

ARTICLE

Rainfall pulse regime drives biomass and community composition in biological soil crusts

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Abstract

Future climates will alter the frequency and size of rain events in drylands, potentially affecting soil microbes that generate carbon feedbacks to climate, but field tests are rare. Topsoils in drylands are commonly colonized by biological soil crusts (biocrusts), photosynthesis-based communities that provide services ranging from soil fertilization to stabilization against erosion. We quantified responses of biocrust microbial communities to 12 years of altered rainfall regimes, with 60 mm of additional rain per year delivered either as small (5 mm) weekly rains or large (20 mm) monthly rains during the summer monsoon season. Rain addition promoted microbial diversity, suppressed the dominant cyanobacterium, *Microcoleus vaginatus*, and enhanced nitrogen-fixing taxa, but did not consistently increase microbial biomass. The addition of many small rain events increased microbial biomass, whereas few, large events did not. These results alter the physiological paradigm that biocrusts are most limited by the amount of rainfall and instead predict that regimes enriched in small rain events will boost cyanobacterial biocrusts and enhance their beneficial services to drylands.

KEYWORDS

arid lands, biocrusts, climate change, cyanobacteria, rainfall variability, soil microbiomes

INTRODUCTION

Precipitation is a master driver of terrestrial ecosystems that fuel plant and microbial activities (Beier et al., 2012; Ru et al., 2018). Intensified variance in regional and global hydrological cycles (Donat et al., 2016; Marvel & Bonfils, 2013; Nolan et al., 2018; Seddon et al., 2016) is reshaping precipitation regimes by altering the amount and timing of rain events (Beier et al., 2012; Knapp et al., 2015). Biotic responses to these changes remain understudied (Beier et al., 2012; Heisler-White et al., 2008; Wang et al., 2020) because few large-scale, long-term experiments manipulate precipitation size and frequency (Knapp et al., 2002; Robertson et al., 2009; Ru et al., 2018;

Suttle et al., 2007; Vargas et al., 2012). This lack of research is particularly acute for microbes (Nielsen & Ball, 2015; Zhou et al., 2018), despite their central functions in carbon feedbacks to climate change (Classen et al., 2015; Rudgers et al., 2020), demonstrable sensitivity (Fernandes et al., 2018), short generation times (Karaoz et al. 2018; Nemergut et al., 2013; Wallenstein & Hall, 2012), and critical roles as primary producers, decomposers, and mitigators of soil erosion. This knowledge gap prompted a recent “warning to humanity” that charged the scientific community to improve the understanding of microbial responses to climate change (Cavicchioli et al., 2019).

The microbial knowledge gap is among the widest for dryland ecosystems, which constitute ~45% of Earth’s

terrestrial surface and support ~40% of the human population (Burrell et al., 2020; Pravalie, 2016). Biological soil crusts (biocrusts) are topsoil microbial (and sometimes cryptogamic) assemblages that have crucial roles in the fertility and stability of drylands (Belnap et al., 2016). Estimated to cover ~12% of the global terrestrial surface, they contribute ~15% of the net primary production and half of the N-fixation of global land surfaces (Elbert et al., 2012; Rodriguez-Caballero et al., 2018). Biocrusts additionally mediate climate feedbacks through soil albedo (Couradeau et al., 2016) and atmospheric dust entrainment (Belnap & Gillette, 1997). Therefore, critical ecosystem services are tethered to how biocrust microbes will respond to climate perturbations in the future.

Past work has suggested that biocrust types vary in their sensitivity to precipitation regimes (Fernandes et al., 2018; Johnson et al., 2012; Reed et al., 2012; Steven et al., 2015; Zhang et al., 2018), requiring greater biogeographic replication and attention to microbial community composition to attain robust predictions. In relation to the extent and diversity of biocrusts, studies are few and of low taxonomic resolution. Here, we filled knowledge gaps for altered precipitation regimes, for which local predictions included reduced rain event size and increased frequencies of summer rainfall events, but stationary total growing season precipitation. Theoretically, this future regime should be detrimental to biocrusts because transitions from dormancy under drought to activity with rains have large metabolic costs (Harel et al., 2004; Rajeev et al., 2013). Starting in 2007, our rainfall experiment added many, small rain events (Many small) or few, large rain events (Few large) during the summer monsoon to test this theoretical prediction for biocrust microbes in the Chihuahuan Desert.

METHODS

Study design

The monsoon rainfall manipulation experiment (MRME) is located in the northern Chihuahuan Desert, New Mexico, USA (details in Appendix S1). MRME imposed two treatments on top of ambient precipitation that altered rainfall variability (detailed methods: Kwiecinski et al., 2020). Both treatments added water that was treated by reverse osmosis (60 mm; ~25% of the mean) using overhead irrigation with raindrop quality sprinklers (producing drop sizes typical of rain) during July–September. Rain was added as either monthly 20 mm events (Few large) or weekly 5 mm events (Many small). Our water treatment lacked wet deposition ammonium or nitrate because wet deposition is low in our region (Báez et al., 2007), and we intentionally decoupled water

from rainfall chemistry by using reverse osmosis water. The thirteen 14 m × 9 m plots included five replicates of each treatment and three control plots, which received only natural precipitation. Precipitation data from the location of the experiment are presented in Appendix S1: Figure S1, showing precipitation metrics for the monsoon season (June–September) during recent years.

We sampled biocrusts on 28 October 2017 and 23 September 2019. Twenty 6-cm diameter by 1.5 cm deep samples were removed with Petri dishes (schematic, Appendix S1: Figure S2) along two transects per plot (300 samples/year). We sampled controls randomly to attain the same sampling effort as in treatment plots. After sampling, biocrusts were air dried immediately to arrest microbial activity and placed into sterile plastic bags at –80°C.

