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Increased temperature and altered summer precipitation have differential effects on biological soil crusts in a dryland ecosystem

Running title: Biocrust response to climate change variables

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Abstract

Biological soil crusts (biocrusts) are common and ecologically important members of dryland ecosystems worldwide, where they stabilize soil surfaces and contribute newly fixed C and N to soils. To test the impacts of predicted climate change scenarios on biocrusts in a dryland ecosystem, the effects of a 2-3 ºC increase in soil temperature and an increased frequency of smaller summer precipitation events were examined in a large, replicated field study conducted in the cold desert of the Colorado Plateau, USA. Surface soil biomass (DNA concentration), photosynthetically active cyanobacterial biomass (chlorophyll *a* concentration), cyanobacteria abundance (quantitative PCR assay), and bacterial community composition (16S rRNA gene sequencing) were monitored seasonally over two years. Soil microbial biomass and bacterial community composition were highly stratified between the 0-2 cm depth biocrusts and 5-10 cm depth soil beneath the biocrusts. The increase in temperature did not have a detectable effect on any of the measured parameters over two years. However, after the second summer of altered summer precipitation pattern, significant declines occurred in the surface soil biomass (avg. DNA concentration declined 38%), photosynthetic cyanobacterial biomass (avg. chlorophyll *a* concentration declined 78%), cyanobacteria abundance (avg. gene copies/g soil declined 95%), and proportion of *Cyanobacteria* in the biocrust bacterial community (avg. representation in sequence libraries declined 85%). Biocrusts are important contributors to soil stability, soil fertility, and plant performance, and the complete loss of biocrusts under an altered precipitation pattern associated with climate change could have significant, negative impacts on stability and productivity of the dryland ecosystem at a regional scale.

Introduction

Dryland ecosystems constitute about 40% of the Earth's terrestrial land mass, and include arid, semiarid, alpine and polar regions (Belnap, 2003c). Sparse plant cover is characteristic of dryland ecosystems, and plant interspaces can comprise up to 70% of the ground surface. The soil surfaces are colonized by biological soil crusts (hereafter termed biocrusts), a photosynthetic and diazotrophic community comprised of cyanobacteria, other bacteria, fungi, lichens and mosses. Filamentous soil cyanobacteria ramify through the soil surface, physically gluing soil particles together to form a surface shield that stabilizes the soil and regulates soil hydrologic processes (Belnap, 2003a, Belnap, 2006). The C and N inputs mediated by dryland biocrusts significantly impact soil nutritional status and subsequent plant health (Belnap, 2003d, Housman *et al.*, 2006).

Dryland ecosystems have adapted to endure limited moisture availability and nutritional status, as well as a wide range of temperature extremes. However, periods of biological activity in dryland ecosystems are tightly linked to seasonal temperature and pulses of moisture availability (Austin *et al.*, 2004, Cable & Huxman, 2004, Huxman *et al.*, 2004, Schwinning & Sala, 2004), making them potentially highly vulnerable to small shifts in temperature and/or precipitation (Dietz *et al.*, 2004, Lioubimtseva *et al.*, 2005, Thomas *et al.*, 2004, Weltzin *et al.*, 2003).

Global and regional climate models predict increases in temperature and altered precipitation patterns over the next 30-100 years for drylands in most parts of the world (Solomon *et al.*, 2007). In the cool desert ecosystem of the Colorado Plateau, Southwest USA, multiple models consistently predict temperature increases of up to 6 °C in this region (e.g., (Cayan *et al.*, 2010, Christensen *et al.*, 2004). The magnitude and direction of predicted

precipitation changes is not consistent and remains uncertain (Solomon *et al.*, 2007, Weltzin *et al.*, 2003). Precipitation patterns in this region are bimodal, consisting of monsoon rainfall events in late summer and early fall, and infrequent rain or snow events in the winter and spring. The most likely precipitation shifts predicted for this region are a decrease in winter precipitation and/or an increase in frequency of small rainfall events during the summer, but with little change in total summer moisture (Weltzin *et al.*, 2003). Recent regional surveys and laboratory manipulation experiments of cyanobacterial biocrusts from the Colorado Plateau suggest that the temperature and precipitation changes predicted for this dryland may have detrimental effects (Belnap *et al.*, 2004, Belnap *et al.*, 2006, Lange *et al.*, 1998). To test the effects of increased soil temperature (2-3 ºC) and/or increased frequency of summer precipitation events, we established a long term, manipulative field experiment in a natural dryland ecosystem near Moab, UT, USA in October 2005. The impacts of the separate and combined treatments on the plants, mosses, soil microfauna, and soil microbiota are being monitored. Here we report the effects of increased temperature, increased frequency of summer precipitation, and the combination on the cyanobacterial biocrusts. We predicted that both the increased temperature and altered precipitation pattern would have a negative effect on the cyanobacterial biocrust but we could not predict a time course for the biocrust responses.

Materials and Methods

Field site description. The field experiment was located in Porcupine Canyon (GPS: 38.67485º N, 109.41637º W; elevation: 1,310 m), just north of Moab, Utah, USA, and treatments began in October 2005. The site landscape is dominated by undisturbed, pinnacled soil biocrusts (Belnap, 2003a), with patches of moss (mostly *Syntrichia caninervis*) and lichens (mostly

Collema tenax). The bunchgrass *Hilaria jamesii* and the shrub *Atriplex confertifolia* were present at the field site (one to three plants per experimental plot). Sampling for the current study was confined to the non-plant interspaces. The soil is classified as a Lithic Torriorthent, a relatively undeveloped fine sandy loam ranging in depth from 10 to 15 cm, with bedrock exposed in some places. Because the field site is located on a natural drainage gradient of approx. 10% slope, the experimental plots were arranged as a randomized blocked design, with five blocks located along the drainage gradient. Each treatment plot was 2 × 2.5 m^2 in size. Samples collected over time were removed in serial rows, leaving one half of each plot undisturbed for long term (5 – 20 yr) measurements.

Four treatment plots were randomized within each of the five blocks. First, heated plots (coded IR for Infrared Radiation) were warmed using a MRM-1208 (120V, 800W 6.7A, 35in) lamp with a modified reflector (Kalglo Electronics Co. Inc; Bethlehem, PA, USA) to achieve surface soil temperatures averaging 2º C above ambient temperatures through each day and night. Second, plots treated with the altered precipitation pattern (coded W for Watered) received a two-fold increase in frequency, but half the size, of the 40-year local means as recorded by the National Oceanic and Atmospheric Administration (http://www.noaa.gov), during the summer months (June to September). To achieve this, each W plot was sprayed with 6 L of water dispersed over the entire plot surface area (equivalent to a 1.2 mm event 2-3 times a week; a total addition of 48 mm in 2006 and 43.2 mm in 2007). Soils returned to their pre-moistened water content after 20 to 30 minutes following each wetting event. Third, the IR and W treatments were applied in combination (coded IRW). The fourth treatment was an ambient control (coded LC for Lamp Control) where a non-functioning IR lamp was suspended over the plot as in the IR treatments. A fifth, natural plot was delineated in each block (with no overhead lamp structure).

