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Resource Legacies and Priming Regulate Microbial Communities in Antarctica's Dry Valleys

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ABSTRACT

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Multiple mechanisms control bacterial community structure but two in particular, the "legacy" of past environmental conditions, and the "priming" of bacteria to respond to seasonal or reoccurring fluctuations in resources, have the potential to determine both bacterial communities, as well as, temporal shifts in active bacterial taxa. To begin to evaluate the legacy effects of resources on microbial communities, we added four limiting resources annually (i.e., water only; C-mannitol + water; N-NH_4NO_3 + water; and C, N + water) and measured shifts in bacterial community composition after seven years in a cold desert ecosystem in the McMurdo Dry Valleys, Antarctica. Further, to investigate the ecological significance of priming, we conducted a series of stable isotope probing experiments (i.e., ^{18}O-DNA SIP with ^{18}O-labeled water, ^{13}C-DNA SIP with ^{13}C-labeled mannitol, ^{15}N-DNA with ^{15}N- NH_4NO_3, and a combined C and N SIP) and characterized the responding (i.e., isotopically labeled) and seed bank (i.e., unlabeled) bacterial communities. We performed each of the SIPs in soil microcosms corresponding to a single resource manipulation (e.g., ^{13}C-labeled mannitol in C addition soils). We hypothesized that all long-term additions of nutrients and water will lead to a distinct bacterial community—a legacy effect due to the nutrient and water impoverished state of Antarctica soils. We also hypothesized that the stronger the legacy effects demonstrated by a specific community the more adapted or primed bacterial species will be to take advantage of the resource and respond.

As hypothesized, resource additions created distinct bacterial legacy but to different degrees among the treatments. The extent of the resource legacy effects was greatest in the CN, intermediate in water and N, and lowest in C communities relative to the control communities, suggesting that C induced changes in communities were intensified by tandem N additions and that water alone created a more distinct legacy than water and C additions combined. Contrary to our hypothesis, the stronger the legacy effects, the less adapted or primed the community was to take advantage of resource additions. For example, the CN treatment that induced the greatest effect on bacterial communities had the lowest number of species (20.9%) in common between the responding and seed bank communities. This inverse relationship may be due to only two species (i.e., *Arthrobacter,* Actinobacteria and *Massilia,* Betaproteobacteria) really being primed to take advantage of CN and these species constituting over 75% of the seed bank community. Water, N, and C additions had similar levels of priming with 38.4%, 41.4%, and 36.3% of the responding species being present in the seed bank community, respectively. But of these three treatments, only the priming with water resulted in a unique responding community, suggesting that water, a universal bacterial resource, was enough to prime bacteria. Furthermore, water generates the most diverse responding community of all the resources with stemming from all of the fourteen dominant phyla. We did find patterns of ecological coherence among the responders, especially in the major responders (i.e., responders that increased in relative recovery by at least ten-fold). These responders were predominantly found in only three phyla (i.e.,

Actinobacteria, Bacteriodetes, and Gammaproteobacteria) regardless of resource addition. Alternatively minor responders (i.e., responders that increased in relative recovery at least two-fold) were contained in fourteen different phyla with specific taxa stimulated by CN (i.e., Betaproteobacteria) and N and water (i.e., Deltaproteobacteria). Further, resource additions elicited responses from 37% of bacterial species with species specializing on a specific resource (e.g., Chloroflexi) or being a generalist (e.g., Planctomycetes and Gammaproteobacteria). Our results offer the first direct links between legacy and priming effects on bacterial community composition and demonstrate that these mechanisms are not always complimentary leading to the formation of similar communities but may both be essential to maintain the high levels of bacterial diversity. Further, all resources produced elicited responders that were either specialists of generalists demonstrating that even bacteria in the extreme environment of Antarctica respond to pulses of resources.

Keywords: Antarctica, soil ecology, bacteria, microbial ecology, soil, stable isotope probing, target metagenomics, 454 pyrosequencing

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1. INTRODUCTION

1.1 Antarctica's bacterial environment

Antarctica's soils are remote, pristine, and extreme, offering a simplified environment for understanding fundamentals of microbial ecology (Adams *et al*., 2006, Barrett *et al*., 2007; Wierzchos *et al*., 2012). At the bottom of the world lies a series of cold deserts known as the McMurdo Dry Valleys, which represent the largest ice-free area (~4800 km^2) on the continent. Of the less than 10 cm precipitation that falls across these landscapes annually, much of it is rapidly sublimated causing free liquid water to be scarce (Fountain, 1999, Doran *et al*., 2002, Barrett *et al*., 2007). Furthermore, water is in the form of ice throughout most of the year as the deserts experience fewer than 50 days above 0°C (Doran *et al*., 2002). These extreme conditions limit the types of organisms found in soil and lake environments and their metabolic activity by removing liquid water from the environment before life can even access it (Doran *et al*., 2002). The predominant organisms that are able to cope with the limited water and freezing temperatures are microorganisms: cyanobacteria, algae, bacteria, fungi, yeasts, protozoan, and a limited number of metazoan invertebrates (Friedmann *et al*., 1993; Alger *et al*., 1997; Laybourn-Parry *et al*., 1997; Freckman and Virginia, 1997; Priscu *et al*., 1999; Cowan *et al*., 2002; Bamforth *et al*., 2005). The lack of biological activity on a macro scale (i.e., the lack of higher plants and animals) causes physical processes to dominate geochemical stoichiometry of soil and aquatic ecosystems (Barrett *et al*., 2007) and microorganisms, like bacteria, reside in a state of dormancy until they are activated by water.

1.2 Antarctica resource limitations and soil microorganisms

In the presence of water, microbial metabolic activity is limited by resource availability, in particular, C and N. Much like other deserts, microorganisms in the Dry Valleys are limited by the availability of organic C substrates (Connon *et al*., 2007; Bell *et al*., 2008). Organic C found in the Dry Valleys commonly comes from the legacy of paleo-lacustrine microbial detritus in metamorphic rock, and from algal mats that are freeze dried and transported via wind, commonly in the form of mannitol (Burkins *et al*., 2000; Barrett *et al*., 2005, Barrett *et al*., 2006). Bacteria in Antarctic soils experience extreme oligotrophic conditions with C substrates available in only the basic organic forms as biomass is continually recycled. Soil N also stimulates bacterial activity (Schimel *et al*., 2001; Carreiro *et al*., 2005; Nemergut *et al*., 2008). The limited amount of soil N is predominantly present as inorganic N, suggesting that N becomes available through physical processes, specifically marine aerosols deposition, rather than biotic inputs (Barrett *et al*., 2003, Barrett *et al*., 2007). The ecological importance of water, C, and N to Antarctica microbial activity is profound as it is in many other ecosystems, but the effects or "legacy" of these nutrients on bacterial communities are only beginning to be explored.

1.3 Resource legacy and priming effects on microbial community structure

Legacies of past environmental conditions are evident in the soil chemistry across multiple ecosystems and may play an essential role in structuring bacterial communities and ecosystem processes. For example, bacteria may acclimate to nutrient pulses, creating an advantage that allows them to respond more quickly and efficiently to additional pulses (Schimel, 2007). Legacy effects have been studied in microbial communities in response to stress events, i.e., drought, rewetting, and freezing events (Larsen *et al*., 2002; Walker *et al*.,

2006; Schimel *et al*., 2007; Sawicka, *et al*., 2010; Evans and Wallenstein, 2012; Göransson *et al*., 2013).

One mechanism causing legacy in microbial communities may be described as a "priming effect" where bacteria in the microbial community are adapted to specific resource conditions and are poised or queued to take advantage of ecosystem pulses of these resources. Once primed, the subsequent microorganism responding to resources may cause a shift in the microbial composition where the responding bacteria become more dominant (Blagodatskaya and Kuzyakov, 2008). C and N have been found to prime bacteria, which increased microbial activation, DOC concentrations, and resulted in a shift in bacteria (Kuzyakov, 2010; Bastida *et al*., 2013).

The combination of legacy and priming has enormous implications for structuring microbial communities and the potential to alter ecosystem processes. These two mechanisms may control the temporal fluctuations in communities as legacies of past environmental conditions shape the baseline community or "seed bank" of microorganisms that reside in the environment and priming may create reoccurring patterns within the community as seasonal reoccurring resource conditions dictate temporal fluctuations in the community.

1.4 Responding bacteria and ecological coherence

Identifying responding bacteria that are influenced or primed by resources in Antarctica is complicated. The dry, cold climate preserves DNA integrity long after the decease of the organism (Adams *et al*., 2006). One approach that has been successfully used for assigning functional identity to active microbial taxa is stable isotope probing (SIP). This technique involves the addition of an isotopically labeled resource (e.g., $^{13}CH_4$) to an environmental sample (Radajewski *et al*., 2002). Following a sufficient incubation period, isotopically enriched

macromolecules (e.g., lipids or nucleic acids) can be extracted and linked to a targeted microbial process such as methane oxidation (Morris *et al*., 2002), contaminant degradation (Leigh *et al*., 2007), nitrogen fixation (Buckley *et al*., 2008), and pulses of CO_2 following soil rewetting (Aanderud, 2011). Therefore, stable isotope probing (SIP) provides a solution to the problem of contaminating active microbial communities with recalcitrant DNA hanging around in the environment.

Identifying the responding bacteria following resource addition also allows for the investigation of patterns of ecological coherence among bacteria. Ecological coherence occurs as bacteria from similar phylogenetic backgrounds respond similarly to environmental fluctuations or conditions (Philippot *et al.*, 2010). Coherence may occur at any taxonomic level, from the species to the phylum, and has explained bacterial phyla responses to temporal fluctuations such as pH, salinity, C and N availability, (Nemergut *et al*., 2008; Philippot *et al.*, 2010). Bacterial species and phyla are simplified in Antarctica, but many of these taxa are found across the world and not only in extreme environments (Niederberger *et al*., 2008; Fierer *et al.*, 2012). Therefore, finding patterns among responding bacteria to nutrients and water will help assign ecological importance to taxa in multiple ecosystems.

1.5 Research objective

The purpose of this study was to investigate legacy effects of resource additions on microbial communities and identify the bacteria that are "primed" to respond to pulses of these resources in an Antarctica cold desert. To evaluate the legacy effects of resources on microbial communities we added resources (i.e., water, C, N, and CN) and a control treatment in a replicated field experiment and measured shifts in community composition with target metagenomics of 16S rDNA. All nutrient additions were performed with water in the same

amount as added in the water treatment. We also evaluate the priming effects of these additions on responding bacteria and patterns of ecological coherence among the taxa with multiple forms of SIP (i.e., ^{13}C-DNA SIP with ^{13}C-labeled mannitol, a sugar alcohol, ^{15}N-DNA with ^{15}N-NH4NO3, and ^{18}O-DNA SIP with $H_2{}^{18}O$) followed by evaluating the responding (i.e., isotopically labeled) and seed bank (i.e., unlabeled) communities with target metagenomics of 16S rDNA of the responding and seed bank communities. We performed each of the SIPs in soil microcosms corresponding to a single resource manipulation (e.g., ^{13}C-labeled mannitol in C addition soils). By evaluating both legacy and priming we will be able to better identify patterns of ecological coherence among bacterial taxa. We hypothesized that all long-term additions of nutrients and water will lead to a distinct bacterial community—a legacy effect due to the nutrient and water impoverished state of Antarctica soils. We also hypothesized that the stronger the legacy effects demonstrated by a specific community the more adapted or "primed" bacterial species will be to take advantage of the resource and respond.

2. MATERIALS AND METHODS

2.1 Site Description

We conducted our study at the McMurdo Dry Valleys Long-Term Ecological Research (MCM LTER) site located in Victoria Land, Antarctica (77°43.496'S - 162°18.741'E). Soils in the Dry Valleys are derived from tills enriched in granites, sandstones, dolerites, and meta-sedimentary rocks that range from Holocene to Miocene in age, they are alkaline, coarse textured, low in organic matter, and contain high concentrations of soluble salts and high pH (Barrett *et al*, 2007, Bockheim, 1997). Soil chemistry and texture reflect a legacy of paleolake environments influenced by glacial tills and mountain erosion (Burkins *et al*., 2000; Wada *et al*., 1981; Wall and Virginia, 1999; Barrett *et al*., 2007). The soils are poorly weathered and typically >95% sand (Barrett *et al*., 2004).