Chlorophyll *a* and scytonemin areal concentrations

We subsampled each Petri dish with one 1 cm diameter × 1 cm deep soil core, pooling, and homogenizing all cores into one composite sample per plot and year. From this homogenate, we aliquoted 0.25 g for DNA processing (please refer to DNA extraction and 16S rRNA gene copy number determination) and used the remainder for pigment extraction (Appendix S1: Figure S3). Details can be found in Appendix S1.

DNA extraction and 16S rRNA gene copy number determination

We used 0.25 g of composite sample for community DNA extraction with MoBio® Power Soil DNA extraction kits. The number of 16S rRNA genes from qPCR estimated absolute population sizes. Details can be found in Appendix S1.

Illumina sequencing and bioinformatic analyses

Bacterial and archaeal community analysis was performed using next generation sequencing on a MiSeq Illumina platform. After sequencing, demultiplexed paired-end FASTQ files were imported into QIIME2.10 (Bolyen et al., 2019). Analyses used the DADA2 plugin (Callahan et al., 2016) to create a feature table with representative sequences (ASVs). ASVs are exact sequences combined with an error model for the sequencing run. Because these are exact sequences, generated without clustering or reference databases, ASV results can be readily compared between studies using the same target

region. (library construction and sequencing details provided in Appendix S1). Raw sequence data are publicly available under BioProject No. PRJNA633650 at the National Center for Biotechnology Information (NCBI).

Statistics

Alpha-diversity

To obtain ASV-level alpha-diversity metrics (Shannon–Weiner diversity index, Pielou Evenness and Faith PD taxon richness), we used the phylogenetic alpha metrics plugin in QIIME2. We also determined alpha-diversity for absolute abundance (sequencing coupled with qPCR) at the phylum level for Bacteria and Archaea and at the genus/species level for cyanobacteria. Taxonomic resolution was deeper for cyanobacteria because they were the focus of this study (<https://github.com/FGPLab/cydrasil>). To calculate richness (S), the Shannon–Weiner diversity index (H'), and its evenness counterpart ($J = H'/\ln(S)$) we used the *vegan* package (Dixon, 2003) in R (R Core Team, 2020).

Composition and beta-diversity

To obtain ASV-level beta-diversity, we applied permutational multivariate analysis of variance (perMANOVA) to compare centroids of rainfall treatments against controls, using weighted UniFrac (a phylogenetic quantitative matrix) from the beta-diversity plugin on QIIME2. To determine whether treatments altered beta-diversity through the dispersion in composition among plots within a treatment, we used permDISP on the Bray–Curtis similarity matrix with 10,000 permutations and Holm–Bonferroni corrections. To visualize treatment effects, we used non-metric multidimensional scaling (nMDS) analysis of the Bray–Curtis similarity matrix with 500 random re-starts. To determine which taxa contributed most to treatment divergence, we used SIMPER analysis in a one-way design for treatment, run separately for each year (Clarke & Gorley, 2009). Further statistical details are in Appendix S1.

RESULTS

Interannual variation was moderate for community composition but large for microbial biomass

Natural interannual variation was captured by comparing control plots between 2017 and 2019. Phylum-level bacterial/archaeal community composition was typical of

biocrusts in general and consistent with a different experiment at this location (Fernandes et al., 2018). Although composition was similar between years (Figure 1a), the relative proportion of non-photosynthetic taxa differed inter-annually, and Crenarchaeota were detected only in 2019. Cyanobacteria were always the most abundant phylum, followed in order by (alpha)Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi and Bacteroidetes (Figure 1a—Control). Cyanobacterial community composition in controls was typical of “light” biocrusts from North American drylands and also consistent between years (Figure 1b—Control). The bundle-forming, non-heterocystous *Microcoleus vaginatus* accounted for >80% of cyanobacterial reads. Several clades in the “*M. steenstrupii*” complex were also present. According to recent taxonomic advances, the *M. steenstrupii* complex will be referred as Coleofasciculaceae, and the clades within it referred to as the corresponding genera (Fernandes et al., 2021). Heterocystous cyanobacteria, such as *Scytonema* spp. or *Nostoc* spp., which make crusts “dark” through their sunscreen pigment scytonemin, were not detected in the control plots (Figure 1b—Control).

Although microbial community composition in control plots was consistent between years, community size was not. The areal concentration of 16S rRNA gene copies was six-fold larger in 2019 than in 2017 (14 ± 1.1 vs. 2.3 ± 0.1 billion copies cm^{-2} ; Table 1). Cyanobacterial abundance followed a similar pattern, with 3.9 ± 2.4 billion copies cm^{-2} in 2019, but 1.2 ± 0.4 in 2017. Consistently, the areal concentrations of Chl *a*, a proxy for cyanobacterial biomass, was significantly greater in control plots in 2019 than 2017, although the difference was not quite two-fold (Table 1).

Given the large interannual variation in community size, we analyzed bacterial, archaeal and cyanobacterial community composition (Figure 1) not only as relative proportions (Figure 1a,b, right), but also as absolute abundance determined by qPCR (Figure 1a,b, left). Relative community composition differed significantly between years for Bacteria and Archaea (phylum-level perMANOVA, pseudo- $F = 25.09$, $p = 0.013$) and for primary producers (cyanobacteria genus/species level perMANOVA, pseudo- $F = 5.33$, $p = 0.046$; Figure 2). Differences in absolute composition, however, were striking for both Bacteria (Figure 1a left, perMANOVA, pseudo- $F = 17.47$, $p = 0.008$) and cyanobacteria (Figure 1b left, perMANOVA, pseudo- $F = 11.15$, $p = 0.024$). Therefore, we focused on absolute abundances determined by qPCR for the evaluation of treatment effects.

