Soil microbial communities in an Antarctic water track: identifying potential ecological optiums in a hyperarid Mars-analog environment

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Abstract

Polar permafrost environments commonly feature “water tracks,” areas of heightened soil moisture in which water is routed downslope above an ice-cemented permafrost interface. Water tracks are active sites of chemical weathering and possible hotspots of biological activity. Occurring in cold, arid environments, water tracks have seasonal and morphological similarities to recurrent slope lineae (RSL) streaks observed on steep equator-facing slopes of Mars. Water tracks are also potential analogs of ephemeral streams and rivers that likely featured in the cold, icy climate of the late Noachian period of early Mars. This study aims to characterize microbial communities within water track soils, looking specifically at community structures and predicted functional differences. Prior studies of water tracks in the McMurdo Dry Valleys in Antarctica have identified increased organic matter and biotic respiration of CO₂ within track soils. To identify possible ecological optiums within the studied water track in East Antarctica, community composition was compared at various elevations within the track and in off-track control soils. Community composition was not found to vary significantly between track and control soils, with Actinobacteria, Chloroflexi and Proteobacteria dominant across all sampling sites; however, gene expression within these clades may increase significantly with hydration, a hypothesis that will be tested as part of future work with transcriptomics. Interestingly, we found significant proportions of unclassified taxa in collected samples (over half of the results at the family level). Further study will expand what is known about the functional diversity of these unique Martian analog environments.

Introduction

The consistently cold and dry climate of ice-free regions in East Antarctica and the McMurdo Dry Valleys (MDV) warrants their use as analogs for both modern and historical “cold and icy” climates of Mars. Understanding how hydrological processes impact biotic processes and biota in these hyperarid Martian analog environments will inform the search for both past and present life on Mars. Defining the biological implications of hydrological features in permafrost environments will also shed light on how life survives when pushed to its limit as well as inform projections of how climate change will impact Antarctica’s soil microbiome. Of specific interest are “water tracks” – areas of heightened soil moisture in otherwise hyperarid soils – which can be activated through positive feedbacks induced by increased solar radiation and episodic warming events (Levy 2018).

Water tracks are persistent features of polar permafrost environments and are characterized by downslope groundwater flow through active layer soils above the permafrost interface. At present, water tracks are thought to occupy up to five percent of soil-covered land area of the MDV (Ball and Levy 2015). Incidence of water tracks may increase in Antarctica due to
enhanced active layer thickening or enhanced regional snowmelt with climate change (Ball and Levy 2015).

Occurring in hyperarid, hypothermal environments, water tracks are morphologically and spectroscopically similar to recurrent slope lineae (RSL) occurring on steep southern-hemisphere slopes of Mars (Levy 2011). RSL are thought to be similarly formed by the shallow percolation and flow of liquid water or water-based brine, lengthening when maximum daily surface temperatures exceed freezing (Levy 2012). Sources of water track brines include snowmelt, ground ice melt, and direct absorption of atmospheric water vapor by hygroscopic salts. The latter of these three sources is a process known as deliquescence, thought to be a possible source of RSL fluid generation on Mars (McEwen 2011). Water tracks are also a potential analog of ephemeral streams and rivers in the hydrologic system of early Mars, consistent with a “cold and icy” Noachian climate (Head and Marchant 2014).

As active sites of chemical weathering, water tracks contain brines enriched in solutes, potentially forming local ecological optiums in moist, lower-salinity regions (Levy 2011). However, the effect of water tracks on Antarctic soil biota is complicated due to the competing influences of heightened moisture and salinity (Ball and Levy 2015). Compared to their better-studied counterparts in Arctic permafrost environments, water tracks in the McMurdo Dry Valleys (MDV) are wetter and more saline. Located in a water-limited ecosystem, Antarctic soil microbiomes are highly sensitive to changes in soil chemistry and water content. Increased soil water content can foster heightened biological activity, as indicated by bio-volume and biotic fluxes of CO$_2$ (Ball and Levy 2015). However, a steep rise in salinity due to track activation can also cause adverse effects to osmotic regulation by soil biota, limiting biological activity.

As a result, activation of water tracks in the MDV has a highly variable impact on soil habitability and biology, forming either low-salinity hot spots or highly saline dead zones (Ball and Levy 2015). Potential determinants of this outcome are soil type and the presence of surficial water flow (Ball and Levy 2015). Understanding this variable impact of water track formation will inform how soil biota respond to climate-induced changes in the local hydrology.

