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A LANDSCAPE ON THE THRESHOLD OF CHANGE: PATTERNS OF SOIL MICROBIAL ECOLOGY ALONG DYNAMIC GEOMORPHIC AND HYDROLOGIC FEATURES IN A POLAR DESERT

Kelli L. Feeser
University of New Mexico

David J. Van Horn
University of New Mexico

Heather N. Buelow
University of Notre Dame

Daniel R. Colman
The University Of Montana

Theresa A. McHugh
Carroll College

Kelli L. Feeser

Candidate

Biology

Department

This dissertation is approved, and it is acceptable in quality and form for publication:

Approved by the Dissertation Committee:

Dr. Donald O. Natvig, Chairperson

Dr. David Van Horn

Dr. Robert Sinsabaugh

Dr. Becky Ball

A LANDSCAPE ON THE THRESHOLD OF CHANGE: PATTERNS OF SOIL MICROBIAL ECOLOGY ALONG DYNAMIC GEOMORPHIC AND HYDROLOGIC FEATURES IN A POLAR DESERT

by

KELLI L. FEESER

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ABSTRACT

The McMurdo Dry Valleys (MDV) of Antarctica are on the threshold of widespread landscape scale change due to increasing temperature and solar radiation and altered hydrology: buried ice is melting, the soil active layer is thickening, thermokarst features are developing along streams, water tracks are expanding, and lake levels are rising. These changes will impact the microbial communities found in each of the affected habitats. The purpose of this work is to first, understand the spatial distribution of soil bacteria in the MDV, specifically investigating the scale-dependent effects of environmental heterogeneity, and second, to perform surveys and coupled experiments to document and assess the impacts of these changes on the associated microbial communities. Our results suggest that this region harbors highly adapted, endemic communities that are susceptible to changing environmental conditions.

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INTRODUCTION

The unique properties of water (Finney et al., 2004) make its presence an essential component of habitats capable of sustaining life on earth (Rothschild and Mancinelli, 2001; Stevenson et al., 2015). Considered the coldest, driest, most oligotrophic desert on the planet (Hopkins et al., 2006; Craig et al., 2010), the McMurdo Dry Valleys (MDV), Victoria Land, Antarctica (77° 30' S, 163° 00' E) comprise one of the harshest environments on Earth. Fresh water availability is the key limiting factor that drives ecosystem functioning in the MDV. In the hyper-arid MDV, complex microbial communities with significant biomass occur in a variety of habitats that receive liquid water including streams (McKnight et al., 1999; Kohler et al., 2015; Van Horn et al., 2016), lakes (Priscu et al., 1998; Takacs and Priscu, 1998), cryoconites (Porazinska et al., 2004; Foreman et al., 2007; Telling et al., 2014), wetted soils surrounding aquatic ecosystems (Moorhead et al., 2003; Zeglin et al., 2011; Niederberger et al., 2015), and in typically dry soils that receive transient water inputs from snow fall events (Lee et al., 2012; Van Horn et al., 2013, 2014; Schwartz et al., 2014). Water-rich terrestrial habitats composed of glacier runoff zones, stream hyporheic zones, and lake margins comprise ~1% of the MDV area. Linking these well-described high-productivity regions is a permafrost-dominated “cryptic” (hidden) watershed that routes the flow of water, nutrients, salt, and heat through MDV soils in subsurface meltwater conduits called water tracks (Ball and Levy, 2015).

The MDV are a microbe-dominated ecosystem, as extreme conditions prohibit the existence of higher plants and animals. Soil bacteria constitute the vast majority of the total diversity found in the MDV (Freckman and Virginia, 1997; Connell et al., 2006;

Buelow et al., 2016). These bacterial communities are responsible for processing nutrients and organic matter (Gregorich et al., 2006) and supporting higher organisms (Treonis et al., 1999). The structure and function of these microbial communities are highly responsive to changing conditions of temperature, moisture, and resource supply (Hopkins et al., 2006; Tiao et al., 2012; Buelow et al., 2016).

The MDV are on the threshold of widespread landscape scale change due to increasing temperature and solar radiation and altered hydrology: buried ice is melting, the soil active layer and water tracks are expanding, thermokarsts are developing along streams, and lake levels are rising (Fountain et al., 2014). The expansion of wetting and thaw is a major environmental change that is beginning to shape MDV terrestrial ecosystems, however the response of microbial communities to this form of environmental change is unknown. The purpose of my dissertation is to first, to understand the spatial distribution of soil bacteria in the MDV, specifically investigating the scale-dependent effects of environmental heterogeneity, and second, to perform surveys and coupled experiments to document and assess the impacts of these changes on the associated microbial communities.

Before the impacts of these landscape-scale change can be understood, we must first determine how the environment structures the microbial communities of the MDV. The simplified trophic structure and high spatial physiochemical heterogeneity of MDV soils provides an ideal model system in which to study natural, but low complexity, microbial community-environment interactions to better understand the underlying scale-dependent processes that organize the communities. To better understand the relationship between bacterial species distribution patterns and the environmental factors that shape

them, my first chapter, entitled "Local and Regional Scale Heterogeneity Drive Bacterial Community Diversity and Composition in a Polar Desert," utilized a spatially-stratified examination of the inherent edaphic gradients within the cold, dry, and oligotrophic polar desert ecosystem of the MDV (Feeser et al., 2018). This study focused on the relationships among edaphic pH and EC gradients on bacterial communities as they vary across local, lake basin, and regional scales. This was the first study to use the inherent edaphic gradients within soil polygons to investigate the effects of spatial scale, environmental heterogeneity, and landscape context on bacterial community structure. Our findings corroborated previous studies of soil bacterial communities within the lake basins of Taylor Valley by describing high spatial variability and linking soil microbe community structure to edaphic geochemical gradients, (Barrett et al., 2006; Niederberger et al., 2008; Smith et al., 2010; Zeglin et al. 2011; Lee et al. 2012; Sokol et al., 2013; Van Horn et al., 2013).

My second chapter, entitled "Stratification of Bacterial Communities Within the Soil Active Layer in the McMurdo Dry Valleys, Antarctica," explored the profiles of microbial communities within the soil active layer of the MDV. Permafrost in the MDV is ubiquitous in both dry and ice-cemented forms, with active layer depths ranging from a few cm at the highest elevations to one meter near sea level. Although the landscape in this region has been considered stable over millennia, ad-hoc field observations have documented extreme geomorphic changes in the valley bottoms over the past decade. Permafrost underlies the soils in the MDV, with an overlying active soil layer that thaws during summer months. Active layers in the Arctic and Antarctic are expanding at a rate of ~1 cm per year (Åkerman and Johansson, 2008; Burn and Zhang, 2009; Callaghan et

al., 2010; Guglielmin and Cannone, 2012; Guglielmin et al., 2014). These changes are likely to impact the biological soil communities and the functions they perform, as increased available water from ice melt and the interaction of this water with nutrient rich soils alter the supply of basic resources. In this study, I surveyed the microbial communities along the soil profile in soil pits and conducted reciprocal transplant experiments where near surface soils were incubated at lower soil horizons, and vice versa. I focused on the importance of depth, which controls factors including temperature, soil moisture, and resource availability (Stomeo et al., 2012), in structuring the microbial communities in the active layer in both wet and dry soils.

Recent observations of widespread shallow groundwater flow (Levy et al., 2011), and groundwater discharge during discrete warm events (Lyons et al., 2005; Harris et al., 2007; Ball and Virginia, 2012) has highlighted the importance of groundwater within this polar ecosystem. Water tracks are narrow bands of high soil moisture that route meltwater downslope in polar regions, through broad, permafrost-lined channels in the subsurface, without surface flow (McNamara et al., 1999; Levy et al., 2011). Water tracks are well documented as a major feature of the Arctic landscape and soil ecosystem (e.g., Hastings et al., 1989; Bowden et al., 2008; McNamara et al., 2008). However, in Antarctica, water track processes are just beginning to be understood. To date, studies of water tracks in the MDV have largely focused on the hydrology and geochemistry of these features. The response of soil communities to the disturbance from water tracks is a largely unexplored frontier. My third chapter, entitled "Drivers of Soil Bacterial Community Structure in Antarctic Water Tracks", was motivated by a desire to understand the current impact of water tracks on soil microbial communities and to

predict the consequences of climate-induced warming. Rising global temperatures are expected to lead to altered precipitation and promote the melting of ice reserves, resulting in the expansion and creation of new water tracks in the MDV. This purpose of this chapter was to the document microbial diversity, community structure, and abundance in water track soils and compare these communities to adjacently located off-track soil communities, assess the relative importance of soil water content and salinity in structuring these communities to determine if water tracks represent microbial biological hotspots or saline dead zones, and evaluate functional genomic capacity and universal metagenomics traits to better understand the metabolic and ecological strategies of these communities. Given that water tracks are a significant component of the Dry Valley landscape, understanding their ecological role in structuring soil communities and the biogeochemical processes they conduct constitutes an increase in our understanding of basic ecological processes in the Dry Valleys. Additionally, this work informs predictions of future warming that will increase water transport in water tracks.

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CHAPTER 1: LOCAL AND REGIONAL SCALE HETEROGENEITY DRIVE BACTERIAL COMMUNITY DIVERSITY AND COMPOSITION IN A POLAR DESERT

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Local and Regional Scale Heterogeneity Drive Bacterial Community Diversity and Composition in a Polar Desert

*Kelli L. Feeser[1], David J. Van Horn[1], Heather N. Buelow[1], Daniel R. Colman[1], Theresa A. McHugh[2], Jordan G. Okie[3], Egbert Schwartz[4] and Cristina D. Takacs-Vesbach[1]**

[1] Department of Biology, University of New Mexico, Albuquerque, NM, United States, [2] Department of Biological Sciences, Colorado Mesa University, Grand Junction, CO, United States, [3] School of Life Sciences, School of Earth and Space Exploration, Arizona State University, Tempe, AZ, United States, [4] Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ, United States

The distribution of organisms in an environment is neither uniform nor random but is instead spatially patterned. The factors that control this patterning are complex and the underlying mechanisms are poorly understood. Soil microbes are critical to ecosystem function but exhibit highly complex distributions and community dynamics due in large part to the scale-dependent effects of environmental heterogeneity. To better understand the impact of environmental heterogeneity on the distribution of soil microbes, we sequenced the 16S rRNA gene from bacterial communities in the microbe-dominated polar desert ecosystem of the McMurdo Dry Valleys (MDV), Antarctica. Significant differences in key edaphic variables and alpha diversity were observed among the three lake basins of the Taylor Valley (Kruskal–Wallis; pH: $\chi^2 = 68.89$, $P < 0.001$, conductivity: $\chi^2 = 35.03$, $P < 0.001$, observed species: $\chi^2 = 7.98$, $P = 0.019$ and inverse Simpson: $\chi^2 = 18.52$, $P < 0.001$) and each basin supported distinctive microbial communities (ANOSIM $R = 0.466$, $P = 0.001$, random forest ratio of 14.1). However, relationships between community structure and edaphic characteristics were highly variable and contextual, ranging in magnitude and direction across regional, basin, and local scales. Correlations among edaphic factors (pH and soil conductivity) and the relative abundance of specific phyla were most pronounced along local environmental gradients in the Lake Fryxell basin where Acidobacteria, Bacteroidetes, and Proteobacteria declined while Deinococcus–Thermus and Gemmatimonadetes increased with soil conductivity (all $P < 0.1$). Species richness was most strongly related to the soil conductivity gradient present within this study system. We suggest that the relative importance of pH versus soil conductivity in structuring microbial communities is related to the length of edaphic gradients and the spatial scale of sampling. These results highlight the importance of conducting studies over large ranges of key environmental gradients and across multiple spatial scales to assess the influence of environmental heterogeneity on the composition and diversity of microbial communities.

Keywords: environmental heterogeneity, 16S rRNA genes, gradient analysis, spatial scale, polar desert, McMurdo Dry Valleys

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International Medical University,
Malaysia

****Correspondence:***
Cristina D. Takacs-Vesbach
cvesbach@unm.edu

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INTRODUCTION

Understanding the controls on the distribution of organisms has been one of the fundamental goals of ecology for the past century. Numerous studies suggest that this distribution is neither uniform nor random, but instead spatially patterned (Legendre and Fortin, 1989; Ettema and Wardle, 2002; O'Brien et al., 2016). However, the factors that control this patterning are complex, multifaceted, and include abiotic characteristics, biotic interactions, and stochastic events. Additionally, many ecological processes that influence the distribution, abundance, and interactions of species are scale-dependent, including the flow of individuals within an environment, the impacts and extent of disturbances, and variation in environmental conditions and habitability (Leibold et al., 2004). Spatial variation in environmental conditions, referred to here as environmental heterogeneity, is often cited as the primary driver of biodiversity (Stein et al., 2014; Coyle and Hurlbert, 2016). Environmental heterogeneity increases resource diversity and provides opportunities for niche partitioning and speciation events (Stein et al., 2014), allowing for increased species coexistence (MacArthur and Levins, 1964; Coyle and Hurlbert, 2016). However, there is an inherent tradeoff between environmental heterogeneity and diversity: as niche opportunities increase, the effective area available for each species decreases and the probability of stochastic extinctions rises (Allouche et al., 2012). Consequently, environmental heterogeneity is an important factor for species coexistence, persistence, and diversification (Stein et al., 2014).

Recent evidence suggests that environmental heterogeneity strongly impacts the distribution of microbial communities. However, these relationships are complex and contingent on several factors. First, there are the confounding effects of spatial scale.

Larger spatial areas generally encompass greater environmental heterogeneity which can come in the form of longer gradient lengths (e.g., wider ranges of conditions) or harsher gradient severity (e.g., more extreme conditions). Thus, as environmental heterogeneity is inextricably linked with spatial scale, patterns observed at small scales do not necessarily correspond to those found at larger scales (Franklin and Mills, 2009; Geyer et al., 2013; Van Horn et al., 2013; Bar-Massada and Wood, 2014; Stein et al., 2014). Additionally, because environmental heterogeneity typically involves simultaneous changes in numerous abiotic and biotic parameters, the impacts of one variable on microbial community structure may become overwhelmed by the impacts of other variables, creating threshold effects and difficulties in resolving drivers of community change. Clades within communities also respond differently to various environmental factors because their diverse physiological adaptations to resource limitations or environmental severity result in differences in relative fitness (Franklin and Mills, 2009; Geyer et al., 2013). Finally, a related complexity is the presence of "contextual effects," i.e., the observation that relationships between environmental factors and communities depend on geographic context (Van Horn et al., 2013). Therefore, multi-scale analyses along environmental gradients and across various landscape contexts are necessary to understand the abstruse dynamics of scale-dependent ecological processes that structure microbial communities.

A recent review of environmental heterogeneity-diversity studies noted that soil habitats are particularly underrepresented in the literature (Stein et al., 2014), despite the critical importance of soil microbial communities to ecosystem functioning (Cavigelli and Robertson, 2000). This limited understanding is due to the extremely high diversity of these systems, which often contain thousands of microbial species per-gram of soil (Torsvik et al., 1996), the physical and chemical complexity and heterogeneity of soil habitats (Nannipieri et al., 2003), and the frequency of stochastic disturbances which result in the formation of complex spatial patterns (O'Brien et al., 2016). Spatially structured communities have been observed at continental scales (Fierer et al., 2009; Lauber et al., 2009; Barberán et al., 2012; Fierer et al., 2012, 2013) to centimeter scales (Morris, 1999; Grundmann and Debouzie, 2000; Franklin and Mills, 2009; O'Brien et al., 2016), but the underlying ecological mechanisms remain difficult to decipher. Two master variables frequently implicated in controlling microbial diversity and community composition are pH and salinity. Several studies have suggested that the most influential variable on soil microbial community composition is pH (Fierer and Jackson, 2006; Baker et al., 2009; Lauber et al., 2009; Smith et al., 2010) while others have suggested salinity (Lozupone and Knight, 2007; Zeglin et al., 2011; Lee et al., 2012). We propose that differences in scale-dependent environmental heterogeneity may underlie these conflicting conclusions, and we suggest that determining the impacts of environmental gradient lengths and severity are crucial to unlocking this puzzle.

The soils of the McMurdo Dry Valleys (MDV) are an ideal natural laboratory to investigate the relationships between microbial community structure, edaphic characteristics, and scale. Considered the coldest, driest, most oligotrophic desert on Earth (Hopkins et al., 2006; Cary et al., 2010), the MDV are a microbe-dominated ecosystem, as extreme conditions prohibit the existence of higher plants and animals. In the absence of vegetation and biotic interactions such as herbivory, MDV soils are shaped into distinct spatial patterns by physicochemical factors including moisture, salinity, pH, and carbon availability (Barrett et al., 2004; Lee et al., 2012; Van Horn et al., 2013, 2014; Okie et al., 2015). MDV soils contain relatively low levels of biodiversity providing an ideal model system with which to investigate community-environment interactions. Invertebrates are rare and phyla include only the protozoa, rotifers, tardigrades, nematodes and *Collembola*. At many sites, only one species of nematode, the endemic *Scottnema lindsayae* are found (Freckman and Virginia, 1997; Barrett et al., 2004). Fungal and archaeal community distribution is similarly patchy (Arenz and Blanchette, 2011; Richter et al., 2014) and while bacteria are ubiquitous, their diversity is on average, approximately one third of that found in most other soils (Van Horn et al., 2013, 2014).

Previous research has leveraged the existence of patterned ground formations or soil polygons in the MDV and elsewhere to investigate the role of geomorphic history (Brinkmann et al., 2007) and physicochemical variation across multiple spatial

scales (Barrett et al., 2004) on soil biodiversity, although the former study was focused on cyanobacteria and the latter on invertebrates. Strong physical and biogeochemical gradients form along soil polygons that are related to nematode abundance and biodiversity variation. However, a similar, systematic investigation of MDV soil bacterial communities has not been conducted, despite their ubiquity across the MDV landscape (Takacs-Vesbach et al., 2010) and that multiple studies have shown that MDV soil bacterial communities are active under *in situ* conditions (Schwartz et al., 2014; Buelow et al., 2016). Understanding the effects of environmental heterogeneity is crucial to predicting the controls on the diversity and distribution of soil microbial communities. However, untangling these relationships is complicated by landscape contexts and spatial-scale dependent evolutionary and ecological mechanisms. Using soil polygons as the organizing framework across multiple biogeochemically diverse basins is an ideal approach to address environmental heterogeneity. The goal of this study was to explore the effects of edaphic pH and electrical conductivity (EC) gradients on bacterial community diversity and composition as they vary across regional, watershed basin, and local scales. Specifically, we examined the degree to which these key edaphic gradients structure microbial communities by disentangling the impacts of (1) spatial scale, (2) edaphic gradients, and (3) related threshold effects on microbial distribution patterns.

MATERIALS AND METHODS

Site, Sampling Description, and Chemical Analysis

The McMurdo Dry Valleys, Victoria Land, Antarctica (77° 30′ S, 163° 00′ E) comprise one of the harshest environments on Earth, with air and surface soil temperatures averaging between −15 and −30°C and extremes ranging from −60 to 25°C on the soil surface (Doran et al., 2002). The MDV are the largest ice-free zone in continental Antarctica (Fountain et al., 1999) with a total area of 22,700 km^2 and an ice-free area of 4,500 km^2 (Levy, 2013). The MDV receive little precipitation (<10 cm snow per year; Keys, 1980), most of which is lost through sublimation (Clow et al., 1988). The low soil moisture and precipitation results in an accumulation of salts and high pH in the upper soil stratum (Bockheim, 1997). Physicochemical gradients across the MDV are especially heterogeneous owing to its diverse glacial history (Lee et al., 2012). The hyper-arid mineral soils are primarily categorized as Anhyorthels or Anhyturbels and contain very little organic matter (Bockheim, 1997). Furthermore, the soils of the MDV are subjected to frequent freeze-thaw cycles that create physical sorting of rocks and soil particles and cause the expansion and contraction of permafrost layers 0.2–0.5 m below the ground surface (Kessler et al., 2001; Bockheim, 2002; Kessler and Werner, 2003). These processes create patterned ground formations, termed soil polygons. The polygons are clearly distinguished by intersecting troughs along their margins, and are a prominent landscape feature useful for geometrically scaling local ecological information (Barrett et al., 2004). Within polygons, significant differences in soil chemistry have been detected that are presumably due to naturally occurring edaphic heterogeneity and/or soil polygon mechanics, i.e., cryoturbation (Bockheim, 2002; Barrett et al., 2004).

Soils were aseptically collected from polygons during the austral summer of 2012 from the three major hydrological basins of the Taylor Valley: Bonney, Fryxell, and Hoare. A map of approximate sampling locations and major topographical features is provided in the **Supplementary Figure S1**. Eight soil polygons with an approximate radius of 6 m were randomly selected within each lake basin. Polygons within the Bonney basin were between 380 and 410 m from the lake margin, polygons within the Hoare basin 270–280 m, and polygons within the Fryxell basin were approximately 120 m away. Within each polygon, five samples were aseptically collected with sterilized scoops to a depth of approximately 10 cm along radial transects beginning from the trough edge to the center (at 0, 0.4, 0.8, 2, and 6 m), for a total of 120 samples. Soils were collected into sterile Whirl-Pak bags. Within 24 h, soils for molecular analysis were subsampled into sterile tubes by preserving approximately 10 g of soil with an equal volume of sucrose lysis buffer (Giovannoni et al., 1990). Samples were stored at −20°C until extraction. Soil pH was determined on 1:2 soil/deionized water extracts using an Orion pH probe. EC of 1:5 soil/water extracts was measured with a Yellow Springs Instrument 3100 conductivity meter.

DNA Extraction, Sequencing, and Sequence Analysis

DNA from 0.7 g of soil was extracted using the cetyltrimethylammonium bromide (CTAB) method (Hall et al., 2008; Mitchell and Takacs-Vesbach, 2008). Barcoded amplicon pyrosequencing of 16S rRNA genes was performed as previously described (Dowd et al., 2008; Van Horn et al., 2013, 2014) using V6 universal bacterial primers 939F 5′ TTG ACG GGG GCC CGC ACA AG-3′ and 1492R 5′-GTT TAC CTT GTT ACG ACT T-3′ on a Roche 454 FLX instrument using Roche titanium reagents following the manufacturer's instructions.

The 16S rRNA gene sequences were quality filtered, denoised, screened for PCR errors, and chimera checked using default parameters in AmpliconNoise (Quince et al., 2011). The Quantitative Insights into Microbial Ecology (QIIME) pipeline was used to analyze the 16S rRNA gene sequences (Caporaso et al., 2010a). Unique 16S rRNA gene sequences or operational taxonomic units (OTUs) were identified using the 97% DNA identity criterion using UCLUST (Edgar, 2010). A representative sequence was chosen from each OTU and aligned using the PyNAST aligner (Caporaso et al., 2010b) and the Greengenes core set (version 13.8) (DeSantis et al., 2006). Taxonomic assignments of the OTUs were made using the Ribosomal Database Classifier program (Wang et al., 2007).

All measures of community diversity (observed species, inverse Simpson, Good's coverage, Bray–Curtis, and Jaccard distances) and composition were performed with randomly selected subsets of 500 sequences per sample to standardize for varying sequencing efforts across samples. Raw sequence data from this study are available through the NCBI Sequence Read Archive as PRJNA436435. The individual sff files from this

study were assigned the accession numbers SAMN08624939–SAMN08625045.

Statistical Analysis

The normality of pH, EC, and alpha diversity distributions were assessed using Shapiro–Wilk tests. Significant differences in pH, EC, and alpha diversity data were assessed using non-parametric Kruskal–Wallis rank sum tests followed by *post hoc* pairwise Tukey honest significant difference (HSD) tests, corrected for multiple comparisons.

Data were pooled among all three lake basins for regional scale analysis. Patterns in microbial communities among and within lake basins (basin scale) were analyzed using non-metric multidimensional scaling (NMDS) using Bray–Curtis and Jaccard distances. Differences in microbial community composition (i.e., Bray-Curtis distances) were assessed by an Analysis of Similarity (ANOSIM) test with 999 permutations to assess significance. In addition, we investigated the degree to which microbial community profiles were associated with environmental factors by using the Random Forests classification algorithm (Breiman, 2001) implemented in QIIME's supervised_learning.py command with 10-fold cross-validation on a rarefied OTU table (−e 500) that was filtered to remove OTUs with less than 10 sequences. Performance of the random forests classifier is reflected by the ratio of baseline error to the estimated generalization error, ratios of 2 or greater indicate that the classifier is at least twice as accurate as random guessing. Phylum level patterns were investigated using linear regression analysis of relative abundance correlations along the radial transects of polygons.

Canonical correspondence analysis (CCA) was used to identify significant environmental variables that explained the variance of the OTU-level community structure. CCA is a constrained analysis that only partitions variation that can be explained by environmental factors while using chi-square distances to perform weighted linear mapping (Oksanen et al., 2010). CCA is considered a robust and valuable analysis for ecological data because it performs well with skewed species distributions, noise, interrelated environmental variables, and violations of assumptions (Palmer, 1993). The statistical significance of explanatory variables was assessed via the adonis test, as implemented in the vegan R package (Oksanen et al., 2010). Adonis is a permutational (n = 999) multivariate analysis of variance test that partitions distance matrices among sources of variation. The significance of CCA model constraints were assessed by the permutation test function anova.cca. CCA tests were run when considering communities at the regional and basin scales. NMDS and CCA tests were conducted using the Vegan library (Oksanen et al., 2016) in the R programming environment.

We considered the degree and significance of spatial structuring on community-environmental relationships across regional, lake basin, and local scales via Mantel and partial Mantel tests (Mantel, 1967; Smouse et al., 1986; Legendre and Legendre, 2012). Mantel tests were conducted to assess spatial auto-correlation using Jaccard- (community composition) and Euclidean- (observed species, pH, EC, spatial) based distance matrices. Spatial distance matrices were based on geographic coordinates at the regional and basin scales and on distance from the polygon trough at local scales. Partial Mantel tests were used to compute the correlations among edaphic variables and community composition and richness while controlling for the effects of spatial structure. Mantel and partial Mantel tests were implemented using Spearman correlations within the Vegan library (Oksanen et al., 2016) and significance of results were assessed via permutational analyses (n = 999). Additional analysis of spatial structuring was performed using these distance matrices to create a multivariate Mantel correlogram.

We investigated the relative influence of pH versus EC on species richness using a sliding window model. To do this, we created windows (i.e., subsets) of 10 samples across each edaphic gradient and then ran the frame across the entire gradient, stepping by one sample at a time. For example, samples were ordered from lowest to highest pH and the first window contained samples 1–10 while the second window consisted of samples 2–11. The process was repeated along the EC gradient. Linear regressions of observed species in relation to pH + EC were calculated for each window and relative contributions of the edaphic variables to explainable variability (R^2) were assessed using the relaimpo R package (Grömping, 2006). We used the recommended "lmg" metric, which provides a decomposition of the model explained variance into non-negative contributions while removing the effects of regression variable ordering. Results were visualized by plotting rectangles representative of the windows shaded by the proportion of variability explained along each edaphic gradient after normalization of overlapping regions. Normalization was conducted by averaging the relative contribution of each respective edaphic gradient in intervals of 5 µS/cm and 0.05 pH units.

Lastly, the effects of pH and EC as drivers of microbial community diversity and composition were assessed by Spearman rank correlations. P-values of environmental factors were adjusted for multiple comparisons using the Benjamini and Hochberg (1995) method.

RESULTS

Sequencing Results

Pyrosequencing of 16S rRNA gene libraries resulted in 680,727 reads (6,275 ± 4,540 reads per sample, n = 107 samples), with a mean length of 393 ± 28 bp. Samples were rarified to 500 sequences per sample to account for uneven sequencing depth among samples. When data from all samples were considered together (regional scale), a total of 25,449 and 5,092 OTUs (97% sequence similarity) were identified in the non-rarefied and rarefied datasets, respectively. The Good's coverage statistic of the rarified dataset ranged from 0.66 to 0.97 with an average of 0.80, indicating that the majority of diversity was detected in most samples (Good, 1953; Kemp and Aller, 2004). The rarified dataset included 5,092 OTUs that were primarily assigned to the phyla Acidobacteria (average of 28% relative abundance), Actinobacteria (9%), Bacteroidetes

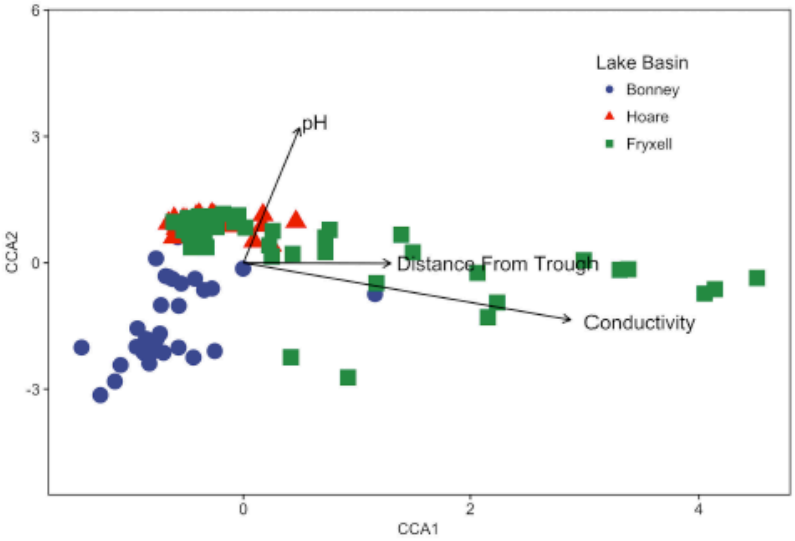

FIGURE 1 | Canonical correspondence analysis (CCA) of soil samples demonstrates that community composition differs both among and within basins and that the role of conductivity, distance to trough, and pH in driving within-basin variation differs among basins. Arrows indicate direction and magnitude of significant environmental factors affecting bacterial community structure.

(19%), Deinococcus–Thermus (8%), Gemmatimonadetes (5%), and Proteobacteria (9%).

