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ERDC 6.2 Geospatial Research and Engineering (GRE) ARTEMIS STO-R DUST-CLOUD

Heterotrophic Microbial Communities in Biological Soil Crusts

Responses to Temperature and Precipitation

Stacey J. Doherty, Sandra L. LeGrand, Karen L. Foley, Shelby A. Rosten, Robert M. Jones, Andmorgan R. Fisher, Masoumeh Sikaroodi, Pat Gillevet, and Robyn A. Barbato

August 2018

Biological soil crusts (BSCs) before and after wetting

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Final Report

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Under ERDC 6.2 Geospatial Research and Engineering (GRE) Applied Research Program's Army Terrestrial-Environmental Modeling and Intelligence System Science Technology Objective—Research (ARTEMIS STO-R), "Dynamic Undisturbed Soils Testbed to Characterize Local Origins and Uncertainties of Dust (DUST-CLOUD)" (WIC 4433FB/U4357514) and "Dynamic Representation of Terrestrial Soil Predictions of Organisms' Response to the Environment (DRT-SPORE)" (WIC 05423J), and ERDC 6.1 GRE "Resolving the Size Distribution of Mineral Dust" (WIC 2469K1/U4357455)

Abstract

Mineral dust affects many of Earth's processes (e.g., radiative forcing, nutrient distribution, and hydrology) and poses a risk to Army maneuverability and situational awareness. Understanding soil processes and how these relate to potential dust emission is of increasing concern as adverse effects of dust become more prevalent. Biological soil crusts (BSCs), commonly found at the soil surface in arid and semiarid regions of the world, protect soils from wind and water erosion. While there is a rich understanding of the behaviors of photosynthetic organisms within BSCs, they are only part of the community. Understanding the other component, the nonphotosynthetic microorganisms and their response to environmental stimuli (i.e., temperature and moisture), will improve dust forecasting models and current soil-stabilization methods.

We conducted a laboratory incubation study to investigate the effects of simulated precipitation events and cooling on the nonphotosynthetic microbial community that made up approximately 40% of the bacterial community in our samples. Our results show how temperature and moisture influence the diversity and resilience of the microbial community and its structure.

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Preface

This study was conducted for the Assistant Secretary of the Army for Acquisition, Logistics, and Technology under the U.S. Army Engineer Research and Development Center (ERDC) 6.2 Geospatial Research and Engineering (GRE) Applied Research Program's Army Terrestrial-Environmental Modeling and Intelligence System Science Technology Objective—Research (ARTEMIS STO-R), "Dynamic Undisturbed Soils Testbed to Characterize Local Origins and Uncertainties of Dust (DUST-CLOUD)" (WIC 4433FB/U4357514) and "Dynamic Representation of Terrestrial Soil Predictions of Organisms' Response to the Environment (DRTSPORE)" (WIC 05423J), and ERDC 6.1 GRE "Resolving the Size Distribution of Mineral Dust" (WIC 2469K1/U4357455).

The work was performed by the Biogeochemical Sciences Branch (CEERD-RRN) of the Research and Engineering Division (CEERD-RR), U.S. Army ERDC Cold Regions Research and Engineering Laboratory (CRREL). At the time of publication, Dr. Justin Berman was Chief, CEERD-RRN; and Mr. J. D. Horne was Chief, CEERD-RR. The Deputy Director of ERDC-CRREL was Mr. David B. Ringelberg, and the Director was Dr. Joseph L. Corriveau.

We would like to thank the research staff at the Jornada Experiment Range, specifically Dr. Nicholas Webb and Mr. Brad Cooper, for site access. We would also like to thank Ms. Ashley Mossell, CRREL, for soils analysis support.

COL Ivan P. Beckman was the Commander of ERDC, and Dr. David W. Pittman was the Director.

Acronyms and Abbreviations

ANOVA	Analysis of Variance
ARTEMIS	Army Terrestrial-Environmental Modeling and Intelligence System
bp	Base Pair
BSC	Biological Soil Crust
C	Control Samples (no water addition during study)
CO_2	Carbon Dioxide
CRREL	Cold Regions Research and Engineering Laboratory
D	Dry Samples (initially dry during incubation and received moisture partway through study)
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleotide Triphosphate
DRTSPORE	Dynamic Representation of Terrestrial Soil Predictions of Organisms' Response to the Environment
DUST-CLOUD	Dynamic Undisturbed Soils Testbed to Characterize Local Origins and Uncertainties of Dust
EPS	Exopolysaccharides
ERDC	Engineer Research and Development Center
GRE	Geospatial Research and Engineering
GWC	Gravimetric Water Content
JER	Jornada Experimental Range
$MgSO_4$	Magnesium Sulfate
OTU	Operational Taxonomic Unit
PCoA	Principal Coordinates Analysis

PCR	Polymerase Chain Reaction
PGM	Personal Genome Machine
QIIME	Quantitative Insights Into Microbial Ecology
rDNA	Recombinant Deoxyribonucleic Acid
SEM	Scanning Electron Microscopy
STO-R	Science Technology Objective—Research
USDA-ARS	United States Department of Agriculture's Agricultural Research Service
W	Wet Samples (received moisture at the start of incubation)
WSC	Wet Sand Control

1 Introduction

1.1 Background

Biological soil crusts (BSCs) are created by diverse communities of microorganisms inhabiting the first few millimeters of surface soil. The primary colonizers of these communities, cyanobacteria, secrete sticky exopolysaccharides (EPS) that bind soil particles together into crusts (Bowker et al. 2010; Baran et al. 2015). BSC presence can influence carbon and nutrient cycling (Elbert et al. 2012; Porada et al. 2014); alter terrain albedo (Rutherford et al. 2017); modulate soil hydrology (Belnap 2003; Austin et al. 2004); and strongly enhance soil aggregate formation and soil stabilization, reducing the potential for soil erosion and dust emission (Bowker 2007). BSCs are prevalent and are estimated to cover more than 35% of the Earth's land surface (Bu et al. 2014), including arid and semiarid regions (Belnap 2003) and temperate regions with limited plant cover (Belnap et al. 2001). Climate conditions are highly variable at sites containing BSCs, requiring these organisms to be tolerant of extreme temperature conditions (Belnap et al. 2001). Given their functional role in terrain processes and abundant coverage of land surface, BSCs likely play a key role in climatic and biogeochemical processes on both local- and global-scales (Ferrenberg et al. 2017). Specifically, on local scales, effective Army maneuverability and situational awareness rely heavily on the fundamental understanding of how BSCs affect soil processes and soil stability. In weak and highly erodible soils where BSCs are not prevalent, lofted particles negatively impact mobility, sensor performance, and human health.

The lifecycle of BSCs in arid regions has been shown to be directly tied to the availability of water (Austin et al. 2004; Belnap 2001; Belnap et al. 2001), typically measured through CO_2 (carbon dioxide) efflux of the system. BSCs grow when cyanobacteria immediately below the soil surface become wetted and stretch their filaments throughout the soil substrate (Belnap 2006). As the soil dries, the cyanobacteria filaments retract, leaving sheath material behind (Belnap 2003). It is estimated that BSCs are active less than 10% of the time given limited precipitation in arid regions (Lange et al. 1994). Over time, the soil particles become increasingly bound together as different species of cyanobacteria experience cycles of wetting and drying and leave behind dense networks of polysaccharide

sheaths (Belnap 2006). Once the soil is somewhat stabilized by this process, lichens and moss will colonize and grow above the soil surface (Belnap 2006). The lichens then provide anchoring structures for fungal materials, which extend into the top few millimeters of soil (Belnap et al. 2001). Many genera and species have been found worldwide, including *Microcoleus vaginatus*, *Psora decipiens*, *Collema tenax*, *Collema coccophorum*, and *Catapyrenium squamu* (Belnap 2003). This suggests that there are key microbial members constituting BSCs, and this may help in understanding the biogeography of BSCs and their role in climatic and biogeochemical processes on a global scale.

A developed BSC contains both autotrophic microbes like cyanobacteria, algae, and lichens, which harness their energy from photosynthesis, and heterotrophic organisms like fungi, other bacteria, archaea, and microfauna, which use organic carbon sources for energy. The heterotrophic microorganisms within BSCs are much more diverse yet less abundant than their counterparts; however, very little is known regarding the ecology and function of these organisms, primarily how they might enhance EPS production (Bowker et al. 2010). These two different types of microbes will eventually develop an interdependence within the BSC. For example, autotrophic BSC elements rely on nitrogen produced by the heterotrophic microbes to grow; and the heterotrophic microbes, in turn, require products of photosynthesis and decomposed organic BSC materials to produce nitrogen (Belnap 2001; Bowker et al. 2010). Furthermore, Castillo-Monroy et al. (2011) describe BSC systems as microcosms where the autotrophic components behave somewhat like vascular plants, providing nutrients, energy, and habitat for the heterotrophic organisms, which function like rhizosphere bacteria.

