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Characterization of Growing Bacterial Populations in McMurdo Dry Valley Soils through Stable Isotope Probing with ^{18}O -water

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Abstract

Soil microbial communities of the McMurdo Dry Valleys, Antarctica (MDV) contain representatives from at least fourteen bacterial phyla. However, given low rates of microbial activity, it is unclear whether this richness represents functioning rather than dormant members of the community. We used stable isotope probing (SIP) with ^{18}O -water to determine if microbial populations grow in MDV soils. Changes in the microbial community were characterized in soils amended with H_2 ^{18}O and H_2 ^{18}O -organic matter. Sequencing the 16S rRNA genes of the heavy and light fractions of the bacterial community DNA show that DNA of microbial populations was labeled with ^{18}O -water, indicating these microorganisms grew in the MDV soils. Significant differences existed in the community composition of the heavy and light fractions of the H_2 ^{18}O and H_2 ^{18}O -organic matter amended samples (Anosim $P < 0.05$ of weighted Unifrac distance). Control samples and the light DNA fraction of the H_2 ^{18}O amended samples were dominated by representatives of the phyla Deinococcus-Thermus, Proteobacteria, Planctomyces, Gemmatimonadetes, Actinobacteria and Acidobacteria, whereas Proteobacteria were more prevalent in the heavy DNA fractions from the H_2 ^{18}O -water and the H_2 ^{18}O -water-organic matter treatments. Our results indicate that SIP with H_2 ^{18}O can be used to distinguish active bacterial populations even in this low organic matter environment.

Introduction

The McMurdo Dry Valleys (MDV), Antarctica experience extreme environmental conditions including low temperatures, minimal available water, and intense ultraviolet radiation inputs. As a consequence, this is a microbially dominated ecosystem; higher plants

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and animals are absent, and a limited diversity of protozoans (Bamforth *et al.*, 2005; Fell *et al.*, 2006) and invertebrates are endemic to the region (Freckman & Virginia, 1997; Freckman & Virginia, 1998; Treonis *et al.*, 1999). Initial investigations of MDV bacterial communities using culturing techniques yielded only a handful of unremarkable bacterial isolates, leading to the conclusion that these soils were essentially sterile and inhabited solely by exogenous organisms incapable of functioning under these conditions (Horowitz *et al.*, 1969; Horowitz *et al.*, 1972). However, with the application of molecular biology techniques to investigate MDV soil biodiversity, a surprising level of bacterial richness has been revealed (Cary *et al.*, 2010; Lee *et al.*, 2012; Van Horn *et al.*, 2013) compared to the eukaryotic community. Studies of 16S rRNA gene diversity have detected relatively diverse communities with representatives from numerous phyla including Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Cyanobacteria, Deinococcus-Thermus, Gemmatimonadetes, Firmicutes, Beta, Delta, and Gamma-Proteobacteria, and Verrucomicrobia (Aislabie *et al.*, 2006; Smith *et al.*, 2006; Aislabie *et al.*, 2008; Niederberger *et al.*, 2008; Wood *et al.*, 2008; Babalola *et al.*, 2009; Pointing *et al.*, 2009). Surveys of available data suggest abiotic factors such as surface stability, moisture, temperature, landscape position and historical context as well as biotic factors including competition, predation, and UV induced mutation are key drivers of MDV soil bacterial diversity and community structure (Cary *et al.*, 2010; Takacs-Vesbach *et al.*, 2010; Van Horn *et al.*, 2013).

However, it remains unclear if the microbial populations detected in the McMurdo Dry Valley soils are active and growing. Perhaps the bacterial richness is illusory, a relic of aerial transport, deposition, and preservation of exogenous bacterial cells. Soil respiration measurements indicate that microbial populations are active in MDV soils but it is unclear if all microbial populations respire (Parsons *et al.*, 2004; Ball *et al.*, 2009). A review of microbial communities in MDV soils (Cary *et al.*, 2010) argues that identifying bacterial populations capable of growth or activity in MDV soils is critical to understanding the role of the bacterial communities in this microbially dominated ecosystem.