Spatial Scale, Edaphic Gradients and Microbial Distributions

The relationships between edaphic gradients and the distribution of bacteria were investigated at three spatial scales; regional, basin, and local. The CCA model constructed at this geographic scale (**Table 1** and **Figure 1**) explained 17.1% of the variability of the OTU-level community matrices, leaving 82.9% of the variation unexplained (model $P = 0.001$). Additionally, adonis tests revealed that lake basin origin accounted for 25% of the community variance, EC for 14%, pH for 3%, and distance from the trough for 2% (all P-values ≤ 0.05). Mantel tests indicated significant spatial structuring of community and environmental variables at the regional scale (**Table 2**). Partial Mantel tests at the regional level indicated significant correlations between pH and community composition and between EC and both community composition and richness (**Table 3**). Similar results were obtained using tests based on both Jaccard and Bray–Curtis community dissimilarity matrices, so only results derived using Jaccard are reported. Additional evidence of spatial autocorrelation is evident in a Mantel correlogram (**Supplementary Figure S3**).

TABLE 1 | Results of canonical correspondence analysis performed at the regional and basins scales.

Test	Statistical measure	Regional	Bonney	Hoare	Fryxell
CCA	Total Inertia	5.56	1.72	0.34	1.33
	Constrained Proportion	0.17	0.11	0.33	0.42
	Unconstrained Proportion	0.83	0.89	0.67	0.58
Anova(cca)	Model χ^2	0.68	0.18	0.11	0.56
	Model F-value	9.38	1.02	5.48	8.42
	Model P-value	0.001***	0.56	0.001***	0.01***
adonis	Lake Basin R^2	0.25***	–	–	–
	Conductivity R^2	0.14***	0.03	0.17***	0.41***
	pH R^2	0.03***	0.02	0.06*	0.04*
	Dist. from trough R^2	0.02**	0.03	0.14***	0.04*
	Residual R^2	0.57	0.92	0.64	0.51

*$P \leq 0.1$, **$P \leq 0.01$, ***$P \leq 0.001$.

At the basin scale, soil pH and EC varied significantly (Kruskal–Wallis; pH: $P < 0.001$, EC: $P < 0.001$) (**Table 4**). The pH values were least alkaline in the Bonney Basin (mean 8.77, ±SE 0.06), intermediate in the Fryxell Basin (mean 9.57, ±SE 0.08), and most basic in the Hoare Basin (10.03, ±SE 0.04) (**Table 4**). In contrast, soil EC values were lowest in Hoare Basin (144 µS/cm, ± SE 10), intermediate in Bonney Basin (361 µS/cm, ±SE 54), and highest in Fryxell Basin (788 µS/cm, ±SE 135) (**Table 4**). Alpha diversity also varied significantly among basins (Kruskal–Wallis; observed species: $P < 0.05$ and inverse Simpson: $P < 0.001$) (**Table 4**). The highest average microbial richness, as measured by the number of observed species (OTUs), was found in the Lake Hoare basin (166 ± 6) and the lowest in the Lake Fryxell basin (136 ± 7). The inverse Simpson diversity index, which incorporates species evenness, was highest (43.31 ± 4.55) in the Lake Bonney basin soils and lowest (20.68 ± 2.16) in the

Lake Fryxell Basin soils. Both indices indicated that the Fryxell basin communities were the least diverse.

Differences were observed in the overall taxonomic composition of the soils from the various basins. Soils from Lake Bonney Basin had the most even distribution of phyla, containing 4-15% relative abundances of Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes, Planctomycetes, Proteobacteria, Verrucomicrobia, and Deinococcus–Thermus (**Supplementary Figure S2**). Lake Fryxell basin soils were dominated by Acidobacteria (28%), Bacteroidetes (24%), and Deinococcus–Thermus (15%). Acidobacteria were especially dominant in the Lake Hoare basin soils (43%), which also included Bacteroidetes (18%), Actinobacteria (9%), and Verrucomicrobia (7%). Bacterial community composition varied significantly among lake basins as evidenced by statistically significant clustering by basin (ANOSIM R statistic = 0.47, $P = 0.001$) using both Bray–Curtis (Legendre and Gallagher, 2001) and Jaccard distances (**Figure 2**), in addition to high random forests classification ratios (ratio of 14.1). Basin-specific CCA models were only significant for communities within the Lake Hoare and Fryxell basins (**Table 1**). EC explained the most variation in the Lake Fryxell basin (adonis: $R^2 = 0.41$), while pH and distance from the trough only explained 4% of variation. Within the Hoare basin, EC explained 17% of variation, distance from the trough explained 14%, and pH accounted for 6%. Partial Mantel tests at the regional level indicated significant correlations between pH and community composition in all three basins, EC and composition in Hoare and Fryxell basins, and EC and richness in Fryxell Basin (**Table 3**).

At the local scale, soil pH varied significantly along the polygon transects only in the Lake Bonney basin (Kruskal–Wallis; $P = 0.0358$) (**Figure 3** and **Supplementary Table S1**), increasing from an average of 8.45 to 9.04 from the trough to the polygon center. Soil EC only varied significantly along transects in the Lake Fryxell basin (Kruskal–Wallis; $P = 0.036$), increasing from 228 µS/cm at the trough to 1,190 µS/cm in the center. Along the polygon transects, the highest number of observed species was found within the Lake Hoare polygon troughs

TABLE 2 | Spearman correlation coefficients of Mantel tests assess spatial structure in each edaphic property and each aspect of microbial community structure.

Scale	Location	pH	EC	Composition	Richness
Regional		0.50***	0.10**	0.44***	0.03*
Basin	Bonney	0.01	−0.01	0.28***	NS
	Hoare	0.10*	0.10*	0.13*	NS
	Fryxell	0.04	0.03	−0.01	0.14**
Local	B_1	0.83*	0.06	−0.17	0.38
	B_2	ND	ND	ND	ND
	B_3	ND	ND	ND	ND
	B_4	ND	ND	ND	ND
	B_5	0.84*	−0.03	−0.22	−0.61
	B_6	0.08	0.75**	0.27	0.02
	B_7	0.75*	−0.27	0.13	−0.23
	B_8	0.03	0.03	0.60	0.77*
	H_1	0.41	0.11	0.72*	0.42
	H_2	−0.05	0.64**	0.05	−0.06
	H_3	0.09	−0.09	0.14	0.77
	H_4	−0.17	−0.26	0.17	−0.18
	H_5	0.58*	0.86*	0.64*	−0.01
	H_6	0.20	0.71	0.83	−0.35
	H_7	0.06	−0.43	0.26	0.89*
	H_8	−0.17	−0.03	0.07	0.01
	F_1	0.12	0.92**	0.37	0.92**
	F_2	−0.20	0.99*	0.70*	0.84*
	F_3	0.22	−0.32	0.35	0.46*
	F_4	0.71*	0.03	0.02	0.11
	F_5	0.20	0.38*	−0.18	0.42*
	F_6	0.01	−0.26	0.41	−0.05
	F_7	0.22	−0.30	0.56	0.59*
	F_8	0.05	0.27	0.12	0.35

*Local scale key indicates basin_polygon number. *$P \leq 0.1$, **$P \leq 0.01$, ***$P \leq 0.001$, ND, not determined due to small sample size.*

TABLE 3 | Partial Mantel test results, reporting Spearman correlation coefficient between each edaphic variable and aspects of community structure when controlling for geographic distance.

		Composition		Richness	
Scale	Location	pH	EC	pH	EC
Regional		0.22***	0.41***	0.01	0.23***
Basin	Bonney	0.14*	0.12	0.00	−0.08
	Hoare	0.31***	0.35***	−0.07	0.01
	Fryxell	0.13*	0.63***	0.13	0.57***
Local	B_1	0.10	0.07	0.58	0.72*
	B_2	ND	ND	ND	ND
	B_3	ND	ND	ND	ND
	B_4	ND	ND	ND	ND
	B_5	−0.42	−0.48	−0.07	−0.78
	B_6	0.20	−0.12	0.06	−0.09
	B_7	−0.36	−0.01	−0.07	−0.40
	B_8	0.66	0.66	−0.71	−0.71
	H_1	0.41*	0.76**	−0.42	0.05
	H_2	0.46	0.46	−0.50	−0.10
	H_3	−0.22	−0.25	0.57	0.33
	H_4	0.26	0.88**	−0.24	0.00
	H_5	0.36	0.02	0.27	−0.05
	H_6	−0.15	−0.13	0.90*	−0.50
	H_7	0.51	0.43	−0.04	0.22
	H_8	0.09	0.08	−0.50	−0.13
	F_1	−0.29	0.62*	0.20	0.92**
	F_2	0.24	0.71*	−0.70	−0.62
	F_3	0.17	−0.36	0.55	−0.29
	F_4	−0.46	0.56*	−0.62	0.69*
	F_5	−0.20	0.93**	−0.29	0.96*
	F_6	0.52*	0.64*	0.26	0.61
	F_7	−0.13	0.02	−0.19	−0.39
	F_8	−0.47	0.71**	−0.55	0.76*

*Local scale key indicates basin_polygon number. *$P \leq 0.1$, **$P \leq 0.01$, ***$P \leq 0.001$, ND, not determined due to small sample size.*

while the lowest was in the center of Lake Fryxell polygons. Lake Bonney basin transects generally had the highest inverse Simpson values and Lake Fryxell basin transects had the lowest. Both metrics decreased toward the center of polygons within the Lake Fryxell basin (Kruskal–Wallis; observed species: $P = 0.0006$, inverse Simpson: $P = 0.0028$). The relative composition of phyla along the polygon transects within Bonney and Hoare basins were stable and indistinguishable (random forests ratios of 1.0 and 1.7, respectively, **Figure 4**). In contrast, communities along the transects in the Fryxell basin soils diverged significantly (random forests ratio of 2.1) as Deinococcus–Thermus and Gemmatimonadetes increased in relative abundance toward the center of the polygons while Acidobacteria and Bacteroidetes decreased. Populations of Acidobacteria and Actinobacteria were inversely correlated, as were Bacteroidetes versus Deinococcus–Thermus and Gemmatimonadetes (**Table 5**). Both Bacteroidetes and Proteobacteria, as well as Gemmatimonadetes and Deinococcus–Thermus were positively correlated. Correlations between soil chemistry and community structure at the local level were only significant for some transects in some basins. In the Fryxell basin, significant correlations were observed between community composition and EC along six out of eight transects, and between richness and EC at four out of eight transects (**Table 3**). Raw edaphic and alpha diversity values are reported in **Supplementary Table S2**.

Threshold Effects and Phylum-Specific Patterns

Our sliding window model constructed at the regional scale simultaneously assessed the effects of pH and EC on richness and indicated that EC generally explained the majority of variation in the number of observed species (**Figure 5**). EC was the more influential edaphic variable across intervals from 80–165, 290–640, and at 1000 µS and above (**Figure 5B**). In contrast, pH explained more variation in small windows from 8.95–9.1 to 10.05–10.15 (**Figure 5A**).

Phylum-specific spearman rank correlations were calculated to determine the proportion of variance in alpha diversity and phyla relative abundance that could be explained by distance from the polygon trough, pH, and EC (**Figure 6**). Significant correlations between distance from trough and alpha diversity were found within the Lake Hoare and Fryxell basin soils based on positive correlations between distance and observed species and negative correlations between distance and inverse Simpson (all $P < 0.05$). Significant correlations to phyla relative abundance were also only found in the Hoare and Fryxell lake basins. In all cases where a phylum was significantly correlated to an edaphic gradient in more than one basin, the direction of the correlation was consistent between basins; for example, Deinococcus–Thermus was positively correlated with EC in both Hoare and Fryxell basins (**Figure 6**).

TABLE 4 | Mean ± standard error of soil geochemical properties and alpha diversity values for soils collected from each lake basin.

Parameter	Lake basin	Minimum	Maximum	Range	Mean	*SE*
pH*	Bonney	7.64	9.33	1.69	8.77^{a}	0.06
	Hoare	9.39	10.40	1.01	10.03^{c}	0.04
	Fryxell	8.40	10.35	1.95	9.57^{b}	0.08
Conductivity*	Bonney	119	1636	1517	361^{a}	54
	Hoare	56	296	40	144^{a}	10
	Fryxell	86	2808	2722	788^{b}	135
Observed species*	Bonney				146^{ab}	9
	Hoare				166^{b}	6
	Fryxell				136^{a}	7
Inverse Simpson*	Bonney				43.3^{c}	4.6
	Hoare				30.9^{b}	2.3
	Fryxell				20.7^{a}	2.2

*Ranges of edaphic factors (minimum–maximum, difference) are reported inside parentheses. *Indicates significant difference in raw data among basins ($P < 0.05$). Basin means with same superscript letter are not statistically different after adjusting for multiple comparisons.*

DISCUSSION

To better understand the relationship between bacterial species distribution patterns and the environmental factors that shape them, we performed a spatially stratified examination of the inherent edaphic gradients within the cold, dry, and oligotrophic polar desert ecosystem of the MDV, Antarctica. This study focused on the relationships among edaphic pH and EC gradients on bacterial communities as they vary across local, lake basin, and regional scales. The simplified trophic structure and high spatial physiochemical heterogeneity of MDV soils provides an ideal model system in which to study natural, low-complexity microbial community-environment interactions and thus better understand the underlying scale-dependent processes that structure these communities. By minimizing the effects of biotic interactions, we can more directly address the poorly understood impacts of environmental heterogeneity and spatial scale on microbial community composition and diversity. Our findings corroborate previous studies of soil bacterial communities within the lake basins of Taylor Valley by describing high spatial variability and linking soil microbial community structure to edaphic geochemical gradients (Barrett et al., 2006; Niederberger et al., 2008; Smith et al., 2010; Zeglin et al., 2011; Lee et al., 2012; Sokol et al., 2013; Van Horn et al., 2013). However, this is the first study to use the inherent edaphic gradients within soil polygons to investigate the effects of spatial scale, environmental heterogeneity, and landscape context on bacterial community structure.

Environmental Gradients at Different Scales

Edaphic gradients, which strongly affect the spatial patterning of soil microbial communities (Barrett et al., 2006; Fierer and Jackson, 2006; Lozupone and Knight, 2007; Niederberger et al., 2008, 2015; Smith et al., 2010; Zeglin et al., 2011; Lee et al., 2012; Sokol et al., 2013; Van Horn et al., 2013), occur across different

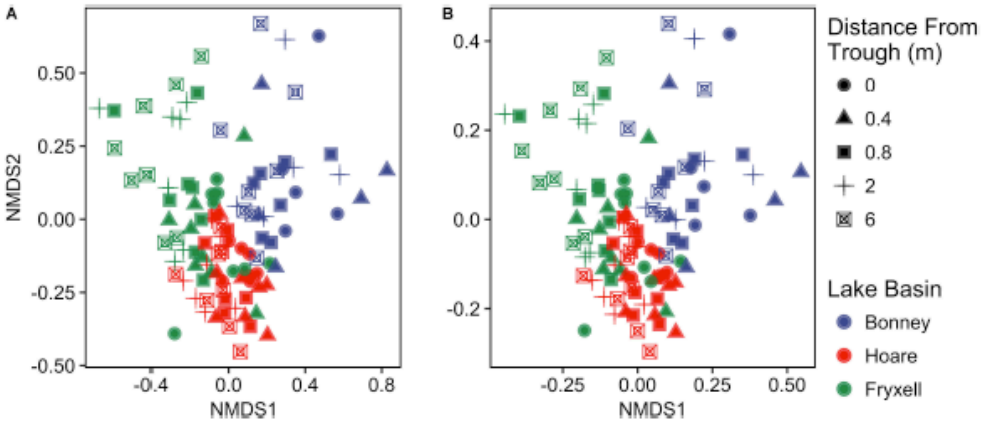

FIGURE 2 | Non-metric multidimensional scaling plots created using **(A)** Bray–Curtis and **(B)** Jaccard distances show distinctiveness of communities among basins.

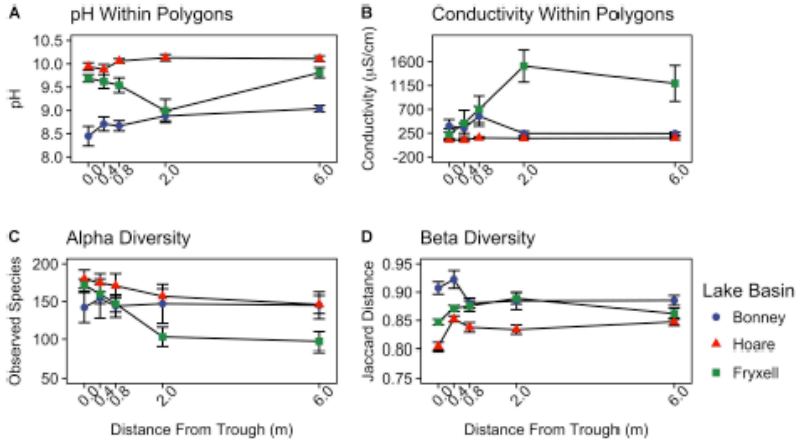

FIGURE 3 | Basin scale spatial variability of **(A)** pH, **(B)** conductivity, **(C)** alpha diversity (observed species), and **(D)** beta diversity (Jaccard distance) (mean ± standard error) along radial transects of polygons. Beta diversity was calculated by pooling samples from each distance from the trough within each lake basin.

spatial scales due to variation in the underlying drivers producing these gradients. For example, broad-scale edaphic gradients can be caused by differences in topography, climate, and geologic history that occur across landscapes (Jenny, 1941; Sinsabaugh et al., 2008; Townsend et al., 2008). The variations in edaphic conditions that we observed between lake basins in the MDV were most clearly illustrated by the absence of overlap between samples from different lake basins in our pH versus EC bi-plots (**Figure 5**). These observations are an example of landscape-scale gradients likely due to differences in parent geology, glacial till sequence, and paleo-lacustrine organic matter deposition (Péwé, 1960; Burkins et al., 2000).

While broad-scale gradients create a general template for the formation of biological communities, fine-scale factors such as soil structure, microclimates, topography, and transition zones between habitats (ex. riparian zones), can superimpose local-scale gradients on top of regional patterns (Ettema and Wardle, 2002). In the MDV, fine-scale gradients in edaphic factors including soil moisture, organic matter, pH, EC, ions, and nutrients have been observed near snowpacks (Gooseff et al., 2003; Van Horn et al., 2013), lake margins (Northcott et al., 2009; Zeglin et al., 2009), hyporheic zones (Barrett et al., 2009; Northcott et al., 2009; Zeglin et al., 2009; Niederberger et al., 2015), ponds (Moorhead et al., 2003), and mummified seals (Tiao et al., 2012). In this study, the effects of the physical processes inherent to polygon formation, (e.g., frost-sorting and aeolian deposition, Kessler et al., 2001; Bockheim, 2002; Kessler and Werner, 2003; Barrett et al., 2004) created

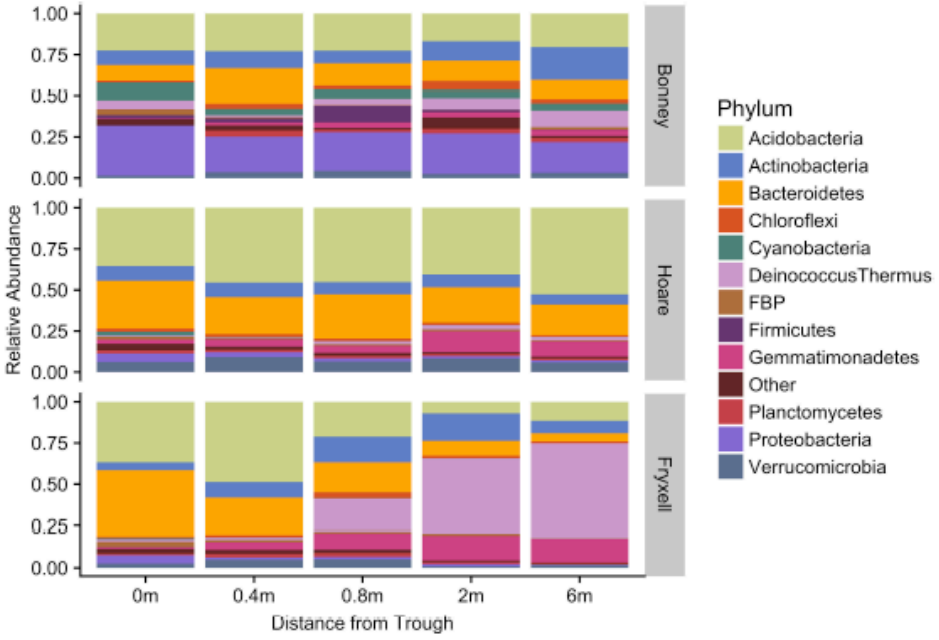

FIGURE 4 | Median relative abundance of top phyla within basins along polygon radial transects (filtered to minimum 1% average relative abundance).

local-scale gradients within some (Fryxell), but not all (Bonney and Hoare) basins. Thus, both the basin- and local-scale gradients for pH and EC provided an opportunity to study the effects of edaphic gradients on microbial community diversity and composition.

Bacterial Community Responses to Gradients

In this study, we focused on bacterial community responses to pH and EC gradients because these edaphic parameters are master drivers of microbial community structure and diversity (Fierer and Jackson, 2006; Lozupone and Knight, 2007). High soil salinity increases water limitation by controlling total ion concentrations, and therefore the total water potential of the soil. Salinity can exert additional biological stress on soil microbes by osmotically increasing intracellular ion concentrations to potentially toxic levels resulting in decreases in respiration, critical enzyme activity, and nitrogen and carbon cycling (Frankenberger and Bingham, 1982; Zahran, 1997). The exact mechanism by which pH exerts its effects on microbes is less well defined, though two possible explanations have been proposed. First, pH may be an amalgam of other edaphic characteristics such as nutrient concentrations, cationic metal solubility, organic matter content, moisture, and salinity, and it is these variables that directly impact the soil communities (Lauber et al., 2009). Alternatively, severe pH gradients may create a selective advantage for species with increased tolerance to pH extremes (Lauber et al., 2009).

As described above, our sampling encompassed both broad- and local-scale variation in these primary edaphic variables, along with the subsequent impacts to bacterial community structure. Similar to other studies (e.g., Fierer and Jackson, 2006; Lauber et al., 2009), broad-scale environmental differences created distinct communities in spatially distant, but cohesive,

TABLE 5 | Statistical metrics of significant linear regressions comparing phyla relative abundances along polygon transects within the Fryxell basin.

Independent variable	Dependent variable	*X* – Intercept	Correlation coefficient	R^2
Acidobacteria	Actinobacteria	0.43**	−1.12*	0.74*
Bacteroidetes	Gemmatimonadetes	0.31***	−2.12**	0.93**
Bacteroidetes	Proteobacteria	0.01	0.25*	0.73*
Bacteroidetes	Deinococcus–Thermus	0.26***	−0.70*	0.82*
Gemmatimonadetes	Deinococcus–Thermus	0.03*	0.31*	0.82*

*$P \leq 0.1$, **$P \leq 0.01$, ***$P \leq 0.001$.

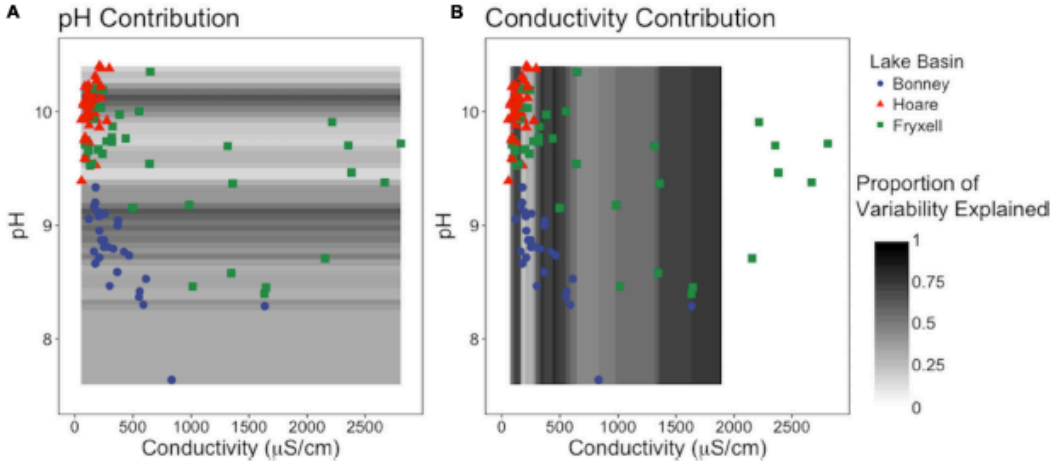

FIGURE 5 | Sliding window model: Relative contributions of pH and conductivity to explainable variation in observed species across **(A)** the pH gradient and **(B)** the conductivity gradient. Models reveals that EC is generally more influential to community diversity and composition, but when EC is low, pH is more important.

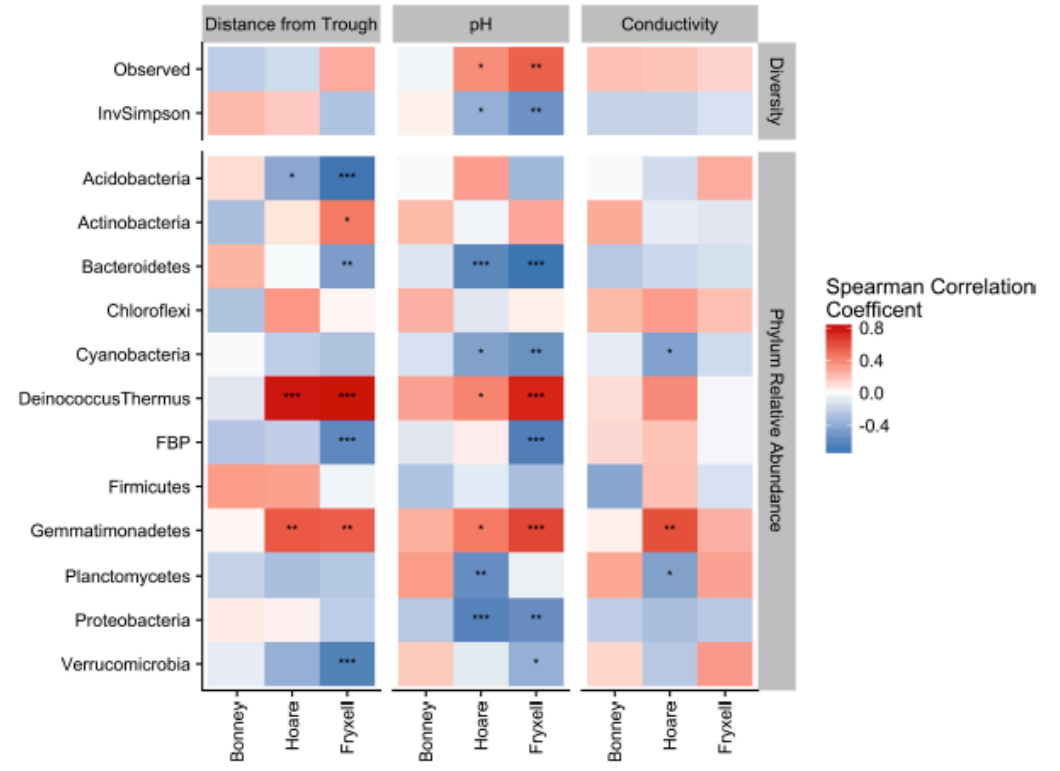

FIGURE 6 | Assessing relative influence of edaphic factors over alpha diversity **(top)** and major phyla relative abundances **(bottom)** using Spearman rank correlations, revealing that phyla tend to respond in a similar direction regardless of basin. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$. *P*-values of distance from trough, pH, and conductivity adjusted for multiple comparison using Benjamini and Hochberg (1995).

areas (lake basins) based on ordination analyses (**Figures 2, 5**). In particular, the Lake Bonney communities were differentiated from those in the other two lake basins, and the Lake Bonney soils had the lowest minimum, maximum, and mean soil pH. This result, coupled with the overall low within- and high between-basin variation in pH, suggests that this variable is particularly important in shaping the baseline community found in different regions of the MDV. Superimposed upon the broad-scale pH pattern, local polygon-scale conductivity gradients appear to drive local community composition, with predictable increases in Deinococcus–Thermus and Gemmatimonadetes and decreases in Acidobacteria, Bacteroidetes, and Proteobacteria with increasing EC (**Table 5** and **Figures 3, 4**). These findings were particularly strong in Lake Fryxell basin, indicating that edaphic variations may elicit sufficient physiological stress to result in community changes due to environmental filtering and competitive interactions, as observed elsewhere (Weiher and Keddy, 2001).

Our findings of both local- and broad-scale edaphic and community patterns, contrast with several previous studies both within and outside of the MDV that found diverse or non-existent patterning at local scales and coherent patterns at larger scales (Barrett et al., 2004; O'Brien et al., 2016). We propose that a key to understanding this discrepancy is the degree of edaphic heterogeneity captured within our study. More specifically, not only is the presence of gradients important in structuring microbial communities, but the length or severity of the gradient is also crucial. For example, Lake Fryxell basin soils have the highest average EC (788 ± 135 μS) and range (86–2808 μS) (**Table 4**) and the bacterial communities at Lake Fryxell were the most highly responsive to EC (**Figures 1, 5**). Furthermore, the strength of relationships among edaphic conditions and microbial communities depended on the magnitude of the heterogeneity exhibited at each scale that was analyzed (**Table 1**). Although the magnitude of relationships among community structure and edaphic factors varied among basins, the direction of change remained constant (**Figure 6**). This implies that, regardless of location, phyla are responding similarly to edaphic conditions. However, because the core communities were different due to the broad-scale underlying factors, communities from different basins did not appear to converge even at the extreme end of the edaphic ranges.

Our results suggest that Deinococcus–Thermus was likely the only phylum thriving in the high EC soils of the Lake Fryxell polygon centers. These organisms have previously been associated with low productivity soils (Niederberger et al., 2008). Deinococcus–Thermus had the largest variation across all scales, second only to Proteobacteria (**Supplementary Figure S2**). Interestingly, Proteobacteria have been identified as constituting a substantial proportion of the active communities in the MDV by studies using stable isotope probing (Schwartz et al., 2014) and RNA sequencing (Buelow et al., 2016). Thus, we can surmise that Proteobacteria and Deinococcus–Thermus are active and adapting to environmental conditions within our study system. Furthermore, the two aforementioned activity-based studies indicated that Acidobacteria and Bacteroidetes were largely inactive. Our study found comparably low variation for these phyla across spatial scales, indicating that perhaps they (or their relic DNA) had a more cosmopolitan distribution that decreased in relative abundance when environmental conditions better suited more specialized taxa, i.e., high EC selection of Deinococcus–Thermus.