1.2 Objective

To date, most BSC research has focused on autotrophic microbes and their response to precipitation events and climate stressors. Bu et al. (2014) found that soil moisture was the most important factor affecting BSC cyanobacteria development as compared to shading and nutrient amendments. Another study found that photosynthetic rates of BSC-associated lichens were negatively affected by both desiccation and too much hydration (Lange et al. 1998). This held true for the range of temperatures tested, from 2°C to 41°C.

Processes in which heterotrophic BSC microbes contribute to soil productivity and behavior, however, are still not fully understood. The goal of our study was to measure the response of heterotrophic BSC microbe activity to changes in temperature and moisture. Furthermore, we examined the effects of these environmental inputs to soil strength metrics.

1.3 Approach

We conducted a laboratory incubation study to investigate the effects of simulated precipitation events and cooling on the heterotrophic microbial community. BSC samples were collected from a study site at the Jornada Experimental Range in Las Cruces, New Mexico, USA. Samples were subjected to environmentally relevant inputs and microbial respiration rates and were monitored throughout the incubation. At specific times, BSCs were destructively sampled to capture shifts in the microbial community structure and to measure compressive strength and soil properties of the BSCs.

2 Material and Methods

2.1 Sample collection

Biological soil crusts were collected from a study site (32.534946, −106.718999, 1317 m) at the Jornada Experimental Range (JER), Las Cruces, New Mexico, in May 2017 (Figure 1). The JER is located in the Chihuahua Desert and experiences an average annual rainfall of 245 mm (Wainright 2006). Average springtime temperature ranges from a minimum of 8°C to a maximum of 22°C (Wainright 2006). The study site was established in April 2013 primarily for wind erosion research (Webb et al. 2016) and is maintained by the United States Department of Agriculture's Agricultural Research Service (USDA-ARS).

Figure 1. Map of the sampling location, JER, Las Cruces, New Mexico.

The site is located on a loam soil with both physical and biological soil crusts and is surrounded by sparse burrograss (*Scleropogon brevifolius*

Phil.) (Webb et al. 2016). The BSCs collected were classified as smooth crusts, which are typically found in hot deserts where soils do not freeze and are mainly composed of cyanobacteria, algae, and fungi (Belnap et al. 2001). Lichens, cyanobacteria, and filamentous *Nostoc* was visually observed on the surface of the BSCs (Figure 2).

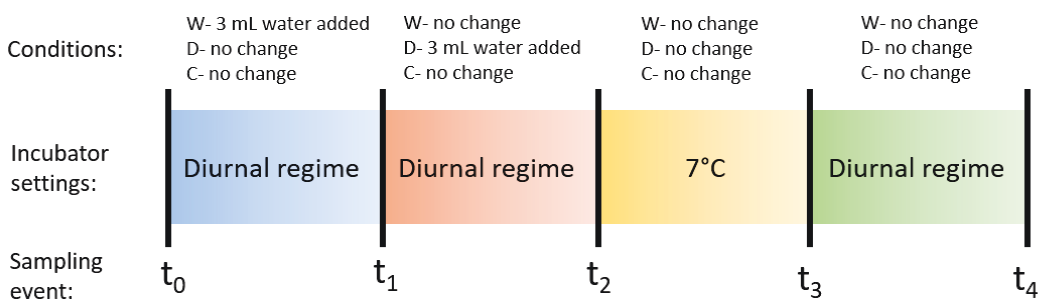
Figure 2. Study site with BSCs surrounding the site edge.

The BSCs were sampled from the area surrounding the experimental site (Figure 2). Samples were collected from three different locations at the site to ensure a more representative sampling of the area. BSCs were collected using aseptic tools. In brief, a 10 × 10 cm square metal scoop was cleaned with 70% ethanol, RNase AWAY, and DNase AWAY before the top 3 cm of soil were extracted from a given sampling location. The BSCs were then lifted off of the scoop to leave behind loose soil under the crust surface and placed in sterile petri dishes and sealed. Samples were placed on ice immediately after sampling and subsequently refrigerated until shipment back to the laboratory, after which they were stored at 7°C in the dark.

2.2 Incubation study

To determine the response of the heterotrophic microorganisms to precipitation events and cooling, we conducted an incubation study with discrete sampling events to capture shifts in the microbial community structure, strength, and soil properties. BSCs were subjected to moisture and temperature changes throughout the study (Figure 3). The study consisted of three different conditions: water addition at the start of the study to one set of BSCs (W), water addition after seven days to the other set of BSCs (D), and no water addition at any point during the study (C). An empty well and a wet sand control (WSC) were also included in the study to serve as laboratory controls. Wetting only a subset of the BSCs during the first incubation phase allowed for direct comparison of wet and dry BSCs under the same temperature regime. The D condition crusts were wetted for the second incubation phase to determine if the crusts would respire at similar rates and harbor a similar dominant community as the W crusts. One third of the crusts were not wetted at any point during the study to represent a control experiencing the same temperature regimes as wetted crusts but limited moisture. This allowed us to compare moisture and temperature inputs on the BSCs.

Figure 3. Conceptual diagram of the experiment detailing conditions, incubation phases, and destructive sampling events.



The BSCs were carefully subsampled with effort to minimize disturbance. Approximately 4 g of sterile sand was placed in the bottom of each well, and a sterile glass fiber filter was set on top for the sample to rest on. This decreased the headspace volume in the sample vessel and also provided a means for slow moisture addition. Thirty-six BSCs chunks were placed in sterile well plates designed to hold approximately 17 cm^3 of sample (Figure 4). On average, 6 g of BSC were in each well.

Figure 4. Example plate layout showing BSC chunk on the sterile filter.

Once the plates were assembled, one third of the BSCs were wetted with 3 mL of filter-sterilized water to simulate a precipitation event. Water was pipetted below the sample and into the sand, which allowed the BSC to take up the water through capillary action, ensuring adequate pore water content throughout the sample. Samples were placed to hydrate in an incubator set at 7°C for one hour, after which sample photographs were taken using a DiMAGE Z10 dermatology camera (Konica Minolta, Tokyo, Japan) to document immediate changes in BSC appearance upon wetting (Figure 5).

Figure 5. BSC before (*left*) and after (*right*) the addition of moisture.

Well plates were connected to a Micro Oxymax Respirometer (Columbus Instruments, Columbus, OH, USA) and placed in a dark incubator to

measure constant heterotrophic CO_2 efflux throughout incubation. The incubator was programed to reflect a diurnal cycle of the typical daily high and low temperatures for New Mexico in March, 9.7°C for 12 hours and 21.3°C for 12 hours. Samples were incubated for 6 days and then destructively sampled in triplicate before conditions were altered for the next incubation phase. This was repeated for a total of four samplings. BSCs not used for the experimental set up were used for baseline assessment analysis. At each destructive sampling event, the BSC was transferred to a clean well for strength measurements and then mixed thoroughly in a sterile Whirlpak bag. A small amount of the sample was transferred to a sterile microcentrifuge tube for DNA (deoxyribonucleic acid) analysis, and the remaining sample was saved for soil properties analysis. Samples were stored at 4°C for soil properties analysis and −80°C for DNA analysis.

2.2.1 SEM imaging

Scanning electron microscopy (SEM) was used to obtain surface images of the BSCs to visualize microbial components contributing to soil stabilization. Images were taken using a Phenom ProX microscope (PhenomWorld, Eindhoven, Netherlands). Images were selected to visualize the microbial structures within the BSC and how this may affect soil stabilization.

2.2.2 Soil properties

Soil properties were measured throughout the study to evaluate changes in soil moisture and organic matter content. Soil moisture was analyzed gravimetrically (gravimetric water content, GWC) by heating a known mass of a sample at 105°C for 24 hours in an oven. The sample was first measured at room temperature, it was heated in the oven, and the mass was measured again. Percent GWC was calculated on a dry mass basis.

Soil organic matter content was measured using an adapted protocol from Storer (1984). Dried soil used for gravimetric water content was used for organic matter content determination. The mass of the dried soil was noted and then heated in a muffle furnace at 360°C for 2 hours. The mass was immediately measured once the temperature dropped below 150°C. Percent loss on ignition was calculated using the difference in mass between the two temperatures.

2.2.3 Strength analysis

Unconfined compressive strength was measured at each sampling event to determine changes in BSC strength due to water and temperature inputs. Samples were moved to a sterile well that had 10 g of sterile sand at the bottom. Unconfined compressive strength was measured using a pocket penetrometer fitted with the ¼ in. tip. The tool was cleaned with 70% ethanol between samples. Results were reported in units of kg cm^{-2}.