2.2 Nutrient and water manipulations

To investigate the legacy effects on ecosystem processes and soil ecology caused by nutrient and water (W) additions, we created nutrient treatments where W, C, N, and CN were added to soils annually, beginning in 2003. Specifically, we added C as mannitol, ($C_6H_{14}O_6$), N as NH_4NO_3, CN as mannitol and NH_4NO_3, and W in a complete randomized block design (4 additions C, N, CN, W and a control × 8 replicates = 40 experimental units). Each experimental unit (1 m × 1 m) received nutrients or water annually at a rate of 15g C- $C_6H_{14}O_6$ m^{-2}, and/or, 2.5g N- NH_4NO_3 m^{-2} in 5.6 L or water m^{-2}. The experiment, initiated in 2003, is part of a larger long-term stoichiometry experiment in multiple valleys of the MCM LTER, but for this study we chose to focus on soils from Fryxell basin. For more details on the study site please see www.mcmlter.org.

2.3. Soil chemistry and respiration

To determine effects of nutrient and water additions on microbial activity, we measured soil chemistry characteristics and soil respiration *in situ* two weeks after nutrient additions. Soils for chemical analyses were conducted on a homogenized, sieved (2 mm), composite (3 subsamples) soil sample from all nutrient and water additions (5 addition treatments x 8 replicates = 48 samples). Soil was collected using a plastic scoop to a 10 cm depth and kept at 5°C until processing within 48 hours of collection. We measured electrical conductivity (Orion 160 meter) and pH (VWR 8015 meter, VWR) on soil subsamples. N was extracted with KCL, filtered through Whatman #42 filter paper, and measured on a Lachat Quikchem 8500 (Lachat Instruments, Loveland, CO). P was filtered and extracted using NaHCO3 and measured on a Lachat. Total C and N were measured on a Carlo Erba NA 1500 N Elemental Analyzer (Carlo Erba Instruments, Milan, Italy). Total organic carbon was measured on a Shimadzu TOC-5000A (Shimadzu Corporation, Columbia, MD) and total N was on a Lachat following Kjeldahl digestion. We tested for the effect of the additions on our response variables (i.e., DOC, N, and NH_4^+ and NO_3^- concentrations, soil respiration, total N, total C, PO_4, conductivity, pH, soil moisture) using ANOVA (SAS PROC GLM) and Tukey's HSD test to identify significant differences among the treatments.

We measured soil CO_2 flux using a LI-COR 8100 (Lincoln, NE) fitted with a soil respiration survey chamber. Plots were fitted with a 10-cm diameter PVC collar that created a seal with the soil. We also measured soil temperature at each plot in the surrounding soil using a soil temperature probe Type E and soil moisture using a Theta probe Model ML2.

2.4. Legacy effects of nutrient and water on bacterial community structure

To evaluate the legacy effects of our nutrient and water additions on bacterial communities, we characterized soil bacteria at the time of spring-thaw using bar-coded pyrosequencing. We randomly selected three replicates from the nutrient and water treatments and control soils and removed the soils for pyrosequencing approximately two-weeks after nutrient and water additions. Soils were kept at -20°C until total genomic DNA was extracted from 0.5 g of soil using the PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA, USA). We extracted DNA in triplicate and pretreated the soils with PowerWater® Sterivex™ solution (MoBio PowerWater Kit, MoBio, Carlsbad, CA, USA) to prevent DNA from adhering to soil particles. Details of the pyrosequencing procedures are described in detail elsewhere (Hamady *et al*., 2008; Fierer *et al*., 2009). Briefly, we amplified the V4-V5 region of the 16S rRNA gene using the bacterial specific primer set 515F and 806R with unique 12-nt error correcting Golay barcodes. rDNA was amplified in 25ul PCR reactions containing 5µl of 5X KAPA2G Buffer A, 0.5µl of dNTPs, 1.25µl of 515F and 806R, and 0.1µl of KAPA2G Robust HotStart DNA Polymerase (5units/µl, Kapa Biosystems, Inc., Woburn, MA) under the following PCR conditions: an initial denaturation step at 94ºC for 3 minutes followed by 35 cycles of denaturation at 94ºC for 45 seconds, annealing at 50ºC for 30 seconds, and an extension at 72ºC for 90 seconds. The amplified rDNA was purified using Agencourt AMPure XP PCR Purification (Beckman Coulter Inc., Brea, California, USA) and quantified using a Quant-iT^{TM} PicoGreen dsDNA Kit (Invitrogen Corporation, Carlsbad, California, USA) to create approximately equimolar concentrations prior to pyrosequencing. Samples were sequenced at the Brigham Young University DNA Sequencing Center (http://dnasc.byu.edu/) in a 454 Life Sciences Genome Sequence FLX instrument (Roche, Branford, Connecticut, USA). We analyzed

all sequences using mothur open-source, expandable software (Schloss, P.D., *et al*., 2009). Only sequences that were > 200 base pairs in length were included in the analysis to assure the accuracy and quality of pyrosequencing. OTU identification was performed using Megablast at a minimum coverage of 99%, and minimum pairwise identity of 97%. The phylogenetic identities of sequences were aligned against the SILVA database (http://www.arb-silva.de/). Template alignment was done using Kmer searching, pairwise alignment with the de-gapped template using Needleman-Wunsch Gotoh and Blastn algorithms, and reinserted gaps using the NAST algorithm.

To determine the legacy of seven-years of nutrient and water additions on bacterial community structure, we performed a principle coordinate analysis (PCoA) on all treatments and controls and compared the resulting ordinations against each other using permutational multivariate analysis of variance (PERMANOVA). The PCoA used an abundance-based distance matrix that was relativized by individual samples to compensate for any differences in sample amplification during pyrosequencing and included all 2,930 OTUs present in our soils. The PCoA used BrayCurtus similarity coefficient for the ordination of treatments and the control. We used PERMANOVA (Aanderud *et al*., 2013; Anderson, 2001) to assess the extent of the legacy effects between the treatments and control. PERMANOVA was implemented with the function *adonis* in the vegan package (Community Ecology Package, R package version 2.0-7) of the R Statistics Environment (R Development Core Team 2013). Individual PERMANOVA models were created for each treatment by control combination. Lastly, we evaluated if the nutrient and water additions were driving the changes in bacterial community composition using redundancy analysis (RDA). The RDA used the same relativized data matrix as the PCoA and places the soil

chemistry values in the ordination as a vector. RDA was implemented with the function *adonis* in the vegan package of the R Statistics Environment (R Development Core Team 2008).

2.5. Stable isotope probing for responding bacteria to nutrient and water

To characterize the bacteria responding to nutrient and water additions, we performed a series of DNA-SIP experiments followed by bar-coded pyrosequencing. We performed ^{13}C-DNA, ^{15}N-DNA, a combination of ^{13}C-DNA and ^{15}N-DNA, and ^{18}O-DNA SIP on treatment soils to simulate an addition of nutrients and water and evaluate the responding soil taxa. Specifically, we performed ^{13}C-DNA SIP on C treatment using ^{13}C-manitol, ^{15}N-NH_4NO_3, and ^{18}O-H_2O (98 atom% ^{13}C, Omicron Biochemistry Inc., South Bend, IN, 99 atom% ^{15}N, and ^{18}O, Isotech, Sigma-Aldrich, St. Louis, MO, USA), in microcosms. Soils for each SIP were stepped up from -20°C to -10°C to -5°C, and finally to 4°C, each temperature held for 24 hours, to reduce stress on organisms. Storage and treatment of soils coincides to MCM-LTER protocol to maintain microbial integrity. Microcosms consisted of a 40 mL borosilicate glass vials with polypropylene, silicone-septa screw caps. Each microcosm contained 5 g of soil. We incubated all soils at 5°C for 7 days to allow for sufficient time for microbial response but to still maintain Antarctica's conditions, which typically doesn't have more than 7 days consecutively above freezing temperatures. We initiated the experiment by evenly applying 750 µL of water with added nutrients, or ^{18}O-labeled at the same nutrient application rate as in the field. Microcosms were uncapped at day 4 of the incubation and the headspace in the microcosm was allowed to equilibrate with atmospheric conditions to prevent anoxia and to reduce the buildup of CO_2 generated by microorganisms. Total genomic DNA was extracted from each microcosm using a PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA, USA) as described in section 2.4.

2.6. Legacy effects of nutrient and water on bacterial community structure

We followed the DNA SIP procedure outlined by Neufeld *et al*. (Neufeld *et al*., 2007). At least 1µg of genomic DNA was loaded into 4.7 mL OptiSeal pollyallomer tubes (Beckman Coulter Inc., Brea, CA, USA) containing cesium chloride (VWR molecular grade) and gradient buffer (0.1 M Tris-HCL, 0.1 M KCL, 1 mM EDTA, pH 8) (with a buoyant density 1.725 g mL^{-1} (Neufeld *et al*., 2007). The CsCl mixture was prepared in bulk before each centrifugation with each 4.7 mL tube receiving approximately 4.5 mL of CsCl, 0.979 mL of gradient buffer, and 150 µl of DNA or AccuGENE® Molecular Biology Water (Lonza, Rockland, ME, USA). We performed isopycnic centrifugation on balanced tubes (within 0.005mg) in a TLA 100.4 rotor with a Beckman Optima TL Ultracentrifuge (Beckman Coulter Inc., Brea, CA). Isotopically labeled 'heavy' and unlabeled 'light' DNA were separated at a speed of 58,000rpm (177,000g_{av}). The SIPs were centrifuged for different durations to maximize separation for each treatments: ^{15}N-DNA SIP = 66 hours (Buckley, 2007), ^{13}C-DNA SIP= 48 hours (Neufeld *et al*., 2007), and ^{18}O-DNA SIP= 72 hours (Aanderud *et al*., 2011a); the sample containing both ^{13}C- and ^{15}N-DNA SIP= 66 hours.

We retrieved isotopically labeled and unlabeled DNA in 30 fractions (120 µL each) from each SIP using a fraction recovery system (Beckman Coulter Inc., Brea, CA, USA) connected to a single-syringe infusion pump (Cole-Parmer, Vernon Hills, IL, USA). The pump delivered mineral oil at a rate of 28.2ml/hour (Aqua Solutions, Deer Park, TX, USA). We incorporated two blanks without DNA in each ultracentrifugation to calculate the density of each fraction using a digital refractometer (Reichert AR200). We then precipitated the DNA with 30% PEG (Sambrook and Russell, 2001, Neufeld *et al*., 2007) and DNA in all fractions was resuspended in 20 µL of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8).

To identify fractioned densities that contained the majority of the DNA, we performed qPCR assays to determine the distribution of DNA within the gradient and select labeled and unlabeled DNA for pyrosequencing. On all 30 fractions from each SIP run, we performed qPCR assays on a Mastercycler EP Realplex qPCR machine (Eppendorf, Hamburg, Germany) using SYBRGreen to detect the abundance of bacterial DNA using primers that target regions of the 16S rDNA. Each 12µl reaction contained 5.4µl KAPA SYBR Fast qPCR Master Mix (KAPA Biosystems), 0.2µl of each primer (10µM Invitrogen), 5.2µl molecular grade water, and 2µl of DNA template DNA. PCR conditions were 5 minutes at 95ºC followed by 40 cycles of 95ºC for 1 minute, annealing temperature for 1 minute, and extension at 72ºC for 1 minute. Annealing temperatures varied between primers. The final extension was set at 72ºC for 7 minutes. Melting curves for each product were conducted to confirm amplification. We generated qPCR standards from a soil bacterium using the TOPO TA Cloning® Kit (Invitrogen). We extracted plasmids from transformed cells (Qiagen Sciences, Germantown, MD, USA) that we used for our standard curve, which captured a range of $10^1 – 10^8$ copies/µL. The coefficients of determination (r^2) for our assays ranged from 0.97 and 0.99, while amplification efficiencies fell between 0.93 and 0.99. Based on melting curve analyses, we found no evidence for primer dimers. The resulting quantities of rRNA gene copies were relativized by expressing gene copies as a ratio of the maximum quantity of copies per treatment replicate. For each DNA-SIP, we used the same fractions from the density gradients in our pyrosequencing effort. The responding bacterial community or isotopically labeled, ‘heavy’ DNA was sequenced from three fractions ranging in density from 1.746-1.736 g/mL, and the seed bank bacterial community or unlabeled, ‘light’ DNA was sequenced from three fractions ranging in density from 1.728-1.718 g/mL.