These were included in the seasonal and depth comparisons presented here but were not used in the treatment comparisons.

Environmental parameters. Soil moisture and temperature were monitored continuously at the surface and 5 cm depth in all plots. Local weather (air temperatures, RH, PAR, wind speed and direction) and rainfall was also continually monitored. Soil chemistry was analyzed from all plots in October 2005 when the plots were established (Supplementary Table 1).

Soil collection and storage for bacterial community analyses. Soil samples were collected at five time points over the first year of the experiment; in October 2005 (at the onset of treatments), January 2006, May 2006, July 2006, and September 2006. The second year of the experiment, samples were collected three times; in December 2006, May 2007 and September 2007. Samples were also collected after a third year in September 2008. In this region, May is a time when native plants are most actively growing and September follows the hot, dry summer conditions when plants and presumably biocrust constituents are most stressed.

At each sampling time, three biocrust/soil samples were collected from three depths, from each treatment plot in blocks number 1, 3 and 5, which spanned the field site drainage gradient. In preliminary surveys, we found the three blocks chosen for soil sampling to be equally variable and indistinguishable from the two blocks not sampled (which were designated primarily for the larger scale and more destructive plant, moss and microfauna assessments).

Individual soil samples were collected in a stratified manner. First, surface biocrusts were collected by scooping crusted soil to 1 cm depth (about 2 × 2 cm area for about 5 g in total). A 2.5 cm diameter soil corer was used to collect approximately 5 g soil samples from 5 and 10 cm depths directly beneath each biocrust sample. The soil was not uniformly $\geq$ 10 cm deep, and we

were unable to collect 10 cm samples in a few of the plots. Immediately upon collection, each sample was placed on dry ice for transport to the laboratory, where it was stored at -70 ºC.

Soil chlorophyll a concentration. Soil chlorophyll *a* concentration was used as a proxy for photosynthetically active cyanobacterial biomass. Surface biocrust samples (0 to 0.5 cm deep) were collected at the Fall and Spring time points for chlorophyll *a* analyses. Extraction in acetone and analysis on HPLC was carried out as described by Bowker *et al.* (Bowker *et al.*, 2002). Five to ten replicate measurements were taken from each treatment for each sampling time.

DNA extraction and quantification. The FastDNA Spin Kit for Soil (Q-BIOgene; Irvine, CA, USA) was used to extract DNA from soil samples, using a bead mill homogenizer (Biospec Products; Bartlesville, OK, USA) for 3 min set to medium speed. The PicoGreen Quant-IT assay (Invitrogen; Carlsbad, CA, USA) was used to quantify extracted DNA. Each sample was diluted 1:10 in nuclease free water and assayed in triplicate using a BioTek Fluorometer (FL600; Winooski, VT, USA). Nucleic acids were checked for quality by visual inspection of ethidium bromide stained agarose gels (Kodak gel Logic 200 Imaging System).

Bacterial 16S rRNA gene sequence libraries. Clone libraries of 16S rRNA gene sequences were constructed to monitor changes in the total bacterial community in the biocrusts, and in soils 5 and 10 cm below the biocrusts. To analyze the bacterial community at different soil depths over time, two soil samples from each depth were selected from the control plots (in field block 3) for cloning. For comparison of treatments over time, one sample per treatment was selected from the biocrust (in field block 3). PCR amplifications were carried out using the bacterial specific 27F primer (5'-AGA GTT TGA TCM TGG CTC AG-3') (Lane *et al.*, 1985) and the universal reverse primer 787Rb (5'-GGA CTA CNR GGG TAT CTA AT-3') (Kuske *et*

al., 2006) in triplicate 50 µL reactions, each containing 15 ng template DNA, 10 µL 0.1 M trehalose (Sigma-Aldrich, St. Louis, MO) 5 µL GeneAmp 10× PCR buffer (Applied Biosystems, Foster City, CA), 4 µL 10 mM dNTP's (Applied Biosystems), 1 µL each primer at 50 µM, 0.5 µL 20 mg mL^{-1} BSA (Roche, Indiapolis, IN), and 2.5 U AmpliTaq DNA Polymerase LD (Applied Biosystems). Cycling conditions included an initial denaturation at 95 ºC for 5 min, 35 cycles of amplification (55 ºC for 45 s, 72 ºC for 90 s and 94 ºC for 30 s), followed by a final annealing (55 ºC for 45 s) and extension (72 ºC for 10 min) step. The three reactions were then pooled and purified using the QiaQuick Gel Extraction kit (Qiagen; Valencia, CA USA).

Clone libraries for sequencing were generated using the TOPO® TA Cloning® Kit (with pCR®2.1-TOPO®) and chemically competent TOP10 *Escherichia coli* (Invitrogen) following the manufacturers instructions. Clones were bi-directionally sequenced using Sanger technology and assembled in Sequencher v4.7 (GeneCodes; Ann Arbor, MI, USA), where each sequence was manually edited and those of low quality were removed. Each data set was oriented in BioEdit (Hall, 1999). Putatively chimeric sequences were detected using Bellerophon (DeSantis *et al.*, 2006) and removed from the datasets. Sequences were assigned to bacterial phyla using the RDPquery tool (Dyszynski & Sheldon, 2007) with some unassigned sequences determined by direct comparison to the RDP II database (Cole *et al.*, 2007). Prior to any comparison of the data, each sequence library was normalized to 70 sequences. Sequences generated through this study have been deposited to GenBank under accession numbers JQ400201 – JQ403018.