2.2.4 Respiration analysis

Soil respiration was measured throughout the study as an indicator of heterotrophic microbial activity. The rate of CO_2 efflux was measured through headspace gas approximately every 4 hours with the Micro-Oxymax Respirometer (Columbus Instruments, Columbus, OH, USA). The instrument performed measurements on a closed system. After placing the samples into an incubator, they were connected to the respirometer for 31 days, only being disconnected to apply treatments and to sample at designated time points. CO_2 efflux was reported using the surface area of the BSC to obtain units of mg C-CO_2 m^{-2} day^{-1}. BSC area was determined by uploading into Adobe Photoshop CC 2017 (San Jose, CA, USA) photographs of each crust. An outline of the well was drawn using the lasso tool, and the histogram panel was used to obtain the pixel count of the plot. Areas containing BSC were selected using the color range selection tool, and the histogram panel was used to obtain a pixel count of the BSC areas. The ratio of BSC area pixel count to well area pixel count was acquired and multiplied by the known area of the well to obtain the area of the BSC.

2.2.5 Molecular analysis and sequencing

DNA sequencing was used to evaluate changes in microbial community structure throughout the incubation study. DNA was extracted using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) and analyzed on the Qubit 3.0 Fluorometer (ThermoFisher Scientific, Grand Island, NY, USA) for concentration. A dilution of the extracted DNA (about 10 ng) was used as the template in a polymerase chain reaction (PCR) using Amplitaq Gold polymerase (Applied Biosystems, Foster City, CA, USA) and universal bacterial primers: L27F (5′AGAGTTTGATCMTGGCTCAG3′) forward and 355R (5′ACTCCTACGGGAGGCAGC3′) reverse primers. These primers amplify the first two variable regions of the 16S rDNA (recombinant DNA)

gene, which has shown to be sufficient for identifying bacterial taxa in a microbial community sample.

The primers were made as fusion primers and included the specific adapters for sequencing with PGM (Personal Genome Machine) (Ion Torrent technology, ThermoFisher Scientific, Grand Island, NY, USA). The forward primers also included 8 bp* tags (to differentiate samples in the sequencing pool), and reverse primers had a Fam label attached (for fingerprinting the PCR products before sequencing as quality control). The PCR mix consisted of a final concentration of 1x PCR Gold buffer, 2.0 mM $MgSO_4$ (magnesium sulfate), 0.2 mM of each dNTPs (deoxyribonucleotide triphosphate), 0.5 μM of each forward and reverse primer, and 0.5 units of Taq Gold polymerase in a 20 μl volume reaction. The PCR was done on a 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA) that was programmed for an initial denaturation step at 95°C for 11 min followed by 35–45 cycles of denaturation at 95°C for 30 s, annealing at 48°C for 30 s, extension at 72°C for 2 min, a 30 min final extension at 72°C, and then kept at 4°C. The PCR product was visualized on a 1% agarose gel with ethidium bromide.

For fingerprinting, the PCR products were diluted according to their intensity based on agarose gel electrophoresis and mixed with ILS-600 size standards (Promega, Madison, WI, USA) and HiDi Formamide (Applied Biosystems, Foster City, CA, USA). The diluted samples were separated on an ABI 3130xl fluorescent capillary sequencer (Applied Biosystems, Foster City, CA, USA) and analyzed using the Genemapper software package (Applied Biosystems, Foster City, CA, USA). The duplicate PCR products were checked and confirmed, and the best PCR was selected for pooling into one sample for sequencing. The duplicate PCRs are usually done with different dilutions of DNA, so the best dilution is selected for sequencing after visualizing the products on the capillary. The pooled PCR product was purified twice using Agencourt AMPure magnetic beads (Beckman Coulter, Brea, CA, USA) to ensure elimination of short products and primers. The purified products were visualized on 1% agarose gel and quantified using a DTX880 Multimode Fluorescent detector (Beckman Coulter, Brea, CA, USA) with excitation at 485 nm and emission at 535 nm. The appropriate amount of purified product was calculated and used in an emulsion

* Base pair

PCR for sequencing. The sequencing was done on a PGM using Ion Torrent technology (Applied Biosystems, Foster City, CA, USA) based on the manufacturer's protocols.

Sequences were processed using the open source software package Quantitative Insights Into Microbial Ecology (QIIME) (Caporaso et al. 2010) to obtain the taxonomy of the bacterial community within the samples. Quality control was performed to trim adapters, to remove barcodes and assign sample identifiers, and to filter out low-quality sequences. To obtain taxonomic identity, sequences that passed the quality control procedure were run through the open reference operational taxonomic unit (OTU) picking pipeline using uclust (Edgar 2010) and the GreenGenes gene reference database (DeSantis et al. 2006). In brief, an initial OTU assignment was done based on a matching identity of 97%. Unsuccessful matches were then compared amongst themselves, and a representative sequence was compared to the database again. Phylogenetic comparisons were made using the identified sequences with FastTree (Price et al. 2010). The final outputs were a .biome file of OTUs and a phylogenetic tree file, which were then analyzed with the QIIME core diversity script. As part of the beta analysis was done in this script, a principal coordinates analysis (PCoA) plot was generated using the weighted unifrac (Lozupone and Knight 2005) metric. This script also generated a summary of the taxa present in the samples.

Known autotrophic phyla were separated from the taxa summary so that the heterotrophic components could be analyzed. The taxa summary was imported into JMP 11.0 (SAS Institute Inc., Cary, NC, USA) where analysis of variance (ANOVA) and All-Pairs Tukey tests were run to test for significant differences between sample groups. The groups were based on condition or time point.

Sequences belonging to each time point were extracted and analyzed in QIIME individually with the same procedure. This allowed the condition of the samples to be compared at each time point separately.

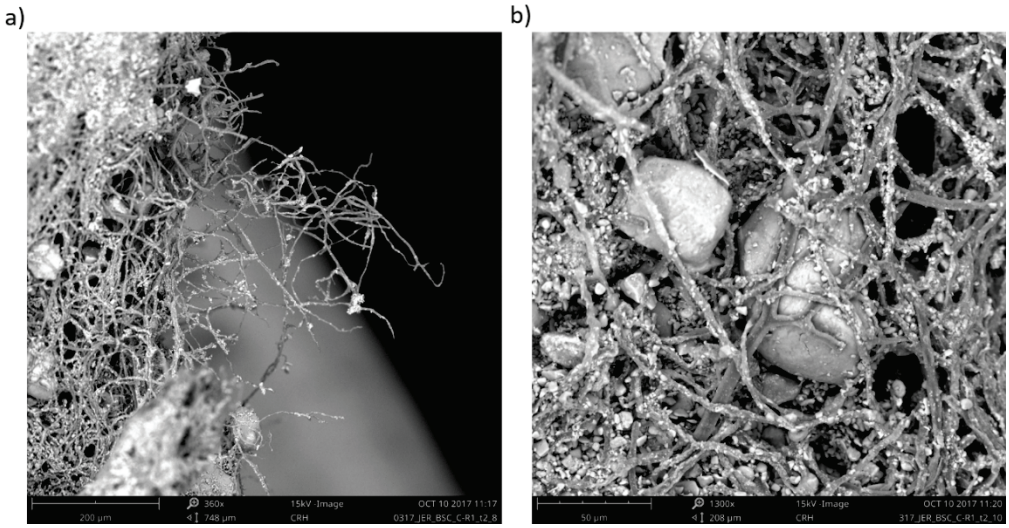
3 Results

We tested the effects of wetting and temperature change through an incubation study and observed increased respiration rates under warmer and wetter conditions. We also observed microbial resilience after temperature stress. Additionally, the bacterial community composition shifted according to the different conditions tested. Overall, soil moisture content had a significant effect on the heterotrophic community composition, strength of the BSCs, and microbial activity and diversity.

3.1 SEM imaging

We used SEM imaging as a visualization tool for observing the microbial contributions to soil stabilization. Sheath material from the cyanobacteria was present in all of the samples imaged. Figure 6 shows example images of one BSC sample. The edges of the BSC samples reveal the anchoring structures of the surface colonizing lichens (Figure 6a). These became exposed at the edges when the small section of BSC was broken off for imaging. We could also see these structures without microscopic assistance when adhering the sample to the SEM pedestal. In the center of the BSC, the cyanobacteria sheath material stretched throughout the surface like a web (Figure 6b). Small soil particles were located directly on the sheath material, which demonstrated their adhesion potential. Larger soil particles could be seen underneath the sheaths, trapped within the BSC.

