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# Exposure to predicted precipitation patterns decreases population size and alters community structure of cyanobacteria in biological soil crusts from the Chihuahuan Desert

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## Summary

**Cyanobacteria typically colonize the surface of arid soils, building biological soil crust (biocrusts) that provide a variety of ecosystem benefits, ranging from fertilization to stabilization against erosion. We investigated how future scenarios in precipitation anticipated for the Northern Chihuahuan Desert affected abundance and composition of biocrust cyanobacteria in two grassland ecosystems. Scenarios included a decrease in precipitation and a delay of monsoon rainfall. After three years, both treatments negatively affected cyanobacteria, although the effects of monsoon delay were milder than those of decreased precipitation. Mature biocrusts in black grama grassland suffered severe losses in cyanobacterial biomass and diversity, but compositionally simpler biocrusts in blue grama-dominated grassland maintained biomass, only suffering diversity losses. This could be partially explained by the differential sensitivity of cyanobacterial taxa: nitrogen-fixing Scytonema spp. were the most sensitive, followed by phylotypes in the Microcoleus steenstrupii complex. Microcoleus vaginatus was the least affected in all cases, but is known to be very sensitive to warming. We predict that altered precipitation will tend to prevent biocrusts from reaching successional maturity, selecting for *M. vaginatus* over competing *M. steenstrupii*, among pioneer biocrust-formers. A shift towards heat-sensitive *M. vaginatus* could ultimately destabilize biocrusts when precipitation changes are combined with global warming.**

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## Introduction

Water is the most significant limiting factor in arid terrestrial ecosystems worldwide (Nemani *et al.*, 2003), including in the Southwestern USA (Heisler-White *et al.*, 2008). Consequently, periods of biological activity in such drylands are tightly linked to seasonal pulses in moisture availability (Schwinning and Sala, 2004). In the Northern Chihuahuan desert, for example, the bulk (> 60%) of annual precipitation falls within the summer monsoon season (Peters *et al.*, 2015). Climate model predictions of global warming typically call for altered precipitation patterns over the next 100 years; however, even within a single biogeographical province, the direction and magnitude of such changes remains uncertain (also see review by Sala *et al.*, 2000; Collins *et al.*, 2013). For the US Southwest, most studies predict an increase in drought severity, resulting from either decreased precipitation and/or increased evaporation (Kunkel *et al.*, 2003; Cayan *et al.*, 2010; Ault *et al.*, 2014; Petrie *et al.*, 2014; Cook *et al.*, 2015). Additionally, studies on potential changes in precipitation patterns and timing (e.g., Cook and Seager, 2013) have predicted a progressive delay in the onset of summer monsoonal rains.

Such changes have the potential to affect all the biota of desert ecosystems, including the widespread microbial communities that live on desert soils, known as biological soil crusts (biocrusts). Biocrusts are ecologically important biotic components of arid lands (see reviews by Eldridge and Greene, 1994; Belnap *et al.*, 2016). These are photosynthetically driven microbial communities that occupy the

topsoil of often large plant interspaces and carry out a variety of biogeochemical processes. Their activities closely track the availability of liquid water through precipitation, because they are only active when wet (Rajeev *et al*., 2013). Biocrusts are typically composed of cyanobacteria (Garcia-Pichel *et al*., 2001) but sometimes also eukaryotic algae, lichens or mosses (Bates *et al*., 2010) as primary producers, accompanied by a variety of chemotrophic bacteria (Nunes da Rocha *et al*., 2015), archaea (Soule *et al*., 2009) and fungi (Bates *et al*., 2010). Biocrusts are responsible for a substantial portion of primary production in arid lands (Elbert *et al*., 2012), and are typically desiccation resistant (Peli *et al*., 2011; Rajeev *et al*., 2013). Given that biocrust are absent from the most extremely arid climates (Pointing and Belnap, 2012), a worsening of desiccation stress, could easily bring these extremophiles close to the limit of their adaptability, making biocrusts potentially vulnerable to decreased or otherwise modified precipitation regimes.

Several studies have assessed the effect of drought or altered precipitation on moss biocrusts (Austin *et al*., 2004; Johnson *et al*., 2012; Yeager *et al*., 2012). Altered precipitation patterns, alone or together with warming treatments, promoted moss mortality and returned a well-developed crust back to an early successional stage, containing mostly cyanobacteria, within 10 years of treatment (Johnson *et al*., 2012; Reed *et al*., 2012; Ferrenberg *et al*., 2015). These studies, however, did not look specifically at cyanobacterial populations, only reporting on generic biomass proxies like total chlorophyll *a* concentration or visually determined percentage cover. And yet, cyanobacteria play a prominent role in biological soil crusts (Pietrasiak *et al*., 2013; Strauss *et al*., 2016), one that differs among different species. Filamentous non-nitrogen fixing cyanobacteria, like *Microcoleus vaginatus* and *Microcoleus steenstrupii, for example*, are often the pioneer crust formers, increasing organic matter and reducing wind and water erosion (Zhang *et al*., 2006; Garcia-Pichel *et al*., 2009; Grote *et al*., 2010). After soil stabilization, other cyanobacteria that fix nitrogen, secondarily colonize biocrusts and contribute significantly to the nitrogen pool of these otherwise low nutrient arid land soils (Yeager *et al*., 2004; Johnson *et al*., 2005; Yeager *et al*., 2012).

