Biocrust Responses to Altered Precipitation Regimes

by

Vanessa Moreira Câmara Fernandes

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Graduate Supervisory Committee:

Ferran Garcia-Pichel, Chair
Jennifer Rudgers
Osvaldo Sala
Christopher Penton

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ABSTRACT

Desert organisms lead harsh lives owing to the extreme, often unpredictable environmental conditions they endure. Climate change will likely make their existence even harsher. Predicting the ecological consequences of future climate scenarios thus requires understanding how the biota will be affected by climatic shifts. Biological soil crusts (biocrusts) are an important ecosystem component in arid lands, one that covers large portions of the landscape, improving soil stability and fertility. Because cyanobacteria are biocrust's preeminent primary producers, eking out an existence during short pulses of precipitation, they represent a relevant global change object of study. I assessed how climate scenarios predicted for the Southwestern United States (US) will affect biocrusts using long-term, rainfall-modifying experimental set-ups that imposed either more intense drought, a seasonally delayed monsoon season, or a shift to smaller but more frequent precipitation events. I expected drought to be detrimental, but not a delay in the monsoon season. Surprisingly, both treatments showed similar effects on cyanobacterial community composition and population size after four years. While successionally incipient biocrusts were unaffected, mature biocrusts lost biomass and diversity with treatment, especially among nitrogen-fixing cyanobacteria. In separate experiments, I assessed the effect of rainfall with modified event size and frequency after a decade of treatment. Small, frequent rainfall events surprisingly enhanced the diversity and biomass of bacteria and cyanobacteria, with clear winners and losers: nitrogen-fixing *Scytonema* sp. benefited, while *Microcoleus vaginatus* lost its dominance. As an

additional finding, I could also show that water addition is not always beneficial to biocrusts, calling into question the notion that these are strictly water-limited systems. Finally, results interpretation was severely hampered by a lack of appropriate systematic treatment for an important group of biocrust cyanobacteria, the "*Microcoleus steenstrupii* complex". I characterized the complex using a polyphasic approach, leading to the formal description of a new family (Porphyrosiphonaceae) of desiccation resistant cyanobacteria that includes 11 genera, of which 5 had to be newly described. Under the new framework, the distribution and abundance of biocrust cyanobacteria with respect to environmental conditions can now be understood. This body of work contributes significantly to explain current distributional patterns of biocrust cyanobacteria and to predict their fate in the face of climate change.

DEDICATION

to my parents, Antônio José e Valéria

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TABLE OF CONTENTS

Page

LIST OF TABLES ix

LIST OF FIGURES x

CHAPTER

1 – DISSERTATION INTRODUCTION 1

Functional Roles of Biological Soil Crusts 1

Biocrust Microbial Community 2

Adaptations of Biocrust Cyanobacteria to Water Limitation 4

Climate Change and its Potential Effects on Biocrust Communities 6

Cyanobacteria Phylogeny and its Ecological Implications 9

Dissertation Research Objective 13

Approach 13

Dissertation Structure 14

Figures 18

References 21

2 – EXPOSURE TO PREDICTED PRECIPITATION PATTERNS DECREASES POPULATION SIZE AND ALTERS COMMUNITY STRUCTURE OF CYANOBACTERIA IN BIOLOGICAL SOIL CRUSTS FROM THE CHIHUAHUAN DESERT 31

Abstract 32

CHAPTER Page

Introduction 33

Methods 36

Results 42

Discussion 47

Figures 53

Supplementary Information 58

References 61

3 – ALTERED RAINFALL REGIMES RESULT IN DISTINCT BIOLOGICAL SOIL CRUST COMMUNITIES 67

Abstract 68

Introduction 70

Methods 74

Results 81

Discussion 87

Tables 96

Figures 99

Supplementary Information 102

References 114

CHAPTER Page

4 – PORPHYROSIPHONACEAE, FAM. NOV., A MONOPHYLETIC HOME FOR THE "MICROCOLEUS STEENSTRUPII" COMPLEX AND OTHER DESICCATION-TOLERANT FILAMENTOUS CYANOBACTERIA 123

Abstract 124

Introduction 126

Methods 129

Results 139

Discussion 150

Formal Descriptions 159

Figures 168

Supplementary Information 175

References 187

5 – CONCLUSIONS 194

Main Point (s) from each Chapter and Dissertation Contribution (s) 194

References 198

REFERENCES 199

APPENDIX

A. WRITTEN PERMISSIONS FOR USE OF COPYRIGHTED WORK 221

LIST OF TABLES

Table Page

1. Bacteria/Archaeal and Cyanobacterial Population Size in Biocrusts by Treatment and Sampling Year. 96

2. Permanova Analysis of Population Sizes (on Bray Curtis Matrix) Showing Significance Among Treatments, Sampling Years and Interaction (Treatment X Year), as well as Pairwise Comparisons Among Treatments for either Data Compiled for both Years or Separated by Year. 97

3. Bacteria/Archaea Alpha-Diversity Indices (Diversity, Richness and Evenness) at the ASV Level of Taxonomic Resolution, for both Years, and Separated by Year. Significances were Measured Using Anova and Kruskal-Wallis Posthoc Tests. . 98

4. Cyanobacteria Alpha-Diversity Indices (Diversity, Richness and Evenness) at the ASV Level of Taxonomic Resolution, for both Years and Separated by Year. Performed Using Qiime2 Phylogenetic Alpha-Diversity. Significances were Measured Using Anova And Kruskal-Wallis Posthoc Tests. 98

LIST OF FIGURES

Figure Page

1. Macrostructure of Biocrusts. (A): a Biocrust from a Silty Soil from the Great Basin Desert. (B) A Topcrust Biocrust from a Sandy Soil in the Great Basin Desert can hold its own Against Gravity and can be Easily Peeled of Soil..... 18

2. Biological Succession for Biocrusts in North America, Conceptualized Progressing from Left to Right. Bundle-Forming Cyanobacteria (Depicted as Black Filaments, whit Sheaths in Gray) Colonizes Bare Soil and Stabilzes it, Enabeling the Colonization by Heterocystous Cyanobacteria (Brown Globules and Rods), with Eventual Colonization by Lichens and Mosses..... 18

3. Photomicrographs of Pioneer Bundle-Forming Biocrust Cyanobacteria from the Genus Microcoleus Isolated from the Great Basin Desert. (A-B) *Microcoleus vaginatus* Filaments. (C-D) Rope-Like Structures From Strain of the "*Microcoleus steenstrupii* Complex". 19

4. Photomicrographs of Sessile Heterocystous Cyanobacteria from Biocrusts. (A) *Scytonema* sp. Strain Isolated from the Great Basin Desert. (B) *Nostoc* sp. Strain Isolated from the Great Basin Desert. (C) *Tolypothrix* sp. Strain Isolated from the Chihuahuan Desert..... 19

5. Photomicrograph of *Nostoc* sp. Strain with Scytonemin Excretion Indicated by the Brown Pigmentation. 20

Figure Page

6. 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 Analyzed 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. 53

7. 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 ($P<0.05$). 54

8. 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 Analyzed 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. 55

9. Non-Metric Multi-Dimensional Scaling Comparison of Cyanobacterial Community Composition in Untreated Plots. 56

10. Non-Metric Multi-Dimensional Scaling Comparison of Cyanobacterial Community Composition Between the Treatments in each one of the Sites..... 57

Figure Page

11. Bacterial Abundance and Community Structure in Control and Treatment Biological Soil Crusts as Determined by High- Throughput 16S rRNA Gene Analyses Coupled to q-PCR, as Well as Relative Abundance. 99

12. Cyanobacterial Abundance and Community Structure in Control and Treatment Biological Soil Crusts as Determined by High- Throughput 16S rRNA Gene Analyses Coupled to q-PCR, as well as Relative Abundance. 100

13. Non-Metric Multidimensional Scaling (nMDS) Comparison of Bacterial/Archaeal (Left) and Cyanobacterial (Right) Community Composition Between the Treatments..... 101

14. Maximum Likelihood Tree Reconstruction of the Natural Occurring Diversity of the *Microcoleus steenstrupii* Complex Based on Short Sequences (~ 300bp) of the 16S rRNA Gene Showing the Diversity of Clades Present Inside this Group as Seen in Field Studies..... 168

15. Bayesian Phylogenetic Reconstruction of the *Microcoleus steenstrupii* Complex Based on 16S rRNA Gene Sequences Showing the Affiliations of the 16S rRNA Gene Sequences Obtained from the 15 Investigated Strains (in bold). 169

16. Photomicrographs of the Filamentous, Non-Heterocystous Cyanobacterial Strains Investigated Grown in JM Medium at 25°C. Strain Denominations are Indicated in Each Panel. Bar 5 µm. 170

Figure Page

17. Temperature Range at Which the Studied Cyanobacterial Strains can grow or Survive. Colored Rectangles Indicate Positive Growth; Hatched Rectangles Indicate Stasis (No Growth, but no Obvious Deterioration)..... 171

18. Growth Yield of Strains in the Porphyronsiphonaceae Family (Divided by Genus) in the Upper and Lower Ranges of Temperature. 172

19. Shifts in Community Composition Along a Climatic Gradient. Relative Distribution of Genera From the Porphyronsiphonaceae Family Defined by Evolutionary Placements done Using an Evolutionary Placement Tool (epa-ng) on Cydrasil. Some Patterns are Clear as some Genera Increases or Decrease with Aridity. 173

20. Correlations Between the Logit Transformed Relative Proportion of Porphyronsiphonaceae Family Genera and Two Climatic Factors: Mean Annual Temperature (MAT) and Mean Annual Precipitation (MAP). 174

1 – DISSERTATION INTRODUCTION

Functional roles of biological soil crusts

Biological soil crusts (hereafter referred to as biocrusts) (Figure 1) are ecologically important biotic components of arid lands (see reviews at Eldridge and Greene 1994, Weber et al. 2016a). These are photosynthetically driven microbial assemblages that develop in areas where light can penetrate directly to the soil without the limitation of plant coverage and litter, so that they become dominant communities in drylands. Because drylands cover nearly 45% of the Earth continental area (Prăvălie 2016) and are predicted to expand due to increased aridity caused by climate change (Seager et al. 2007, Seager and Vecchi 2010a, Nolan et al. 2018), biocrusts matter not only locally, but also globally. Biocrusts are important not only because they cover large portions of drylands, but also because they play key roles in the global biogeochemical cycles of carbon and nitrogen (Housman et al. 2006, Elbert et al. 2012a). The global carbon stock of biocrusts has been calculated to be about 54 x 10_{12} g (Garcia-pichel et al. 2006) and to be responsible for 15% of the net primary production around the globe (Elbert et al. 2012a, Rodriguez-Caballero et al. 2018). Dinitrogen fixation of biocrusts accounts for half of the overall N fixation of terrestrial surface, being estimated to be at 49 Tg/yr (Elbert et al. 2012b, Rodriguez-Caballero et al. 2018). Besides, biocrusts are also capable of enriching the soil in other macro and micronutrients, such as P, Mg, Na, K and Ca by trapping dust particles (Reynolds et al. 2001), so that the presence of biocrusts enhances soil fertility relative to uncrusted soils (Harper and Pendleton 1993, Harper and Belnap 2001). Additionally, biocrusts provide soil surface stabilization, protecting it against wind

(Belnap and Gillette 1997, Zhang et al. 2006) and water erosion (Gaskin and Gardner 2001).

While the benefits of biocrust communities to desert ecosystems is very clear, some other effects of biocrusts can be both positive or negative, depending on the scale studied or the type of biocrust, among other factors. Biocrusts communities influence hydrological processes due to changes in soil roughness and porosity (Belnap 2006) and can positively or negatively affect soil water retention and runoff depending on the spatial scale and soil properties (Verrecchia et al. 1995), although further studies are needed to fully understand these effects (Chamizo et al. 2016). The effects of biocrusts on seed germination and survival and growth of seedlings of vascular plants were shown to be positive (Defalco et al. 2001, Godínez-Alvarez et al. 2012), negative (Zaady et al. 1997) or sometimes have no effect (Megill et al. 2011, Godínez-Alvarez et al. 2012) depending on species and environment characteristics (Havrilla et al. 2018). Following establishment, biocrusts were shown to have an overall positive effect on plant growth (Defalco et al. 2001, Pendleton et al. 2003, Langhans et al. 2009), but recent studies suggest net competition between plants and biocrusts, especially under altered precipitation patterns (Zhang et al. 2016b, Dettweiler-Robinson et al. 2018).

Biocrust microbial community

Biocrusts are usually divided among successional stages, where cyanobacterial-dominated biocrusts (incipient or “light” and more mature or “dark”) comprise the initial stages of development, followed by lichen or moss dominated biocrusts (Yeager et al.

2004, Housman et al. 2006, Nejidat et al. 2016) (Figure 2). However, studies shown that in many unaltered environments, terminal succession of biocrusts can be cyanobacteria-dominated (Yeager et al. 2004, Couradeau et al. 2016, Muñoz-Martín et al. 2018). Cyanobacteria are key players to most biocrust developmental stages, because they are the pioneer organisms, extremely important for soil stabilization and further colonization. Whenever lichen or moss biocrusts do develop, it is likely that they are preceded by one or more cyanobacterial or algal-dominated stages.

In North American arid lands, colonization of bare soils is initiated by motile cyanobacteria, such as *Microcoleus vaginatus* and those in the *Microcoleus steenstrupii* complex (Zhang 2005, Pointing and Belnap 2012a) (Figure 3). *Microcoleus* species in biocrusts also form bundles that are composed of many trichomes together within a polysaccharide sheath, forming rope-like structures that are capable of binding soil particles together (Garcia-Pichel and Wojciechowski 2009). The vertical (Pringault and Garcia-Pichel 2004) and horizontal (Sorochkina et al. 2018) gliding migration of these organisms forms a weave of extracellular sheaths that are left behind and stabilizes loose soils, forming a crust (Figure 1). By means of this soil stabilization, other bacteria (Garcia-Pichel et al. 2001, Nagy et al. 2005), archaea (Soule et al. 2009b), and fungi (Bates and Garcia-Pichel 2009) can settle, becoming part of the biocrust community and this stage, which usually referred to as “light crusts” in literature (Weber et al. 2016b). Biocrusts succession can eventually lead to the colonization of sessile heterocystous cyanobacteria. The three most common clades of such cyanobacteria are *Nostoc* spp., *Tolypothrix* spp. and *Scytonema* spp. (Yeager et al. 2007) (Figure 4). These cyanobacteria

not only supply nitrogen through atmospheric nitrogen-fixation, but also protect biocrusts from UV radiation by producing the sunscreen pigment, scytonemin (Garcia-Pichel and Castenholz 1991a) (Figure 5). Due to its dark color, production of scytonemin brings significant abiotic changes in albedo and soil surface temperatures, that in turn, can lead to changes in biocrust community composition through a biophysical feedback (Couradeau et al. 2016). At this stage, biocrusts are referred to as "dark crusts". In mature "dark" crusts, cryptogamic populations of lichens and/or mosses (Belnap et al. 2001, Ullmann and Büdel 2001) may colonize, depending of temperature and moisture conditions (Garcia-Pichel 2003). Biocrusts succession can be altered by a diversity of stressors, such as fire (Bowker et al. 2004, Shen et al. 2016) and sand deposition (Weber et al. 2016c), as well as different environments that select for distinct communities of organisms, such as fog and dew deserts (Lalley and Viles 2008) and mesic climates (Read et al. 2011).