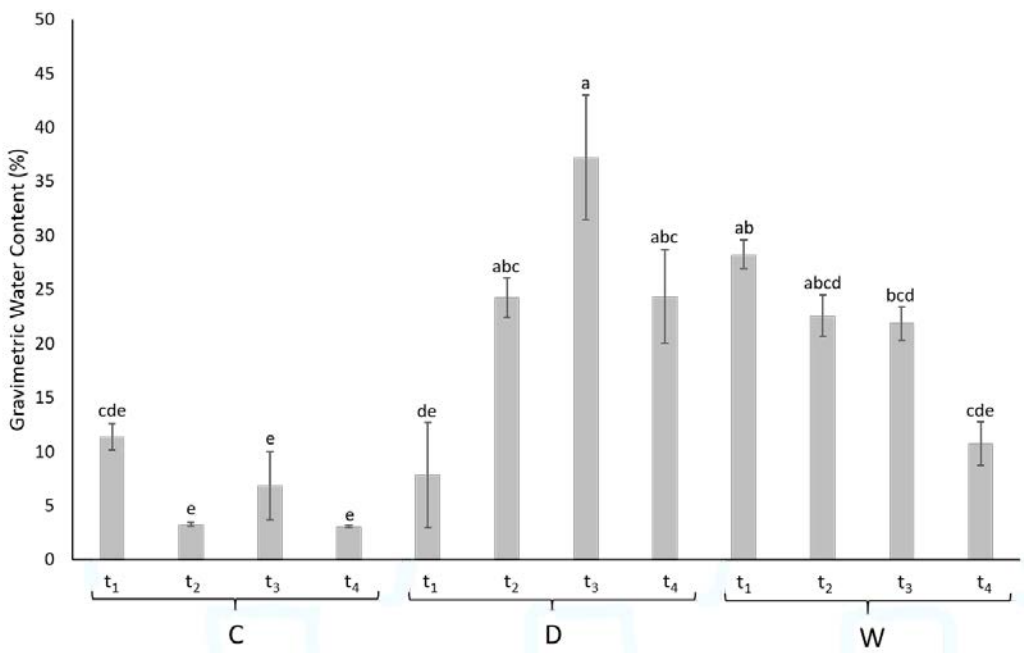
Figure 6. Select SEM images of a BSC sample showing (*a*) lichen anchoring structures at the edge of the BSC and (*b*) cyanobacteria sheath material stabilizing soil particles.



3.2 Soil properties

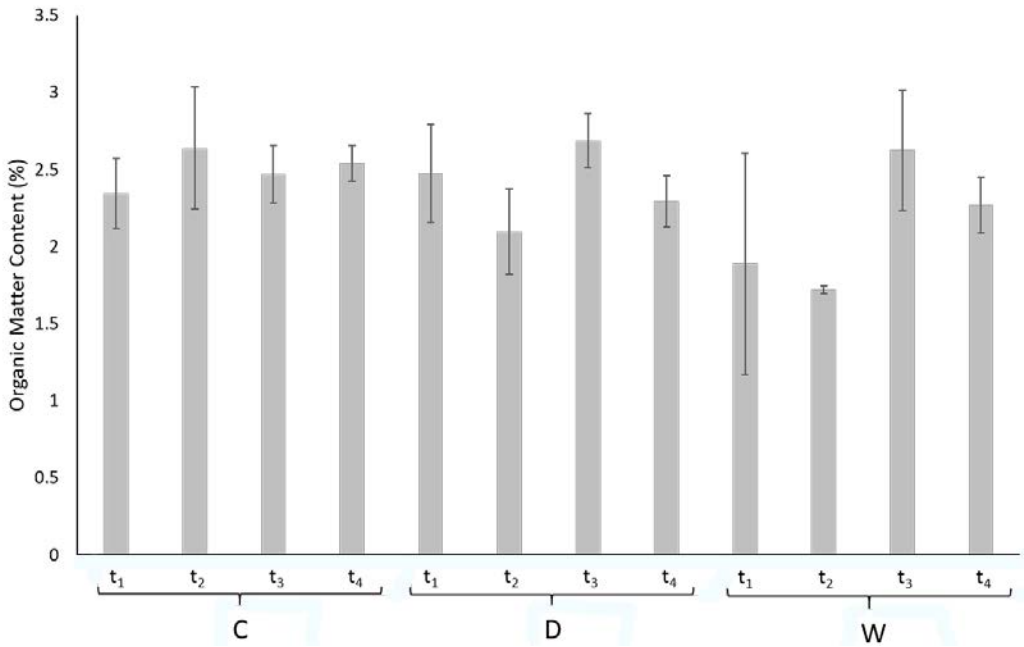
We measured soil properties to evaluate changes in water content and organic matter content throughout the study as these parameters have been shown to affect respiration and community composition (Barbato et al. 2015; Chowdhury et al. 2011; Howard and Howard 1993; Manzoni et al. 2012; Riveros-Iregui et al. 2007). The GWC of the BSCs prior to receiving moisture ranged from 3% to 11%, with a median value of 10% (Figure 7). After the addition of 3 mL of water, the GWC reached an average of 28%, which was significantly higher than the starting dry condition ($p < 0.0001$, Figure 7). The samples that were wetted at the start of incubation (W) dried out by approximately 38% throughout the study. The samples that were wetted after the first sampling event (D) experienced an increase in GWC at t_3, but then decreased in the subsequent sampling event (Figure 7). Better contact between the bottom of the BSC and the filter paper, giving more surface area to uptake water, may have caused less drying in the D crusts. Moisture loss after water addition was expected due to the headspace air being refreshed by the instrument each time it was analyzed, causing increased evaporation.

Figure 7. Gravimetric water content. Bars are an average of three replicates; error bars indicate standard error. Letters of significance indicate values that are significantly different from one another ($p < 0.0001$).



Organic matter content remained constant throughout the study at approximately 2.5% (Figure 8). There was no significant difference between organic matter in the samples at any time point.

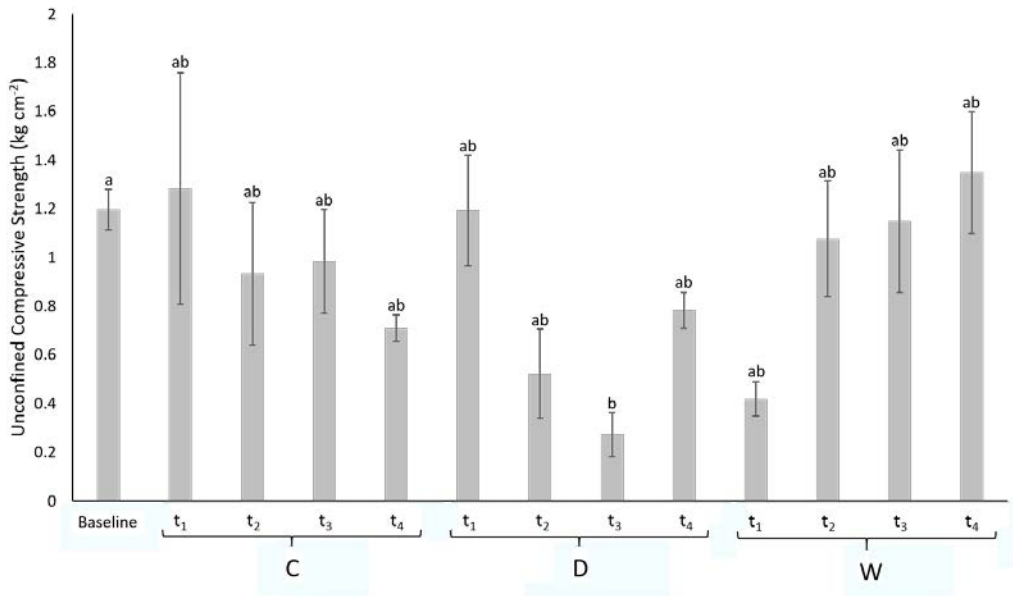
Figure 8. Soil organic matter content. Bars are an average of three replicates; error bars indicate standard error.



3.3 Strength analysis

We measured unconfined compressive strength to determine how environmental inputs may contribute to BSC strength. The C and D samples at t_1 showed similar average strength measurements; however, there was more variability in this measurement as indicated by large standard error bars (Figure 9). Compressive strength of sample D-t_3 was significantly (p = 0.0062) lower than the baseline measurement. This sample also had the highest water content as shown in Figure 7.