Pyrosequencing for the responding and seed bank community followed the same procedure as outlined in section 2.4.

2.7. Ecological coherence patterns of responding bacteria to nutrient and water

To identify ecological coherence patterns of responding bacteria, the priming effects of the nutrients and water on the priming of active bacteria, and links between these taxa to the legacy effects of our treatments on microbial community composition, we compared the bacterial responding (i.e., isotopically labeled) and seed bank (i.e., unlabeled) from the phyla to the species or OTU level. To assess taxonomical responses to the nutrient additions and rewetting, we: first, calculated the relative recovery of either each OTU or the phyla and subclass taxonomical level, second, created a response ratio of relative recoveries, and, third, evaluated taxonomical differences between treatments for taxa with a response ratio measuring the increase in relative recovery from the seed bank to responding community. Relative recovery of OTUs was measured as the total number of OTUs in a given OTU or phyla divided by the total number of OTUs present in each treatment replicate. To evaluate phyla-level patterns, we used ANOVA (SAS PROC GLM) and Tukey's HSD tests to test for differences among the total number of major and minor responding taxa in each phyla and the total relative recovery of these responding taxa in the responding bacterial communities. We also performed a PCoAs on the seed bank responding bacterial communities and tested for differences in these communities using PERMANOVA as explained in section 2.4. Last, we created Venn diagrams to identify the level of priming that occurred in each treatment.

To investigate patterns at the species level, we created a phylogenetic tree of species found in all treatment soils using the Interactive Tree of Life (iTOL) web server (Letunic and Bork, 2007) and added bars around the tree to identify minor and major responders. Last, we

quantified changes in bacterial OTU richness using Chao-1 diversity estimation and OTU diversity using mothur among the treatments (Chao, 1984; Aanderud and Lennon, 2011b) using ANOVA and Tukey's HSD tests.

3. RESULTS

3.1. Effects of nutrient and water manipulations on the soil environment

After seven-years, the nutrient manipulations affected inorganic N concentrations, P availability, and dissolved organic C. In general, the N additions from both the N and CN treatment increased soil inorganic N and the mannitol additions from the C treatments only influenced organic C levels (Table 1). For example, NH_4^+ concentrations increased at least one-hundred and sixty-one-times and NO_3^- concentrations increased at least sixteen-times in N and CN treatment soils. DOC concentrations were higher in C (0.06 mg C kg^{-1} soil) and CN (0.04 mg C kg^{-1} soil) than control soils (0.03 mg C kg^{-1} soil). However, the total soil C and N remained the same after seven years of nutrient and water additions. Last, soil conductivity increased due to CN additions with conductivity increasing from 219 dS/cm in control to 301 dS/cm CN soils.

3.2. Legacy effects of nutrient and water on bacterial community structure

The N, CN, and W manipulations created a lasting legacy on bacterial community structure but only the CN additions altered soil respiration. The bacterial communities differed among the nutrient and water treatments (PERMANOVA: $F_{(4)} = 2.00$ P=0.023) and the extent of the legacy effects of the manipulations was greatest in the CN communities (PERMANOVA: $F_{(2)} = 5.14$ P=0.01) followed by W (PERMANOVA: $F_{(1)} = 2.90$ P=0.038) and N communities (PERMANOVA: $F_{(1)} = 3.61$ P =0.019), and lowest in the C communities (PERMANOVA: $F_{(1)} = 1.94$ P =0.063) relative to the control communities (Figure 1). Based on a redundancy analysis the CN and N treatments were associated with the soil chemistry parameters that were altered by their additions (i.e., total C and N, inorganic N species, and DOC (Supplemental figure 1)). Alternatively, C, W, and control bacterial communities were associated with pH and soil

conductivity. Even with the alterations in community structure among the nutrient and water additions, soil respiration only increased in CN soils (ANOVA: $F_{(4)} = 5.78$ $P = 0.03$, (Supplemental Figure 2). Soil respiration was 2.3-times higher in CN than control soils.

3.3. Stable isotope probing for responding bacteria to nutrient and water additions

All of our different forms of SIP created bacteria that were isotopically labeled and clearly distinct from unlabeled DNA. For example, the ^{18}O-DNA SIP caused the buoyant density of rDNA to increase to an average of 1.74 g/mL within 72 hours (Figure 2). There was always a substantial quantity of unlabeled rDNA present in the less dense CsCl fractions (i.e., 12-14), which presumably reflected bacterial taxa that did not respond positively to rewetting. The ^{13}C-DNA, ^{15}N-DNA demonstrated the same separation (data not shown). The responding rDNA communities were pyrosequenced from three fractions with densities 1.746-1.736 g/mL and the seed bank rDNA communities were pyrosequenced from three fractions with densities 1.728-1.718 g/mL. Within the 30-fraction gradient, the density of 1.735 g ml^{-1} consistently separated isotopically labeled from unlabeled rDNA.

3.4. Priming of responding bacteria to treatment additions

All additions primed the bacterial community to respond to specific nutrient and water but to different degrees (Figure 3). To understand priming effects from past treatments, we measured the responding species that are also found in the seed bank. The N, W and C additions had similar priming effects with 29.2%, 26.6%, and 27.7% of the species being shared by responders and seed bank taxa, respectively. CN additions only had 17.0% species in common between the responding and seed bank communities. To further investigate priming effects, we compared labeled communities to seed bank communities (Figure 4). Shifts in community

composition occurred in labeled W treatments (PERMANOVA: $F_{(2)}$= 4.20, *P*=0.009) and CN treatments (PERMANOVA: $F_{(2)}$= 4.48, *P*=0.004) but were not significant in C (PERMANOVA: $F_{(2)}$= 2.08, *P*=0.124) or N (PERMANOVA: $F_{(1)}$= 1.5, *P*=0.336) treatments.

3.5. Bacterial responders to nutrient and water at the phyla level

The nutrient and water treatments stimulated responses from different phyla. All microbes that were isotopically labeled did respond to either nutrient or water additions but the response did not necessarily change the labeled community from the unlabeled community. To identify the differences between responding bacterial taxa we relied on a response ratio that categorized responders into minor responding taxa (i.e., experienced at least a two-fold increase in relative recovery from the seed bank to responding community) and major responding taxa (at least a ten-fold increase in relative recovery from the seed bank to responding community).

All major responders were in seven phyla with the most responders across the treatments occurring in Acidobacteria, Actinobacteria, and Bacteriodetes (Table 2). Of the major responding taxa, the water additions stimulated the highest number of bacteria-a total of 16, while the nutrient treatments only induced 2 to 10 advanced responders. The contribution of advanced responding taxa did not differ between treatments and the contribution of these taxa never exceeded 18% of the responding community (Figure 5). We used 360,702 total sequences and 67,408 unique sequences in our 24 samples with an average read length of 260 base pairs.

Of the minor responding taxa, the N and W additions stimulated the highest number of taxa, while the CN additions stimulated the fewest number of taxa. The N treatment stimulated 170 ±10 taxa and the water treatment stimulated 120 ±10 taxa (Figure 5). The minor responding taxa were contained in fourteen different phyla (Table 2). Most of the responders in N and W treatments were in the phyla: Actinobacteria, Acidobacteria, and Proteobacteria, subclasses:

Alphaproteobacteria, and Betaproteobacteria with higher numbers occurring in both these treatments than the CN treatment. Furthermore, of the remaining nine phyla, either W or N treatment soils contained the highest number of responding species. The minor responding taxa in N soils comprised upwards of 86% ±10 of the responding community, while the responding species in W soils comprised only 37% ±10. The CN treatment stimulated only 45 species—a decrease of at least 60% of the responding taxa from W and N treatments. The minor responders in the CN treatments only occurred in nine phyla with over 50% of the species occurring as Actinobacteria.

The responding community was different from the seed bank community for all the treatments ($F_{(3)}$= 2.00, *P*=0.023). Total species richness decreased with responding bacteria (ANOVA: $F_{(4)}$ = 5.78 *P* = 0.03, Supplemental Figure 3) as did species diversity (P=0.007, $F_{(3)}$=6.12). CN treatments have the lowest diversity in responding taxa ($F_{(1)}$=4.62, *P*=0.05,).

3.6. Bacterial responders to nutrient and water at the species level

The nutrient and water additions elicited responses from 37% of bacterial species found in Antarctica soils and of these species certain ones were specialists (i.e., responding specifically to a certain nutrient or water) or generalists. For example, some Actinobacteria species (i.e., Solirubrobacterales and *Arthrobacter*) responded to each treatment, but Deltaproteobacteria only responded to W and N additions (Figure 6). Deinococcus showed a high response to W and N but only two taxa responded to the C and CN treatments. Chloroflexi didn't have any responders to CN treatments but responded heavily to W, C, and N. Planctomycetes and Gammaproteobacteria showed a high affinity for all the treatments. The following taxa responded to all 4 nutrient additions: phylum Actinobacteria, genus *Arthrobacter*; phylum Deinococcus, genus *Truepera*; class Gammaproteobacteria, genus *Pseudomonas*; class

Betaproteobacteria, family Oxalobactera; and class Alphaproteobacteria, family Shpingomonadaceae. Betaproteobacteria, genus *Massilia,* both responded ten-fold to N and CN additions. Actinobacteria, order Solirubrobacterales responded ten-fold to W, N, and CN additions. N and W had the greatest number of responders, followed by C and CN. Interestingly, while CN had the lowest number of unique responders, the abundance of responders makes up 75% of the total recovery (Figure 4).

4. DISCUSSION

Bacteria in Antarctica's Dry Valleys must survive hypersaline soils, dark winter months, and harsh summer UV radiation (Freckman and Virginia, 1997; Bargagli *et al.*, 1999; Cowan *et al*., 2002; Sinclair *et al*., 2003; Wall, 2005; Adams *et al*., 2006; Barrett *et al*., 2006; Wierzchos *et al*., 2012) before they even contend with the hyper-arid climate and oligotrophic conditions. Of all the bacteria species living in this extreme environment, only 37% responded to our resource treatments. Our additions (i.e., C, N, and CN) were primarily geared to stimulate the activity of chemooorganoheterophs, while the water addition acted as a universal stimulant for all bacteria. Therefore, the remaining 63% of bacteria may have different metabolism requirements, besides water, needed to respond in this environment. Additionally some of the bacterial DNA that we found in seed bank communities may be just preserved DNA that resides in the soil long after the death of the organism (Adams *et al*., 2006). Even with these limitations, we did find that all the resource treatments, except the addition of C alone, created a resource legacy and altered community structure, suggesting that C induced changes in communities were intensified by tandem N additions and that water alone created a more distinct legacy than water and C additions combined. Legacy effects did not necessarily prime the community to respond to their specific nutrient addition, demonstrating that legacy may structure seed bank communities but resource priming has the potential to create unique communities of responders. We did find patterns of ecological coherence among the responders, especially in the major responders (i.e., responders that increased in relative recovery from the seed bank to the responding community by at least ten-fold). These responders were predominantly found in only three phyla (i.e., Actinobacteria, Bacteriodetes, and Betaproteobacteria) regardless of resource addition. Alternatively, minor responders (i.e., responders that increased in relative recovery from the seed

bank to the responding community by at least two-fold but less than ten-fold) were found in fourteen phyla.