Increased rainfall can, but does not necessarily, increase microbial abundance

Abundance of Bacteria and Archaea was largest in the Many small rainfall treatment relative to controls but

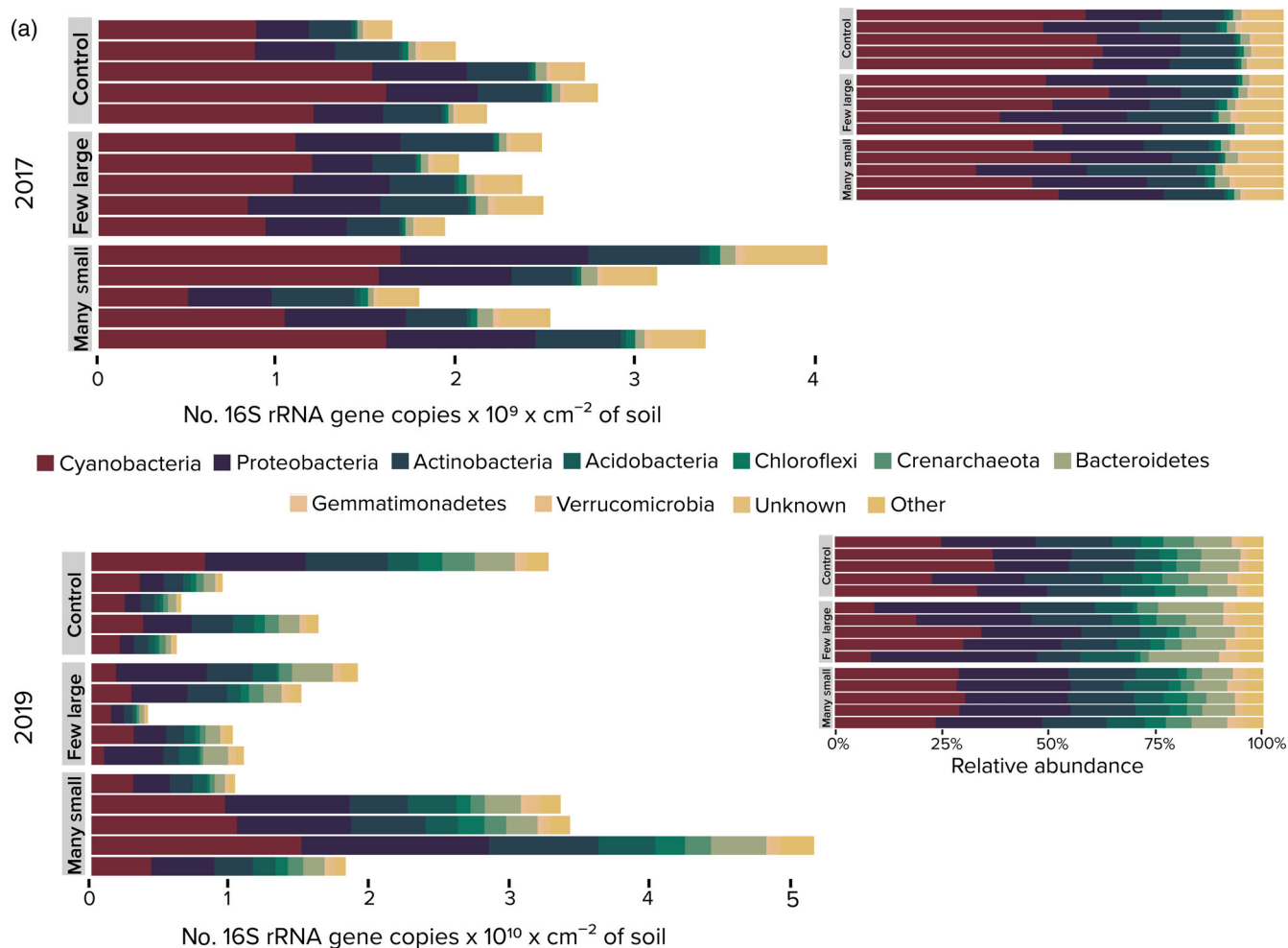


FIGURE 1 Microbial abundance and community structure in control and rain treatment biological soil crusts as determined by high-throughput 16S rRNA gene analyses coupled to qPCR, as well as relative abundance (top right side in each panel). Five replicate plots/samples were analyzed for each treatment. Each bar is a replicate. Note that the scales are not the same for the 2 years. (a) Bacteria/Archaea. Phylogenetic assignments were based on *blast* to the Greengenes database and carried to the phylum level. (b) Cyanobacteria; phylogenetic assignments were based on *blast* to Cydrasil to the genus or species level, as feasible.

was unaffected or reduced by rain addition in the Few large regime (Table 1). The addition of Many small rains had a larger impact in the year of plenty (2019), when community size was more than double over controls, than in 2017 when communities in the Many small treatment were only ~30% larger than controls. Some treatment effects were marginally non-significant ($0.1 < p < 0.05$; Table 1) because of the high variance in community data and low replication ($n = 5$ plots) that were trade-offs against the large plot size. For cyanobacteria, the Many small treatment either had no effect (2017) or significantly enhanced (2019, $p = 0.02$) 16S rRNA gene-based abundance over controls, whereas Few large rains caused moderate declines over controls (Table 1). Chl *a*-based estimates of cyanobacterial biomass indicated greater biomass in both rain additions than controls (Table 1), although in 2019, the Few large

treatment did not significantly differ from controls at $p < 0.05$.

