RESEARCH ARTICLE

Characterization of growing bacterial populations in McMurdo Dry Valley soils through stable isotope probing with ^{18}O-water

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Received 16 November 2013; revised 21 February 2014; accepted 24 April 2014. Final version published online 22 May 2014.

DOI: 10.1111/1574-6941.12349

Editor: John Priscu

Keywords
stable isotope probing; McMurdo Dry Valley; soil bacterial diversity.

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 shows that DNA of microbial populations was labeled with ^{18}O-water, indicating these micro-organisms 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 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, 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, 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, 2008; Smith *et al.*, 2006; 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 micro-organisms 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, 2009). Water is universally used as a substrate by micro-organisms 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 nonlabeled 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 nonlabeled fraction and through analysis of, for instance, 16S rRNA gene sequences the growing micro-organisms in an environmental sample can be identified. Thus, SIP with $H_2{}^{18}O$ may provide important insights into the identity of growing micro-organisms 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, 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.* (2014). 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 to 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% to 0.07% 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 μS cm^{-1} (± 4) as described in Van Horn *et al.* (2014).

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 *c.* 12 cm, homogenized by mixing thoroughly with the sterilized scoop, and placed into 15-mL Falcon tubes to roughly the 10 mL line. Additionally, *c.* 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.*, 2014). 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 (0.6–25%, 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 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, and 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 cm^3 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 (*c.* 35 r.p.m.) 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 h 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 (200 mM Tris pH 8.0, 200 mM KCl, 2 mM EDTA) and 0.5 μL of 10 000× 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 (Beckman-Coulter) with a TLA-110 rotor at 65 000 r.p.m. [176 000 ***g*** at the average radius (r_{ave})] and 20 °C for at least 72 h. 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 $H_2{}^{18}O$ ('water' and 'organic matter' additions), while only one band was present in tubes with DNA extracted from the control samples which were incubated with $H_2{}^{16}O$ (Fig. 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 microliters of water were added to each sample together with 10 μg of glycogen and 800 μL of isopropanol and the DNA was precipitated through centrifugation at 14 549 ***g*** for 30 min. 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) 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.

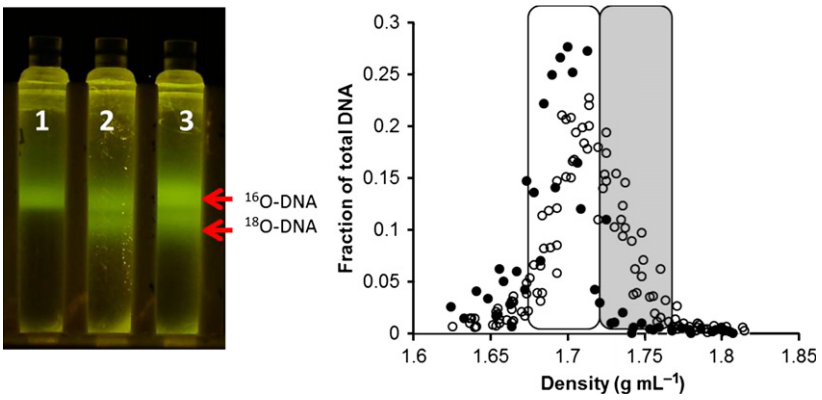

Fig. 1. Image of ultracentrifuge tubes, on the left, taken after isopycnic centrifugation showing a single nonlabeled DNA band in tube 1, a control sample to which no isotopes were added, and two DNA bands in tubes 2 and 3, samples to which $H_2{}^{18}O$ was added. The graph on the right shows the relationship 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 (●).

Pyrosequencing and sequence analysis

For each of the 20 DNA fractions (an ^{18}O and a ^{16}O 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 $MgCl_2$, 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 two-way 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 > 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 nonlabeled DNA (light) that was sequenced had a density between 1.683 and 1.720 g mL^{-1} (Fig. 1). The labeled DNA is hereafter referred to as the heavy fraction, while the nonlabeled 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 nonenriched water. In the samples to which $H_2{}^{18}O$ and organic matter was added 19.8% (± SE 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% (± SE 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 = 325–4431) and the total number of unique OTUs observed among all 20 samples was 1841. Coverage estimates for the individual samples ran-

ged 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 = 109–524) 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) were 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 (Supporting information).

