the mineral permafrost were similar to the mean BX and CBH activities recorded in permafrost from under MAT vegetation in Sagwon Hills, Alaska, USA (Lynch et al., 2023). The mean hydrolytic EEA in the soils at my study sites was generally lower than that in non-permafrost systems that have high percentages of organic matter. The mean CBH activity across all sites was about 8 times lower than the CBH activity of samples taken from sphagnum peatlands in Michigan, USA (Wiedermann et al., 2017) and about 4 times lower than in a forested bog in Minnesota, USA (Rewcastle et al., 2020), but on the same order of magnitude as that in drained peat soils in the Netherlands (Brouns et al., 2016).

When considering EEA on a per gram dry soil basis, both hydrolytic enzymes (BX and CBH) responded to soil horizon in a 2-way ANOVA considering the effects of age and horizon (Appendix Table D7). However, once the EEA was normalized to account for the bulk C in each sample, and considered on a per gram C basis, these patterns disappeared. When considering EEA per gram C, the hydrolytic EEA does not respond significantly to age or soil horizon (Appendix Table D5). This suggests that in active layer and permafrost, the potential activity of hydrolytic enzymes is correlated with carbon availability in the soil and may decline with depth if carbon availability declines with depth, which is supported by previous work (Schnecker et al.,

2014, 2015). Microbial communities may be producing these enzymes specifically in response to the availability of carbon, which is supported by the result that the hydrolytic EEA was higher in organic permafrost and organic active layer, and lower in mineral permafrost and mineral active layer, even though the organic permafrost is deeper than mineral active layer (Figure 3b and 3c). Glacial drift was not a significant explanatory variable for hydrolytic EEA per gram C in most instances, as the carbon availability that correlates with the differences in hydrolytic EEA varied more across soil horizons than it does across glacial drift (Appendix Figure A2).

In contrast to the hydrolytic EEA, mean POX activity per gram dry soil did not vary with depth (Figure 2c) and responded significantly to few variables. The POX activity was 2-3 times higher than the mean POX activity recorded in active layer and permafrost in boreal black spruce forests in Alaska, USA (Waldrop et al., 2010), though the POX activity in both studies was less than 10 μmol g $soil^{-1}$ hr^{-1}. POX activity at the study sites was about half of the POX activity in a fen in the Netherlands and about the same as the POX activity in a bog in the Netherlands (Brouns et al., 2016). Phenol oxidase activity can be inhibited by low pH (Tahvanainen & Haraguchi, 2013); the lower values at my study sites may be partially caused by the acidity of the soils. Additionally, my study includes the EEA of samples that were taken from deeper, frozen mineral permafrost soils, while these other studies focused on surface peat and bog layers with more organic material.

POX activity per gram dry soil did not respond significantly to soil horizon or age in a 2-way ANOVA, though it did respond to the interaction term (Appendix Table D7). On a per gram dry soil basis, it appears that the mean POX activity at each individual site is similar regardless of depth or soil horizon. The lack of significant response to depth for the POX activity per gram dry soil is quite interesting. It contradicts my hypothesis that potential activity would be highest

in organic soils, and also suggests that microbial communities throughout this depth profile are producing the same amount of this oxidative enzyme regardless of the environmental variables that change with depth. This is in line with some previous research on phenol oxidase in permafrost, which found that phenol oxidase activity did not change with depth even when hydrolytic enzymes changed with depth (Gittel et al., 2014b; Schnecker et al., 2015), and sometimes even increased slightly with depth (Varsadiya et al., 2022). A potential cause of this finding is that unlike hydrolytic enzymes, oxidative enzymes like phenol oxidase are not produced to target specific compounds or nutrients (Sinsabaugh, 2010), and as such are not responding to changes in carbon or other nutrients with depth. Additionally, this EEA remained constant throughout the soil profile even though the microbial community diversity and abundance decreased with depth, suggesting that either the microbes that produce phenol oxidase do not decrease with depth, or that many different types of microbes are able to produce phenol oxidase (Gittel et al., 2014b).

Microbial community diversity in permafrost at these sites is most strongly driven by depth and does not seem to directly respond to glacial drift.

At the phylum level, bacterial alpha diversity decreased with depth (Figure 4b), which is in line with other studies of bacterial communities in permafrost (Müller et al., 2018; Ollivier et al., 2014; Steven et al., 2008; Tripathi et al., 2018). Archaeal alpha diversity at the phylum level did not show any significant response to depth. Bacterial diversity was greatest in the organic active layer across sites, as expected (Deng et al., 2015; Frank-Fahle et al., 2014; Ollivier et al., 2014; Steven et al., 2008; Wang et al., 2022), and lowest in the mineral permafrost (Figure 5d). In a 2-way ANOVA, the alpha diversity of the bacterial community responded to both soil horizon and glacial drift (Appendix Table E1).

The beta diversity of communities was also responsive to depth. With a Mantel test, I observed that samples that are further apart in depth also have dissimilar microbial communities. In addition, in a principal coordinates analysis (PCoA) ordination, microbial communities grouped by both soil horizon and depth (Figure 6). While each variable was significant when considered alongside age in a PERMANOVA, depth explained 29.334% of variation in the composition of microbial communities (Table 6) and soil horizon explained 13.038% of variation (Table 7). Unfortunately, because only one organic permafrost sample was successfully sequenced, it's impossible to draw conclusions about whether or not the microbial communities in organic permafrost samples differ from communities in other soil horizons. This would have been particularly interesting when considering the question of how cryoturbation might impact the composition of microbial communities in permafrost. Previous work has shown that microbial communities in cryoturbated organic soils can be as abundant as in unburied topsoils (Gittel et al., 2014a; Schnecker et al., 2014). I could not test this pattern in this study, but I hope to re-sequence the organic permafrost samples to better understand how cryoturbation might be impacting microbial communities across depth and glacial drifts. Regardless, the significant response of microbial communities to depth is as expected, because important environmental characteristics such as pH, carbon, and nitrogen availability can vary strongly with depth.

Glacial drift did not have as strong of an effect as depth in structuring microbial communities. The site effect was significant in PERMANOVAs and explained 4.904% of variation, but this was much less powerful than the depth effect, which explained 29.334% of variation, (Table 6) or the soil horizon effect, which explained 13.038% of variation (Table 7). Additionally, a Mantel test considering age as a continuous variable did not show a significant effect of age. Studies in this region of Alaska observed landscape age controls on microbial

activity, but they also noted that this correlated with a pH boundary, where older soils were more acidic than younger soils (Whittinghill & Hobbie, 2011). In this study, all sites were located under moist acidic tundra regardless of their age (Table 1), so while the oldest site was slightly more acidic than the youngest site, pH is not likely to be confounding the response of the microbial community to age. To further confirm this, when pH was included in PERMANOVAs with depth, age, or soil horizon, it only explained about 2% of the variation and was not statistically significant (Appendix Table E5).

Microbial community composition, diversity, and carbon-degrading activity at these sites are driven primarily by depth and carbon availability.

Extracellular enzyme activity and microbial community composition both had limited responses to glacial drift, suggesting that when the pH is constant across a glacial history gradient, glacial drift does not strongly structure microbial community diversity and activity. In the absence of major pH differences with historical glacial drift in this region of northern Alaska, it may not be necessary to focus on glacial drift as a categorical variable structuring microbial community diversity; this may be helpful for future analyses given the complicated glacial history of the landscape (Figure 1). Microbial community diversity and potential EEA are instead most responsive to carbon availability and depth, with the exception of the oxidative enzyme activity, which was consistent throughout the soil profile and did not respond to these variables.

Microbial EEA and community diversity all responded to soil horizon. Soil horizons were defined by the carbon content or mineral nature, and the presence of ice, so these environmental characteristics might be most important for microbial community dynamics. The hydrolytic EEA was slightly more responsive to soil horizon than the microbial community, which may be because the hydrolytic EEA is driven specifically by the presence of carbon, and because the microbial community response to organic permafrost could not be observed due to

the smaller dataset. Resequencing these samples and analyzing the permafrost microbial communities in finer detail along the depth gradient will help me understand if the strong response of hydrolytic EEA to carbon availability in the permafrost is correlated with microbial diversity. More diverse and abundant microbial communities may produce more extracellular enzymes. Alternatively, microbial communities in these soils may always be able to produce hydrolytic enzymes where carbon is available, regardless of microbial community diversity.