While morphological, geochemical, and basic biological analyses of Antarctic water tracks have been conducted in the MDV, microbial community composition within Antarctic water tracks has not yet been analyzed. Additionally, water tracks occurring in ice-free regions outside of the McMurdo Dry Valleys have been understudied, with nearly all analyses thus far concentrated in the Taylor Valley. Samples for this study were collected along a water track in Queen Maud Land, East Antarctica, a comparatively understudied dry valley ecosystem similar to the MDV. The track was located on a well-exposed North-facing slope South of Lake Untersee below a hanging snow deposit. Samples were collected at the ice-cemented permafrost interface at four different elevations, with controls taken from sites > 50 meters adjacent to surface moisture at approximately equivalent elevations.

DNA and RNA extracted from these samples will be used to characterize microbial communities within water track soils, their metabolic potential, and their gene expression. Evaluating the community composition of soil microbiota will help determine potential correspondence between structural or functional differences and higher moisture and salinity within track soils. To
identify ecological optimums within the studied water track, metagenomes will be analyzed from representative samples within and outside of the track at the permafrost interface. Untersee is a hyperarid dry valley region similar to the MDV, with peak seasonal and daily temperatures above 0°C despite average annual temperatures well below freezing (Andersen et al 2015). Samples were collected in mid-December when temperatures were above freezing and liquid water was present. Seasonality of water tracks is mimicked by RSL which begin lengthening when maximum daily surface temperatures reach 297 ± 5 Kelvin (Grimm et al 2013).

Incidence of water tracks in the MDV has been correlated with an increase in insolation driven melting of active layer soils. Positive feedbacks driving this melting in the MDV have been attributed to an increase in solar energy between 1991-2000, coupled with a warm episode in the summer of 2001-2002 (Levy et al 2018) The average solar flux at Lake Untersee from 2008-2013 was 99.2 ± 0.6 W/m². The mean annual temperature for this same period was -10.6° ± 0.6°C, with annual degree-days above freezing ranging from 7.0 to 48.8 (Andersen et al 2015).
Models suggest that the late Noachian climate of Mars may have been characterized by a hydrologic system horizontally stratified by a globally continuous cryosphere (Head and Marchant 2014). Insights may be gained about transfers from the cryosphere to the hydrosphere occurring during punctuated warming events by using Antarctic terrestrial analogs. Determining the distribution and stability of water within these hydrologic systems thus has important implications for the identification of environments conducive to life.

Methods

Sample collection
Samples analyzed in this study were collected across five sites at varying elevations along and adjacent to the study water track. Soil samples were collected from the surface and at the permafrost interface in triplicates using ashed copper spoons and sterile plastic spatulas. Samples were stored in sterile cryotubes, Whirl-Paks (Nasco, Fort Atkinson, WI, United States) and glass jars. Samples were immediately put on ice and transported back to the field site for storage in a cryoshipper (Cryo Associates, Inc., Gaithersburg, MD, United States) at -190° C for the remainder of the field season. Samples were transported to Georgetown University (Washington, DC, United States) in the cryoshipper and kept at -80° C until processed.

Community DNA purification
DNA was extracted from each site in replicate using the QIAGEN PowerSoil Pro Kit (Qiagen, Inc., Valencia, CA) per the manufacturer’s protocol, eluted in PCR Grade Water (Sigma Aldrich, St. Louis, MO, United States) and stored at -80° C until processing. DNA yields were quantified with Qubit dsDNA high sensitivity assays (Thermo Fisher Scientific, Waltham, MA). Extraction with yields > 0.5 ng/μL were submitted for sequencing, with a total of 10 extractions meeting this requirement. Sixteen additional extractions were performed but produced no amplifiable DNA, likely due to low biomass.

Amplicon sequencing
Libraries for amplicon sequencing were prepared with primers 515F (5'-GTGYCAAGCMGCCGCGGTAA-3’) and 806R (5’-GGACTACNVGGGTWTCTAAT-3’) as described by Caporaso et al. (2011) to target the V4 region of prokaryotic 16 SSU rRNA. A total of 10 samples were sequenced. All sequencing was performed with the MiniSeq instrument (Illumina, San Diego, CA).

Data analysis
Several Quantitative Insights into Microbial Ecology 2 (QIIME2) and VSEARCH functions were wrapped into a reusable pipeline using a custom script (Caporaso 2010, Caporaso 2011). It begins by using VSEARCH’s ‘-fastq_mergepairs’, tolerating up to 10 base-pair differences and as few as 12 overlapping base-pairs. This overlap length was found to be crucial in recovering a reasonable number of fragments with data derived from the MiniSeq library. After barcodes were extracted and sequences were demultiplexed, each library was individually interrogated for primer sequences using VSEARCH’s ‘-search_oligodb’ and a FASTA file containing Illumina primers. The 5’-end was trimmed by 19 base-pairs and the 3’-end was stripped of 20 base-pairs. QIIME2’s deblur/denoise function was used to remove potential chimerae using a trim length of 120. QIIME2’s pick_otus.py was used to identify OTUs de novo at 97% sequence similarity from the chimera-filtered fragments using a trained dataset. Alpha and beta diversity measures were derived using the QIIME2 diversity package.