Cyanobacteria-specific 16S rRNA gene quantitative PCR assay. SYBR Green quantitative PCR (qPCR) was carried out on DNA extracted from all biocrust samples, and on 5 cm depth soil samples collected from control plots, to measure the soil cyanobacteria abundance at the two depths, and across the experimental treatments over time. Prior to amplification, all

samples were normalized to a DNA concentration of 25 ng DNA μL^{-1} and diluted 10 and 100-fold in nuclease free water. Each sample was amplified in triplicate 30 µL reactions, using the cyanobacteria-specific forward primer 359F (5'-GGG GAA TYT TCC GCA ATG G-3'), modified from (Nübel *et al.*, 1997) with reverse primers 781Ra (5'-GAC TAC TGG GGT ATC TAA TCC CAT T-3') and 781Rb (5'GAC TAC AGG GGT ATC TAA TCC ATT T-3') (Nübel *et al.*, 1997). Each reaction contained 15 µL SYBR Green SuperMix (Bio-Rad Inc.; Hercules, CA, USA), 7.5 µM the forward primer, 3.75 µM each reverse primer, and 1 µL template DNA. The following thermal profile was carried out on a Bio-Rad iCycler: initial denaturation at 95º C for 5 min, 40 cycles of amplification (95 ºC for 1 min, 62 ºC for 1 min and 72 ºC for 1 min), a final extension step at 72 ºC for 10 min and a melt curve (reading for 10 s $cycle^{-1}$, beginning at 55 ºC and increasing 0.5 ºC $cycle^{-1}$ for a total of 80 cycles).

A standard curve for the qPCR assay was generated using a field-representative mixture of cyanobacterial sequences. The cyanobacteria primers described above for qPCR were used to amplify cyanobacteria 16S rRNA genes from each soil sample collected October 2005. The PCR amplicons were pooled across treatments and field replicates and were cloned as described above for the gene sequence libraries. DNA was extracted from a set of pooled clones using the QIAprep Spin Kit (Qiagen), linearized by digesting with *ScaI* (New England BioLabs; Ipswich, MA, USA), and quantified using PicoGreen dye. Plasmid DNA was diluted to 10^9 gene copies μL^{-1}. Standard concentrations in the assay were run in triplicate from 10^1 to 10^8 gene copies $reaction^{-1}$, yielding a log-linear curve.

Statistical analyses. After preliminary analyses found no significant block trends in the qPCR, chlorophyll *a*, or sequence datasets, the data from all three blocks was treated as nine replicate samples for each soil depth, in each treatment, for each time point. ANOVA and t-tests

were implemented using the JMP software package (v5.1, SAS; Cary, NC, USA). Significant differences were determined if the resulting P value was less than 0.05. If ANOVA testing revealed significant differences among the data points, mean separations were carried out using Tukey's HSD test. Replicated data are represented as averages ± 1 SE. Relative abundance and composition of major phyla in the 16S rRNA gene clone/sequence libraries was compared using a Manhattan distance matrix based on the relative abundance of each phylum and visualized using nonmetric multidimensional scaling plots (MDS) using the R software program (version 2.11.1; www.r-project.org).

Results

Field Site Characteristics and Environmental Measurements. Ambient air temperatures recorded at the field site were a minimum of -6.98 ºC in winter and a maximum of 38.5 ºC in summer over the first two years of the experiment (data not shown). Surface biocrust temperatures ranged from 1.04 ± 0.15 ºC to 38.8 ± 0.56 ºC in control plots (Figure 1a). Soil temperatures at 5 cm depth were generally 1 ºC warmer in winter and 1 ºC cooler in summer than the surface biocrust temperatures (Figure 1b). The IR lamps increased surface and 5 cm depth soil temperatures on average 2.5 ºC with a minimum increase of 1 ºC (Figure 1c, 1d).

Precipitation at the field site was measured from August 2005 through October 2007 (Figure 1e). Mean precipitation was 22.5 ± 4.7 mm mo^{-1}, with a minimum of 0.5 mm in August 2005 and a maximum of 118.9 mm in October 2006. (The long-term average is 19.1 mm mo^{-1} for yrs 1889 – 2006 for this site.) Two individual rainfall events (28 mm and 41 mm) accounted for 60% of the rainfall in the wettest month (October 2006) of the experimental period. Measured soil moisture (not shown) generally followed monthly precipitation trends (Figure 1e). The

additional summer precipitation applied as an experimental variable was too small to be detected in the soil moisture measurements.

Influence of soil depth on bacterial community biomass and composition. Most of the soil microbial biomass, as estimated by extracted DNA, was located in the top 1 cm of the biocrust (Figure 2). The amount of DNA extracted from the control biocrusts ranged from 10.4 ± 1.1 to 22.8 ± 1.3 µg DNA g^{-1} soil (n = 9). In comparison, DNA extracted from 5 cm deep samples ranged from 5.0 ± 0.37 to 6.0 ± 0.35 µg DNA g^{-1} soil (n = 9). Similar concentrations of extractable DNA were measured in samples from 10 cm below the soil surface (data not shown). Across the eight sampling times between October 2005 and September 2007, soil cyanobacteria concentration was significantly higher in the biocrust (range: 7.29 ± 3.5 × 10^8 to 2.73 ± 0.26 × 10^9 gene copies g^{-1} soil) than in 5 cm deep soil (range 3.97 ± 2.7 × 10^5 to 1.52 ± 0.62 × 10^8 gene copies g^{-1} soil; pairwise t-test for each month, $P < 0.0001$, except for September 2007, $P < 0.01$). Results for samples collected at 10 cm were similar to those collected at 5 cm (data not shown).

Sequences from the phylum *Cyanobacteria* were a major component of the surface biocrust rRNA gene clone libraries, accounting for 50% ± 2.2% (range 26% – 90%) of the sequences generated with general bacterial primers (Figure 3a). The next most abundant phyla were the *Actinobacteria*, *Bacteriodetes*, *Chloroflexi* and *Proteobacteria*. In contrast, *Cyanobacteria* represented only 8.0% ± 1.3% (range 0.0% – 39%) of the libraries from 5 cm deep soil when averaged across all time points (Figure 3b). Libraries derived from October 2005 in 5 cm depth soil samples contained more *Cyanobacteria* than all subsequent 5 cm deep samples. This was probably due to our initial soil coring method at that time point, in which soils from the upper strata were inadvertently mixed with collections from below. After reviewing the first time point data, we revised our sampling strategy for subsequent collections to that

described in the Methods section. *Cyanobacteria* were rarely detected in 5 cm depth soil rRNA gene clone libraries generated from the 2006 and 2007 time points (2.32% ± 0.76%). Instead, these libraries were dominated by a variety of *Actinobacteria*, with large constituencies of *Bacteriodetes*, *Chloroflexi* and *Proteobacteria*.