Conclusion

In glacial drifts of different ages located under moist acidic tundra in northern Alaska, I observed that microbial community dynamics were more responsive to carbon availability and depth than the glacial history of a given site. In soil horizons with more carbon, microbial communities were more diverse, and hydrolytic enzyme activity per gram dry soil was higher regardless of depth. In deeper soil horizons, microbial communities were less diverse, and hydrolytic enzyme activity decreased if carbon availability decreased with depth. The exception was organic permafrost layers, which had higher hydrolytic EEA; it remains to be seen if microbial communities in deeper, carbon-rich organic permafrost layers are more diverse despite the depth in the soil. Oxidative enzyme activity per gram dry soil was constant across depth profiles and glacial drifts, suggesting that microbial communities do not produce phenol oxidase in response to carbon availability in these soils. The response of hydrolytic enzyme production to carbon availability suggests that microbial communities throughout the soil profile can use hydrolytic enzymes to break down carbon, despite the diminished microbial community diversity and frozen conditions deep within the permafrost. The observation of microbial activity throughout the soil profile and microbial community turnover with depth demonstrates that these

are dynamic systems that merit continued attention, especially given the large amounts of carbon stored in permafrost.

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APPENDICES

Appendix A: Supplementary site characteristics

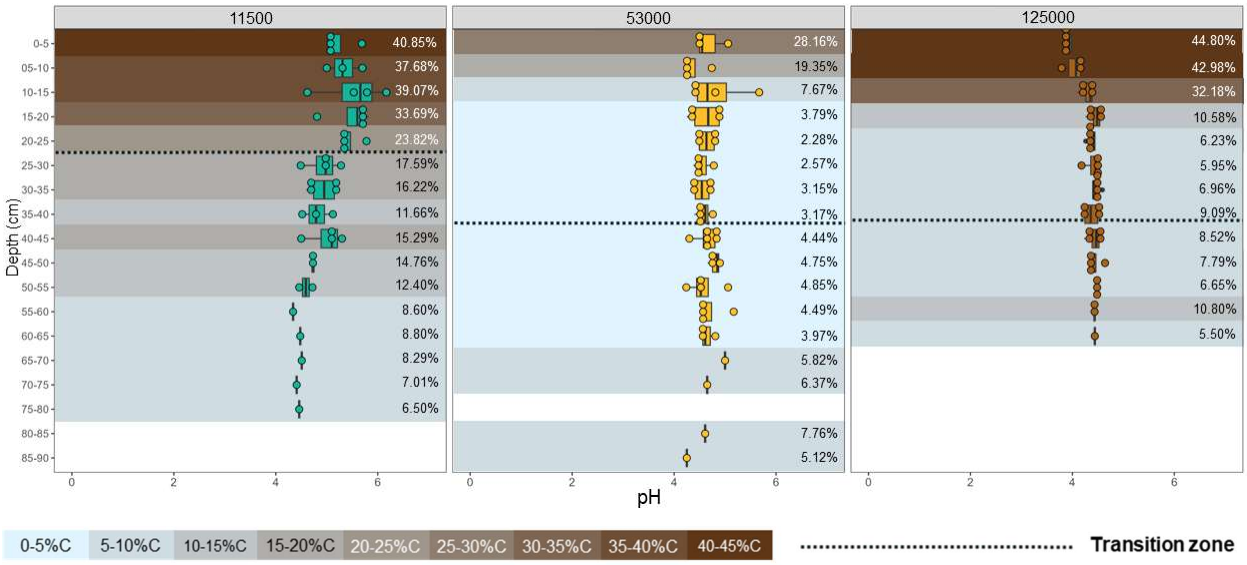

Figure A1. pH at each study site throughout the soil profile. Each point represents a sample. The box displays the median between the 25th and 75th percentiles. The whiskers extend to display a 95% confidence interval. The dashed line represents the transition zone between active layer and permafrost. The percent C values on the right of each panel represent the mean percent C of all samples taken in each 5-cm increment at each site. The color scale represents the gradient from low percent C in light blue to high percent C in dark brown.

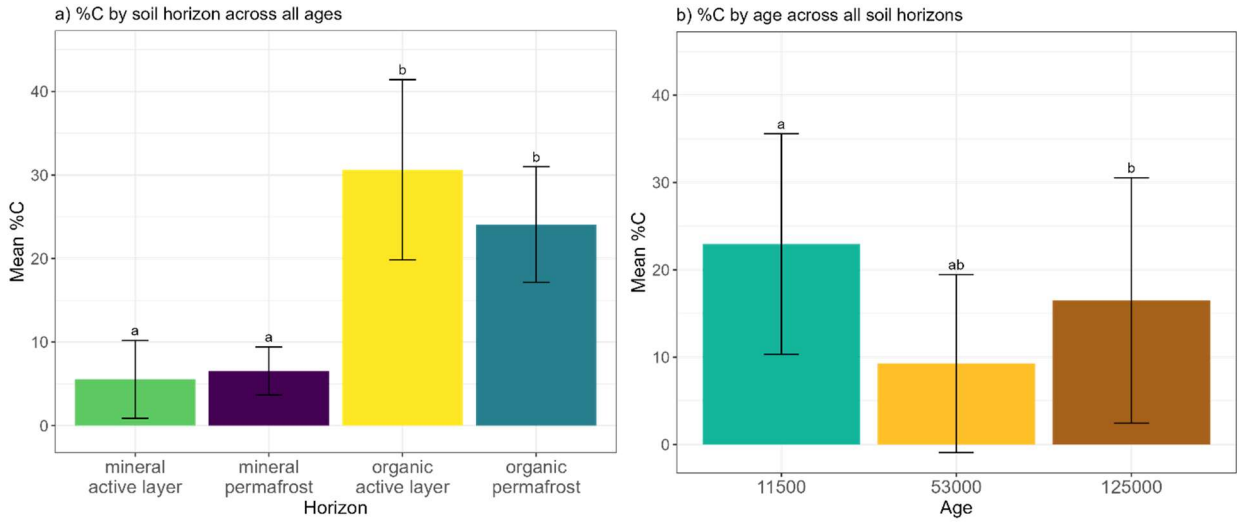

Figure A2. Differences in the mean percent C by age and by soil horizon. Error bars represent one standard deviation. a) Percent C was higher in the organic active layer and permafrost than in the mineral active layer and permafrost. b) Percent C varied with glacial drift through the full soil profile: it was highest at Itkillik II, the youngest glacial drift, second highest at Sagavanirktok River, the oldest glacial drift, and lowest at Itkillik I, which was between the other two glacial drifts in age.

Appendix B: Enzyme assay methods

Table B1. Hydrolytic enzyme assay layout, used for BX & CBH assays. All quantities are in µl. Buff = Sodium acetate buffer, Sub = substrate, Std = MUB standard, S# = sample.

Blank	**Ref Std**	**Neg Cont**	**S1 Quench**	**S1 Control**	**S1 Assay**	**S2 Quench**	**S2 Control**	**S2 Assay**	**S3 Quench**	**S3 Control**	**S3 Assay**
200 Buff 50 Buff	200 Buff 50 Std	200 Buff 50 Sub	200 S1 50 Std	200 S1 50 Buff	200 S1 50 Sub	200 S2 50 Std	200 S2 50 Buff	200 S2 50 Sub	200 S3 50 Std	200 S3 50 Buff	200 S3 50 Sub
200 Buff 50 Buff	200 Buff 50 Std	200 Buff 50 Sub	200 S1 50 Std	200 S1 50 Buff	200 S1 50 Sub	200 S2 50 Std	200 S2 50 Buff	200 S2 50 Sub	200 S3 50 Std	200 S3 50 Buff	200 S3 50 Sub
200 Buff 50 Buff	200 Buff 50 Std	200 Buff 50 Sub	200 S1 50 Std	200 S1 50 Buff	200 S1 50 Sub	200 S2 50 Std	200 S2 50 Buff	200 S2 50 Sub	200 S3 50 Std	200 S3 50 Buff	200 S3 50 Sub
200 Buff 50 Buff	200 Buff 50 Std	200 Buff 50 Sub	200 S1 50 Std	200 S1 50 Buff	200 S1 50 Sub	200 S2 50 Std	200 S2 50 Buff	200 S2 50 Sub	200 S3 50 Std	200 S3 50 Buff	200 S3 50 Sub
200 Buff 50 Buff	200 Buff 50 Std	200 Buff 50 Sub	200 S1 50 Std	200 S1 50 Buff	200 S1 50 Sub	200 S2 50 Std	200 S2 50 Buff	200 S2 50 Sub	200 S3 50 Std	200 S3 50 Buff	200 S3 50 Sub
200 Buff 50 Buff	200 Buff 50 Std	200 Buff 50 Sub	200 S1 50 Std	200 S1 50 Buff	200 S1 50 Sub	200 S2 50 Std	200 S2 50 Buff	200 S2 50 Sub	200 S3 50 Std	200 S3 50 Buff	200 S3 50 Sub
200 Buff 50 Buff	200 Buff 50 Std	200 Buff 50 Sub	200 S1 50 Std	200 S1 50 Buff	200 S1 50 Sub	200 S2 50 Std	200 S2 50 Buff	200 S2 50 Sub	200 S3 50 Std	200 S3 50 Buff	200 S3 50 Sub
200 Buff 50 Buff	200 Buff 50 Std	200 Buff 50 Sub	200 S1 50 Std	200 S1 50 Buff	200 S1 50 Sub	200 S2 50 Std	200 S2 50 Buff	200 S2 50 Sub	200 S3 50 Std	200 S3 50 Buff	200 S3 50 Sub