Adaptations of biocrust cyanobacteria to water limitation

Water is thought to be the most limiting factor for terrestrial ecosystems around the world (Nemani et al. 2003). Deserts are especially water limited and are referred to as "water-controlled" ecosystems (Noy-Meir 1973), as periods of biological activity in drylands are tightly linked to seasonal pulses in moisture availability (Schwinning and Sala 2004). While temperature, solar radiation and nutrient inputs in desert ecosystems are fairly stable over the year, rainfall events are infrequent, discrete and quite unpredictable. In arid regions that are only 10-50 rainy days a year, occurring in 3-15 rain events or

clusters of rainy days (Sala and Lauenroth 1982), followed by long periods of dryness (Knapp et al. 2008).

Precipitation is thought to control biocrust distribution and composition from global to local scales, and it is cited as the most limiting factor for biocrust development (Weber et al. 2016b). Biocrusts organisms are able to utilize short periods of activity during infrequent precipitation events, then survive desiccation by relying on the unique capabilities of their vegetative cells to enter a dormant state and rapidly resuscitate upon wetting (Angel and Conrad 2013, Rajeev et al. 2013, Karaoz et al. 2018). Adaptations requiring the formation of spores and subsequent germination upon wetting are absent or quite rare in biocrusts. To survive desiccation and respond rapidly to precipitation, biocrust cyanobacteria possess a set of physiological adaptations, such as the accumulation of compatible solutes like trehalose and sucrose (Rajeev et al. 2013, Baran et al. 2017) and the production of exopolysaccharides that slow the speed of water loss (Couradeau et al. 2018).

Pioneer bundle-forming *Microcoleus* spp. have the capability to migrate below the surface to find refuge from the extreme UV-solar radiation and erosional abrasion (Garcia-Pichel and Pringault 2001, Pringault and Garcia-Pichel 2004, Yeager et al. 2007), increasing their overall survival. Once moisture availability increases, these cyanobacteria can move up to the surface of biocrusts, responding as fast as 30 minutes after a wetting event (Garcia-Pichel and Pringault 2001). *Microcoleus* spp. also form bundles that allows these cyanobacteria to colonize bare soils (Garcia-Pichel and Wojciechowski 2009), but their bundles may also serve as a buffer zone that helps to

slow down cell dehydration, enabling water transfer to the cyanobacterial cells (Couradeau et al. 2018). Sessile nitrogen-fixing cyanobacteria, such as *Nostoc* spp., *Tolypothrix* spp. and *Scytonema* spp. lack the ability to move down the soil to protect themselves against desiccation and solar radiation. These cyanobacteria instead produce scytonemin, a UV-sunscreen pigment, that is excreted and deposited in the extracellular polysaccharide sheaths of these cyanobacteria and serves as a shield from solar damage to the microbial community at large (Garcia-Pichel and Castenholz 1991b, Soule et al. 2009b). Additionally, biocrust cyanobacteria are able to rapidly resuscitate and turn on their photosynthesis machinery upon wet-up and later, to prepare for desiccation by entering a dormant state once drying down (Garcia-Pichel and Belnap 1996a, Rajeev et al. 2013).

Climate change and its potential effects on biocrust communities

Climate change will influence temperature and rainfall patterns across the globe (IPCC 2014, Seddon et al. 2016, Nolan et al. 2018). Dryland ecosystems will not be exempt from these changes and are expected to become even harsher environments (Bernstein et al. 2008, Loarie et al. 2009). In particular, the southwestern United States is predicted to experience an increase in temperature of about 1 °C per decade (Seager and Vecchi 2010b). Climate models also 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, Dai 2013, Ault et al. 2014, Petrie et al. 2014, Cook et al. 2015), while research into precipitation patterns and timing (Cook and Seager 2013) has predicted a progressive delay in the onset of summer monsoonal rains. Other models do not predict changes in total annual precipitation, but changes in the *size* of summer rainfall events (Weltzin et al. 2003, Bernstein et al. 2008). For example, in the Northern Chihuahuan Desert, 60% of annual precipitation falls during the summer monsoon season (June-September), when a small number of *large* events are responsible for most of the annual precipitation (Petrie et al. 2014, Peters et al. 2015). Smaller individual rain events without declines in total rainfall, like those predicted, would shorten the period during which soils remain wet during each rain event.

The effect of climate change on biocrusts is of special interest as biocrusts are dynamic consortia, capable of rapid responses to changing environmental conditions through changes in community structure, which are likely to control ecosystem processes critical to controlling functional traits at the ecosystem scale (Maestre et al. 2015, Rodríguez-Caballero et al. 2018). Despite the capacity of these communities to thrive under the marked variations in temperature and precipitation typical of desert ecosystems, their biological activity is strongly linked to seasonal temperature and moisture content, making them susceptible to changes in these environmental factors in the long term (Johnson et al. 2012, Reed et al. 2012, Steven et al. 2015).

Several studies have assessed the effects of altered precipitation event-size on moss-dominated biocrusts (Johnson et al. 2012, Reed et al. 2012, Ferrenberg et al. 2015)

and found that, alone or together with warming treatments, shortening event size increased moss mortality (Reed et al. 2012), and within 10 years of treatment, forced a well-developed biocrust back to an early successional stage of mostly cyanobacteria (Ferrenberg et al. 2015). These studies led to the prevailing paradigm that increased frequency of small rain events, predicted by some climate models, will be detrimental to biocrusts. More recent reports from the same long term experiment showed that cyanobacteria increased in relative abundance and biomass under small rainfall events of 1.2 mm after 6 years of treatment (Steven et al. 2015), showing that cyanobacteria might actually thrive under smaller, more frequent rainfall events.

Many studies have demonstrated that biocrust cyanobacteria are extremely well-adapted to specific temperature ranges. For example, biogeographic surveys across the western US showed that the distribution of pioneer species of cyanobacteria in biocrust is dependent on temperature, with *Microcoleus vaginatus* dominating colder locations, while clades of the *M. steenstrupii* complex dominate hotter locations (Garcia-Pichel et al. 2013a). In the same way, nitrogen-fixing cyanobacteria in biocrusts were found to have temperature preferences, with *Scytonema* sp. dominating in hot locations and *Nostoc* spp. together with *Tolypothrix* spp. dominating mild and cold temperature locations (Giraldo-Silva et al. 2020).

It is clear that cyanobacteria have a differential sensitivity to temperature, however it is unclear how altered precipitation patterns will affect their role in biocrust formation and dryland fertilization. Studies on smaller, more frequent rainfall events only reported differences in total cyanobacteria biomass, without exploring how different taxa

respond to the changes (Ferrenberg et al. 2015, Steven et al. 2015), or have focused on moss-dominated biocrusts. Because different cyanobacterial taxa contribute distinct services to the ecosystem, understanding how different taxa respond to precipitation changes will help us better understand the large-scale consequences for soil erodibility or fertility, of small-scale community shifts. Additionally, to my knowledge, no prior studies have assessed how biocrusts respond to increased drought or to a delay in the rainy season, two other possible scenarios predicted for future desert climates.

Cyanobacteria phylogeny and its ecological implications

Cyanobacteria, previously known as blue-green algae, are one of the most diverse and widely distributed phyla of bacteria. Among the prokaryotes, they are considered among the most important microorganisms on Earth, given their ability to perform oxygenic photosynthesis. Because of their traditional assignment as algae, the classification of these organisms was created by phycologists, following the rules of the Botanical Code (Stafleu et al. 1972). Thuret (1875) (Thuret 1875), Bornet & Flahaut (1887-1888) (Bornet 1888, Bornet and Flahault 1888) and Gomont (1982) (Gomont 1892) wrote the first comprehensive taxonomic monographs for cyanobacteria, recognized by phycologists. In 1932, Geitler (Geitler 1925) provided an updated taxonomic review and determination manual that become the first taxonomical system recognized by both microbiologist and phycologists. Here, the discriminatory properties for the description of both genera and species were either structural or ecological. Types were represented by

herbarium specimens or, failing these, by description and illustrations. Cyanobacterial cultures were not recognized as valid type material under the Botanical Code.

Later, utilizing the argument that cyanobacteria are unquestionably prokaryotes, another taxonomical system was proposed introducing the bacteriological criteria to cyanobacteria taxonomy (Waterbury and Stanier 1977, Krumbein 1979, Rippka et al. 1979). In this new system, cultured cyanobacteria are usually assigned the name of genus with a strain code and are the basic taxonomic unit. The current edition of the Bergey's Manual of Systematic Bacteriology includes compiled information from both bacteriological and phycological sources (Castenholz 2001).

With the advent of electron microscopy and molecular and genetic methods for characterization of cyanobacteria, many proposals on how to organize and describe cyanobacteria were made simultaneously, with Cyanobacteria being continually revised for the last twenty years. The practice of classifying cyanobacteria by morphological characteristics is now widely recognized as inefficient, as a number of the morphological characteristics used to define higher taxa (tapering, polarity, types of branching, presence of akinetes, among others) have apparently arisen and/or been lost several times during the evolution of modern species and genera (Gugger and Hoffmann 2004, Komárek et al. 2013). This phenomenon is due to the complex evolutionary history of cyanobacteria, possible achieved by horizontal gene transfer (Schirrmeister et al. 2011, Shih et al. 2013).

A coherent taxonomical system for cyanobacteria is essential for ecological studies, as correct, consistent identification is required in order to understand the role of a particular cyanobacterium in nature or effects of the environment on cyanobacteria biodiversity. The

most comprehensive study of cyanobacterial species is known to be the polyphasic approach of Colwell (Colwell 1970). This approach included assessing morphological, phylogenetic and ecological features, thereby preventing the naming of species based on only one criterion, such as morphology; a practice that has led to non-coherent taxa, such as *Leptolyngbya* spp. (Perkerson et al. 2011), *Microcoleus steenstrupii* (Boyer et al. 2002) and *Geitlerinema* spp. (Komárek et al. 2014).

In biological soil crusts, pioneer bundle-forming cyanobacteria have been traditionally assigned to the genus *Microcoleus*, due to their morphological similarities. However, the epithet '*Microcoleus steenstrupii*' has long been recognized as a supra-generic entity. The genus *Microcoleus* was initially described by Gomont in 1982 (Gomont 1892) with *Microcoleus vaginatus* (Vaucher) Gomont ex Gomont presented as the type species for the genus. Later, in the work of J.B Petersen, a new species, *Microcoleus steenstrupii*, was described differing from *Microcoleus vaginatus* by the absence of calyptra and the presence of cross walls constrictions, among other morphological differences (Petersen 1923).

In 2002, Boyer et. al. studied the genus *Microcoleus* using 16S rRNA gene based phylogenetic distances and found that *Microcoleus steenstrupii* was phylogenetically distant from the type species for the genus *Microcoleus* (*Microcoleus vaginatus*) and that the genus was most likely composed of many different species or genera (Boyer et al. 2002). Two reasons were pointed to reach this conclusion: 1) The great genetic distance from the type species, *M. vaginatus* and 2) the low similarity between the different sequences of *Microcoleus steenstrupii* found in nature, with variability between strains

sometimes being evident in non-variable regions of the 16S rRNA gene (Boyer et al. 2002). Following Boyer's study, the salt-tolerant species in the *Microcoleus* genus, *M. chthonoplastes*, was re-described as *Coleofasciculus chthonoplastes* (Siegesmund et al. 2008) and assigned to the family Phormidiaceae, separate from *Microcoleus vaginatus*, which remains in the family Oscillatoriaceae. Here, Siegsmund et al. 2008 suggested that *Microcoleus* species should be divided into these two families (Phormidiaceae and Oscillatoriaceae).

In 2013, Strunecký et al. revised and redescribed the genus *Microcoleus* and described a new family, the family Microcoleaceae, excluding the phylogenetic distant species with constricted cross walls, such as *M. paludosus* and *M. steenstrupii* (Strunecký et al. 2013). However, no new genus or family was proposed for these species. In 2014, Komárek et. al. proposed a new system of classification of Cyanobacteria, advocating for evolutionary history, monophyletic groups and the use of the polyphasic approach (Komárek et al. 2014). The proposed classification system, however, did not resolve the classification of the *Microcoleus steenstrupii* complex.

Because of their role as pioneers and soil stabilizers of biocrusts, members of the traditionally defined *Microcoleus* genus were frequently the focus of studies on biocrust cyanobacteria. First studies on climate change effects on biocrust cyanobacteria showed that *M. vaginatus* dominates colder locations, while *M. steenstrupii* dominate hotter locations (Garcia-Pichel et al. 2013a). In this case, responses could occur even though the *Microcoleus steenstrupii* complex was being treated as a single species. Later, studies simulating extreme drought found that *M. vaginatus* was more tolerant to drought, but that

different clades within the *Microcoleus steenstrupii* complex had different responses to drought, showing that the clades inside the complex also had apparently diverging ecological traits (Fernandes et al. 2018). Therefore, to better understand the ecological responses of *M. steenstrupii* clades, we needed to first resolve their evolutionary relationships and taxonomy, so as to better understand their responses to the environment and their consequences for desert ecosystems.

Dissertation research objective

My overarching objective was to investigate the effects of climate change on cyanobacterial biocrust community structure, focusing specifically on the effects of predicted alterations on precipitation patterns and, consequently, on water availability, to understand the possible consequences of these abiotic changes to desert ecosystems.

Approach

I focused on cyanobacteria because i) they are usually the biocrust pioneer organisms, ii) their relative abundance within the community has the potential to modify soil properties such as soil stability, nutrient availability and water retention iii) they affect the biogeochemical cycling of C (carbon) and N (nitrogen), and iv) their inherent relevance in the context of climate change. Long term studies on climate change conducted at Sevilleta Long Term Ecological Research (located in the Northern Chihuahuan Desert) station were chosen to study the effects of predicted precipitation changes due to the presence of biocrust in on-going experiments. Previous studies focused on the effects of

climate change on plants and soil nutrients, but no studies had examined changes in cyanobacterial community structure, creating an opportunity for collaboration and funding for my research.

My first tasks were to study the effects of drought, delayed monsoon and smaller, more frequent rainfall events on biocrust microbial community structure by collecting biocrust samples from two on-going long-term climate change studies in the Chihuahuan Desert. After discovering differential responses among the clades of the *Microcoleus steenstrupii* complex to altered precipitation patterns and water availability, I directed my focus to resolve the taxonomy of this group of cyanobacteria. Using our cyanobacterial tree, Cydrasil, I investigated the possible clades present inside the *Microcoleus steentrupii* complex and developed new approaches to isolate distinct clades of the complex from biocrust communities. This in-depth knowledge of the biology of microbial biocrust species allowed me to propose the first comprehensive system of taxonomy of the *Microcoleus steenstrupii* complex.

Dissertation structure

My dissertation is comprised of this introductory chapter, followed by three research chapters that are structured as stand-alone published or publishable manuscripts. Finally, the document has a general conclusions chapter.

Chapter 1. Dissertation introduction

This chapter introduces the biological soil crust community, its core microbial components and its dynamics. It also reviews the main factors impacting biocrust disturbance and the current state of cyanobacteria phylogeny.

Chapter 2. Exposure to predicted precipitation patterns decreases population size and alters community structure of cyanobcateria in biological soil crusts from the Chihuahuan Desert (Published in Environmental Microbiology, impact factor: 5.147)

This chapter presents the impacts of a delayed monsoon season or a chronic drought in the bacterial community structure and population sizes of biocrusts from the Chihuahuan Desert, with focus on cyanobacteria. We presented sequencing data combined with the total number of 16S rRNA gene copies obtained with qPCR to arrive at population sizes for each bacterial/cyanobacteria taxon; a technique that allowed us to show both declines on the total population sizes and changes to relative abundance. We focused on the responses of cyanobacteria and showed differential responses by distinct taxa, with *M. vaginatus* being more resistant to drought, while nitrogen-fixing cyanobacteria, such as *Scytonema* spp., were most negatively affected by both drought and a delay in the monsoon season.