Rainfall regime reorganized the composition of microbial communities

Bacteria and Archaea assemblage

At the fine resolution of ASVs, rainfall additions significantly altered bacterial and archaeal community composition (Appendix S1: Table S1) even when coarse, phylum-level composition was only marginally affected ($p = 0.09$; Appendix S1: Table S2), nevertheless consistently in both years (Figure 2, Appendix S1: Table S2; perMANOVA, Year \times Treatment, $p > 0.07$). Altered beta-diversity did not underlie changes in microbial diversity or community

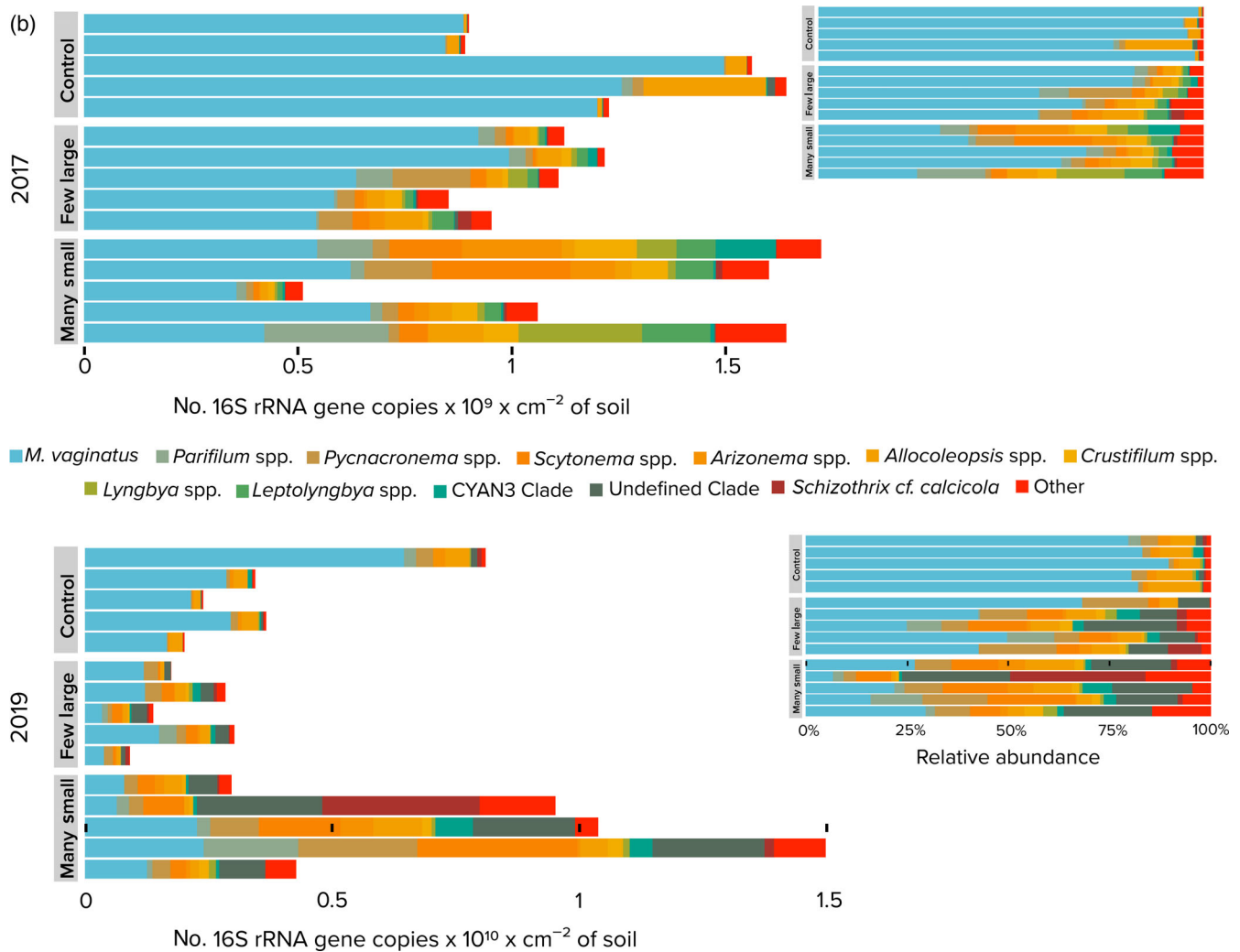


FIGURE 1 (Continued)

composition because treatments did not increase dispersion among plots (Appendix S1: Table S4). Among phyla, cyanobacteria responded most strongly to the rainfall regime. Cyanobacteria were most abundant in absolute terms in the Many small treatment and least abundant in the Few large treatment, and their contributions to community dissimilarity at the phylum level ranged from 21% to 47%. Cyanobacteria were followed in rank importance by Proteobacteria, then by Actinobacteria (Appendix S1: Table S3). Proteobacteria contributed less than half as much to community dissimilarity as cyanobacteria (12.5%–27.7%; Appendix S1: Table S3) and responded more to any rain addition than to the rainfall regime. Similar to cyanobacteria, Proteobacteria were most abundant in the Many small treatment (Appendix S1: Table S3, Figure 1a). Within the Proteobacteria, the most responsive ASVs were members of the Methylobacteraceae, and within the Actinobacteria, responsive taxa were in the Geodermatophilaceae and Micromonosporaceae.

Cyanobacterial assemblage

At the genus/species level, cyanobacteria significantly diverged in composition between the Many small and Few large treatments ($p < 0.02$, Appendix S1: Table S2; Figures 1a and 2) and between each rainfall treatment and the controls (all $p < 0.03$, Appendix S1: Table S2; Figures 1b and 2). These differences persisted at the ASV level (Appendix S1: Table S1). Cyanobacteria composition also responded more strongly to altered precipitation regime in 2019 than in 2017 (Appendix S1: Table S2; perMANOVA, Year \times Treatment, $p < 0.05$), and more strongly to the addition of Many small than Few large monsoon rain events (Figure 2). Because rain treatments did not differ in community dispersion within a given year (all $p > 0.33$), the perMANOVA result was not driven by differences in community divergence among the treatments and met the assumption of multivariate homogeneity of variances among treatments, excepting

TABLE 1 Biocrust bacterial/archaeal and cyanobacterial community size by treatment and sampling year.