Actively growing microorganisms in environmental samples may be identified through stable isotope probing with H_2^{18}O , water in which the oxygen atom contains 8 protons and 10 neutrons (Schwartz, 2007; Schwartz, 2009). Water is universally used as a substrate by microorganisms and incorporated into a range of biomolecules including nucleotides. When cells divide they replicate their DNA, building a new strand that will contain ^{18}O atoms if the cells were exposed to H_2^{18}O . The labeled DNA is denser than non-labeled DNA and these two fractions can be separated on a cesium chloride density gradient generated in an ultracentrifuge. Subsequently, the labeled DNA fraction can be recovered separately from the non-labeled fraction and through analysis of, for instance, 16S rRNA gene sequences the growing microorganisms in an environmental sample can be identified. Thus SIP with H_2^{18}O may provide important insights into the identity of growing microorganisms in MDV soils. The purpose of this study was to test if sufficient DNA, in MDV soil samples incubated in the field with H_2^{18}O at ambient temperatures, could be labeled and, if so, to identify the bacteria that grew during the incubation.

Materials and Methods

Site Description

This study was conducted along the southern shore of Lake Fryxell (77°37'S, 163°12-13'E) in Taylor Valley, McMurdo Dry Valleys (MDV), Antarctica (approximately 1 km from the lake shore and 2.25 km from the F6 field camp) and represents the “low” salinity site described in Van Horn *et al.* (In final review). The Fryxell basin experiences only 25.5 days above freezing annually, with soil temperatures averaging -18.4°C (Doran *et al.*, 2002). Precipitation in the basin ranges from 20-37 mm annually (Fountain *et al.*, 2010), though sublimation and ablation rates are high throughout the MDV (Clow *et al.*, 1988). Soil organic matter is also low, typically ranging from only 0.005-0.07 percent organic carbon throughout Taylor Valley (Burkins *et al.*, 2001), though it can peak to 1% under microbial mats or other biological hotspots (Geyer *et al.*, 2013). Percent organic carbon at this specific study site is 0.027 (± 0.006). Percent nitrogen is 0.006 (± 0.002), pH is 9.0 (± 0.1), and conductivity is $105 \mu\text{S cm}^{-1}$ (± 4) as described in Van Horn *et al.* (In final review).

Sampling and Treatments

Field experiments took place during the austral spring and summer of 2010-2011. Prior to sampling the desert pavement was removed from the soils. Soils were then collected with a sterile scoop to a depth of ~ 12 cm, homogenized by mixing thoroughly with the sterilized scoop, and placed into 15 mL Falcon tubes to roughly the 10 mL line. Additionally, ~ 1.5 L of homogenized soil was transported to the lab for chemical analysis. One of three treatments was randomly assigned to each of the samples: control (three samples, added H_2^{16}O water), water addition (three samples, added H_2^{18}O water), or organic matter addition (four samples, added H_2^{18}O -organic matter). The organic matter addition was a leachate (2.8 g L^{-1} dissolved organic carbon) prepared from native cyanobacterial mat from Lake Fryxell and filter sterilized (Van Horn *et al.*, In final review). Control, water, and organic matter samples were amended to achieve 10% soil moisture by weight (based on weight to volume ratios determined in the laboratory) to ensure that enough ^{18}O label was present to perform SIP. This level of soil moisture represents a gravimetric moisture content intermediate to the range found in the lower Taylor Valley area adjacent to our plot (0.6 to 25%, average=9.5%, $n=12$). Soil moisture content of the samples at the time of amendment was approximately 0.1%, though it should be noted that this plot experiences moisture input from an adjacent snow patch, which are common across the MDV landscape (Eveland *et al.*, 2013) and transient wetting events due to periodic snowfall. For organic matter treatments, this addition increased bulk soil organic carbon by roughly 10%. Treatments were added once at the start of the incubation by using a sterile syringe to add the water to the tube and capping and shaking to thoroughly wet all of the soil. A sterile needle was then used to pierce the cap to allow air exchange with the atmosphere. The tubes were then placed in ground, matching the soil level in the tubes with the surrounding ground level. Because the tubes were capped with only a syringe-sized hole to maintain the soils exposed to air, soil moisture was maintained throughout the 30-day incubation period. Tubes were removed from ground in January 2011 and stored at -20°C immediately with sucrose lysis buffer (Giovannoni *et al.*, 1990) added to saturation. Soil pH, salinity, total nitrogen, soil organic carbon, were determined as described by Van Horn, *et al.* (2013).