Chapter 3. Increased rainfall variability promotes higher diversity in biological soil crusts cyanobacteria

This chapter presents the effects of smaller, more frequent rainfall events on biocrust microbial community structure with a focus on cyanobacteria. Experimental plots were submitted to rainfall additions of either 20 mm or 5 mm events, to a total of 60 mm of rainfall addition on summer monsoon months. Therefore, we also analyzed the effects of water addition in biological soil crusts, by comparing treatment plots to controls without rainfall addition. Unexpectedly, rainfall addition was not always beneficial to biocrust microbial community, opposing the paradigm that biocrusts are water limited. Smaller, more frequent rainfall events showed to be mostly beneficial to biocrusts community, increasing total diversity of bacteria/archaea, as well as cyanobacteria.

Chapter 4. Decomplexing the *Microcoleus steenstrupii* complex

This chapter proposes the first comprehensive taxonomy of the *Microcoleus steenstrupii* complex. Here, by using the polyphasic approach of Colwell (Colwell 1970), we evaluated morphological, phylogenetic and ecological features of this complex to provide better taxonomical assignment. We studied 15 strains within the *Microcoleus steenstrupii* complex by sequencing their full 16S rRNA gene, analyzing their morphological features and the presence of pigments, as well as their responses to different temperatures. A new family of terrestrial, non-heterocystous, desiccation tolerant cyanobacteria is described to resolve the *Microcoleus steenstrupii* complex. The new family, called Porphyrosiphoneceae, is composed by 11 genera, five of which are described for the first

time in here (*Sociatus*, *Parifilum*, *Xeronema*, *Crassifilum* and *Crustifilum*). The coherence of these newly assigned genera is tested using a meta-analysis of a large set of molecular surveys of biocrust cyanobacteria.

Chapter 5. Dissertation conclusions

The conclusions chapter summarizes the main findings discovered during the execution of each of the chapters that comprise my dissertation. It emphasizes the contributions of my work to the field of microbial ecology and physiology, and how the generated knowledge further impacts biocrust research.

Figures

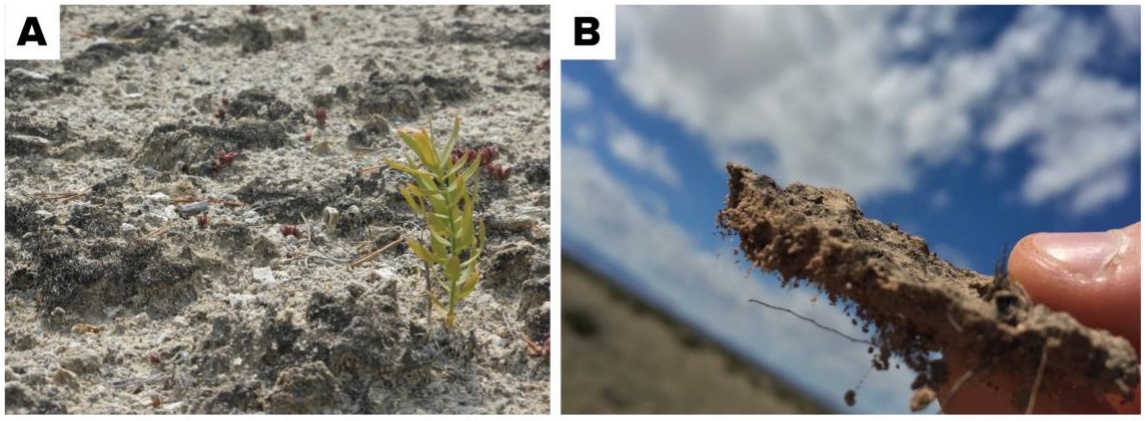
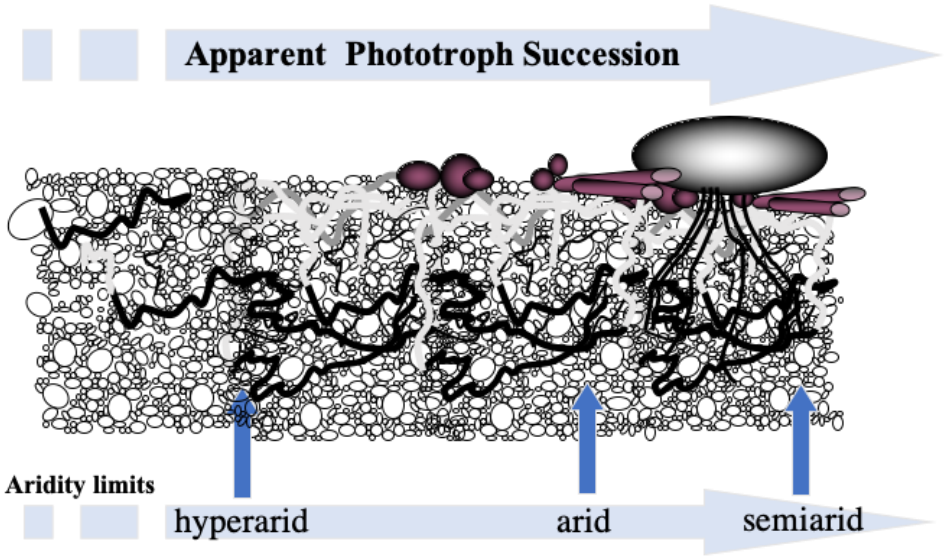


Figure 1. Macrostructure of biocrusts. (**a**): A biocrust from a silty soil from the Great Basin Desert. (**b**) A topcrust biocrust from a sandy soil in the Great Basin Desert can hold its own against gravity and can be easily peeled of soil. Photo credit: Sergio Velasco-Ayuso.



Adapted from Garcia-Pichel (2002) Encyc. Environ. Microbiol

Figure 2. Biological succession for biocrusts in North America, conceptualized progressing from left to right. Bundle-forming cyanobacteria (depicted as black filaments, whit sheaths in gray) colonizes bare soil and stabilzes it, enabeling the colonization by heterocystous cyanobacteria (brown globules and rods), with eventual colonization by lichens ans mosses.

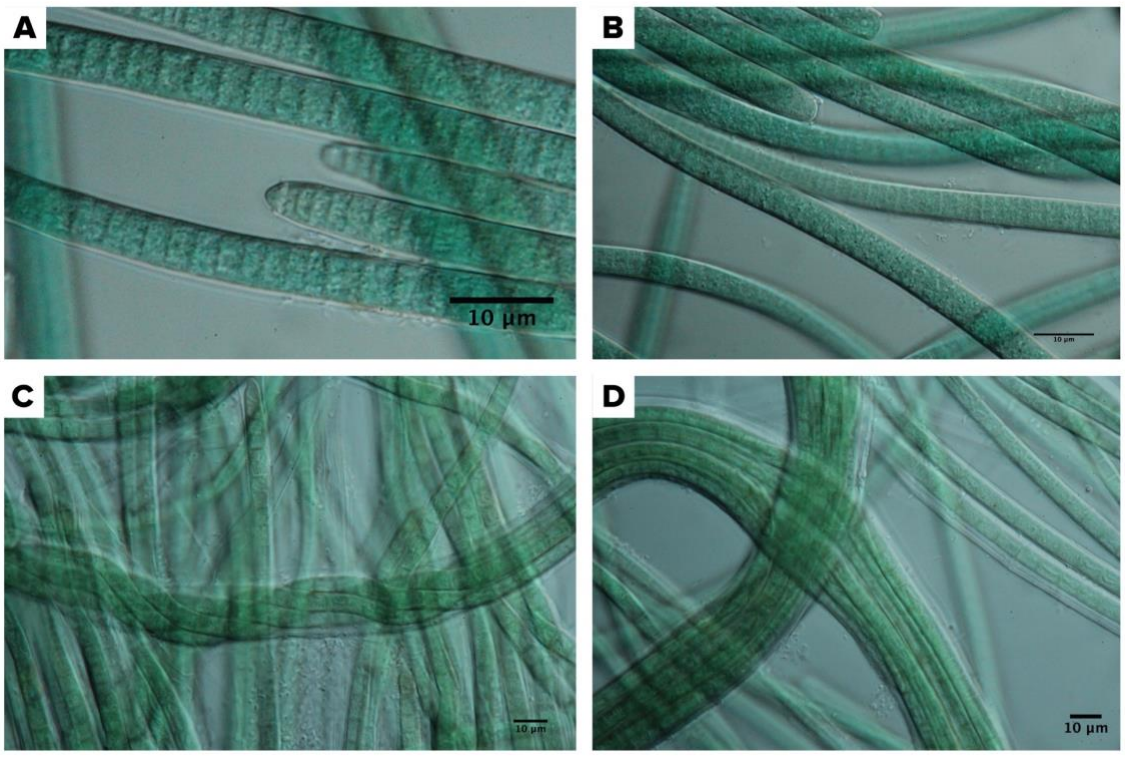


Figure 3. Photomicrographs of pioneer bundle-forming biocrust cyanobacteria from the genus *Microcoleus* isolated from the Great Basin Desert. (**a-b**) *Microcoleus vaginatus* filaments. (**c-d**) Rope-like structures from strain of the "*Microcoleus steenstrupii* complex". Scale bars are 10 µm. Photo credit: Ana Giraldo-Silva.

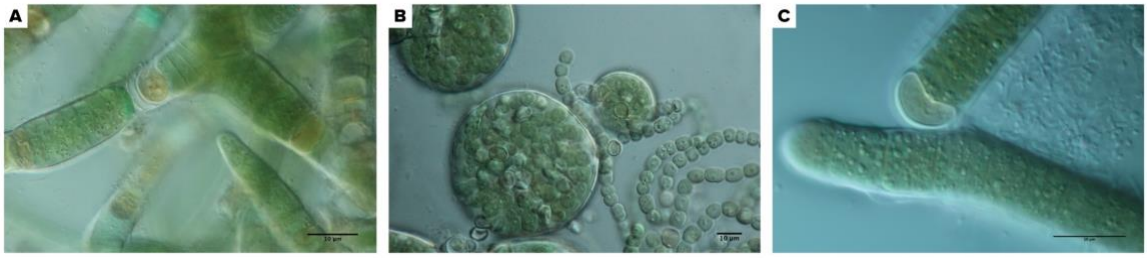


Figure 4. Photomicrographs of sessile heterocystous cyanobacteria from biocrusts. (**a**) *Scytonema* sp. strain isolated from the Great Basin Desert. (**b**) *Nostoc* sp. strain isolated from the Great Basin Desert. (**c**) *Tolypothrix* sp. strain isolated from the Chihuahuan Desert. Scale bars are 10 µm. Photo credit: Ana Giraldo-Silva.

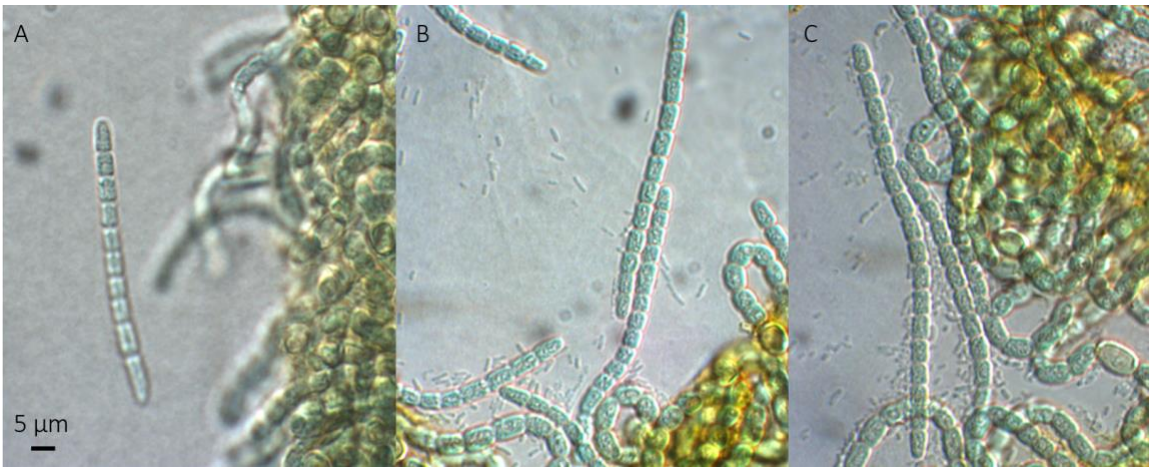


Figure 5. Photomicrograph of *Nostoc* sp. strain with scytonemin excretion indicated by the brown pigmentation. Scale bars are 5 µm. Photo credit: Kevin Klicki.

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2 – 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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Authors:

Vanessa M. C. Fernandes, Náthali Maria Machado de Lima, Daniel Roush, Jennifer Rudgers, Scott L. Collins and Ferran Garcia-Pichel

Abstract

Cyanobacteria typically colonize the surface or arid soils, building biological soil crust (biocrusts) that provide a range 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 the pioneer biocrust-formers. A shift toward heat-sensitive *M. vaginatus* could ultimately destabilize biocrusts when precipitation changes are combined with global warming.

Key words: Biological soil crusts, Biocrusts, climate change, precipitation, drought, cyanobacteria, nitrogen fixation.

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 dry lands 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 (Cayan et al. 2010; Ault et al. 2014; Cook et al. 2015; Kunkel et al. 2003; Petrie et al. 2014). 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.

Biological soil crusts (biocrusts) are ecologically important biotic components of arid lands (see review 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 algae, lichens or mosses (Bates et al.

2010b) as primary producers, accompanied by a variety of chemotrophic bacteria (da Rocha et al. 2015), archaea (Soule et al. 2009a), and fungi (Bates et al. 2010). Biocrusts are responsible for a substantial portion of primary production in arid lands (Elbert et al. 2012a), and are typically desiccation resistant (Peli et al. 2011, Rajeev et al. 2013). Given that biocrust are absent from the most extremely arid climates, 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 (Garcia-Pichel and Wojciechowski 2009; Zhang et al. 2006; Grote et al. 2010). After soil stabilization, other cyanobacteria that are nitrogen-fixing, secondarily

colonize biocrusts and contributing significantly to nitrogen pool of these otherwise low nutrient aridland soils (Yeager et al. 2004; Yeager et al. 2012; Johnson et al. 2005).

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.

Methods

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, the largest "hot" desert in North America. 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 (Garcia-Pichel and Belnap 1996b, 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 are early succession

crusts, mostly composed of filamentous, non-nitrogen fixing cyanobacteria (Garcia-Pichel et al. 2001, Zhang et al. 2006). To sample crusts, five 1 cm diam. x 1 cm deep soil cores were randomly taken at each of the plots, for a total of 300 samples (5 cores x 10 plots x 3 treatment x 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 1991a) 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 analyzed 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 ~3 min. The slurry was transferred to a plastic centrifuge tube, the volume was adjusted to 20mL with 90% acetone, vortexed, and allowed to extract for 24h 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, New York, USA), we used qPCR (quantitative real-time PCR) with universal (bacteria+ archaeal) 16S rRNA gene primer set (338F 5′-ACTCCTACGGGAGGCAGCAG-3′, 518R 5′-GTATTACCGCGGCTGCTGG-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), the total area used for extraction. The number of 16s rRNA genes obtained by qPCR was later used to arrived at total population sizes for each phylum or taxon as determined by illumine sequencing and bioinformatics 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 (2x150 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 (Operational Taxonomic Units) 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). In order 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 variables. 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 3 restarts and 9999 iterations.

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

Results

Sites differed in biocrust compostion

Total bacterial/archaeal population size did not differ significantly between the two grassland sites. Figure 6 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 was $7.4 \pm 2.9 \times 10^5$ 16S rRNA gene copies cm^{-2}. It was $1.2 \pm 0.8 \times 10^6$ 16S rRNA gene copies cm^{-2} at Site 2. 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 generalist archaeal phylum, Euryarchaeota. 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. 2013b). Bacterial community structure at the phylum level did not differ significantly between the two sites (PERMANOVA p=0.08; Fig. 6a - 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 7), which were also much higher in Site 1. These two findings support the conclusion 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. 2003b, Housman et al. 2006, Couradeau et al. 2016).