Together, these results suggest several consistent patterns with respect to interactions between environmental gradients and bacterial community structure. First, sufficiently long gradients allowed a greater number of niches (and therefore a greater number of taxa), resulting in variation in community composition along the gradients. These niches may be due to differences physiological stresses or other factors correlated to the abiotic gradient (Okie et al., 2015). We found that at any scale, there was potential for thresholds of effect: we observed local-scale thresholds within the Fryxell lake basin soils, but basin-scale thresholds in pH between Lake Bonney and Lake Hoare soils. Additionally, these edaphic factors did not work in isolation, but instead interacted synergistically. Further investigation into the relative contributions of EC and pH to microbial richness at the regional-scale revealed the dominance of EC (**Figure 5B**), while pH explained more variation in small windows that correspond to EC levels below 500 μS (**Figure 5A**). This not only highlights the importance of gradient severity, but reveals that when EC is low, pH is more influential to community diversity and composition. Thus, it appears that environmental gradient length organizes soil bacterial communities. This conclusion is especially interesting considering the discrepancies over the suggested dominant environmental drivers reported in the literature. For example, Fierer and Jackson (2006) suggest that soil bacterial diversity is primarily correlated to pH, whereas Lozupone and Knight (2007) found diversity strongly correlates to soil salinity, but not pH. Our study encompassed almost neutral to very basic soils, ranging from ∼7.6 to 10.4 while Fierer and Jackson (2006) studied a pH range of 3.5 to 9, and did not consider soil salinity. We note that it is plausible that microbial communities are more responsive to acidity than alkalinity due to the complexities involved in the physiological adaptations toward acidophily (Colman et al., 2017). The pH and salinity ranges in the Lozupone and Knight (2007) study are not reported, and samples were binned into "saline" and "non-saline" groups. Thus, it is possible there are no actual contradictions in the conclusions given by these research groups but that these reportedly differing results were due to limitations of the edaphic gradients present in the different studies.

CONCLUSION

These findings stress the importance of a spatially explicit experimental design and recognition of the inherent gradients across a variety of spatial scales. In particular, effort is needed to sample the entirety of gradients present at each relevant spatial scale before reaching conclusions about the distribution of organisms or relationships among communities and environmental factors. Failure to do so may lead to spurious inferences or results that are artifacts of limited and unrepresentative data. Random sampling with the objective

of capturing an unbiased representation of soil heterogeneity may capture edaphic ranges but will not capture the local structure needed to inform our understanding of ecological processes. For instance, we captured clear patterns at scales less than 6 m in Lake Fryxell soils because we had sufficient EC gradients to elicit physiological response. The substantial local EC gradients resulted from the physical processes of polygon formation and significantly affected larger-scale patterning. While environmental extremes may be less frequent, they play an important role in structuring biota across the landscape, even in the physiologically challenging environment posed by the MDV. Furthermore, we observed coherent local-scale patterns because the bacterial communities were (1) diverse, (2) active, and (3) adapted to the local environment. Barrett et al. (2004) did not observe clear local-scale patterns, potentially because the edaphic gradients sampled did not impart sufficient physiological stress on the nematodes, or perhaps because the eukaryotic community was not diverse enough and many samples did not contain organisms. In contrast, the ubiquity of bacteria in these soils allows us to see an additional aspect of environment-community interactions, that is, the effects of environmental filtering across finer spatial scales.

We conclude that a combination of local-scale polygon mechanisms as well as regional-scale geological histories drove changes in edaphic gradients that played a large role in determining the microbial community composition and diversity within the McMurdo Dry Valleys of Antarctica. Our results suggest that the relative importance of pH versus EC in structuring microbial communities is contextually related to the length and severity of edaphic gradients and the spatial scale of sampling, creating a framework in which to interpret conflicting literature.

AUTHOR CONTRIBUTIONS

Funding was secured by CT-V, DVH, and ES. DVH conceived and designed the experiments. DVH, DC, TM, and HB performed the fieldwork. KF processed the samples for DNA sequencing. DVH, CT-V, and KF analyzed the data. KF, DVH, and CT-V wrote the manuscript with input from all the authors.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2018.01928/full#supplementary-material

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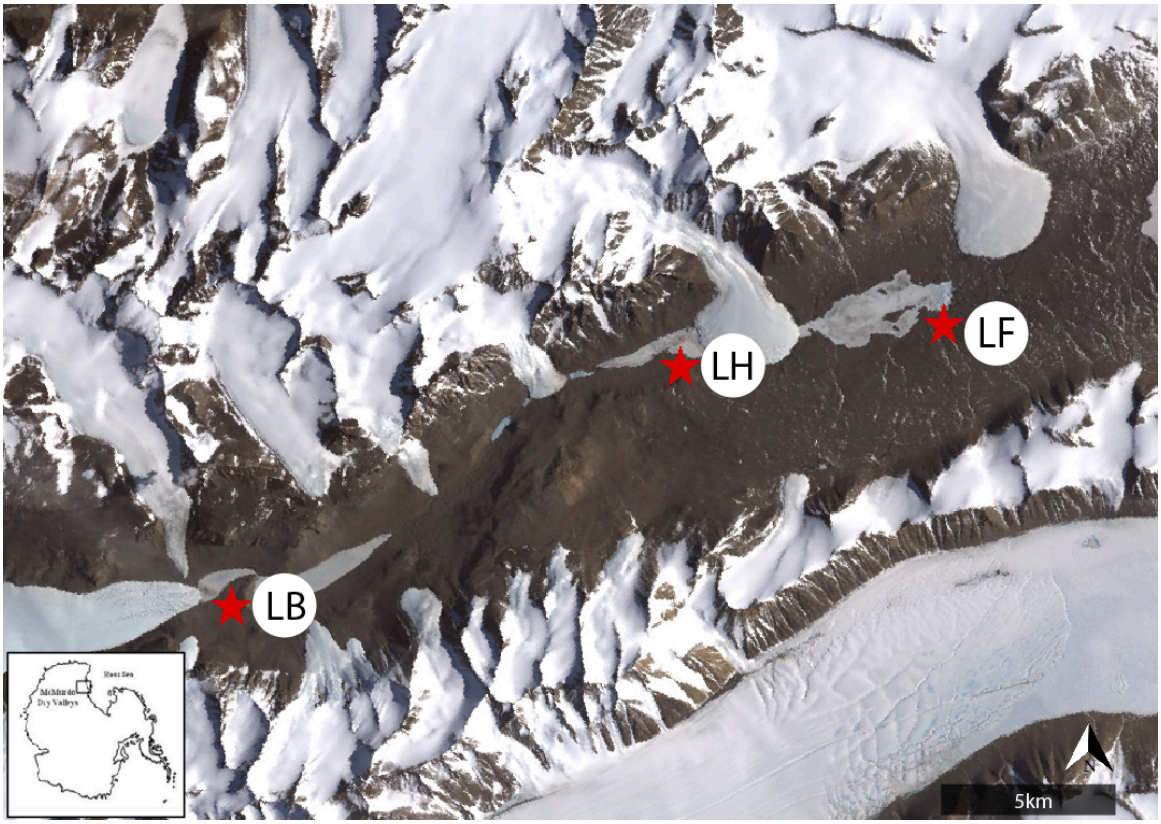
Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

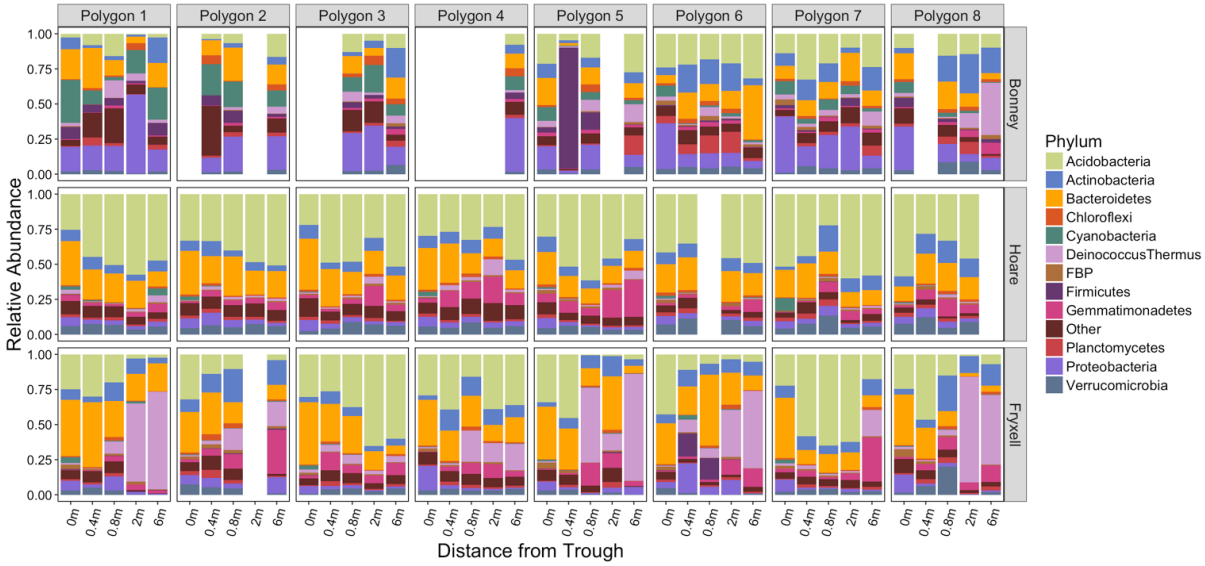
APPENDIX A. CHAPTER 1 SUPPLEMENTARY MATERIAL

Supplemental Figure 1.



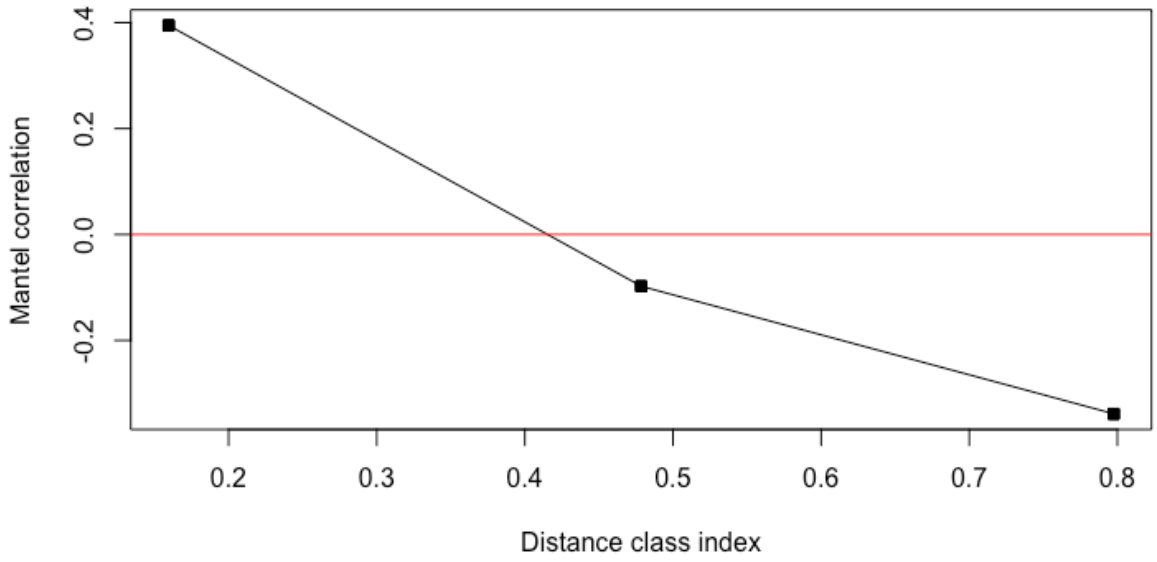
Map of sampling locations. Red stars indicate approximate locations of polygons within the Lake Bonney (LB), Lake Hoare (LH), and Lake Fryxell (LF) basins. Source: This image was acquired by Landsat 7's Enhanced Thematic Mapper plus (ETM+) instrument on December 18, 1999. Image by Robert Simmon, based on data provided by the NASA GSFC Oceans and Ice Branch and the Landsat 7 Science Team. Image retrieved from: https://earthobservatory.nasa.gov/images/2140/mcmurdo-dry-valleys on June 6th, 2018.

Supplemental Figure 2.



Relative abundance of major phyla

Supplemental Figure 3.



Mantel Correlogram using Jaccard distance matrix

Supplemental Table 1. Mean ± standard error of soil geochemical properties and alpha diversity values of soils collected from each distance from the polygon trough within each lake basin. Means with the same superscript letter are not statistically different after adjusting for multiple comparisons.

Lake Basin	Distance from Trough (m)	pH	Conductivity (μS)	Observed Species	Inv. Simpson
Bonney	0	8.45^{a} ± 0.21	388 ± 119	142 ± 20	41.6 ± 11.3
	0.4	8.71^{ab} ± 0.15	345 ± 96	154 ± 26	39.7 ± 13.1
	0.8	8.67^{ab} ± 0.11	571 ± 187	144 ± 15	56.5 ± 9.6
	2	8.88^{ab} ± 0.11	246 ± 43	147 ± 26	35.4 ± 12.3
	6	9.04^{b} ± 0.07	243 ± 29	145 ± 18	40.0 ± 7.9
Hoare	0	9.94 ± 0.08	127 ± 23	180 ± 12	40.9 ± 6.0
	0.4	9.88 ± 0.11	121 ± 20	175 ± 12	31.8 ± 4.5
	0.8	10.06 ± 0.05	162 ± 22	171 ± 16	35.9 ± 5.1
	2	10.13 ± 0.07	152 ± 15	157 ± 10	22.4 ± 4.5
	6	10.11 ± 0.06	158 ± 29	146 ± 12	25.8 ± 3.3
Fryxell	0	9.69 ± 0.08	228^{a} ± 108	172^{c} ± 8	30.7^{b} ± 3.7
	0.4	9.62 ± 0.15	437^{ab} ± 246	160^{c} ± 13	25.6^{b} ± 4.8
	0.8	9.54 ± 0.16	698^{ab} ± 253	147^{bc} ± 11	23.8^{bc} ± 5.0
	2	8.99 ± 0.25	1517^{b} ± 302	103^{ab} ± 13	13.9^{bc} ± 2.8
	6	9.81 ± 0.11	1190^{b} ± 339	97^{a} ± 13	9.2^{ac} ± 3.0

Supplemental Table 2. Raw soil geochemical results and alpha diversity values of soils collected from each distance from the polygon trough within each lake basin. Data are excluded from samples with less than 500 sequencing reads.

Lake Basin	Polygon Number	Distance from Trough (m)	pH	Conductivity (μS)	Observed Species	Inv. Simpson
Bonney	1	0	8.47	299	147	20.1
		0.4	8.42	558	170	69.8
		0.8	8.37	552	148	27.3
		2	9.17	163	60	6.4
		6	9.33	178	90	18.8
	2	0				
		0.4	9.06	119	174	16.2
		0.8	8.53	612	143	61.7
		2				
		6	8.87	248	125	42.7
	3	0				
		0.4				
		0.8	8.74	466	115	47.2
		2	8.79	331	134	15.3
		6	8.66	176	131	58.0
	4	0				
		0.4				
		0.8				
		2				
		6	9.04	371	112	16.8
	5	0	7.64	832	172	73.2
		0.4	8.30	589	51	1.3
		0.8	8.29	1636	114	37.4
		2				
		6	9.10	255	164	68.6
	6	0	8.72	209	98	25.1
		0.4	8.95	210	202	54.8
		0.8	8.87	226	214	101.4
		2	8.59	364	177	76.9
		6	8.90	367	212	38.3

	7	0	8.67	177	98	24.7
		0.4	8.81	249	174	56.3
		0.8	8.81	287	134	44.9
		2	8.77	163	214	38.2
		6	9.20	175	160	64.6
	8	0	8.77	421	196	65.1
		0.4				
		0.8	9.12	199	232	75.6
		2	9.08	206	150	40.4
		6	9.12	196	80	12.5
Hoare	1	0	10.12	221	219	54.5
		0.4	9.73	123	195	32.9
		0.8	10.00	122	203	41.0
		2	10.04	110	137	7.6
		6	9.53	165	169	31.9
	2	0	9.93	186	195	57.1
		0.4	9.53	177	202	36.2
		0.8	9.99	166	221	46.0
		2	9.88	122	207	40.5
		6	10.17	116	173	24.8
	3	0				
		0.4	10.10	141	181	14.5
		0.8	10.11	159	177	28.1
		2	10.31	175	189	41.0
		6	10.06	136	180	25.1
	4	0	10.13	145	208	46.0
		0.4	10.22	223	194	43.7
		0.8	10.27	188	203	43.9
		2	10.40	211	177	32.6
		6	10.18	184	196	39.5
	5	0	10.14	114	198	49.3
		0.4	10.22	87	144	15.9
		0.8	10.23	114	122	14.3
		2	10.38	226	139	20.4
		6	10.38	296	130	14.2

	6	0	9.75	82	144	39.9
		0.4	10.08	76	212	51.1
		0.8				
		2	10.06	74	116	18.3
		6	10.18	117	147	25.5
	7	0	9.59	84	154	23.0
		0.4	9.39	57	161	33.2
		0.8	9.93	108	155	52.6
		2	9.97	107	108	8.6
		6	9.97	95	141	17.9
	8	0	9.93	56	142	15.7
		0.4	9.77	87	114	26.7
		0.8	9.92	275	113	25.3
		2	9.95	125	132	25.9
		6	9.86	210	155	33.8
Fryxell	1	0	9.71	86	208	50.2
		0.4	9.54	149	192	20.9
		0.8	9.73	319	181	47.5
		2	8.40	1632	87	10.1
		6	9.46	2383	45	2.6
	2	0	9.69	120	192	39.6
		0.4	9.74	271	197	55.8
		0.8	9.77	317	167	34.3
		2				
		6	9.7	2355	60	9.1
	3	0	9.95	152	155	26.1
		0.4	10.03	222	186	26.2
		0.8	9.87	323	170	30.9
		2	10.20	165	114	6.63
		6	10.19	240	103	3.7
	4	0	9.75	92	190	33.0
		0.4	9.63	236	178	22.9
		0.8	9.76	437	153	22.0
		2	9.54	643	144	16.1

	6	9.97	383	145	10.8
5	0	9.72	115	153	19.7
	0.4	9.59	93	139	18.8
	0.8	9.91	2215	86	5.0
	2	9.37	1360	111	22.0
	6	9.38	2669	64	2.8
6	0	9.94	160	171	32.1
	0.4	8.71	2156	91	29.0
	0.8	8.58	1346	115	9.6
	2	8.45	1646	97	18.7
	6	9.70	1316	80	5.5
7	0	9.18	984	163	21.5
	0.4	9.67	200	129	9.3
	0.8	9.53	120	148	12.9
	2	8.46	1014	124	14.0
	6	10.00	553	99	11.2
8	0	9.66	115	141	23.3
	0.4	10.04	169	166	21.9
	0.8	9.15	499	154	28.2
	2	9.72	2808	55	2.4
	6	10.35	649	106	4.9

CHAPTER 2: STRATIFICATION OF BACTERIAL COMMUNITIES WITHIN THE SOIL ACTIVE LAYER IN THE MCMURDO DRY VALLEYS, ANTARCTICA

Kelli L. Feeser[1], David J. Van Horn[1], Jordan G. Okie[2], Becky A. Ball[3], Andrew G. Fountain[4], Michael N. Gooseff[5], Joseph Levy[6], Maciej K. Obryk[7], and Cristina D. Takacs-Vesbach[1]

[1]Department of Biology, University of New Mexico, Albuquerque, NM, USA

[2]School of Earth & Space Exploration, Arizona State University, Tempe, AZ, USA

[3]School of Mathematical and Natural Sciences, Arizona State University at the West Campus, Glendale, AZ, USA

[4]Department of Geology, Portland State University, Portland, OR, USA

[5]Department of Civil, Environmental, and Architectural Engineering, University of Colorado, Boulder, CO, USA

[6]Department of Geology, Colgate University, 13 Oak Ave., Hamilton, NY, USA

[7]U.S. Geological Survey Cascades Volcano Observatory, Vancouver, WA, USA

Abstract

The McMurdo Dry Valleys (MDV) of Antarctica are underlain by a unique combination of ice-cemented and dry-frozen permafrost, with an overlying active soil layer that thaws during summer months. The soil active layer is expected to expand as temperatures increase, altering conditions for soil microbial communities. We used a combination of survey and experimental approaches to assess the importance of depth in structuring the microbial communities within the active layer of both wetted and dry soils. Samples from the surface sediments were generally more diverse (~7,500 OTUs) than samples near the soil-permafrost interface (~5,000 OTUs), however patterns varied by basin. Surface samples generally showed enrichments of Cyanobacteria and Proteobacteria and corresponding lower abundances of Acidobacteria and Actinobacteria. Relationships among community structure and depth also depended on the relative position of the sampling sites to water tracks. In the surface sediments, enrichment of Cyanobacteria (15-22%) was only found in the wetted, downslope sites while the dry, upslope sites contained < 2% Cyanobacteria and the dominant phyla were Acidobacteria (60% in LHSS) and Proteobacteria (92% in WHC). In additional to soil surface community differences among wet and dry sites, the uniformity of taxonomic profiles of samples taken from 5 cm to the soil-permafrost interface also varied by the relative position to a water track. In the wet, downslope sites of both basins, generally uniform and stable community compositions were found, whereas the horizon profiles in the dry, upslope sites were variable. Our results suggest that this region harbors highly adapted endemic communities that are susceptible to changing conditions.

Introduction

The surface of the terrestrial cryosphere is comprised of two strata, an upper "active layer" of seasonally thawed, biologically active soil, and an underlying zone of consistently frozen rock, sediment, and soil termed permafrost. Close to a quarter of the terrestrial surface of the Northern Hemisphere and high elevation and latitude portions of the Southern Hemisphere are composed of this active layer/permafrost combination. These layers host cold-adapted microbial communities uniquely adjusted to this ecological habitat. The majority of organisms are found in the active layer [1, 2], which contains a dynamic, transitional continuum physically connecting the permanently frozen region to the soil surface. Active layer thickening and near-surface permafrost thawing is predicted to occur as a consequence of rising global temperatures [1, 3, 4]. Active layers in the Arctic and Antarctic are expanding at a rate of ~1 cm per year [5–9]. These changes are likely to impact the biological soil communities and the functions they perform, as increased available water from ice melt and the interaction of this water with nutrient rich soils alter the supply of basic resources. Comparably little is known about the distribution and characteristics of permafrost and the active layer in the Antarctic in contrast to the Arctic [10, 11]. However, a clear understanding of Antarctic soil active layer is necessary because it is one of the most important areas for detecting the impact of climate change in the terrestrial cryosphere [11]. Furthermore, there is a need to explore the contribution of the Antarctic permafrost and active layer in the global carbon cycle as well as understand their role in dynamic climate feedback systems.

The McMurdo Dry Valleys (MDV), the largest ice-free zone in Antarctica [12], are on the threshold of widespread landscape scale change due to increasing temperature

and solar radiation and altered hydrology [13, 14]. This harsh polar desert is home to some of the most extreme conditions on Earth [15, 16]. In the past decade, dramatic landscape-scale change has already occurred in the (MDV). Glaciers have deflated, thermokarst slumps have formed near lakes and streams, lake levels have risen, and rivers have become incised. The MDV are underlain by a unique combination of ice-cemented and "dry-frozen" (ice-free) permafrost, with an overlying active soil layer that thaws during summer months [10]. MDV permafrost is susceptible to warming-related degradation—either directly through slumping of melt-lubricated sediments and surface ablation by sublimation-driven ice removal [17, 18], or indirectly, as ice-free permafrost sediments are preferentially removed by warming-induced fluvial erosion [19].

Microbes are the dominant life form in the MDV with the vast majority of the total diversity found among soil bacteria [20–22]. These bacterial communities are responsible for processing nutrients and organic matter [23] and supporting higher organisms [24]. The structure and function of these microbial communities are highly responsive to changing conditions of temperature, moisture, and resource supply [16, 22, 25]. While microbes can grow and divide within permafrost, the low temperatures greatly reduce these rates and thus the active layer likely contains the bulk of the microbial activity. The soil active layer is expected to expand as temperatures increase, altering conditions for soil microbial communities [13]. The active soil layer in the MDV ranges from ~20 to 70 cm in depth [10]. Temperatures in the active layer fluctuate dramatically on a diurnal and seasonal basis and based on aspect, soil moisture, and depth [2].

While previous research into MDV active layer communities suggests that the surface and near-surface depths harbor more microbial diversity and metabolic activity

than the permafrost soil interface [2], additional study using updated sequencing approaches is required to thoroughly profile the microbial communities within this environment. Temperatures within the soil surface are dynamic, fluctuating on both a daily and seasonal basis [2]. Furthermore, increasing depth in the soil active layer is associated with colder, more stable temperatures, increasing relative humidity, and decreasing soil organic matter [2].

In this study, we used a combination of survey and experimental approaches to assess the importance of depth, which controls factors including temperature, soil moisture, and resource availability [2], in structuring the microbial communities in the active layer. We surveyed the microbial communities along the soil profile in soil pits associated with both water tracks and dry soils. Our first aim was to explore community dynamics in terms of bacterial composition and alpha and beta diversity along vertical soil horizons within the active layer to better understand the environmental determinants of community structure. We characterized the variation in community structure by partitioning the beta diversity into turnover (species replacement) and nestedness (differences in richness) components, allowing us to elucidate mechanisms of community assembly along the vertical soil profiles. Our second aim was to analyze how the relationships among taxonomy, diversity and depth vary in wet versus dry soil. We sampled 'upslope' profiles higher along the valley walls located in dry soil adjacent to nearby water tracks and 'downslope' pits within a water track, representing wetted soils. Our final aim involved reciprocal transplant experiments with near surface soils and soils from the permafrost/active layer boundary to see if changes in microbial community structure could be detected in a relatively short period of time. These surveys and

experiments were conducted under the premise that a more thorough understanding of the factors that structure these communities will enable a more rapid and accurate detection of future climate-induced changes.

Material and Methods

Sampling description and study design

Survey sampling was co-located with two active layer monitoring stations installed in 2014 at Worm Herder Creek (77°43.509' S, 162°18.694' E), and a water track on the south shore of Lake Hoare, southwest of Canada Glacier (77°38.54' S, 162°58.715' E) (Figure 1). At each location four soil pits were dug at the downslope/upslope locations (8 pits total) to sample the microbial communities and soil properties at 5 cm intervals until the permafrost layer was encountered. In each pit, approximately 20 grams of soil were collected for microbial community structure analysis (16S rRNA gene sequencing), with a total of 85 samples collected.

Experimental approach:

Reciprocal transplant experiments were conducted along the soil profiles in one upslope and one downslope pit at each of the two sampling locations. In each of the two experimental pits, four samples of soil from the top 5-cm layer (warm, highly variable temperatures) were placed in falcon tubes and moved to the bottom layer (cold stable temperatures) and vise-versa. The caps on the tubes were loosely attached to allow gas exchange. Additionally, four samples from the top and bottom were placed in falcon tubes and re-inserted in their original depths to control for tube effects. The pits were

backfilled and the tubes were collected and preserved after ~1.5 months of incubation at the new temperature regime. A total of 64 experimental samples were collected.

DNA extraction, 16S rRNA gene sequencing and analysis

Within 24 hours of collection, soils for molecular analysis were subsampled into sterile tubes by preserving approximately 10 g of soil with an equal volume of sucrose lysis buffer [26]. Samples were stored at -20 °C until extraction. DNA from 0.7 g of soil was extracted using the cetyltrimethylammonium bromide (CTAB) method [27, 28]. Dual-index paired-end amplicon sequencing of 16S rRNA genes was performed as previously described [29–31] using V6 universal bacterial primers 939F 5' TTG ACG GGG GCC CGC ACA AG-3' and 1492R 5'-GTT TAC CTT GTT ACG ACT T-3' on an Illumina MiSeq.

The 16S rRNA gene sequences were trimmed and quality filtered using Sickle [32] and paired-end reads were aligned and merged via PANDAseq [33]. The Quantitative Insights into Microbial Ecology (QIIME) pipeline was used to analyze the reconstructed gene sequences [34]. Unique 16S rRNA gene sequences or operational taxonomic units (OTUs) were identified by the 97% DNA identity criterion using UCLUST [35]. A representative sequence was picked from each OTU and aligned using the PyNAST aligner [36] and the Greengenes core set (v. 13_8, [37]) and given taxonomic assignments using the Ribosomal Database Classifier program [38].

Survey and experimental data were processed separately. All measures of community diversity (observed species, inverse Simpson, Good's coverage, Bray-Curtis, and Jaccard distances) and composition were performed with randomly selected subsets of 1,189 sequences per-sample, to standardize for varying sequencing efforts across

samples. Data from this project have been described on the Antarctic Master Directory (https://gcmd.nasa.gov/r/d/Biological_responses_to_landscape_change_in_the_McMurdo_Dry_Valleys), sequence data are available via the National Center for Biotechnology Information (NCBI, SRA accession: PRJNA525069, Temporary Submission ID: SUB5239758, Release date: 2019-03-08, accession numbers SAMN11041036 to SAMN11041351), and edaphic data have been submitted to the McMurdo LTER data manager for immediate release.

qPCR

Extracted DNA from a subset of experimental samples was also used for real-time quantitative PCR (qPCR). Standards for qPCR were obtained using DNA extracted from *Escherichia coli* cultures that had undergone two consecutive rounds of PCR to ensure the final product contained no genomic DNA. DNA was amplified using the prokaryotic universal primer set developed by Takahashi et al. 2014 [39] – Pro341F: 5'-CCT ACG GGN BGC ASC AG-3' and Pro805R: 5'-GAC TAC NVG GGT ATC TAA TCC-3'. Amplified DNA was cleaned using UltraClean PCR Clean-Up Kits (MoBio Corporation, Carlsbad, CA, USA) and quantitated by Qubit assay (Invitrogen, Carlsbad, CA, USA). The purified DNA solution was serially diluted 10-fold to give solutions ranging from 10^3 to 10^{10} copies/µL (given a fragment length of 465 base pairs). The standard dilution series was used to generate a standard curve that was applied to estimate the copy number for each qPCR reaction. Reactions were run in triplicate using the SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad, California, USA) using previously described settings [39]. Briefly, the conditions were initial denaturation at 95 °C for 30 seconds, followed by 35 cycles of 5 s at 95°C, 20 s at 60 °C, and 20 s at 72 °C. A melt

curve was run at the end of the reaction, from 60 to 95 °C, increasing 0.5 °C every 5 s cycle. Cycle threshold values of triplicate reactions were averaged and the copy numbers of samples were calculated from the linear region of the standard curve. Due to the variability of copy number per-bacterium, our estimates of copy number per-sample be interpreted as relative estimates of biomass change per location.