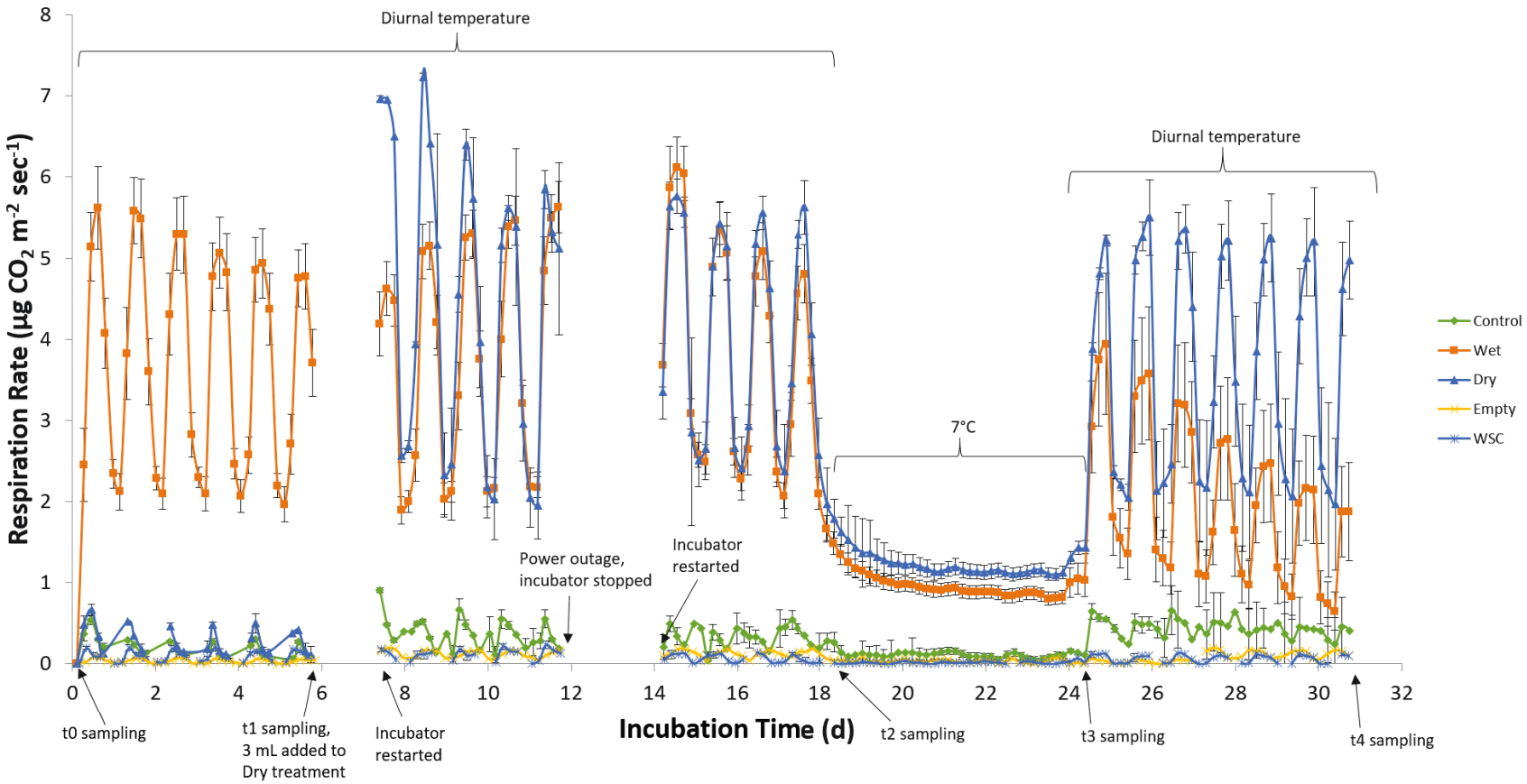
Figure 9. Unconfined compressive strength measurements. Each bar is an average of three replicates; error bars indicate standard error. Letters of significance indicate values that are significantly different from one another (p = 0.0062).



3.4 Respiration analysis

Microbial activity was evaluated using CO_2 efflux throughout the incubation as a measure of the heterotrophic respiration rate (Figure 10). Microbial respiration mimicked the diurnal incubation scheme, with higher respiration rates occurring at higher temperatures and lower respiration rates occurring at lower temperatures. Specifically, microbial activity was highest at 21.3°C and lowest during the temperature stress of 7°C. Respiration rates during the high temperature of the diurnal cycle were typically three times higher than at the low temperature. When crusts remained dry, the respiration rate did not exceed 1 μg CO_2 m^{-2} s^{-1}. There was an increase in microbial activity after the addition of water at the start of incubation and on day eight when water was added to the originally dry crusts. Wet crusts were five times more active than dry crusts; respiration rates reached 7 μg CO_2 m^{-2} s^{-1} in some instances. During the cold temperature stress, respiration rates dropped significantly for all BSC treatments. Once the diurnal temperature scheme resumed, BSC microbes exhibited similar respiration rates as what occurred prior to the cold temperature stress, indicating their resilience to dramatic changes in temperature. Interestingly, the BSCs that were initially dry (D) respired significantly (p = 0.0434) more than the wet (W) BSCs, suggesting that the community that experienced a recent wetting event was in fact more resilient.

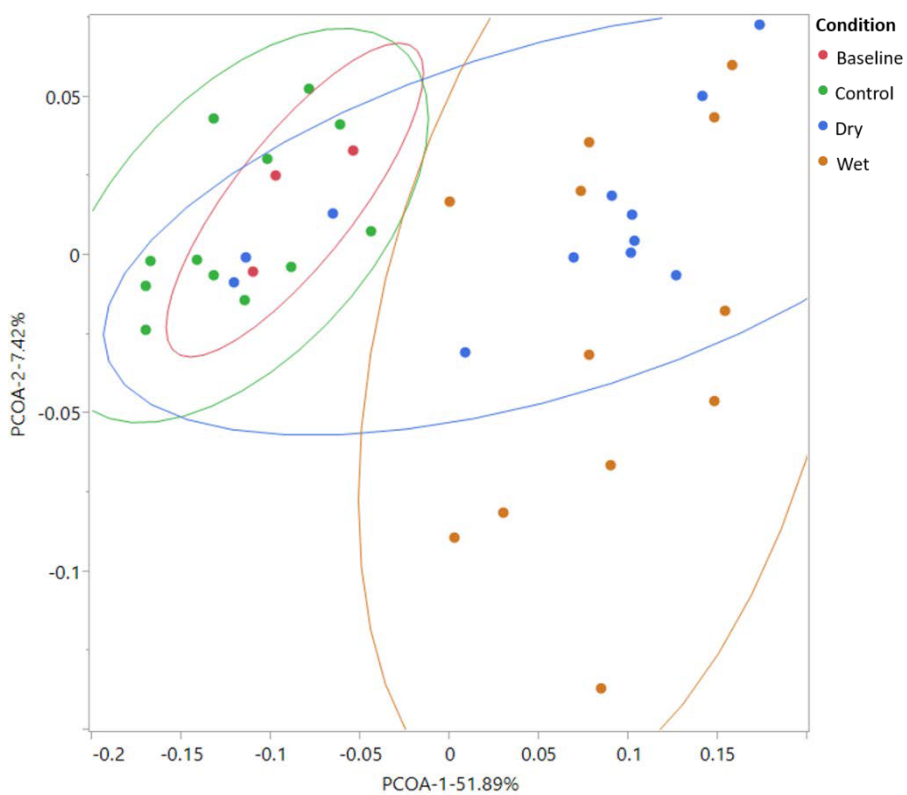
Figure 10. Respiration rate of BSCs throughout the incubation. Points indicate average respiration measurements from three replicates. Bars indicate standard error.



3.5 Microbial community composition

Both temperature and moisture affected the composition of the heterotrophs in the BSCs in this study. Baseline samples collected from the field were analyzed to assess the initial BSC community. Figure 11 shows that the heterotrophs in the baseline closely clustered, suggesting that these bacterial communities were more similar to one another. As expected, the heterotrophs within the control samples (C) that were dry throughout the incubation clustered with those in the baseline BSCs (Figure 11), indicating that the dry conditions stabilized the microbial community. As the C BSCs experienced temperature changes, the community started to shift slightly. The communities in the BSCs that experienced different moisture regimes (D and W) shifted and became more distinct as they experienced changes in temperature and moisture (Figure 11). The large density ellipses in the D and W samples suggest variability in the community composition (Figure 11). Interestingly, when the D samples were wetted, the communities shifted towards the W samples (Figure 11).

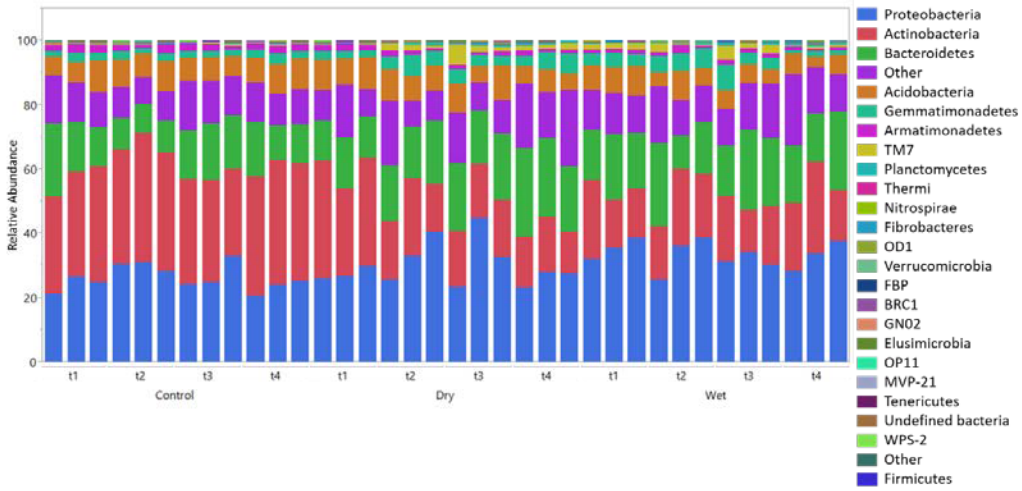
Figure 11. PCoA of heterotrophic organisms. Each point represents one sample; ellipses indicate 95% confidence interval of each sample set.