4.1 Legacy effects on bacterial communities and soil characteristics

As hypothesized, we found that all of the long-term additions of resources, except the C addition alone, created a distinct legacy on soil bacteria. The extent of the legacy effects was greatest in CN, intermediate in water and N, and lowest in C communities relative to the control communities. N was essential for bacterial communities to shift in response to additions of mannitol. Inorganic N availability is relatively low in Antarctica with soils NH_4^+ and NO_3^- levels often an order of magnitude lower in Antarctica than other deserts (Bell *et al*., 2008). The N in Antarctica soils represent thousands of years of atmospherically derived salts accumulating in soils where leaching and denitrification are prevented by the arid climate and low biotic activity (Bockheim, 1997; Barrett *et al*., 2007). Furthermore, the soils in the study area where we conducted our experiment, the Taylor Valley, contain significantly lower N concentrations than soils from the neighboring valleys. Together these soil N characteristics help explain why N treatments resulted in significant microbial responses (Barrett *et al*., 2007). N additions are known to elicit varying responses in soil heterotrophic processes including increasing the turnover of organic C constituents, inducing higher respiration rates, and shifting microbial community composition (Schimel *et al*., 2001; Carreiro *et al*., 2005; Nemergut *et al*., 2008). We also found that the addition of N altered soil respiration as well as microbial community structure. The CN treatment was the only treatment that demonstrated a soil respiration higher than the respiration rate in control soil—a three-fold increase in respiration from N alone.

We also investigated the legacy of the resource addition on soil chemistry. Unlike the resource legacies on community composition, only soil N additions in the N and CN treatments created a lasting impact on the soil. In general, the N additions from both the N and CN treatment increased the two inorganic N forms by more than sixteen-times in the N and CN addition soils- the N additions actually increased total soil N in the CN additions soils. Taken together, the amount of N necessary to create a legacy effect in the microbial communities was exceeded by the addition of N and this resource then accumulated in the soils and was left in excess.

The addition of the universal resource requirement for all life, water, created a more distinct legacy on bacterial communities than C additions alone. Water legacy effects on bacterial communities have been documented in multiple ecosystems stemming from drought and soil rewetting (Larsen *et al*., 2002; Walker *et al*., 2006; Schimel *et al*., 2007), and freeze-thaw events during winter months (Aanderud *et al.,* 2011b). Furthermore, legacies associated with water have been found in hot deserts, Arctic tundra, and other arid environments (Schimel *et al*., 2007; Bell *et al*., 2008). There is little doubt that water is the most prominent limiting resource in Antarctica soils (Barrett *et al*., 2007).

4.2 Priming effects on responding communities

Contrary to our hypothesis, the stronger the legacy effects the less adapted or primed the community was to take advantage of resource additions. We measured the effects of priming by looking for shifts in the community following the addition of specific resource following SIP and calculating the percentage of shared taxa that were present in the responding and the seed bank communities (or the shared numbers of OTU's in our Venn diagrams divided by the total number

of OTU's in the seed bank community x 100). We then compared these results against our ranking of legacy effects of the resources. Through this comparison, we found that the CN treatment with the greatest legacy had the lowest priming effect on bacteria, only sharing 17.0% between the responding and seed bank communities. However, the priming of CN soils resulted in a unique responding community compared to the seed bank community. The reason for this reduction in priming effect on bacteria may be due to the drastic reduction of species richness and diversity among the responders. Although all responding communities experienced a decline in richness and diversity, the CN responding community had three-times lower species richness and seven-times lower diversity in the CN addition and control soils. Basically, the N allowed for specific soil bacteria to dominate the community and create a unique microbial community. The two most dominant species in the responding community were an *Arthrobacter species* in the phylum Actinobacteria and a *Massilia species* in the Betaproteobacteria. These two species combined constituted 75% of the overall responding community. Of the other additions, W, N and C all had similar levels of priming, 26.6%, 29.2%, and 27.7% respectively. But of these three treatments, only the priming of water resulted in a unique responding community compared to the seed bank community. Thus, water additions alone were enough to prime bacteria. The priming by water actually generated the most diverse community of all responding communities with major responders occurring in six different phyla and minor responders occurring in all of the fourteen dominant phyla present in our soils. Therefore, water pulses caused a diverse set of bacteria to become metabolically active and grow. Pulses of water may originally acts as a stress to bacteria as they contend with regulating intracellular osmolytes to avoid cell rupture (Kieft *et al*., 1987; Schimel *et al*., 2007). But if bacteria survive, they rapidly become metabolically active. Although water limitations in most cold deserts are caused by drying-rewetting cycles,

Antarctica bacteria suffer a different type of drought—one of ice. The continual freezing and thawing cycle of water still induce osmotic stresses and may be just as important in structuring bacterial communities. It is unclear which stress, drying or freezing, has the greatest effect on soil bacterial community composition. As the global climate warms, precipitation regimes are also being altered (Karl *et al*., 1995; IPCC, 2007; Zhang *et al*., 2007) and these shifts may alter the bacteria composition of Antarctica. These changes include alterations in snowfall timing, magnitude, and variability. In tandem with global temperatures warming, shifts in snowfall magnitude, in particular, have the potential to decrease the frequency of freeze-thaw cycles and increase the time free water is available for microbes to become metabolically active. Based on our results, changes in temperature and water availability will create microbial communities to increase in diversity and experience compositional shifts of activated bacteria.

4.3 Phylum and species responses to resource additions

There was evidence that phyla in responding communities specialized to certain resources or were more stimulated by multiple resources. For example, Actinobacteria, Bacteriodetes, Chloroflexi, and subclasses Gammaproteobacteria, Betaproteobacteria, were generalists and demonstrated responses to all resource additions. Together, these phyla represent upwards of 50% of the responding community. Of the intermediate responders Acidobacteria, Gemmatimonadetes, Planctomycetes, and Verrucomicrobia specialized on water and N additions, while Firmicutes were only present in the C and water addition soils. We also found generalists and specialists at finer taxonomical resolutions. For example, the psychotrophic genus, *Arthrobacter,* represented the major responder in CN soils, constituting upwards of 45% of recovered Actinobacteria. This species is resistant to starvation and desiccation and can

specialize in arid and extreme soils (Boylen, 1973; Fredrickson *et al*., 2004). *Arthrobacter* were present in all of our treatment soils but never to the overwhelming abundance as found in CN soils. A strain of *Arthrobacter,* Strain GP3T, is isolated from penguin guano, specifically Adelie penguins (*Pygoscelis adeliae*) from Ardley Island, Antarctica (Wang *et al*., 2009), suggesting that this species is particularly adapted to compete in cold environments and take advantage of enriched organic sources of C and N. Also in the Actinobacteria, the Rubrobacteridae, a subclass that comprises 32.8% of recovered Actinobacteria in responding CN communities, are extremely radiotolerant and often found in extreme environments (Ferreira *et al*., 1999; Shravage *et al*., 2007). *Rubrobacter* in Rubrobacteridae, is known for its extreme radiotolerance and association with hot springs (Ferreira *et al*., 1999). Although Antarctica represents a very different environment from hot springs, the radiation resistance may explain the bacteria's resilience. *Chloracidobacterium*, a genus that makes up 67.9% of Acidobacteria, is a photosynthetic extremophile specialized on the N treatments (Bryant *et al*., 2007). *Massilia,* a genus representing 54.3% of responding Betaproteobacteria demonstrated a specialization to N and CN treatments. Thermomicrobia, a class that represents 8.1% of Chloroflexi specialized on the water additions.

5. CONCLUSIONS

Our results offer insights into the legacy effects of various resources on bacterial communities, describe relationships between legacy and priming mechanisms that create both long-term and short-term temporal shifts in community composition, and categorize bacterial phyla and species by their degree of responsiveness (i.e., major versus minor responders) and specialization to certain resources (generalist versus specialist). We found that legacy and priming effects are not always complimentary, leading to formation of similar communities but may both be essential to maintain the high levels of bacterial diversity. All resources produced elicited responders that were either specialists or generalists demonstrating that even bacteria in the extreme environment of Antarctica respond to pulses of resources.

6. REFERENCES

Aanderud ZT, Jones SE, Schoolmaster DR, Jr., Fierer N, & Lennon JT (2013) Sensitivity of soil respiration and microbial communities to altered snowfall. *Soil Biology & Biochemistry* 57:217-227.

Aanderud ZT & Lennon JT (2011a) Validation of heavy-water stable isotope probing for the characterization of rapidly responding soil bacteria. *Applied and Environmental Microbiology* 77(13):4589-4596.

Aanderud ZT, Schoolmaster DR, Jr., & Lennon JT (2011b) Plants mediate the sensitivity of soil respiration to rainfall variability. *Ecosystems* 14(1):156-167.

Adams BJ*, et al.* (2006) Diversity and distribution of Victoria Land biota. *Soil Biology & Biochemistry* 38(10):3003-3018.

Aiken G, McKnight D, Harnish R, & Wershaw R (1996) Geochemistry of aquatic humic substances in the Lake Fryxell Basin, Antarctica. *Biogeochemistry* 34(3):157-188.

Aislabie JM, Jordan S, & Barker GM (2008) Relation between soil classification and bacterial diversity in soils of the Ross Sea region, Antarctica. *Geoderma* 144(1-2):9-20.

Alger AS, Stoermer EF, & McKnight DM (1997) Factors influencing algal abundance and species distribution in meltwater streams in Taylor Valley, Antarctica. *Phycologia* 36(4):1-1.

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26(1):32-46.

Bamforth SS, Wall DH, & Virginia RA (2005) Distribution and diversity of soil protozoa in the McMurdo Dry Valleys of Antarctica. *Polar Biology* 28(10):756-762.

Barrett JE, Gooseff MN, & Takacs-Vesbach C (2009) Spatial variation in soil active-layer geochemistry across hydrologic margins in polar desert ecosystems. *Hydrology and Earth System Sciences* 13(12):2349-2358.

Barrett JE*, et al.* (2006) Terrestrial ecosystem processes of Victoria Land, Antarctica. *Soil Biology & Biochemistry* 38(10):3019-3034.

Barrett JE*, et al.* (2007) Biogeochemical stoichiometry of Antarctic Dry Valley ecosystems. *Journal of Geophysical Research-Biogeosciences* 112(G1):12.

Barrett JE, Virginia RA, Parsons AN, & Wall DH (2005) Potential soil organic matter turnover in Taylor Valley, Antarctica. *Arctic Antarctic and Alpine Research* 37(1):108-117.

Barrett JE, Virginia RA, Parsons AN, & Wall DH (2006) Soil carbon turnover in the McMurdo Dry Valleys, Antarctica. *Soil Biology & Biochemistry* 38(10):3065-3082.

Barrett JE, Virginia RA, & Wall DH (2002) Trends in resin and KCl-extractable soil nitrogen across landscape gradients in Taylor Valley, Antarctica. *Ecosystems* 5(3):289-299.

Barrett JE*, et al.* (2004) Variation in biogeochemistry and soil biodiversity across spatial scales in a polar desert ecosystem. *Ecology* 85(11):3105-3118.

Bastida F*, et al.* (2013) Can the labile carbon contribute to carbon immobilization in semiarid soils? Priming effects and microbial community dynamics. *Soil Biology & Biochemistry* 57:892-902.

Beilke AJ & Bockheim JG (2013) Carbon and nitrogen trends in soil chronosequences of the Transantarctic Mountains. *Geoderma* 197:117-125.

Bell C, McIntyre N, Cox S, Tissue D, & Zak J (2008) Soil microbial responses to temporal variations of moisture and temperature in a Chihuahuan Desert Grassland. *Microbial Ecology* 56(1):153-167.

Blagodatskaya E & Kuzyakov Y (2008) Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. *Biology and Fertility of Soils* 45(2):115-131.

Blecker SW*, et al.* (2006) Phosphorus fractions in soils of Taylor Valley, Antarctica. *Soil Science Society of America Journal* 70(3):806-815.

Bockheim JG (1997) Properties and classification of cold desert soils from Antarctica. *Soil Science Society of America Journal* 61(1):224-231.

Bomblies A, McKnight DM, & Andrews ED (2001) Retrospective simulation of lake-level rise in Lake Bonney based on recent 21-year record: indication of recent climate change in the McMurdo Dry Valleys, Antarctica. *Journal of Paleolimnology* 25(4):477-492.