Cyanobacterial community structure (Fig. 8) differed greatly between the two sites (PERMANOVA, Pseudo-F=3.7995, df=1/18, p=0.016). 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 multi-generic. 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. 8a), 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. 7). Also, cyanobacterial Shannon's diversity was significantly higher, by 44.8%, in Site 1 (p=0.023) (Table S1). Indeed, 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. 8b). 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. 8a and 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. 8b and Table S2).

Differences between sites were further analyzed via PERMANOVA to compare the cyanobacterial community structure at both sites and to understand the drivers of variation, which turned out to be significantly different (PERMANOVA, Pseudo-F=3.7995, df=1/18, p=0.016). 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. 9).

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}; Fig. 6a and Fig. 7a). This shift was accompanied by a significant, absolute increase in the abundance of other phyla (Proteobacteria, Bacteroidetes and Actinobacteria, being the most pronounced; Fig. 6a). 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 OTUs – Table S1), but only in Site 1 ($p<0.0001$; Fig. 6b). 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 7), 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. 7a, 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 (Fig 7a). *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 8-fold 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 quite sensitive to drought in Site 1 (declining to 1-5% of respective control levels; 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 10a). 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 (Table S2; Note that no *Scytonema* populations were present there). Drought in Site 2 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 where tested statistically with PERMANOVAs and are shown in Fig 10.

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. 6a). Delayed monsoon caused a decrease to all taxa of cyanobacteria, although not significantly so for every taxon (Table S2). These patterns were mirrored in the Chl *a* concentration (Fig 7). 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. 8a and 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 structure or in the population size of any particular taxon (Fig. 8b and 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, characterized by mature 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 regarless 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 autochtonous 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; Table 2*), 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 typical incipient, "light" crust, nitrogen fixation, while likely present (Johnson et al. 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. Of course, other explanations could be called up, such as the presence of naturally 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.

It was surprising to us to see 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 to 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, 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 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 pioneer species in biological soil crusts in desert ecosystems across the Southwestern US (Garcia-Pichel and Wojciechowski 2009). It has been shown that *M. steenstrupii* is the dominant species 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 *M. steenstrupii* that was 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 advantages 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 1991a, 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 species-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 et al. (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.

Figures

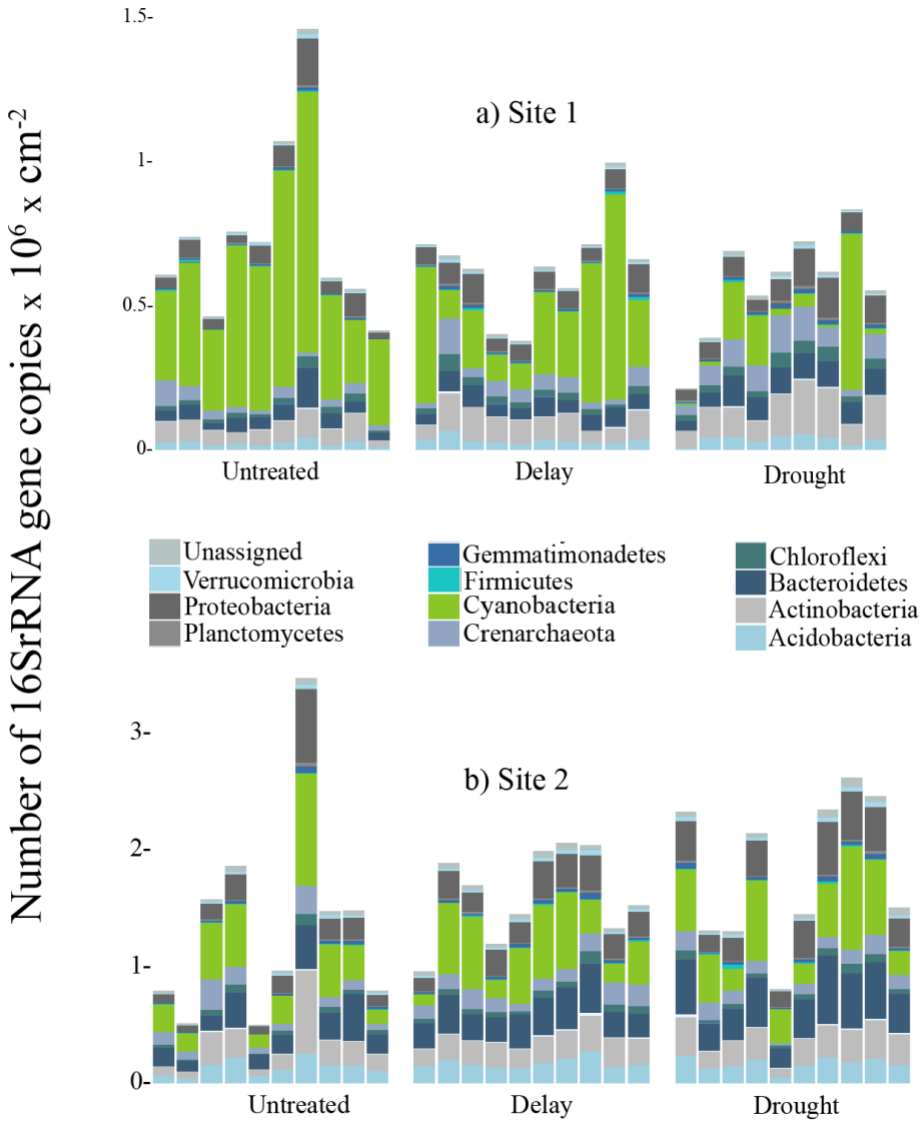

Figure 6. Bacterial abundance and community structure in control and treatment biological soil crusts at 2 sites as determined by high-throughput 16S rRNA gene analyses coupled to q-PCR. Ten independent plots were analyzed for each treatment and site, and each bar represents an independent plot. Phylogenetic assignments for each OTU were based on blast to the Greengenes database and carried to the Phylum level.

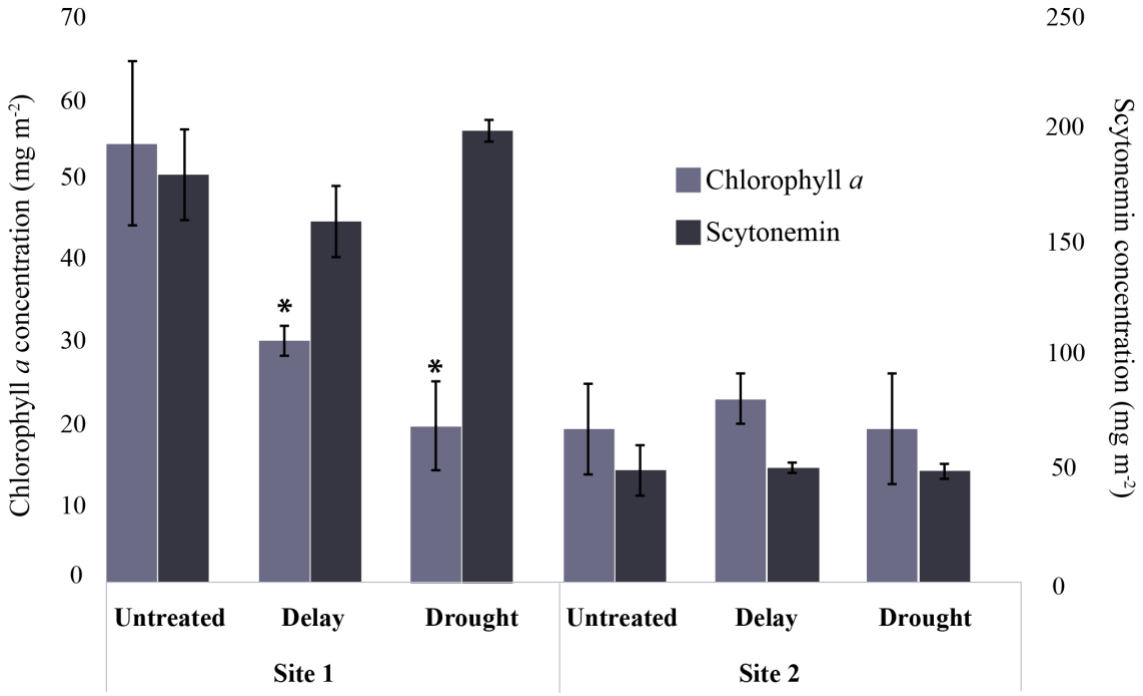

Figure 7. Areal concentrations of biomarker pigments Chl a and scytonemin in biological soil crusts for controls and treatments at both sites. Error bars are ± s.d. (n = 9). Asterisks denote significant difference from control ($p<0.05$).

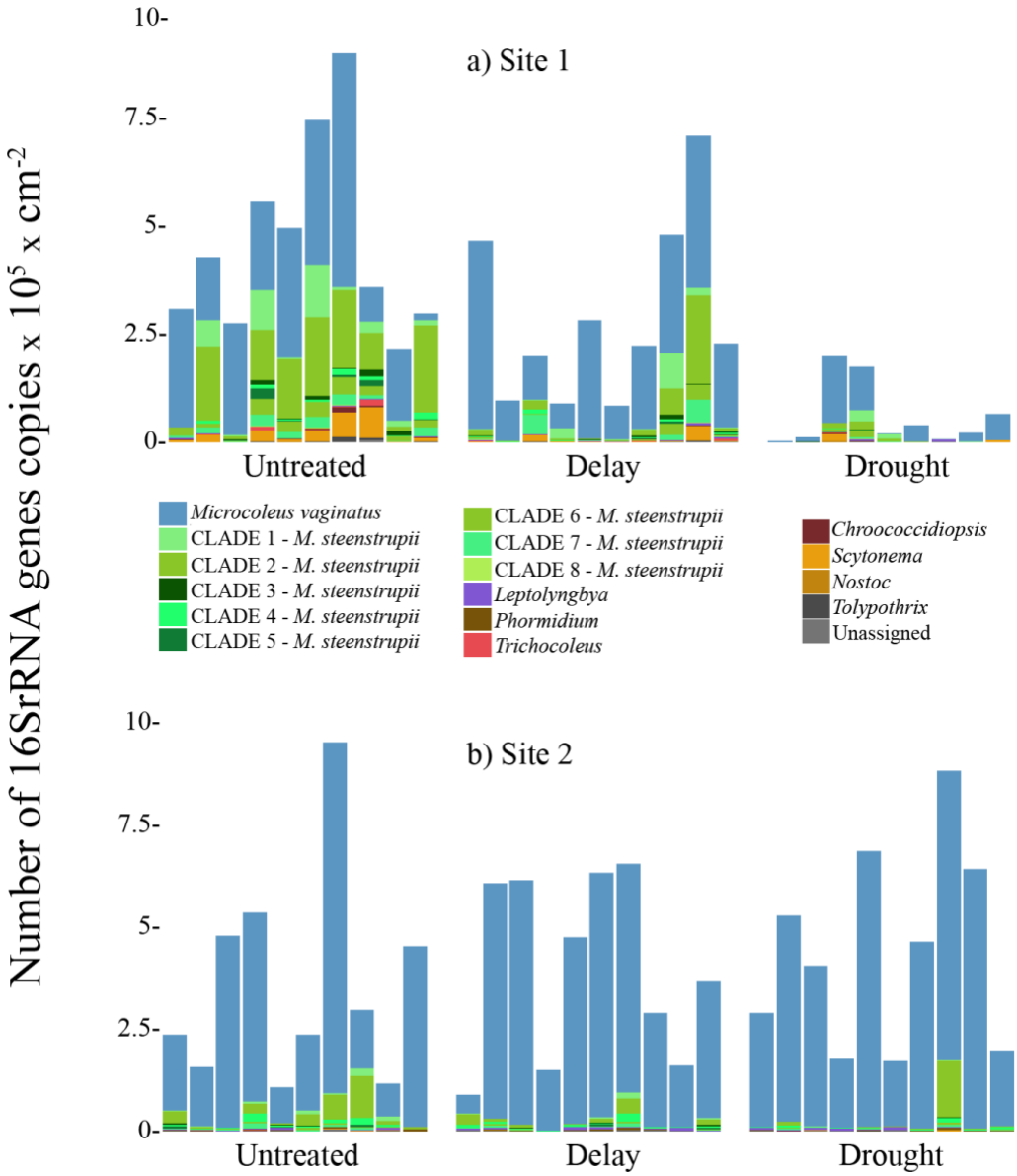

Figure 8. Cyanobacterial abundance and community structure in control and treatment biological soil crusts at 2 sites, as determined by high-throughput 16S rRNA gene analyses coupled to q-PCR. Ten independent plots were analyzed for each treatment and site, and each bar represents an independent plot. 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.

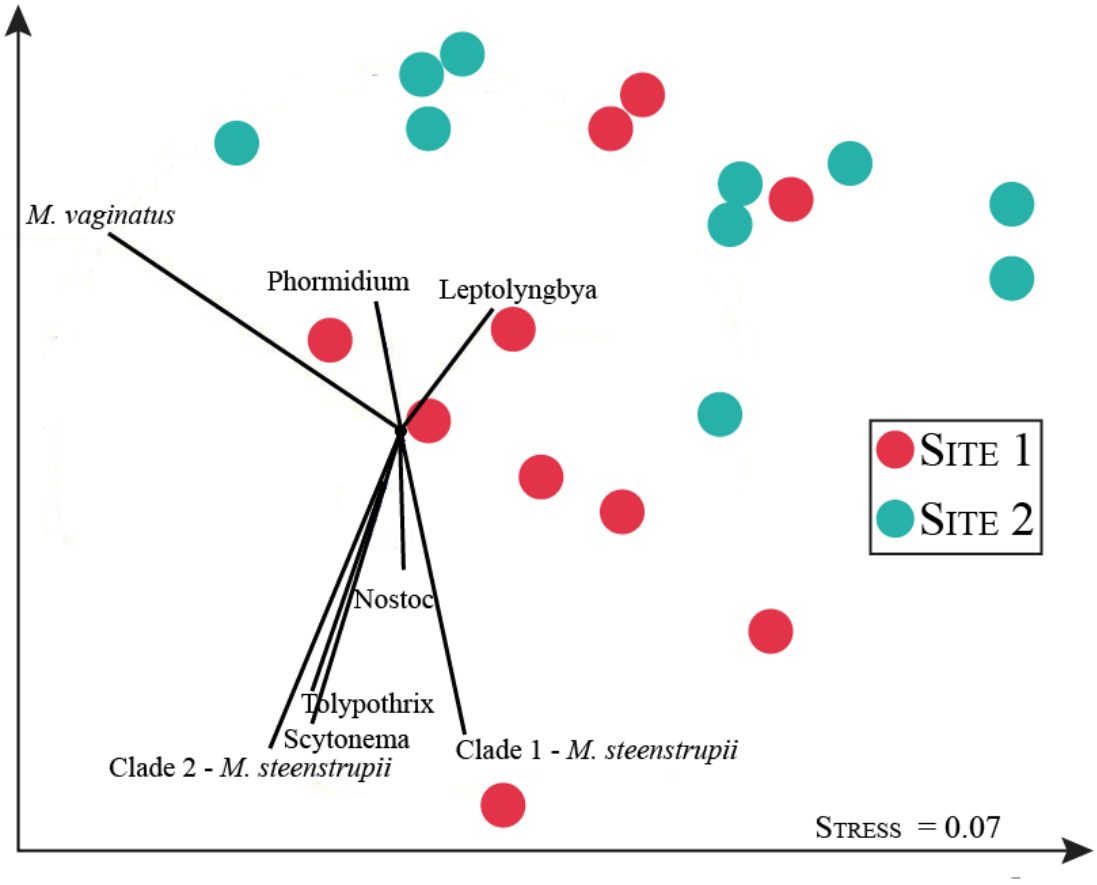

Figure 9. Non-metric Multi-Dimensional Scaling comparison of cyanobacterial community composition in untreated plots. The nMDS ordination was based on Bray-Curtis similarity of cyanobacterial OTUs. Data points are color coded by site. Taxon vectors size represents their importance along each nMDS axis. 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.