Analysis of the heterotrophic phyla showed that members from the phyla Proteobacteria and Actinobacteria were the most abundant in the BSCs (Figure 12). Members from the phylum Actinobacteria were the most abundant in the samples that never received moisture (C and D-t1 samples). There was a shift in the most abundant phylum from Actinobacteria to Proteobacteria once the BSCs received moisture. There was also a slight increase in mem-bers of the Bacteroidetes after moisture addition (Figure 12).

Statistical analysis was conducted through one-way ANOVA and all-pairs Tukey-Kramer comparison to test significant changes in heterotrophic composition throughout the incubation. The relative abundance of Actinobacteria was significantly ($p < 0.0001$) higher in samples that had not received any moisture input. As the sample GWC increased, the relative abundance of Actinobacteria decreased. In general, relative abundance of the phylum Gemmatimonadetes was significantly ($p = 0.0001$) lower in samples that had not received moisture addition or those that may have dried out during incubation, particularly W-t_4. The relative abundance of the Fibrobacteres phylum was significantly ($p = 0.0015$) higher in samples that received moisture for the entirety of incubation (W-t_3 and W-t_4). In general, the relative abundance of Fibrobacteres was much lower in samples that never received moisture addition.

Figure 12. Relative abundance of heterotrophic phyla.



A closer investigation into the heterotrophic bacterial community composition at each time point shows interesting trends (Figure 13). At t_1, there was clear separation between the W community and the D and C communities (Figure 13a). This was expected as the W condition had received

moisture at this point, and the D and C conditions were essentially replicate samples up to this time point. Statistical analysis of significant phyla between conditions at t_1 also showed differences in community structure as a function of moisture addition. Relative abundance of the phyla Proteobacteria ($p = 0.0067$), Gemmatimonadetes ($p = 0.0158$), and candidate phylum TM7 ($p < 0.0001$) were all higher after incubating under wet conditions (W). Alternatively, members from the phylum Actinobacteria ($p = 0.0110$) were lower after wet incubation when compared to their dry counterparts. Both Armatimonadetes ($p = 0.0119$) and Nitrospirae ($p = 0.0442$) were more abundant in the control samples than those that experienced moisture. Planctomycetes was significantly ($p = 0.0435$) higher in the wet condition than in the control samples.

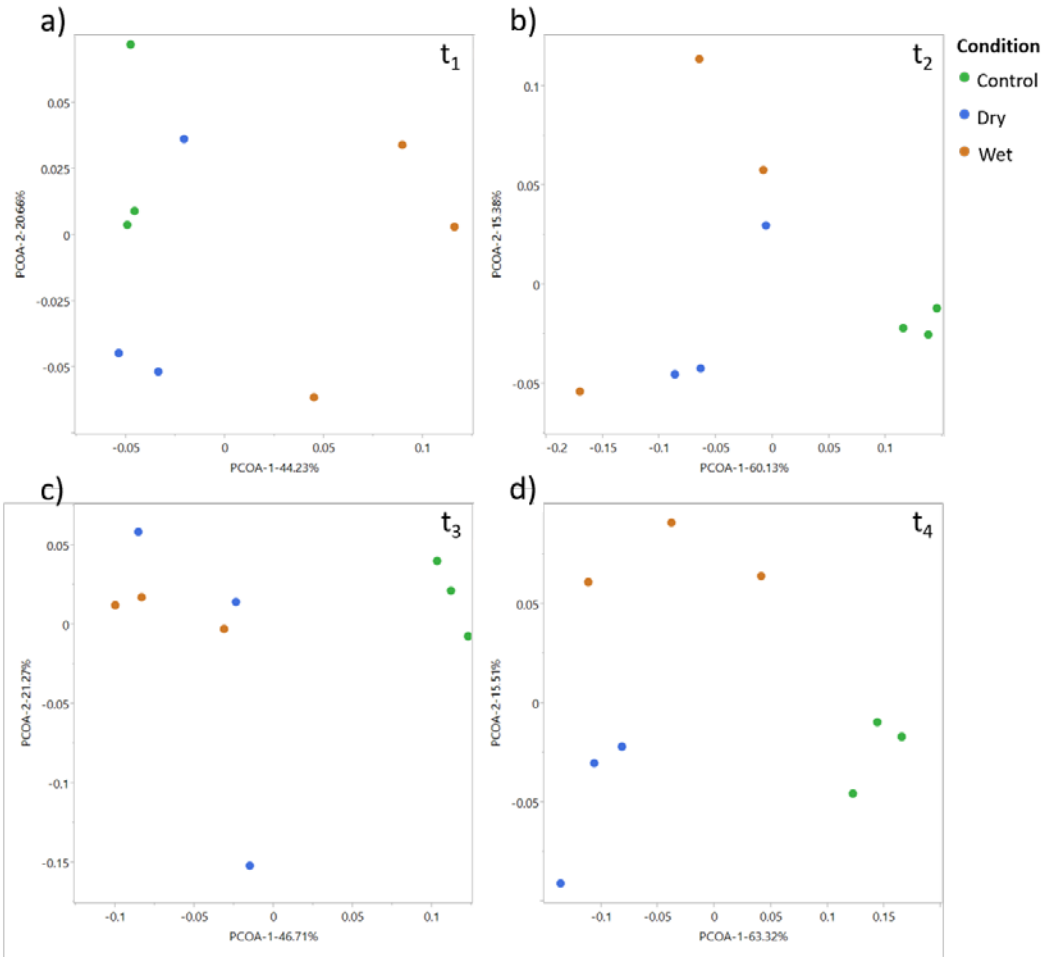
The C condition shows clustering distant from the D and W conditions at t_2 (Figure 13b) because both D and W BSCs received moisture inputs prior to this sampling event, where the C BSCs did not. Statistical analysis of the relative abundance of the phyla revealed a similar trend. Again, Actinobacteria was significantly ($p = 0.0015$) higher; and conversely, Gemmatimonadetes was significantly ($p = 0.0079$) lower in BSCs that did not receive moisture addition. There was also a significantly ($p = 0.0070$) higher relative abundance of the Thermi phylum in the dry BSCs.

After the temperature stress, t_3, there was a similar separation of conditions as seen in t_2 where the W and D samples clustered separately from the C samples (Figure 13c). The Actinobacteria phylum once again had a significantly ($p = 0.0020$) higher relative abundance in the dry samples when compared to those that received moisture. There was also a significantly ($p = 0.0050$) higher abundance of the Nitrospirae phylum in the D condition samples. The relative abundance of candidate phylum OD1 was significantly ($p = 0.0048$) lower in the dry samples after the temperature stress.

At the final sampling event, there was a distinct community for each condition (Figure 13d). Actinobacteria remained significantly ($p = 0.0011$) higher in the BSCs that remained dry throughout the incubation. Armatimonadetes was also significantly ($p = 0.0037$) higher in the dry samples compared to the wetted. Planctomycetes was significantly ($p = 0.0087$) higher in the wetted samples than the dry. The Fibrobacteres ($p = 0.0021$) and candidate OP11 ($p = 0.0094$) phyla were significantly higher in the W

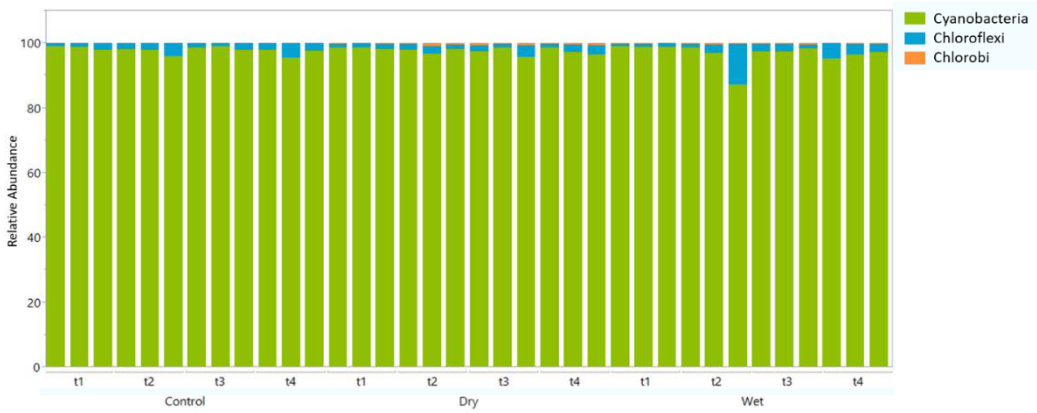
condition at the end of incubation. The Proteobacteria phylum was significantly (p = 0.0208) higher in the W condition when compared to the control samples that never received moisture.