Seasonal and year-to-year patterns observed during the two-year field study. Control plot soil samples were used to document seasonal and year-to-year patterns in soil DNA concentration, soil chlorophyll *a* concentration, cyanobacteria abundance (by qPCR) and bacterial community composition over the two-year experiment. DNA concentrations varied by sampling point, but did not follow a repeatable seasonal pattern in the surface biocrust or the 5 cm deep soils (Figure 2, Table S2). The most notable trend was a significant increase of biocrust DNA concentrations at the December 06 and May 07 time points relative to the other six time points (Figure 2, Table S2), and a parallel increase in cyanobacteria 16S rRNA gene copy number (F-test, P = 0.0012) at the May 07 time point. After an initial drop in chlorophyll *a* concentrations between Oct 2005 and May 2006, the spring values trended higher than the following autumn values (Figure 4b). Little overall change in bacterial community composition could be attributed to season in either the surface biocrust or the 5 cm deep soil (Figure 3).

Biocrust response to increased temperature and altered precipitation pattern

We found no significant effects of increased temperature on the biocrust DNA concentration (Figure 4a), soil cyanobacteria abundance (Figure 4b), chlorophyll *a* concentration (Figure 4c), or the proportion of cyanobacteria sequences in 16S rRNA gene clone libraries (Figure 5) over the two-year study. We also found no effect of the altered summer precipitation pattern after the first summer of application on the biocrusts (September 2006 sampling; Figures 4 and 5). However, the chlorophyll *a* concentrations were significantly lower (44.4 % reduction)

in the altered precipitation plots (W and IRW) than the lamp control plots in May 2007 (W + IRW: 4.34 ± 0.50 µg chlorophyll *a* g^{-1} soil; F-test, P = 0.041; Figure 4c). After the second summer of altered precipitation pattern, all of the measured parameters showed significant declines in the W and IRW treatment plots (September 2007 panels in Figures 4 and 5). Compared to the control plots, DNA concentrations from the W and IRW treatments were reduced by 38% (F-test, P = 0.017; Figure 4a). The soil cyanobacteria concentration (16S rRNA gene copies g^{-1} soil) was reduced by 95% under altered precipitation pattern (F-test, P = 0.005; Figure 4b), and the chlorophyll *a* concentration declined by 78% (F-test, P = 0.0002; Figure 4c).

Bacterial community composition at the phylum (Figure 5) or family level (data not shown) was consistent (ie. replicate field samples were as variable within a treatment as between the treatments) until after the second summer of altered precipitation. Although the proportion of cyanobacteria sequences comprised 25.7% to 90.0% of the 16S rRNA gene libraries across the treatments from October 2005 through May 2007, this was reduced in the September 2007 libraries to only 1.43% to 11.4% of the bacterial 16S rDNA sequences in the W and IRW plots, respectively (Figure 5). This low percentage of cyanobacteria sequences was confirmed with two additional replicate libraries from each of the altered precipitation treatments at this time point (14% ± 6.9% cyanobacteria sequences, final n = 6 clone libraries from precipitation treatment plots).

Distance matrices were constructed from phylum-level percent composition data, with all of the 16S rRNA gene libraries from all three depths at all five time points (n = 49) and plotted using non-metric MDS. Soil surface biocrust libraries (n = 29) were easily distinguishable from the 5 and 10 cm depth libraries (n = 10; Figure 6). When the six libraries generated from the September 2007 W and IRW samples were plotted on the same graph, they generally ordinated

in an intermediate space between the biocrust and 5 or 10 cm deep soil samples. This suggested that the precipitation-affected biocrust soils lost surface biocrust phyla but still remained compositionally distinct from the deeper soils.

Discussion

The biocrusts colonizing the interspaces of this dryland are composed of cyanobacteria-dominated mats interspersed with patches of moss and cyanolichens. In contrast to our prediction, the 2-3 °C increase in soil temperature did not result in any detectable effect on the cyanobacterial biocrust biomass, cyaobacterial abundance or gross bacterial community composition over the two-year study period. Similarly, no temperature effect was detected in the mosses that covered about 25% of each plot surface in the first two years (Reed, 2012, Zelikova *et al.*, 2012). A regional survey conducted in the Colorado Plateau over seven years (1996 – 2003) showed that a 6 °C increase in maximum summer temperatures over that time period resulted in a significant decrease in biocrust lichens (Belnap *et al.*, 2006). The biocrust bacterial communities have presumably adapted to wide fluctuations in temperature, which typically range from 30-35 °C highs in the summer months to below zero in the winter months (Figure 1). Any potential impacts of slightly increased temperature may only become apparent during times when soil moisture is adequate for microbial activity (i.e., during summer or winter pulses of precipitation) and may take several years to be detected in the soil microbial community (Shaver *et al.*, 2000).

Regional increases in air temperature may have direct effects on the dryland soils, by drying them more quickly after rain events, or may have broader effects by altering the timing and length of the seasons. In the dryland systems of the Colorado Plateau, the active season for

biocrusts and many plants is the fall through spring when soil moisture is recharged by fall monsoons and winter snow. It is possible that even slight temperature increases could induce seasonal shifting toward an earlier spring and a longer summer season. These potential interactive effects will require further long-term studies to effectively document.

Our study documented a rapid decline in the biocrusts in response to an altered pattern of summer precipitation, where total moisture was increased by about 45 mm over the summer (about a 20% increase over the 40 yr average for this area) through a doubling of the number of small precipitation events (1.2 mm each). After only two years, the altered precipitation pattern had a dramatic negative impact on the cyanobacterial community that was initially detected using molecular and biochemical assays. Measures of soil cyanobacteria abundance (by qPCR), photosynthetically active cyanobacterial biomass (by chlorophyll *a* concentration), and proportion of the *Cyanobacteria* in the total bacterial community consistently showed significant declines across the field experiment after two years (Figures 4 and 5). The most sensitive harbinger of the decline was the chlorophyll *a* measurement, which showed a significant decline in the spring after one summer of the altered precipitation pattern (Figure 4c). Thus the combination of molecular and biochemical measures used here provided early-warning of the decline before the cyanobacterial biocrusts visibly disappeared. The cyanobacterial biocrusts did not recover with subsequent year altered precipitation treatments, and Figure 7 illustrates the dramatic changes in soil surface that were apparent after five years of altered precipitation pattern.

The timing and frequency of precipitation in dryland ecosystems are critical triggers for animal, plant, and soil biological activity. Our field study shows that biocrust cyanobacteria are sensitive to an altered pattern of small rainfall events in summer. A prior greenhouse study

conducted on biocrusts exposed to six months of an increased precipitation frequency showed reduced C and N fixing activity and reduced UV-protective and chlorophyll *a* pigment production (Belnap *et al.*, 2004). Biocrust cyanobacteria and lichens readily absorb moisture from small precipitation events and begin respiration almost immediately, and the net compensation point (the time at which carbon gained from photosynthesis equals that lost during respiration) in these organisms is not usually reached within 30 minutes at the temperatures relevant to our study (Belnap *et al.*, 2004, Housman *et al.*, 2006, Lange *et al.*, 1998). Since the small precipitation events applied in our field study usually dried within 30 minutes, it is likely that photosynthetic activity ceased before the cyanobacteria reached their net compensation point. We hypothesize that repetition of this cycle likely resulted in eventual C starvation, and thus death of the cyanobacteria.