Year	Bacteria/Archaea			Cyanobacteria			Chlorophyll <i>a</i>		
	(10 ¹⁰ 16S rRNA gene copies cm ⁻²)			(10 ⁹ gene copies cm ⁻²)			(mg m ⁻²)		
	Control*	Few large*	Many small*	Control*	Few large*	Many small*	Control*	Few large	Many small*
2017	0.2 ± 0.1 a	0.2 ± 0.0 a	0.2 ± 0.0 a [†]	1.2 ± 0.4 a	1.1 ± 0.1a	1.3 ± 0.5 a	39 ± 7.5 a	80 ± 21 b	86 ± 20 b
2019	1.4 ± 1.1 a	1.2 ± 0.5 a	3.0 ± 1.6 a [†]	3.9 ± 2.4 a	2.0 ± 0.9 b	8.4 ± 4.8 a [†]	66 ± 6.5 a	87 ± 26 a	116 ± 32 a [†]

Note: Asterisks indicate significant differences between years using *t*-tests with *p*-values corrected for multiple response variables. For each microbial group and parameter, differing letters represents a significant difference according to ANOVA with Tukey–Kramer post hoc tests within each year ($p < 0.05$; $n = 5$). Daggers indicate $0.1 > p > 0.05$ against other treatment means.

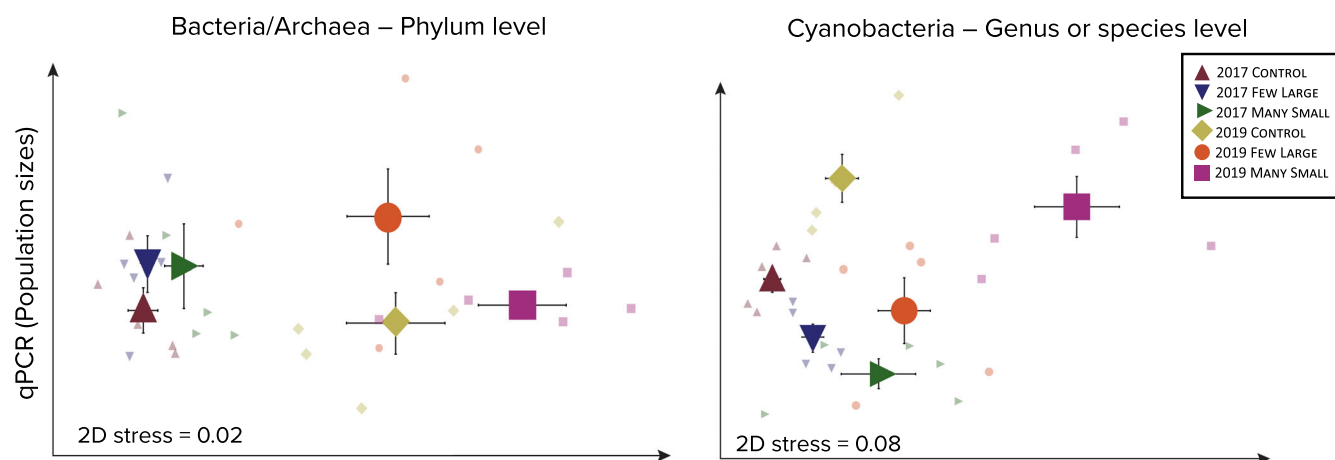


FIGURE 2 Non-metric multidimensional scaling (nMDS) comparison of absolute abundance (qPCR) bacterial/archaeal (left) and cyanobacterial (right) community composition between the treatments. The nMDS ordination was based on Bray–Curtis similarity. Data points are color coded by treatment and year (and centroid with scatter for each treatment is in intense colors).

one case of increased dispersion within the Many small treatment in 2019 (Appendix S1: Table S4). The cyanobacterial taxon most responsible for divergence in community composition was *M. vaginatus*, which declined 35-fold to 71-fold in the Few large treatment and 54-fold in the Many small treatment relative to controls (Appendix S1: Table S5, Figure 1b). In contrast, genera in the family Coleofasciculaceae (*Parifilum*, *Arizonema*, and a new clade with no cultured representatives) benefited most from the addition of Many small rains, and somewhat less from Few large events. For example, in 2017, *Parifilum* spp. was 18-fold more abundant in the Many small treatment than in controls, and six-fold more abundant in the Few large treatment than in controls (Appendix S1: Table S5, Figure 1b). Similarly, in 2019, *Parifilum* spp., was 10-fold more abundant in Many small than in controls, and 20% greater in Many small than Few large. The heterocystous, surface dwelling *Scytonema* spp., which was not detected in control plots in 2017 or 2019 (Appendix S1: Table S5, Figure 1b), comprised 1%–14% of cyanobacterial reads in

the Few large treatment and 3%–22% in the Many small treatment, depending on sampling year. Concentrations of the heterocystous biomarker, scytonemin, corroborated this change. In both years, there was 170% more scytonemin in plots with Many small rains than Few large rains (Appendix S1: Figure S5) and on average, both rainfall addition treatments combined had 250% more scytonemin than controls.