DNA Extraction

Soils (15 g total) from each of the 10 samples were extracted following the cetyltrimethylammonium bromide (CTAB) method described in Mitchell & Takacs-Vesbach (2008), modified for larger samples and to include a bead beating step. Briefly, 1 cm³ of 0.1 mm diameter Zirconia Silica beads (BioSpec Products), 1250 µL of 1% CTAB, and 100 µg and 1 mg each of proteinase K and lysozyme, respectively, were added to 5 g of preserved sample. Samples were incubated with continuous vertical rotation (~35 rpm) at 37 °C for 0.5 h. Sodium dodecyl sulfate was added (final concentration 2%) and samples were returned to the laboratory rotator for 0.5 hour at 60 °C. Samples were then bead-beated on a vortexor for 5 min at the medium setting. Nucleic acids were extracted with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1), followed by an extraction with chloroform and precipitated in 95% ethanol after the addition of 0.1 volume 3 M sodium acetate. Nucleic acid was washed once in 70% ethanol, air dried, and resuspended in 40 µL 10 mM Tris, pH 8.0. Replicate DNA extracts were combined for the isopycnic centrifugation.

Isopycnic Centrifugation of DNA Extracted from Soil

The extracted DNA was combined with 3.6 ml of cesium chloride (1.9 g mL⁻¹), 0.3 ml of gradient buffer (200mM Tris pH 8.0, 200mM KCl, 2mM EDTA) and 0.5 µL of 10,000X SYBR green I (Invitrogen Corporation, Carlsbad CA) and added to an optiseal ultracentrifuge tube (Beckman-Coulter, Fullerton CA). Centrifugation was performed using an Optima MAX benchtop ultracentrifuge (Beckmann-Coulter, Fullerton CA) with a TLA-110 rotor at 65,000 rpm (176,000 g at the average radius (r_{ave})) and 20°C for at least 72 hours. After centrifugation the tubes were photographed while illuminated with a blue light. Two visually distinct bands of DNA appeared in centrifuge tubes with DNA extracted from soil incubated with H₂ ¹⁸O ('water' and 'organic matter' additions), while only one band was present in tubes with DNA extracted from the control samples which were incubated with H₂ ¹⁶O (Figure 1). A needle was inserted into the bottom of the tube and the contents of the tube were recovered in 16 separate fractions. The density of each fraction was measured with a digital refractometer (Reichert). Four hundred µl of water was added to each sample together with 10 µg of glycogen and 800 µl of isopropanol and the DNA was precipitated through centrifugation at 13,000 rpm for 30 minutes. The precipitate was washed with 70% ethanol, resuspended in 100 µl of water, of which 5 µl was used for measuring DNA concentration with a Qubit assay (Invitrogen Corporation, Carlsbad CA) according to the instructions of the manufacturer. Sufficient DNA was recovered from the samples, including the heavy fractions of the no-isotope controls, for further DNA analysis.

Pyrosequencing and Sequence Analysis

For each of the 20 DNA fractions (an ¹⁸O and a ¹⁶O fraction for each sample), barcoded amplicon pyrosequencing of 16S rRNA genes was performed by a single step PCR. PCRs were performed in triplicate 25 µL reactions containing 0.25 mM forward and reverse fusion primer, 0.25 mM dNTP (each), 1× Platinum PCR buffer (Lifetech), 1.5 mM MgCl₂, 1 U Platinum Taq Polymerase (Lifetech) and 2 µL of DNA template. Fusion primers were designed so that the forward primer consisted of the Roche adapter A, followed by a 10 base error-correcting barcode for multiplexing (Hamady *et al.*, 2008), and the universal bacterial

primer 939F 5' TTG ACG GGG GCC CGC ACA AG-3'. The reverse primers included the Roche adapter B, followed by the reverse PCR primer 1492R 5'-GTT TAC CTT GTT ACG ACT T-3'. The thermal cycler program included an initial 5 min denaturation at 95 °C, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s, and extension for 30 s at 72 °C. A final 7 min extension completed the PCR. PCR amplicons were purified using the Mo-bio Gel Purification Kit following the manufacturer's instructions, quantified spectrophotometrically, and combined in equimolar concentrations for multiplexed pyrosequencing. Sequencing template was quantitated fluorometrically using a picogreen dye kit, assayed for quality and fragment length on an Agilent Bioanalyzer DNA 1000 chip before library preparation using Roche titanium reagents and titanium procedures. All samples from this study were run on one half region of a sequencing plate, with no more than 96 samples total per region. Pyrosequencing was performed on a Roche 454 FLX instrument following manufacturer's protocols.