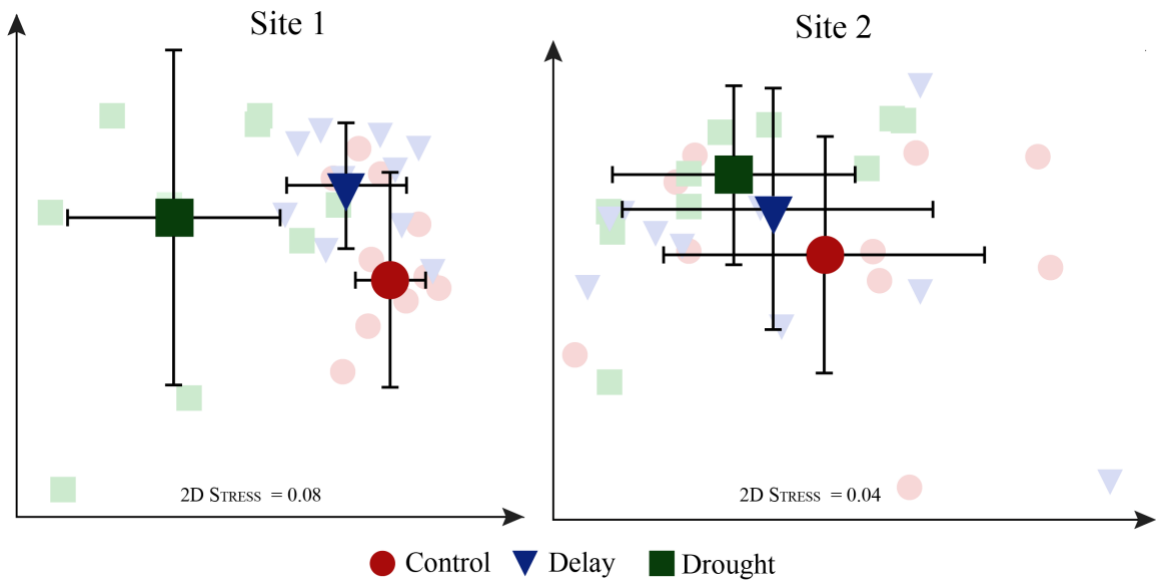

Figure 10. Non-metric Multi-Dimensional Scaling comparison of cyanobacterial community composition between the treatments in each one of the sites. The nMDS ordination was based on Bray-Curtis similarity of cyanobacterial OTUs. Data points are color coded by treatment (and centroid with scatter for each treatment are 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-$F=0.967$, $df=27$).

Supplementary Information

Supporting Tables

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).

Phylogenetic Level	Site	Treatment	Shannon diversity index	Number of OTUs
Bacteria	**1**	**Untreated**	6.52±0.81	3620±575
Bacteria	**1**	**Delayed monsoon**	7.12±1.22	4086±661
Bacteria	**1**	**Drought**	8.52±1.10	4414±651
Bacteria	**2**	**Untreated**	8.44±0.63	5751±737
Bacteria	**2**	**Delayed monsoon**	8.10±0.71	5641±778
Bacteria	**2**	**Drought**	8.23±0.72	5076±625
Cyanobacteria	**1**	**Untreated**	3.01±1.21	144±19
Cyanobacteria	**1**	**Delayed monsoon**	1.68±1.14	109±31
Cyanobacteria	**1**	**Drought**	1.76±0.83	59±38
Cyanobacteria	**2**	**Untreated**	1.66±0.89	95±29
Cyanobacteria	**2**	**Delayed monsoon**	0.66±0.32	74±23
Cyanobacteria	**2**	**Drought**	0.53±0.36	60±19

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 is 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. * = p<0.05

Taxon	SITE 1				SITE 2			
	$100\ x\ \frac{drought}{untreated}$		$100\ x\ \frac{delay}{untreated}$		$100\ x\ \frac{drought}{untreated}$		$100\ x\ \frac{delay}{untreated}$	
		p		***p***		***p***		***p***
Leptolyngbya	NA	NA	NA	NA	88%	0.6852746	162%	0.4580063
***M. steenstrupii* – Clade 1**	4%	0.0921817	32%	0.2835033	15%	0.4550958	20%	0.4146054
***M. steenstrupii* – Clade 2**	1%	0.00149*	10%	0.01686*	13%	0.7128716	48%	0.4639239
***M. steenstrupii* – Clade 4**	4%	0.002392*	10%	0.01863*	52%	0.3220705	159%	0.7443986
***M. steenstrupii* – Clade 5**	1%	0.1177240	21%	0.2930683	NA	NA	NA	NA
***M. steenstrupii* – Clade 6**	3%	0.007849*	15%	0.06023	15%	0.2750914	32%	0.5015658
***M. steenstrupii* – Clade 7**	2%	0.7582020	20%	0.8204537	73%	0.7411368	55%	0.9484710
M. vaginatus	15%	0.005065*	60%	0.868132	208%	0.5203165	157%	0.7723141

Phormidium	NA	NA	NA	NA	46%	0.9995737	127%	0.9979148
Scytonema	1.5%	0.03417*	10%	0.07474	NA	NA	NA	NA

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3 – ALTERED RAINFALL REGIMES RESULT IN DISTINCT BIOLOGICAL SOIL CRUST COMMUNITIES

Coauthors have acknowledged the use of this manuscript in my dissertation

Authors:

Vanessa M. C. Fernandes, Jennifer Rudgers, Scott L. Collins and Ferran Garcia-Pichel

Abstract

Future climates around the globe are likely to include altered precipitation regimes that differ in the frequency and size of precipitation events. These changes could have large impacts on microbial communities that influence biogeochemical cycles. However, experimental manipulations of the precipitation regime have been rare, particularly in dryland ecosystems, which cover 45% of terrestrial land area. We quantified the abundance, composition and diversity of bacteria in biological soil crusts to investigate long-term effects of alternative rainfall regimes on dominant microbes in drylands. Rainfall treatments added 60 mm rain per year either as twelve 5 mm events (small-frequent events) or as three 20 mm events (large-infrequent events) during the summer monsoon. We sampled the experiment following 10 and 12 years of recurrent treatments. The addition of rainfall (both regimes) did not consistently increase bacterial/archaeal or cyanobacterial biomass, as measured by 16S rRNA gene abundance, suggesting the surprising result that microbial abundances are not always water-limited. However, the regime of small-frequent rain events consistently increased microbial and cyanobacterial biomass and cyanobacterial diversity when compared to larger, less frequent rainfall additions or to controls. Small-frequent rains also consistently promoted the nitrogen-fixing cyanobacterium *Scytonema* spp. and reduced the dominance of *Microcoleus vaginatus* relative to members of the *M. steenstrupii* complex. These results contradict the paradigm that biocrusts are most limited by water and predict that regimes of small-frequent rain events will favor cyanobacterial biocrusts, potentially enhancing their beneficial services to desert ecosystems.

Key words: Biocrusts, rainfall addition, climate change, cyanobacteria.

Introduction

Climate change will influence precipitation patterns globally (IPCC 2014, Seddon et al. 2016, Nolan et al. 2018), including in drylands, potentially making these ecosystems more stressful for the local biota (Bernstein et al. 2008, Loarie et al. 2009). Ecological impacts of climate change are expected to be negative and significant (Maestre et al. 2015, Rodríguez-Caballero et al. 2018).

The potential for climate disruptions, such as altered precipitation regimes, to alter microbial communities sparked a recent "warning to humanity" about the importance of improving understanding of microbial responses to climate change (Cavicchioli et al. 2019). Microbes can undergo swift community shifts in response to environmental change due to their short generation times, making it critical to predict their biology under alternative climate scenarios. Through networks of interactions with plants and other organisms, microbes can affect the resistance (ability to remain unchanged) of ecosystems to climate disruption or mediate ecological resilience to change (ability to recover from disturbance) (Rudgers et al. 2020). Furthermore, microbial populations can create feedbacks to climate in two important ways. First, soil microbes can alter primary production, thereby influencing the magnitude of the plant-based carbon (C) sink (e.g., Sulman et al. 2019). Second, soil microbes drive decomposition and soil C, affecting respiration and the release of C back to the atmosphere (Classen et al. 2015, Song et al. 2019).

An important gap in understanding microbial responses to climate change is tracking microbial responses in dryland ecosystems. Drylands constitute ~45% of Earth's

terrestrial surface (Pravalie 2016); thus, climate-impacts on their biodiversity will have glocal scale implications. In addition, dryland soils store approximately 470 Pg organic C within the top 1 m of soil (Plaza et al. 2018), which constitutes up to one-third of the estimated global soil organic carbon pool (Stocker et al. 2013, Plaza et al. 2018, Friedlingstein et al. 2019).

Dryland microbial communities are dominated by biological soil crusts that inhabit the topsoil (Weber et al. 2016a) and provide important ecosystem services. Biocrusts are responsible for 15% of the terrestrial net primary production as well as half of the terrestrial N fixation(Elbert et al. 2012a, Rodriguez-Caballero et al. 2018). Their presence mitigates soil erosion and fugitive dust formation, a crucial service for ecosystem stability and public health (Middleton 2017).

Biological soil crusts (biocrusts) are topsoil microbial assemblages in drylands (Weber et al. 2016a). They are initiated by growth of pioneer filamentous cyanobacteria (Couradeau et al. 2019), which physically stabilize the soil(Garcia-Pichel and Wojciechowski 2009) , allowing eventual colonization by other cyanobacteria (Yeager et al. 2007), bacteria, archaea and fungi (Bates and Garcia-Pichel 2009, Soule et al. 2009a) , even lichens and mosses (Lange et al. 1997, Ullmann and Büdel 2001) in developed stages of biocrust succession.

Water availability is thought to be the single most important limitation for biocrust productivity (Ullmann and Büdel 2001, Pointing and Belnap 2012b, Bowker et al. 2016) because these microbial communities are only active when wet (Brock 1975, Rajeev et al. 2013). Logically, their fate may be quite sensitive to changes in precipitation

patterns (Evans and Wallenstein 2012, Rajeev et al. 2013). However, the number of experimental studies that measure biocrust or other dryland microbe responses to precipitation manipulations is relatively small given the large terrestrial surface area and microbial diversity encompassed by biocrusts worldwide (see review in Reed et al. 2016). For example, decade-long experiments on moss-dominated biocrusts (Johnson et al. 2012, Reed et al. 2012, Ferrenberg et al. 2015) showed that additional small rain events bring about severe moss mortality (Reed et al. 2012) and a regression to cyanobacteria-dominated early successional stages (Ferrenberg et al. 2015),(Steven et al. 2015). In our prior work, four years of drought reduced cyanobacteria abundance up to 95% in one location but had little effect in a second site that was dominated by a different microbial assemblage (Fernandes et al. 2018). This work suggests that microbial taxa will differ in responsiveness to precipitation manipulations, requiring altered rainfall regime experiments to understand microbial responses in biocrusts that are not moss-dominated.

Climate model predictions for the Southwestern US include changes in rainfall patterns, a trend supported by long-term climate data (Cayan et al. 2010, Cook and Seager 2013, Ault et al. 2014, Petrie et al. 2014, Cook et al. 2015). A key prediction is for an invariant *total* growing season precipitation coupled to a decrease in the *size* of summer rainfall events (Weltzin et al. 2003, Bernstein et al. 2008). The future functioning of biocrusts thus will depend in part on how they fare under such a scenario of more, but smaller precipitation events (Zhang 2005, Housman et al. 2006, Garcia-Pichel and Wojciechowski 2009). Theoretically, the shift should be detrimental, since transitions from activity to dormancy are associated with a metabolic cost (Harel et al.

2004, Rajeev et al. 2013). However, the documentation thus far pertains only to biomass or productivity and focused on moss-dominated biocrusts. Thus, understanding of potential effects of shifts in the rainfall regime on microbial community diversity and composition is incomplete.

And yet, biocrust cyanobacteria are demonstrably and differentially sensitive to minor changes in environmental conditions. For example, the distribution of pioneer, biocrust-forming cyanobacteria depends nimbly on temperature, with *Microcoleus vaginatus* dominating colder locations, and clades of the *M. steenstrupii* complex more prevalent elsewhere (Garcia-Pichel et al. 2013a). Similarly, among nitrogen-fixing cyanobacteria, *Scytonema* sp. becomes more dominant while *Nostoc* spp. and *Tolypothrix* spp. lose ground with increasing temperature (Giraldo-Silva et al. 2020). With respect to drought, *M. vaginatus* seems to be more tolerant than specific clades in the *M. steenstrupii* complex or heterocystous cyanobacteria (Fernandes et al. 2018).

We sought to fill knowledge gaps concerning biocrust microbial community responses to changes in the rainfall regime, with a focus on cyanobacteria as primary producers. We used long-term rainfall manipulations that imposed additions of many, small rain events (small-frequent) or a few, large events (large-infrequent) during the summer monsoon to assess responses in biocrusts from the Northern Chihuahuan Desert.

Methods

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 averages 234 mm, most of which falls during the summer monsoon (July-September). The vascular plant assemblage is dominated by black-grama grass (*Bouteloua eriopoda*). Biocrusts commonly grow in the interspaces between plants and include both light (early successional) to dark (mid successional) cyanobacterial types, with few lichens or mosses (Garcia-Pichel and Belnap 1996b, Yeager et al. 2004).

The Monsoon Rainfall Manipulation Experiment (MRME) imposes two treatments to probe the impacts of increased precipitation variability (detailed design methods are in https://sevlter.unm.edu/node/2106). Both treatments add precipitation (60 mm; some 25% over typical) during the three months of summer monsoon (July-September), either as 20 mm of rain once per month (3 large rain events), or as 5 mm events of rain weekly (many small rain events). Thirteen 14 x 9 m plots are divided into 5 replicates of each treatment and 3 control plots, the latter receiving naturally occurring precipitation only. All plots are at an altitude of 1538 m and have soils of uniform texture (sandy loam/sandy clay loam).

We sampled biocrusts in October 2017 and September 2019 near the end of the monsoon season. Twenty 6-cm in diameter by 1.5 cm deep samples were taken with Petri

dishes (a schematic representation of our spatial sampling approach is in Fig. S1) along two transects in each experimental plot, placed to avoid plot boundaries and areas directly under sprinklers. Control plots were oversampled randomly to attain the same sampling effort as in the treatment plots. In total, 300 samples were processed per year. After sampling, biocrusts were immediately air dried to stop microbial activity, placed inside self-sealing plastic bags, and stored at -80 °C until analysis.

Chlorophyll *a* and scytonemin areal concentrations

We subsampled each Petri dish with one 1 cm diameter x 1 cm deep soil core, pooling and homogenizing all cores from a plot to a single composite sample. From this homogenate, we aliquoted 0.25 g for DNA processing (see below) and the remainder was used for pigment extraction (Fig. S2). 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 1991a) concentration was measured as a proxy for the biomass of heterocystous cyanobacteria. Aqueous acetone pigment extraction and spectrophotometric quantification was performed following (Sorochkina et al. 2018, Giraldo-Silva et al. 2018). Pigment concentrations were calculated using the trichromatic equations developed by Garcia-Pichel and Castenholz (1991) (Garcia-Pichel and Castenholz 1991a) to de-convolute each pigment's contribution to absorbance. Concentrations are reported as mass per soil surface (mg m^{-2}).