Given the primary role of cyanobacteria in biocrust formation and function, we investigated whether experimentally imposed drought versus a seasonal delay in monsoon rainfall affected cyanobacterial abundance and the structure of their communities in soil crusts. We leveraged the large-scale, Extreme Drought in Grassland Experiment (EDGE) at the Sevilleta National Wildlife Refuge in New Mexico, to examine effects on soil crusts during the third year of this on-going, long-term experiment.

## Results

### *Sites differed in biocrust composition*

Total bacterial/archaeal population size did not differ significantly between the two grassland sites (ANOVA, df = 62, $F = 90.304$, $p = 0.096$). Figure 1 depicts the population size of the bacterial/archaeal communities, as well as the absolute taxonomic composition at the phylum level, as obtained by combining Illumina sequencing of 16S rRNA genes and qPCR quantification of total 16SrRNA copies (see Materials and Methods). Bacterial/archaeal areal population size at Site 1 (with 'dark' biocrusts and black grama vegetation) was $7.4 \pm 2.9 \times 10^5$ 16S rRNA gene copies $cm^{-2}$ and $1.2 \pm 0.8 \times 10^6$ 16S rRNA gene copies $cm^{-2}$ at Site 2 (with 'light' biocrusts and blue grama vegetation). These are typical of levels found in previous biocrusts studies.

Overall, bacterial composition at both sites was dominated by Cyanobacteria, followed, in descending order of numerical importance, by Actinobacteria, Proteobacteria, Bacteroidetes, Acidobacteria, Verrucomicrobia and the

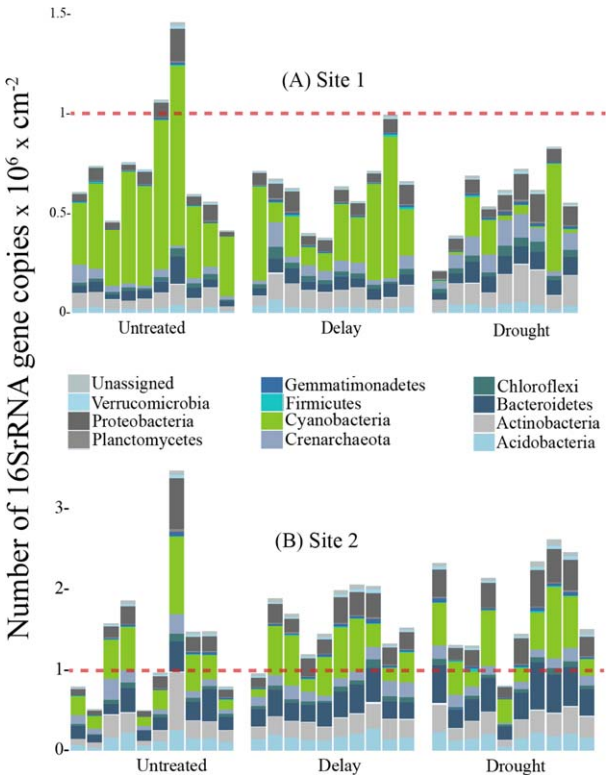


**Fig. 1.** Bacterial abundance and community structure in control and treatment biological soil crusts at 2 sites as determined by high-throughput 16S rRNA gene analyses coupled to q-PCR. Ten independent plots were analysed for each treatment and site, and each bar represents an independent plot. Phylogenetic assignments for each OTU were based on blast to the Greengenes database, and carried to the Phylum level. Note that the scales are not the same for the two sites.

generalist archaeal phylum, Crenarchaeota. These results are similar to those observed in prior studies on biological soil crusts (Nagy *et al.*, 2005; Gundlapally and Garcia-Pichel, 2006; Steven *et al.*, 2013). Bacterial community structure at the phylum level did not differ significantly between the two sites (PERMANOVA df = 1/18, pseudo-$F = 50.067$ $p = 0.08$; Fig. 1A and B). However, in comparing population sizes of major individual phyla between sites, cyanobacteria had significantly larger populations, by 58%, in Site 1 ($p = 0.0017$). This was consistent with alternative, indirect proxy measurements of cyanobacterial biomass (areal Chl *a* concentration; Fig. 2), which were also much higher in Site 1. These two findings support our assumption that biocrusts at Site 1 were successionally more mature than at Site 2, because higher relative abundance of cyanobacteria and total biomass is a trait of well-developed crusts (Garcia-Pichel *et al.*, 2003; Housman *et al.*, 2006; Couradeau *et al.*, 2016).