Community composition

Beta diversity patterns

Differences between treatments and fractions were observed by PCoA of weighted Unifrac distances (Lozupone & Knight, 2005) among samples (Fig. 2). 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 side of the same axis, PC1, which explained 61.35% of the variation, all the organic matter amended samples clustered together, along with the heavy DNA fraction from the $H_2{}^{18}O$-water addition. However, the clusters of samples from the light DNA fraction of the organic matter addition and the heavy DNA fraction from the $H_2{}^{18}O$-water addition were significantly different (ANOSIM $P < 0.1$, See Table S2 for complete ANOSIM results). Similar patterns of community composition differences were observed in the unweighted Unifrac analysis (data not shown), though most clusters were not statistically significant by ANOSIM.

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 (Fig. 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.

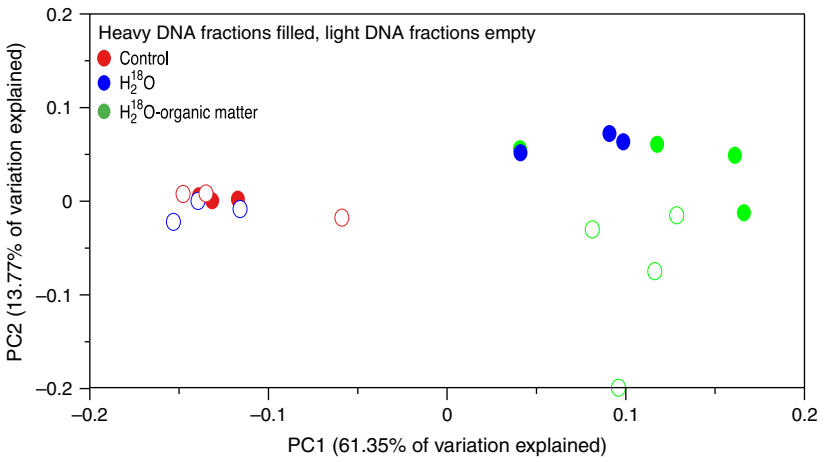

Fig. 2. Average relative abundance of phyla among the light and heavy DNA fractions from the three treatments appeared to group into three distinct groups (Types I–III). Type II was represented by samples from the heavy fractions of the $H_2{}^{18}O$-water and the $H_2{}^{18}O$-organic matter ($H_2{}^{18}O$-OM) 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 $H_2{}^{18}O$ water treatment.

Genus level differences

The heavy fractions of the samples were dominated by a selected 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, Sporichtyaceae, 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 (Fig. 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 Bacteriodetes 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 *et al.* (2008) found Gammaproteobacteria in high productivity soils. They also detected members of the Deinococcus-Thermus

Table 1. Abundance, in% of total sequences, of dominant taxa in the heavy fractions of samples not exposed to heavy isotopes or exposed to $H_2{}^{18}O$ or $H_2{}^{18}O$ and Carbon