Amplicon pyrosequences were quality filtered, denoised, screened for PCR errors, and chimera checked using default settings in AmpliconNoise and Perseus (Quince *et al.*, 2011). The Quantitative Insights into Microbial Ecology (QIIME, as MacQIIME v. 1.7.0-20130523) pipeline was used to analyze alpha and beta diversity of the DNA sequence data (Caporaso *et al.*, 2010). Unique operational taxonomic units (OTUs, i.e., unique DNA sequences or amplicon types) were identified by the 97% DNA identity criterion using the uclust OTU picker (Edgar, 2010) in QIIME. A set of representative DNA sequences was randomly chosen for each unique OTU in QIIME (pick_rep_set.py) and used for all subsequent analyses. Taxonomic assignment of the OTUs was determined by alignment with the GreenGenes (gg_13_5) dataset (DeSantis *et al.*, 2006). Alpha (Good's coverage, Chao1, Faith's phylogenetic diversity, Shannon, and Simpson (1-D)) and beta diversity (unweighted and weighted Unifrac) analyses were performed on randomly selected subsets of 300 DNA sequences per sample 1000 times to standardize for varying sequencing efforts across samples. One-way and Twoway analysis of variance (ANOVA) was used to determine if alpha diversity differed by isotope or sample type and isotope. Unweighted and weighted Unifrac (Lozupone & Knight, 2005) distance matrices were generated and principal coordinate analysis (PCoA) was performed as implemented in QIIME by the beta_diversity_through_plots.py script (-e 300). Statistical significance of differences in community structure by sample type was determined using ANOSIM in PRIMER (Clarke & Gorley, 2006). Groups were designated as significantly different when the global test was significant ($P < 0.05$), the pairwise test was significant ($P < 0.10$, due to the small sample size), and the R statistic was greater than 0.40.

All raw sequence data from this study are available through the NCBI Sequence Read Archive. The individual raw sff files from this study were assigned the accession numbers SAMN02402205 through SAMN0240224 under Bioproject PRJNA228945.

Results

Isopycnic Centrifugation

The labeled DNA (heavy) ranged in density between 1.728 and 1.768 g mL⁻¹, while the non-labeled DNA (light) that was sequenced had a density between 1.683 and 1.720 g mL⁻¹

(Figure 1). The labeled DNA is hereafter referred to as the heavy fraction while the non-labeled DNA is described as the light fraction. This terminology is used to indicate that we did not directly measure the isotopic composition of the DNA fractions but identified them by their buoyant densities. A greater fraction of the DNA from samples to which H_2^{18}O was added was present in the heavy fraction than in control samples exposed to non-enriched water. In the samples to which H_2^{18}O and organic matter was added 19.8% ($\pm$ standard error of 3.0%) of the DNA on average was recovered in heavy fractions. In samples to which only H_2^{18}O was added 23.1% ($\pm$ standard error of 2.6%) of the DNA on average was recovered in heavy fractions while only 2.2% of the DNA from control samples to which H_2^{16}O was added was detected in heavy fractions.

Alpha Diversity

Pyrosequencing of the 20 samples resulted in 30,909 16S rRNA gene sequences after quality filtering, denoising, and chimera removal. The average number of sequences per sample was 1545 (range=325 to 4431) and the total number of unique OTUs observed among all 20 samples was 1841. Coverage estimates for the individual samples ranged from 68% to 90% (average=78%), indicating that a majority of the OTU richness was detected in all samples. There was no significant difference in the coverage or alpha diversity metrics between the heavy and light DNA fractions (ANOVA $P < 0.05$), despite a lower depth of sequencing in the light fractions. Average Chao1 richness was 268 (range=109 to 524) and did not differ statistically among samples ($P > 0.05$) by isotope fraction (heavy vs. light), treatment (control, water, and organic matter addition), or treatment by isotope (e.g., control H_2^{18}O vs. control H_2^{16}O). However, although richness did not differ significantly among the samples and their fractions, in the heavy DNA fractions, Shannon diversity and phylogenetic diversity (Faith's phylogenetic diversity) was higher in the control samples relative to the water and organic matter treatments ($P < 0.05$). Within treatment differences were only detected by the Shannon diversity index, which was higher in the heavy DNA fraction of the control, compared to the light fraction of the control, but lower in the heavy DNA fraction of the water treatment relative to the light DNA fraction of the water treatment ($P < 0.05$). No statistically significant differences were found in the Simpson diversity measurements among any of the samples or their fractions. Sequencing and alpha diversity metrics are summarized in Table S1.