Hydrolytic enzyme activity calculation

Final activity (nmol g^{-1} h^{-1}) = $\frac{NF\ x\ 125\ ml}{EmC\ x\ 0.2\ ml\ x\ time\ (hr) x\ soil\ mass\ (g)}$

Where:

NF (Net fluorescence) = $(\frac{Sample\ assay\ RFU - Sample\ control\ RFU}{QC})$ – Substrate control RFU

QC (Quench coefficient) = $\frac{Quench\ RFU - Sample\ control\ RFU}{Standard\ RFU}$

125 ml = volume buffer added

EmC (Emission coefficient) = $\frac{Standard\ RFU}{0.5\ nmol\ standard\ added\ to\ well}$

0.2 ml = volume of soil slurry added to each well

Time (hr) = length of incubation

Soil mass (g) = mass of soil sample incorporated into soil slurry

Table B2. Oxidative enzyme layout, used for POX assays. All quantities are in µl.
Buff = Sodium acetate buffer, Sub = substrate, Std = MUB standard, S# = sample, BLK = blank.

BLK	**Neg. Control**		**S1 Control**	**S1 Assay**	**S1 Assay**	**S2 Control**	**S2 Assay**	**S2 Assay**	**S3 Control**	**S3 Assay**	**S3 Assay**
200 Buff 50 Buff	200 Buff 50 Sub		200 S1 50 Buff	200 S1 50 Sub	200 S1 50 Sub	200 S2 50 Buff	200 S2 50 Sub	200 S2 50 Sub	200 S3 50 Buff	200 S3 50 Sub	200 S3 50 Sub
200 Buff 50 Buff	200 Buff 50 Sub		200 S1 50 Buff	200 S1 50 Sub	200 S1 50 Sub	200 S2 50 Buff	200 S2 50 Sub	200 S2 50 Sub	200 S3 50 Buff	200 S3 50 Sub	200 S3 50 Sub
200 Buff 50 Buff	200 Buff 50 Sub		200 S1 50 Buff	200 S1 50 Sub	200 S1 50 Sub	200 S2 50 Buff	200 S2 50 Sub	200 S2 50 Sub	200 S3 50 Buff	200 S3 50 Sub	200 S3 50 Sub
200 Buff 50 Buff	200 Buff 50 Sub		200 S1 50 Buff	200 S1 50 Sub	200 S1 50 Sub	200 S2 50 Buff	200 S2 50 Sub	200 S2 50 Sub	200 S3 50 Buff	200 S3 50 Sub	200 S3 50 Sub
200 Buff 50 Buff	200 Buff 50 Sub		200 S1 50 Buff	200 S1 50 Sub	200 S1 50 Sub	200 S2 50 Buff	200 S2 50 Sub	200 S2 50 Sub	200 S3 50 Buff	200 S3 50 Sub	200 S3 50 Sub
200 Buff 50 Buff	200 Buff 50 Sub		200 S1 50 Buff	200 S1 50 Sub	200 S1 50 Sub	200 S2 50 Buff	200 S2 50 Sub	200 S2 50 Sub	200 S3 50 Buff	200 S3 50 Sub	200 S3 50 Sub
200 Buff 50 Buff	200 Buff 50 Sub		200 S1 50 Buff	200 S1 50 Sub	200 S1 50 Sub	200 S2 50 Buff	200 S2 50 Sub	200 S2 50 Sub	200 S3 50 Buff	200 S3 50 Sub	200 S3 50 Sub
200 Buff 50 Buff	200 Buff 50 Sub		200 S1 50 Buff	200 S1 50 Sub	200 S1 50 Sub	200 S2 50 Buff	200 S2 50 Sub	200 S2 50 Sub	200 S3 50 Buff	200 S3 50 Sub	200 S3 50 Sub

Oxidative enzyme activity calculation

Final activity (µmol g^{-1} h^{-1}) = $\frac{Final\ abs\ x\ 125\ ml}{EC\ x\ 0.2\ ml\ x\ time\ (hr) x\ soil\ mass\ (g)}$

Where:

Final abs = Mean Assay abs – Substrate Control abs

125 ml = volume buffer added

EC (Extinction coefficient) = 7.9 abs/µmol product per well; EC determined by lab

0.2 ml = volume of soil slurry added to each well

Time (hr) = length of incubation

Soil mass (g) = mass of soil sample incorporated into soil slurry

Appendix C: Challenges with microbial community data

As discussed in the Methods section, there were two main problems with the sequencing of the microbial communities. First, 14 of 42 extraction blanks were sequenced with over 10,000 reads, suggesting that there were contamination issues in the plating of the soil samples. Second, only 54 of 148 samples were successfully sequenced, suggesting that there may be issues with either inhibitors in the soil or challenges with the DNA plate extraction method. Additionally, only one organic permafrost sample was successful, meaning that soil horizon had to be excluded from several analyses. As such, the analysis of the microbial community data should be considered preliminary and not definitive. The team will be moving forward with re-extracting the samples from this project with a different DNA extraction method and will re-sequence the samples to compare with this preliminary work and offer a more robust understanding of the microbial community at these sites.

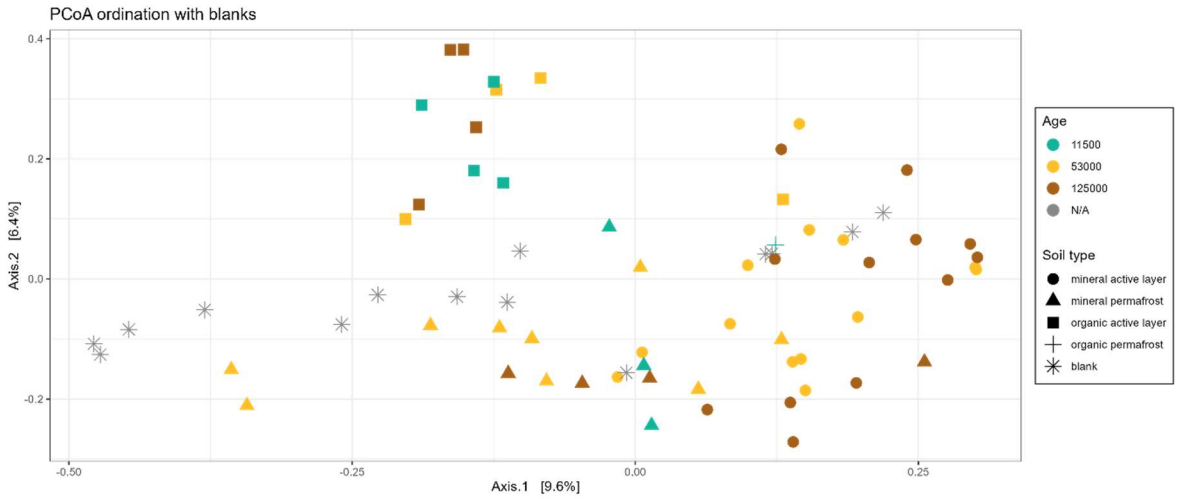

Figure C1. Principal coordinates analysis (PCoA) ordination that includes the 14 contaminated blanks that sequenced along with the successful sample data. All of these blanks had >10,000 reads.