In laboratory manipulation studies of dryland mosses (*Syntrichia, (Reed, 2012); Grimmia (Alpert & Oechel, 1985)*) and the cyanolichen, *Collema tenax* (Lange *et al.*, 1998), similar patterns of C loss have been observed with small repeated wetting events. For example, Reed *et al.* (Reed, 2012) recently showed that a 1.2 mm pulse of moisture resulted in a C loss in the dominant moss at this site, *Syntrichia ruralis*, while a 5 mm pulse of moisture that provided a longer hydration time, resulted in C gain.

The cyanobacterial biocrusts, mosses, and lichens in drylands can constitute 70% to nearly 100% of the ground cover. Collectively, these nutritionally enrich the interspace soils between widely-spaced plants, and loss of the biocrust is expected to have major negative effects on the nutritional status of the C- and N-limited dryland soil. Although C fixation rates in drylands are relatively small (Garcia-Pichel F., 1996, Lange, 2003), the vast expanses of drylands globally make their contribution a significant factor in global carbon budgets.

Loss of the N-fixing cyanobacteria from the dryland ecosystem has been shown to negatively impact soil N content and health of surrounding plants (Belnap, 2003b, Kuske *et al.*, 2011). Recently, loss of biocrust mosses was shown to reduce microbial N pools and propel a switch from ammonium-dominated to a nitrate-dominated N cycle (Reed, 2012). Changes in dryland precipitation patterns and their consequent detrimental effects on interspace biocrust organisms could therefore result in accelerated N loss and differential utilization by plants and soil fauna (Barger *et al.*, 2005, Barger *et al.*, 2006, Evans, 1999, McCalley & Sparks, 2009).

The biocrusts are also critical to stabilize dryland soil surfaces. Loss of this stabilizing 'cap' has been shown to result in increased soil erosion that can have multiple consequences at the regional scale. Soil erosion depletes nutrients at the source and fertilizes sink areas. Dust emissions can have negative health effects. Changes in snowpack albedo can accelerate melt rates, affecting the quality, quantity and timing of water in rivers (Painter *et al.*, 2010). Collectively, such surface changes over large areas can have major ramifications for terrestrial and atmospheric conditions that drive regional weather patterns. Thus, the rapid decline in soil cyanobacteria and biocrusts documented here under an altered precipitation pattern, and their slow recovery rates from physical or physiological disturbance, indicates that altered summer precipitation could rapidly drive changes in dryland surface soils that contribute important, lasting regional feedbacks to the climate system.

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Supporting Information Legends

Table S1: Soil chemistry at the field site. Electrical conductivity is reported as meq100 g^{-1} and ionic components are listed as µg g^{-1} soil unless otherwise noted.

Table S2. Biomass in biocrust and 5 cm deep soil as measured by extractable DNA over the two-year experiment. Averages are followed by standard deviation of the mean for each observation. The Oct 05 time point measurements were calculated from 15 replicate field samples and the other time point measurements were calculated from 18 replicate field samples. Values followed by the same letter were not significantly different by season using Tukey's HSD mean separation procedure ($\alpha < 0.05$).

Figure Legends

Figure 1: Soil temperature and precipitation measurements at the field site across two years from November 2005 through September 2007, averaged by month. Data shown are averages ± 1 SE. Temperature measures are the average of 10 soil probes for each treatment. Panel a: Soil surface temperature (ºC) from control plots. Panel b: soil temperature at 5 cm depth (ºC) from control plots. Panel c: Soil surface temperature differential (δºC) between the lamp control (LC) plot and each of the treatment plots. Panel d: Soil temperature at 5 cm depth differential (δºC) between the lamp control (LC) plot and each of the treatment plots. Panel e: Natural precipitation at the field site (mm per month). Treatments: IR lamp – rectangles; Watered – ovals; IR lamp + watered – triangles).

Figure 2: DNA extracted from biocrust (black bars) and 5 cm deep soil beneath the biocrust (grey bars) from October 2005 through September 2007. Values are averages ± 1 SE of 15 field replicate samples for the Oct 05 sampling date and 18 field replicate samples for the other dates. DNA concentration was significantly higher in biocrusts than in the 5 cm deep soil at all time points (pairwise t test, Oct 05 $p = 0.0006$, Sept 07 $p = 0.0132$, all other time points $p < 0.0001$).

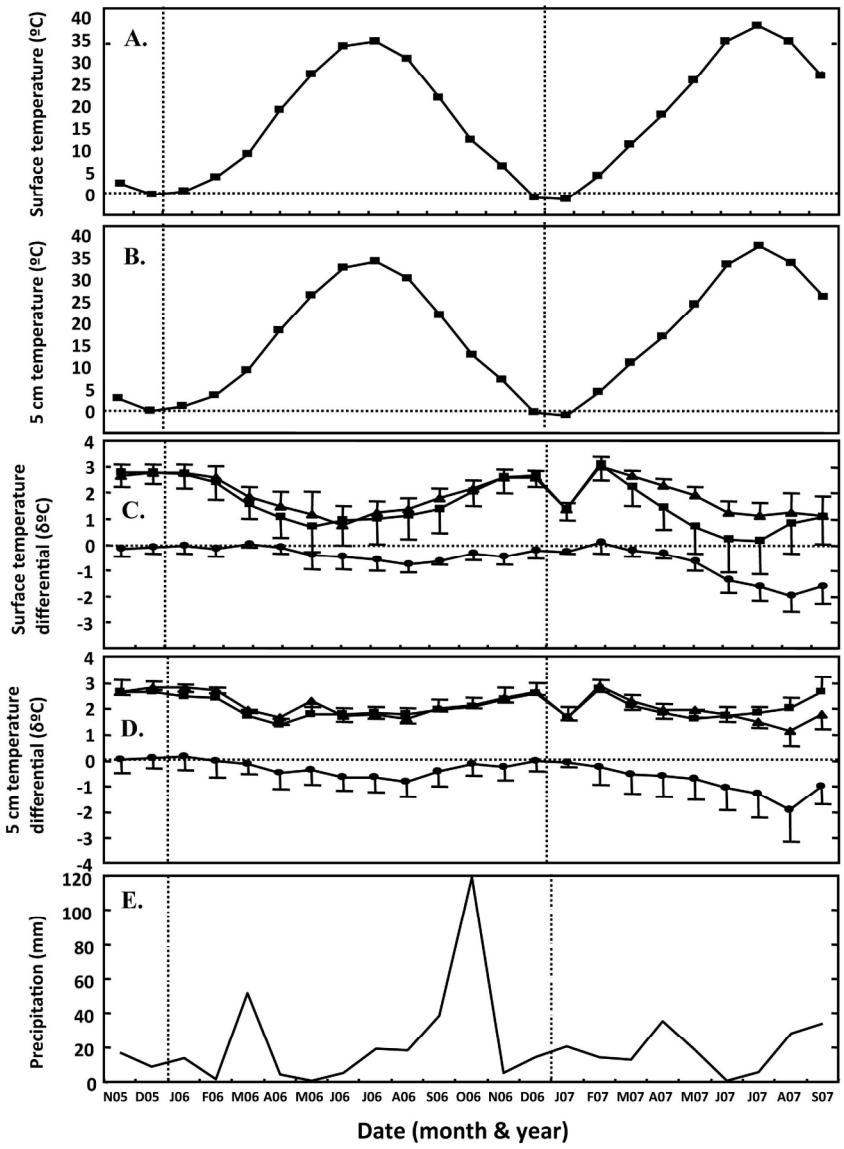
Figure 3: Phylum-level bacterial community composition in duplicate field soil replicates from biocrusts (panel a), and 5 cm deep soil beneath the biocrusts (panel b), in control plots from October 2005 through September 2007. Data are shown as percent contribution of major phyla to the 16S rRNA gene sequence library (n=70 sequences per library).