Boylen CW (1973) Survival of Arthrobacter-Crystallopoietes during prolonged periods of extreme desiccation. *Journal of Bacteriology* 113(1):33-37.

Brussaard L*, et al.* (1997) Biodiversity and ecosystem functioning in soil. *Ambio* 26(8):563-570.

Bryant DA*, et al.* (2007) Candidatus Chloracidobacterium thermophilum: An aerobic phototrophic acidobacterium. *Science* 317(5837):523-526.

Buckley DH, Huangyutitham V, Hsu S-F, & Nelson TA (2008) N-15(2)-DNA-stable isotope probing of diazotrophic methanotrophs in soil. *Soil Biology & Biochemistry* 40(6):1272-1283.

Burkins MB, Virginia RA, Chamberlain CP, & Wall DH (2000) Origin and distribution of soil organic matter in Taylor Valley, Antarctica. *Ecology* 81(9):2377-2391.

Carreiro MM, Sinsabaugh RL, Repert DA, & Parkhurst DF (2000) Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. *Ecology* 81(9):2359-2365.

Cary SC, McDonald IR, Barrett JE, & Cowan DA (2010) On the rocks: the microbiology of Antarctic Dry Valley soils. *Nature Reviews Microbiology* 8(2):129-138.

Chao A (1984) Nonparametric-estimation of the number of classes in a population. *Scandinavian Journal of Statistics* 11(4):265-270.

Chu HY*, et al.* (2010) Soil bacterial diversity in the Arctic is not fundamentally different from that found in other biomes. *Environmental Microbiology* 12(11):2998-3006.

Connon SA, Lester ED, Shafaat HS, Obenhuber DC, & Ponce A (2007) Bacterial diversity in hyperarid Atacama Desert soils. *Journal of Geophysical Research-Biogeosciences* 112(G4).

Cowan DA, Russell NJ, Mamais A, & Sheppard DM (2002) Antarctic Dry Valley mineral soils contain unexpectedly high levels of microbial biomass. *Extremophiles* 6(5):431-436.

Cowan DA*, et al.* (2011) Hypolithic communities: important nitrogen sources in Antarctic desert soils. *Environmental Microbiology Reports* 3(5):581-586.

Dhanasekaran S, Doherty TM, Kenneth J, & Grp TBTS (2010) Comparison of different standards for real-time PCR-based absolute quantification. *Journal of Immunological Methods* 354(1-2):34-39.

Dixon P (2003) VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science* 14(6):927-930.

Doran PT*, et al.* (1999) Dating Quaternary lacustrine sediments in the McMurdo Dry Valleys, Antarctica. *Palaeogeography Palaeoclimatology Palaeoecology* 147(3-4):223-239.

Doran PT*, et al.* (2008) Hydrologic response to extreme warm and cold summers in the McMurdo Dry Valleys, East Antarctica. *Antarctic Science* 20(5):499-509.

Doran PT*, et al.* (2002) Antarctic climate cooling and terrestrial ecosystem response. *Nature* 415(6871):517-520.

Ferreira AC*, et al.* (1999) Characterization and radiation resistance of new isolates of Rubrobacter radiotolerans and Rubrobacter xylanophilus. *Extremophiles* 3(4):235-238.

Fierer N*, et al.* (2012) Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. *Proceedings of the National Academy of Sciences of the United States of America* 109(52):21390-21395.

Fierer N & Jackson RB (2006) The diversity and biogeography of soil bacterial communities. *Proceedings of the National Academy of Sciences of the United States of America* 103(3):626-631.

Fierer N, Schimel JP, & Holden PA (2003) Influence of drying-rewetting frequency on soil bacterial

community structure. *Microbial Ecology* 45(1):63-71.

Foreman CM*, et al.* (2011) When a habitat freezes solid: microorganisms over-winter within the ice column of a coastal Antarctic lake. *Fems Microbiology Ecology* 76(3):401-412.

Fountain AG*, et al.* (2012) The disappearing cryosphere: impacts and ecosystem responses to rapid cryosphere loss. *Bioscience* 62(4):405-415.

Fountain AG*, et al.* (1999) Physical controls on the Taylor Valley ecosystem, Antarctica. *Bioscience* 49(12):961-971.

Fredrickson JK*, et al.* (2004) Geomicrobiology of high-level nuclear waste-contaminated vadose sediments at the Hanford Site, Washington State. *Applied and Environmental Microbiology* 70(7):4230-4241.

Friedmann EI, Kappen L, Meyer MA, & Nienow JA (1993) Long-term productivity in the cryptoendolithic microbial community of the Ross Desert, Antarctica. *Microbial Ecology* 25(1):51-69.

Goeransson H, Godbold DL, Jones DL, & Rousk J (2013) Bacterial growth and respiration responses upon rewetting dry forest soils: Impact of drought-legacy. *Soil Biology & Biochemistry* 57:477-486.

Gordon DA, Priscu J, & Giovannoni S (2000) Origin and phylogeny of microbes living in permanent Antarctic lake ice. *Microbial Ecology* 39(3):197-202.

Harris KJ, Carey AE, Lyons WB, Welch KA, & Fountain AG (2007) Solute and isotope geochemistry of subsurface ice melt seeps in Taylor Valley, Antarctica. *Geological Society of America Bulletin* 119(5-6):548-555.

Hery M, Herrera A, Vogel TM, Normand P, & Navarro E (2005) Effect of carbon and nitrogen input on the bacterial community structure of Neocaledonian nickel mine spoils. *Fems Microbiology Ecology* 51(3):333-340.

Hirsch P (1986) Microbial life at extremely low nutrient levels. *Advances in space research: the official journal of the Committee on Space Research (COSPAR)* 6(12):287-298.

Hodson A*, et al.* (2008) Glacial ecosystems. *Ecological Monographs* 78(1):41-67.

Hogg ID*, et al.* (2006) Biotic interactions in Antarctic terrestrial ecosystems: Are they a factor? *Soil Biology & Biochemistry* 38(10):3035-3040.

Hopkins DW*, et al.* (2006) Controls on the distribution of productivity and organic resources in Antarctic Dry Valley soils. *Proceedings of the Royal Society B-Biological Sciences* 273(1602):2687-2695.

Hunt HW, Fountain AG, Doran PT, & Basagic H (2010) A dynamic physical model for soil temperature and water in Taylor Valley, Antarctica. *Antarctic Science* 22(4):419-434.

Intergovernmental Panel on Climate Change. 2008. Observed and projected changes in climate as they relate to water. Bates BC, Kundzewicz ZW, Wu S, Palutikof JP, editors. Climate Change and Water. Technical Paper of the Intergovernmental Panel on Climate Change. Intergovernmental Panel on Climate Change Secretariat, Geneva. p13–31. www.ipcc.ch/ipccreports/tp-climate-change-water.htm.

Janssen PH, Yates PS, Grinton BE, Taylor PM, & Sait M (2002) Improved culturability of soil bacteria and isolation in pure culture of novel members of the divisions Acidobacteria, Actinobacteria, Proteobacteria, and Verrucomicrobia. *Applied and Environmental Microbiology* 68(5):2391-2396.

Jari Oksanen, F. Guillaume Blanchet, Roeland Kindt, Pierre Legendre, Peter R. Minchin, R. B. O'Hara, Gavin L. Simpson, Peter Solymos, M. Henry H. Stevens and Helene Wagner (2013). vegan: Community Ecology Package. R package version 2.0-7. http://CRAN.R-project.org/package=vegan.

Jin VL & Evans RD (2007) Elevated CO2 increases microbial carbon substrate use and nitrogen cycling in Mojave Desert soils. *Global Change Biology* 13(2):452-465.

Johnson JB, Peat SM, & Adams BJ (2009) Where's the ecology in molecular ecology? *Oikos* 118(11):1601-1609.

Kennedy AD (1993) Water as a limiting factor in the Antarctic terrestrial environment- a biogeographical synthesis. *Arctic and Alpine Research* 25(4):308-315.

Kieft TL, Soroker E, & Firestone MK (1987) Microbial biomass response to a rapid increase in water potential when dry soil is wetted. *Soil Biology & Biochemistry* 19(2):119-126.

Knoepfle JL, Doran PT, Kenig F, Lyons WB, & Galchenko VF (2009) Particulate organic and dissolved inorganic carbon stable isotopic compositions in Taylor Valley lakes, Antarctica: the effect of legacy. *Hydrobiologia* 632(1):139-156.

Kunisawa T (2011) The phylogenetic placement of the non-phototrophic, Gram-positive thermophile 'Thermobaculum terrenum' and branching orders within the phylum 'Chloroflexi' inferred from gene order comparisons. *International Journal of Systematic and Evolutionary Microbiology* 61:1944-1953.

Kuzyakov Y (2002) Review: Factors affecting rhizosphere priming effects. *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde* 165(4):382-396.

Kuzyakov Y (2006) Sources of CO2 efflux from soil and review of partitioning methods. *Soil Biology & Biochemistry* 38(3):425-448.

Kuzyakov Y (2010) Priming effects: Interactions between living and dead organic matter. *Soil Biology & Biochemistry* 42(9):1363-1371.

Kuzyakov Y, Friedel JK, & Stahr K (2000) Review of mechanisms and quantification of priming effects. *Soil Biology & Biochemistry* 32(11-12):1485-1498.

Kuzyakov Y & Gavrichkova O (2010) REVIEW: Time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls. *Global Change Biology* 16(12):3386-3406.

Larsen KS, Jonasson S, & Michelsen A (2002) Repeated freeze-thaw cycles and their effects on biological processes in two arctic ecosystem types. *Applied Soil Ecology* 21(3):187-195.

Lauber CL, Hamady M, Knight R, & Fierer N (2009) Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. *Applied and Environmental Microbiology* 75(15):5111-5120.

LaybournParry J*, et al.* (1997) The microbial plankton of Lake Fryxell, southern Victoria Land, Antarctica during the summers of 1992 and 1994. *Polar Biology* 17(1):54-61.

Letunic I & Bork P (2007) Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. *Bioinformatics* 23(1):127-128.

Letunic I & Bork P (2011) Interactive Tree Of Life v2: online annotation and display of phylogenetic trees made easy. *Nucleic Acids Research* 39:W475-W478.

Lyons WB*, et al.* (2000) Importance of landscape position and legacy: the evolution of the lakes in Taylor Valley, Antarctica. *Freshwater Biology* 43(3):355-367.

Lyons WB*, et al.* (2003) Surface glaciochemistry of Taylor Valley, southern Victoria Land, Antarctica and its relationship to stream chemistry. *Hydrological Processes* 17(1):115-130.

Magalhaes C*, et al.* (2012) At limits of life: Multidisciplinary insights reveal environmental constraints on biotic diversity in continental Antarctica. *Plos One* 7(9).

Moorhead DL*, et al.* (1999) Ecological legacies: Impacts on ecosystems of the McMurdo Dry Valleys. *Bioscience* 49(12):1009-1019.

Morgan-Kiss RM, Priscu JC, Pocock T, Gudynaite-Savitch L, & Huner NPA (2006) Adaptation and acclimation of photosynthetic microorganisms to permanently cold environments. *Microbiology and Molecular Biology Reviews* 70(1):222-+.

Nemergut DR*, et al.* (2005) Structure and function of alpine and arctic soil microbial communities. *Research in Microbiology* 156(7):775-784.

Nemergut DR*, et al.* (2008) The effects of chronic nitrogen fertilization on alpine tundra soil microbial

communities: implications for carbon and nitrogen cycling. *Environmental Microbiology* 10(11):3093-3105.

Neufeld JD, Dumont MG, Vohra J, & Murrell JC (2007) Methodological considerations for the use of stable isotope probing in microbial ecology. *Microbial Ecology* 53(3):435-442.

Neufeld JD*, et al.* (2007) DNA stable-isotope probing. *Nature Protocols* 2(4):860-866.

Neufeld JD, Wagner M, & Murrell JC (2007) Who eats what, where and when? Isotope-labelling experiments are coming of age. *Isme Journal* 1(2):103-110.