Figure C2. Non-metric multi-dimensional scaling (NMDS) ordination that shows how microbial community data from this project compares to rhizosphere microbial community data from another project from the same sites. In a), the samples with an age of N/A are blanks from this project. In b), the samples with a depth of N/A are the rhizosphere microbial community data.

Appendix D: Supplementary extracellular enzyme activity results

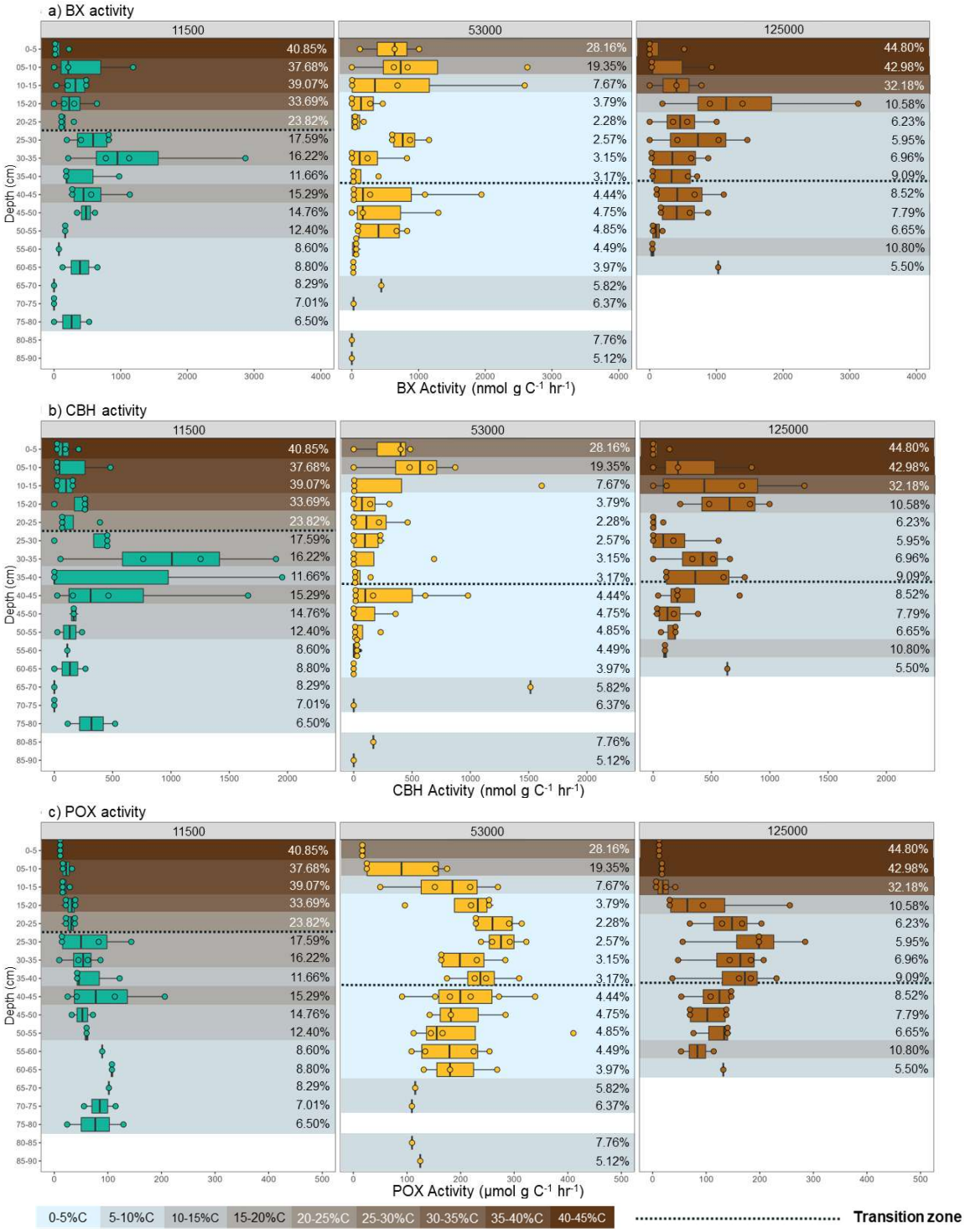

Figure D1. EEA in nmol (BX, CBH) or µmol (POX) per gram C per hour. Percent C values represent the mean percent C of samples taken in each 5-cm increment at each site. The data of each individual core can be viewed in Appendix Figure D2. Table D1 shows the means of each enzyme grouped by age. Table D2 shows the means of each enzyme when grouped by soil horizon. A 2-way ANOVA assessing the response of EEA to the categorical variables of age and depth is available in Table D5.

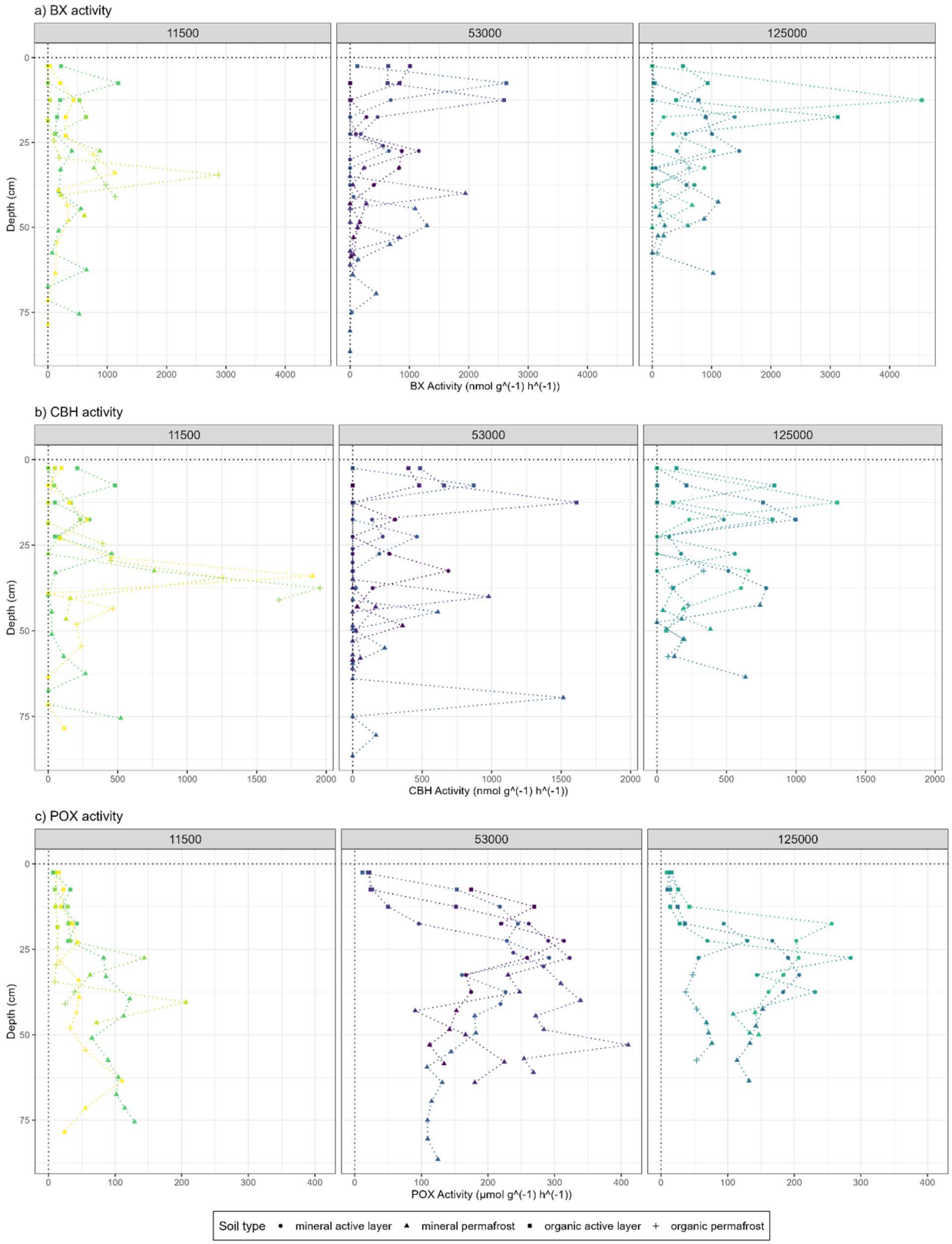

Figure D2: EEA in nmol (BX and CBH) or µmol (POX) per gram C for individual cores. 4 soil cores were taken at each site, and not all cores were the same depth.