Figure 4: Treatment effects on the surface soil biomass (panel a), cyanobacteria abundance (panel b), photosynthetically active cyanobacterial biomass (panel c) sampled at five time points over two years. Treatment codes: lamp control (LC, open bars), increased temperature (IR, light grey bars), altered summer precipitation pattern (W, medium grey bars), and both altered temperature and precipitation (IRW, black bars). Panel a: Extractable DNA (µg DNA g^{-1} soil; n = 9 measured samples per treatment), Panel b: Cyanobacterial 16S rRNA gene qPCR results (16S rDNA copy number g^{-1} soil; n = 9) plotted on a log scale. Panel c: Extractable chlorophyll *a* over time and treatment (µg pigment g^{-1} soil; n = 5-10). Values shown are averages ± 1 SE (n = 9). Within each time point, ANOVA and mean separation using Tukey's HSD test was conducted. The ANOVA F-test results are displayed beneath sets of bars for each time point. If significant ($\alpha < 0.05$) then lowercase letters were added within the bars of that sampling time to show the results of the Tukey's HDS test. Nsd = no significant differences.

Figure 5: Phylum-level bacterial community composition in representative treatment plots from biocrusts from October 2005 through September 2007. Data are shown as percent contribution of the major phyla to the 16S rRNA gene library (n=70 sequences per library).

Figure 6: Non-metric MDS plot showing the relationships between the phylum level bacterial community composition of the biocrust bacterial communities (circles, n = 23), the 5 and 10 cm depth bacterial communities (squares, n = 9 each) in the control plots over two years, and the biocrust communities after two summers of altered precipitation pattern (stars, n = 6). (The two open squares are 5 cm depth soil samples that are outliers to the rest of the group, collected in Oct 05 prior to revising the sampling strategy to avoid incidental mixing).

Figure 7: View of the soil surface in a lamp control plot and an altered precipitation plot five years after the experiment was initiated. The cyanobacterial biocrusts are absent in the altered precipitation plot.



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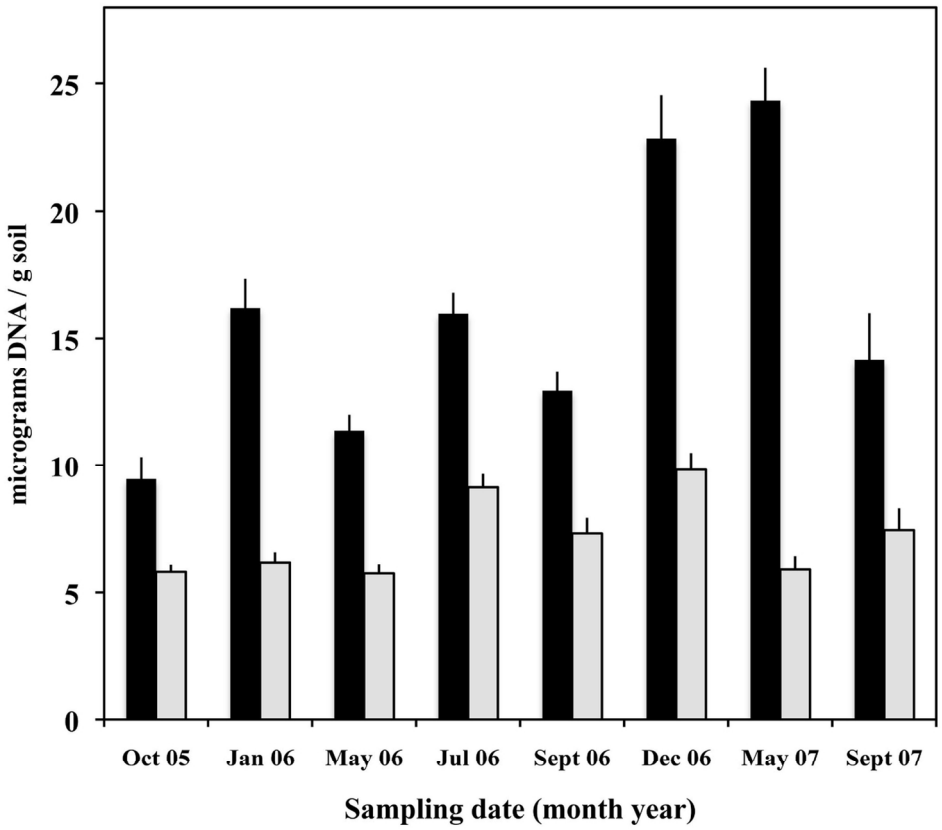
171x236mm (300 x 300 DPI)