Statistical analysis

Differences among alpha diversity (measured by the chao1 index) and sampling depths were assessed using non-parametric Kruskal-Wallis tests. Patterns in microbial communities among locations and sampling depths were visualized using nonmetric multidimensional scaling (NMDS) using Bray-Curtis distances. The statistical significance of explanatory variables was assessed via the adonis test, as implemented in the vegan R package [40]. Adonis is a permutational (n=999) multivariate analysis of variance test that partitions distance matrices among sources of variation. These analyses were conducted using the phyloseq [41] and vegan packages [40] within the R programming environment.

Changes in total beta diversity were also explored along the gradient of sampling depths by decomposing this variation into its constitutive components. Communities can differ in composition, and therefore beta diversity, owing to species turnover (species replacement) and nestedness (species loss) [42]. Species turnover arises when species replace each other, while nestedness occurs when a site with lower alpha diversity contains a subset of taxa from a more diverse (i.e. species rich) site. Thus, turnover and nestedness represent two ends of a continuum, and total beta diversity is comprised of one or both components. We leveraged recently proposed analytical framework [43, 44]

and used Sorensen's dissimilarity (βsor) to compute total beta diversity, Simpson's index (βsim) to measure turnover, and then subtracted βsor minus βsim to get βsne, the nestedness proportion of total beta diversity. Beta diversity analyses were performed using the betapart package in R [45].

Results

Survey – relationships among taxonomy, diversity, and depth

Sequencing of the 16S rRNA genes present in the survey samples collected from a total of eight soil pits per basin resulted in a total of 454,483 reads (5,347 ± 6,476 reads per sample (± s.d.), $n = 85$ samples). These reads comprised 151,931 OTUs. After rarefaction, 37,209 OTUs remained ($n = 73$ samples). The Good's coverage statistic of the rarified dataset ranged from 0.28 to 0.60, with an average of 0.42 ± 0.07 suggesting adequate sequencing depth was achieved [46, 47].

Samples from the surface sediments were generally more diverse (median Chao1 richness estimates of ~7,500 OTUs) than samples from the bottom of the active layer (median Chao1 richness estimates of ~5000 OTUs), however patterns varied by basin. Within LHSS sites, there were significant differences in diversity within sampling depths (all $P < 0.05$) with a strong inverse correlation found between diversity and depth (Figure 2A) in all eight LHSS pits (SI Fig 1). Within WHC sites, diversity responded variably with depth, increasing in some pits and displaying a "U-shaped" pattern in others (SI Fig 1). No significant differences in diversity were found between depths in the WHC pits. NMDS ordinations suggested that the surface bacterial communities are distinct from the

communities found at depth (Figure 2B). Phyla-level relative abundance patterns confirmed the distinctiveness of surface samples (Figure 2C, SI Fig 2). Surface samples generally showed enrichments of Cyanobacteria and Proteobacteria and corresponding lower abundances of Acidobacteria and Actinobacteria. In contrast, samples from 5-30 cm were dominated by Acidobacteria, followed by Proteobacteria and Actinobacteria. Pits dug in the upslope site of LHSS were the exception to this trend; Proteobacteria increased with depth while Acidobacteria decreased (Figure 2C).

Beta diversity analysis revealed a modest increase in beta diversity with depth ($R^2_{adj} = 0.09$, $P = 0.01$) (Figure 3A). Partitioning the total beta diversity into turnover and nestedness components as a function of depth revealed that nearly all the variation was due to species turnover ($R^2_{adj} = 0.096$, $P = 0.001$) (Figure 3B) with no significant variation ($R^2_{adj} = 0.003$, $P > 0.1$) attributed to nestedness (Figure 3C).

Survey – community-depth relationships vary by soil moisture

We investigated how microbial community-depth relationships varied within wet versus dry soils by sampling profiles in both dry, upslope sites that were located adjacent to nearby water tracks and in wet, downslope sites located directly inside of water tracks. In an NMDS ordination combining all basins, soil moisture condition (dry upslope versus wet downslope), and depths showed clear separation not only of the surface samples, but also distinct clustering by soil condition (Figure 4). Adonis tests confirmed that while depth was most important to structuring these communities ($F = 1.50$, $R^2 = 0.12$, $P < 0.001$), soil condition was the second most influential single variable ($F = 4.81$, $R^2 = 0.06$, $P < 0.001$). The surface layers of the downslope sites in both basins were significantly enriched in Cyanobacteria, while the upslope site at WHC was

overwhelming dominated (> 90%) by Proteobacteria (Figure 2C). As previously mentioned, a contrasting pattern was found in the upslope site in LHSS where surface samples hosted ~5% Proteobacteria and ~60% Acidobacteria. With the exception of the enrichment of Cyanobacteria in the surface samples, the taxonomic profile of communities was relatively unchanging with depth in the downslope sites in both basins. However, the taxonomic profile of upslope sites was dynamic and variable. In the LHSS upslope site, Proteobacteria increased with depth and Acidobacteria decreased, while in the WHC upslope site, a more variable pattern was observed (Figure 2C).

Experimental reciprocal transplants

Sequencing of the 16S rRNA genes present in the experimental samples resulted in a total of 218,178 reads (3,698 ± 2,849 reads per sample (± s.d.), n = 59 samples). These reads comprised 73,402 OTUs. After rarefaction, 27,466 OTUs remained (n = 55 samples). The Good's coverage statistic of the rarified dataset ranged from 0.34 to 0.64, with an average of 0.45 ± 0.07, again suggesting adequate sequencing depth was achieved [46, 47].Reciprocal transplants of soil from 5 to 30 cm and vice versa did not appear to impact community diversity or composition (Figure 5). Results from qPCR, although few in number, indicate shallower communities might have higher bacterial abundance than deeper communities (average ± s.d. 16S copy number, downslope 5 cm: 354 ± 144, upslope 5 cm: 331 ± 167, upslope 30 cm: 205 ± 83).

Discussion

The goal of this project was to assess the biological consequences of geomorphic, climate-induced changes to the soil active layer within the McMurdo Dry Valleys (MDV)

of Antarctica. In this study, we profiled microbial communities in these vulnerable habitats, and we used experimental approaches to determine the response of communities to disturbances. Specifically, we surveyed the microbial communities along the soil profile in both wet and dry soil pits and conducted reciprocal transplant experiments where near surface soils were incubated at lower soil horizons, and vice versa. Our results suggests that surface communities are unique in comparison to deeper samples and that relationships among communities and depth are dependent on soil moisture. Together these surveys and experiments suggest that this region harbors highly adapted endemic communities that are susceptible to changing conditions.

As in many other studies of the MDV soils [22, 48–51] and Arctic soils [52–54], this study found a predominance of Acidobacteria, Actinobacteria, and Proteobacteria (Figure 2). When comparing community profiles by depth, the most striking aspect was the distinct clustering of surface-level samples (Figure 2). The surface samples were generally more diverse than samples from the bottom of the active layer and held taxonomically distinct compositions, typically with greater abundances of Cyanobacteria and Proteobacteria. As photoautotrophic organisms, the presence of Cyanobacteria in the soil surface layers, where they have the greatest access to photosynthetically active radiation, is expected. Such cyanobacterial communities are known colonizers of cold soils in the Arctic and Antarctic [55] and are thought to play a primary role in key functional processes related to carbon and nitrogen cycling [56–58]. The presence of significant percentages of Proteobacteria in the soil surface layers is of interest, as stable isotope probing [49] and metatranscriptomic sequencing [22] have identified this group of bacteria as a key portion of the active community in MDV soils. Additionally, Stomeo

et al. (2012) reported that the greatest metabolic activity was found at the soil surface [2], and thus our results support that the surface communities contain active assemblages of primary producers (Cyanobacteria) and heterotrophic consumers (Proteobacteria). As temperature decreases with depth [2], we suggest that this represents an increase in environmental stress, as demonstrated by the general decrease in alpha diversity (Figure 2A) and biomass (as measured by ATP titres and DNA yields) noted previously [2]. This supports a recently proposed [59] and tested [60, 61] hypothesis that environmental stress increases the challenges associated with maintaining physiological homeostasis and acquiring sufficient nutrient resources, resulting in lowered growth rates, increased environmental filtering, and declining alpha diversity as response to narrower niche widths. The beta diversity patterns we observed showing of a weak (slope of 0.0028, adjusted $R^2 = 0.09$) but significant ($P = 0.01$) increase in beta diversity with depth along the vertical soil profile (Figure 3A), also support this hypothesis. Partitioning the total beta diversity into turnover and nestedness components as a function of depth revealed that nearly all the variation was due to species turnover ($R^2_{adj} = 0.096$, $P = 0.001$) (Figure 3B) with no significant variation ($R^2_{adj} = 0.003$, $P > 0.1$) attributed to nestedness (Figure 3C). Species turnover, as opposed to nestedness, has been described along naturally occurring gradients of environmental stress [62], again confirming that depth acts as a stressor to bacterial communities in the soil active layer. This stress-gradient hypothesis suggests that the turnover is a result of environmental filtering selecting for specialist taxa adapted to localized conditions [62].

The finding that the community members known to be the most active were found either on the soil surface or in the top 5 cm of the soil profile, with significant changes in

composition and diversity in comparison to the 5-10 cm horizon (Figs 2 & 4), has clear implications for future soil active layer sampling in the MDV. To date, many MDV soil studies have collected and homogenized soil from the top 10 cm of soil as part of their standard soil sampling protocol [20, 50, 63–66]. Our results indicate that this may combine active and less-active surface and subsurface communities, obscuring patterns in both the vertical structure of these soil microbial communities and in cross-site comparisons of the surface communities. We therefore suggest that future MDV soil sampling plans should take these results into consideration.

Relationships among community structure and depth also depended on the position of the sampling sites relative to water tracks. Upslope sites were located in dry soil, adjacent to water tracks while downslope sites were located inside wetted, water track soil. We classified the upslope sites as dry because, in addition to the deliberate sampling away from upslope water tracks, upslope habitats in the MDV are known to be dehydrated by solar radiation which causes the rapid evaporation of permafrost meltwater [16]. In contrast, the downslope sites were located inside water tracks. Water tracks are defined as narrow, cryptic hydrological systems that transport shallow groundwater from high elevations to the valley floor through the soil active layer [67, 68] and are found in both the Arctic [69, 70] and Antarctic [67, 68, 71]. Our finding that samples clustered by soil moisture condition (wet versus dry) as opposed to by lake basin is of interest (Figure 4). Several studies in MDV soils suggest that different basins host distinctive bacterial communities [48, 50, 72], however, Niederberger et al. (2015) [73] and other previous studies of water track communities [74] also noted distinct clustering of arid and wet soils. As previously discussed, the surface samples in every basin and site clustered

separately and were taxonomically unique in comparison to the deeper samples, although differences in surface samples were noted between wet and dry sites. Enrichment of Cyanobacteria (15-22% relative abundance) was only found in the wetted, downslope sites (Figure 2C). Increased abundance of Cyanobacteria in wetted soil of the MDV is expected [73, 75] as conditions likely promote *in-situ* primary production [76]. In contrast, the upslope sites contained < 2% Cyanobacteria and the dominant phyla were Acidobacteria (60% in LHSS) and Proteobacteria (92% in WHC). Variable responses in the relative abundance of Cyanobacteria and Acidobacteria have also been observed in Arctic water tracks [77], where Cyanobacteria often, but not always increased in relative abundance inside water tracks and Acidobacteria was more abundant inside in one of three sites.

In addition to soil surface community differences among wet and dry sites, the uniformity of taxonomic profiles of samples taken from 5 cm to the soil-permafrost interface also varied by the relative position to a water track (Figure 2C). In the downslope sites of both basins, generally uniform and stable community compositions were found among the soil horizons, whereas the horizon profiles in the upslope sites were variable. In the LHSS dry upslope sites, Proteobacteria increased with depth while Acidobacteria decreased. In the WHC upslope sites, the proportions of Acidobacteria, Actinobacteria, Proteobacteria, and Verrucomicrobia varied and no consistent pattern was seen with increasing depth. We hypothesize that the wetted, downslope sites are more homogenized because the increased soil moisture and the flow of water through these soil profiles allows for movement of bacteria, nutrients, and other solutes throughout the soil

column. In the drier, upslope sites, the communities are more stratified by depth because there is little to no water-mediated mechanisms for dispersal.

The differences in community structure among the lake basins could be due to differing characteristics of the water tracks sampled in each basin. In the MDV, water flux in the water tracks is highly variable [67]. This variability in flow rate has significant consequences for edaphic conditions, as areas with lower flow rates experience more interactions between the groundwater and rocks/soil, resulting in higher soil salinity, while fast moving tracks are associated with the flushing of solutes and relatively low soil salinity [67, 68, 78]. The water track in WHC experiences intermittent overland flow which disperses and dilutes solutes [79] while the water track in LHSS is reported to have a lower water flux and concomitantly higher salinity [68].

Reciprocal transplants of soil did not appear to impact community diversity or composition (Figure 5), suggesting that changing the temperature regime alone for moderate durations had little impact on the soil bacterial communities, and that other factors, or longer time scales, are responsible for the impact of depth on community diversity and structure. It is likely that the slow growth rates inherent to the region prevented the detection of significant community changes within the six-week experiment.

This project fits within a larger context of research within the MDV dedicated to understanding the drivers of baseline soil community dynamics, documenting landscape-scale disturbances, and assessing the impact of those disturbances on the associated biological communities. Major activities related to understanding the basic structure and drivers of MDV biological communities include the exploration of microbial

communities in MDV streams [80], in soil communities along salinity gradients [61, 81], and associated with soil polygon features [50]. Microbial community responses to water and organic matter amendments reveal that climate change is likely to induce the replacement of endemic taxa adapted to the cold, dry, and oligotrophic conditions with generalist, copiotrophic taxa [22]. The findings described in this study suggest that, as in other soil habitats in the MDV, the soil active layer supports unique microbial communities that vary distictively with depth, soil moisture, and landscape position, suggesting high degrees of local adaptation. These active layer communities, along with communities associated with stream riparian areas experiencing thermokarst activity, lake margin soils which are becoming inundated due to lake level rise, and previously dry hill slope soils that become saturated due to water track expansion, are all likely to be significantly impacted by the increase in water and associated resources.

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Figure legends

Figure 1. Map of sampling locations. Red x's indicate approximate locations of water tracks within Worm Herder Creek (WHC) and the south shore of Lake Hoare (LHSS). Source: This image was acquired by Landsat 7's Enhanced Thematic Mapper plus (ETM+) instrument on December 18, 1999. Image by Robert Simmon, based on data provided by the NASA GSFC Oceans and Ice Branch and the Landsat 7 Science Team. Image retrieved from: https://earthobservatory.nasa.gov/images/2140/mcmurdo-dry-valleys on June 6th, 2018.

Figure 2. Survey diversity of composition. Diversity and composition of active layer survey communities by depth and location. (A) Boxplots of the chao1 diversity measures show significantly decreasing diversity with depth in LHSS and no relationship between diversity and depth in WHC samples. Significance was assessed using non-parametric Kruskal-Wallis tests. (B) Non-metric multidimensional scaling (NMDS) plots created using Bray-Curtis distances matrices show stratification of soil microbial communities by depth. (C) Mean relative abundance of bacterial phyla revealed that communities were dominated by Acidobacteria and Proteobacteria. Surface-level (00 cm) samples are visually distinctive from deeper samples. Phyla with less than 1% relative abundance among any of the sample were binned into the < 1% relative abundance category.

Figure 3. Survey beta diversity analyses. (A) Total beta diversity of survey samples along each depth is measured by Sorensen's dissimilarity (βsor) while the turnover component

(B) of total beta diversity is given by Simpson's dissimilarity (βsim). Dissimilarity due to nestedness (C) was computed as Sorensen's minus Simpson's indices (βsne).

Figure 4. Non-metric multidimensional scaling (NMDS) plots created using Bray-Curtis distances matrices show stratification of soil microbial communities by depth and location. Significance of categorical variables including basin (LHSS versus WHC), site (DownSlope versus UpSlope), basin_site, and depth was assessed using adonis.

Figure 5. Experimental diversity and composition. Diversity and composition of experimental active layer samples by depth, treatment, and location. Samples are labeled by the depth of their origin. Treatment samples were taken from their original depth and transplanted from 5 cm to 30 cm or vice versa. (A) Boxplots of the chao1 diversity measures show that deeper samples tend to be less diverse. (B) Non-metric multidimensional scaling (NMDS) plots created using Bray-Curtis distances matrices show stratification of soil microbial communities by depth but minimal clustering by treatment. (C) Mean relative abundance of bacterial phyla revealed that communities were dominated by Acidobacteria and Proteobacteria. Phyla with less than 1% relative abundance among any of the sample were binned into the < 1% relative abundance category. Experimental samples tend to be most influenced by their original depth with no significance shifts seen in the treatment samples, indicating that the incubation period within this study was likely insufficient for any substantial effects on community structure to be detected.

Figure 1.

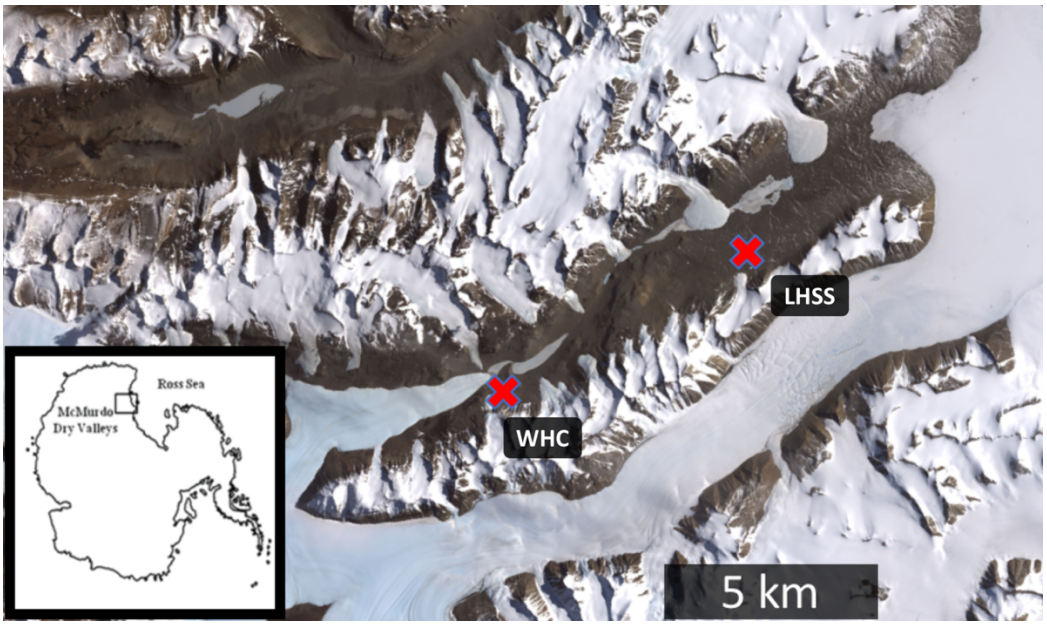


Figure 2.

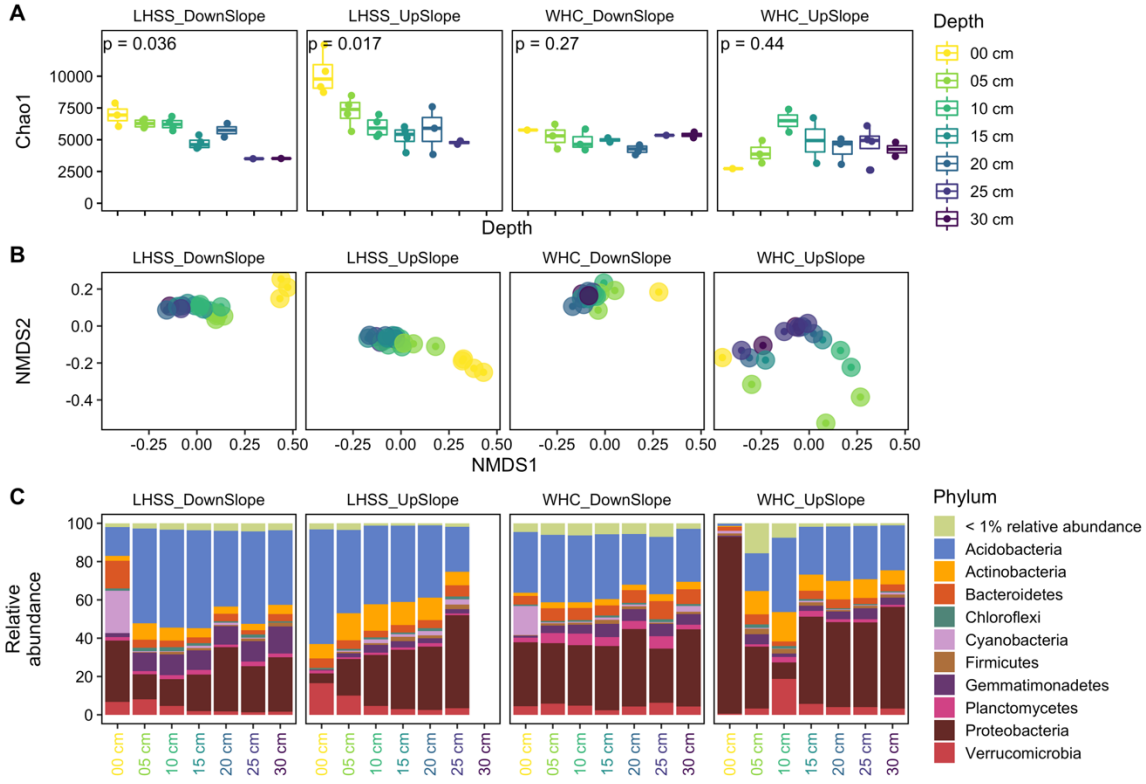

A
LHSS_DownSlope
LHSS_UpSlope
WHC_DownSlope
WHC_UpSlope
p = 0.036
p = 0.017
p = 0.27
p = 0.44
Chao1
10000
7500
5000
2500
0
Depth
Depth
00 cm
05 cm
10 cm
15 cm
20 cm
25 cm
30 cm
B
NMDS2
0.2
0.0
-0.2
-0.4
-0.25
0.00
0.25
0.50
NMDS1
C
Relative abundance
100
80
60
40
20
0
Phylum
< 1% relative abundance
Acidobacteria
Actinobacteria
Bacteroidetes
Chloroflexi
Cyanobacteria
Firmicutes
Gemmatimonadetes
Planctomycetes
Proteobacteria
Verrucomicrobia

Figure 3.

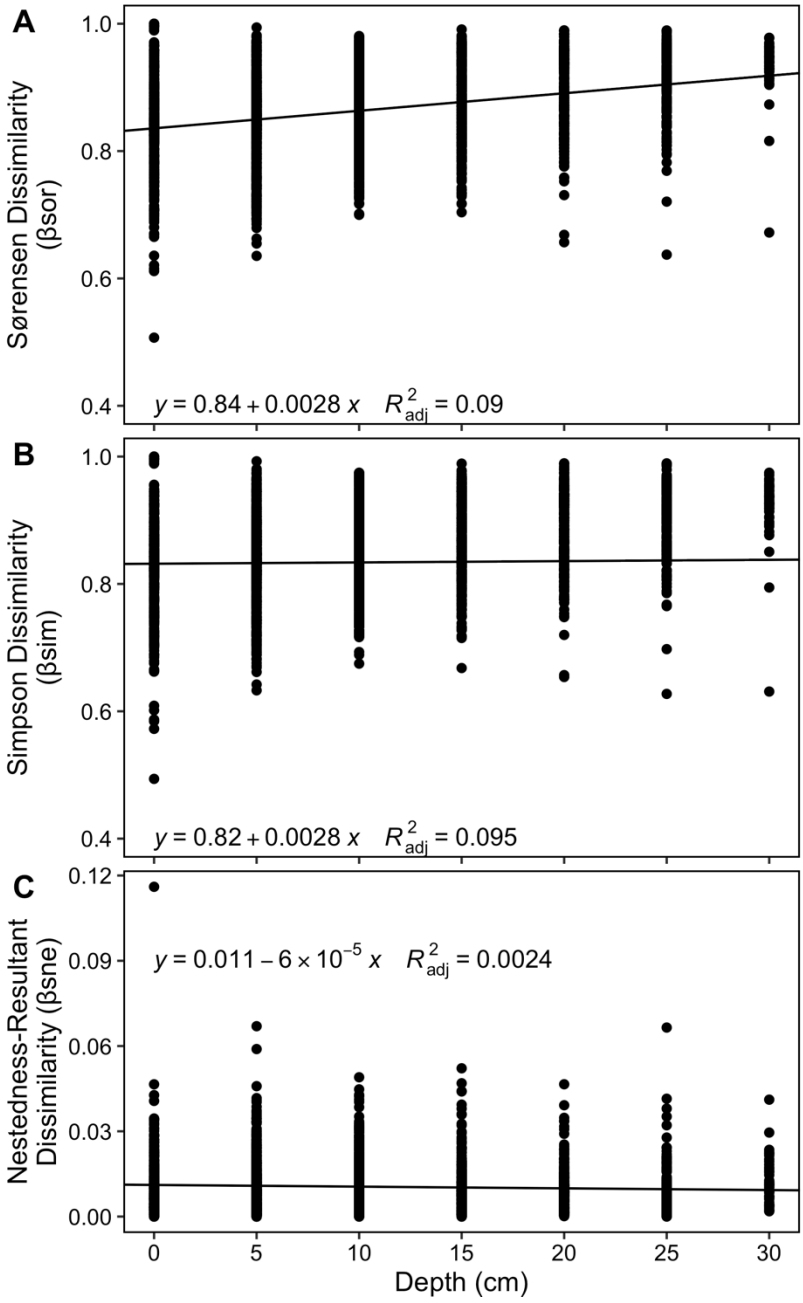

A
Sørensen Dissimilarity (βsor)
1.0
0.8
0.6
0.4
$y = 0.84 + 0.0028\,x \quad R^2_{adj} = 0.09$
B
Simpson Dissimilarity (βsim)
1.0
0.8
0.6
0.4
$y = 0.82 + 0.0028\,x \quad R^2_{adj} = 0.095$
C
Nestedness-Resultant Dissimilarity (βsne)
0.12
0.09
0.06
0.03
0.00
$y = 0.011 - 6 \times 10^{-5}\,x \quad R^2_{adj} = 0.0024$
0
5
10
15
20
25
30
Depth (cm)

Figure 4.

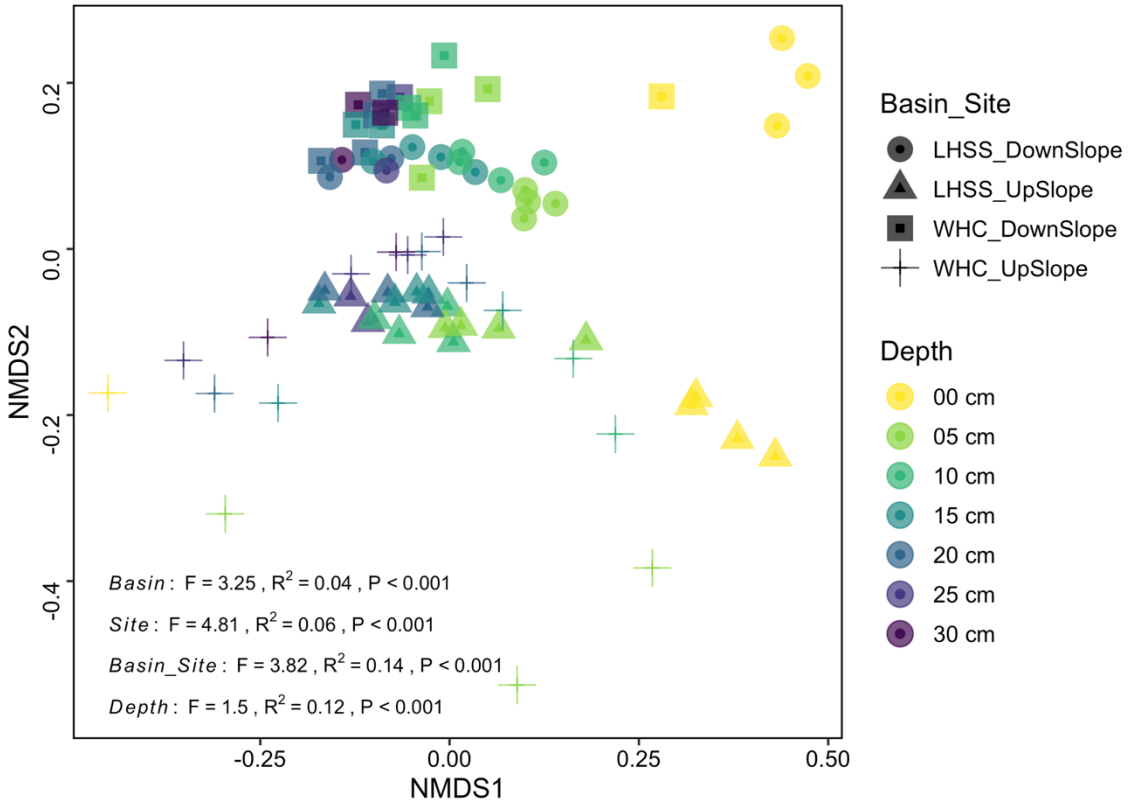

NMDS2
0.2
0.0
-0.2
-0.4
-0.25
0.00
0.25
0.50
NMDS1
Basin: F = 3.25 , R2 = 0.04 , P < 0.001
Site: F = 4.81 , R2 = 0.06 , P < 0.001
Basin_Site: F = 3.82 , R2 = 0.14 , P < 0.001
Depth: F = 1.5 , R2 = 0.12 , P < 0.001
Basin_Site
LHSS_DownSlope
LHSS_UpSlope
WHC_DownSlope
WHC_UpSlope
Depth
00 cm
05 cm
10 cm
15 cm
20 cm
25 cm
30 cm

Figure 5.