Figure 13. PCoA of conditions at (*a*) t_1, (*b*) t_2, (*c*) t_3, and (*d*) t_4.



We also conducted an analysis of the known autotrophic phyla. Though the BSCs were incubated in the dark, photosynthetic bacteria were still detected in the samples because we used a DNA technique to assess community composition. The autotrophic components made up approximately 65% of the total bacterial community sequenced in our samples. These cells were likely resting during the incubation due to the absence of light. Cyanobacteria was by far the most abundant autotrophic phyla in our samples, accounting for more than 90% abundance (Figure 14). Members of the Chloroflexi and Chlorobi phyla were also present in our BSC samples. The Chlorobi became more abundant with moisture addition, but its overall abundance was much lower than the other two photosynthetic phyla tested.

Figure 14. Bar chart of known autotrophic phyla

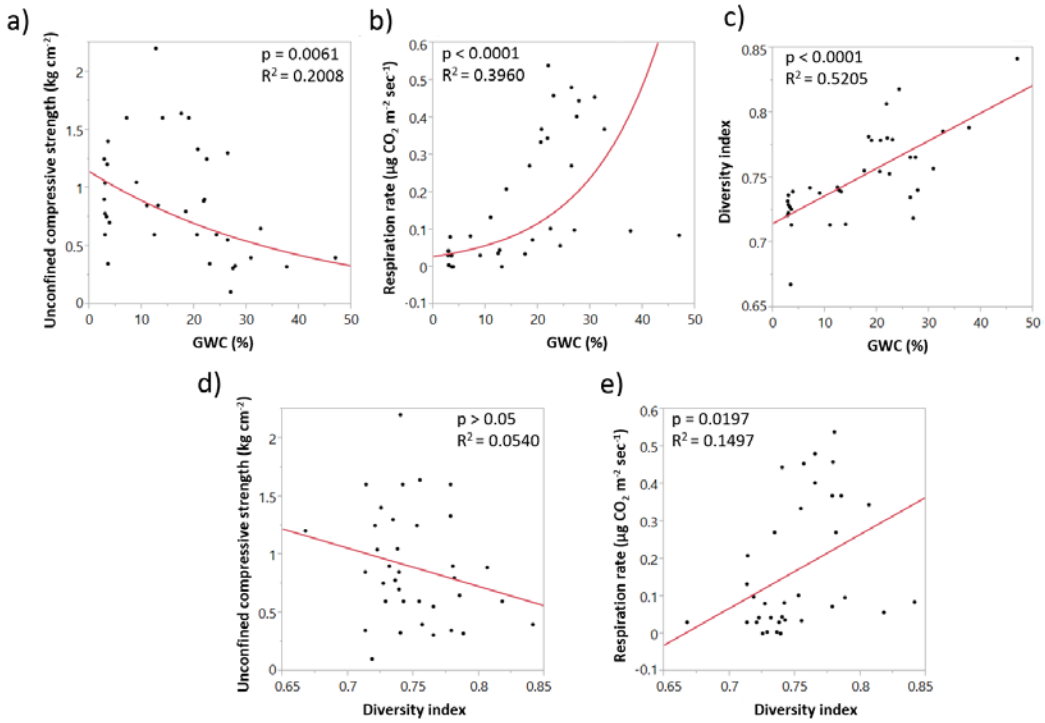


3.6 Regression models

We used regression models to investigate potential correlations in the collected data. Regression fits were chosen using JMP 11.0 software (SAS Institute Inc., Cary, NC, USA, 1989–2007). We used the Shannon diversity index (Morris et al. 2014) to calculate phylogenetic diversity of our samples. This diversity index allowed for the comparison between the microbial and physical attributes (e.g., community datasets, microbial respiration, and soil strength).

There was a weak yet significant ($p = 0.0061$) logarithmic relation between BSC strength and water content (Figure 15a). The unconfined compressive strength decreased as a function of increasing water content of the sample. This was evident during sampling when the penetrometer easily pushed into the surface of the sample without clearly breaking the surface crust as with the dry samples. There was a significant ($p < 0.0001$) logarithmic relationship between microbial activity and water content of the sample (Figure 15b). The respiration rate increased exponentially when water content increased. In addition to increasing microbial activity, water content also significantly ($p < 0.0001$) increased the microbial diversity of the samples (Figure 15c). The diversity index did not show significant linear correlation with our strength measurements (Figure 15d); however, we saw a significant ($p = 0.0197$) positive correlation with microbial respiration and diversity (Figure 15e).

Figure 15. Regression models of log-transformed regression of (*a*) strength as a function of water content and (*b*) respiration rate at the time of sampling as a function of water content. Linear regression of (*c*) the Shannon diversity index as a function of water content, (*d*) strength as a function of the Shannon diversity index, and (*e*) the respiration rate at the time of sampling as a function of the Shannon diversity index.



4 Discussion

BSCs are small but complex systems that greatly affect the erodibility of desert surface soils. Many studies have examined BSCs in various desert environments, with a focus on the photosynthetic component of the BSCs (Belnap et al. 2001, 2004). Our objective was to reveal patterns in the other component of BSCs, the heterotrophs, and how physical inputs (i.e., the timing of wetting and cold-shock events) governed their activity and composition.

We found that the microbial heterotrophs in the BSCs responded to both temperature and moisture. Heterotrophic activity, as measured by microbial respiration, was heightened with warmer temperatures and increased water content. Studies show BSC organisms are only metabolically active when wet (Belnap 2001; Belnap et al. 2001; Belnap et al. 2004); however, in our study, BSCs also respired in the dry samples, albeit at low levels (Figure 10). This indicates that at least a portion of the microbial community remains active during periods of dry conditions rather than every member becoming dormant. The dry samples in this study did, however, have between 3% and 11% moisture, which likely influenced the respiration. Heterotrophic respiration mirrored the diurnal temperature cycle, becoming higher in the warm periods of incubation and lower under cool conditions. Thomas et al. (2011) observed similar patterns in in situ microbial activity under temperature change, where CO_2 efflux was lowest during the coolest part of the day, right after dawn. We found that when the BSC samples received moisture addition, they became five times more active than their dry counterparts (Figure 10). Thomas et al. (2011) also found that moisture addition increased the CO_2 efflux in soils with BSC cover by two- to eightfold, depending on the site, making moisture the primary factor regulating soil respiration. Other studies of BSCs collected from various locations in Botswana, Africa, found significant increases in soil respiration after wetting when compared to dry crusts under the same conditions (Wang et al. 2007; Lane et al. 2013).

Our tests showed that wetted BSCs remained active during the cold temperature stress of 7°C for 6 days. The dry crusts had similar respiration rates to the control samples, indicating little to no respiration was occurring. Once the temperature was resumed to the initial diurnal cycle, the second set of wetted crusts (D) were able to resume similar respiration rates as those before the stress. The crusts that were wetted from the start

of the study (W) only had about half the response of before the stress. This indicates that the BSCs that experienced more recent moisture addition were more resilient after an extreme cold event than the BSCs that were wetted from the start of the study. Being able to respond to extreme temperature changes is very important for BSC organisms as site temperatures can range from 70°C to −20°C in the same location (Belnap et al. 2001). These W samples had lower GWC values at the final sampling, which may have contributed to the overall decline in respiration rates. These results suggest that increased moisture content may lead to microbial resilience after a cold temperature stress. Grote et al. (2010) investigated the effects of water content and temperature on the heterotrophic respiration, finding that temperature had a greater effect than water content when incubated at lower temperatures (less than 15°C). As temperatures increased, water content contributed more to changes in respiration. We also saw less of a difference in respiration rate between the wetted and dried crusts during the cold incubation when compared to the diurnal cycle.

When investigating relationships between microbial activity, water content, and microbial diversity, we found that higher moisture and diversity lead to significantly increased respiration rates (Figure 15b and e). Typically, more diverse communities are considered to be more resilient. Low microbial diversity in BSCs has been attributed to the extreme environments from which they come, where long periods of desiccation limit carbon inputs, which heterotrophic organisms depend on (Nagy et al. 2005). Our observations of low diversity indices at low water contents support this concept. Differences in BSC diversity has been attributed to the degree of crust maturity from crusts collected in the Colorado Plateau (Gundlapally and Garcia-Pichel 2006). The BSCs used in our study were all assumed to be of the same maturity level, as signs of disturbed areas were limited.

The heterotrophic microbial community structure changed with both temperature and moisture inputs, with moisture being the more important driver. Figure 11 shows the heterotrophic community shift due to temperature as evidenced by the C condition samples. These samples never experienced moisture during the incubation but still shifted from the starting community (baseline). However, moisture was the main driver of microbial diversity during incubation. Figure 11 shows D and W samples shifting away from the baseline community and becoming more distant within a

given condition. A correlation plot of diversity index as a function of moisture shows a significant relationship between the two variables (Figure 15c). Sample water content lead to increases in microbial diversity.