Cyanobacterial community structure (Fig. 3) differed greatly between the two sites (PERMANOVA, Pseudo-$F = 3.7995$, df = 1/18, $p = 0.016$) (Fig. 4). Both communities were dominated by non-nitrogen fixing, filamentous cyanobacteria allied to *Microcoleus vaginatus* and multiple clades of the *M. steenstrupii* complex. The latter taxon could be considered one species based on morphology, but phylogenetic work has shown it to consist of a diverse group, likely monophyletic but multigeneric. For this reason, we have maintained various *M. steentrupii* clades as individual taxonomic entities of relevance in our analyses. However, Site 1 contained dark cyanobacterial crusts and large populations of nitrogen-fixing sessile cyanobacteria, such as *Nostoc* spp. and *Scytonema* spp. (Fig. 3A), which are correlated with the presence and production of the cyanobacteria pigment scytonemin. These results are in line with the 72.3% higher aerial concentration of scytonemin in Site 1, when compared to Site 2 (Fig. 2). Also, cyanobacterial Shannon's diversity was significantly higher, by

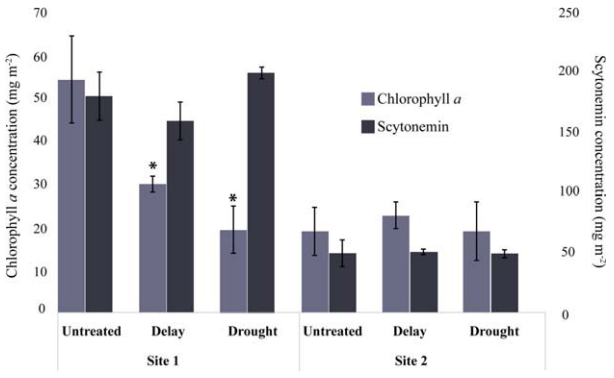


**Fig. 2.** Areal concentrations of biomarker pigments Chl *a* and scytonemin in biological soil crusts for controls and treatments at both sites. Error bars are ± s.d. ($n = 9$). Asterisks denote significant difference from control ($p < 0.05$)

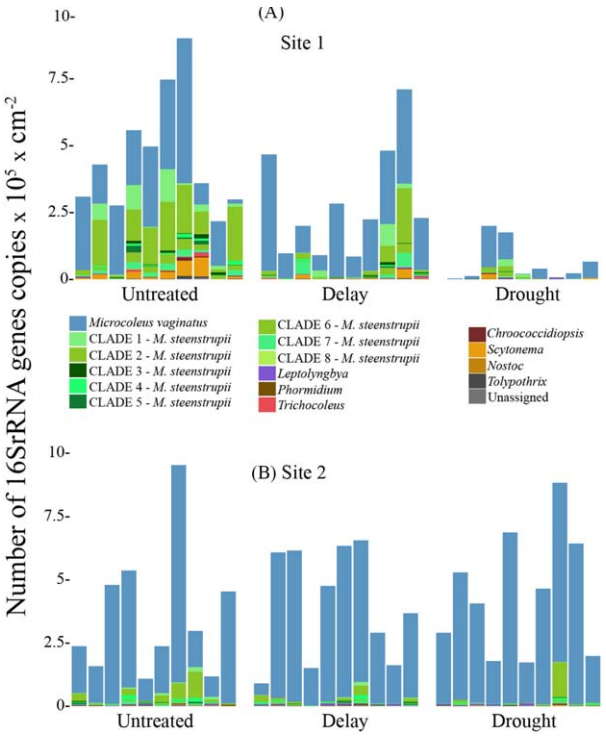


**Fig. 3.** Cyanobacterial abundance and community structure in control and treatment biological soil crusts at 2 sites, as determined by high-throughput 16S rRNA gene analyses coupled to q-PCR. Ten independent plots were analysed for each treatment and site, and each bar represents an independent plot. Phylogenetic assignments for each OTU were based on blast to an in-house biocrust cyanobacteria database, and carried to the Genus or species level, as feasible.

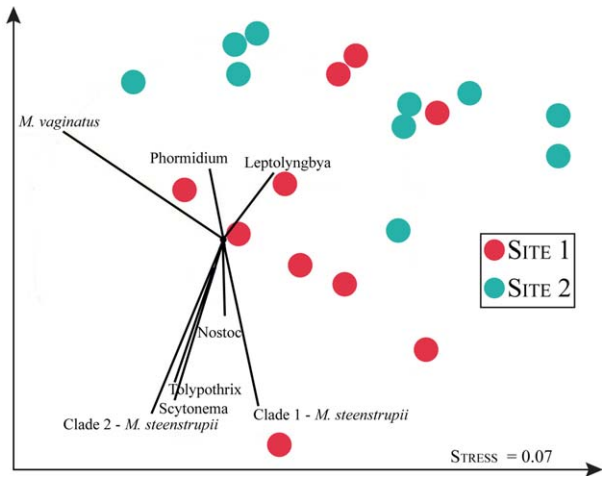


**Fig. 4.** Non-metric multidimensional scaling (nMDS) comparison of cyanobacterial community composition in untreated plots. The nMDS ordination was based on Bray-Curtis similarity of cyanobacterial OTUs. Data points are color coded by site. Taxon vector size represents their importance along each nMDS axis. This analysis clearly separated the communities of Site 1 and Site 2 and the presence of nitrogen fixing heterocystous cyanobacteria was one of the most important differences.