DNA extraction and 16S rRNA gene copy number determination

We used 0.25 g of composite sample (obtained as explained in the previous section) for community DNA extraction with the MoBio® Power Soil DNA extraction kit. After fluorometric determination of DNA concentration in the extract (Qubit, Life Technologies, New York, USA), we used qPCR (quantitative real-time PCR) with universal (bacteria+ archaea) 16S rRNA gene primer set (338F 5′-ACTCCTACGGGAGGCAGCAG-3′, 518R 5′-GTATTACCGCGGCTGCTGG-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 number of 16S rRNA genes obtained by qPCR was later used to arrive at absolute population sizes for each taxon of interest as determined by Illumina sequencing and bioinformatics analyses (see below).

16S rRNA library construction and next generation Illumina sequencing

Bacterial/archaeal community analysis was performed via 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(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 (2x150 paired-end) following manufacturer's specifications.

Bioinformatic analyses and phylogeny

Demultiplexed paired-end FASTQ files were imported into Qiime2.10 (Bolyen et al. 2019) under default parameters. Analyses were done using the DADA2 plugin (Callahan et al. 2016) to create a feature table with representative sequences (ASVs) and their frequency of occurrence. To remove highly variable positions, sequences were aligned with the MAFFT program (Katoh and Standley 2013). Because the two years differed in average sequencing depth (2017= 115,000 reads/sample; 2019= 16,000 reads/sample); the number of reads per sample was constrained to a common value of 10,000 reads/sample using a random draw to maintain a comparable sampling effort. Singleton and doubleton ASVs were excluded from downstream processing. Rarefaction curves for each year showed that Shannon diversity reached a plateau in all determinations, even when the observed OTUs did not in 2017 determinations (Supplementary Material Fig. S3).

Initial taxonomical assignments of ASVs were done with the Naive Bayes classifier trained on the Greengenes 13.8 release, where sequences were trimmed to include 250 bases from the V4 region, bound by 515F/806R primers (McDonald et al.

2012). The resulting feature table was used for calculation of phylogenetic alpha-diversity indices in QIIME2.

Additionally, all ASVs assigned to the Phylum cyanobacteria or plastids (sensu Garcia-Pichel et al, 2020 (Garcia-Pichel et al. 2020) were subject to individual, full phylogenetic scrutiny against the curated cyanobacterial database, Cydrasil, to produce accurate assignments at high level of resolution, following the default workflow (https://github.com/FGPLab/cydrasil). Resulting placements were visualized with iTOL3 server (Letunic and Bork 2007). Once all ASVs were defined and taxonomically assigned, we built abundance matrices for both relative abundance and absolute (qPCR-adjusted) abundance for further use in diversity and community structure analyses.

Raw sequence data are publicly available under BioProject number PRJNA633650 at NCBI.

Statistics

Alpha diversity. To obtain ASV-level alpha-diversity metrics (Shannon-Weiner Diversity, Pielou Evenness and Faith PD taxon richness), the phylogenetic alpha metrics plugin in QIIME2 was used. Kruskal-Wallis tests were run to test significance between treatments and controls. Analysis for diversity metrics was performed separately for all ASVs (Bacteria/Archaea), as well as for cyanobacterial ASV's (excluding plastids). Additionally, we determined alpha-diversity at the phylum level for Bacteria/Achaea and at the level of genus/species for Cyanobacteria (Cydrasil assignment dataset), using the

vegan package (Dixon 2003) to calculate richness (*S*), the Shannon-Weiner diversity index (*H'*), and its evenness counterpart ($J = H' / \ln(S)$), building general linear mixed effects models using the lmer function in package lme4 (Bates et al. 2015) for each metric. Models included the fixed, categorical effects of treatment and year as well as the random effect of plot (nested in treatment) to account for lack of independence of repeated samples taken from the same plot. We obtained chi-square test statistics from analysis of deviance on each model using car (Fox and Weisberg 2019). If treatment or treatment × year interactions were significant, we decomposed the differences in diversity among the three treatments within each year using planned contrasts of the estimated model means (emmeans, Lenth 2018).

Beta diversity. To obtain ASV-level beta-diversity, permutational multivariate analysis was used to compare centroids and dispersions of rainfall addition treatments and controls. perMANOVA was performed on weighted unifrac (a phylogenetic quantitative matrix) using the beta-diversity plugin on QIIME2. Additionally, we determined beta-diversity at the phylum level for Bacteria/Achaea and at the level of genus/species for Cyanobacteria (Cydrasil assignment dataset) in Primer v. 6 (Clarke and Gorley 2009) to calculate and compare centroids and dispersions. Models included the fixed effects of year, treatment and treatment × year, and the random, repeated effect of plot (nested in treatment) as explained above for alpha-diversity. Treatment effects were tested over the variation between plots. Models were run on two datasets (i) bacterial/archaeal phylum composition as absolute abundances of each phylum and (ii) cyanobacterial genus/species composition as absolute abundance of each taxon. For each

analysis, similarity matrices were created using the Bray-Curtis distance metric, and statistical analyses used 10,000 permutations. If treatment or treatment × year interactions were significant, we then decomposed treatment effects using the perMANOVA contrast function among the three treatments. We evaluated treatment effects within each year with pairwise tests using 10,000 permutations, correcting for multiple comparisons according to the sequential Holm-Bonferroni method (Holm 1979). To determine whether treatments altered beta-diversity, i.e., the dispersion in composition among plots within a treatment, we used permDISP on the Bray-Curtis similarity matrix with 10,000 permutations, with Holm-Bonferroni corrections for pairwise tests among treatments.

For visualization of community composition, we used non-metric multidimensional scaling analysis (NMDS) of the Bray-Curtis similarity matrix with 500 random re-starts. To determine which taxa were most responsible for variations in microbial composition, we used SIMPER analysis in a one-way design for treatments and controls, applied separately to each year (Clarke and Gorley 2009).

Results

Rainfall treatments consisted of either adding one large event of 20 mm or four small events of 5 mm per month of the monsoon season (July-September). For simplicity these treatments will be referred as "Large" and "Small", respectively. Controls received only naturally occurring precipitation, with no additions. All plots were sampled in 2017 (after 10 years of treatment) and 2019 (after 12 years).

Controls plots and interannual variability

Phylum-level bacterial/archaeal community composition was typical of biocrusts in general (Nagy et al. 2005, Gundlapally and Garcia-Pichel 2006, Steven et al. 2013b), and consistent with previous analyses from the same region (Fernandes et al. 2018). Phylum-level community composition was similar between years. Cyanobacteria were always the most abundant phylum, followed in abundance by (alpha)Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi and Bacteroidetes (Fig. 1 – Control), in this order. Crenarchaeota were detected only in 2019. Cyanobacterial community composition in control plots was also typical of so-called "light" biocrust from North American arid lands (Nagy et al. 2005, Gundlapally and Garcia-Pichel 2006, Steven et al. 2013b) and consistent between sampling years (Fig. 2 – Control). The bundle-forming, non-heterocystous *Microcoleus vaginatus* accounted for more than 80% of the cyanobacterial reads. Several clades falling within the "*Microcoleus steenstrupii"* complex of genera were also present, but heterocystous cyanobacteria, such as *Scytonema* spp. or *Nostoc* spp. (Yeager et al. 2007), which make crusts "dark" through the synthesis of the

sunscreen pigment scytonemin, were not detected in the control plots (Figure 2 – Control). Bacterial/Archaeal and cyanobacterial community composition (Figs. 1 and 2) are presented in relative (Fig. 1-2, right) terms, and in absolute terms (Fig. 1-2, left), after adjusting relative composition based on total 16S rRNA gene copies obtained from the DNA extracts through qPCR in each.

While community composition was consistent in both sampling years, total population size was not. The areal concentration of 16S rRNA gene copies was six-fold higher in 2019 than in 2017 (14 ± 1.1 vs. 2.3 ± 0.1 billion copies per cm^2; Table 1). This was also true for cyanobacterial populations, which reached 3.9 ± 2.4 billion copies per cm^2 in 2019, but only 1.2 ± 0.4 in 2017, between 3 and 4-fold less. Consistently, the areal concentrations of Chl *a* in the biocrusts, a proxy for cyanobacterial biomass, was also significantly higher in 2019 control plots, although the difference here did not quite reach two-fold (Table 1, Figure S4).

Treatment effects on microbial population sizes

Populations of bacteria/archaea were positively affected by the Small treatment but unaffected or negatively affected by the Large treatment with respect to controls (Table 1 and Table S1). The impact of the Small treatment was much larger in the year of plenty (2019), when populations more than doubled, than in 2017, when they were only about 30% larger than those of controls. Given the variance in the dataset, however, the differences were only marginally significant , and for clarity we have given significance level of $p < 0.1$ in (Table S1, but no treatment effects were significant at $p < 0.05$ (Table

1). For cyanobacteria, the Small treatment either had no effect (2017) or increased (2019) 16S rRNA gene-based population size over controls, significantly so when assessed at $p < 0.1$, but not at $p < 0.05$. The Large treatment caused moderate population declines over controls even at $P < 0.05$ (Table S1). Chl *a*-based cyanobacterial population estimates showed a different trend, with positive effects both rain additions over controls ($P < 0.1$), although the difference in the 2019 Large treatment was non-significant at $P < 0.05$ (Table 1).

Treatment effects on microbial diversity

Bacterial community. Rainfall additions did not significantly alter phylum-level biocrust bacterial/archaeal composition at $P < 0.05$, but only at $P < 0.1$ (Treatment $P = 0.0886$; Treatment × Year = 0.0752), but there were some clear changes in composition (Figure 1). Composition responded similarly to rainfall addition in both sampling years (Table 2, perMANOVA, Year X Treatment, $P > 0.07$, Fig.1). When assessed using ASV-level data, bacterial/archaeal composition also did not significantly change with rainfall addition (Table S2). Only when assessed at the ASV-level and comparing each year separately, rainfall additions significantly altered bacterial/archaeal community composition compared to controls at $p< 0.05$, but treatments did not significantly differ from each other (Table S2).

A representation of similarity distance between the treatments and control on both years is shown in Figure 3 with stress values of less than 0.1, indicating a well resolved representation of the true similarity matrix. Cyanobacteria was the phylum responding

most strongly to treatment, but Proteobacteria and Actinobacteria were also very responsive, ranking second or third (Table S3). Cyanobacterial contributions to community dissimilarity ranged from 21 to 47%, while proteobacterial contributions were less than half those of cyanobacteria (Table S3). Cyanobacteria were most abundant in absolute terms in the Small treatment and least abundant in the Large treatment. In contrast, Proteobacteria responded more to rain addition (i.e., compared to controls) than to the type of rainfall addition, although absolute abundance, as with Cyanobacteria, was still highest in the Small treatment. (Table S3, Fig. 1).

Cyanobacterial community. Cyanobacterial communities at the genus/species level significantly diverged in composition between the Small and Large treatments ($P < 0.02$, Table 2; Figs. 2 and 3) and between each rainfall treatment and the controls (all $P < 0.03$, Table 2; Figs. 2 and 3). These differences persisted when assessed at the ASV-level (Table S2). They also responded more strongly to altered precipitation regime in 2019 than in 2017 (Table 2, perMANOVA, Year X Treatment, $P < 0.05$; Fig. 3), and more strongly to the Small treatment. To confirm that these changes were not due to increased dispersion on cyanobacterial community, we also analyzed the dispersion of the community (Table S4) and found that the only case where we had significant differences was in the Small treatment in 2019. Although patchiness increased in treatment plots (Table S4), this was clearly not the main driver of changes in diversity and community composition.

The cyanobacterial taxon most responsible for divergence in community composition was *Microcoleus vaginatus*, which was 1.5 to 3.5-fold more abundant in controls than in the Large treatment, depending on year, and 0.5 to 2.2-fold more abundant in controls than in the Small treatment (Table S5, Fig. 2). This cyanobacterium was clearly negatively affected by water additions, regardless of pulse size. In contrast, three clades in the *M. steenstrupii* complex (SON57, HS024, and a new clade with no cultured representatives) benefited most from the Small treatment, and somewhat less from the Large treatment. For example, in 2017 the "*M. steenstrupii* SON57 Clade" was 18-fold more abundant in the Small treatment than in control plots, and 6-fold more abundant in Large treatment than in controls. (Table S5, Fig. 2). For this clade, water additions were a plus, and the smaller and more frequent pulses were better. Similarly, in 2019, *M. steenstrupii* clade SON57, was 10-fold more abundant in the Small treatment than in controls (and 20% more so in the Small than in the Large treatment). The heterocystous, surface dwelling *Scytonema* spp., which was not detected in control plots in either 2017 or 2019 (Table S5, Fig. 2) accounted for 1 to 14% of the cyanobacterial reads in the Large treatment plots and for 3 to 22% in the Small treatment plots, depending on sampling year. The content of the heterocystous biomarker, scytonemin, corroborated this change, being significantly higher in rainfall addition treatments than in controls in both years, and more so in the Small treatment, which presented significantly higher concentration than controls and the Large treatment (Fig. S4).

Diversity indices. When assessed at the ASV level, water addition resulted in significant increases in both diversity and evenness of bacteria/archaea, but richness was not affected

by treatments (Table 3 – Treatment). Highest values were attained with the Small treatment. When comparing between years, 2019 had the largest values of Shannon diversity and Taxon richness, although diversity between Small treatments was not significantly different between years (Table 3 – Treatment x Year). When determined at the phylum level, there was only significant differences if looking at years separately (Table S6). Shannon diversity and evenness also increased with rainfall addition in 2017, but not in 2019. Phylum richness did not change, since most phyla were present in all treatments, with the exception of Crenarchaeota that was only present in 2019 (Table S6). Therefore, although the same trends occurred with the phylum-level data as with the ASV-level determinations, the latter gave a stronger response.

Cyanobacterial alpha-diversity (diversity, evenness and richness) at the ASV's level was significantly enhanced by both rainfall addition treatments over controls, again the Small treatment had the largest values across both years (Fig, 2; Table 4 – Treatment). Cyanobacterial diversity metrics tended to be higher overall in 2019 (Table 4 – Treatment/Year), except for the Small treatment, that showed no differences between years (Table 4 – Treatment/Year). Treatments were significantly different from each other. When assessed at the genus/species level, cyanobacteria showed similar trends to those seen at the ASV level (Table S7), with the only exception being taxon richness, which was not significantly different between treatments at this coarser phylogenetic level (Table S7).

Discussion

The case for limitation of biocrusts by water availability.

While the experimental set-up was ultimately intended to address the effects of rainfall variability, it was pragmatically constructed to impose *added* variations in rainfall regime, above naturally occurring precipitation. This made it necessary to include untreated control plots. Logically, comparisons between control and treatment plots do not answer questions regarding rainfall variability *per se*, but rather give us a glimpse of the magnitude of water limitation on various biocrust parameters. Also, because natural precipitation varies strongly from year to year in the US Southwest deserts (Knapp et al. 2002, Kunkel et al. 2003), data from two different years enhance the robustness of our results. Indeed, in biocrust population terms, 2019 represented a year of plenty over 2017. Interestingly, however, this did not correlate with total precipitation or precipitation pulse size during the year (nor biennium) preceding sampling, which was in fact slightly higher in 2017, according to local meteorological records (271 mm in 2017 vs. 256 mm in 2019, https://sevlter.unm.edu). Average precipitation event size for the water year was 4.6 mm in 2017 and 4.1 mm in 2019. Together, these results suggest that the biocrusts may not have been strictly limited by water.