Understanding known functional attributes of key members of the community provides insight to how the BSCs might be affected or enhanced by environmental inputs. The most abundant phyla in all samples were Proteobacteria, Actinobacteria, Bacteroidetes, Acidobacteria, and Gemmatimonadetes. This is consistent with studies conducted on BSCs from the Sonoran Desert and Colorado Plataeu, which also found members of these phyla to be the most abundant (Kuske et al. 1997; Nagy et al. 2005). Several isolates from the Proteobacteria phylum have been shown to produce pigmentation, which can help the BSCs survive under extreme ultraviolet exposure (Couradeau et al. 2016; Gundlapally and Garcia-Pichel 2006). Pigmented organisms decrease surface albedo and increase surface soil temperature. Changes to the pigmented community members would result in changes in biogeochemical processes as these depend greatly on soil temperature (Couradeau et al. 2016).

Actinobacteria has been attributed to playing a significant role in carbon cycling in BSC communities (Gundlapally and Garcia-Pichel 2006). Many members of the Actinobacteria phylum are also mycelial (produce hyphae) and therefore may also contribute to crust formation and maintenance much like cyanobacteria (Gundlapally and Garcia-Pichel 2006). Mycelial bacteria also likely contribute to soil stabilization.

Members of the Bacteroidetes phylum are commonly found in BSCs from various regions (Gundlapally and Garcia-Pichel 2006; Maier et al. 2014). Several cultured organisms from this phyla, as well as those from Proteobacteria, have been shown to produce a mucoid resulting from production of exopolysaccharides (Gundlapally and Garcia-Pichel 2006). This mucoid likely contributes to crust formation and soil stabilization. Bacteroidetes has been shown to thrive in high-carbon environments where Acidobacteria prefers low-carbon environments (Fierer et al. 2007). There was a higher relative abundance of Bacteroidetes members in our samples throughout the entirety of incubation, indicating there was potentially a high source of carbon in these particular BSCs.

Acidic soils harbor Acidobacteria (Moquin et al. 2012); however, due to low sample mass, we were unable to analyze for pH. Because we observed

high numbers of Acidobacteria, we suspect that pH was low. Maier et al. (2014) found that below-crust soil communities were largely dominated by Acidobacteria, Verrucomicrobia, Armatimonadetes, Gemmatimonadetes, and Planctomycetes. Fluctuations in the relative abundance of these phyla in our samples may be a result of variations in the thickness of the BSC, where more below-ground soil included in the BSC sample lead to increases in abundance of these particular phyla.

Statistical analysis of differences in microbial phylogeny throughout incubation showed three phyla that were significantly affected by moisture addition. Members of the Actinobacteria phylum were significantly ($p < 0.0001$) more abundant in samples that never received moisture addition during incubation. Barnard et al. (2013) also saw a decrease in Actinobacteria when BSC samples were wetted; and upon drying, the abundance increased again. Both the Gemmatimonadetes and Fibrobacteres phyla showed significant ($p = 0.0001$ and $p = 0.0015$) increases in relative abundance after moisture addition. Members of the Gemmatimonadetes phyla typically make up about 2% of soil bacterial communities and have shown adaption to dry soils (DeBruyn et al. 2011). Interestingly, DeBruyn et al. 2011 found that the relative abundance of Gemmatimonadetes was inversely correlated to soil moisture content, where we saw an increase in this phyla with moisture addition. Little is known about the members of the Fibrobacteres phyla, with few or no cultured organisms (Janssen 2006). These phyla represent a small portion of the total abundance of heterotrophic bacteria in our samples.

Further investigation into community differences between conditions at each time point corroborated our findings and provided insight into the community differences at the end of incubation. At t_1, there was a community shift induced by moisture added to the W samples (Figure 13a). Both t_2 and t_3 showed separation between the control samples that were kept dry and those that received moisture (W and D). This suggests that the heterotrophic community converged after moisture input and that wetted and dry BSCs respond differently to temperature stress. By t_4, there were three distinct communities, clustered by condition. We also saw three levels of respiration at t_4 (Figure 10), which may be a function of the different microbial communities.

We measured unconfined compressive strength to determine if environmental inputs (i.e., temperature and moisture) would change the surface

soil strength of BSCs. We observed similar strength measurements to those conducted by Belnap et al. (2009) where the average strength measurement of dry crusts was approximately 1 kg cm^{-2}; however, Belnap et al. (2009) did not conduct wet measurements in the study. We saw a significant (p = 0.0062) difference between the strength of wettest sample, D-t_3, and our baseline measurements. A correlation assessment of water content and unconfined compressive strength showed a significant (p = 0.0061) logarithmic relationship between the two variables. Higher water content in the samples led to decreases in strength. Even though wet crusts showed decreased compressive strength, the dust emission potential likely also decreased as the presence of moisture creates capillary forces between soil particles (Fécan et al. 1998). In future work, cohesive strength between soil particles would be a superior metric for determining BSC strength, particularly because of exopolysaccharide production of the microorganisms that make up the BSC matrix.

5 Conclusion

Through a laboratory incubation study, we investigated the effects of precipitation events, diurnal temperature changes, and cooling on the heterotrophic microbial community of biological soil crusts. Our findings indicate that both temperature and, more importantly, moisture affect microbial activity. BSC production is directly regulated by moisture events when the microbial members are metabolically active. The microbial community was five times more active after receiving moisture and was three times more active during the warm temperature portion of the diurnal cycle. The BSCs that experienced a delayed moisture input were able to resume higher respiration rates after a cold temperature stress than their wetted counterparts, suggesting resilience to dramatic changes in temperature.

Shifts in the heterotrophic microbial community structure provided insight on key members that are directly affected by the environmental inputs tested. Microbial community structure shifted according to temperature changes and water addition during the incubation when compared to baseline assessments. We specifically investigated microbial diversity in response to moisture as it is an important factor for maintaining multiple ecosystem functions. In this incubation study, the microbial diversity significantly increased with greater moisture content, indicating moisture is an important environmental factor for this system. Members of the phyla Gemmatimonadetes and Fibrobacteres showed significant increases in relative abundance after receiving moisture. Alternatively, members of the Actinobacteria phylum significantly decreased with moisture addition. These results suggests that members of these phyla are significantly affected by the availability of water. Investigation of the known functional attributes of the phyla affected by moisture addition provided insight on potential key microbial players that could be targeted in future work to enhance soil stabilization.

6 Future Work

This study provided insight on the environmental triggers, particularly moisture addition, of certain members of the BSC heterotrophic community. Further investigations are needed to look into how heterotrophic members affect local dust emission potential, carbon and nitrogen cycling, hydrology, and soil strength as a function of environmental inputs. Of particular interest is how BSC microbes contribute to exopolysaccharide production and how these materials then contribute to soil stabilization and other physical processes. Fungal communities would also be of interest as these members aid in the stabilization of the lichens through anchoring structures. Increased knowledge of these very important biological processes would potentially allow for stimulation of these microbial members to increase their production of sticky materials to strengthen the surface crust. Development of alternative measurements of crust strength would also greatly improve our understanding of the binding capacity of BSCs and how this is affected by environmental inputs, which would then improve dust forecasting models.

Most BSC studies have been conducted in arid or semiarid ecosystems, particularly of the western United States, Australia, and Israel (Belnap et al. 2001). Little is known regarding BSCs of South America and Asia, mostly due to remote locations and difficulty translating local literature (Belnap et al. 2001), and alpine and arctic BSCs. There is still a need for better characterization of ecology, cover, and function on a global scale to estimate the potential impacts on biogeochemical processes and climate (Ferrenberg et al. 2017) and the influence they have on dust emission potential. Incorporating the role of BSCs into dust emission models is difficult without global coverage of location and diversity. However, similar structure and function of BSC taxa occur both locally and globally (Belnap et al. 2001), which may assist with incorporating BSCs into the models.

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14. ABSTRACT
Mineral dust affects many of Earth's processes (e.g., radiative forcing, nutrient distribution, and hydrology) and poses a risk to Army maneuverability and situational awareness. Understanding soil processes and how these relate to potential dust emission is of increasing concern as adverse effects of dust become more prevalent. Biological soil crusts (BSCs), commonly found at the soil surface in arid and semiarid regions of the world, protect soils from wind and water erosion. While there is a rich understanding of the behaviors of photosynthetic organisms within BSCs, they are only part of the community. Understanding the other component, the nonphotosynthetic microorganisms and their response to environmental stimuli (i.e., temperature and moisture), will improve dust forecasting models and current soil-stabilization methods.

We conducted a laboratory incubation study to investigate the effects of simulated precipitation events and cooling on the nonphotosynthetic microbial community that made up approximately 40% of the bacterial community in our samples. Our results show how temperature and moisture influence the diversity and resilience of the microbial community and its structure.

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