Community Composition

Beta Diversity Patterns. Differences among treatments and fractions was observed by PCoA of weighted Unifrac distances (Lozupone & Knight, 2005) among samples. The heavy and light DNA fractions from control samples (incubated with unlabeled water) clustered together with the light DNA fractions from the water addition treatments (incubated with H_2^{18}O). On the opposite

Phylum level differences—The average relative abundance of phyla among the light and heavy DNA fractions from the three treatments clustered into three distinct types (Figure 3). The first group was represented by samples from the light and heavy DNA fraction from the control treatment (no isotope) and the light DNA fraction from the water addition (H_2^{18}O only treatment). These samples were dominated by the phyla Deinococcus-Thermus, Proteobacteria, Planctomyces, Gemmatimonadetes, Actinobacteria and Acidobacteria. The

second type was represented by the heavy DNA fractions from the labeled water (H_2^{18}O -water) and the organic matter (H_2^{18}O -water + organic matter) treatments. The phylum Proteobacteria was approximately three times more abundant in these samples than in the light or heavy DNA fractions from the control treatment or the light DNA fraction from the H_2^{18}O water treatment. The final group contained the microbial community amplified from the light DNA fraction of the H_2^{18}O -organic matter treatment. These samples contained nine times more Firmicutes, specifically members of the family Planococcaceae, than the samples represented by groups one and two.

Genus level differences—The heavy fractions of the samples were dominated by a select group of bacterial families. Table 1 shows the families that were more abundant in heavy DNA fractions from samples treated with H_2^{18}O or H_2^{18}O -organic matter than in the heavy DNA fraction of the no isotope control. These numbers only show the relative abundance within the sequencing data set and do not account for the approximately tenfold difference in DNA concentration between the heavy fractions of the labeled treatments and the no-isotope control. The families Oxalobacteraceae, Xanthomonadaceae, Rhodobacteraceae, Sporichthyaceae and Micrococcaceae were especially dominant in the heavy DNA fractions derived from soils incubated with H_2^{18}O . When organic matter was added to the incubation, 16S rRNA genes from members of the family Comamonadaceae were more abundant in the heavy fraction. In stable isotope probing experiments, bacterial populations that grow rapidly, regardless of their abundance in soil, will have high ratios of 16S rRNA gene abundance in the heavy fraction over the light fraction referred to here as growth ratios. Table 2 shows the microbial genera that had the highest growth ratios in our study. The same families that dominated the heavy DNA fractions (Table 1) also had very high growth ratios with the exception of the Xanthomonadaceae. They were abundant in the heavy DNA fraction but also the light DNA fraction of soils incubated with isotopes so their growth ratio was low. The genus *Kaisobacter* did have a high growth ratio even though it represented less than 1% of the sequences in the heavy fractions taken from soils incubated with isotopes.

Discussion

The bacterial communities in soils of the MDV are diverse and contain representatives of at least 14 different phyla of bacteria (Aislabie *et al.*, 2006; Cary *et al.*, 2010). The microbial communities represented by 16S rRNA genes recovered from the light and heavy DNA fractions from our control soils to which no isotopes were added can appropriately be compared to published phylogenetic surveys that did not use H_2^{18}O stable isotope probing. As indicated by the ordination analyses (Figure 2) the communities in these unlabeled light and heavy DNA fractions were very similar to each other. In our study we detected 23 different bacterial phyla. There are still relatively few next generation sequencing studies in the literature and many of the phyla we detected beyond the 14 previously described in the literature were present in very low abundances. As seen in other terrestrial environments, Often the most dominant phyla in MDV soils are the Acidobacteria, Actinobacteria and Bacteroidetes (Cary *et al.*, 2010). Our control soils were dominated by Deinococcus-Thermus, Proteobacteria, Planctomyces, Actinobacteria, Acidobacteria and

Gemmatimonadetes while Bacterioidetes only represented a small proportion of the community. Interestingly, Proteobacteria are often underrepresented in MDV soils relative to temperate soils (Cary *et al.*, 2010) though we found Proteobacteria to comprise a substantial fraction of the bacterial community in this study's soils, indicating that bacterial communities vary across MDV soils. Niederberger and co-workers (2008) found Gammaproteobacteria in high productivity soils. They also detected members of the Deinococcus-Thermus phylum, which were well represented in the soils of our study, associated with low productivity soils.