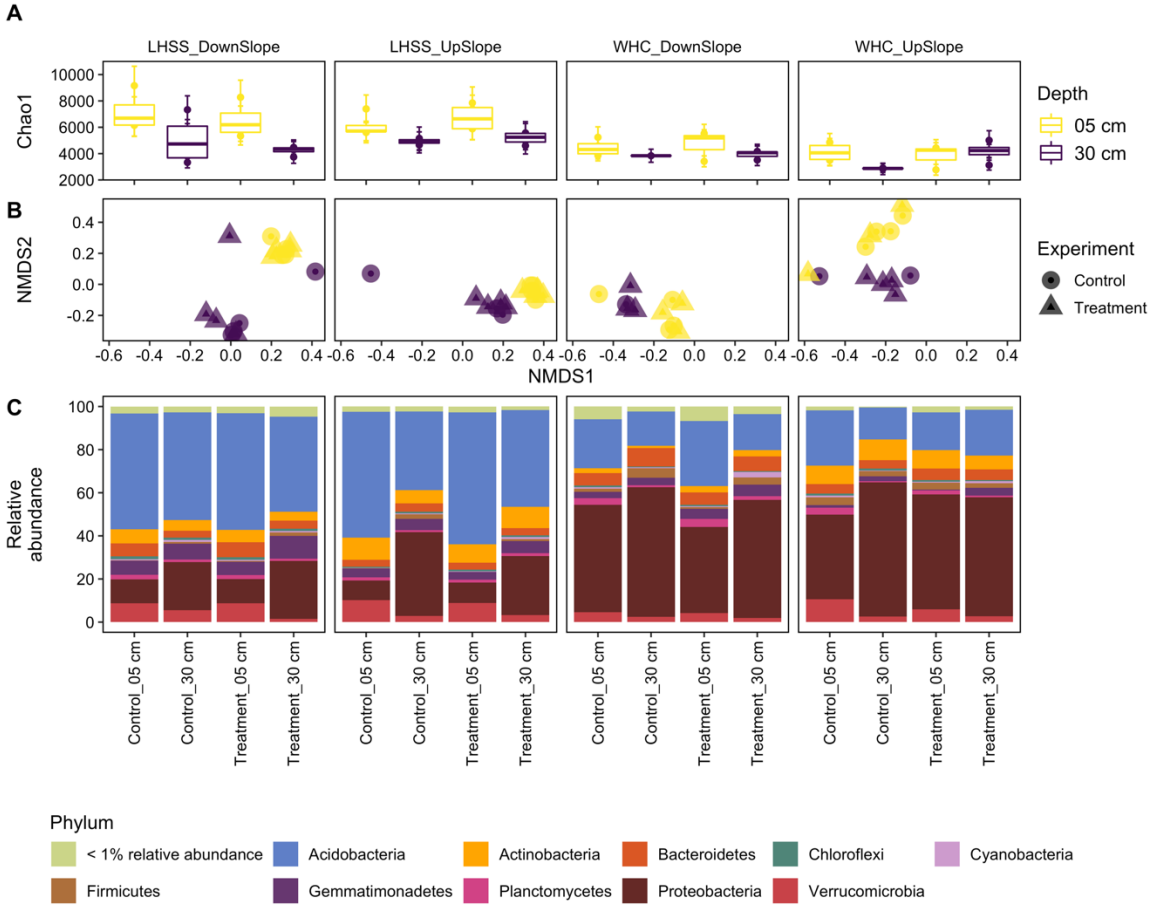
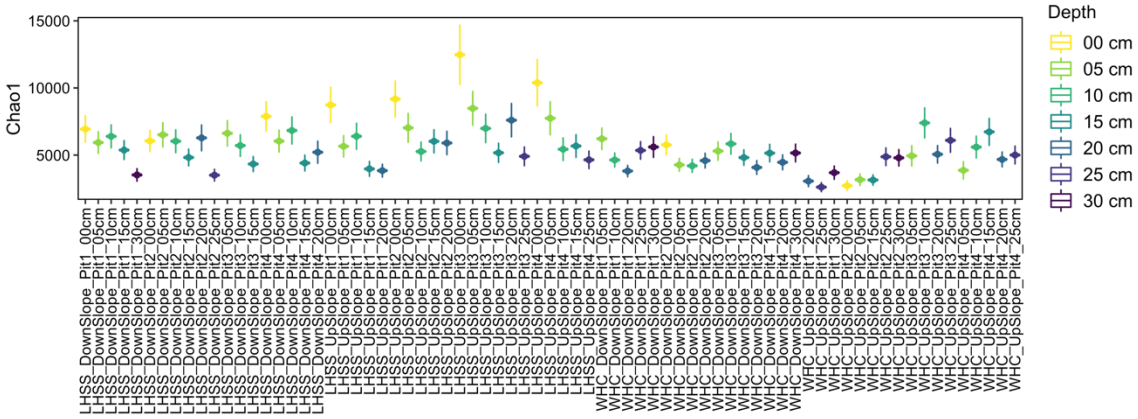

A
LHSS_DownSlope
LHSS_UpSlope
WHC_DownSlope
WHC_UpSlope
Chao1
10000
8000
6000
4000
2000
Depth
05 cm
30 cm
B
NMDS2
0.4
0.2
0.0
-0.2
-0.6 -0.4 -0.2 0.0 0.2 0.4
NMDS1
Experiment
Control
Treatment
C
Relative abundance
100
80
60
40
20
0
Control_05 cm
Control_30 cm
Treatment_05 cm
Treatment_30 cm
Phylum
< 1% relative abundance
Acidobacteria
Actinobacteria
Bacteroidetes
Chloroflexi
Cyanobacteria
Firmicutes
Gemmatimonadetes
Planctomycetes
Proteobacteria
Verrucomicrobia

Table 1. qPCR results from WHC experiment (each sample run in duplicate, 4 reps per group). qPCR was unsuccessful for the DownSlope control at 30 cm.

Site	Experiment	Depth (origin)	Depth (incubation)	Average (± s.d.) 16s copy number
DownSlope	Control	5 cm	5 cm	354 ± 144
	Treatment	5 cm	30 cm	461 ± 255
	Control	30 cm	30 cm	n/a
	Treatment	30 cm	5 cm	118 ± 7
UpSlope	Control	5 cm	5 cm	331 ± 167
	Treatment	5 cm	30 cm	356 ± 228
	Control	30 cm	30 cm	205 ± 83
	Treatment	30 cm	5 cm	209 ± 106

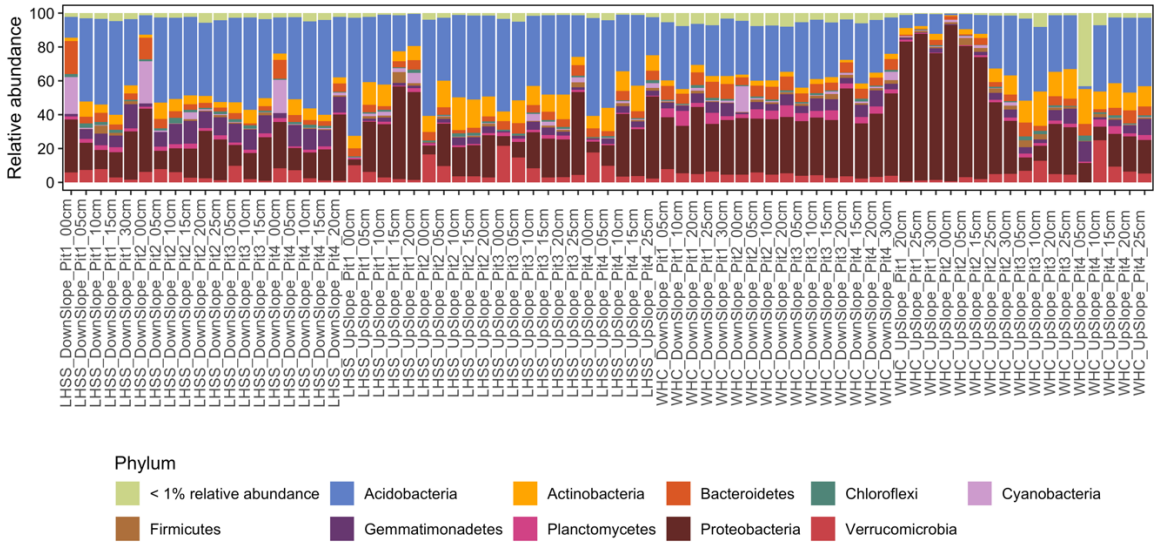
APPENDIX B. CHAPTER 2 SUPPLEMENTARY MATERIAL

Supplemental Figure 1.



Results of chao1 diversity measures by individual survey samples.

Supplemental Figure 2.



Phyla relative abundance of individual survey samples. Phyla with less than 1% relative abundance among any of the sample were binned into the < 1% relative abundance category.

CHAPTER 3: DRIVERS OF SOIL BACTERIAL COMMUNITY STRUCTURE IN ANTARCTIC WATER TRACKS

Kelli L. Feeser[1], David J. Van Horn[1], Jordan G. Okie[2], Becky A. Ball[3], Andrew G. Fountain[4], Michael N. Gooseff[5], Joseph Levy[6], Maciej K. Obryk[7], and Cristina D. Takacs-Vesbach[1]

[1]Department of Biology, University of New Mexico, Albuquerque, NM, USA

[2]School of Earth & Space Exploration, Arizona State University, Tempe, AZ, USA

[3]School of Mathematical and Natural Sciences, Arizona State University at the West Campus, Glendale, AZ, USA

[4]Department of Geology, Portland State University, Portland, OR, USA

[5]Department of Civil, Environmental, and Architectural Engineering, University of Colorado, Boulder, CO, USA

[6]Department of Geology, Colgate University, 13 Oak Ave., Hamilton, NY, USA

[7]U.S. Geological Survey Cascades Volcano Observatory, Vancouver, WA, USA

Abstract

Water availability is a key limitation of ecosystem function in the McMurdo Dry Valleys, Antarctica. Climate change in this region is projected to alter precipitation and promote melting of ice reserves, resulting in the creation and expansion of water tracks that transport water, solutes, and heat downslope. We utilized surveys and experiments to describe the microbial communities associated with two of these understudied landscape features. In the first track, diversity (Chao1; means of 4438/2593, $P < 0.005$) and abundance (16S copy number; means of 20055/10266, $P = 0.076$) were higher inside the track, corresponding to decreased salinity (EC; means of 59/307 μS, $P < 0.001$). Salinity did not vary significantly across the second track, although in the middle reach, diversity and abundance decreased inside where salinities were elevated (Chao1; means of 1638/4223, $P < 0.001$; 16S copy number; means of 1462/8522, $P = 0.017$). Overall, salinity was negatively correlated to diversity ($\rho = -0.43$, $P < 0.001$) and abundance ($\rho = -0.32$, $P = 0.003$). Metagenomic analysis revealed that communities were overwhelming dominated by Bacteria, although a greater proportion were observed in outside track samples (average ± s.d inside: 98.4 ± 0.3%; outside: 99.1± 0.03%, $P \leq 0.001$). Metagenomic trait-based analyses confirmed that communities in the wetted, more copiotrophic sites, had larger average genomes sizes (inside: 5.4 ± 0.14 Mbp; outside: 4.8 ± 0.23 Mbp, $P \leq 0.001$), although unexpectedly, lower GC content (frequency of guanine and cytosine nucleotides, inside: 61 ± 2%; outside: 67 ± 1%, $P \leq 0.001$). Experiments designed to simulate changes in water and solutes associated with the expansion of water track systems into dry soils suggested that decreased moisture and elevated salt content

increased the abundance of Acidobacteria and decreased Proteobacteria. These results expose the vulnerability of endemic dry soil communities to future climate change.

Introduction

The unique properties of water (Finney 2004) make its presence an essential component of habitats capable of sustaining life on earth (Rothschild and Mancinelli 2001, Stevenson et al. 2015). In the hyper-arid McMurdo Dry Valleys (MDV) in Antarctica, complex microbial communities with significant biomass occur in a variety of habitats that receive liquid water including streams (McKnight et al. 1999, Kohler et al. 2015, Van Horn et al. 2016), lakes (Priscu et al. 1998, Takacs and Priscu 1998), cryoconites (Porazinska et al. 2004, Foreman et al. 2007, Telling et al. 2014), wetted soils surrounding aquatic ecosystems (Moorhead et al. 2003, Zeglin et al. 2011, Niederberger et al. 2015), and in typically dry soils that receive transient water inputs from snow fall events (Lee et al. 2012, Van Horn et al. 2013, Schwartz et al. 2014, Van Horn et al. 2014). Recent observations suggest that an additional water-rich habitat exists in the MDV that has been largely overlooked: shallow groundwater. While a few early reports documented shallow groundwater movement in some areas (Wilson 1979, Cartwright and Harris 1981), the substantial portion of the soils that are dry-frozen (Bockheim et al. 2007) and the absence of obvious inputs to groundwater flow supported the assumption that shallow groundwater movement played a limited role in the landscape-scale hydrology of this region.

Within the past decade, sampling of meltwater seeps (Harris et al. 2007, Ball and Virginia 2012) and wetted soils occurring in linear depressions on hillslopes (Levy et al.

2011, Gooseff et al. 2013, Ball and Levy 2015), confirmed the presence of water tracks in the MDV. Water tracks are features that were first described in Arctic landscapes (Hastings et al. 1989, McNamara et al. 1999) and are defined as areas of water-saturated soil in topographic lows which drain hillslopes in the absence of overland flow. An investigation of the channel network in an arctic watershed found that the distribution of fully developed streams channels on the landscape was lower than expected given common slope-area scaling relationships (McNamara et al. 1999). The authors proposed that this was due to the reduced occurrence of erosion in permafrost dominated soils, and that in upland portions of the watershed, subsurface water tracks replaced streams as a means of draining slopes (McNamara et al. 1999). Isotopic analyses suggests that the water in arctic water tracks is derived from snow patches and ice melt from the upper layer of permafrost (Paquette et al. 2018). These areas contain altered vegetation and increased plant biomass (Hastings et al. 1989), stimulate biogeochemical cycling (Oberbauer et al. 1991, McNamara et al. 2008), and harbor distinct microbial communities with high proportions of Cyanobacteria and Acidobacteria (Steven et al. 2013).

Water tracks in the MDV are estimated to cover ~4% of the surface area and transport water during the austral summer when an active layer forms within the top meter of the permafrost (Levy et al. 2011). The water within these saturated soils (Levy et al. 2011, Gooseff et al. 2013) is derived from snow patches, ground ice, and to a lesser extent buried massive ice (Levy et al. 2011). Water flux in Antarctic water tracks can be either very slow, with estimated transport times from the top to the bottom of up to 75-150 years (Levy et al. 2011), or rapid when inputs and slopes are high. This variation in

flow allows for significant water/rock interaction in slow flowing tracks creating high soil salinity, and flushing of soils and low salinity in fast moving tracks (Levy et al. 2011, Gooseff et al. 2013, Ball and Levy 2015). The biological communities in these water tracks appear to respond differently to these two types of environments, with low nematode abundance and microbial biomass in saline water tracks, and elevated microbial biomass and carbon mineralization rates in low salinity tracks (Levy et al. 2014, Ball and Levy 2015).

While MDV water tracks have received some recent attention, no studies to date have investigated the diversity and structure of the bacterial communities living in these habitats. The responsiveness of microbes in this region to water, salt, and organic matter is well documented (Schwartz et al. 2014, Van Horn et al. 2014, Buelow et al. 2016, Aanderud et al. 2018, Feeser et al. 2018) and suggests that Antarctic water tracks may be a previously unexplored biological hotspot. Additionally, the current extent of water tracks in this area is likely to increase as temperatures warm and melting rates increase (Fountain et al. 2014) and thus the extent of this habitat may expand rapidly in the near future. This project leverages 16S rRNA gene sequencing, quantitative PCR (qPCR), and PCR-free whole genome sequencing within a series of surveys and experiments to explore the effects of these edaphic conditions on soil microbial communities.

The specific goals of this project were to 1) document microbial diversity, community structure, and abundance in water track soils and compare these communities to adjacently located off-track soil communities, 2) assess the relative importance of soil water content and salinity in structuring these communities to determine if water tracks represent microbial biological hotspots or saline dead zones, and 3) evaluate functional

genomic capacity and universal metagenomics traits to better understand the metabolic and ecological strategies of these communities. This study presents the most thorough and deeply sequenced whole genome dataset from the McMurdo Dry Valleys to date, representing an unbiased and comprehensive view of microbial community dynamics within this extreme polar ecosystem.

Material and Methods

Sampling description and study design

This study included both survey and experimental approaches and was conducted on two water tracks within the Taylor Valley of the McMurdo Dry Valleys, Victoria Land, Antarctica (77° 30' S, 163° 00' E). One water track, Worm Herder Creek (WHC), was located on the south shore of Lake Bonney and the second water track on the south shore of Lake Hoare (LHSS). Three reaches were identified along the length of each water track and designated as upper, middle, and lower water track reaches (here abbreviated as UWT, MWT, and LWT, respectively). Within each reach, three transects with five sampling points were established, spanning the water track to include dry soils on either side of the track and three within-track samples (see Map Fig). The transects ranged in width from 10 to 78 meters. At each sampling point, soils were homogenized to 10cm depth and aseptically sampled for bacterial community structure (sequencing of the 16S rRNA gene), bacterial community abundance (qPCR), soil chemistry, and natural carbon and nitrogen stable isotope abundances. A total of 90 samples were collected (2 water tracks x 3 reaches x 3 transects x 5 samples per transect). A subset of 20 samples were collected for metagenomic analysis.

For the experimental portion of the project, dry soil from two survey sampling sites (the outer right sample on transect 3 on the middle reach of WHC and the outer left sample on transect 2 at the upper reach of LHSS), was collected, homogenized (an ~20x20x5 cm area), and distributed into 20-15ml Falcon tubes with perforated caps. The 20 tubes from each site were divided into five sets. One set of four tubes served as a control, one set received sterile filtered deionized water (calculated to bring the soil water content to ~15% by weight), two sets received water plus salt (NaCl) in concentrations calculated to elevate the soil salinity from ~100 μS to 500 and 1000 μS, and a final set received sterile filtered water from the water track. The tubes were incubated for ~1.5 months. A total of 40 samples were collected for the experimental approach.

Chemical analysis

Within 24 hours of collection, soils for molecular analysis were subsampled into sterile tubes by preserving approximately 10 g of soil with an equal volume of sucrose lysis buffer (Giovannoni et al., 1990). Samples were stored at -20 °C until extraction. Soil pH was determined on 1:2 soil/deionized water extracts using an Orion pH probe. Electrical conductivity of 1:5 soil/water extracts was measured with a Yellow Springs Instrument 3100 conductivity meter. Approximately 20g of material were subsampled weighed and dried for 24 hours at 100 degrees C. Soil moisture content was calculated as wet mass – dry mass / dry mass. Dry soil samples were ground with a mortar and pestle into a fine powder and then weighed into tin capsules (~15 mg) for isotope analysis. A step-wise acidification process was utilized to remove carbonates from the soils as described by Walthert et al. (2010). Briefly, 50 μL of 1% HCl was added to each open capsule. In desiccators, the capsules were exposed for 24 h to the vapor from 100 mL of

32% HCl in a beaker. All samples were again wetted with 50 μL of 1% HCl and put in the desiccator, where they were exposed for 32 h to the vapor from 100mL of 37% HCl. Samples were then dried in a drying oven for 3 days at 35°C–40°C. Carbon ($\delta^{13}C$) isotope values were measured using a Costech 4010 elemental analyzer coupled to a Thermo Scientific Delta V isotope ratio mass spectrometer at the University of New Mexico Center for Stable Isotopes (Albuquerque, NM). Stable isotope data are expressed as δ values using the equation $\delta^{13}C = [(R_{Sample} - R_{Standard})/R_{Standard}] \times 1000$, where R_{Sample} and $R_{Standard}$ are the ratios of ^{13}C /^{12}C for each sample and standard. The internationally accepted standard for $\delta^{13}C$ is Vienna Pee Dee Belemnite (V-PDB). The units are expressed as permil (‰). Internal lab reference materials included organic sediment standards with $\delta^{13}C$ values (± SD) of -24.5 ± 0.6. Analytical precision was estimated via repeated (within-run) measurements of these reference materials calibrated to internationally accepted standards; within-run standard deviation for all reference materials was ≤ 0.2‰. We also measured the weight percent carbon and nitrogen concentrations of each sample.

DNA extraction, 16S rRNA gene sequencing and analysis

DNA from 0.7 g of soil was extracted using the cetyltrimethylammonium bromide (CTAB) method (Hall et al., 2008; Mitchell and Takacs-Vesbach 2008). After ethanol precipitation, DNA was resuspended using 25 μL of 10 mmol Tris. Dual-index paired-end amplicon sequencing of 16S rRNA genes was performed as previously described (Caporaso et al. 2011, 2012, Dowd 2008, Kozich et al. 2013) using V6 universal bacterial primers 939F 5’ TTG ACG GGG GCC CGC ACA AG-3’ and 1492R 5’-GTT TAC CTT GTT ACG ACT T-3’ on an Illumina MiSeq.

The 16S rRNA gene sequences were trimmed and quality filtered using Sickle (Joshi and Fass 2011) and paired-end reads were aligned and merged via PANDAseq (Masella et al., 2012). The Quantitative Insights into Microbial Ecology (QIIME) pipeline was used to analyze the reconstructed gene sequences (Caporaso et al. 2010a). Unique 16S rRNA gene sequences or operational taxonomic units (OTUs) were identified by the 97% DNA identity criterion using UCLUST (Edgar 2010). A representative sequence was picked from each OTU and aligned using the PyNAST aligner (Caporaso et al. 2010b) and the Greengenes core set (v. 13_8, DeSantis et al. 2006) and given taxonomic assignments using the Ribosomal Database Classifier program (Wang et al 2007).

Survey and experimental data were processed separately. All measures of community diversity (observed species, inverse Simpson, Good's coverage, Bray-Curtis, and Jaccard distances) and composition were performed with randomly selected subsets of 1000 (survey) or 1347 (experiment) sequences per sample to standardize for varying sequencing efforts across samples. Raw sequence data from this study are available through the NCBI Sequence Read Archive as PRJNA525069. The individual fastq files from this study were assigned the accession numbers SAMN11041036 to SAMN11041351.

qPCR

Extracted DNA was also used for real-time quantitative PCR (qPCR). Standards for qPCR were obtained using DNA extracted from *Escherichia coli* cultures that had undergone two consecutive rounds of PCR to ensure the final product contained no genomic DNA. DNA was amplified using the prokaryotic universal primer set developed

by Takahashi et al. 2014 – Pro341F: 5'-CCT ACG GGN BGC ASC AG-3' and Pro805R: 5'-GAC TAC NVG GGT ATC TAA TCC-3'. Amplified DNA was cleaned using UltraClean PCR Clean-Up Kits (MoBio Corporation, Carlsbad, CA, USA) and quantitated by Qubit assay (Invitrogen, Carlsbad, CA, USA). The purified DNA solution was serially diluted 10-fold to give solutions ranging from 10^3 to 10^{10} copies/μL (given a fragment length of 465 base pairs). The standard dilution series was used to generate a standard curve that was applied to estimate the copy number for each qPCR reaction. Each 75 μL qPCR reaction contained 3 μL of forward and reverse primers, 37.5 μL SSOAdvanced Universal SYBR Green Supermix (Bio-Rad Laboratories, Inc., Bio-Rad, Hercules, CA, USA), 25.5 μL sterile PCR water and 6 μL of template DNA. Template DNA was a 1:10 dilution of originally extracted DNA. Reactions were run in triplicate using previously described settings (Takahashi et al. 2014). Briefly, the conditions were initial denaturation at 95 °C for 30 seconds, followed by 35 cycles of 5 s at 95°C, 20 s at 60 °C, and 20 s at 72 °C. A melt curve was run at the end of the reaction, from 60 to 95 °C, increasing 0.5 °C every 5 s cycle. Cycle threshold values of triplicate reactions were averaged and the copy numbers of samples were calculated from the linear region of the standard curve. Due to the variability of copy number per bacterium, our estimates of copy number per sample can only be appropriately interpreted as relative estimates of biomass change per location.

Metagenomic sequencing

A subset of 20 samples including 1-3 outside-track and 3 inside-track samples from the upper and lower reaches of LHSS and upper and middle reaches of WHC were selected for metagenomic analysis. This subset comprised 8 inside-track and 12 outside-

track samples. PCR-free metagenomic sequencing was performed on extracts of environmental DNA (>1500 ng) from the subset of samples. Sequencing was conducted on an Illumina NovaSeq using 2 × 150 paired-end sequencing. A minimum of 20 million paired-end reads (minimum length of 150bp) were generated for each sample, providing sufficient coverage to identify the metabolic potential of key representatives from the soil communities. Quality control processing was conducted using FASTQC. Paired reads were discarded in the following situations: when either read contained adapter contamination; when either read contained uncertain nucleotides more than 10 percent; when either read contained more than 50 percent low quality nucleotides (base quality less than 5), or when read lengths were less than 150 bp. Fastq sequence files were uploaded to MG-RAST (Meta Genome Rapid Annotation using Subsystem Technology; v4.0) server at the Argonne National Laboratories (Keegan et al. 2016) for merging, processing, and annotation. Merged fastq files were processed using the pipeline options: unassembled, allowing for replicates, and filtered to a minimum phred score of 15. These fastq sequence files are publicly available on MG-RAST under project mgp91657 with MG-RAST ID numbers mgs773624, mgs773964, mgs773967, mgs773973, mgs773976, mgs773979, mgs773982, mgs773985, mgs773988, mgs773991, mgs773994, mgs773997, mgs774000, mgs774003, mgs774006, mgs774009, mgs774012, mgs774015, mgs774018, and mgs774021.

Potential rRNA genes with a cut-off of 70% identity to ribosomal sequences were identified (Rognes et al. 2016) and sequences were clustered at 97% identity (Fu et al. 2012). After removal of rRNA sequences, putative protein coding features were predicted using FragGeneScan (Rho et al. 2010) and clustered at 90% identity. Sequences passing

MG-RAST pipeline quality controls were then annotated using the non-redundant hierarchical M5nr database (Wilke et al. 2012) and SEED Subsystems (Overbeek et al. 2005) databases for taxonomic and functional analyses, respectively. All annotations were made following the MG-RAST default settings of e-value ≤1e - 5, identity ≥60%, and alignment length ≥15 base pairs. Mean GC content was calculated within MG-RAST. Average Genome Size (AGS) and Number of Genomes (NGs) were calculated as described by Pereira-Flores et al (2019). Briefly, the command line tool ags.sh was used to annotate 35 single copy genes universally present in prokaryotes (Raes et al. 2007). The NGs were computed as the mean coverage of the 35 single copy genes and the AGS was computed by dividing the total number of base pairs by the NGs.

Statistical analysis

Patterns in microbial communities among locations and treatments were assessed using nonmetric multidimensional scaling (NMDS) using Bray-Curtis distances. Differences in microbial community composition were analyzed by Random Forests (RF) classification algorithm (Breiman 2001) implemented within the randomForest package using 1,000 trees on rarefied OTU tables (-e 1000) that were filtered to remove OTUs with less than 10 sequences. Performance of the random forests classifier is reflected by the out-of-bag (OOB) error. RF was also used to regress OTU tables against quantitative edaphic data. The statistical significance of explanatory variables was assessed via the adonis test, as implemented in the vegan R package (Oksanen 2010). Adonis is a permutational (n=999) multivariate analysis of variance test that partitions distance matrices among sources of variation. Canonical correspondence analysis (CCA) was used to identify significant environmental variables that explained the variance of the OTU-

level community structure. The significance of CCA model constraints were assessed by the permutation test function anova.cca. NMDS and CCA tests were conducted using the phyloseq (McMurdie and Holmes 2013) and vegan packages (Oksanen et al., 2016) within the R programming environment.

Differences in the relative abundance of metagenomic taxa and metagenomic traits (AGS, NGs, and GC content) were assessed using the Wilcoxon test. After MG-RAST annotation, results were analyzed in R using the phyloseq (McMurdie and Holmes, 2013) and DESeq2 (Love et al. 2014) packages. Differences in the abundance of taxa and the variance in expression of transcripts were characterized using the DESeq2 parameters fitType = "local" and an adjusted p-value threshold of 0.05 to calculate log2 fold changes between inside- and outside-track samples. Taxonomy was assigned to subsystems that were significantly over-abundant in inside track samples.

Results

Edaphic chemistry

Values of basic soil chemistry parameters for inside versus outside water track samples were significantly different in some, but not all reaches. Inside-track soil pH values were significantly lower for all LHSS reaches ($P < 0.05$, Table 1), however, results for the WHC reaches were mixed (Table 1). Electrical conductivity (EC) was significantly lower for the within water track samples at all three of the WHC reaches ($P < 0.05$, Table 1), while no differences were found between on and off-track samples at the LHSS reaches. Off-track average soil water content was lower for all six reaches

(Table 1, Figure 1) but this difference was significant ($P < 0.05$) for four out of the six total reaches.

We attempted to measure both $\delta^{15}N$ and $\delta^{13}C$, as well as percent carbon and nitrogen, however most samples had nitrogen concentrations below the detection limit, so here only carbon data is reported. All reaches had lower $\delta^{13}C$ values outside the track, and in 5 of 6 reaches this trend was significant ($P < 0.05$, Table 2). Significantly higher concentrations of organic carbon were found in inside tracks in the Upper LHSS, Upper WHC, and Middle WHC reaches ($P < 0.05$, Table 2).

Amplicon sequencing results and bacterial abundance, diversity, and taxonomy

Sequencing of the 16S rRNA genes present resulted in a total of 761,026 reads (6,395 ± 5,323 reads per sample (± s.d.), $n = 119$ samples), with a mean length of 505 ± 43 bp. The Good's coverage statistic of the rarified dataset ranged from 0.25 to 0.87, with an average of 0.57 ± 0.14 suggesting adequate sequencing depth was achieved (Good 1953, Kemp and Aller 2004).

Bacterial abundance, as estimated by quantifying the 16S gene copy number, was significantly higher ($P < 0.05$, Table 3) for samples within versus outside the water track at one reach per water track and was significantly higher ($P < 0.05$, Table 3) for samples outside of the water track at the LHSS middle reach only. Similar trends were observed for bacterial diversity, as measured by chao1 richness estimates and the by the number of observed OTUs. Diversity tended to be highest inside tracks (significant in WHC 2 reaches), although chao1 was higher outside in the Middle LHSS reach ($P < 0.05$, Table 3, Figure 2).

