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

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Resource Legacies and Priming Regulate Microbial Communities in

Antarctica’s Dry Valleys

Sabrina Deni Saurey

A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of

Master of Science

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ABSTRACT

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

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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$_4$NO$_3$ + 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$_4$NO$_3$, 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 communities, 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 km²) 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.,
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 CO₂ 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-NH$_4$NO$_3$, 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 \(\times 8\) replicates = 40 experimental units). Each experimental unit (1 m \(\times\) 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 \( \text{NH}_4^+ \) and \( \text{NO}_3^- \) concentrations, soil respiration, total N, total C, \( \text{PO}_4^- \), conductivity, pH, soil moisture) using ANOVA (SAS PROC GLM) and Tukey’s HSD test to identify significant differences among the treatments.

We measured soil CO2 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 25μl 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 94°C for 3 minutes followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 50°C for 30 seconds, and an extension at 72°C 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™ 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 Bray-Curtis 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 \textit{adonis} in the vegan package of the R Statistics Environment (R Development Core Team 2008).

2.5. \textit{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-$\text{NH}_4\text{NO}_3$, and $^{18}$O-$\text{H}_2\text{O}$ (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 $\mu$L 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 Masterscycler 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: $F_{(4)} = 5.78 \quad P = 0.03$, (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 $170 \pm 10$ taxa and the water treatment stimulated $120 \pm 10$ 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 Oxalobacteria; 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


7. TABLES

**Table 1.** Soil chemistry from nutrient and water treated soils

<table>
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<th></th>
<th>Control</th>
<th>C</th>
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<th>N</th>
<th>CN</th>
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<tr>
<td><strong>PH</strong></td>
<td>9.74±0.15</td>
<td>9.86±0.12</td>
<td>9.87±0.05</td>
<td>9.46±0.2</td>
<td>9.56±0.17</td>
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<td><strong>Conductivity (µS/cm)</strong></td>
<td>219.87±42.07</td>
<td>224.44±40.89</td>
<td>182.89±45.70</td>
<td>185.72±18.23</td>
<td>300.85±97.43</td>
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<td><strong>NH₄–N (µg/g)</strong></td>
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<td>0.04±0.01a</td>
<td>0.27±0.05a</td>
<td>17.41±1.64b</td>
<td>12.92±2.63b</td>
</tr>
<tr>
<td><strong>NO₃NO₂–N (µg/g)</strong></td>
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<td>0.09±0.03a</td>
<td>1.07±0.57a</td>
<td>22.14±4.2b</td>
<td>10.39±2.53a</td>
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<td><strong>PO₄–P (µg/g)</strong></td>
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<td>2.74±0.39</td>
<td>2.15±0.37</td>
<td>2.46±0.23</td>
<td>4.18±0.74</td>
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<td><strong>Total C (%)</strong></td>
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<td>0.14±0.01</td>
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<td><strong>Total N (%)</strong></td>
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<td>0.005±0.001a</td>
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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

<table>
<thead>
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<td>Acidobacteria</td>
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<td>2.67 ±0.67 b</td>
<td>13.5 ±0.50 a</td>
<td>0.33 ±0.33 c</td>
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<td>Actinobacteria</td>
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<td>13.7 ±2.85 c</td>
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<td>Alphaproteobacteria</td>
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<td>13.0 ±2.89 a</td>
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<td>13.5 ±2.50 a</td>
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<td><strong>Major responders</strong></td>
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</table>

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.
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.