Family	Genus	No Isotope	$H_2{}^{18}O$	$H_2{}^{18}O$ and Carbon
Micrococcaceae	*Other1*	0.3 (0.2)	2.5 (0.7)	0.7 (0.2)
Micrococcaceae	*Other2*	0.3 (0.2)	2.7 (1.0)	1.7 (0.9)
Sporichthyaceae	*Other*	1.1 (0.4)	3.3 (1.4)	1.8 (1.0)
Xanthomonadaceae	*Lysobacter*	2.7 (1.3)	7.2 (2.4)	1.0 (0.4)
Xanthomonadaceae	*Other*	5.4 (0.7)	11.5 (3.2)	4.9 (1.2)
Rhodobacteraceae	*Paracoccus*	1.0 (0.6)	3.4 (1.2)	1.6 (0.6)
Oxalobacteraceae	*Janthinobacterium*	2.5 (0.2)	25.6 (7.8)	16.8 (2.0)
Planococcaceae	*Paenisporosarcina*	0.1 (0.1)	0.1 (0.1)	2.5 (0.6)
Planococcaceae	*Planomicrobium*	1.3 (1.3)	0.0 (0.0)	4.5 (4.4)
Oxalobacteraceae	*Other*	0.2 (0.0)	1.5 (0.5)	6.7 (0.8)
Oxalobacteraceae	*Other*	0.0 (0.0)	1.1 (0.5)	2.5 (0.8)
Comamonadaceae	*Polaromonas*	1.3 (0.3)	6.0 (1.7)	23.2 (6.3)
Comamonadaceae	*Variovorax*	0.1 (0.1)	0.4 (0.4)	1.8 (0.5)
Comamonadaceae	*Other*	1.1 (0.4)	2.7 (0.0)	4.3 (0.8)

Standard errors are shown in parentheses. Taxa on top are more abundant in heavy fractions derived from samples exposed only to $H_2{}^{18}O$ than in heavy fractions from samples exposed to $H_2{}^{18}O$ and carbon, while taxa on the bottom are more abundant in heavy fractions from soil exposed to $H_2{}^{18}O$ and carbon than in heavy fractions from samples exposed to $H_2{}^{18}O$ alone. The taxa account for 68.1% of the total sequences in heavy fractions from soil exposed to $H_2{}^{18}O$ and 73.9% of the total sequences in heavy fractions from soil exposed to $H_2{}^{18}O$ and Carbon.

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 $H_2{}^{18}O$ or $H_2{}^{18}O$ and Carbon

Family	Genus	No Isotope	$H_2{}^{18}O$	$H_2{}^{18}O$ and Carbon
Micrococcaceae	*Other*	1.3	8.5	1.8
Micrococcaceae	*Other*	0.5	25.4	15.2
Gemmataceae	*Gemmata*	0.0	4.5	0.3
Rhodobacteraceae	*Paracoccus*	1.0	29.0	3.7
Comamonadaceae	*Polaromonas*	0.9	21.7	9.2
Comamonadaceae	*Other*	5.3	23.6	8.6
Oxalobacteraceae	*Janthinobacterium*	3.0	24.4	0.9
Oxalobacteraceae	*Other*	0.2	19.9	1.7
Sporichthyaceae	*Other*	0.8	11.3	11.8
Sphingomonadaceae	*Kaistobacter*	1.8	1.8	6.5
Comamonadaceae	*Methylibium*	1.2	4.1	8.2
Oxalobacteraceae	*Other*	*	12.5	19.3
Xanthomonadaceae	*Other*	1.9	2.4	4.2

Taxa on top have higher growth rates in samples exposed only to $H_2{}^{18}O$ than in samples exposed to $H_2{}^{18}O$ and carbon, while taxa on the bottom have higher growth rates in soil exposed to $H_2{}^{18}O$ and carbon than in samples exposed to $H_2{}^{18}O$ alone.
*Growth rate could not be calculated because taxon was not detected in the light fraction.