There are now at least three different lines of evidence that show microbial communities in MDV soils are active. First, several studies have quantified soil respiration rates and shown that although respiration rates are small and largely abiotically driven (Shanhun *et al.*, 2012), microorganisms in MDV soils do actively respire. Burkins and coworkers (2001), for instance, detected carbon dioxide efflux rates of approximately $1.0 \mu\text{moles s}^{-1}\text{m}^{-2}$ at several sites in the Taylor Valley, while Parsons, *et al.* (2004), also working in the Taylor Valley, found efflux rates as high as $0.15 \mu\text{moles s}^{-1}\text{m}^{-2}$. Secondly, microbial communities in MDV soils change when their environment is manipulated *in situ*. For instance, when Tiao, *et al.* (2012) moved a mummified seal carcass, the bacterial communities in soils newly covered by the carcass became more similar to the bacterial communities originally present under the seal carcass before it was moved. These communities were different from bacterial communities in control soil samples that were not covered. For such change to occur bacterial populations likely grew, although it is possible for a community to change via death as well. Additionally, when water and an organic matter leachate were added to soil at three Taylor Valley sites of varying salinity soil respiration, extracellular enzyme activities, and community composition were all significantly affected (Van Horn *et al.*, In final review). The third line of evidence comes from this study, which shows that DNA of microorganisms in MDV soils incubated *in situ* with H_2^{18}O can be labeled with ^{18}O . Incorporation of ^{18}O into DNA requires enzymatic activity (Richards & Boyer, 1966) and is correlated with growth rates (Blazewicz & Schwartz, 2011). High rates of incorporation of ^{18}O into DNA, as is required for SIP experiments, requires construction of a new strand of DNA during DNA replication and thus only occurs when cells divide.

There remain few studies that characterize microbial populations capable of growth in the McMurdo Dry Valleys. In the study on the impact of a seal carcass on soil microbial communities, a *Psychrobacter* sp. (a member of the Gammaproteobacteria) and the Planococcaceae family, part of the phylum Firmicutes, dominated the new microbial community underneath the moved seal carcass (Tiao *et al.*, 2012). In our study Proteobacteria also grew rapidly and dominated the heavy DNA fractions of soil incubated with H_2^{18}O (water and organic matter treatments) but they were not restricted to only the γ -subdivision of Proteobacteria. Rather members of Alphaproteobacteria (the family Rhodobacteraceae), Betaproteobacteria (the families Oxalobacteraceae and Comamonadaceae) and Gammaproteobacteria (the family Xanthomonadaceae) all grew during the incubations. We did detect a *Psychrobacter* species in our data set but it was not present in all heavy fractions and, when detected, comprised less than 0.1% of the community. In our study Firmicutes dominated the light DNA fractions, especially of

samples amended with organic matter, and were less abundant in the heavy fractions and consequently had low growth ratios.

It is more likely for a population to be dominant in a heavy fraction if it is also dominant at the start of incubation with H_2^{18}O because the number of divisions per cell does not have to be very high. In contrast, a cell of a rare taxonomic group must divide many times for the taxon to become dominant in the heavy DNA fraction. By taking the ratio of the abundance of a population in the heavy DNA fraction over the abundance in the light DNA fraction it is possible to identify smaller populations, which may not dominate the heavy DNA fraction but still have high growth rates. The genus *Kaistobacter*, which grew especially well in organic matter amended soils, had a high growth rate but was not a dominant member of the heavy fraction. The growth rate is susceptible to an artifact due to GC content of a microorganism's genome. DNA sequences with a high GC content are denser than sequences with a low GC content. GC content can impact the buoyant density of DNA by as much as 0.04 g/mL (Rolfe & Messelson, 1959). Consequently high GC DNA sequences are more likely to occur in the heavy DNA fraction even when not labeled with heavy isotopes and conversely are less likely to be detected in the light DNA fraction leading to artificially high growth rates.