Nicolaus B*, et al.* (1992) Isolation of extremely holotolerant cocci from Antarctica. *Fems Microbiology Letters* 99(2-3):145-149.

Niederberger TD*, et al.* (2008) Microbial community composition in soils of Northern Victoria Land, Antarctica. *Environmental Microbiology* 10(7):1713-1724.

Parsons AN, Barrett JE, Wall DH, & Virginia RA (2004) Soil carbon dioxide flux in Antarctic dry valley ecosystems. *Ecosystems* 7(3):286-295.

Philippot L*, et al.* (2009) Spatial patterns of bacterial taxa in nature reflect ecological traits of deep branches of the 16S rRNA bacterial tree. *Environmental Microbiology* 11(12):3096-3104.

Philippot L*, et al.* (2009) Spatial patterns of bacterial taxa in nature reflect ecological traits of deep branches of the 16S rRNA bacterial tree. *Environmental Microbiology* 11(12):3096-3104.

Quaiser A*, et al.* (2003) Acidobacteria form a coherent but highly diverse group within the bacterial domain: evidence from environmental genomics. *Molecular Microbiology* 50(2):563-575.

Radajewski S*, et al.* (2002) Identification of active methylotroph populations in an acidic forest soil by stableisotope probing. *Microbiology-Sgm* 148:2331-2342.

Sabacka M*, et al.* (2012) Aeolian flux of biotic and abiotic material in Taylor Valley, Antarctica. *Geomorphology* 155:102-111.

Sambrook J & Russell DW (2001) Molecular cloning: A laboratory manual. *Molecular cloning: A laboratory manual*.

Sawicka JE, Robador A, Hubert C, Jorgensen BB, & Bruchert V (2010) Effects of freeze-thaw cycles on anaerobic microbial processes in an Arctic intertidal mud flat. *Isme Journal* 4(4):585-594.

Schimel DS*, et al.* (2001) Recent patterns and mechanisms of carbon exchange by terrestrial ecosystems. *Nature* 414(6860):169-172.

Schimel J, Balser TC, & Wallenstein M (2007) Microbial stress-response physiology and its implications for ecosystem function. *Ecology* 88(6):1386-1394.

Schloss PD*, et al.* (2009) Introducing mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Applied and Environmental Microbiology* 75(23):7537-7541.

Shravage BV, Dayananda KM, Patole MS, & Shouche YS (2007) Molecular microbial diversity of a soil sample and detection of ammonia oxidizers from Cape Evans, Mcmurdo Dry Valley, Antarctica. *Microbiological Research* 162(1):15-25.

Simmons BL*, et al.* (2009) Terrestrial mesofauna in above- and below-ground habitats: Taylor Valley, Antarctica. *Polar Biology* 32(11):1549-1558.

Sinclair BJ, Klok CJ, Scott MB, Terblanche JS, & Chown SL (2003) Diurnal variation in supercooling points of three species of Collembola from Cape Hallett, Antarctica. *Journal of Insect Physiology* 49(11):1049-1061.

Townsend-Small A, McClelland JW, Holmes RM, & Peterson BJ (2011) Seasonal and hydrologic drivers of dissolved organic matter and nutrients in the upper Kuparuk River, Alaskan Arctic. *Biogeochemistry* 103(1-3):109-124.

Ugolini FC (1988) Antarctica- soils, weathering processes and environment- Campbell,IB, Claridge, GGC. *Nature* 332(6163):404-404.

Wada E, Shibata R, & Torii T (1981) N-15 abundance in Antarctica- origin of soil-nitrogen and ecological implications. *Nature* 292(5821):327-329.

Walker VK, Palmer GR, & Voordouw G (2006) Freeze-thaw tolerance and clues to the winter survival of a soil community. *Applied and Environmental Microbiology* 72(3):1784-1792.

Wall DH & Virginia RA (1999) Controls on soil biodiversity: insights from extreme environments. *Applied Soil Ecology* 13(2):137-150.

Wang FP, Gai YB, Chen MX, & Xiao X (2009) Arthrobacter psychrochitiniphilus sp nov., a psychrotrophic bacterium isolated from Antarctica. *International Journal of Systematic and Evolutionary Microbiology* 59:2759-2762.

Wierzchos J, de los Rios A, & Ascaso C (2012) Microorganisms in desert rocks: the edge of life on Earth. *International Microbiology* 15(4):172-182.

Zeglin LH, Sinsabaugh RL, Barrett JE, Gooseff MN, & Takacs-Vesbach CD (2009) Landscape distribution of microbial activity in the McMurdo Dry Valleys: Linked biotic processes, hydrology, and geochemistry in a cold desert ecosystem. *Ecosystems* 12(4):562-573.

Zhang, X, Zwiers FW, Hegerl GC, Lambert FH, Gillett NP, Solomon S, Stott PA, Nozawa T. 2007. Detection of human influence on twentieth-century precipitation trends. Nature 448:461–465.

7. TABLES

Table 1. Soil chemistry from nutrient and water treated soils

	Control	C	W	N	CN
PH	9.74±0.15	9.86±0.12	9.87±0.05	9.46±0.2	9.56±0.17
Conductivity (µS/cm)	219.87±42.07	224.44±40.89	182.89±45.70	185.72±18.23	300.85±97.43
NH_4 –N (µg/g)	0.08±0301a	0.04±0.01a	0.27±0.05a	17.41±1.64b	12.92±2.63b
NO_3NO_2 –N (µg/g)	0.64±0.30a	0.09±0.03a	1.07±0.57a	22.14±4.2b	10.39±2.53a
PO_4 –P (µg/g)	3.63±0.86	2.74±0.39	2.15±0.37	2.46±0.23	4.18±0.74
Total C (%)	0.14±0.01	0.14±0.01	0.12±0.01	0.14±0.02	0.15±0.01
Total N (%)	0.004±0.001a	0.005±0.001a	0.004±0.001a	0.009±0.001ab	0.010±0.001b
Total Organic C (%)	0.03±0	0.06±0.01	0.03±0	0.03±0	0.04±0.01

Data are mean (± SEM, n=8) with different letters indicating significant differences between treatments (P <0.05) based on ANOVA.

Table 2. Responding bacteria based on OTU's from 16S rDNA pyrosequencing data

	C	W	N	CN
Intermediate responders				
Acidobacteria	1.00 ±0 c	2.67 ±0.67 b	13.5 ±0.50 a	0.33 ±0.33 c
Actinobacteria	40.7 ±4.18 b	39.3 ±6.74 b	64.0 ±4.00 a	13.7 ±2.85 c
Alphaproteobacteria	8.00 ±0.58 a	13.0 ±2.89 a	10.0 ±3.00 a	0 b
Bacteriodetes	6.67 ±1.20 b	12.0 ±2.65 a	13.5 ±2.50 a	1.67 ±0.67 b
Betaproteobacteria	7.00 ±1.00 b	11.33 ±0.88 a	9.00 ±2.00 ab	3.00 ±0.58 c
Chloroflexi	2.33 ±0.33 ab	4.33 ±0.88 ab	5.00 ±2.00 a	1.67 ±1.20 b
Cyanobacteria	0 b	0.67 ±0.33 b	2.50 ±0.50 a	0.67 ±0.67 b
Deltaproteobacteria	0.67 ±0.67 b	4.33 ±0.88 a	1.00 ±0 b	0 b
Deinococcus	1.00 ±0 bc	2.67 ±0.33 a	1.50 ±0.50 b	0.33 ±0.33 c
Firmicutes	1.33 ±33 a	2.33 ±0.67 a	0 b	0 b
Gammaproteobacteria	4.00 ±1.00 ab	6.67 ±1.20 a	6.50 ±1.50 a	2.67 ±0.33 b
Gemmatimonadetes	0 b	5.33 ±0.67 a	5.00 ±1.00 a	1.00 ±0.58 b
Planctomycetes	2.00 ±0 b	1.33 ±0.88 b	9.00 ±2.00 a	0 b
Verrucomicrobia	1.00 ±0.58 c	2.33 ±0.33 b	4.50 ±0.50 a	0 c
Major responders				
Acidobacteria	0 c	4.0 ±0 a	1.00 ±0 b	0 c
Actinobacteria	2.00 ±0 a	0 ±0 b	3.00 ±0 a	0.67 ±0.67 b
Bacteriodetes	0 c	2.33 ±0.33 a	2.00 ±0 a	1.00 ±0 b
Betaproteobacteria	0 c	2.00 ±0 a	0 c	1.00 ±0 b
Chloroflexi	0 b	1.00 ±0 a	0 b	0.33 ±0.33 b
Gammaproteobacteria	0 c	2.00 ±0 a	1.00 ±0 b	0.67 ±0.33 b
Gemmatimonadetes	0	1.00 ±0	0	0.67 ±0.67

The number of responding bacteria in thirteen of the most highly represented bacterial taxa (phyla and Proteobacteria classes) from the addition treatments. The intermediate and major responders are based on a response ratio from the OTUs from 16S rDNA pyrosequencing data. The response ratio was calculated by dividing the relative recoveries of responding taxa in each phyla or subclass from isotopically labeled rDNA by the relative recoveries of seed bank or unlabeled rDNA of taxa in the given phyla or subclass. Data are mean (± SEM, n=3) with different letters indicating significant differences among treatments (P <0.05) based on ANOVA.

8. FIGURES

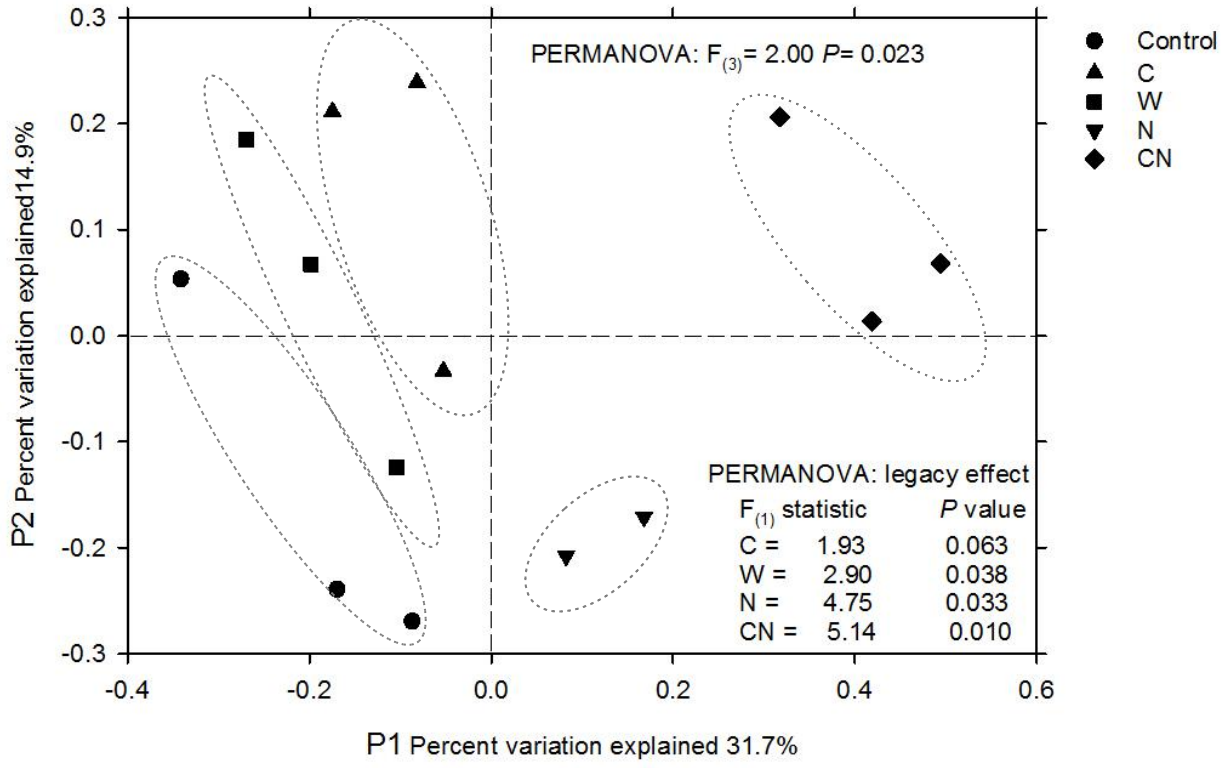


Figure 1. Nutrient and water legacy effects on bacterial communities. Principle component analysis (PCoA) plot of bacterial species composition in carbon (C), nitrogen (N), C and N, and water additions. Communities (*n*=3) are based on operational taxonomic units (OTU) using 97% similarity cut-offs from 16S rDNA pyrosequenced libraries. Significant differences between treatments and control communities are based on permutational multivariate analysis of variance (PERMANOVA) comparisons with attending F statistic and *P* value. The overall PERMANOVA results are in the upper right-hand corner of the figure and the PERMANOVA results comparing each addition treatment to the control is in the lower right-hand corner.