Additions of Many small rain events prominently boosted microbial diversity

Bacteria and archaea

Water additions enhanced the diversity and evenness of Bacteria and Archaea at the ASV level, but rainfall regime did not affect ASV richness (Figure 3a). The Many small treatment supported the largest ASV diversity in both years (Figure 3a). However, at the phylum level, Shannon diversity and evenness increased with any

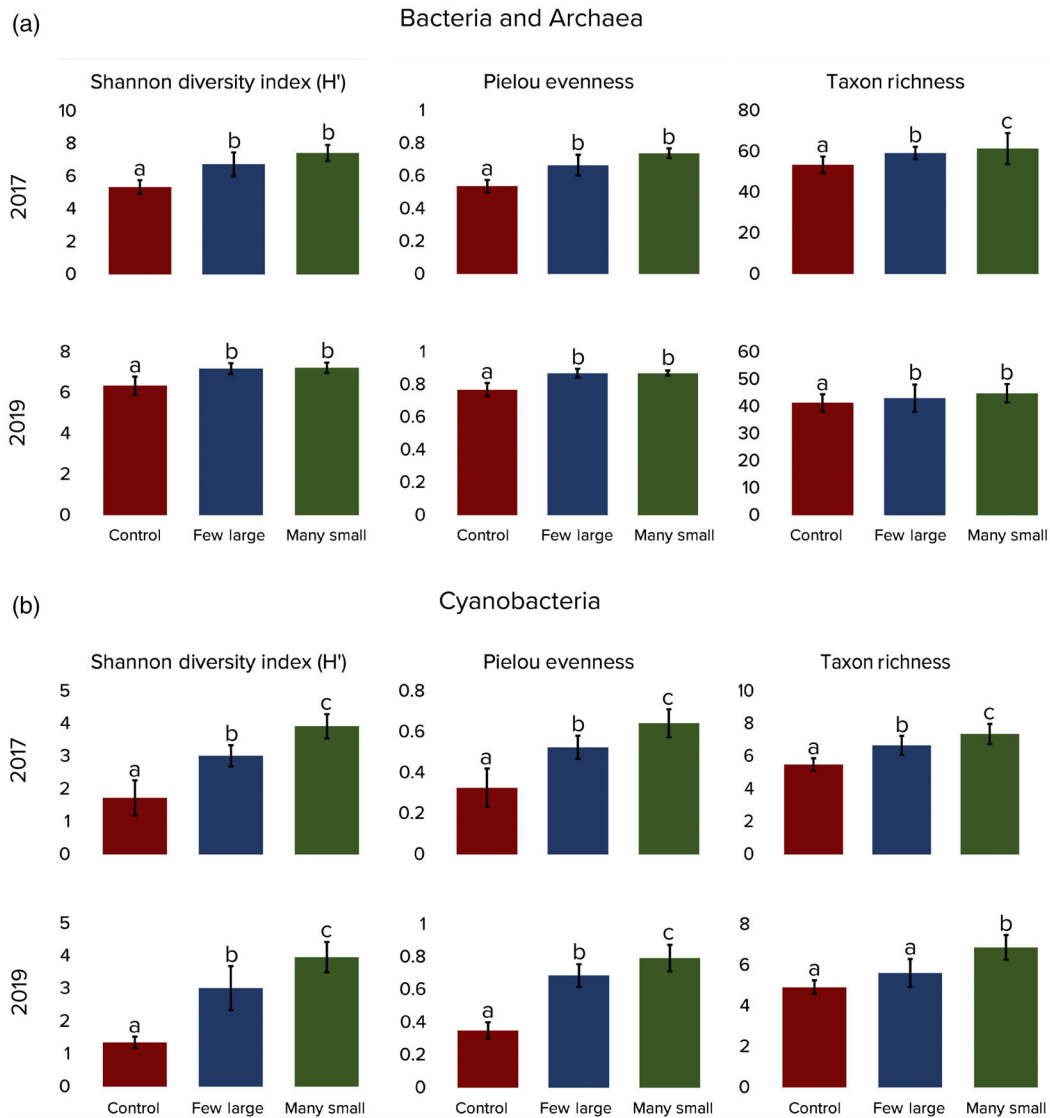


FIGURE 3 Biocrust alpha-diversity indices (diversity, richness and evenness) at the ASV level of taxonomic resolution. Bacteria and Archaea (a) and cyanobacteria (b). Performed using QIIME2 phylogenetic alpha-diversity. Significant differences among treatments from ANOVA/Kruskal–Wallis post hoc tests are indicated by different letters.

rainfall addition in 2017, but not in 2019 (Appendix S1: Table S6).

Cyanobacteria

Both rain additions boosted the ASV alpha-diversity of cyanobacteria (diversity, evenness, richness, Figure 3b) over controls, but the largest cyanobacterial diversity occurred with Many small rains in both years (Figures 1b and 3b). At the genus/species level, cyanobacteria had similar trends to the ASV level, except for richness, which did not significantly differ among treatments at coarser phylogenetic resolution (Appendix S1: Table S7).

DISCUSSION

The case for limitation of biocrusts by water availability

Our experiment revealed that the magnitude and frequency of rain pulses—which define the rainfall pulse regime (Collins et al., 2008)—mattered as much, if not more, than the total amount of water input. Therefore, our findings partially contradict the paradigm that deserts are fundamentally “water-controlled” ecosystems (Noy-Meir, 1973), in which the net amount of water input is paramount (Nemani et al., 2003). Because biocrust organisms are only active when wet, precipitation amount is hypothesized to control biocrust composition

(Evans & Lange, 2003; Johnson et al., 2005). Precipitation is often cited as the most limiting factor for biocrust development because of the physiological limitation of biocrusts according to Liebig's principles (von Liebig, 1855; Yeager et al., 2012; Yahdjian et al., 2011; Hooper & Johnson, 1999; Peterjohn & Schlesinger, 1990). In contrast, for most microbial responses in our study, differences between Many small and Few large regimes were greater or equal in magnitude to the differences between either water addition and controls. Furthermore, contrary to the current paradigm, precipitation additions did not always benefit biocrusts. Total microbial biomass estimators had only weak responses to few high-volume additions, regardless of sampling year. Specifically, for primary producers, Few large rains suppressed biomass, whereas high-frequency, small rains boosted biomass. Interestingly, studies assessing plant productivity in our same experiment returned a similar conclusion, that rainfall regime was more important than the amount of rain in affecting plant above-ground biomass (Thomey et al., 2011). Together, our study and that of Thomey et al. (2011) pointed to co-limitation and strong legacy effects of precipitation regime on dryland communities.