Water is thought to be one of the most important limiting factors to microbial growth in MDV soils and increased moisture has been shown to promote the growth of specific microbial populations as observed through changes in abundance of the 16S rRNA gene without isotopic labeling (Van Horn *et al.*, In final review). Soil moisture content in our study was raised to 10%, which is representative of soil conditions surrounding streams and lakes or melting snow patches. At this level of water input we observed substantial labeling of DNA. The diversity of bacteria represented by the heavy fractions was high with a large number of phyla detected, suggesting that the bacteria did not experience excessive moisture stress but that increased available water favored some members of the community. Members of the bacterial families Oxalobacteraceae, Rhodobacteraceae, Sporichthyaceae and Micrococcaceae grew well in water amended soil samples as observed both in this labeling study as well as through the total abundance of 16S rRNA genes (Van Horn *et al.*, In final review). These families are limited to two bacterial phyla: Proteobacteria and Actinobacteria. Members of Oxalobacteraceae have been detected in cold environments previously, including the species *Glaciimonas immobilis* isolated from alpine glacier cryoconites (Zhang *et al.*, 2011). Over two-thirds of the species within the family Rhodobacteraceae originate from marine environments and they are often associated with algal populations (Gutierrez *et al.*, 2011). *Antarctobacter*, a genus within Rhodobacteraceae, has been isolated from saline lakes in Antarctica (Labrenz *et al.*, 1998). The soils in the MDV can have high saline contents and algae, together with cyanobacteria, are the primary producers in this system so it may not be surprising that Rhodobacteraceae play an important role in MDV soils. Members of the Micrococcaceae family have been characterized in Antarctic soils before. *Micrococcus antarcticus*, a psychrophilic bacterium, was isolated from the Chinese Great-Wall station on King George island in Antarctica (Liu *et al.*, 2000). It should be noted that 95% of MDV soil surfaces are only moistened by periodic snowfalls, which may provide limited moisture for short periods (Campbell *et al.*,

1998). Thus, this experiment does not allow us to draw conclusions regarding growth of bacterial populations in the driest MDV soils receiving very transient moisture inputs.

While the growing community in H₂ ¹⁸O-organic matter amended soil was similar to that of the soil incubated with only H₂ ¹⁸O, as evidenced by the ordination analysis (Figure 2) organic matter did select for particular populations. Specifically, two members of the family Comamonadaceae, were more abundant in the heavy DNA fraction when organic matter and water were added to the soil than when only water was added. Members of the Comamonadaceae have also been shown as significant components of microbial communities in permanently cold subglacial environments in Antarctic and Arctic settings (Lanoil *et al.*, 2009).

In summary, we recovered labeled DNA from McMurdo Dry Valley soil samples amended with H₂ ¹⁸O, indicating that bacterial populations are capable of growth in the cold soils of Antarctica when amended with water. Not all bacterial populations we detected in our samples grew during the incubations. Members of the families Oxalobacteraceae, Xanthomonadaceae, and Micrococcaceae grew in soils to which H₂ ¹⁸O was added. When soils were amended with H₂ ¹⁸O-organic matter, a bacterial community similar to the H₂ ¹⁸O only was detected. In

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Image of ultracentrifuge tubes, on the left, taken after isopycnic centrifugation showing a single non-labeled DNA band in tube 1, a control sample to which no isotopes were added, and two DNA bands in tubes 2 and 3, samples to which H_2^{18}O was added. The graph on the right shows the relationship between density of the fractions and the fraction of total DNA recovered from an ultracentrifuge tube. Heavier DNA fractions, outlined in the gray box, taken from samples incubated with H_2^{18}O (○) contained a greater fraction of total DNA than in samples incubated without isotopes (●).

Figure 2.

Principal coordinate analysis of weighted Unifrac distances. Significant differences were detected among the communities by DNA fraction and treatment (Anosim $P < 0.05$). Similar, but less significant patterns were detected with unweighted Unifrac distances (data not shown).

Figure 3.

Average relative abundance of phyla among the light and heavy DNA fractions from the three treatments appeared to group into three distinct groups (Type I- Type III). Type II was represented by samples from the heavy fractions of the H₂¹⁸O-water and the H₂¹⁸O-organic matter (H₂¹⁸O-OM) treatments, which was enriched in Proteobacteria relative to the light or heavy DNA fractions from the control treatment or the light DNA fraction from the H₂¹⁸O water treatment.