Table D1. Mean EEA per gram C by age. Letters in a column denote statistically different groups for the mean of that EEA across the 0 - 60 cm core, as per Tukey HSD. One standard deviation is reported in parentheses.

	Mean BX activity (nmol gC^{-1} hr^{-1})	Mean CBH activity (nmol gC^{-1} hr^{-1})	Mean POX activity (μmol gC^{-1} hr^{-1})
11500	392.9 (501.8) *a*	301.4 (477.3) *a*	53.0 (44.5) *a*
53000	935.9 (4029.0) *a*	583.1 (2874.7) *a*	191.7 (92.8) *b*
125000	636.2 (933.0) *a*	409.1 (880.6) *a*	100.6 (77.1) *c*

Table D2. Mean EEA per gram C by soil horizon. Letters in a column denote statistically different groups for that EEA across the 0 - 60 cm core, as per Tukey HSD. One standard deviation is reported in parentheses.

	Mean BX activity (nmol gC^{-1} hr^{-1})	Mean CBH activity (nmol gC^{-1} hr^{-1})	Mean POX activity (μmol gC^{-1} hr^{-1})
Organic active layer	683.5 (708.1) *a*	319.3 (282.8) *a*	47.0 (59.2) *b*
Mineral active layer	466.0 (214.9) *a*	172.0 (120.7) *a*	190.0 (64.6) *a*
Organic permafrost	666.4 (556.5) *a*	620.4 (639.2) *a*	33.7 (17.0) *b*
Mineral permafrost	412.1 (240.5) *a*	270.3 (282.5) *a*	133.7 (70.1) *a*

Table D3. Results of 2-way ANOVA examining the response of EEA per gram C to age and depth. Age and depth are treated as categorical variables. Age is the estimated age of the glacial drift at each site. Depth is binned in 5-cm increments. Two outliers are excluded in this analysis.

			Df	Sum sq	Mean sq	F value	Pr (>F)	Significance
Full core (0-60 cm)	BX activity (nmol gC^{-1} hr^{-1})	age	2	444832	222416	0.489	0.615	
		depth	11	6714771	610434	1.342	0.214	
		age:depth	22	10431042	474138	1.042	0.423	
		Residuals	95	43219748	454945			
	CBH activity (nmol gC^{-1} hr^{-1})	age	2	414165	207083	1.44	0.242	
		depth	11	2314740	210431	1.463	0.159	
		age:depth	22	3541735	160988	1.119	0.342	
		Residuals	95	13666286	143856			
	POX activity (µmol gC^{-1} hr^{-1})	age	2	492492	246246	62.084	<2.00E-10	***
		depth	11	263898	23991	6.049	2.05E-07	***
		age:depth	22	94035	4274	1.078	0.384	
		Residuals	95	376801	3966			
Active layer (0 -30 cm)	BX activity (nmol gC^{-1} hr^{-1})	age	2	2594697	1297348	2.128	0.13	
		depth	5	3790962	758192	1.244	0.303	
		age:depth	10	5679992	567999	0.932	0.513	
		Residuals	51	31091658	609640			
	CBH activity (nmol gC^{-1} hr^{-1})	age	2	175912	87956	0.847	0.435	
		depth	5	682479	136496	1.315	0.273	
		age:depth	10	1359447	135945	1.309	0.251	
		Residuals	51	5295354	103830			
	POX activity (µmol gC^{-1} hr^{-1})	age	2	262714	131357	42.425	1.41E-11	***
		depth	5	208775	41755	13.486	2.18E-08	***
		age:depth	10	82389	8239	2.661	0.0107	*
		Residuals	51	157907	3096			
Permafrost (30 - 60 cm)	BX activity (nmol gC^{-1} hr^{-1})	age	2	922636	461318	1.648	0.204	
		depth	5	2121185	424237	1.516	0.205	
		age:depth	10	1748329	174833	0.625	0.784	
		Residuals	43	12034070	279862			
	CBH activity (nmol gC^{-1} hr^{-1})	age	2	1719829	859915	4.438	0.0177	*
		depth	5	1354691	270938	1.398	0.244	
		age:depth	10	870259	87026	0.449	0.9128	
		Residuals	43	8331350	193752			
	POX activity (µmol gC^{-1} hr^{-1})	age	2	188197	94099	19.106	1.16E-06	***
		depth	5	12031	2406	0.489	0.783	
		age:depth	10	12510	1251	0.254	0.988	
		Residuals	43	211778	4925			

Table D4. Results of 2-way ANOVA examining the response of EEA per gram C to age and depth. Age and depth are treated as categorical variables. Age is the estimated age of the glacial drift at each site. Depth is binned in 5-cm increments. Two outliers are included in this analysis.

			Df	Sum sq	Mean sq	F value	Pr (>F)	Significance
Full core (0-60 cm)	BX activity (nmol gC^{-1} hr^{-1})	age	2	9267372	4633686	0.634	0.533	
		depth	11	63015107	5728646	0.784	0.655	
		age:depth	22	1.65E+08	7481739	1.024	0.444	
		Residuals	97	7.09E+08	7306129			
	CBH activity (nmol gC^{-1} hr^{-1})	age	2	2219875	1109938	0.287	0.751	
		depth	11	33746264	3067842	0.794	0.646	
		age:depth	22	87582380	3981017	1.03	0.437	
		Residuals	97	3.75E+08	3865370			
	POX activity (µmol gC^{-1} hr^{-1})	age	2	528497	264249	54.667	<2.00E-10	***
		depth	11	236879	21534	4.455	2.04E-05	***
		age:depth	22	67387	3063	0.634	0.889	
		Residuals	97	468881	4834			
Active layer (0 -30 cm)	BX activity (nmol gC^{-1} hr^{-1})	age	2	27747521	13873760	1.056	0.355	
		depth	5	46291947	9258389	0.704	0.623	
		age:depth	10	1.46E+08	14604194	1.111	0.371	
		Residuals	53	6.97E+08	13142763			
	CBH activity (nmol gC^{-1} hr^{-1})	age	2	11574246	5787123	0.837	0.439	
		depth	5	27468922	5493784	0.794	0.559	
		age:depth	10	76805062	7680506	1.11	0.372	
		Residuals	53	3.67E+08	6916414			
	POX activity (µmol gC^{-1} hr^{-1})	age	2	304029	152014	32.229	6.94E-10	***
		depth	5	185510	37102	7.866	1.34E-05	***
		age:depth	10	53354	5335	1.131	0.357	
		Residuals	53	249987	4717			
Permafrost (30 - 60 cm)	BX activity (nmol gC^{-1} hr^{-1})	age	2	922636	461318	1.648	0.204	
		depth	5	2121185	424237	1.516	0.205	
		age:depth	10	1748329	174833	0.625	0.784	
		Residuals	43	12034070	279862			
	CBH activity (nmol gC^{-1} hr^{-1})	age	2	1719829	859915	4.438	0.0177	*
		depth	5	1354691	270938	1.398	0.244	
		age:depth	10	870259	87026	0.449	0.9128	
		Residuals	43	8331350	193752			
	POX activity (µmol gC^{-1} hr^{-1})	age	2	188197	94099	19.106	1.16E-06	***
		depth	5	12031	2406	0.489	0.783	
		age:depth	10	12510	1251	0.254	0.988	
		Residuals	43	211778	4925			

Table D5. Results of 2-way ANOVA examining the response of EEA per gram C to age and soil horizon. Age and soil horizon are treated as categorical variables. For each enzyme, mean is the mean enzyme activity within the horizon, and sum is the sum of the enzyme activities for all samples from that horizon divided by the depth in centimeters of the horizon.