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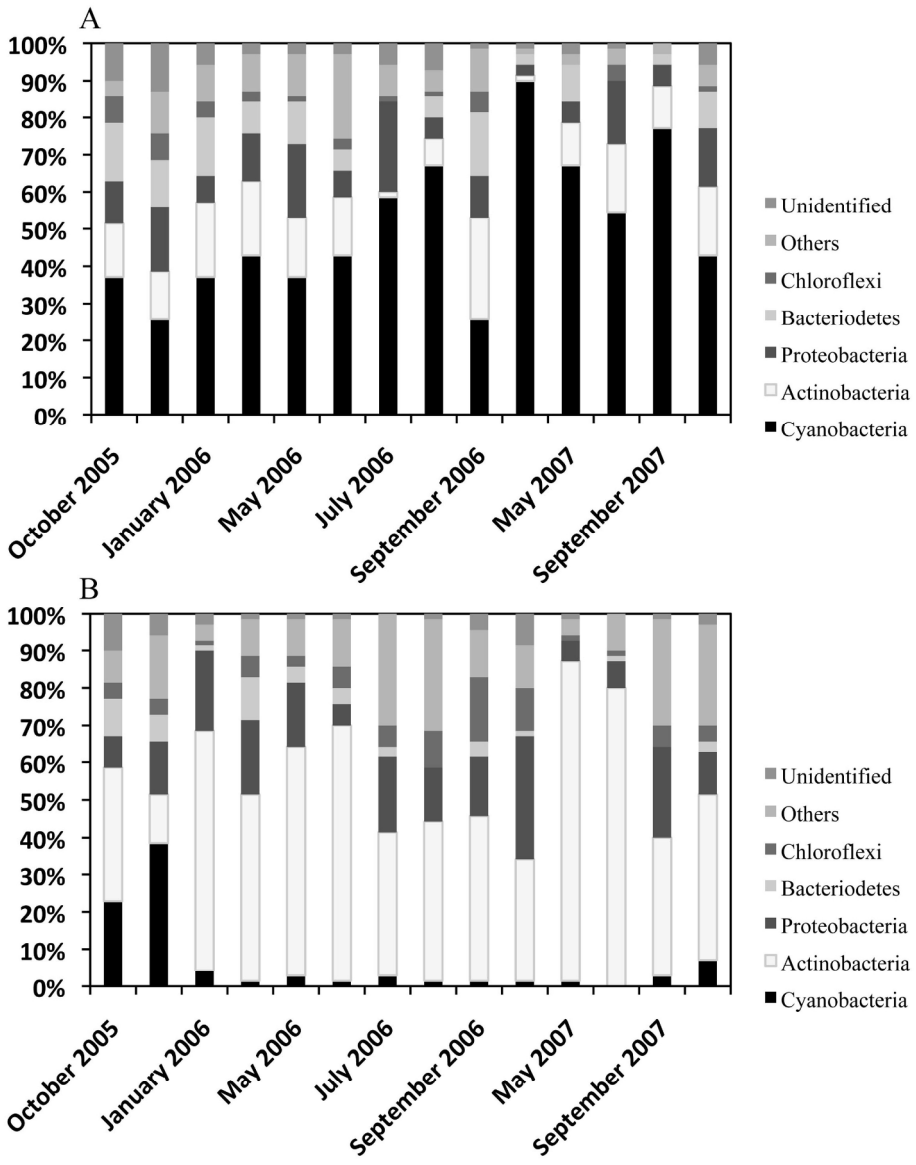
127x112mm (300 x 300 DPI)



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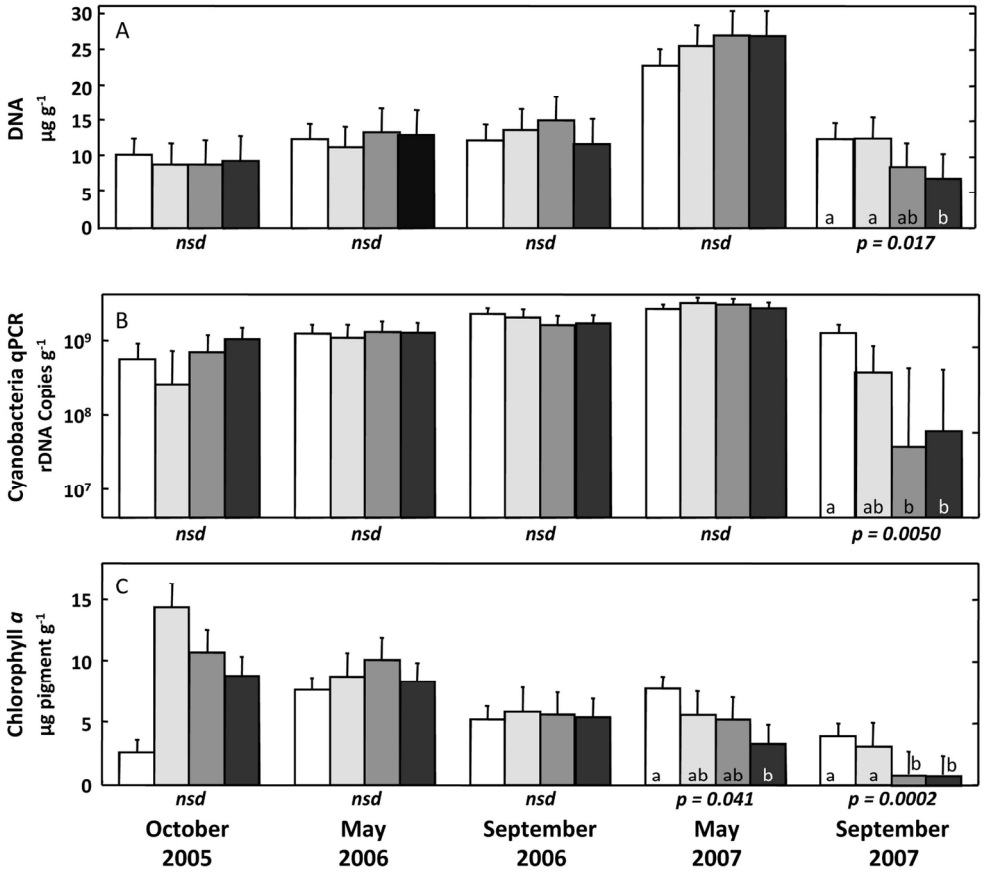
192x241mm (300 x 300 DPI)