44.8%, in Site 1 ($p = 0.023$) (Supporting Information Table S1). Indeed, the incipient 'light' biocrusts site (Site 2) showed no or very small populations of nitrogen fixing cyanobacteria, being strongly dominated by *M. vaginatus*, with lower prevalence of *M. steenstrupii*, *Leptolyngbya* spp. and *Phormidium* spp. (Fig. 3B). The eight most abundant cyanobacterial groups in Site 1, which accounted for 90% of all sequences, in descending order, were: *M. vaginatus*, *M. steenstrupii* – CLADE 2, *M. steenstrupii* – CLADE 1, *Scytonema* spp., *M. steenstrupii* – CLADE 6, *M. steenstrupii* – CLADE 7, *M. steenstrupii* – CLADE 4 and *M. steenstrupii* – CLADE 5 (Fig. 3A and Supporting Information Table S2). For Site 2, these groups were composed of *M. vaginatus*, *M. steenstrupii* – CLADE 2, *M. steenstrupii* – CLADE 4, *M. steenstrupii* – CLADE 1, *M. steenstrupii* – CLADE 7, *M. steenstrupii* – CLADE 6, *Leptolyngbya* spp and *Phormidium* spp. (Fig. 3B and Supporting Information Table S2). The PERMANOVA analysis further showed that nitrogen fixing cyanobacteria (Scytonema, Nostoc and Tolypothrix), and two clades of *M. steenstrupii* (Clades 1 and 2) were determinant for Site 1, while filamentous non-nitrogen fixing cyanobacteria (Leptolyngbya and Phormidium) were uniquely typical from Site 2 (Fig. 4).

### *Bacterial and cyanobacterial responses to drought were strongest in Site 1*

The most conspicuous effect of extreme drought was a significant, 95% decrease of the cyanobacterial abundance relative to the controls at Site 1 ($2.5 \pm 1.7 \times 10^4$ vs. $3.9 \pm 2.2 \times 10^5$ 16S rRNA gene copies $cm^{-2}$; Figs. 1A and 2A). This shift was accompanied by a significant, absolute increase in the abundance of other phyla (Proteobacteria, Bacteroidetes and Actinobacteria, being the most pronounced; Fig. 1A). Thus, despite the compositional shift, drought did not affect total abundance of Bacteria/Archaea. Drought plots contained $6.2 \pm 2.5 \times 10^5$ 16S rRNA gene copies $cm^{-2}$ (vs. the $7.4 \pm 2.9 \times 10^5$ 16S rRNA gene copies $cm^{-2}$ in the controls) at Site 1, and $1.8 \pm 0.6 \times 10^6$ 16S rRNA gene copies $cm^{-2}$ (vs. $1.2 \pm 0.8 \times 10^6$ 16S rRNA gene copies $cm^{-2}$ in the controls) at Site 2. We detected a significant positive effect of drought on bacterial diversity (both Shannon's diversity and number of Operational Taxonomic Units (OTUs) – Supporting Information Table S1), but only in Site 1 ($p < 0.0001$; Fig. 1B). Interestingly, the effects of drought on abundance and diversity were absent at Site 2, where the abundance of cyanobacteria and other bacteria were not significantly different from those of controls. These results are consistent with proxy measurements of cyanobacterial biomass (areal Chl *a* concentration; Fig. 2), which decreased significantly with drought in Site 1, but not in Site 2.

Drought caused a decrease in overall cyanobacteria diversity (Shannon's diversity and number of OTUs) at both grassland sites (Fig. 2A, Supporting Information Table S1) to the relative benefit of *M. vaginatus*. However, the intensity of this effect differed between the sites. Strong declines of *M. vaginatus, M. steenstrupii* clades and *Scytonema* spp. (down to 1.5% of respective control levels) occurred in Site 1, the dark biocrust site (Fig. 2A). *Scytonema* spp. populations were the most sensitive to drought, reaching only $0.3 \pm 2 \times 10^3$ 16S rRNA gene copies $cm^{-2}$ compared to the $1.3 \pm 2.9 \times 10^4$ in control plots, in which they were 43-fold more abundant. *M. vaginatus* populations, were one order of magnitude more resistant to drought than *Scytonema*, attaining eightfold higher abundance in the control plots ($2.32 \pm 1.4 \times 10^5$) than in drought plots ($0.29 \pm 5 \times 10^5$ 16S rRNA gene copies/$cm^2$). *M. steenstrupii* clades were also sensitive to drought in Site 1 (declining to 1–5% of respective control levels; Supporting Information Table S2). The combined effects of a decrease in secondary cyanobacteria and an increase in dominant *M. vaginatus*, was sufficient to detect a significant negative effect of drought on overall cyanobacterial diversity in Site 1 (Fig. 5A). By contrast, the same 66% drought treatment had weaker effects in Site 2, where the decline in population size for most cyanobacterial taxa was much less pronounced, and often so variable among samples as to be non-significant (Supporting Information Table S2; Note that no *Scytonema* populations were present there). Drought in Site 2, the light biocrusts site, caused a slight, but non-significant, relative increase in the abundance of *M. vaginatus*. The significant effects of drought on Site 1 and not significant effects on Site 2 were tested statistically with PERMANOVAs and are shown in Fig. 5.