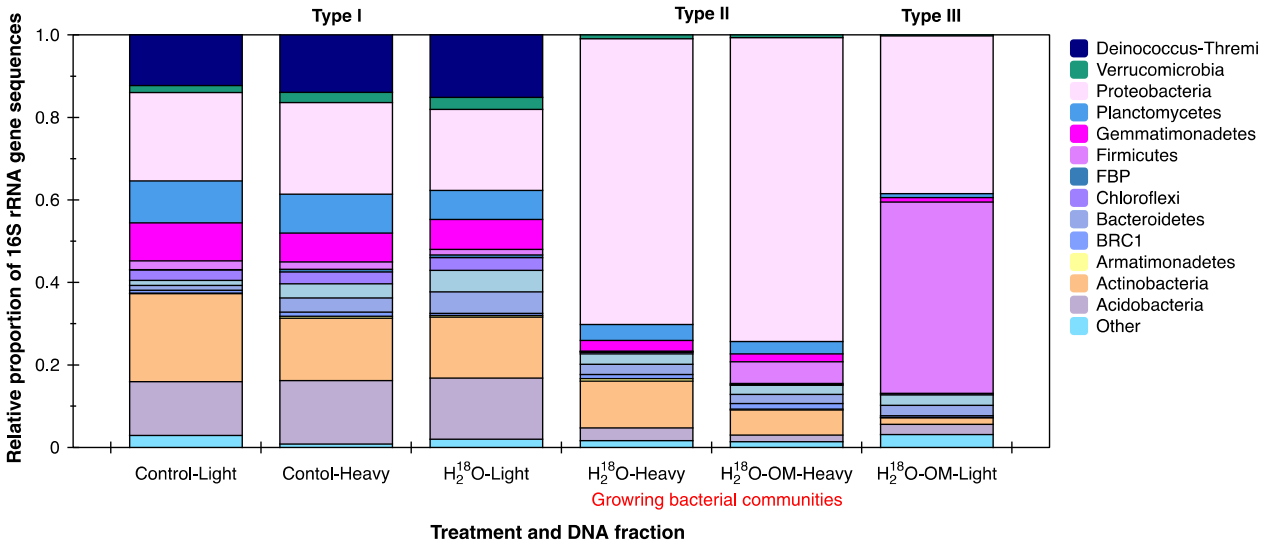

Fig. 3. PCoA of weighted Unifrac distances. Significant differences were detected among the communities by DNA fraction and treatment (ANOSIM $P < 0.05$). Similar, but less significant patterns were detected with unweighted Unifrac distances (data not shown). Open circles represent light fractions while filled circles denote heavy fractions.

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), micro-organisms in MDV soils do actively respire. Burkins *et al.* (2001), for instance, detected carbon dioxide efflux rates of approximately 1.0 μmoles s^{-1} 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 μmoles s^{-1} 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.*, 2014). The third line of evidence comes from this study, which shows that DNA of micro-organisms 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, require construction of a new strand of DNA during DNA replication and thus only occur when cells divide.

There remain few studies that characterize microbial populations capable of growth in the MDV. 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-subdivsion 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 $< 0.1\%$ 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 micro-organism'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^{-1} (Rolfe & Meselson, 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.*, 2014). 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*, *Sporichtyaceae*, 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.*, 2014). 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 $H_2{}^{18}O$-organic matter amended soil was similar to that of the soil incubated

with only $H_2{}^{18}O$, as evidenced by the ordination analysis (Fig. 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 $H_2{}^{18}O$, 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 $H_2{}^{18}O$ was added. When soils were amended with $H_2{}^{18}O$-organic matter, a bacterial community similar to the $H_2{}^{18}O$ only was detected, but the organic matter amendment soils were also enriched with members of the family *Comamonadaceae*. Our results indicate that stable isotope probing with $H_2{}^{18}O$ is a viable technique even in this cold, low bacterial biomass environment, which may offer new insights into the environmental parameters that govern growth of microbial populations in soils from the MDV.

Acknowledgements

This research was funded by NSF OPP grants 0838879 to CTV, 1142102 to CTV and DJVH, 1142096 to ES, 0838922 to JEB, and 0838850 to MNG. Additional support was provided by the McMurdo LTER, NSF OPP grant 1115245. The authors are grateful for logistical support provided by Raytheon Polar Services and PHI, Inc. The Molecular Biology Facility at the University of New Mexico is supported by NIH Grant Number 1P20RR18754 from the Institute Development Award (IDeA) Program of the National Center for Research Resources. The authors have no conflict of interest statement.

Authors' contributions

E. S. and D. J. V. H. contributed equally to this work.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Summary of sequencing results and diversity metrics.

Table S2. ANOSIM results on weighted and unweighted Unifrac distances.