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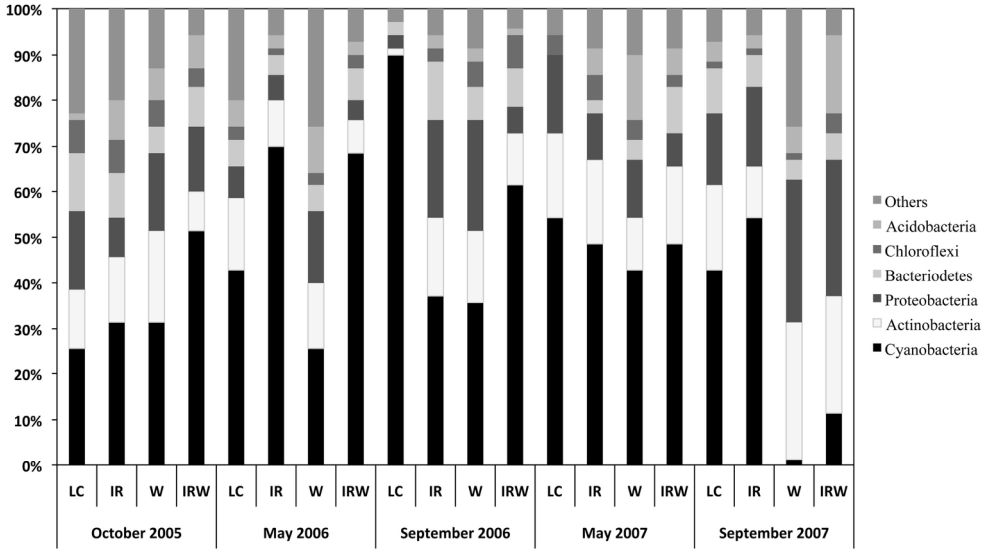
149x131mm (300 x 300 DPI)



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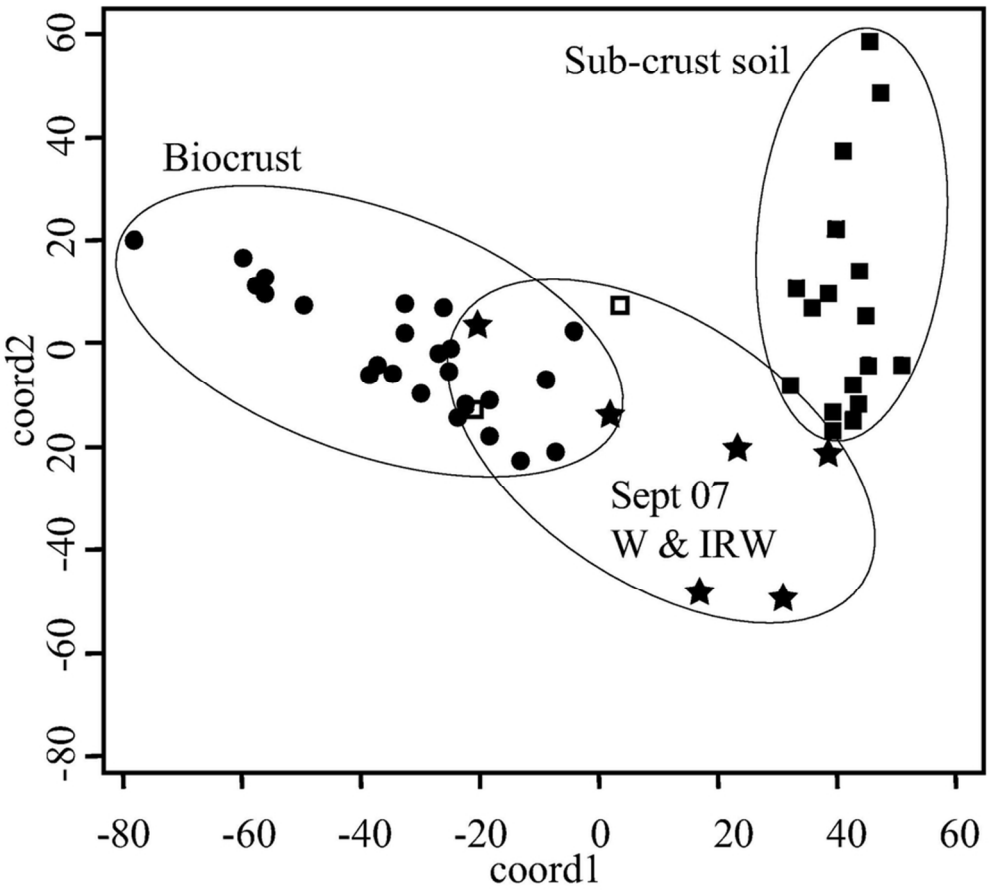

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80x74mm (300 x 300 DPI)

Figure 7: View of the soil surface in a lamp control plot and an altered precipitation plot five years after the experiment was initiated. The cyanobacterial biocrusts are absent in the altered precipitation plot.
55x23mm (300 x 300 DPI)

Supplemental Table 1: Soil chemistry at the field site. Electrical conductivity is reported as meq100 g^{-1} and ionic components are listed as µg g^{-1} soil unless otherwise noted.

	Average	Std Dev
% Sand	61.06	4.74
% Clay	14.1	2.74
% Silt	24.84	3.21
% Organic Matter	0.38	0.22
Electrical Conductivity	6.35	1.13
pH	8.11	0.13
Ca (exchangeable)	2688.48	362.38
$CaCO_3$ (%)	3.07	0.76
Cu (exchangeable)	0.41	0.08
Fe (exchangeable)	2.53	0.49
K (available)	162.05	34.45
K (exchangeable)	151.28	69.93
Mg (exchangeable)	147.97	44.81
Mn (exchangeable)	4.45	0.74
Na (exchangeable)	52.35	13.56
P (available)	6.68	2.83
Total N	46.00	37.04
Zn (exchangeable)	0.18	0.09

Supplemental Table 2. Biomass in biocrust and 5 cm deep soil as measured by extractable DNA over the two-year experiment. Averages are followed by standard deviation of the mean for each observation. The Oct 05 time point measurements were calculated from 15 replicate field samples and the other time point measurements were calculated from 18 replicate field samples. Values followed by the same letter were not significantly different by season using Tukey's HSD mean separation procedure ($\alpha < 0.05$).

Season	Biocrust DNA (μg DNA/g soil)	5 cm soil DNA (μg DNA/g soil)
Oct 05	9.4 (3.4) c	5.8 (1.2) c
Jan 06	16.2 (4.8) b	6.2 (1.7) c
May 06	11.4 (2.6) bc	5.7 (1.7) c
July 06	16.0 (3.5) b	9.1 (2.2) ab
Sept 06	12.9 (3.1) bc	7.3 (2.6) bc
Dec 06	22.9 (7.1) a	9.8 (2.9) a
May 07	24.3 (5.6) a	5.8 (2.2) c
Sept 07	14.2 (7.7) bc	7.5 (3.6) abc