### *Delayed monsoon had weaker effects than drought on bacteria and cyanobacteria*

The delayed monsoon had parallel effects to those of the drought treatment, but they were considerably milder. In Site 1, the delay significantly reduced cyanobacterial abundance, accompanied by significant absolute increases in other phyla (Actinobacteria, Bacteroidetes, Armatiomonadetes and Chlorobi – Fig. 1A). Delayed monsoon caused a decrease to all taxa of cyanobacteria, although not significantly so for every taxon (Supporting Information Table S2). These patterns were mirrored in the Chl *a* concentration (Fig. 2). While the relative order of cyanobacterial sensitivity was the same as we found in drought treatments (*Scytonema* > clades of M. *steenstrupii* > *M. vaginatus*), the overall effects with respect to controls were weaker (10%, 10%–32% and 60% respectively; Fig. 3A and Supporting Information Table S2). Again, as with drought, Site 2 was more impervious than Site 1 to the monsoon delay, showing no significant changes in Chl *a*, community

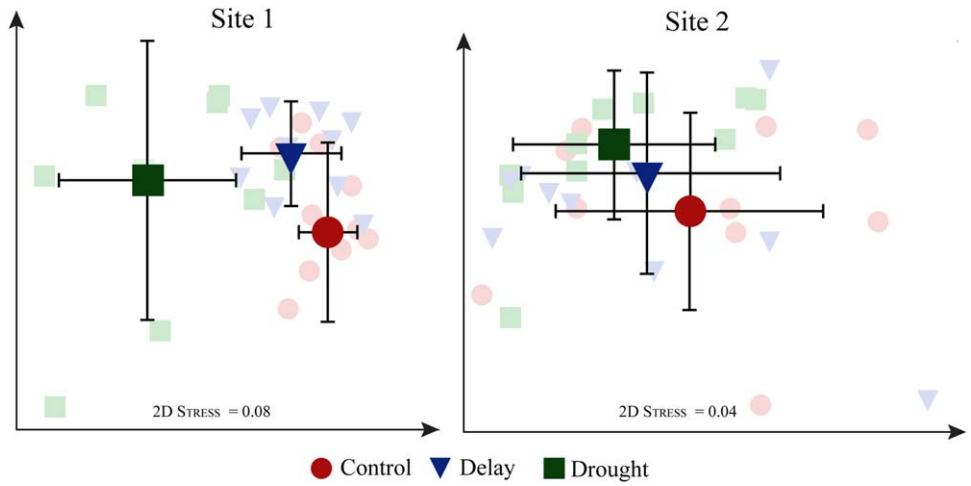


**Fig. 5.** Non-metric multidimensional scaling (nMDS) comparison of cyanobacterial community composition between the treatments in each one of the sites. The nMDS ordination was based on Bray-Curtis similarity of cyanobacterial OTUs. Data points are color coded by treatment (and centroid with scatter for each treatment is in intense colors).
A. Site 1, displaying a significant overall treatment effect (PERMANOVA, $p = 0.0001$, pseudo-$F = 6.24$, df = 27) and all treatments significantly differed from each other in pairwise comparisons ($p < 0.05$).
B. Site 2, where treatment had no significant effects on cyanobacterial composition (PERMANOVA, $p = 0.406$, pseudo-$F = 0.967$, df = 27).

structure or in the population size of any particular taxon (Fig. 3B and Supporting Information Table S2). Altogether, these results were very consistent with the effects seen in drought treatments.

## Discussion

Predicted shifts in the precipitation regime for the Chihuahuan Desert negatively and relatively rapidly affected cyanobacterial soil crusts, causing a generalized decrease in diversity. Both drought and a delayed monsoon showed similar negative effects on the biocrust community, with the latter being less detrimental. The effects observed on the cyanobacterial community varied in intensity between the two sites studied, with Site 1, with complex biocrusts communities in black grama grassland, being more susceptible than Site 2, which supported incipient crusts in blue grama communities. Nonetheless, responses were markedly different among taxa of cyanobacteria, but congruent for each taxon regardless of site, with nitrogen-fixing *Scytonema* spp. being the most sensitive, followed by various phylotypes in the *Microcoleus steenstrupii* complex. *Microcoleus vaginatus* was the least affected in all cases. Interestingly the mortality of cyanobacteria in mature crusts (from our Site 1) was accompanied by an increase in bacterial diversity and richness, and with increases in the population size of several non-cyanobacterial groups. Severe disturbances in soil and sediments are known to enhance bacterial diversity by opening new niches while not decimating existing populations (Galand *et al.*, 2016; Shen *et al.*, 2016; Vuono *et al.*, 2016). In our case, it is easy to imagine how the mortality of primary producers may have redistributed organic carbon to competing groups of heterotrophs.

One could resort to several differential traits between the two sites to seek a mechanistic explanation for the differences in the sensitivity of their biocrust: plant communities and the successional maturity of the crusts did demonstrably differ between sites, and one could seek further differences in geochemical properties or nutrient limitation present in each location. Specific ecological interactions between plant composition and biocrust cyanobacteria that could offer an explanatory basis, however, have not been reported. Since biocrusts rely on autochthonous primary production, and develop in desert soils even without the presence of higher plants, we see it as principally unlikely that plant composition would explain the differential responses we see. While we did not carry out an exhaustive investigation of potentially differentiating edaphic or climatic traits, the soils in the two sites were quite similar in all aspects encompassed in the Sevilleta National Wildlife Reserve database. Lastly, by contrast, we think that the different levels of successional maturity in the sites offer a very plausible explanation to differential sensitivity, as explained in the following.