Contrary to expectations, but consistent with the hypothesis that biocrusts are not always water-limited, the additional precipitation in our experiments did not always lead to beneficial effects on biocrusts. Total microbial biomass estimators responded little to low frequency and high volume, but were enhanced by high-frequency, low volume additions, regardless of sampling year, albeit with marginal non-significance (Tables 1 and

S1). Specifically, for primary producer (cyanobacterial) populations measured with the same proxy (16S rRNA genes), additional precipitation also resulted in divergent outcomes depending on the regime: slight suppression with low-frequency, large additions, but marginally significant enhancement with high-frequency, small additions. And yet, when assessed through Chl *a* concentration as a proxy, both types of additions caused an increase in the biomarker over controls. The discrepancy may have been caused by treatment effects on community structure, given that different species of cyanobacteria have differing contents of Chl *a* (Nelson et al. 2020). In our case, cultivated members of the *M. steenstrupii* complex that became more prominent in water addition treatments have higher content of Chl *a* per cell (by some 14-26 %) than *M. vaginatus*, which relies more heavily on light absorption by phycobilins (Fernandes and Garcia-Pichel, unpublished). Although in many cases chlorophyll *a* concentration is considered to be a good comparative proxy for photosynthetic biomass (Yeager et al. 2012, Sorochkina et al. 2018, Fernandes et al. 2018), in scenarios where major changes in community composition occur, such as here, or when light intensity varies, alternative proxies are recommended (Nelson et al. 2020). Again here, the data suggest that the purported limitation by water may be more complex a driver than originally thought, being also dependent on rain event size and/or frequency. The fact that, in general terms, the effects on population size of both bacteria and cyanobacteria were more pronounced in the year of plenty than in 2017, when populations were apparently more strongly limited by some other factor, is consistent with the notion of, at most, a co-limitation of biocrusts by water availability and another resource.

Our findings are at least, partially contrary to the common belief that deserts are fundamentally "water-controlled" ecosystems (Noy-Meir 1973), and that water is the most limiting factor in arid terrestrial ecosystems worldwide (Nemani et al. 2003). Precipitation is thought to control biocrust distribution and composition from global to local scales, and it is cited as the most limiting factor for biocrust development (Weber et al. 2016a). We show that event size and frequency, rather than exclusively the total amount of precipitation, play roles in biocrust development and distribution, at least at a local scale. Interestingly, studies assessing effects of increased rainfall variability on plant productivity in our same experiment did not find clear responses in plant ANPP with increased precipitation either (Petrie et al. 2018). In more recent data, aboveground ANPP increased in subplots with added nitrogen (data not published). Both our study and Petrie et. al. 2018 point to co-limitation or strong legacy effects on both biocrusts and plants to increased precipitation.

Increased rainfall enhances biocrust microbial diversity

Additional precipitation events significantly enhanced both bacterial and cyanobacterial diversity in both sampling years (Tables 3 and 4), and most intensely in the year of plenty, 2019. The bacterial/archaeal compositional responses were driven most strongly by Cyanobacteria, followed by Proteobacteria or Actinobacteria. In the cyanobacterial communities, differences were driven most strongly by a loss of *M. vaginatus* dominance with water addition, and concurrent increases in a variety of forms in the *M. steentrupii* clade and the heterocystous *Scytonema* spp. Effects of increased precipitation on

cyanobacterial diversity were particularly strong, at least doubling Shannon's diversity, as the communities also increased in evenness. This is consistent with the tenet that decreasing water availability restricts the pool of microbial species able to thrive, hence creating a positive dependency between frequency of wet pulses and microbial diversity metrics (Rothrock and Garcia-Pichel 2005), a tendency with parallels in other extreme environmental gradients (Ward et al. 1998, Nübel et al. 1999, Avrahami and Conrad 2003).

Small-event, more frequent rainfall enhances microbial and cyanobacterial biomass

Strictly, effects of precipitation variability on biocrust communities can be derived only from comparisons of the two rainfall addition treatments, which shared the same total precipitation. Small, more frequent rainfall events significantly increased microbial biomass (measured by 16S rRNA gene copies) in both years (Table 1). Cyanobacteria biomass, measured by 16S rRNA gene copies, as well as chlorophyll *a* areal concentration was significantly higher in 2019 (Table 1, Fig. S4). In 2017, although the increase was not significant for either of the biomass indicators, values were still higher in the Small treatment.

While a minimum pulse size requirement for net growth of biocrust cyanobacteria has not been directly determined, it can be derived from studies by Zhang et al.(Zhang et al. 2018) and Steven et al.(Steven et al. 2015) to be significantly smaller than the 5 mm we imposed, probably below 2 mm. In laboratory incubations, Zhang et. al. found that four additional events of 2 mm resulted in increased net carbon gain in cyanobacterial biocrusts, while in moss-dominated biocrusts only the 5 mm treatment led to net carbon

gain, whereas the 2 mm treatment resulted in net carbon loss (Zhang et al. 2018). Reed et al.(Reed et al. 2012) showed that mosses grow under 5 mm rainfall additions, but do not survive under 1.2 mm rainfall additions. These results are in accordance with our data and show that cyanobacteria biomass increases with smaller rainfall events and suggest that their water limit is lower than that of mosses. It should prove interesting to determine the actual minimum event threshold for cyanobacteria, and if and how much it varies by species.

Smaller, more frequent rainfall enhances microbial and cyanobacterial and diversity

In general, the Small treatment, simulating alterations to rainfall patterns that are consistent with trends over the last 100 years in this region (Petrie et al. 2014), resulted in significant increases in alpha-diversity in time-integrated datasets for both bacteria/archaea and cyanobacteria either when performed at fine taxonomic resolution (ASVs; Tables 3 and 4) or coarse resolution (phyla for bacteria, genus/species for cyanobacteria; Tables S6 and S7). Again here, the most responsive taxa for these changes were cyanobacteria and proteobacteria at the phylum level (Fig. 1; Table S3). Among the primary producers, the main changes in community composition where due to changes in *M. vaginatus* abundance, which actually declined in absolute terms with more variable regimes, while members of the *M. steenstrupii* complex and *Scytonema* spp. sustained net and relative gains (Fig 2; Table S5).

Cyanobacterial winners and losers with a more variable rainfall pattern

Biocrusts cyanobacteria can be divided in two groups that exploit somewhat different niches. One group is the pioneer bundle-formers that colonize bare soils (Garcia-Pichel and Wojciechowski 2009) and are filamentous, motile and cannot fix nitrogen (Housman et al. 2006, Starkenburg et al. 2011) by themselves, instead resorting to symbioses with heterotrophic bacteria (Couradeau et al. 2019). These pioneers typically inhabit the soil a few hundred microns below the surface, although they migrate to the surface during pulses of precipitation (Pringault and Garcia-Pichel 2004). The cyanobacteria within this group are foundational biocrust microbes (Couradeau et al. 2019) and are responsible for initial soil stabilization (Pointing and Belnap 2014). In North American arid lands, pioneer cyanobacteria usually include *Microcoleus vaginatus* and clades of the *M. steenstrupii* complex (Gundlapally and Garcia-Pichel 2006, Garcia-Pichel et al. 2013a, Couradeau et al. 2016). The epithet "*Microcoleus steenstrupii"* was recognized as a supra-generic entity in dire need of taxonomic re-evaluation, because of its genetic diversity, the very deep phylogenetic distance from *M. vaginatus* (the type species for the genus *Microcoleus*) (Machado-de-Lima et al. 2019) and the apparently diverging ecological diversification within this complex of species (Fernandes et al. 2018). For these reasons, we split it into phylogenetically coherent clades in this study. The second group, the heterocystous cyanobacteria, colonize biocrusts after soil has been stabilized by the pioneers and are filamentous, sessile, nitrogen-fixing cyanobacteria. They inhabit the very surface of the biocrusts, and do not migrate. In North American arid lands this group is encompassed largely by *Scytonema* spp., *Nostoc* spp. and *Tolypothrix* spp.

(Yeager et al. 2007). These cyanobacteria not only supply fixed N, but also protect biocrusts from UV radiation by producing the sunscreen pigment scytonemin (Garcia-Pichel and Castenholz 1991a).

Among the pioneer functional group, *M. vaginatus*, the dominant taxon in our control plots, was negatively affected by rainfall additions, with strongest effects from the Small treatment plots, where it lost its dominance. It also lost its dominance in the Large treatment in 2017. By contrast, certain clades (*Ms SON57* clade, for example) belonging to the *M. steenstrupii* complex benefited from the Small treatment. Taken together, members of the *M. steenstrupii* complex become the most dominant taxa in the Small treatment plots, with specific clades benefitting most in different treatments and years. For example, and uniquely in our study, it is clear that the *Pycnacronema* spp. clade was positively affected by the Large treatment during both years of sampling, but not by the Small treatment (Table S5, Fig. 2). *Pycnacronema* is dominant in many biocrusts from the Brazilian savanna (64, 65), characterized by a mean annual precipitation 5 times higher than that of the Chihuahuan Desert that comes largely as intense pulses during the wet season. In the same manner, other clades responded more strongly to the Small treatments, including the *Ms SON57* clade and a novel clade in 2019 (Table S5, Fig. 2). Yet, another clade (*Ms PCC7113* clade) responded similarly to *M. vaginatus*, being sensitive to both rainfall addition treatments during both years of sampling. These results clearly demonstrate differential responses to water availability within the *M. steenstrupii* complex, indicating a previously unappreciated niche delineation within the complex that may prove quite useful to interpret ecological patterns. The contrast between *M.*

vaginatus and the *M. steentrupii* complex with respect to rainfall, creates an interesting trade-off, since *M. vaginatus* and the *M. steenstrupii* complex also have differential responses to temperature, with *M. vaginatus* being psychrotolerant and the *M. steenstrupii* complex (as a whole) being more thermotolerant (Garcia-Pichel et al. 2013a). The two parameters are expected to change concurrently under future climate scenarios, and while their potential interaction remains to be studied, one can foresee that a drier future will benefit *M. vaginatus*, but a hotter one with increased rainfall variability will benefit the *M. steentrupii* complex.

The heterocystous cyanobacteria, *Scytonema* spp., was not present in control plots but appeared with increased rainfall addition treatments, and more strongly so under small pulses (Figs, 2 and S4; Table S5). Hence a more variable rainfall regime in the future may promote biocrust N fixation ability and protection against UV damage. These potential effects on N-fixation are opposite to those of increased temperature (Zhou et al. 2016) and increased drought (Fernandes et al. 2018).

Concluding remarks

Our long-term experiments, conducted on typical cyanobacterial biocrust of the US Southwest, surprisingly pointed to a lack of universality in the paradigm that biocrusts, like arid ecosystems at large, are fundamentally limited by a lack of moisture and that other limitations are secondary. Clearly, such a water limitation was dependent on the status of the biocrust, as judged by the variability between the two years, and on

the mode of delivery of additional moisture. Additional moisture also resulted in communities with higher diversity, opening opportunities for taxa that were apparently less impervious to drought, both among pioneers and secondary colonizers alike. The comparisons of water addition treatments of different event size and frequency revealed that the nature of rainfall delivery has profound effects on both standing stock and diversity in biocrusts. The striking changes in community composition, with clear winners and losers, highlights the risk of using "taxonomic black boxes" in biocrust studies, where potentially functionally relevant shifts in community composition may go unnoticed. The clear differential effects with smaller, more frequent precipitation leading to largely positive effects on both standing stock and diversity, would predict a rosier future for biocrust as far as this parameter is concerned. Unfortunately, a more variable future precipitation regime will not play in isolation, but rather in association with increases in temperature and aridity that are already evident locally and regionally (Rudgers et al. 2018, Maurer et al. 2020), and which can suppress biocrusts. Integrative studies need to be designed that encompass all climate variables simultaneously, as evidence of strong interactions on outcomes when warming is added to pulse size treatments has been presented (Steven et al. 2015).

Acknowledgments

We thank Mariah Patton and Eva Stricker for their help collecting and analyzing pigments data for the 2019 sampling. Partial support was provided by NSF LTREB grant DEB-1856383.

Tables

Table 1. Bacteria/archaeal and cyanobacterial population size in biocrusts by treatment and sampling year. Asterisks indicate significant differences between years using t-tests with p-values corrected for multiple comparisons. For each population type and parameter, differing background color represents significant difference according to ANOVA/Tukey-Kramer post-hoc tests within a single year. ($p < 0.05$; n= 5 ± SD).

	Bacteria/Archaea			Cyanobacteria					
	(10^{10} 16S rRNA gene copies cm^{-2})			16S rRNA (10^{9} gene copies cm^{-2})			Chlorophyll *a* (*mg* m^{-2})		
Year	Control*	Large*	Small*	Control*	Large*	Small*	Control*	Large	Small*
2017	0.2 ± 0.1	0.2 ± 0.0	0.3 ± 0.4	1.2 ± 0.4	1.1 ± 0.1	1.3 ± 0.5	39 ± 7.5	80 ± 21	86 ± 20
2019	1.4 ± 1.1	1.2 ± 0.5	3.0 ± 1.6	3.9 ± 2.4	2.0 ± 0.9	8.4 ± 4.8	66 ± 6.5	87 ± 26	116 ± 32

Table 2. perMANOVA analysis of population sizes (on Bray Curtis matrix) showing significance among treatments, sampling years and interaction (Treatment X Year), as well as pairwise comparisons among treatments for either data compiled for both years or separated by year.

perMANOVA	Bacteria/Archaea absolute abundance (Phylum level)		Cyanobacteria absolute abundance (Genus/species level)	
Effect	Pseudo-*F*	*P*	Pseudo-*F*	*P*
Rain treatment	2.0	0.0886	8.2	**0.0001**
Year	57.4	**0.0001**	18.0	**0.0001**
Treatment x Year	2.4	0.0752	4.0	**0.0003**
Plot (Treatment)	1.2	0.3188	1.2	0.2206
Compiled data for both years				
Control vs. Large	0.8	0.5034	8.9	**0.0077**
Control vs. Small	2.4	0.0975	12.2	**0.0100**
Large vs. Small	2.9	**0.0419**	4.7	**0.0077**
Data for 2017				
Control vs. Large	*n.t.*	*n.t.*	2.6	**0.0166**
Control vs. Small	*n.t.*	*n.t.*	3.1	**0.0080**
Large vs. Small	*n.t.*	*n.t.*	1.4	0.0874
Data for 2019				
Control vs. Large	*n.t.*	*n.t.*	2.7	**0.0076**
Control vs. Small	*n.t.*	*n.t.*	2.7	**0.0072**
Large vs. Small	*n.t.*	*n.t.*	2.3	**0.0080**

n.t = not tested, since initial comparisons of treatments and interaction were not significantly different for Bacteria/Archaea abundance

Table 3. Bacteria/Archaea alpha-diversity indices (diversity, richness and evenness) at the ASV level of taxonomic resolution, for both years, and separated by year. Significances were measured using ANOVA and Kruskal-Wallis posthoc tests.

	Shannon Diversity Index (*H'*)			Pielou Evenness			Taxon Richness		
Both Years	Mean	SD		Mean	SD		Mean	SD	
Control	5.9	0.7	**a**	0.7	0.1	**a**	47.4	7.3	**a**
Large	7.0	0.6	**b**	0.8	0.1	**a**	51.2	9.4	**b**
Small	7.3	0.4	**c**	0.8	0.1	**a**	53.2	10.4	**b**
Treatment/Year	Mean	SD		Mean	SD		Mean	SD	
Control 2017	5.4	0.4	**a**	0.5	0.0	**a**	53.5	4.0	**a**
Large 2017	6.7	0.7	**bc**	0.7	0.1	**b**	59.3	3.0	**b**
Small 2017	7.4	0.5	**d**	0.7	0.0	**c**	61.5	7.6	**c**
Control 2019	6.4	0.4	**b**	0.8	0.0	**d**	41.4	3.2	**b**
Large 2019	7.2	0.3	**c**	0.9	0.0	**d**	43.0	1.8	**d**
Small 2019	7.2	0.2	**cd**	0.9	0.0	**d**	44.9	3.4	**d**

Table 4. Cyanobacteria alpha-diversity indices (diversity, richness and evenness) at the ASV level of taxonomic resolution, for both years and separated by year. Performed using QIIME2 phylogenetic alpha-diversity. Significances were measured using ANOVA and Kruskal-Wallis posthoc tests.