			Df	Sum sq	Mean sq	F	Pr(>F)	Significance
BX activity (nmol gC^{-1} hr^{-1})	mean	age	2	23278	11639	0.055	0.947	
		horizon	3	584509	194836	0.917	0.445	
		age:horizon	5	2097517	419503	1.974	0.114	
		Residuals	28	5950423	212515			
	sum per cm	age	2	591	295	0.043	0.958	
		horizon	3	8870	2957	0.432	0.732	
		age:horizon	5	55273	11055	1.614	0.189	
		Residuals	28	191767	6849			
CBH activity (nmol gC^{-1} hr^{-1})	mean	age	2	417968	208984	2.168	0.1332	
		horizon	3	475814	158605	1.646	0.2013	
		age:horizon	5	1092499	218500	2.267	0.0751	
		Residuals	28	2698473	96374			
	sum per cm	age	2	7161	3580	1.261	0.299	
		horizon	3	2283	761	0.268	8.48E-01	
		age:horizon	5	30159	6032	2.124	0.092	
		Residuals	28	79524	2840			
POX activity (µmol gC^{-1} hr^{-1})	mean	age	2	110917	55458	25.843	4.37E-07	***
		horizon	3	97770	32590	15.187	4.66E-06	***
		age:horizon	5	4322	864	0.403	0.843	
		Residuals	28	60087	2146			
	sum per cm	age	2	5193	2596.3	36.3	1.67E-08	***
		horizon	3	5169	1722.9	24.089	6.54E-08	***
		age:horizon	5	423	84.6	1.183	0.342	
		Residuals	28	2003	71.5			
% C	mean	age	2	1172	585.8	18.06	9.17E-06	***
		horizon	3	4401	1467	45.225	7.30E-11	***
		age:horizon	5	317	63.5	1.957	0.116	
		Residuals	28	908	32.4			

Table D6. Results of 2-way ANOVA examining the response of EEA per gram soil to age and depth. Age and depth are treated as categorical variables. Age is the estimated age of the glacial drift at each site. Depth is binned in 5-cm increments. Two outliers are excluded in this analysis.

			Df	Sum sq	Mean sq	F value	Pr (>F)	Significance
Full core (0-60 cm)	BX activity (nmol g soil^{-1} hr^{-1})	age	2	48863	24432	1.376	0.2575	
		depth	11	405404	36855	2.076	0.0293	*
		age:depth	22	532521	24205	1.363	0.1535	
	CBH activity (nmol g soil^{-1} hr^{-1})	age	2	50584	25292	2.988	0.0551	
		depth	11	114580	10416	1.231	0.2776	
		age:depth	22	259516	11796	1.394	0.1375	
	POX activity (µmol g soil^{-1} hr^{-1})	age	2	11.6	5.793	1.418	0.247	
		depth	11	65.9	5.991	1.467	0.157	
		age:depth	22	67.5	3.069	0.751	0.775	
Active layer (0 -30 cm)	BX activity (nmol g soil^{-1} hr^{-1})	age	2	25152	12576	0.785	0.4614	
		depth	5	232058	46412	2.897	0.0221	*
		age:depth	10	259808	25981	1.622	0.1264	
	CBH activity (nmol g soil^{-1} hr^{-1})	age	2	3202	1601	0.244	0.7842	
		depth	5	68340	13668	2.085	0.082	
		age:depth	10	114682	11468	1.75	0.0942	
	POX activity (µmol g soil^{-1} hr^{-1})	age	2	0.25	0.126	0.024	0.977	
		depth	5	43.04	8.609	1.629	0.169	
		age:depth	10	49.51	4.951	0.937	0.508	
Permafrost (30 - 60 cm)	BX activity (nmol g soil^{-1} hr^{-1})	age	2	166960	83480	4.119	0.0231	*
		depth	5	79707	15941	0.787	0.5651	
		age:depth	10	127275	12728	0.628	0.7816	
	CBH activity (nmol g soil^{-1} hr^{-1})	age	2	143220	71610	6.529	0.00334	**
		depth	5	27751	5550	0.506	0.77005	
		age:depth	10	53792	5379	0.49	0.88692	
	POX activity (µmol g soil^{-1} hr^{-1})	age	2	20.73	10.365	3.948	0.0267	*
		depth	5	22.8	4.559	1.737	0.1467	
		age:depth	10	9.68	0.968	0.369	0.9535	

Table D7. Results of 2-way ANOVA examining the response of EEA per gram soil to age and soil horizon. Age and soil horizon are treated as categorical variables. For each enzyme, mean is the mean enzyme activity within the horizon, and sum is the sum of the enzyme activities for all samples from that horizon divided by the depth in centimeters of the horizon.

			Df	SumOfSqs	R2	F	Pr(>F)	Significance
BX	mean	age	2	17140	8570	0.918	0.41335	
		horizon	3	192895	64298	6.89	0.00178	**
		age:horizon	5	83541	16708	1.79	0.15466	
		Residuals	23	214648	9333			
	sum	age	2	171891	85946	1.491	0.246063	
		horizon	3	1708991	569664	9.884	0.000223	***
		age:horizon	5	353031	70606	1.225	0.329303	
		Residuals	23	1325580	57634			
CBH	mean	age	2	19183	9591	2.777	0.083107	.
		horizon	3	109141	36380	10.534	0.000149	***
		age:horizon	5	46638	9328	2.701	0.046205	*
		Residuals	23	79433	3454			
	sum	age	2	193332	96666	3.372	0.052	.
		horizon	3	1053323	351108	12.246	5.43E-05	***
		age:horizon	5	267553	53511	1.866	0.14	
		Residuals	23	659418	28670			
POX	mean	age	2	4.16	2.0804	0.834	0.447	
		horizon	3	0.26	0.0855	0.034	0.991	
		age:horizon	5	15.27	3.054	1.224	0.33	
		Residuals	23	57.4	2.4957			
	sum	age	2	144.3	72.1	0.747	0.485	
		horizon	3	288.9	96.3	0.997	0.4119	
		age:horizon	5	2231.1	446.2	4.619	0.0046	**
		Residuals	23	2221.9	96.6			