The different responses in the two sites can be at least partly explained by the varying sensitivity of major biocrust cyanobacterial taxa, which are probably a result of their differential capacity to withstand desiccation. Heterocystous, nitrogen-fixing cyanobacteria in the genus *Scytonema* sp. were the most sensitive, followed by the non nitrogen-fixing pioneer cyanobacteria in the *Microcoleus steenstrupii* complex. Mature crusts in Site 1, contained significant

populations of *Scytonema* and thus suffered large diversity and biomass losses. Incipient crust such as those found in Site 2, dominated by the most resistant cyanobacterium, *M. vaginatus*, were less impacted by the treatments, and did not significantly suffer cyanobacterial biomass losses. The loss of biomass in Site 1, with cyanobacterial populations in the drought plots decreasing to 5% of the control plots, however, was likely too severe to be explained simply by the differential sensitivity of the taxa. There was clearly a site-specific effect. In fact, for the two taxa that were in sufficient numbers in both sites so as to establish a robust comparison (*M. vaginatus* and *M. steentrupii;* Supporting Information Table S2), sensitivity to the same treatment was about an order of magnitude higher in Site 1 than in Site 2. We propose that the differences might have been caused by a cascade effect due to the demise of nitrogen-fixing cyanobacteria in Site 1, which would have exacerbated the drought effect with nitrogen limitation. In Site 2, colonized by a typically incipient, 'light' crust, nitrogen fixation, while likely present at rates similar to those of mature crusts (Johnson, 2005), was probably being carried out by heterotrophic diazotrophs (Pepe-Ranney *et al.*, 2015) rather than by *Scytonema*, so that this additional nutrient limitation stressor did not come to be relevant. Also, *M. vaginatus* excretes many exometabolites that support an entourage of heterotrophic bacteria surrounding it (Baran *et al.*, 2013). This may have contributed to its relative resistance. Of course, other explanations could be called up, such as the presence of genetically resistant cyanobacterial strains in Site 2 only, strains that one could not tell apart with the genetic resolution employed here. Those alternative explanations, however, remain much less parsimonious for lack of an obvious reason to support them.

We found it surprising that the delayed monsoon treatments caused similar effects to those of drought, even if milder. In principle, there is no physiological reason as to why the timing of rain/activity events should have negative consequences on cyanobacteria, and one could potentially see benefits in receiving rain events at times when temperatures, and evaporation rates, are more moderate. Biological soil crusts are known to be very sensitive to changes in the size of the rain event (Johnson *et al.*, 2012; Ferrenberg *et al.*, 2015), for example, but a delay in the monsoon season was never tested before. Further experimentation will be needed to provide a better understanding of the physiological or ecological basis of this effect. In the meantime, it clearly contributes as an additional source of global change stress for the soil communities in these areas, one that should be considered in future studies.

The experiment and results presented here focused on climate change scenarios regarding one climatic variable. Yet, models predict not just altered precipitation, but also concurrently increasing temperatures. In this regard, our results also point to interesting trade-offs between the two main cyanobacterial taxa, *M. vaginatus* and *M. steenstrupii*, that are known to be the most important pioneers in biological soil crusts in desert ecosystems across the Southwestern US (Garcia-Pichel *et al.*, 2009). It has been shown that *M. steenstrupii* is the dominant group in hot deserts, while *M. vaginatus* dominates cold desert locations (Garcia-Pichel *et al.*, 2013), largely because of their differential sensitivity to temperature, so that global warming would result in the preferential loss of *M. vaginatus* (Garcia-Pichel *et al.*, 2013). And yet, here we found that it was members of the *M. steenstrupii* complex that were 4–5 times more sensitive than *M. vaginatus* to drought, irrespective of its geographic location. If indeed global warming does not only bring about an increase in temperature to the arid lands of the US Southwest, but also increased drought and delayed monsoons, then we could have a situation where *both* major types of crust-forming organisms will be affected, each chiefly by one climatic stressor.

Similarly, studies focusing on increased temperatures have shown that, among the heterocystous cyanobacteria, *Scytonema* spp. was the least sensitive to temperature stress (Garcia-Pichel *et al.*, 2013, Giraldo Silva *et al.*, in prep.), and could be predicted to attain competitive advantage under global warming scenarios. In our experiments, *Scytonema* was decimated by drought and delayed monsoon (incidentally, *Nostoc* spp. and *Tolypothrix* spp. also decreased in population size, with similar sensitivity to that of *Scytonema* spp., although their populations were always low in our crusts; calculations not shown). Nitrogen fixing cyanobacteria are crucial as the entry point of N in secularly nitrogen-limited mature soil crusts (Housman *et al.*, 2006; Strauss *et al.*, 2016). Additionally, nitrogen-fixing cyanobacteria provide photoprotection by producing sunscreen pigments to mature biological soil crusts (Garcia-Pichel and Castenholz, 1991; Abed *et al.*, 2010). The identification of this biotic component of soil crust as the most sensitive to drought, points to specific but indirect biogeochemical effects of global warming. In this case, the effects of drought on nitrogen fixation and cycling potential will likely exacerbate those anticipated for warming, as seen in recent studies on temperature dependence of nitrogen cycling (Zhou *et al.*, 2016). Given the long-term nature of the EDGE experiment, it will be interesting to look into actual effects of drought on nitrogen cycling rates in this set-up.

Our results also point out the value of using compositionally explicit studies of soil crust. Had we only reported black-box type parameters in our study (i.e., Chl *a*, total population sizes), or worse yet, indirect measures of biomass like percentage cover, we would have been left with a perplexing result in which the same treatment had very different effects on different sites, and with no logical model to either explain such differences, or to predict logical

general outcomes. The data on differential responses of different taxa allowed us to fill this gap. Therefore, it seems important to highlight that looking at communities of microbes with explicit compositional metrics is informational and quite helpful.