Results
Community Composition
Community composition did not differ significantly between on- and off-track sampling sites. Microbial communities on and off-track were dominated by similar phylotypes. Actinobacteria were dominant in all samples, with an average relative abundance of 56 percent across control
samples and 58 percent across track samples (Figure 2). Within the phylum *Actinobacteria*, dominant classes included *Thermophilia, Actinobacteria, Rubrobacteria* and *Acidomicrobilia*.

*Proteobacteria* comprised 12 percent of control samples and 9 percent of track samples, while *Chloroflexi* comprised 11 and 12 percent, respectively. This composition is typical of Antarctic soils, which are largely dominated by *Actinobacteria*, with large percentages of *Proteobacteria* and *Chloroflexi* (Bai et al., unpublished). Few samples contained substantial numbers of *Acidobacteria* and *Verrucomicrobia*. The archaeal OTU SCA1145 was present in both control and track samples (2% and 1% relative abundance, respectively).

Actinobacteria are strongly dominant in Antarctic soil communities, likely due to their adaptability to arid environments with high UV radiation exposure (Aislabie et al., 2006, Bull 2011, Rasuk et al., 2017). Widely distributed and thought to be endemic to Antarctic soil communities, Actinobacteria are metabolically diverse. The phylum evolved roughly 2,700 million years ago to break down plant biomass under various environmental extremes, including high temperatures, varying pH and anoxic or aerobic states (Lewin et al., 2017). Adaptations of extremophilic Actinobacteria are well-documented, and their prolonged survival in permafrost sediments has been attributed to their unique structural, biochemical and molecular organization (Kochkina et al., 2001).

Interestingly, a significant percentage of bacteria unclassified at the phylum level were present within each sample (5 to 6% across track and control sites, respectively). At the family level a large proportion of taxa were unclassified, a subset of these are expected to have a percentage identity under the threshold for a new species (97%) as identified by sequencing majority of the 16S rRNA region. These taxa represent promising candidates for future metagenomic and metatranscriptomic study.
Figure 2. KRONA plots illustrating the taxonomic breakdown of sampled communities grouped by on- or off-track location. Community composition was similar between track soils (top) and control soils (bottom). A significant percentage of present taxa were unclassified at the phylum (5-6%) and family level.

Alpha diversity
Rarefaction was performed at a sampling depth of 4000, and rarefaction curves for all five sampling sites were computed (Figure 3). As shown, most samples began to plateau around a sequencing depth of 2000, though deeper sequencing may still reveal new OTUs. Site 8, corresponding to a fully saturated sampling site midway up the track 20 cm from standing water and 20 m from running water, appears to have the highest diversity of the sampling sites. Site 9, a dry control site adjacent to Site 8, but in a slightly elevated area off-track, appears to have the lowest diversity of the sampling sites.

![Rarefaction curve](image)

**Figure 3.** Rarefaction as a measure of alpha diversity. Rarefaction curves for all five sample sites were computed, with samples rarefied to a depth of 4000.

![Taxonomy breakdown](image)

**Figure 4.** Taxonomy breakdown by sample, with correspondence evident within sampling sites. Actinobacteria are dominant in all samples, followed in relative abundance by Chloroflexi and Proteobacteria.
Beta diversity
In order to cluster community structures by site and location within or outside of the track, Bray-Curtis distance measures between the ten soil samples to create a nonmetric multidimensional scaling (NMDS) plot. Samples did cluster by site, with the exception of replicates from Site 5, a control site just below the highest elevation of the water track (Figure 5). Samples differentiated by on- or off-track location also appear to cluster, with track samples grouped along Axes 1 and 3. Inclusion of additional sites and/or replicates is necessary for a statistical analysis of the significance of these clusters.

Figure 5. Bray-Curtis distance measures by sampling site.

Figure 6. Bray-Curtis distance measures differentiated between on- or off-track (control) samples.
Discussion

Though unanticipated, the lack of significant difference between track and control community composition reflects the recent findings of a parallel study by Chan-Yam et al in the MDV (2019). Microbial community composition did not differ between soils within the studied water track in Pearse Valley and control soils, and did not correspond to soil moisture content. Microbial community composition instead differed according to location up- or downslope and depth. Surface microbial communities and upper subsurface soil communities were distinctly clustered, as were wet soils sampled from the downslope of the water track (Chan-Yam et al 2019).