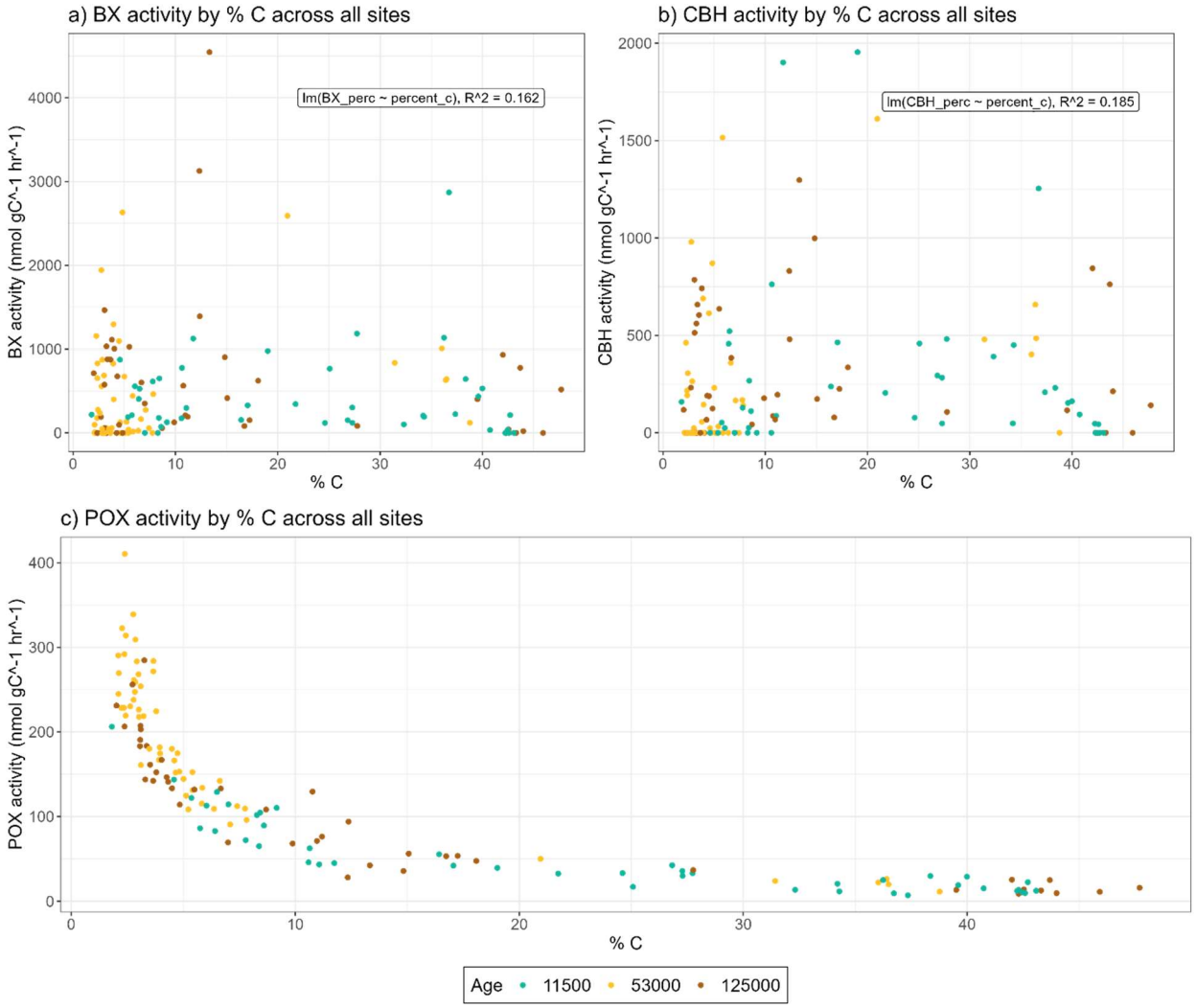

Figure D3. Relationship between percent C in a sample and the potential EEA per g C of that sample for a) BX, b) CBH, and c) POX. There is not a strong relationship between these variables for the two hydrolytic enzymes. The oxidative enzyme POX was a similar value per gram dry soil across all sites, so when divided by percent C and expressed as an activity per gram C, there is a strong relationship between these variables.

Appendix E: Supplementary microbial community results

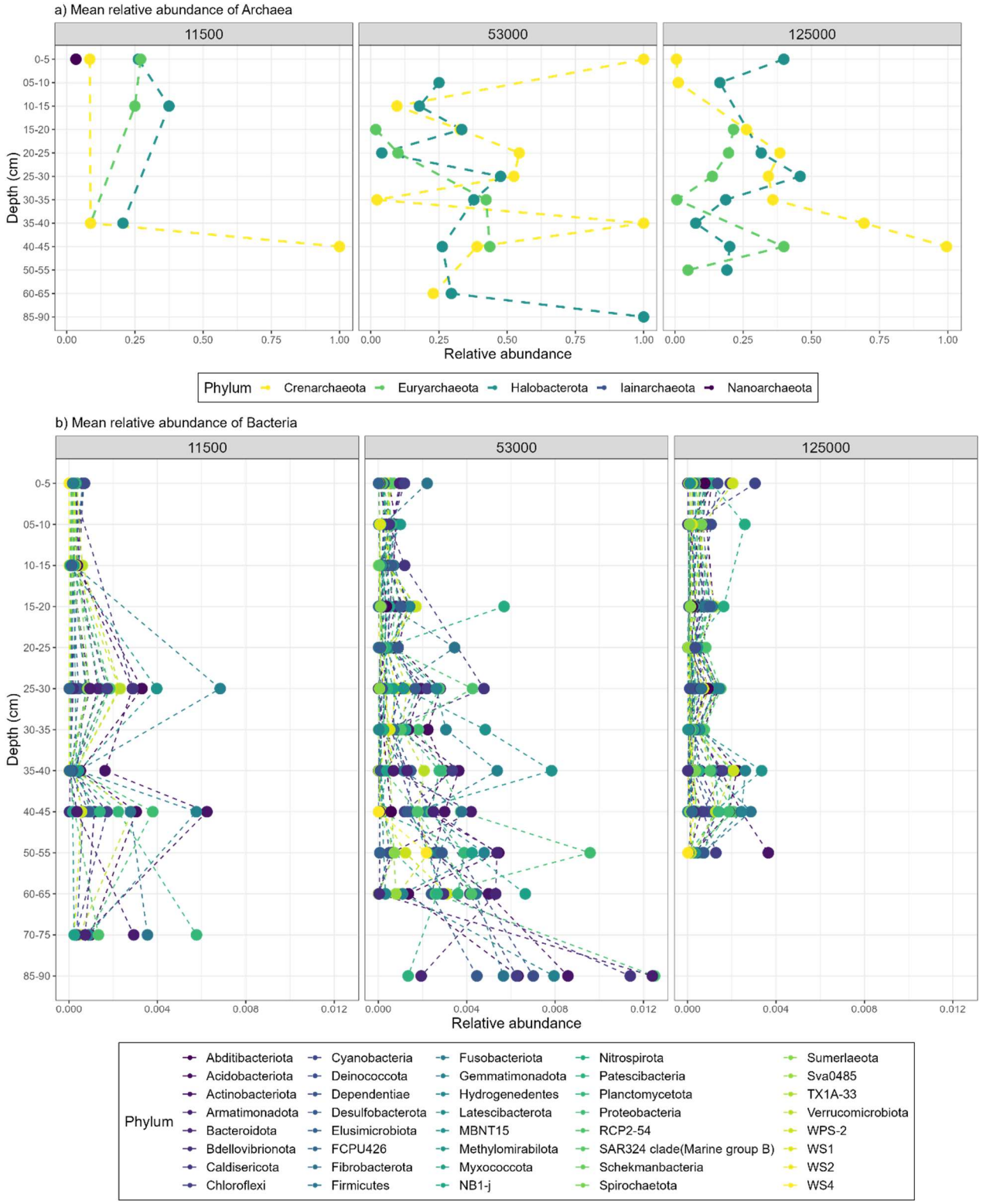

Figure E1. Change in the relative abundance of phyla with depth. In each figure, a complete community sums to 1.00.