With this study, we can conclude that well-developed cyanobacterial crusts are particularly sensitive to anticipated changes in future precipitation regimes, even to alterations of timing (without altering total rainfall precipitation or event size). Services provided by light cyanobacterial crusts, like soil stabilization and carbon fixation are less likely to suffer dramatic changes due to the altered precipitation, although there is a decrease in diversity and an overall decreasing trend in cyanobacterial population sizes with drought. Our results thus reinforce the observations made by others (Reed *et al*., 2012; Ferrenberg *et al*., 2015) that, with climate change, biological soil crusts will be constrained in development and will not be able to reach advanced succession stages, altering the resources and services that these ecosystems provide to desert soils. These studies, complementing those of Garcia-Pichel and colleagues (2013) speak for quite dire effects of global change on cyanobacterial crusts of the US Southwest, predicting significant losses to biocrust pioneer species, accelerated by a collapse in nitrogen cycling.

## Experimental procedures

### *Study design*

The study was conducted at the Sevilleta National Wildlife Refuge (SNWR) and Long-Term Ecological Research site in New Mexico, USA, at the Northern edge of the Chihuahuan Desert. This region is far from marine moisture sources and occupies a position in which mountains scavenge moisture from weather fronts (Petrie *et al*., 2014). Total annual precipitation is 200–300 mm, most of which falls during the summer monsoon. We sampled biological soil crusts in the Extreme Drought in Grassland Experiment (EDGE), which includes two treatments designed to probe the impacts of predicted climate change. One treatment imposes the severe chronic drought expected by the end of this century (Cook *et al*., 2015) by reducing the monsoon rainfall by 66%. A second treatment delays the summer monsoon by collecting all precipitation that falls in July and August and applying it during September and October, thus simulating a delayed monsoon (Cook and Seager, 2013). Each of the two EDGE sites used in this experiment consists of 30 plots, 10 for each treatment and 10 controls, situated at the same altitude (1538 m) with similar soil texture (sandy loam/sandy clay loam). Site 1 is dominated by black grama grass (*Bouteloua eriopoda*) and has well developed, dark cyanobacterial biocrust with few lichens or mosses, which we assume to be in a more advanced successional stage (Garcia-Pichel and Belnap, 1996; Yeager *et al*., 2004). Site 2 supports Plains-Mesa grassland vegetation, dominated by blue grama grass (*B. gracilis*) along with individuals of black grama, dropseed (*Sporobolus* spp.) and sand muhly (*Muhlenbergia arenicola*). Biocrusts at the blue grama site were of the light cyanobacteria type, which we assume to be in an early successional stage, mostly composed of filamentous, non-nitrogen fixing cyanobacteria (Garcia-Pichel *et al*., 2001; Zhang *et al*., 2006). Samples were collected in February 2016 after three years of manipulation of the plots. The treatment years (2013–2015), had average annual rainfall for the region, and although 2015 had a dry summer, the months prior to sampling were wet. Further information on the Sevilleta LTER meteorology can be found on its website (http://sev.lternet.edu/section/met-central). To sample crusts, five 1 cm diameter $\times$ 1 cm deep soil cores were randomly taken at each of the plots, for a total of 300 samples (5 cores $\times$ 10 plots $\times$ 3 treatment $\times$ 2 sites). Soil from each core was placed in a WHIRL-PAK®, immediately frozen by submersion in liquid nitrogen, brought to the lab and kept frozen at $-80$°C until analysis.

### *Chlorophyll a and scytonemin areal concentrations*

Chlorophyll *a* areal concentration was measured as a proxy of photosynthetic biomass (Couradeau *et al*., 2016) and the sunscreen pigment scytonemin (Garcia-Pichel and Castenholz, 1991) concentration was measured as a proxy for the biomass of nitrogen-fixing cyanobacteria. Only 4 of the 10 replicate plots in each treatment and site were analysed for pigments. The 5 samples from each plot were pooled, weighed and aliquoted into triplicates, to assess analytical variability. Each triplicate was ground in 90% aqueous acetone with a mortar and pestle for $\sim$3 min. The slurry was transferred to a plastic centrifuge tube, the volume was adjusted to 20 ml with 90% acetone, vortexed and allowed to extract for 24 h at 4°C in the dark. Extracts were then clarified by centrifugation at 5060 rcf at 5°C for 8 min or until the supernatant was clear. Absorbance spectra were then recorded on a UV-Visible Spectrophotometer (Shimadzu UV-1601) between 350 and 750 nm. Pigment concentrations were calculated using the trichromatic equations developed by Garcia-Pichel and Castenholz (1991) to de-convolute each pigment's contribution to absorbance. Concentrations are reported as mass per soil surface (mg $cm^{-2}$).