Taxonomic data suggests that when data from all sites were grouped together, at the phylum level Cyanobacteria and Proteobacteria were overrepresented, and Acidobacteria were underrepresented within water tracks as compared to the samples taken outside of the water tracks (Figure 3A). However, substantial between-reach variation in taxonomic composition was also observed (Figure 3B). The LHSS Upper and Middle water track reaches both had increased relative abundances of Proteobacteria and Bacteroidetes and decreases in Acidobacteria; the phylum level distributions of taxa from the Lower LHSS and the Upper WHC water tracks were relatively uniform across the transects; and the outside-track samples from the Middle WHC reach had higher relative abundances of Actinobacteria, Deinococcus Thermus, and FBP, while the Lower WHC reach had greater abundances of Proteobacteria within the water tracks (Figure 3B).

Community analyses

Overall, EC was negatively correlated with bacterial abundance, diversity and richness ($P < 0.05$, Table 4). Inside WHC, pH also correlated negatively with bacterial abundance, diversity and richness ($P < 0.05$, Table 4), although relationships with both pH and EC were not significant when samples were analyzed as inside versus outside track. The $\delta^{13}C$ values only correlated significantly to richness within WHC (rho = 0.48, $P < 0.05$, Table 5). Percent organic carbon was strongly and positively correlated with abundance, diversity and richness overall as well as within inside track samples ($P < 0.05$, Table 5).

The RF models accurately classified bacterial communities inside and outside tracks (OOB error = 4.4%, Table 6), however they were less accurate classifying communities by reach (OOB error = 31.9%, Table 6). When subset by reach, 4 models

retained an accurate classification of inside vs outside track (OOB errors from 0-7.14%, Table 6). The NMDS plots show clearly distinct reach-level groupings of samples from inside the water tracks, and a single group that includes the out of track samples from all of the reaches (Figure 4, SI-Figure 1). The RF models support this observation: inside track samples tended to classify well by reach (OOB error = 14.6%, Table 6) while outside track samples did not (OOB error = 67.9%, Table 6). When the inside and outside water track samples were ordinated independently, the outside samples from the Middle and Upper reaches from the WHC water track formed distinct groupings (SI-Figure 1). RF models based on regressing OTU tables against quantitative edaphic variables revealed different patterns within inside track communities compared to outside track communities (Table 7). For example, pH explained very little variance within inside samples (3.7%, Table 7) but more in outside samples 43.8%, Table 7). Conversely, more variance was explained in inside samples when regressing against EC (35.6 % inside vs. 4.9% outside), SWC (60.1% inside vs. 7.8% outside), and percent organic carbon (25.8% inside vs. 0.2% outside, Table 7).

These results were also supported by canonical correspondence analyses which were significant ($P < 0.001$, constrained inertia ranging from 0.30 to 0.41) for all samples together as well as for groupings of the inside and outside track samples (Figure 5). Soil water content (R^2 values of 0.15 and 0.13) and EC (R^2 values of 0.10 and 0.18) were the most strongly correlated edaphic variables for ordinations of all of the samples and the inside track only samples, while pH ($R^2 = 0.11$) and EC ($R^2 = 0.08$) were most strongly correlated with community composition in the outside water track samples (Figure 5).

Experimental results

Chao1 diversity estimates for the experimental treatments suggest that for the WHC water track the control samples were the most diverse while the water and salt additions had decreased diversity (Figure 6). In contrast, in the LHSS water track the samples from the high salt addition had the highest diversity. Changes in the phylum level taxonomy were observed with water and salt additions in both water tracks with increases in the relative abundance of Proteobacteria and decreases in Acidobacteria (Figure 6).

Metagenomic taxonomy results

Metagenomic sequencing resulted in over 149Gb of data and a total of 499,417,893 paired-end sequences. After MG-RAST merging and quality control filters, an average (± s.d.) of 41,336,708 ± 5,750,018 reads per sample were retained for further analysis (range = 32,446,712 – 51,576,972). Of these sequences, an average of 0.17 ± 0.02 % (range = 0.12 – 0.20 %) were rRNA for each sample. Sequences of mRNA able to be annotated as protein-coding averaged 45 ± 5 % (range = 40 – 52 %) of reads per sample. A summary of statistics after MG RAST processing can be found in SI-Figure 2.

At the domain level, samples were overwhelming dominated by Bacteria, although a greater proportion were observed in outside track samples (average ± s.d inside: 98.4 ± 0.3%; outside: 99.1± 0.03%, $P \leq 0.001$) (Figure 7). Similarly, Viruses also had a higher relative abundance in outside track samples (inside: 0.30 ± 0.05%; outside: 0.46 ± 0.08%, $P \leq 0.001$). Inside track samples had higher relative abundance of Archaea (inside: 0.46 ± 0.08%; outside: 0.30 ± 0.05%, $P \leq 0.001$) and Eukaryota (inside: 1.0 ± 0.3%; outside: 0.5 ± 0.2%, $P \leq 0.05$).

Bacterial phyla were dominated by Actinobacteria (inside: 25.5 ± 11.2%; outside: 70.4 ± 5.0%, $P \leq 0.0001$) and Proteobacteria (inside: 33.1 ± 0.5%; outside: 15.0 ± 3.1%, $P \leq 0.0001$) (SI-Figure 3). Of the 10 most abundant bacterial phyla, 9 had significantly higher abundance in the inside track samples (Acidobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Firmicutes, Gemmatimonadetes, Planctomycetes, Proteobacteria, and Verrucomicrobia, all $P \leq 0.01$) while only Actinobacteria was significantly higher in the outside samples ($P \leq 0.0001$).

Ascomycota (20.1 ± 10.2%), Chordata (27.1 ± 22.2%), and Streptophyta (25.8 ± 13.9%) were the most abundant eukaryotic phyla (SI-Figure 4). Of the 10 most abundant eukaryotic phyla only 1 had significantly higher abundance in the inside track samples (Bacillariophyta, inside: 3.8 ± 3.8%; outside: 0.7 ± 0.3%, $P \leq 0.01$) and 3 were more abundance in the outside tracks (Arthropoda, inside: 5.7 ± 2.0%; outside: 11.8 ± 10.2%, $P \leq 0.05$; Ascomycota, inside: 15.4 ± 6.0%; outside: 27.2 ± 11.5%, $P \leq 0.05$; and Nematoda, inside: 1.4 ± 0.6%; outside: 2.5 ± 1.0%, $P \leq 0.05$).

Archaeal phyla were dominated by Euryarchaeota (SI-Figure 5). This phylum was slightly but still significantly more abundant in the outside track samples (inside: 72.4 ± 6.9%; outside: 75.9 ± 0.3%, $P \leq 0.05$). No other archaeal phyla held significantly different relative abundance patterns noted between inside- and outside-track samples.

Metagenomic trait and differential gene abundance

Inside track samples had significantly larger average genome size (AGS, Figure 8) (inside: 5.4 ± 0.14 Mbp; outside: 4.8 ± 0.23 Mbp, $P \leq 0.001$). Outside track samples

had significantly higher number of genomes (NGs) (inside: 1,261 ± 170; outside: 1,550 ± 234, $P \leq 0.01$) and GC content (inside: 61 ± 2%; outside: 67 ± 1%, $P \leq 0.001$).

The SEED Subsystems functional annotations provide four levels of resolution. At the highest level of functional categorization (L1), a total of 28 subsystems were identified in the dataset. Of these, 21 were differentially abundant ($P_{adj} \leq 0.01$) when comparing inside to outside track samples (Figure 9). Subsystems found to be significantly more abundance in the inside track samples include; amino acids and derivatives, cell division and cell cycle, clustering-based subsystems, cofactors, vitamins, prosthetic groups, and pigments, DNA metabolism, dormancy and sporulation, nitrogen metabolism, phages, prophages, transposable elements, and plasmids, protein metabolism, and respiration. Taxonomy assigned to the inside track sequences annotated as these subsystems is shown in Figure 10. Phyla-level relative abundance patterns were generally consistent among subsystems, i.e. the most abundant phylum was Proteobacteria, followed by Actinobacteria and Firmicutes. However, some shifts were observed among subsystems, for example, Firmicutes and Bacteroidetes had higher relative abundances within Dormancy and Sporulation compared to Nitrogen Metabolism while the opposite was seen for Actinobacteria.

Discussion

Previous research has documented diverse and active soil microbial communities in water rich habitats in the MDV (Zeglin et al. 2011, Van Horn et al. 2013, Niederberger et al. 2015), however, no studies to date have explored the abundance, diversity, functional capacity, and structure of communities inhabiting newly described water track

features. The wide range of flow velocities and water residence times in MDV water tracks create varying cross-track edaphic conditions. In this study we document diverse and previously undescribed bacterial communities in Antarctic water tracks and explore how interactions between soil water content and salinity impact the habitability of these moist soils.

Water track edaphic conditions

Recent descriptions of edaphic conditions in MDV water tracks suggest that relatively high flow tracks such as the WHC track flush salts, while low flow tracks including the LHSS track accumulate substantial salt loads (Levy et al. 2011, Gooseff et al. 2013, Ball and Levy 2015). Our finding that EC within the water tracks was consistently lower than outside track soils for all sites and reaches (Table 1) was unexpected, particularly for the Middle Reach in the LHSS, which has previously been described as highly saline (Ball and Levy 2015). While the mean conductivity value for the samples taken from the center of the Middle Reach LHSS water track was four to eight times higher than the EC for one of the outside track samples (Figure 1), the high degree of spatial heterogeneity in MDV soil geochemistry (Barrett et al. 2004, Van Horn et al. 2013, Feeser et al. 2018) likely explains the absence of a significant inside versus outside comparison for this reach. This heterogeneity is particularly relevant given the more limited sampling replication from this study as compared to the other MDV water track studies (Ball and Levy 2015). The generally lower pH values observed within the water tracks have been reported elsewhere (Ball and Levy 2015) and may be a result of either increased soil respiration and subsequent carbonic acid formation due to the elevated SWC and available organic matter (Ball and Levy 2015), or may be due to

flushing of carbonates which elevate soil pH. Interestingly, while there were no consistent across-reach differences in edaphic characteristics for the within versus outside water track samples, in each reach at least one edaphic characteristic varied significantly between inside and outside track samples (Table 1).

16s rRNA gene-based bacterial community abundance, diversity and structure within MDV water tracks

The finding of both significantly elevated and depressed bacterial abundance and diversity within different water track reaches as compared to off track soils (Table 3), strongly suggests that water tracks are not consistent biological hot or cold spots but are instead highly variable habitats that, depending on local conditions, can either foster or restrict biological communities. The strong positive correlation between PctC and both bacterial gene copy number and diversity metrics (Table 5) has been observed in other studies from this region. For example, microbial biomass and diversity were strongly correlated in a study of soils surrounding snow patches in Taylor and Wright valleys (Van Horn et al. 2013), and in a survey of twelve streams from this area, diversity was strongly correlated with ash free dry mass (Van Horn et al. 2016). Thus, as in other habitats in the MDV, diversity appears to be correlated with available energy as predicted by the potential energy hypothesis (Gaston 2000, Hurlbert and Stegen 2014). This control on diversity may be particularly pronounced in the MDV given the scarcity of nutrient and water resources and the low biomass as compared to most other systems on earth.

In contrast to the positive relationship between soil organic carbon and bacterial diversity and abundance, EC was the edaphic characteristic that was the most consistently negatively correlated with abundance and diversity (Table 4). Salinity is a master variable

determining microbial community composition in aquatic and terrestrial environments (Lozupone and Knight 2007). In soils, salinity exerts a primary limitation on water availability, as total water potential is the sum of matrix potential, which is a function of soil composition and texture, and osmotic potential, which is controlled by total ion concentrations. Current MDV data suggests that the soil bacterial communities begin responding negatively (and linearly) to salinity beyond 300 μS (Feeser et al. 2018, Jiang et al. 2019) and that bacterial communities from high salinity soils are depauperate (Van Horn et al. 2014). Additionally, while increased soil salinity limits water availability, rapid water additions to saline soils may also cause dilution stress for microbes, as they must rapidly discharge or utilize osmolytes to avoid cell lysis (Schimel et al. 2007). Thus, increased salinity represents a biological stress that requires evolutionary and/or energetically expensive solutions, limiting abundance and diversity in water tracks that accumulate high salt loads.

The clear patterns we observed in the ordinations of community data suggests that multiple factors structure these communities. The distinct separation between inside and outside water track communities (Figure 4) provides evidence that the significant but varied differences in edaphic characteristics for dry versus wet habitats in each reach resulted in clear environmental selection. This finding is consistent with previous research in the MDV, which demonstrates both correlations between edaphic parameters and microbial community composition for a variety of habitats within this region (Lee et al. 2012, Van Horn et al. 2013, Geyer et al. 2014, Feeser et al. 2018), and the rapid response of communities to changing environmental conditions (Tiao et al. 2012, Schwartz et al. 2014, Van Horn et al. 2014, Buelow et al. 2016, Aanderud et al. 2018).

These findings are also consistent with meta-analyses which indicate that environmental selection is the dominant driver of microbial community composition in non-MDV habitats including aquatic, terrestrial, plant and animal associated, and extreme environments (Hanson et al. 2012).

While the clear separation of inside versus outside water track communities was striking, the patterns present within each of these groups is also marked and of interest. The tight cluster of outside track samples as compared to the widely distributed but distinctly and significantly (Table 6) clustered groups of within track samples (Figure 4) may be due to several potential mechanisms. It is possible that outside track edaphic conditions are more consistent than those of the within track samples (or, stated differently, the total environmental gradient lengths for the outside track samples may be shorter than for inside track samples) leading to a consistent dry-soil community, however, the finding that there was no significant difference in the Average Distance to Median (betadisper) results for the edaphic data for inside versus outside track samples suggests this possibility is unlikely. Alternatively, within track samples may be more active and thus responsive to environmental filtering, producing greater between-reach/track community variation. The finding that outside track community variance was moderately (44%) related to a single edaphic variable (pH), while the variance of inside track communities was strongly to moderately (60-25%) correlated with three variables (EC, SWC, and POC) lends support for the hypothesis that inside track communities are active and responsive to local conditions. Niederberger et al. (2015) found similar tight clustering of bacterial communities from disparate dry soil habitats in the MDV as compared to communities in wetted soils adjacent to glaciers, lakes, and streams which

had higher dissimilarity. While this finding again suggests that wetted communities may be more active, further research in wet versus dry habitats is needed, particularly using techniques capable of identifying active and inactive communities.

The amplicon-based taxonomic data from inside versus outside track communities suggests that while consistent patterns across all tracks/reaches are absent, some trends do exist. For example, the elevated percentage of Proteobacteria within three of the six water tracks is consistent with these communities being active, as this phylum was identified as one of the most responsive to the addition of organic matter and water additions in other MDV soils (Tiao et al. 2012, Van Horn et al. 2014). Additionally, studies using stable isotope probing (Schwartz et al. 2014) and community RNA transcripts (Buelow et al. 2016) identified Proteobacteria in MDV soils as metabolically active and responsive to changing conditions. The increase in cyanobacteria in some reaches was also consistent with findings from other wetted MDV soils (Neiderberger et al. 2015), and suggests that some water track reaches retain sufficient moisture to support wet-adapted communities.

Insights from metagenomic sequencing

Metagenomic sequencing confirmed that this is a bacterially dominated ecosystem with an average of 98.7% of sequences belonging to Bacteria (Figure 7). Every major phylum (those with at least 3% relative abundance in at least 1 sample) had a significant difference (all $P \leq 0.01$) in relative abundance between inside- and outside-track samples. Acidobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Firmicutes, Gemmatimonadetes, Planctomycetes, Proteobacteria, and Verrucomicrobia had higher abundances in the inside track samples while only Actinobacteria were significantly more

abundant in the outside track samples. Thus, it appears that the majority of the phyla find the inside-track conditions to be more favorable, with the exception of Actinobacteria. Similar to other studies within the MDV, Actinobacteria and Proteobacteria constituted the largest fractions of all communities, regardless of location (Schwartz et al. 2014, Buelow et al. 2016, Feeser et al. 2018). Actinobacteria are known to have a cosmopolitan distribution (Babalola et al. 2009) and are important degraders of organic matter (Hayakawa and Nonomura 1987), making them critical mediators of environmental stability (National Research Council 2007). Proteobacteria, on the other hand, have been observed within a substantial proportion of the active communities in the MDV by studies using stable isotope probing (Schwartz et al. 2014) and RNA sequencing (Buelow et al. 2016). These results of declining Actinobacteria in response to increases soil water and organic matter are consistent with other DNA-based (Schwartz et al. 2014, Van Horn et al. 2014) and RNA-based (Buelow et al. 2016) studies in MDV soils.

The most notable difference between the taxonomy of the amplicon versus the metagenomic sequencing was seen in the relative abundances of Acidobacteria and Actinobacteria. Within the amplicon dataset, Acidobacteria had an average relative abundance of 45.0% while Actinobacteria had an average of 3.2% within the metagenome dataset, those values were 2.5 and 43.5%, respectively. This disparity likely suggests a bias due to primer selection.

Metagenomic sequencing can not only provide insight into the functional capacity of microbial communities but also reveal the physiological traits that underpin their ecological strategy within the oligotrophy-copiotrophy continuum. The Average Genome Size (AGS) of organisms is positively correlated with environmental complexity and

represents increased and more diverse metabolic repertoire (Barberán et al. 2012, Guieysse and Wuertz 2012, Okie et al. 2019, Pereira-Flores et al. 2019). As expected, the AGS of the wetter, more organic matter rich inside track samples was larger than that of the drier, more oligotrophic outside track samples (inside: 5.4 ± 0.14 Mbp; outside: 4.8 ± 0.23 Mbp, $P \leq 0.001$) (Figure 8). The relatively copiotrophic environments of the inside track soils provides a more diverse offering of substrates and having larger genomes would likely enable a microbial community to more effectively harvest energy and nutrients, experience higher growth rates, and response to fluctuating environmental conditions. Additionally, the genomes with greater frequencies of the nucleotides G (guanine) and C (cytosine) are thought to require more nitrogen and impose higher energy costs of production and more limited intracellular availability compared to A (adenosine) and T/U (thymine/uridine) (Rocha and Danchin 2002, Okie et al. 2019). Therefore, we expected that oligotrophic environments with lower water and organic matter would favor a smaller AGS and lower GC content. However, we found that the oligotrophic outside track samples had significantly higher GC content (inside: 61 ± 2%; outside: 67 ± 1%, $P \leq 0.001$). As GC content in microbes can vary from ~17% to 75% (Nakabachi et al. 2006, Hildebrand et al. 2010), these values are relatively high, although common of terrestrial bacteria (Foerstner et al. 2005). These results contrast Okie et al. (2019) who found positive correlations between GC content and nutrient enrichment. The unexpected patterns of GC content may be the result of variable taxonomic composition, and although statistically significant, may have little ecological consequence.

Of the 28 total subsystems identified in this dataset, the majority ($n = 21$) were found to be differentially abundant when comparing on- and off-track sites (Figure 9),

indicating that the broad-scale metabolic and ecological strategies of the microbial communities varied significantly among environments. Sequences relating to amino acids metabolism, cell division and cell cycle, proteosomes, ribosomes and recombination-related clusters (clustering-based subsystems), cofactors and vitamins, DNA metabolism, dormancy and sporulation, nitrogen metabolism, phages, protein metabolism and respiration were more highly represented in the wetted, inside track soils. In contrast, sequences relating to cell well and capsule, iron acquisition and metabolism, membrane transport, metabolism of aromatic compounds, motility and chemotaxis, potassium metabolism, regulation and cell signaling, secondary metabolism, sulfur metabolism, and virulence were more less abundant in the wetted, on track soils.

When examining which taxa were responsible for the enriched subsystems in the inside-track samples, phyla-level relative abundance patterns were generally consistent among subsystems, i.e. the most abundant phylum was Proteobacteria, followed by Actinobacteria and Firmicutes (Figure 10). Therefore, there does not appear to be any apparent niche partitioning at the phylum-level, even though some small shifts in the relative abundance of phyla among subsystems were observed. Although Proteobacteria, Actinobacteria, and Firmicutes were the most abundant members, a small but significant amount of Cyanobacteria were also present. Cyanobacteria are known to colonize cold soils in the Arctic and Antarctic (Cowan et al. 2015) and are thought to drive most functional processes related to carbon and nitrogen cycling (Rhodes et al. 2013, Makhalanyane et al. 2014, Makhalanyane et al. 2015). Increased soil water content inside the water tracks was correlated to increased abundance of Cyanobacteria which provide a phototrophic energy source for the community. Cyanobacteria are poised to take

advantage of the decomposition and nutrient recycling activities of Proteobacteria (Varin et al. 2010, Varin et al. 2012). Proteobacteria are seen to have a clear advantage within the relatively moist and organic carbon rich water tracks. The higher representation of genes related to nutrient scavenging (i.e. within amino acids and derivatives, clustering-based subsystems, and nitrogen metabolism) likely reflects the communities' abilities to assimilate and utilize a diverse range of external nutrients (Varin et al. 2010). Thus, these functions enable the co-existence of phototrophs and heterotrophs within this oligotrophic polar desert. Further investigations into the niche partitioning, functional redundancy, and metabolic strategy of finer taxonomic resolutions will be the focus of a future manuscript.

Conclusions

The edaphic conditions found in water tracks in the MDV are highly variable and water track/reach dependent. This variability impacts the microbial community, with some water track reaches harboring more abundant and diverse bacterial communities than off track soils, while other reaches are biologically depauperate. Metagenomic sequenced confirmed that the microbes within this extreme ecosystem are highly influenced by cryptic hydrological features and utilize significantly different broad-scale metabolic and ecological strategies of the microbial communities depending on whether they reside within or outside of a water track. As climate related landscape-scale change occurs in the MDV and water tracks expand, the impact to associated soil communities will depend on a variety of factors including the water track flow rate, solute content, and the ability to support the accumulation of soil organic matter.

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Figure Legends

Figure 1. Relationships among electrical conductivity (EC), reported as μS/cm, pH, percent carbon (PercentC), and chao1

Figure 2. Scatterplots of bacterial abundance and diversity against edaphic conditions. Independent and response variables include among electrical conductivity (EC), reported as μS/cm, pH, soil water content (SWC), reported as the fraction of wet mass – dry mass / dry mass, chao1, and mean 16S rRNA gene copy numbers (reported as copy numbers per μL), based on 1:10 dilutions of template DNA.

Figure 3. Survey phyla relative abundance. (A) Phyla relative abundance inside vs outside water track (global average, SD error bars) and (B) phyla relative abundance by water track position. Phyla with less than 1% relative abundance among any of the sample were binned into the Other category.

Figure 4. NMDS by water track position and location alongside boxplots of alpha diversity (chao1)

Figure 5. Canonical correspondence analysis (CCA) of soil samples demonstrates that community composition differs by location when considering all water track samples (A), only inside track samples (B), and only outside track samples (C). Furthermore, it reveals that the that the relationships among community composition and environmental factors

differs depending on whether the sites are on- or off-track. Arrows indicate direction and magnitude of significant environmental factors affecting bacterial community structure.

Figure 6. Experiment (A) chao1 and (B) phyla relative abundance. Phyla with less than 1% relative abundance among any of the sample were binned into the Other category.

Figure 7. Domain-level classification of metagenomic sequencing revealed that communities were dominated by Bacteria, followed by Eukaryota, Archaea, and Viruses. Significant differences in the relative abundance of the four domains were noted between inside- and outside-track samples (Wilcoxon test, ****: $P \leq 0.0001$, ***: $P \leq 0.001$, **: $P \leq 0.01$, *: $P \leq 0.05$, ns: $P > 0.05$).

Figure 8. Summary statistics of metagenomic traits including AGS (A), NGs (B), and mean GC content (C). Significant differences between inside- and outside-track samples are shown (Wilcoxon test, ****: $P \leq 0.0001$, ***: $P \leq 0.001$, **: $P \leq 0.01$, *: $P \leq 0.05$, ns: $P > 0.05$).

Figure 9. Significant differential subsystem abundance. All significant (p-adjusted value ≥0.01) differential SEED subsystems (L1), expressed as the log2 fold change of sequences in inside vs. outside track samples.

Figure 10. Phylum-level taxonomy of sequences that had significantly greater differential abundance (log2 fold change) in the inside track samples, expressed as the percentage of

total sequences for each differential abundance result. Outside track samples have been excluded. Phyla with less than 3% relative abundance among any of the sample were binned into the < 3% relative abundance category.

Figure 1.

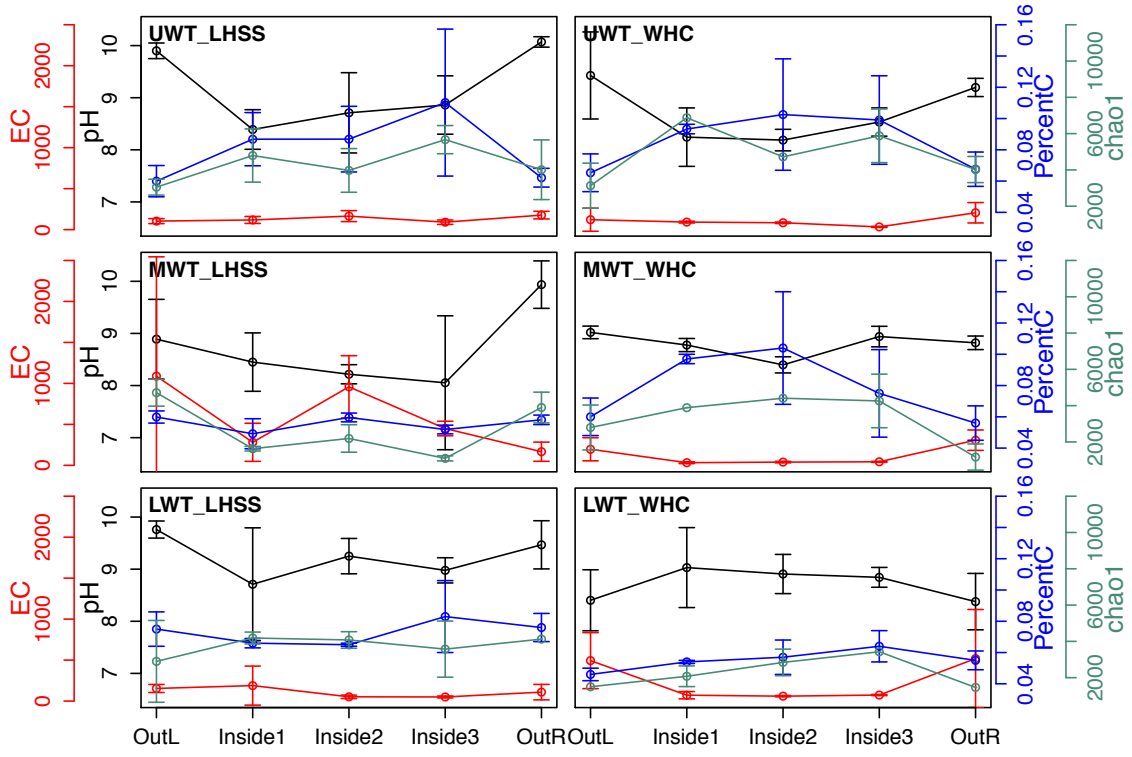

UWT_LHSS
UWT_WHC
MWT_LHSS
MWT_WHC
LWT_LHSS
LWT_WHC
EC
pH
PercentC
chao1
OutL
Inside1
Inside2
Inside3
OutR

Figure 2.

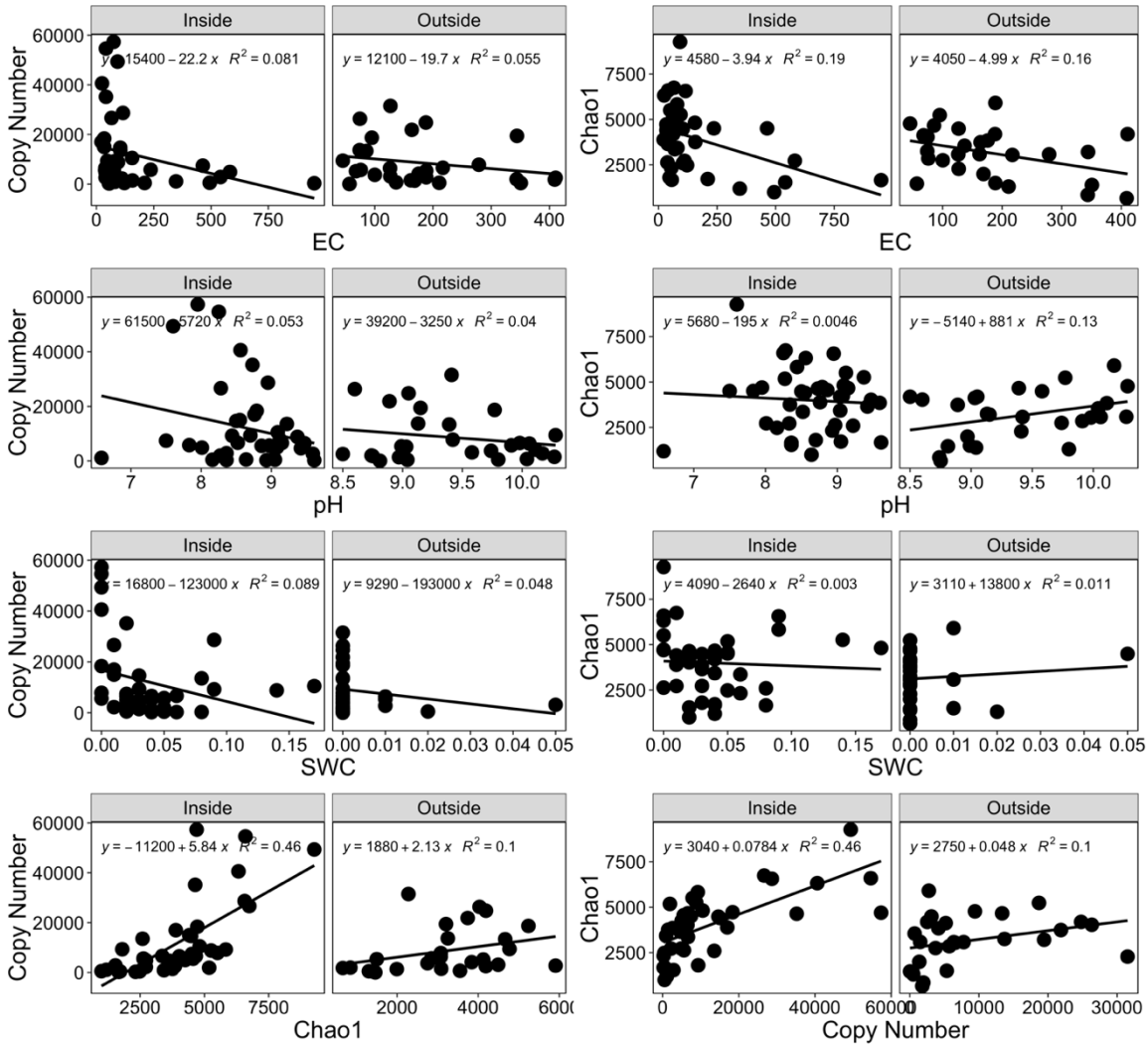

Inside
Outside
Copy Number
Chao1
EC
pH
SWC
y = 15400 – 22.2 x R² = 0.081
y = 12100 – 19.7 x R² = 0.055
y = 4580 – 3.94 x R² = 0.19
y = 4050 – 4.99 x R² = 0.16
y = 61500 – 5720 x R² = 0.053
y = 39200 – 3250 x R² = 0.04
y = 5680 – 195 x R² = 0.0046
y = – 5140 + 881 x R² = 0.13
y = 16800 – 123000 x R² = 0.089
y = 9290 – 193000 x R² = 0.048
y = 4090 – 2640 x R² = 0.003
y = 3110 + 13800 x R² = 0.011
y = – 11200 + 5.84 x R² = 0.46
y = 1880 + 2.13 x R² = 0.1
y = 3040 + 0.0784 x R² = 0.46
y = 2750 + 0.048 x R² = 0.1

Figure 3.