Prior studies of found individual water tracks had non-uniform effects on biomass and in situ soil CO$_2$ flux, independent of soil moisture content (Ball and Levy 2015). These findings challenge the prevailing hypothesis that the higher water content of water track soils are responsible for the increased habitability of water track environments than surrounding water-limited soils. Chan-Yam et al found increased total and culturable biomass and increased biological activity in water track soils. This suggests that these soils are more conducive to biological processes than adjacent dry soils (2019). However, moisture content was not correlated with total cultured cell counts or heterotrophic plate counts, but was positively correlated with respiratory activity of cultured isolates.

The community composition and diversity of Pearse Valley soils highly resemble those found in Untersee track soils. Pearse Valley soils were similarly dominated by cosmopolitan phylotypes and varied only somewhat across samples. *Actinobacteria* (21-75%) was dominant across all samples, followed in relative abundance by *Proteobacteria* (6-26%), *Chloroflexi* (2-14%) and *Bacteroidetes* (1-17%) (Chan-Yam et al 2019). Amending soil moisture content of MDV soils with 10% GWC has been found to effect community composition. Amending MDV soils with 10% water increased the relative abundance of *Actinobacteria*, slightly increased the abundance of *Proteobacteria*, and resulted in the decrease of *Bacteroidetes*, *Cyanobacteria*, and *Firmicutes*.

These community responses were only partially reflected in the findings of this study. *Actinobacteria* increased in abundance in wetter track soils (56% in control, 58% in track), but *Proteobacteria* decreased in abundance (12% in control, 9% in track) and *Bacteroidetes* remained equivalently abundant (2% in control and track). Effects of increased soil moisture on community composition are complicated by competing geochemical factors. The study of Chan-Yam et al suggests that higher soil salinity may diminish composition responsiveness to increased soil moisture content (2019).

Geochemical composition of track soils can be expected to vary significantly as a result of active in situ chemical weathering. Increased salinity within track soils is thought to be a major limiting factor in habitability counteracting heightened soil moisture, with ecological optimums expected in wet lower salinity soils. Viable cell counts of Pearse Valley isolates cultured at 5% NaCl were generally much lower than those cultivated at 0% NaCl, suggesting that these communities were not adapted to the higher salinity expected in downslope dry soils (Chan-Yam 2019).
Excepting pH, other measured soil physiochemical properties did not exhibit consistent effects across microbial biomass, activity and community composition of Pearse Valley soils. Different soil texture grain sizes correlated with total biomass, activity and composition, but not in any obvious pattern. Whereas Ball and Levy found a positive correlation between finer soils and \textit{in situ} CO$_2$ flux (2015), Chan-Yam et al found a positive correlation with \% 0.5-2mm soil grains and a negative correlation with finer \% 0.063-0.25 mm soil grains (2019). Remaining ambiguity about the effect of physicochemical soil properties on biological activity suggests the need to incorporate additional factors. Unmeasured geochemical indicators that may influence C-cycling include \textit{in situ} soil temperatures, soil elemental composition, and C and N content.

Further physiochemical analysis of Untersee track soils will be compared to the genomic data presented in this study. Measured indicators are to include soil volumetric water content and electrical conductivity and C and N content of soil and brine samples (if detectable). These complimentary analyses will expand on the findings of prior MDV studies to explore the possible correlations between physiochemical soil properties and soil microbial activity and diversity.

This potential correspondence could have important implications for determining parameters of habitability within seasonally-wetted hyperarid Martian RSL soils. As the studies of Ball et al and Chan-Yam et al indicate, small amounts of liquid water or brine within tracks have the potential to stimulate microbial activity in cold, arid soils. This suggests that RSL formed by liquid water may be more supportive of microbial life than surrounding dry surface soils. As such, RSL and other runoff slope features of past and present Martian environments should be considered primary targets for the search of life on Mars.

**Conclusion**

The preliminary findings presented in this study suggest that biotic effects of seasonal water track occurrence are not reflected in the community composition of track soils. Soil microbial communities in this study were dominated by similar species but also exhibited significant proportions of unclassified taxa. Antarctic soils more generally feature highly endemic microbial communities with novel physiologies and biochemical processes. These soil microbial communities are highly exceptional compared to those found in global soils (Bai et al, unpublished). Dominated by \textit{Actinobacteria} rather than globally-dominant \textit{Proteobacteria}, Antarctic soil microbes also display greater functional diversity and unique community structures. Less active than Arctic soil ecosystems, Antarctic soil ecosystems are also highly sensitive to changes in soil chemistry and water content (Ball et al 2015). These changes may be instigated and/or compounded by changing climate conditions. Antarctic soil microbial communities have been shown to be adapted and resistant to short-term climatic fluctuations, suggesting functional redundancies (de Scally et al 2016). Further transcriptomic analysis of Untersee track samples would determine whether changes in soil chemistry and water content alter microbial functionality. This transcriptomic analysis would reveal whether physiochemical changes within track soils induce significant differences in gene expression between structurally similar on- and off-track soils.
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