### *DNA extraction and 16S rRNA gene copy number determination*

The 5 sample cores from each plot were pooled and homogenized into a single composite sample. Thereafter, 0.25 g of each homogenate were aliquoted and total DNA

extracted using the MoBio® Power Soil DNA extraction Kit. After fluorometric determination of DNA concentration in the extract (Qubit, Life Technologies, NY, USA), we used qPCR (quantitative real-time PCR) with universal (bacteria+ archaeal) 16S rRNA gene primer set (338F 5′-ACTCCTACGGGAGGCAGCAG-3′, 518R 5′-GTATTACCG CGGCTGCTGG-3′) to determine the number of 16S rRNA gene copies present in each extract. The PCR reaction was performed in triplicate using the Sso Fast mix (Bio-Rad, Hercules, CA, USA) under conditions previously published (Couradeau *et al.*, 2016). The final 16S rRNA gene copy number per unit area of biocrust was determined from the qPCR data (copies/extract) and the total area used for extraction. The number of 16s rRNA genes obtained by qPCR was later used to arrive at total population sizes for each phylum or taxon, by multiplying the total number of genes by the relative abundance of the taxon or phylum at hand, as determined by Illumina sequencing and bioinformatic analyses (see below).

### *16S rRNA library construction and next generation Illumina sequencing*

Bacterial/Archaeal community analysis was performed via commercial next generation sequencing in a MiSeq Illumina platform. Amplicon sequencing of the V4 region of the 16S rRNA gene was performed with barcoded primer set 515f/806r designed by Caporaso *et al.*, (2012) following the Earth Microbiome Project (EMP) protocol (Gilbert *et al.*, 2010) for library preparation. PCR amplifications were done in triplicate, then pooled and quantified using Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen). 240 ng of DNA of each replicate was pooled and cleaned using QIA quick PCR purification kit (QIAGEN). The DNA in the pooled amplificate was quantified by Illumina Library Quantification Kit ABI Prism® (Kapa Biosystems) and diluted with NaOH to a final concentration of 4 nM, then denatured and diluted to a final concentration of 4 pM, and 30% of PhiX was added to the solution. The library was then loaded in the sequencer using the chemistry version 2 (2 × 150 paired-end) following manufacturer's specifications.

### *Bioinformatic analyses and phylogeny*

The forward and reverse read files were concatenated and checked for quality with FASTQC (Andrews, 2010), trimming the sequences that had a Phred quality score $< 30$ with Trimmomatic (Bolger *et al.*, 2014), then pairing and assembling them using Pear with statistical testing, automatically discarding low-probability pairs (Zhang *et al.*, 2014). After splitting the library according to barcode, and removing barcodes, we checked for chimeras using VSEARCH (Rognes *et al.*, 2016). The data set was then processed with QIIME® through the 'pick_open_reference_otus.py' pipeline to pick OTUs using the SortMeRNA protocol (Kopylova *et al.*, 2012) to filter the fragments and using Sumaclust (Schloss, 2016) to compare sequence clusters. We used Greengenes (DeSantis *et al.*, 2006) as the reference database for picking OTUs. We discarded singletons or doubletons that occurred in a single sample. The resulting OTU table was used for taxonomic assignment, and for calculation of diversity indices. Raw sequence data were submitted to NCBI and are publicly available under BioProject number PRJNA394792.

Additionally, all OTUs assigned to cyanobacteria or plastids were subject to individual, full phylogenetic scrutiny against our in-house biocrust cyanobacteria database, to produce more accurate taxonomic assignments at this high level of resolution. For this, representative sequences of each OTU were placed in a pre-assembled reference tree, which had been built using RAxML8 (Stamatakis, 2014) through bootstrap and maximum likelihood workflow on the CIPRES cluster (Miller *et al.*, 2010). To place OTUs, reference sequences for each were aligned within the reference tree using PaPaRa (Berger and Stamatakis, 2011), then placed on the reference tree using the RAxML8 evolutionary placement algorithm (Stamatakis, 2014). The placed sequences were visualized with iTOL3 server (Letunic and Bork, 2007). Once all OTUs were defined and taxonomically assigned, we built an abundance table with all the OTUs and samples, which was then used in the following analyses.

### *Statistical analyses*

We used one-way analysis of variance (ANOVA) to determine treatment effects on population size. The assumptions of a normal distribution of the residuals and equality of variances were tested with Shapiro's and Levene's tests. Significant treatment effects ($p < 0.05$) were further examined with a multiple comparisons test (Tukey's honestly significant difference). Statistical tests used the <car> package (Fox *et al.*, 2013) in R (R Core Team, 2014).

For compositional data, community differences were assessed via permutational multivariate analysis of variance (PERMANOVA). PERMANOVAs were performed on Bray–Curtis distance matrices of relative abundances derived from sequencing and used 9999 permutations. The function betadispar was used to test the variances (PERMDISP) and the PERMANOVAs were performed using the function adonis2, all in the <vegan> package (Dixon, 2017) in 'R' (R Core Team, 2014). A $p$ value of 0.05 was set as the significant threshold for all multivariate statistical analyses. Community composition was visualized with NMDS, using 25 (only 3 were necessary to reach solution) restarts and 9999 iterations.

Diversity core metrics on QIIME® (Caporaso *et al*., 2010) were used to analyse the differences in diversity among treatments and sites and to calculate significant differences.

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## Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Table S1.** Summary of Shannon diversity index and number of OTUs in each sample calculated for all treatments at each site (mean of $n = 10$).

**Table S2.** Treatment effects on Cyanobacteria taxon showed as percentage of the population size on untreated plots compared with both drought and delayed monsoon plots. Data are shown only for the 8 more abundant taxa of each site. ANOVA and *post hoc* Tukey-Kramer test were done for populations of each taxa comparing treatments and *p* values for the *post hoc* tests are shown on the side of each percentage.