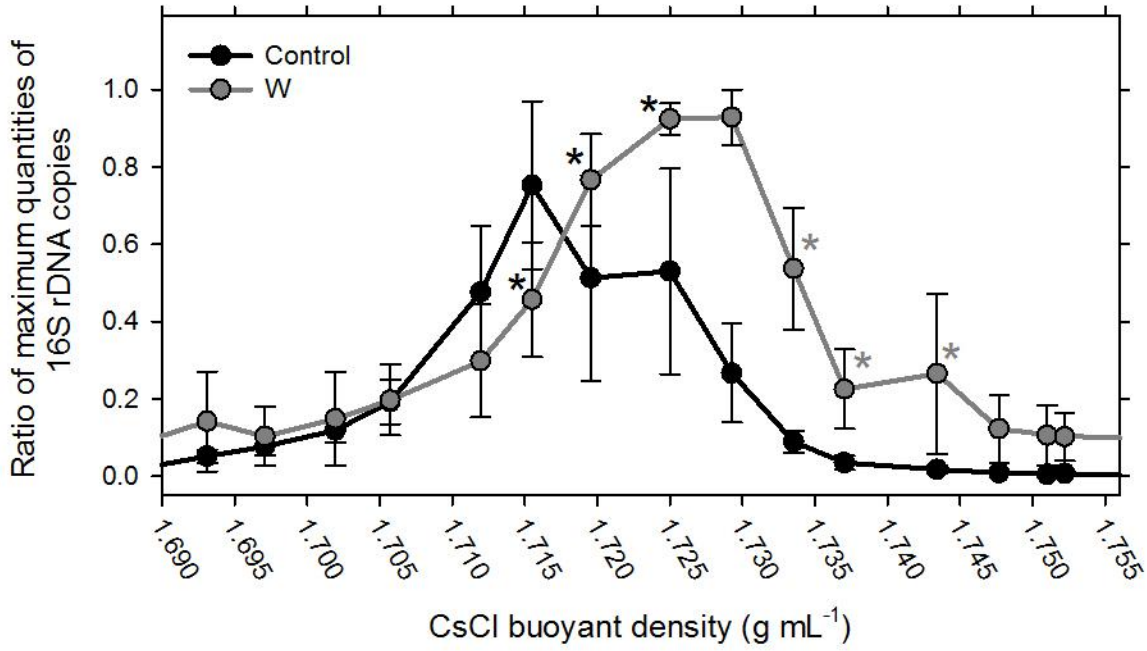


Figure 2. 18O-DNA SIP of 18O and 16O-labeled bacterial rDNA following the addition of $H_2{}^{18}O$ water (W) and rDNA in a soil without an addition (Control). Values are means of the resulting quantities of rRNA gene copies (*n*=3) with ± SEM within cesium chloride (CsCl) gradients that are relativized by expressing gene copies as a ratio of the maximum quantity of copies per rDNA qPCR replicate. The DNA amplified in each density fraction showed distinct separation of labeled (grey asterisks) and unlabeled DNA (black asterisks) to correctly separate and identify microbes that have incorporated the heavy isotope into their DNA structure. Results are from a 7-day laboratory incubation at 5°C. Asterisks depict DNA samples used for target metagenomics.

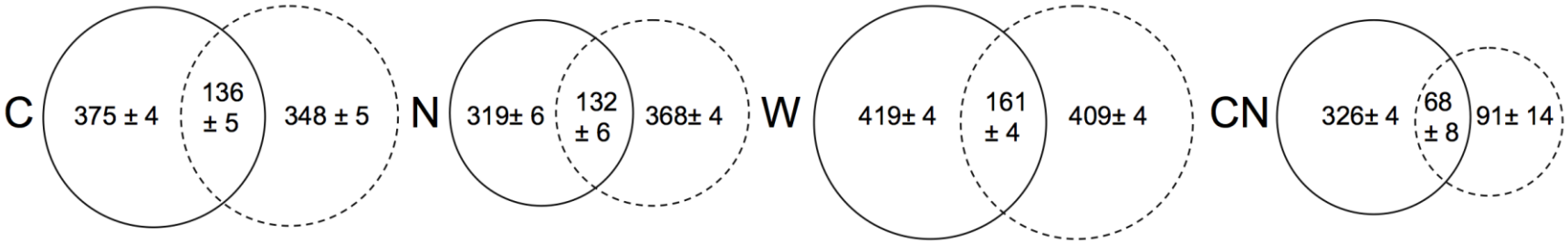


Figure 3. Venn diagrams of bacterial taxa from responding (dotted circles) and seed bank bacteria (solid circles), and the taxa these communities have in common. The number of taxa (n=3) are based on Chao 1 richness estimates of operational taxonomic units (OTU) using 97% similarity cut-offs from 16S rDNA pyrosequenced libraries.

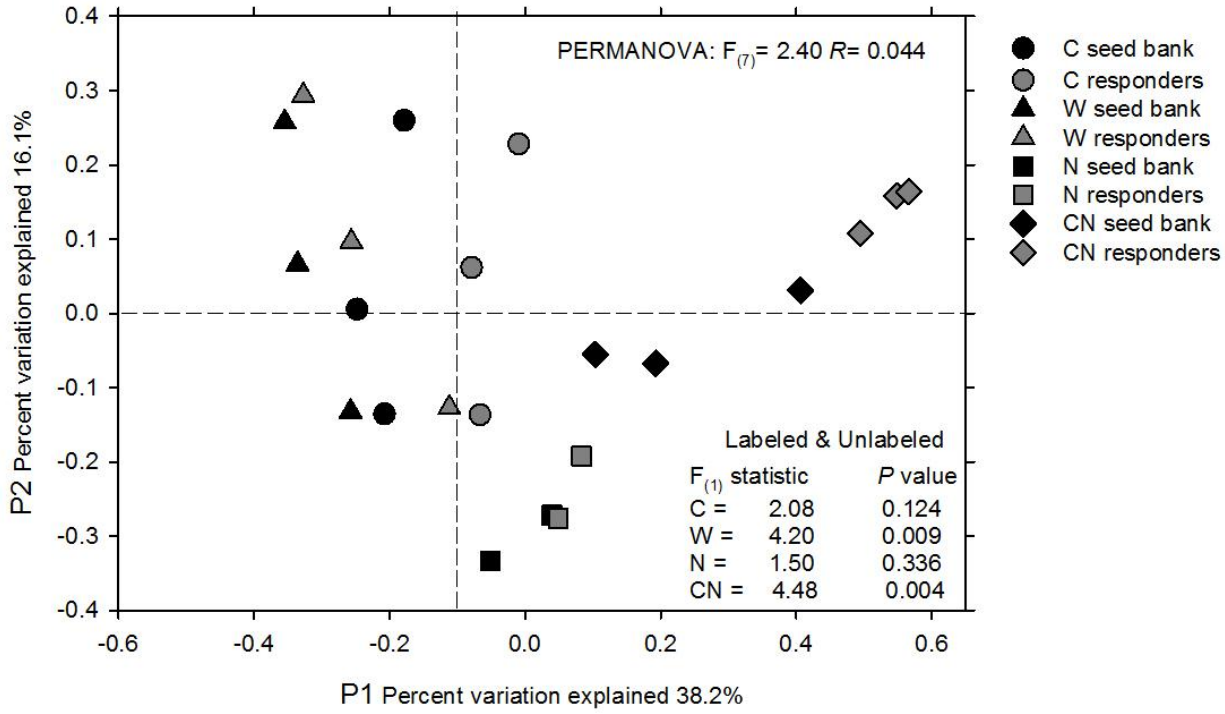


Figure 4. Responding and seed bank community composition from nutrient and water treatment soils. Principle component analysis (PCoA) plot of bacterial communities C, W, CN, and water isotopically labeled and unlabeled rDNA. Communities (n=3) are based on operational taxonomic units (OTU) using 97% similarity cut-offs from 16S rDNA pyrosequenced libraries. Significant differences between responding and seed bank communities are based on PERMANOVA comparisons with attending F statistic and P value in the lower right-hand corner of the figure.

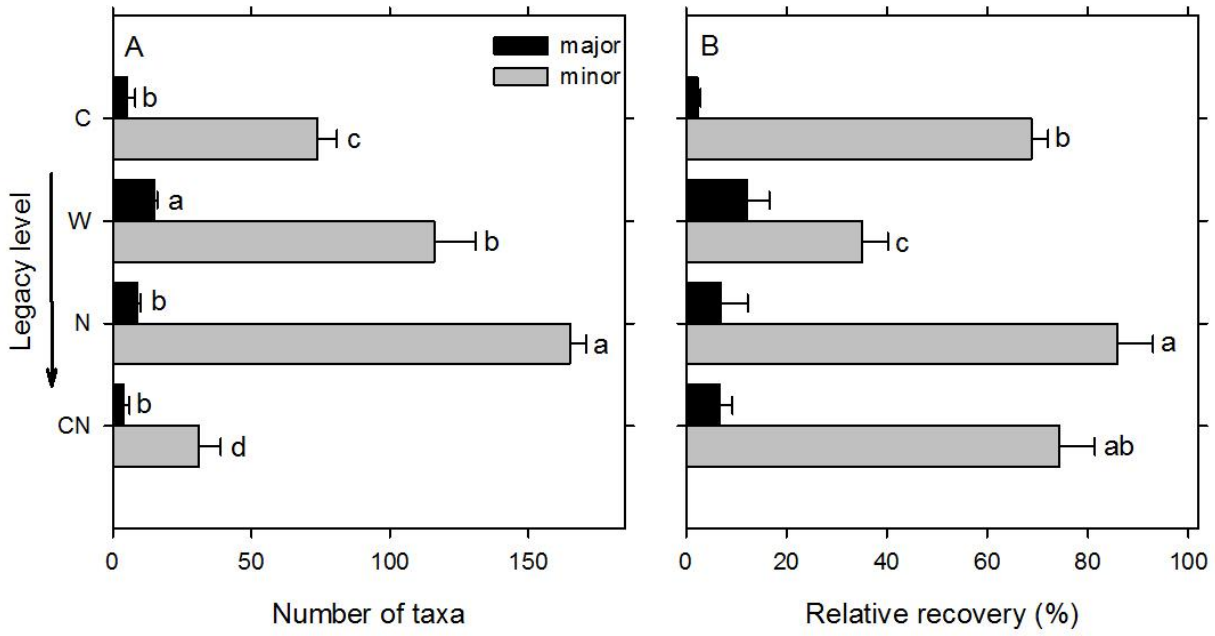


Figure 5. Number of taxa (A) and relative recovery (B) of major and minor responding bacteria in nutrient and water additions. Data (mean ± SEM, *n*=3) are based on the relativized OTUs from 16S rDNA copies from pyrosequencing (isotopically labeled rDNA) and unlabeled (unlabeled rDNA) relative recoveries. Responders were divided into two categories of responders: minor responders—at least doubled relative recovery from labeled and unlabeled rDNA, and major responders—at least a ten-fold relative recovery from labeled and unlabeled rDNA. The response ratio was calculated by dividing the relative recoveries of responding taxa from isotopically labeled rDNA by the relative recoveries of seed bank or unlabeled rDNA taxa.