Data from 2 years relevantly challenged the robustness of our results because natural precipitation varies strongly from year to year in North American deserts (Maurer et al., 2020, Rudgers et al., 2018). In terms of biocrust community size, 2019 represented a year of plenty over 2017, yet the greater importance of rain event regime (size and frequency) over the total amount of precipitation was largely consistent between years. Inter-annual differences in microbial community size did not correlate with either naturally higher mean annual precipitation (2017: 271 mm; 2019: 256 mm) or rain event size (mean event size 2017: 4.34 mm; 2019: 4.11 mm), further supporting the hypothesis that biocrusts respond to more complex aspects of rainfall regime than simple precipitation totals. It is not yet clear why delivery of additional water as large pulses failed to promote microbial growth. Mechanisms may include increased runoff during large rain events, which could reduce percolation and local soil water retention, but this mechanism does not apply at our site where large rain events increase infiltration and retention of soil moisture (Vargas et al., 2012). Moreover, more small rains are likely to expand the cumulative activity period of soil surface microbes, sustaining growth over longer time windows than sparse, but large rain events, by keeping the surface soil moist. Another possible mechanism is the leaching of nutrients into subcrust soils during large rain events. Cyanobacteria-dominated biocrusts leach organic carbon and various forms of nitrogen and phosphorous to the

underlying soil layers (Johnson et al., 2007; Kristina et al., 2022). The rapid leaching of soil nutrients could explain why we did not observe microbial growth under large rainfall events.

Increased rainfall enhanced microbial diversity by suppressing dominant extremophiles

Rain additions, regardless of frequency, did enhance both bacterial and cyanobacterial diversity, most intensely during the year of plenty, 2019. Within cyanobacteria, assemblage change occurred most strongly from the loss of dominance by *M. vaginatus* and increases in members of the Coleofasciculaceae family and heterocystous *Scytonema* spp. These compositional changes support the tenet that severely restricting water availability also restricts the pool of microbial species able to thrive, constraining diversity (Rothrock & Garcia-Pichel, 2005), which parallels patterns from other extreme environmental gradients (Avrahami & Conrad, 2003; Nübel et al., 1999; Ward et al., 1998). In our case, *M. vaginatus* may lose dominance with water addition because it is considered the most resilient against drought among local bacteria taxa, a result confirmed for biocrust cyanobacteria in our prior extreme drought experiment (Fernandes et al., 2018).

Small, frequent rains surpassed large, rare downpours in promoting microbial biomass and diversity

An understanding of the importance of variability in the precipitation regime to biocrust communities derives from comparisons between the two rain addition treatments, which received the same total amount of precipitation. Small, more frequent rains significantly increased microbial biomass in both years over Few large events (Table 1). Cyanobacteria biomass, measured by either 16S rRNA gene copies or as Chl *a* areal concentration was greater under Many small events in both years, and significantly so in 2019 (Table 1; Appendix S1; Figure S5). These results indicated that smaller, more frequent, pulses promoted biocrust development over less frequent, larger ones and therefore that predicted future rainfall scenarios for the area, which are consistent with trends over the last 100 years (Petrie et al., 2014), should promote biocrust cover. Although a minimum pulse size requirement for net cyanobacterial growth has not been directly determined, studies by Zhang et al. (2018) and Steven et al. (2015) suggested that it is probably <2 mm. In laboratory incubations, 2 mm pulses

sufficed for net carbon gain in cyanobacterial biocrusts (Zhang et al., 2018) but not for moss biocrusts. Consistently, Reed et al. (2012) showed that mosses grew with 5 mm rainfall additions but died under 1.2 mm, when cyanobacteria still did well. These differences in desiccation thresholds could have large ecosystem impacts depending on the initial biocrust community composition, as shown by the contrast between our results and those of Reed et al. (2012), in which frequent, small rainfall events decreased the productivity of the system due to moss mortality. This mortality could also affect soil hydrology, soil stabilization, and other ecosystem functions. In our case, because microbes benefited from small rainfall events, we do not predict a loss of function in a future rainfall regime of more, smaller events. Clearly, cyanobacteria have higher desiccation thresholds than mosses, and our results demonstrate that differential adaptations to water availability also exist among biocrust cyanobacteria taxa, with *M. vaginatus* having greater desiccation tolerance than members of the Coleofasciculaceae or *Scytonema* spp. It is becoming clear that the lack of data to build physiological response curves to desiccation is limiting our interpretive and predictive abilities for microbial communities under climate change (please also refer to Rudgers et al., 2020).

Winners and losers under future rainfall regimes

Biocrust cyanobacteria can be divided in two groups that exploit different niches. One gathers the pioneer bundle-formers that colonize bare soils (Garcia-Pichel & Wojciechowski, 2009); these taxa are motile and cannot fix nitrogen by themselves (Starkenburg et al., 2011), resorting to symbioses with heterotrophic bacteria (Nelson et al., 2020b). In North American drylands, pioneer cyanobacteria usually include *M. vaginatus* and the family Coleofasciculaceae (previously known as *M. steenstrupii*) (Couradeau et al., 2016; Garcia-Pichel et al., 2013). The second group colonize biocrusts after soil has been stabilized and are sessile, nitrogen-fixing cyanobacteria. In North American drylands this group is encompassed largely by *Scytonema* spp., *Nostoc* spp. and *Tolypothrix* spp. (Yeager et al., 2007), which supply fixed N.