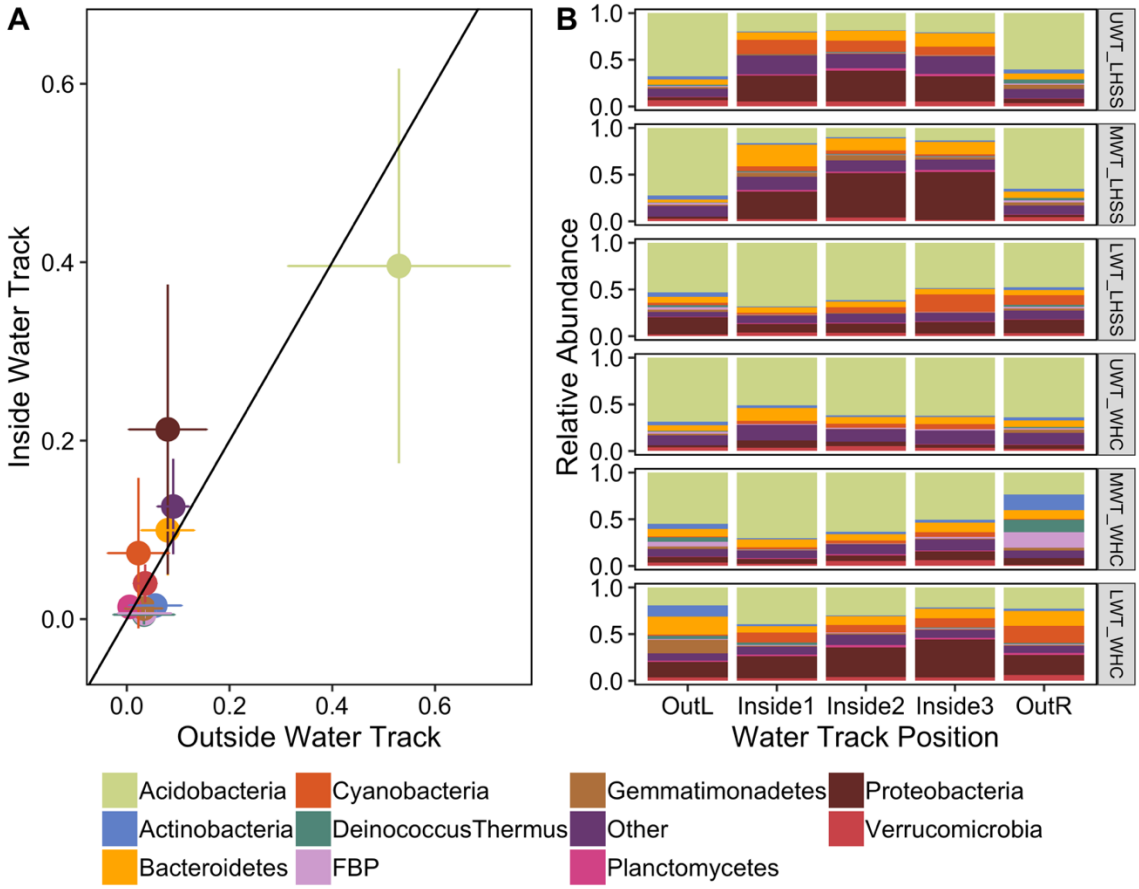

A
Inside Water Track
Outside Water Track
0.0
0.2
0.4
0.6
B
Relative Abundance
1.0
0.5
0.0
UWT_LHSS
MWT_LHSS
LWT_LHSS
UWT_WHC
MWT_WHC
LWT_WHC
OutL
Inside1
Inside2
Inside3
OutR
Water Track Position
Acidobacteria
Actinobacteria
Bacteroidetes
Cyanobacteria
DeinococcusThermus
FBP
Gemmatimonadetes
Other
Planctomycetes
Proteobacteria
Verrucomicrobia

Figure 4.

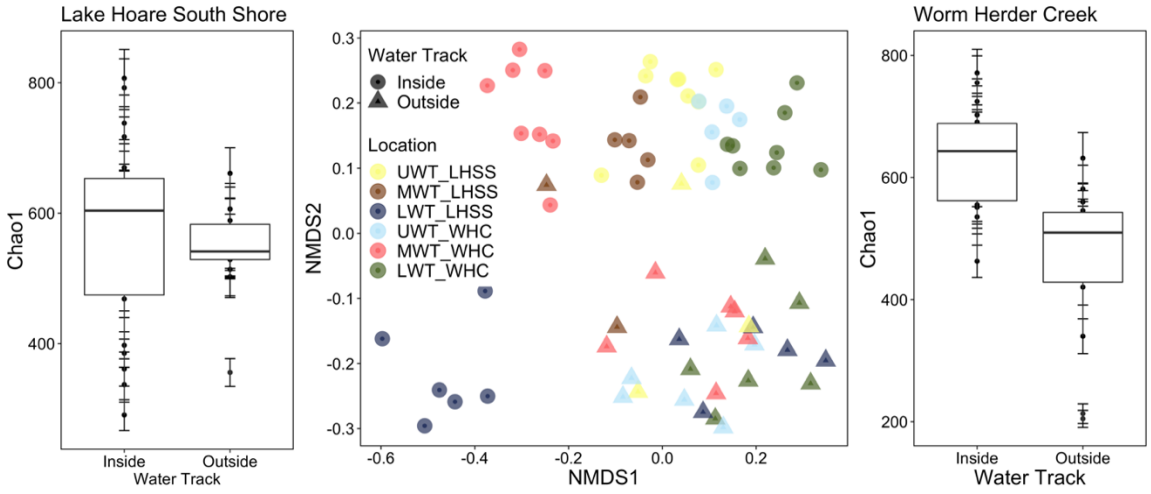

Lake Hoare South Shore
Chao1
800
600
400
Inside
Outside
Water Track
NMDS2
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.6
-0.4
-0.2
0.0
0.2
NMDS1
Water Track
Inside
Outside
Location
UWT_LHSS
MWT_LHSS
LWT_LHSS
UWT_WHC
MWT_WHC
LWT_WHC
Worm Herder Creek
Chao1
800
600
400
200
Inside
Outside
Water Track

Figure 5.

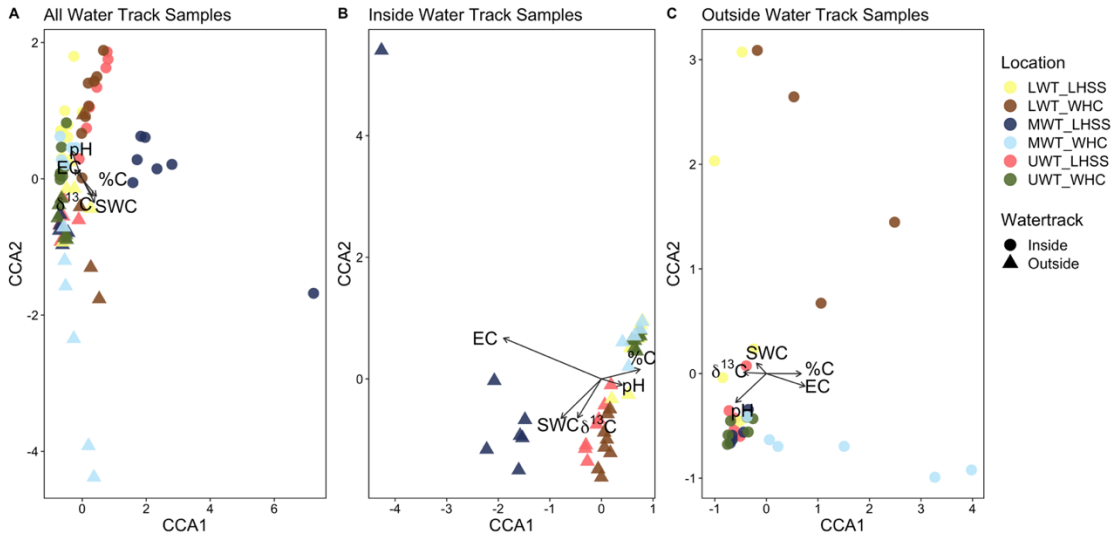

A
All Water Track Samples
B
Inside Water Track Samples
C
Outside Water Track Samples
CCA1
CCA2
pH
EC
%C
δ13C
SWC
Location
LWT_LHSS
LWT_WHC
MWT_LHSS
MWT_WHC
UWT_LHSS
UWT_WHC
Watertrack
Inside
Outside

Figure 6.

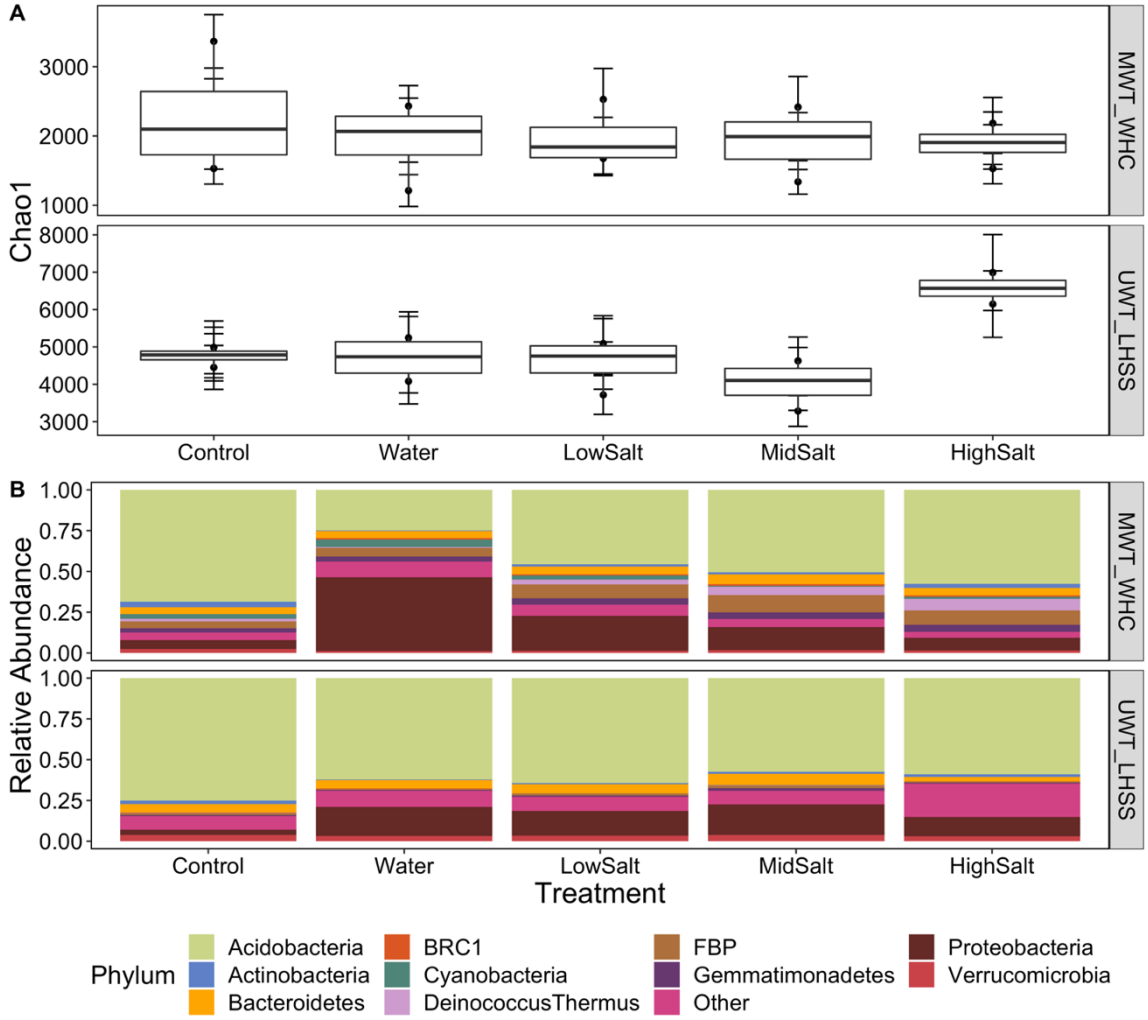

A
Chao1
3000
2000
1000
8000
7000
6000
5000
4000
3000
MWT_WHC
UWT_LHSS
Control
Water
LowSalt
MidSalt
HighSalt
B
Relative Abundance
1.00
0.75
0.50
0.25
0.00
Treatment
Phylum
Acidobacteria
Actinobacteria
Bacteroidetes
BRC1
Cyanobacteria
DeinococcusThermus
FBP
Gemmatimonadetes
Other
Proteobacteria
Verrucomicrobia

Figure 7.

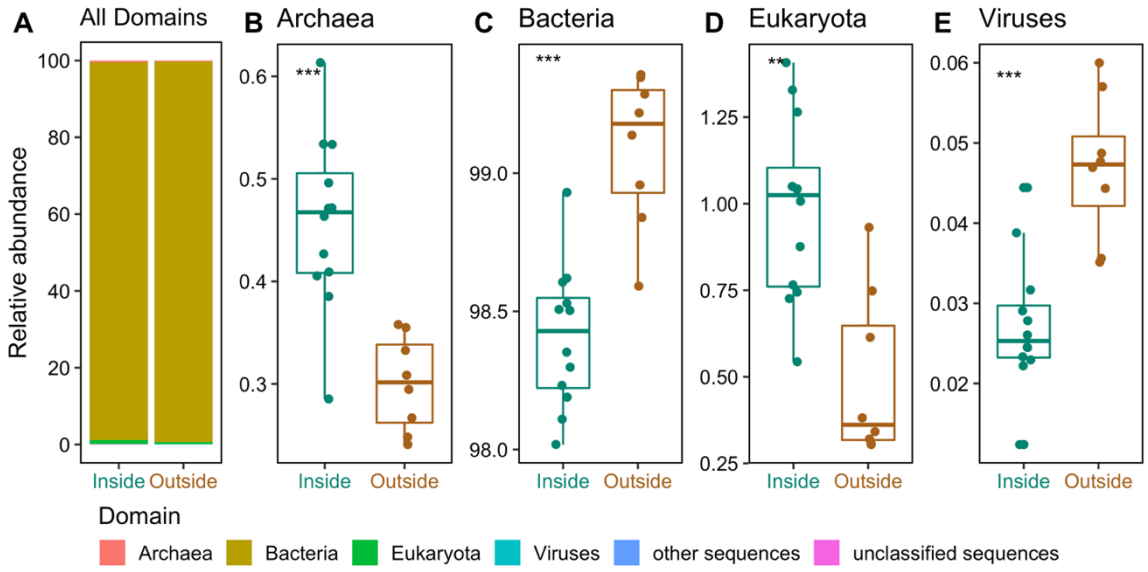

A
All Domains
Relative abundance
100
80
60
40
20
0
Inside
Outside
B
Archaea
***
0.6
0.5
0.4
0.3
C
Bacteria
***
99.0
98.5
98.0
D
Eukaryota
**
1.25
1.00
0.75
0.50
0.25
E
Viruses
***
0.06
0.05
0.04
0.03
0.02
Domain
Archaea
Bacteria
Eukaryota
Viruses
other sequences
unclassified sequences

Figure 8.

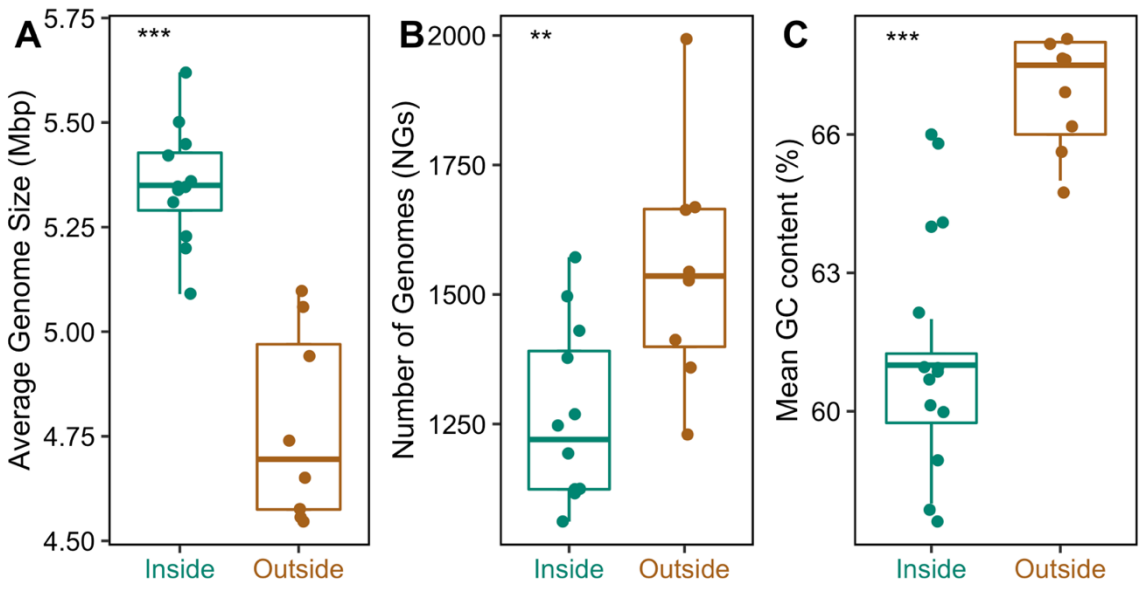

A
***
Average Genome Size (Mbp)
5.75
5.50
5.25
5.00
4.75
4.50
Inside
Outside
B
**
Number of Genomes (NGs)
2000
1750
1500
1250
Inside
Outside
C
***
Mean GC content (%)
66
63
60
Inside
Outside

Figure 9.

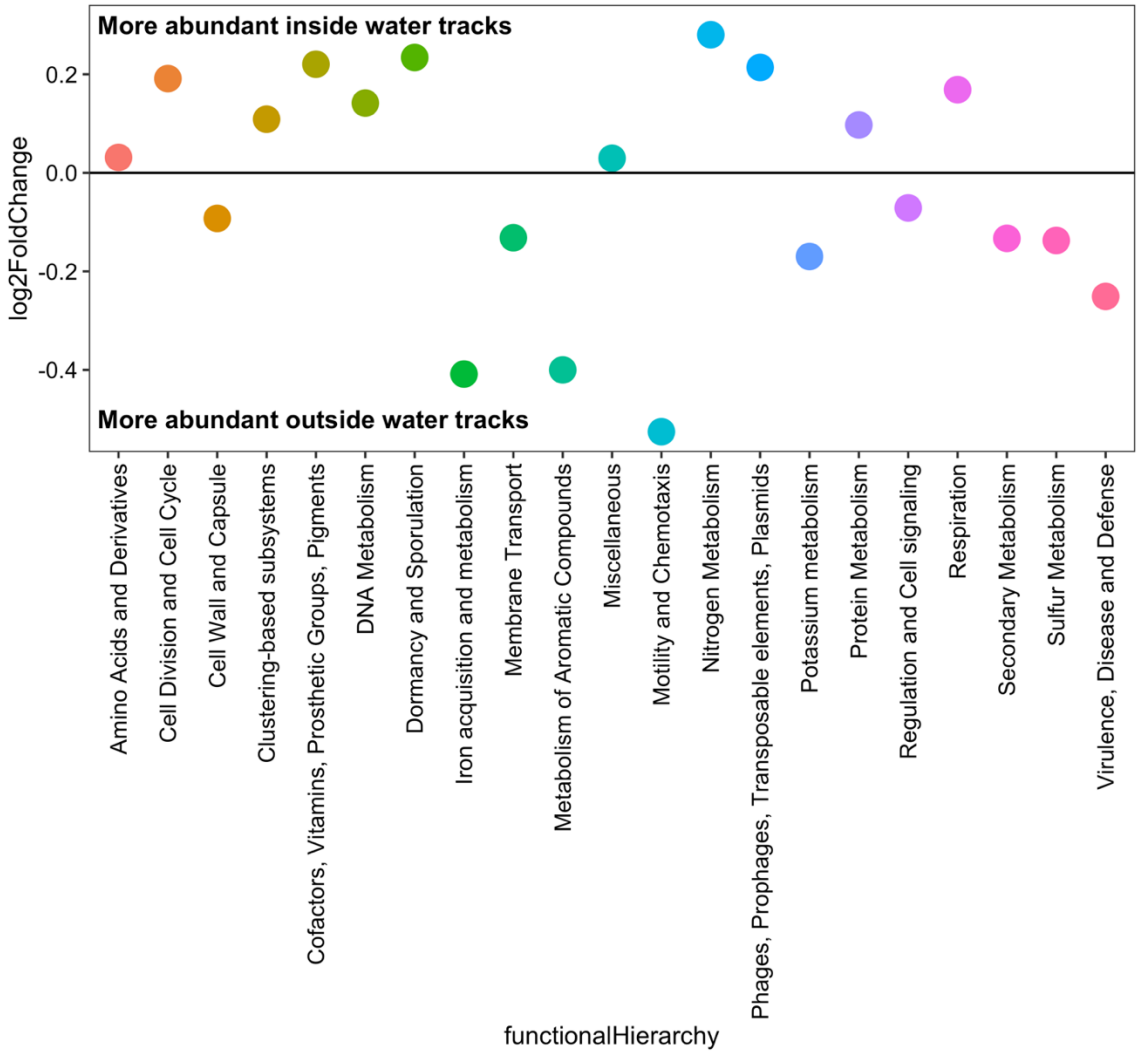

More abundant inside water tracks
More abundant outside water tracks
log2FoldChange
0.2
0.0
-0.2
-0.4
Amino Acids and Derivatives
Cell Division and Cell Cycle
Cell Wall and Capsule
Clustering-based subsystems
Cofactors, Vitamins, Prosthetic Groups, Pigments
DNA Metabolism
Dormancy and Sporulation
Iron acquisition and metabolism
Membrane Transport
Metabolism of Aromatic Compounds
Miscellaneous
Motility and Chemotaxis
Nitrogen Metabolism
Phages, Prophages, Transposable elements, Plasmids
Potassium metabolism
Protein Metabolism
Regulation and Cell signaling
Respiration
Secondary Metabolism
Sulfur Metabolism
Virulence, Disease and Defense
functionalHierarchy

Figure 10.

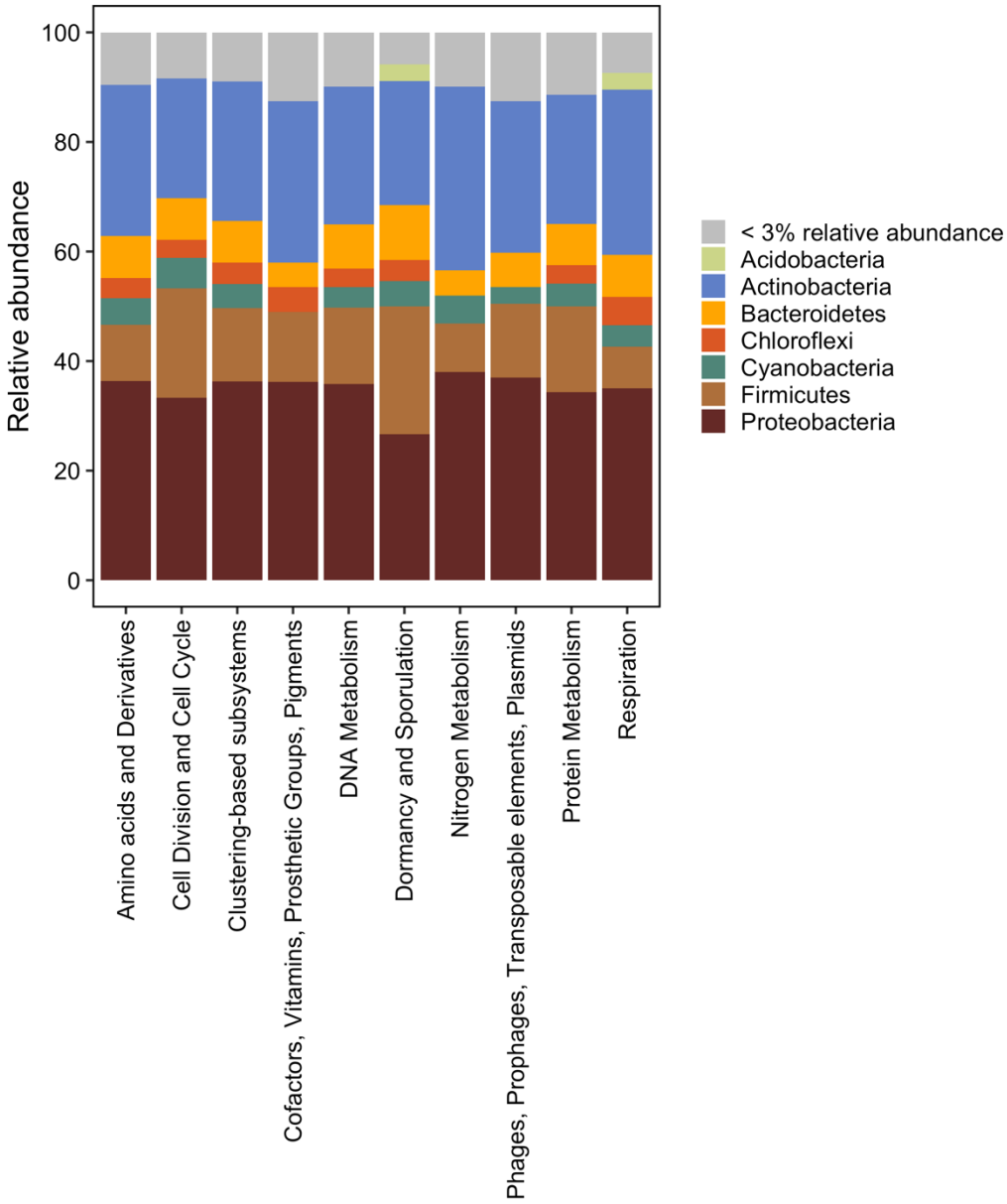

Relative abundance
100
80
60
40
20
0
Amino acids and Derivatives
Cell Division and Cell Cycle
Clustering-based subsystems
Cofactors, Vitamins, Prosthetic Groups, Pigments
DNA Metabolism
Dormancy and Sporulation
Nitrogen Metabolism
Phages, Prophages, Transposable elements, Plasmids
Protein Metabolism
Respiration
< 3% relative abundance
Acidobacteria
Actinobacteria
Bacteroidetes
Chloroflexi
Cyanobacteria
Firmicutes
Proteobacteria

Table 1. Summary statistics of edaphic conditions. Mean ± standard deviation, significance assessed by ANOVA. Electrical conductivity (EC) is reported as μS/cm and soil water content (SWC) is reported as the fraction of wet mass – dry mass / dry mass.

Location	EC				pH				SWC			
	Inside	Outside	F-value	P-value	Inside	Outside	F-value	P-value	Inside	Outside	F-value	P-value
LHSS	263±313	300±621	NS	NS	8.65±0.68	9.67±0.54	28.43	< 0.001	0.057±0.041	0.007±0.012	26.27	< 0.001
WHC	59±27	307±294	19.25	< 0.001	8.65±0.44	8.87±0.57	NS	NS	0.021±0.022	0.001±0.002	15.62	< 0.001
UWT_LHSS	128±53	141±53	NS	NS	8.73±0.52	9.98±0.15	32.22	< 0.001	0.093±0.048	0.003±0.005	20.68	< 0.001
MWT_LHSS	563±380	628±1053	NS	NS	8.24±0.73	9.41±0.80	8.64	0.012	0.048±0.025	0.002±0.004	18.99	< 0.001
LWT_ LHSS	97±138	132±72	NS	NS	8.98±0.63	9.61±0.35	5.04	0.043	0.031±0.011	0.015±0.019	NS	NS
UWT_ WHC	70±31	164±99	7.33	0.018	8.32±0.37	9.31±0.55	17.63	0.001	0.006±0.010	0.0±0	NS	NS
MWT_WHC	39±9	252±134	23.65	< 0.001	8.70±0.28	8.92±0.16	NS	NS	0.010±0.009	0.0±0	7.8	0.015
LWT_ WHC	68±24	506±436	9.39	0.009	8.93±0.45	8.39±0.50	4.72	0.049	0.048±0.013	0.002±0.004	69.19	< 0.001

Table 2. Summary statistics of elemental analysis. Mean ± standard deviation, significance assessed by ANOVA. Elemental variables analyzed include ^{13}C natural abundance isotopes and percent carbon (%C).