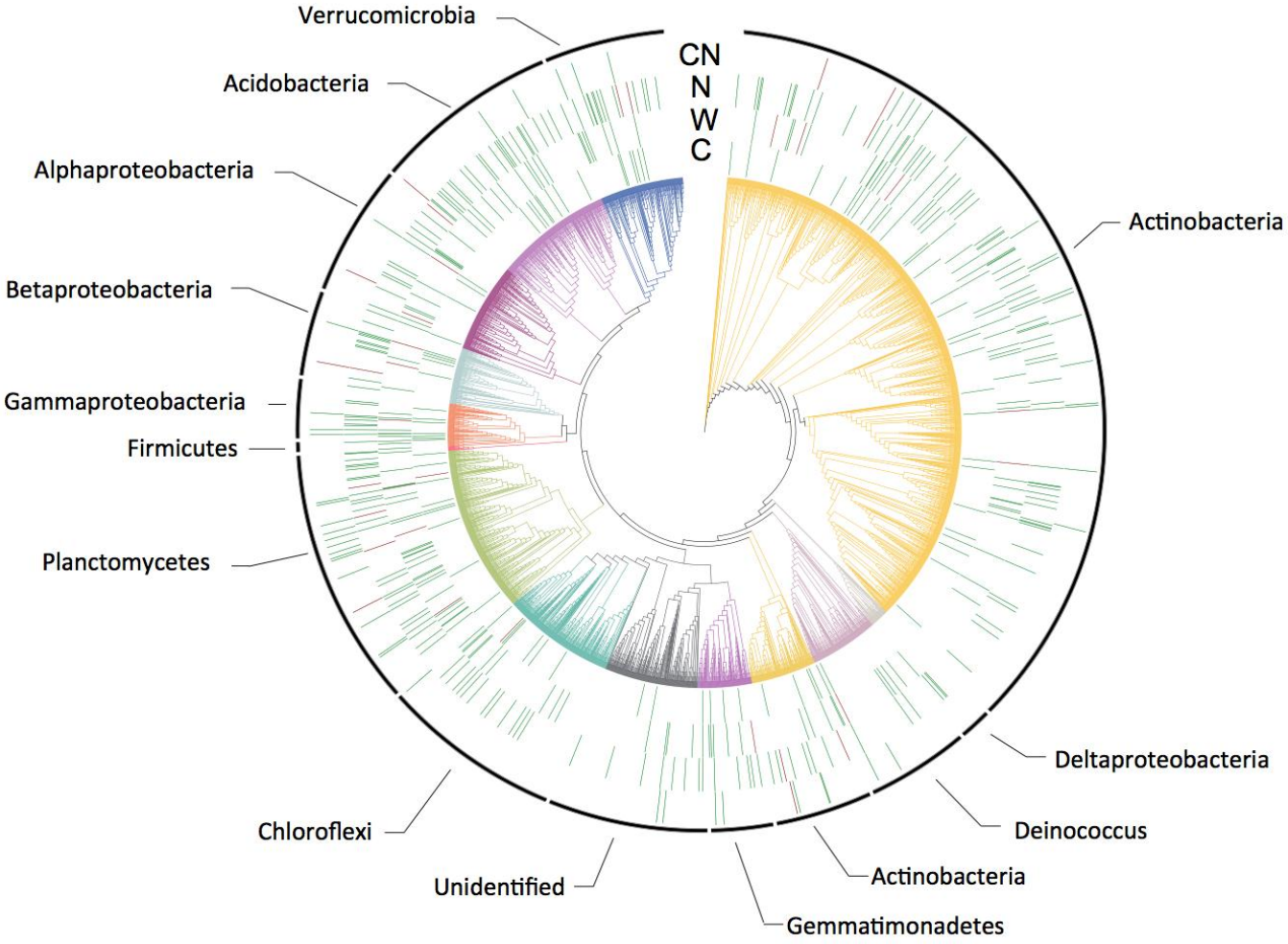
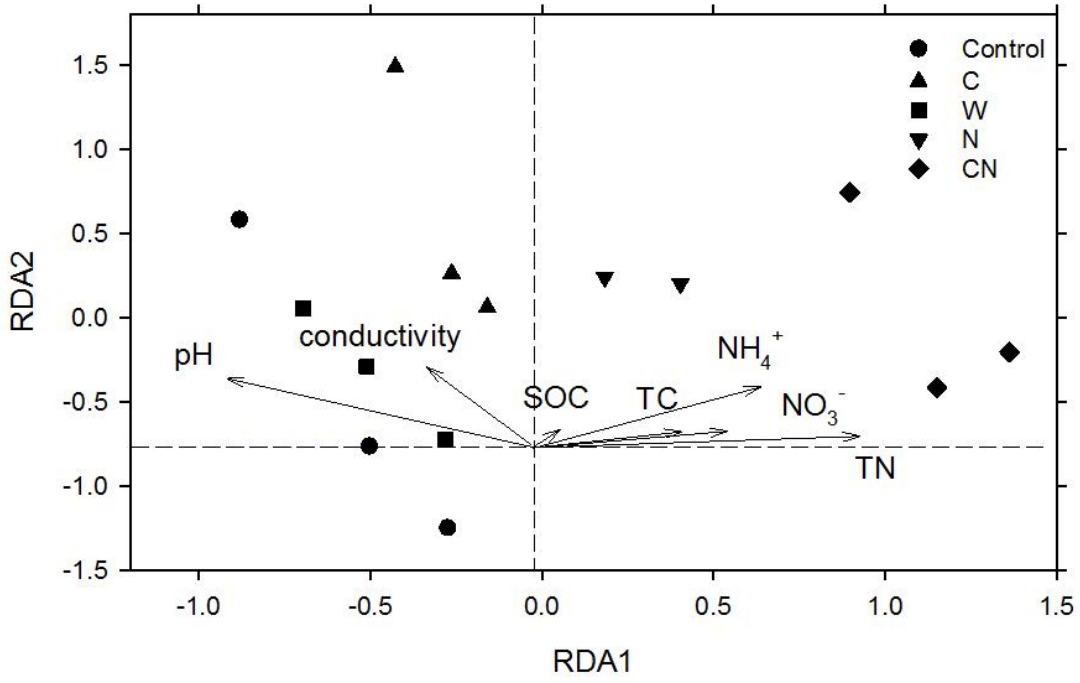
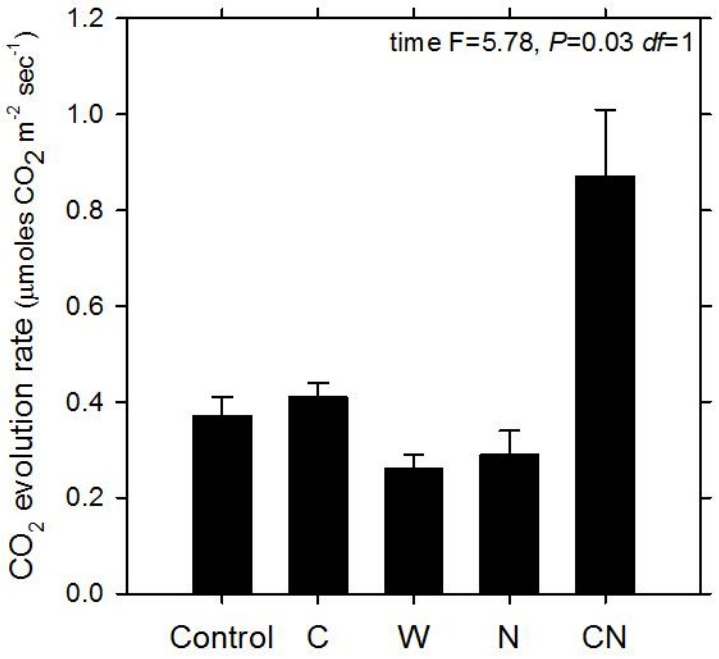


Figure 6. ITOL tree linking responding microorganisms to each treatment. Major responders are represented in red and minor responders are in green. Actinobacteria represent the largest phylum of responding microbes. Phylogenetic tree and bar chart representing the major and minor responding bacterial species in nutrient and water addition soils. The bars on the perimeter of the tree denote responding species. The rings around the tree represent the different treatments (i.e., C, W, N, and CN). Bars are means (n=3) calculated from a response ratio of responding (isotopically labeled rDNA) and unlabeled (unlabeled rDNA) relative recoveries.

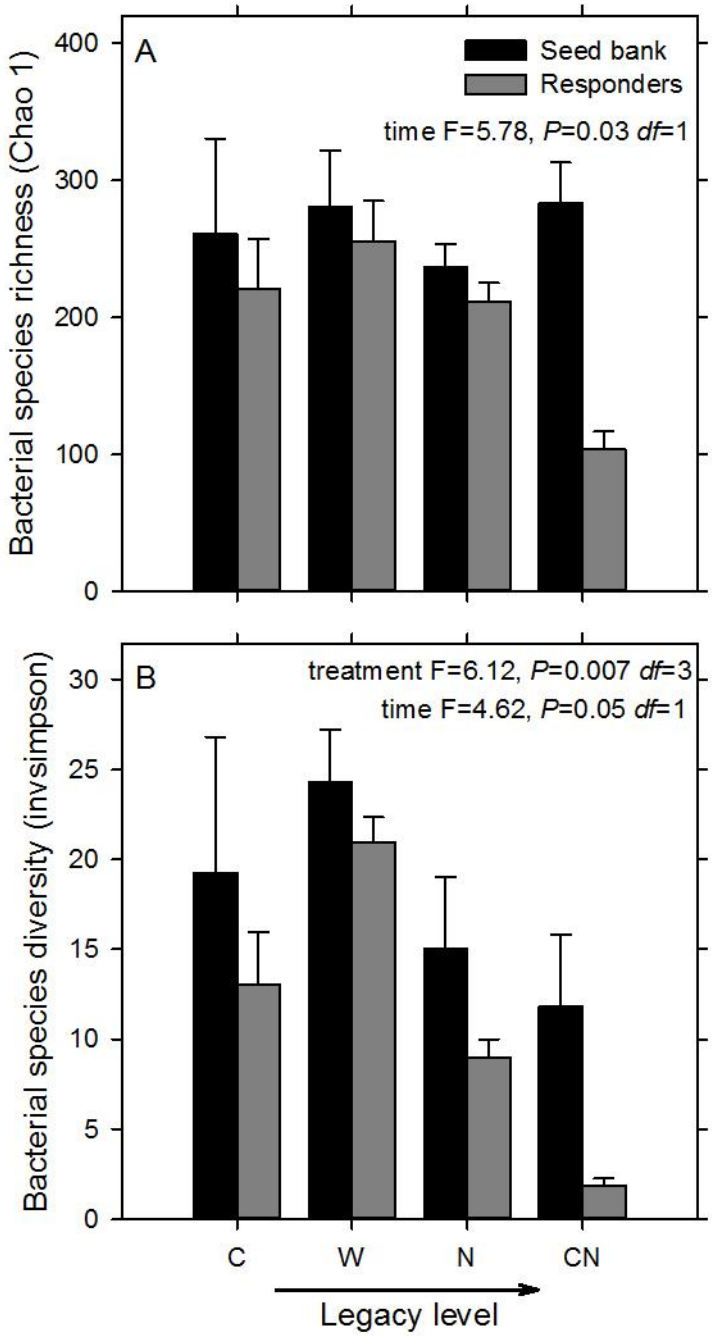
APPENDIX



Supplemental Figure 1. The relationship of bacterial communities and the soil environment using redundancy analysis. The environmental variables include: DOC, NH_4^+ and NO_3^- concentrations, total N, total C, conductivity, and pH.



Supplemental Figure 2. Soil respiration from nutrient and water treated soils. Data are mean (± SEM, *n*=3) with the treatment exhibiting significant effects based on ANOVA.



Supplemental Figure 3. Species richness and diversity in nutrient and water treated soils. Data are mean (± SEM, *n*=3) with the time exhibiting significant effects based on ANOVA.