Among the taxa of the pioneer functional group, *M. vaginatus*, the dominant taxon in our control plots, declined with any rainfall addition, leading to its loss of dominance. In contrast, genera within the Coleofasciculaceae family became dominant with our Many small rains treatment. However, even within this family, genera diverged in their sensitivity to rainfall regimes: for example, *Pycnacronema* spp. uniquely benefited from Few large rains, but not from Many small (Appendix S1: Table S5, Figure 1b). *Pycnacronema* is common in biocrusts of

Brazilian savannas (Machado-de-Lima et al., 2019), characterized by mean annual precipitation five times greater than the Chihuahuan Desert and by large, intense rain pulses during the wet season. Interestingly, experimental additions of large rain events have also been shown to affect plant functional types differentially, promoting forbs over grasses in a tallgrass prairie system (Jones et al., 2016). Our detection of divergent responses to rainfall regime within the Coleofasciculaceae revealed a previously unappreciated niche delineation in this family, improving interpretation of microbial function from taxonomy.

The contrasting responses between *M. vaginatus* and the Coleofasciculaceae to rainfall regimes suggest an interesting life history trade-off because *M. vaginatus* and Coleofasciculaceae also differentially respond to temperature: *M. vaginatus* is more psychrotolerant but less heat tolerant, but the Coleofasciculaceae is more thermotolerant (Garcia-Pichel et al., 2013), but less desiccation tolerant. Both warming and aridity are expected to change concurrently under future climate scenarios (Ault et al., 2014; Dai, 2013), and although their potential interaction remains to be studied, one prediction deriving from our results is that a drier future will benefit *M. vaginatus*, but a hotter one with increased rainfall variability will benefit genera in the Coleofasciculaceae family.

Among the secondary biocrust successional group, *Scytonema* spp., was not detected in controls but appeared in both water addition treatments, although more abundantly under many, small pulses. This important result implies that an altered future rainfall regime will promote two key ecosystem services: biocrust N-fixation ability and protection against UV damage. Given the large global contribution of biocrusts to terrestrial N-fixation, this may have widespread functional significance for global biogeochemical cycles. These potential effects on N-fixation are also opposite to those predicted for climate warming (Zhou et al., 2016) or increased drought (Fernandes et al., 2018). As we attempt to advance our understanding of cyanobacterial responses to complex changes in temperature and rainfall, the interactions between the two factors must not be neglected; studies in which both factors are manipulated concurrently are clearly needed.

In this study, we were able to detect changes in population sizes within cyanobacterial taxa by combining qPCR data and 16S rRNA sequencing data. This approach has been shown to be appropriate whenever total community size is affected (Fernandes et al., 2018). A recent study that analyzed cell size, cell volume and number of 16S rRNA copies (Gonzalez-de-Salceda & Garcia-Pichel, 2021) also points to the advantage of combining these two approaches instead of presenting each separately.

Discordance among metrics of microbial community responses to rain pulses

Not all metrics of microbial biomass yielded congruent measures of treatment effects. For example, both rain additions caused net gains in cyanobacteria judged by Chl *a* concentration. However, 16S rRNA genes indicated a slight decrease in cyanobacteria abundance under Few large rains, but biomass enhancement with Many small rain additions. The apparent discrepancy may be explained by treatment effects on community structure combined with known differences among cyanobacteria species in per cell production of Chl *a* (Nelson et al., 2020a). Particularly, cultivated representatives of the Coleofasciculaceae family (which increased with our water additions) produce more Chl *a* per cell (on the order of 10%–30%) than *M. vaginatus*, which relies more heavily on light absorption by phycobilins (Fernandes & Garcia-Pichel, unpublished). Although in many cases Chl *a* concentration is a good proxy for photosynthetic biomass (Fernandes et al., 2018; Sorochkina et al., 2018; Yeager et al., 2012), in scenarios in which major changes in cyanobacterial community composition occur, such as in this experiment, or when light intensity varies, alternative proxies are recommended (Nelson et al., 2020a). Future efforts to characterize the per cell Chl *a* content for a greater diversity of cyanobacteria taxa will enable precise estimates of the community-weighted mean photosynthetic capacity that results from changes in microbial community composition due to altered climate regimes.

Conclusions

Our long-term experiment, conducted on typical cyanobacterial biocrust types of the southwestern USA in the largest and most endangered desert biome of North America, surprisingly challenged the paradigm that biocrusts, as in dryland ecosystems generally, are fundamentally and principally limited by the total amount of precipitation. Our experimental additions of rain failed to consistently increase biocrust standing stocks, which varied significantly between years but did not correlate with mean annual precipitation. Whether or not rain additions increased biocrust populations hinged on the combination of delivery frequency and rain event size. By contrast, increased precipitation always enhanced microbial diversity by promoting taxa that were less resistant to drought, among pioneers and secondary colonizers alike. The striking changes in cyanobacterial community composition under alternative pulse regimes, with clear winners and losers, highlights the risk of using “taxonomic black boxes” in microbial studies, in which potentially functionally relevant shifts in community composition

may go unnoticed. Such community shifts are not without probable functional consequences, and here, the most relevant is a predicted increase in N-fixation under the many small pulse regime projected to increase regionally (due to an increase in nitrogen-fixing cyanobacteria such as *Scytonema* spp.). Unfortunately, a more variable future precipitation regime will, in association with the warming temperatures and greater aridity already evident locally and regionally (Maurer et al., 2020; Rudgers et al., 2018), tend to suppress biocrusts. Integrative studies of interacting axes of environmental change (Rillig et al., 2019; Steven et al., 2015) will help to contextualize the dramatic shifts in microbial communities that we observed to improve predictions on future consequences for ecological functions.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT


Raw sequence data are available from the NCBI under BioProject ID 633650 at <https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA633650>.

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