	%C				$\delta^{13}C$			
Location	Inside	Outside	F-value	P-value	Inside	Outside	F-value	P-value
LHSS	0.07±0.03	0.07±0.01	NS	NS	-26.5±2.5	-29.4±1.6	18.27	< 0.001
WHC	0.08±0.02	0.06±0.01	14.96	< 0.001	-25.4±2.3	-28.3±1.7	20.74	< 0.001
UWT_ LHSS	0.10±0.03	0.06±0.01	7.25	0.021	-24.2±2.6	-29.6±1.0	22.53	< 0.001
MWT_ LHSS	0.05±0.01	0.06±0	NS	NS	-25.9±1.2	-28.8±2.3	9.56	0.009
LWT_ LHSS	0.07±0.01	0.08±0.01	NS	NS	-28.9±1.1	-29.8±1.3	NS	NS
UWT_ WHC	0.10±0.01	0.07±0.01	27.97	< 0.001	-26.3±2.5	-29.8±0.7	10.65	0.006
MWT_ WHC	0.09±0.03	0.06±0.01	7.66	0.017	-24.7±2.5	-27.9±1.4	8.03	0.015
LWT_ WHC	0.06±0.01	0.05±0.01	NS	NS	-25.1±1.7	-27.1±1.7	4.94	0.045

Table 3. Summary statistics of community diversity and abundance. Mean ± standard deviation, significance assessed by ANOVA. Mean 16S rRNA gene copy numbers are reported as copy numbers per μL, based on 1:10 dilutions of template DNA.

	16S Copy Number				Chao1			
Location	Inside	Outside	F-value	P-value	Inside	Outside	F-value	P-value
LHSS	6,477±6,443	5,304±4,381	NS	NS	3,645±1,572	3,730±1,178	NS	NS
WHC	20,055±19,326	10,266±11,190	3.34	NS	4,438±1,923	2,593±1,314	9.43	0.005
UWT_LHSS	10,968±8,573	4,307±2,329	3.38	NS	4,814±1,229	3,530±1,197	NS	NS
MWT_LHSS	1,462±1,769	8,522±6,322	8.14	0.017	1,638±602	4,223±835	35.65	< 0.001
LWT_LHSS	5,886±1,892	3,283±2,385	5.09	0.044	3,945±870	3,307±1,744	NS	NS
UWT_WHC	32,178±19,268	19,491±10,777	NS	NS	5,694±1,976	3,577±875	5.92	0.032
MWT_WHC	22,814±15,791	6,859±8,867	4.90	0.047	4,214±1,062	1,981±1,271	9.72	0.012
LWT_WHC	1,316±2,364	1,538±2,553	NS	NS	2,652±741	1,480±25	NS	NS

Table 4. Spearman rank correlations of edaphic variables to diversity

		pH x copy #		EC x copy #		pH x chao1		EC x chao1	
Scale	Location	rho	P-value	rho	P-value	rho	P-value	rho	P-value
Overall		-0.12	NS	-0.39	<0.001	-0.07	NS	-0.43	<0.00
Within Basin	LHSS	0.12	NS	-0.40	0.010	0.08	NS	-0.40	0.014
	WHC	-0.36	0.021	-0.32	0.042	-0.40	0.025	-0.52	0.002
Within LHSS	Inside	0.27	NS	-0.34	NS	0.17	NS	-0.39	NS
	Outside	0.08	NS	-0.43	NS	0.13	NS	-0.15	NS
Within WHC	Inside	0.61	0.002	-0.30	NS	-0.42	NS	-0.20	NS
	Outside	0.38	NS	-0.29	NS	0.37	NS	-0.50	NS

Table 5. Spearman rank correlations of elemental variables to diversity. Mean 16S rRNA gene copy numbers are reported as copy numbers per μL, based on 1:10 dilutions of template DNA. Elemental variables analyzed include ^{13}C natural abundance isotopes and percent carbon (%C).

		%C x copy #		$\delta^{13}C$ x copy #		%C x chao1		$\delta^{13}C$ x chao1	
Scale	Location	rho	P-value	rho	P-value	rho	P-value	rho	P-value
Overall		0.66	<0.001	0.01	NS	0.60	<0.001	0.07	NS
Within Basin	LHSS	0.49	0.002	-0.11	NS	0.51	0.002	-0.11	NS
	WHC	0.78	<0.001	0.12	NS	0.68	<0.001	0.29	NS
Within LHSS	Inside	0.85	<0.001	0.02	NS	0.59	0.005	-0.03	NS
	Outside	-0.33	NS	-0.38	NS	0.39	NS	-0.15	NS
Within WHC	Inside	0.83	<0.001	0.33	NS	0.68	0.002	0.23	NS
	Outside	-0.33	NS	-0.38	NS	0.39	NS	-0.15	NS

Table 6. Random Forest classification – OOB estimate of error rate

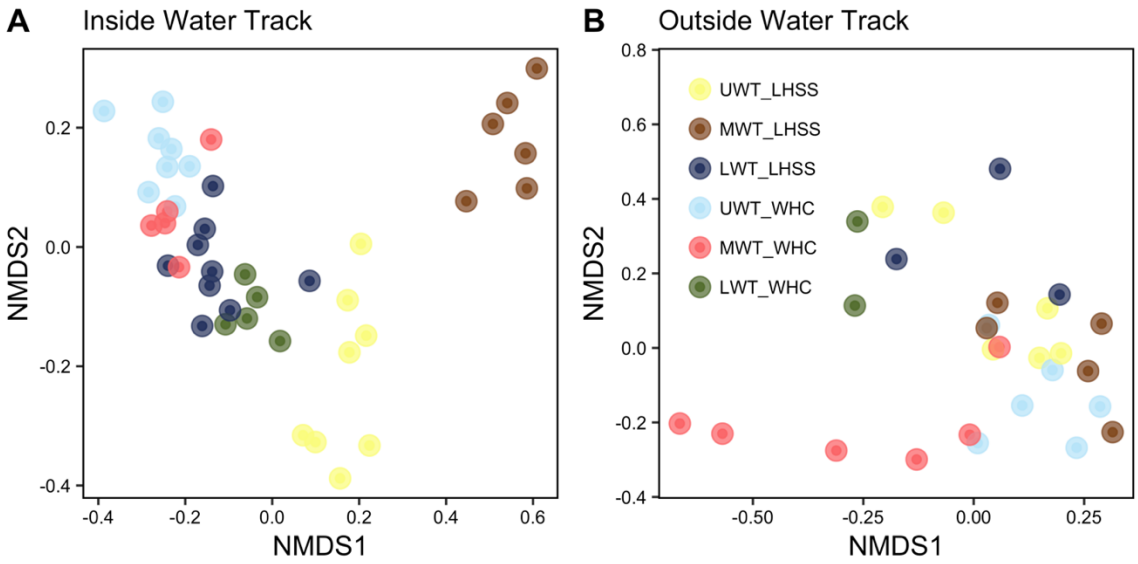
	Inside vs. Outside	By Reach	Inside vs. Outside within Reach
Overall	4.35	31.88	33.33
Inside	-	14.63	-
Outside	-	67.86	-
UWT_LHSS	7.14	-	-
MWT_LHSS	0	-	-
LWT_LHSS	25	-	-
UWT_WHC	0	-	-
MWT_WHC	0	-	-
LWT_WHC	28.57	-	-

Table 7. Random Forest regression – mean of squared residuals (MSR) and percent variance explained (PVE).Edaphic and elemental variables analyzed included pH, electrical conductivity (EC) as reported as μS/cm, soil water content (SWC), reported as the fraction of wet mass – dry mass / dry mass, ^{13}C natural abundance isotopes, and percent carbon (%C).

	pH		EC		SWC		$\delta^{13}C$		%C	
	MSR	PVE	MSR	PVE	MSR	PVE	MSR	PVE	MSR	PVE
Overall	0.27	44.5	16376	38.2	0.0004	60.4	3.46	43.5	0.0003	33.8
Only Inside	0.35	3.7	23542	35.6	0.0005	60.1	4.27	25.5	0.0004	25.8
Only Outside	0.17	43.8	10639	4.9	0.0008	7.8	1.21	26.3	0.0001	0.2

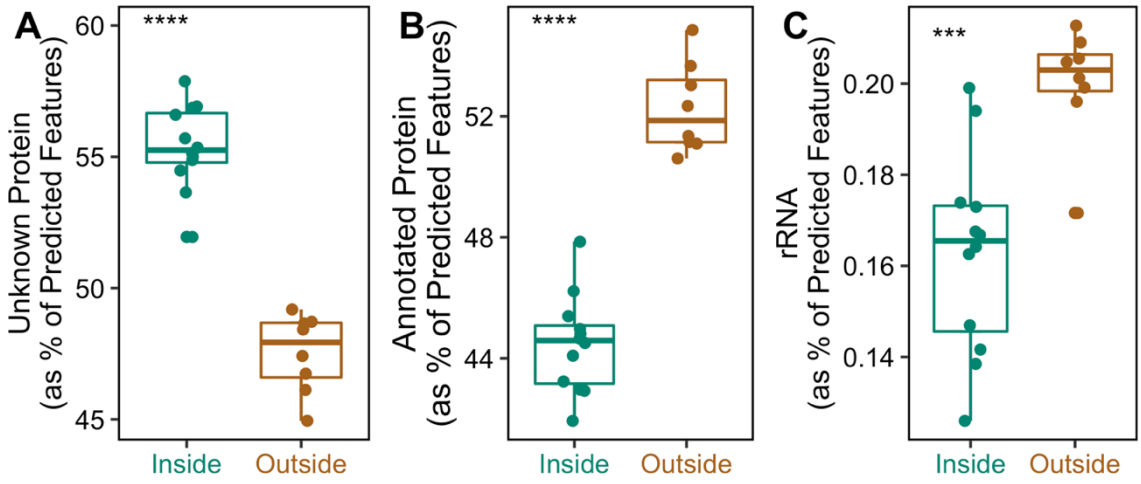
APPENDIX C. CHAPTER 3 SUPPLEMENTARY MATERIAL

Supplemental Figure 1.



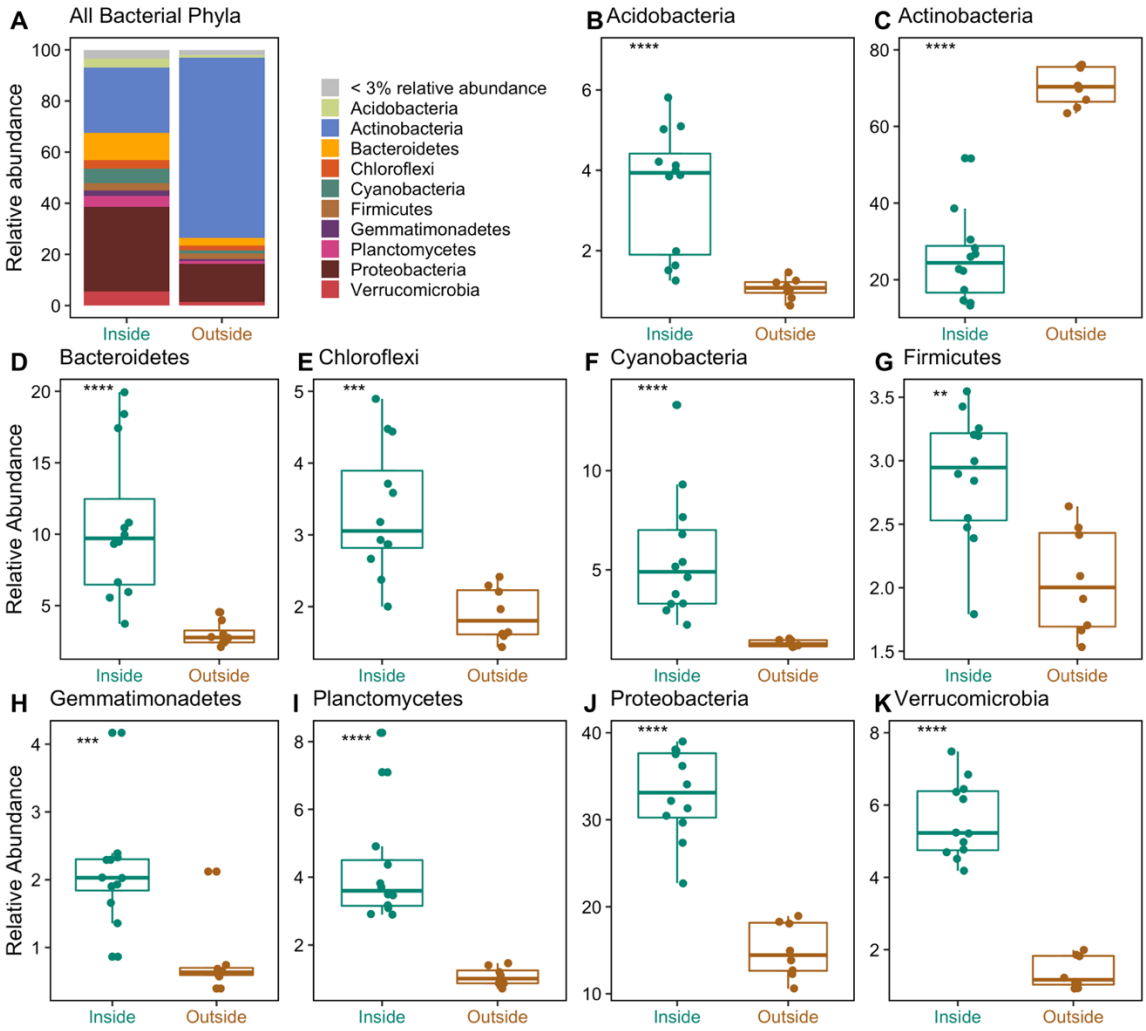
NMDS ordinations within inside (A) and within outside (B) samples

Supplemental Figure 2.



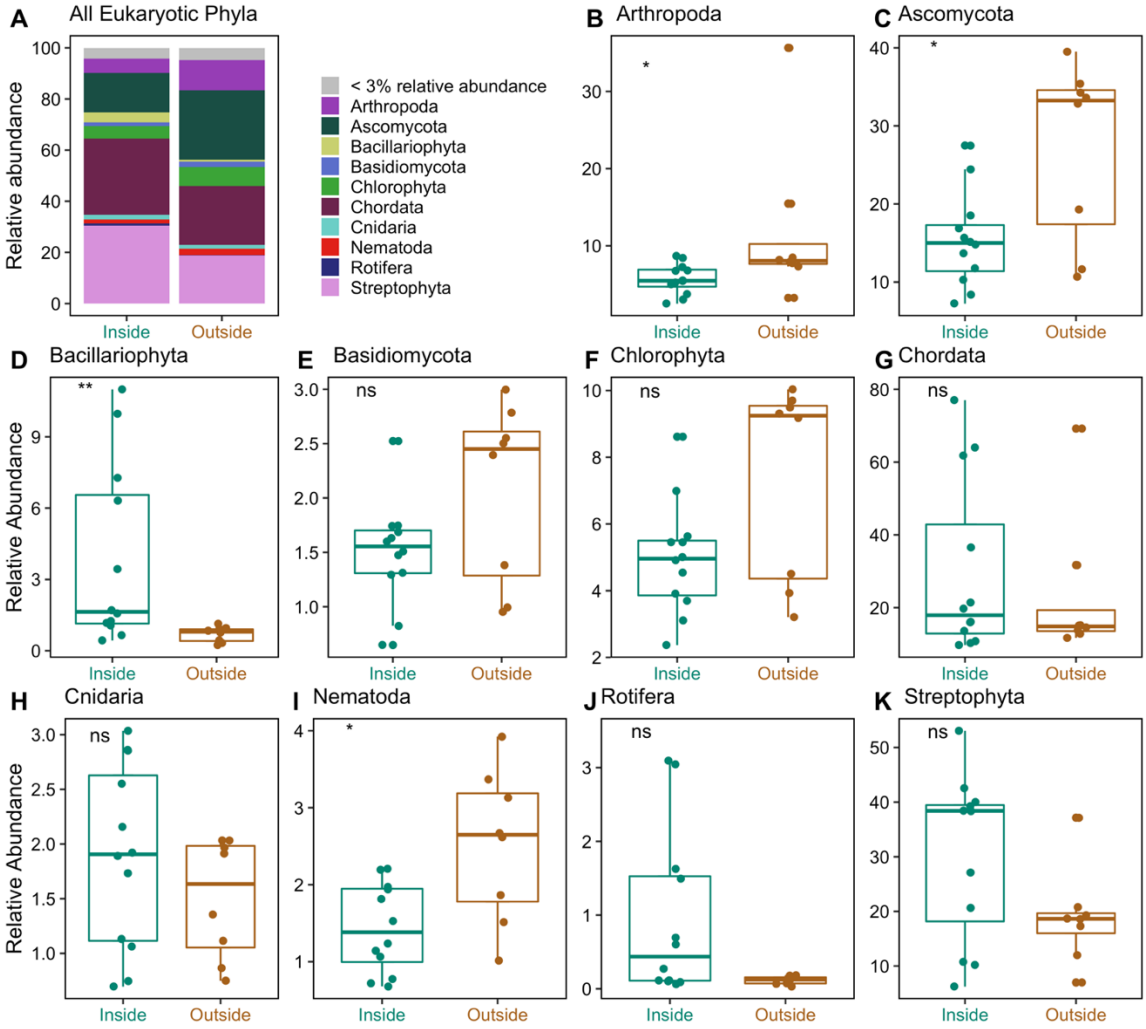
Summary statistics after MG RAST processing comparing the reads annotated as unknown protein (A), annotated protein (B), and rRNA (C). Features are summarized as the percentage of predicted features. Significant differences between inside- and outside-track samples are shown (Wilcoxon test, ****: $P \leq 0.0001$, ***: $P \leq 0.001$, **: $P \leq 0.01$, *: $P \leq 0.05$, ns: $P > 0.05$).

Supplemental Figure 3.



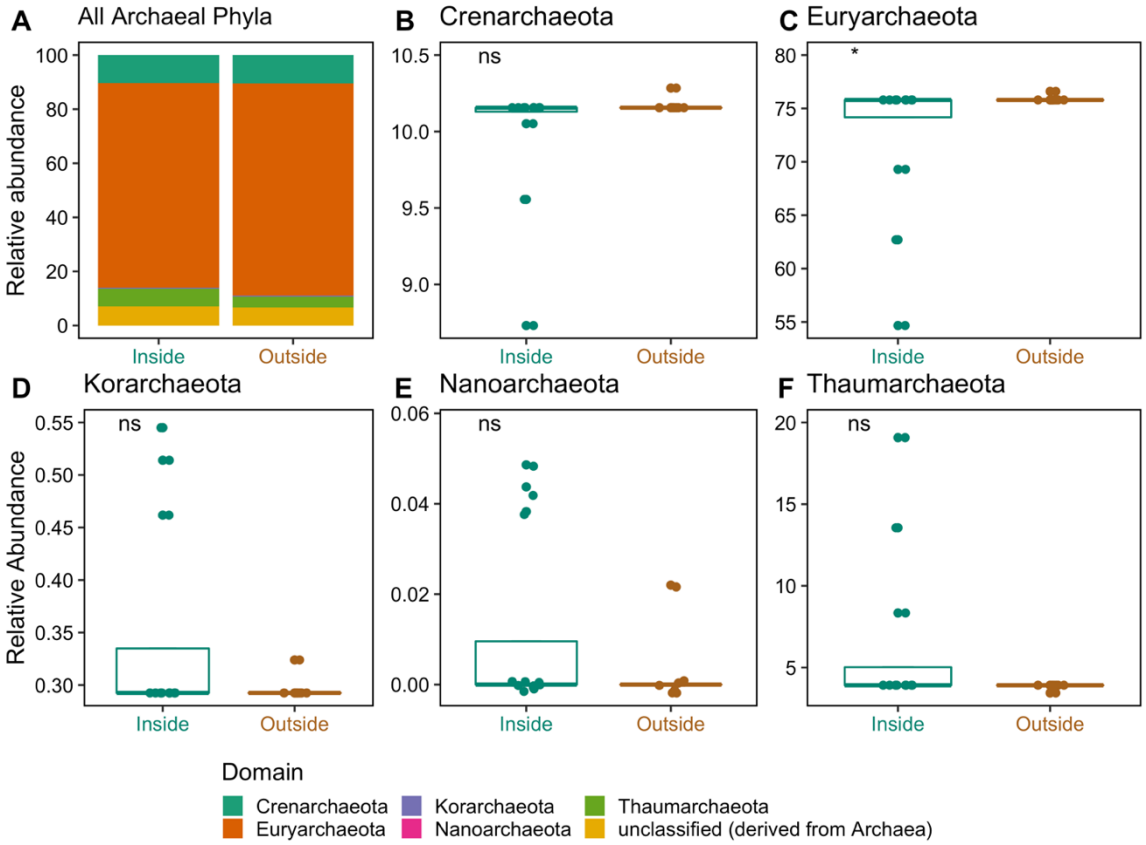
Classification of metagenomic sequences attributed to bacterial phyla revealed that communities were dominated by Actinobacteria and Proteobacteria. Significant differences in the relative abundance of all of the 10 most abundant phyla were noted between inside- and outside-track samples (Wilcoxon test, ****: $P \leq 0.0001$, ***: $P \leq 0.001$, **: $P \leq 0.01$, *: $P \leq 0.05$, ns: $P > 0.05$). Acidobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Firmicutes, Gemmatimonadetes, Planctomycetes, Proteobacteria, and Verrucomicrobia had higher abundances in the inside track samples while only Actinobacteria were significantly more abundant in the outside track samples. Phyla with less than 3% relative abundance among any of the sample were binned into the < 3% relative abundance category.

Supplemental Figure 4.



Classification of metagenomic sequences attributed to eukaryotic phyla revealed that communities were dominated by Ascomycota, Chordata, and Streptophyta. Significant differences in the relative abundance of 4 of the 10 most abundant phyla were noted between inside- and outside-track samples (Wilcoxon test, ****: $P \leq 0.0001$, ***: $P \leq 0.001$, **: $P \leq 0.01$, *: $P \leq 0.05$, ns: $P > 0.05$). Bacillariophyta had higher abundances in the inside track samples while Arthropoda, Ascomycota and Nematoda were significantly more abundant in the outside track samples. Phyla with less than 3% relative abundance among any of the sample were binned into the < 3% relative abundance category.

Supplemental Figure 5.



Classification of metagenomic sequences attributed to archaeal phyla revealed that communities were dominated by Euryarchaeota. The only significant difference observed in the relative abundance was in Euryarchaeota which were slightly higher in the outside track samples. (Wilcoxon test, ****: $P \leq 0.0001$, ***: $P \leq 0.001$, **: $P \leq 0.01$, *: $P \leq 0.05$, ns: $P > 0.05$).

SUMMARY

In the past decade, dramatic landscape-scale change has occurred in the McMurdo Dry Valleys (MDV) Antarctica due to increased temperatures and solar radiation and altered hydrology and energy flux. Glaciers have deflated, thermokarst slumps have formed near lakes and streams, and rivers have become incised. The goal of this project was to assess the biological consequences of these geomorphic changes. I began by utilizing the inherent edaphic gradients within soil polygons to understand the effects of environmental heterogeneity on microbial community structure while disentangling the effects of landscape context and spatial scale. Next, I collected information on how landscape scale disturbance has impacted environmental factors in disturbance prone habitats including the soil active layer and water tracks, surveyed microbial communities in these prone habitats, and used experimental approaches to determine the response of communities to disturbances. To explore these topics, I have leveraged genetic data associated with three key geomorphic and hydrologic features within the MDV: 1) soil polygon, 2) the soil active layer, and 3) water tracks. The primary findings of the three chapters of my dissertation are summarized below.

Chapter 1: Local and Regional Scale Heterogeneity Drive Bacterial Community Diversity and Composition in a Polar Desert

Soils in the MDV are subjected to the frequent freeze-thaw cycles, causing the physical sorting of rocks and soil particles in addition to the expansion and contraction of the permafrost in the soil sub-surface. These processes result in patterned ground formations in the shape of polygons. MDV soil polygons contain inherent edaphic

gradients, due either to naturally occurring environmental heterogeneity or the physical processes inherent to polygon formation. I leveraged these gradients to study the impact of environmental heterogeneity on bacterial community diverse and composition across a variety of biogeochemically-diverse lake basins within the Taylor Valley of the MDV. I found that relationships between community structure and edaphic characteristics were highly variable and contextual, ranging in magnitude and direction across regional, basin, and local scales. Correlations among edaphic factors (pH and soil conductivity) and the relative abundance of specific phyla were most pronounced along local environmental gradients in the Lake Fryxell basin where Acidobacteria, Bacteroidetes, and Proteobacteria declined while Deinococcus–Thermus and Gemmatimonadetes increased with soil conductivity (all $P < 0.1$). Species richness was most strongly related to the soil conductivity gradient present within this study system. I conclude that a combination of local-scale polygon mechanisms as well as regional-scale geological histories drove changes in edaphic gradients that played a large role in determining the microbial community composition and diversity within the McMurdo Dry Valleys of Antarctica. These results suggest that the relative importance of pH versus EC in structuring microbial communities is contextually related to the length and severity of edaphic gradients and the spatial scale of sampling, creating a framework in which to interpret conflicting literature.

Chapter 2: Stratification of Bacterial Communities Within the Soil Active Layer in the McMurdo Dry Valleys, Antarctica

This active layer is expected to expand as temperatures increase, altering conditions for soil microbial communities. In my second chapter, I surveyed the microbial communities within the vertical horizons of the soil profile and conducted reciprocal transplant experiments where near surface soils were incubated at lower soil horizons, and vice versa. These results suggest that surface communities are unique in comparison to deeper samples and that relationships among communities and depth are dependent on soil moisture.

Samples from the surface sediments were generally more diverse (~7,500 OTUs) than samples near the soil-permafrost interface (~5,000 OTUs), however patterns varied by basin. Surface samples generally showed enrichments of Cyanobacteria and Proteobacteria and corresponding lower abundances of Acidobacteria and Actinobacteria. Relationships among community structure and depth also depended on the relative position of the sampling sites to water tracks. In the surface sediments, enrichment of Cyanobacteria (15-22%) was only found in the wetted, downslope sites while the dry, upslope sites contained < 2% Cyanobacteria and the dominant phyla were Acidobacteria (60% in LHSS) and Proteobacteria (92% in WHC).

Beta diversity analyses revealed a slight increase of beta diversity with depth along the vertical soil profile. Partitioning the total beta diversity into turnover and nestedness components as a function of depth revealed that nearly all the variation was due to species turnover, suggesting that variables that correlate with depth act as a stressor to bacterial communities in the soil active layer. These results provide additional support for the stress-gradient hypothesis.

In additional to soil surface community differences among wet and dry sites, the uniformity of taxonomic profiles of samples taken from 5 cm to the soil-permafrost interface also varied by the relative position to a water track. In the wet, downslope sites of both basins, generally uniform and stable community compositions were found, whereas the horizon profiles in the dry, upslope sites were variable. I hypothesize that the wetted, downslope sites are more homogenized because the increased soil moisture and the flow of water through these soil profiles allows for movement of bacteria, nutrients, and other solutes throughout the soil column. In the drier, upslope sites, the communities are stratified by depth because there is little to no water-mediated mechanisms for dispersal.

Reciprocal transplants of soil did not appear to impact community diversity or composition, suggesting that changing the temperature regime alone for moderate durations had little impact on the soil bacterial communities, and that other factors are responsible for the impact of depth on community diversity and structure.

Collectively, these conditions imply that landscapes in the MDV will become progressively unstable in a warming future as heat is increasingly transported to deeper depths in the valley floor, and that these changes are likely to significantly impact the associated microbial communities.

Chapter 3: Drivers of Soil Bacterial Community Structure in Antarctic Water Tracks

Climate change in this region is projected to alter precipitation and promote melting of ice reserves, resulting in the creation and expansion of water tracks that

transport water, solutes, and heat downslope. For my third chapter, I collected samples along transects that spanned three reaches of two water tracks in Taylor Valley, Antarctica. I utilized surveys and experiments to describe the microbial communities associated with these understudied landscape features.

Edaphic conditions found within water tracks were highly variable. This variability impacted the associated microbial communities, with some water track reaches harboring more abundant and diverse bacterial communities than off track soils, while other reaches were biologically depauperate.

In the first track, diversity (Chao1; means of 4,438/2,593, $P < 0.005$) and abundance (16S copy number; means of 20,055/10,266, $P = 0.076$) were higher inside the track, corresponding to decreased salinity (EC; means of 59/307 μS, $P < 0.001$). Salinity did not vary significantly across the second track, although in the middle reach, diversity and abundance decreased inside where salinities were elevated (Chao1; means of 1,638/4,223, $P < 0.001$; 16S copy number; means of 1,462/8,522, $P = 0.017$). Overall, salinity was negatively correlated to diversity ($r = -0.43$, $P < 0.001$) and abundance ($r = -0.32$, $P = 0.003$). The finding of both significantly elevated and depressed bacterial abundance and diversity within different water track reaches as compared to off track soils, strongly suggests that water tracks are not consistent biological hot or cold spots but are instead highly variable habitats that, depending on local conditions, can either foster or restrict biological communities.

The distinct separation between inside and outside water track communities provided evidence that the significant but varied differences in edaphic characteristics for dry versus wet habitats in each reach resulted in clear environmental selection. I

hypothesize that within track samples may be more active and thus responsive to environmental filtering, producing greater between-reach/track community variation. Metagenomic sequenced confirmed that the microbes within this extreme ecosystem are highly influenced by cryptic hydrological features and utilize significantly different broad-scale metabolic and ecological strategies of the microbial communities depending on whether they reside within or outside of a water track. Metagenomic trait-based analyses confirmed that communities in the wetted, more copiotrophic sites, had larger average genomes sizes (inside: 5.4 ± 0.14 Mbp; outside: 4.8 ± 0.23 Mbp, $P \leq 0.001$), although unexpectedly, lower GC content (frequency of guanine and cytosine nucleotides, inside: $61 \pm 2\%$; outside: $67 \pm 1\%$, $P \leq 0.001$).

Experiments designed to simulate changes in water and solutes associated with the expansion of water track systems into dry soils suggested that decreased moisture and elevated salt content increased the abundance of Acidobacteria and decreased Proteobacteria.

Overall, as landscape scale change occurs in the MDV and water tracks expand, the impact to associated soil communities will depend on a variety of factors including the water track flow rate, solute content, and the ability to support the accumulation of soil organic matter.