Table 1

Abundance, in % of total sequences, of dominant taxa in the heavy fractions of samples not exposed to heavy isotopes or exposed to H₂¹⁸O or H₂¹⁸O and Carbon. Standard errors are shown in parentheses. Taxa on top are more abundant in heavy fractions derived from samples exposed only to H₂¹⁸O than in heavy fractions from samples exposed to H₂¹⁸O and Carbon while taxa on the bottom are more abundant in heavy fractions from soil exposed to H₂¹⁸O and Carbon than in heavy fractions from samples exposed to H₂¹⁸O alone. The taxa account for 68.1% of the total sequences in heavy fractions from soil exposed to H₂¹⁸O and 73.9% of the total sequences in heavy fractions from soil exposed to H₂¹⁸O and Carbon.

Family	Genus	No Isotope	H ₂ ¹⁸ O	H ₂ ¹⁸ O and Carbon
Micrococcaceae	<i>Other1</i>	0.3 (0.2)	2.5 (0.7)	0.7 (0.2)
Micrococcaceae	<i>Other2</i>	0.3 (0.2)	2.7 (1.0)	1.7 (0.9)
Sporichthyaceae	<i>Other</i>	1.1 (0.4)	3.3 (1.4)	1.8 (1.0)
Xanthomonadaceae	<i>Lysobacter</i>	2.7 (1.3)	7.2 (2.4)	1.0 (0.4)
Xanthomonadaceae	<i>Other</i>	5.4 (0.7)	11.5 (3.2)	4.9 (1.2)
Rhodobacteraceae	<i>Paracoccus</i>	1.0 (0.6)	3.4 (1.2)	1.6 (0.6)
Oxalobacteraceae	<i>Janthinobacterium</i>	2.5 (0.2)	25.6 (7.8)	16.8 (2.0)
Planococcaceae	<i>Paenisporosarcina</i>	0.1 (0.1)	0.1(0.1)	2.5 (0.6)
Planococcaceae	<i>Planomicrobium</i>	1.3 (1.3)	0.0 (0.0)	4.5 (4.4)
Oxalobacteraceae	<i>Other</i>	0.2 (0.0)	1.5 (0.5)	6.7 (0.8)
Oxalobacteraceae	<i>Other</i>	0.0 (0.0)	1.1 (0.5)	2.5 (0.8)
Comamonadaceae	<i>Polaromonas</i>	1.3 (0.3)	6.0 (1.7)	23.2 (6.3)
Comamonadaceae	<i>Variovorax</i>	0.1 (0.1)	0.4 (0.4)	1.8 (0.5)
Comamonadaceae	<i>Other</i>	1.1 (0.4)	2.7 (0.0)	4.3 (0.8)

Table 2

Growth rates, calculated by dividing the abundance of a sequence in the heavy fraction by the abundance in the light fraction, of select taxa detected in samples exposed to H₂¹⁸O or H₂¹⁸O and Carbon. Taxa on top have higher growth rates in samples exposed only to H₂¹⁸O than in samples exposed to H₂¹⁸O and carbon while taxa on the bottom have higher growth rates in soil exposed to H₂¹⁸O and carbon than in samples exposed to H₂¹⁸O alone.

Family	Genus	No Isotope	H ₂ ¹⁸ O	H ₂ ¹⁸ O and Carbon
Micrococcaceae	<i>other</i>	1.3	8.5	1.8
Micrococcaceae	<i>other</i>	0.5	25.4	15.2
Gemmataceae	<i>Gemmata</i>	0.0	4.5	0.3
Rhodobacteraceae	<i>Paracoccus</i>	1.0	29.0	3.7
Comamonadaceae	<i>Polaromonas</i>	0.9	21.7	9.2
Comamonadaceae	<i>other</i>	5.3	23.6	8.6
Oxalobacteraceae	<i>Janthinobacterium</i>	3.0	24.4	0.9
Oxalobacteraceae	<i>other</i>	0.2	19.9	1.7
Sporichthyaceae	<i>other</i>	0.8	11.3	11.8
Sphingomonadaceae	<i>Kaistobacter</i>	1.8	1.8	6.5
Comamonadaceae	<i>Methylibium</i>	1.2	4.1	8.2
Oxalobacteraceae	<i>other</i>	*	12.5	19.3
Xanthomonadaceae	<i>other</i>	1.9	2.4	4.2

* Growth rate could not be calculated because taxon was not detected in the light fraction.