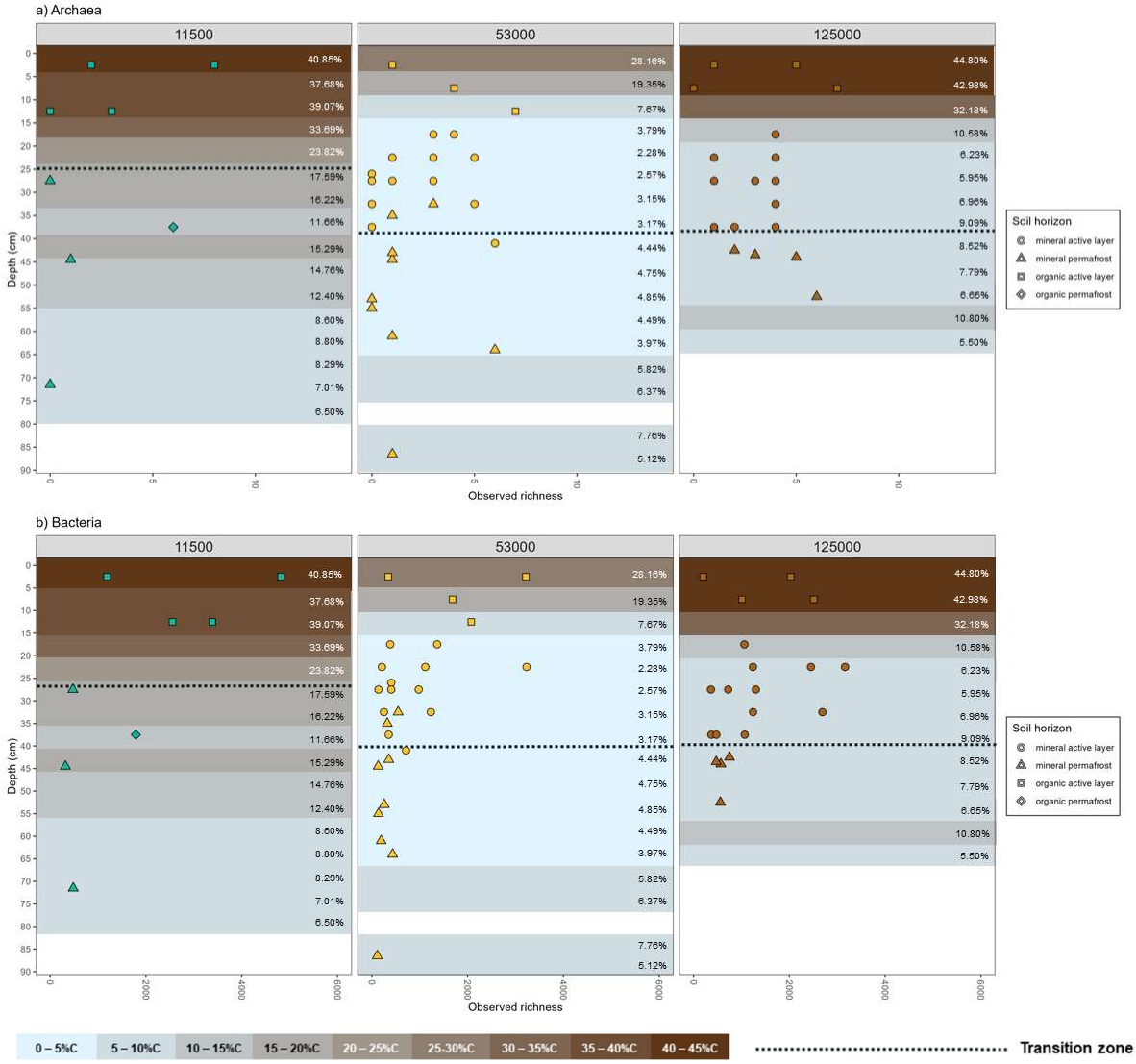

Figure E2. Observed richness of microbial communities with depth at all three sites. The dashed line represents the transition zone between active layer and permafrost. The percent C values on the right of each panel represent the mean percent C of all samples taken in each 5-cm increment at each site. The color scale represents the gradient from low percent C in light blue to high percent C in dark brown.

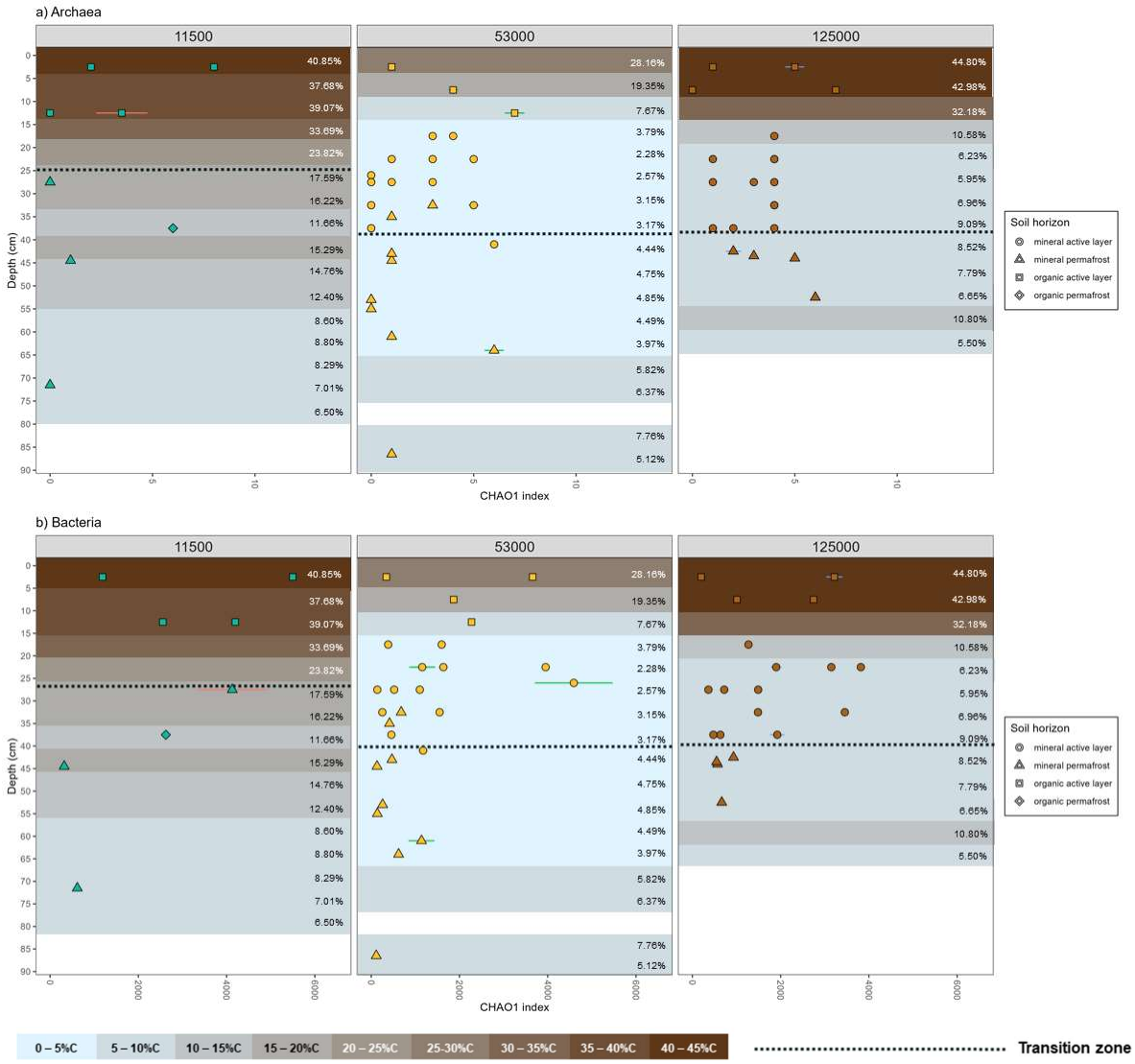

Figure E3. CHAO1 index of alpha diversity of microbial communities with depth at all three sites. The dashed line represents the transition zone between active layer and permafrost. The percent C values on the right of each panel represent the mean percent C of all samples taken in each 5-cm increment at each site. The color scale represents the gradient from low percent C in light blue to high percent C in dark brown.

Table E1. Results of 2-way ANOVA analyzing the response of Shannon index of diversity to the categorical variables of age and soil horizon. The structure of this model was shannon_diversity ~ age * horizon, for first the archaeal community and then the bacterial community.

		Df	Sum Sq	MeanSq	F value	Pr(>F)	Significance
Archaeal community	age	2	1.116	0.558	2.105	0.134	
	soil horizon	2	0.59	0.2948	1.112	0.338	
	age:horizon	3	0.899	0.2997	1.13	0.347	
	Residuals	45	11.931	0.2651			
Bacterial community	age	2	4.562	2.281	3.656	0.0338	*
	soil horizon	2	22.164	11.082	17.762	2.06E-06	***
	age:horizon	3	1.97	0.657	1.053	0.3786	
	Residuals	45	28.076	0.624			

Table E2. Mean and standard deviations of Shannon index of diversity by age for bacterial and archaeal communities. Letters in each column denote statistically significant groups as per Tukey's HSD.

	Bacterial community	Archaeal community
	Mean Shannon index (*H*)	Mean Shannon index (*H*)
11500	5.516 (1.49) *a*	0.43 (0.66) *a*
53000	4.80 (1.05) *a*	0.31 (0.50) *a*
125000	5.33 (0.78) *a*	0.62 (0.49) *a*

Table E3. PERMANOVA analyzing the response of microbial community data to age, depth, and % C. The structure of this model was physeq1_bray ~ (age+depth_bin+percent_c).

	Df	SumOfSqs	R2	F	Pr(>F)	Significance
age	2	1.0498	0.04904	1.4581	0.004	**
depth	12	6.2788	0.29334	1.4535	0.001	***
% C	1	0.3968	0.01854	1.1024	0.257	
Residual	38	13.6795	0.63908			
Total	53	21.405	1			

Table E4. PERMANOVA analyzing the response of microbial community data to age, soil horizon, and % C. The structure of this model was physeq1_bray ~ (age+horizon+percent_c).

	Df	SumOfSqs	R2	F	Pr(>F)	Significance
age	2	1.0498	0.04904	1.4399	0.006	**
horizon	3	2.7907	0.13038	2.5518	0.001	***
% C	1	0.4312	0.02014	1.1827	0.135	
Residual	47	17.1333	0.80044			
Total	53	21.405	1			

Table E5. PERMANOVA analyzing the response of microbial community data to age, depth, pH, and % C. The structure of this model was physeq1_bray ~ (depth_bin+pH+age+percent_c).

	Df	SumOfSqs	R2	F	Pr(>F)	Significance
depth	11	6.0284	0.28777	1.5297	0.001	***
pH	1	0.4528	0.02161	1.2639	0.097	
age	2	0.8156	0.03893	1.1383	0.159	
% C	1	0.3966	0.01893	1.1070	0.276	
Residual	37	13.2553	0.63275			
Total	52	20.9487	1			