	Shannon Diversity Index (*H'*)			Pielou Evenness			Taxon Richness		
Both Years	Mean	SD		Mean	SD		Mean	SD	
Control	1.5	0.4	**a**	0.3	0.1	**a**	5.2	0.5	**a**
Large	3.0	0.5	**b**	0.6	0.1	**b**	6.2	0.8	**b**
Small	3.9	0.4	**c**	0.7	0.1	**c**	7.1	0.6	**c**
Treatment/Year	Mean	SD		Mean	SD		Mean	SD	
Control 2017	1.7	0.5	**a**	0.3	0.1	**a**	5.5	0.4	**a**
Large 2017	3.0	0.3	**b**	0.5	0.1	**b**	6.7	0.6	**b**
Small 2017	3.9	0.4	**c**	0.6	0.1	**c**	7.4	0.6	**c**
Control 2019	1.4	0.2	**a**	0.4	0.1	**a**	4.9	0.3	**a**
Large 2019	3.0	0.5	**b**	0.7	0.1	**c**	5.6	0.1	**a**
Small 2019	4.0	0.5	**c**	0.8	0.1	**d**	6.9	0.6	**bc**

Figures

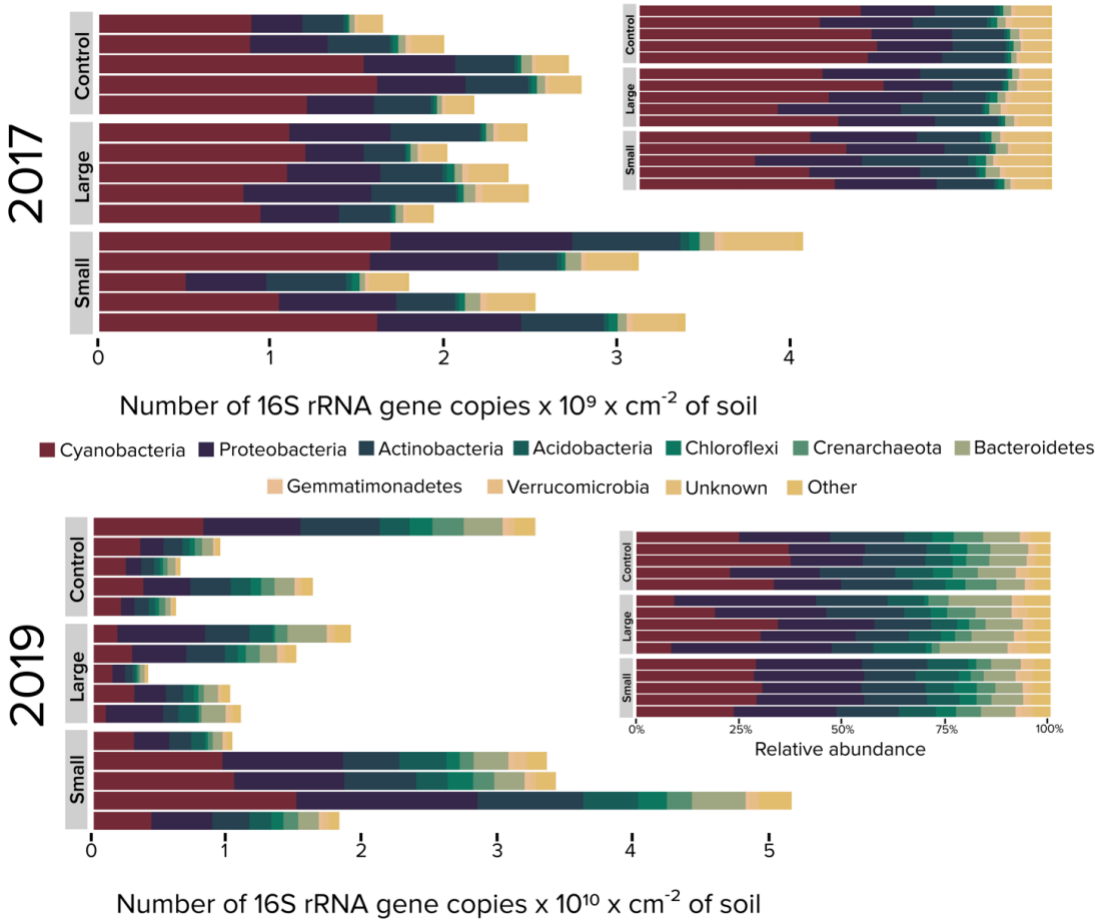

Figure 11. Bacterial abundance and community structure in control and treatment biological soil crusts as determined by high- throughput 16S rRNA gene analyses coupled to q-PCR, as well as relative abundance (top right side). Five replicate plots were analyzed for each treatment and each bar represents a replicate plot. Phylogenetic assignments for each sequence 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 years.

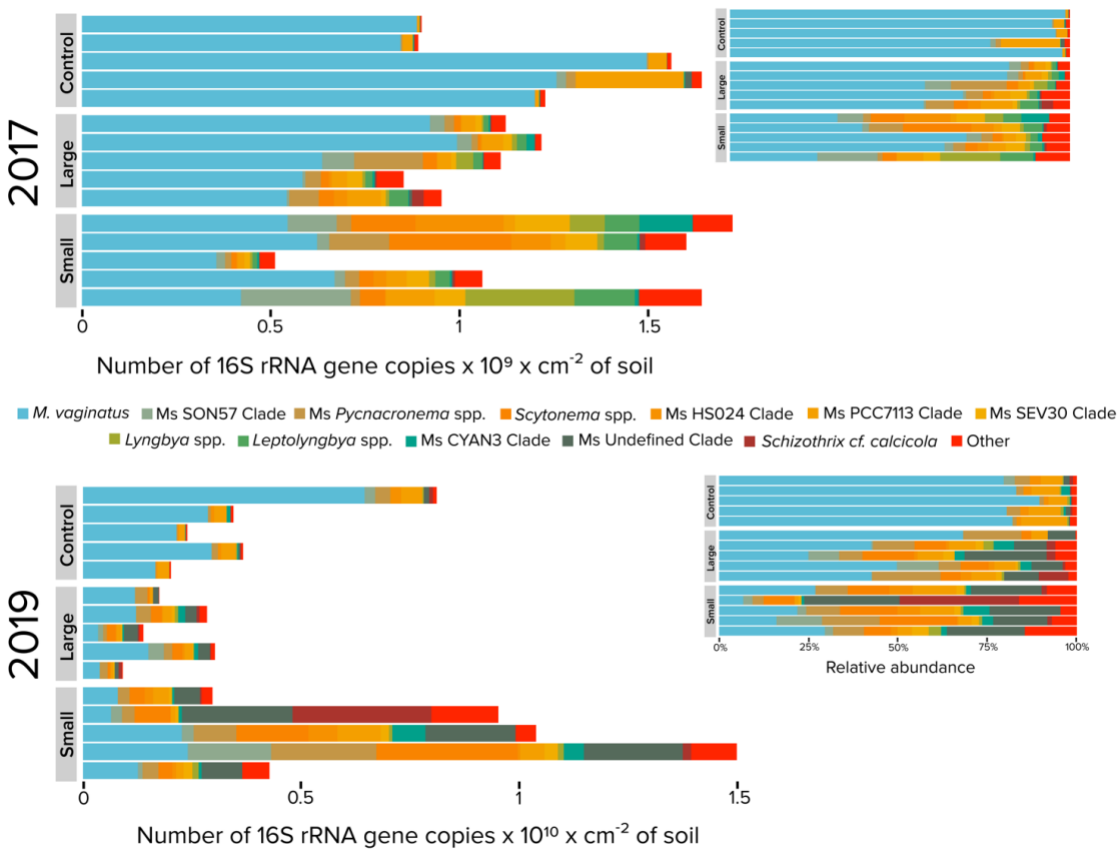

Figure 12. Cyanobacterial abundance and community structure in control and treatment biological soil crusts as determined by high- throughput 16S rRNA gene analyses coupled to q-PCR, as well as relative abundance (top right side). Five replicate plots were analyzed for each treatment and each bar represents a replicate plot. Phylogenetic assignments for each sequence were based on blast to Cydrasil and carried to the Genus or species level, as feasible. Note that the scales are not the same for the two years.

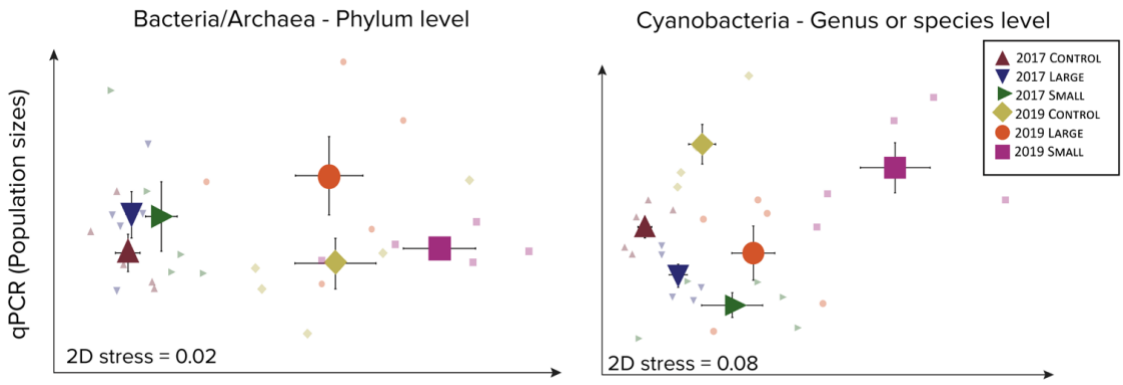

Figure 13. Non-metric multidimensional scaling (nMDS) comparison of bacterial/archaeal (left) and cyanobacterial (right) community composition between the treatments. The nMDS ordination was based on Bray-Curtis similarity. Data points are color coded by treatment and year (and centroid with scatter for each treatment is in intense colors).

Supplementary Information

Table S3. Bacteria/archaeal and cyanobacterial population size in biocrusts by treatment and sampling year. Asterisks indicate significant differences between years using t-tests with p-values corrected for multiple comparisons. For each population type and parameter, differing background color represents significant difference according to ANOVA/Tukey-Kramer post-hoc tests within a single year. ($p < 0.10$; n= 5 ± SD).

Year	Bacteria/Archaea (10^{10} 16S rRNA gene copies cm^{-2})			Cyanobacteria					
				16S rRNA (10^{9} gene copies cm^{-2})			Chlorophyll *a* (*mg* m^{-2})		
	Control*	Large*	Small*	Control*	Large*	Small*	Control*	Large	Small*
2017	0.2 ± 0.1	0.2 ± 0.0	0.3 ± 0.4	1.2 ± 0.4	1.1 ± 0.1	1.3 ± 0.5	39 ± 7.5	80 ± 21	86 ± 20
2019	1.4 ± 1.1	1.2 ± 0.5	3.0 ± 1.6	3.9 ± 2.4	2.0 ± 0.9	8.4 ± 4.8	66 ± 6.5	87 ± 26	116 ± 32

Table S4. perMANOVA analysis of ASVs on Weighted Unifrac (phylogenetic quantitative) matrix showing significance among treatments, as well as pairwise comparisons among treatments separated by year.

perMANOVA	Bacteria/Archaea relative abundance (ASVs level)		Cyanobacteria relative abundance (ASVs level)	
Compiled data for both years	*Pseudo-F*	*P*	*Pseudo-F*	*P*
Control vs. Large	0.75	0.472	13.0	**0.013**
Control vs. Small	1.31	0.213	17.0	**0.005**
Large vs. Small	0.47	0.652	2.47	**0.042**
Data for 2017				
Control vs. Large	3.33	**0.031**	4.97	**0.006**
Control vs. Small	7.38	**0.012**	9.06	**0.011**
Large vs. Small	1.76	0.111	2.64	0.061
Data for 2019				
Control vs. Large	4.23	**0.008**	13.0	**0.013**
Control vs. Small	8.30	**0.009**	17.0	**0.005**
Large vs. Small	1.77	0.136	2.47	**0.042**

Table S5. SIMPER results showing, in ranked order, which phyla contributed the most to the differences in community composition at the phylm level between each pair of treatments in each year.

	2017					
	2017 mean abundance (mean dissimilarity = 14.35)					
Taxon	Control	Large	Dissimilarity	Dissimilarity /SD	Contribution %	Cumulative %
Cyanobacteria	1,244,420,237	1,051,100,513	6.75	1.56	47.06	47.06
Proteobacteria	444,201,268	542,612,732	3.37	1.27	23.51	70.57
Actinobacteria	333,117,286	389,509,994	2.35	1.37	16.4	86.97
Other	161,151,757	175,957,430	0.86	1.41	6.02	92.99
	2017 mean abundance (mean dissimilarity = 21.78)					
Taxon	Control	Small	Dissimilarity	Dissimilarity /SD	Contribution %	Cumulative %
Cyanobacteria	1,051,100,513	1,307,998,836	9.23	2.11	45.09	45.09
Proteobacteria	389,509,994	458,815,772	2.55	1.45	12.47	57.56
Actinobacteria	542,612,732	770,294,003	4.88	1.56	23.84	81.4
Other	161,151,757	282,310,856	2.18	2	10.01	90.92
	2017 mean abundance (mean dissimilarity = 20.47)					
Taxon	Large	Small	Dissimilarity	Dissimilarity /SD	Contribution %	Cumulative %
Cyanobacteria	1,244,420,237	1,307,998,836	9.12	1.33	41.9	41.9
Proteobacteria	444,201,268	770,294,003	6.03	1.8	27.69	69.59
Actinobacteria	333,117,286	458,815,772	2.47	1.24	11.32	80.91
Other	175,957,430	282,310,856	1.95	1.52	9.52	90.92
	2019					
	2019 mean abundance (mean dissimilarity = 37.06)					
Taxon	Control	Large	Dissimilarity	Dissimilarity /SD	Contribution %	Cumulative %
Cyanobacteria	3,918,479,761	1,979,116,071	7.65	1.53	20.65	20.65
Proteobacteria	2,893,476,479	3,590,079,830	9.86	1.5	26.6	47.25
Bacteroidetes	1,245,145,399	1,474,364,798	3.97	1.44	10.7	57.95
Actinobacteria	2,439,137,517	1,808,604,834	5.71	1.41	15.41	73.36
Crenarchaeota	924,816,714	544,387,853	2.24	1.5	6.04	79.4
Acidobacteria	1,025,074,563	1,008,420,779	2.89	1.74	7.79	87.19
Other	617,904,649	663,516,571	1.96	1.82	5.29	92
	2019 mean abundance (mean dissimilarity = 37.06)					
Taxon	Control	Small	Dissimilarity	Dissimilarity /SD	Contribution %	Cumulative %
Cyanobacteria	1,244,420,237	8,405,466,352	20.45	3.72	25.15	25.15
Proteobacteria	444,201,268	7,504,777,866	21.21	8	26.08	51.23
Crenarchaeota	0	1,174,638,792	3.77	3.99	4.63	55.86
Actinobacteria	333,117,286	4,310,599,083	12.01	7.57	14.77	70.63
Acidobacteria	19,996,204	2,496,427,108	7.85	7	9.65	80.28
Bacteroidetes	42,598,323	2,205,647,691	6.63	9.01	8.15	88.43
Chloroflexi	23,644,925	1,214,865,504	3.46	2.58	4.25	93
	2019 mean abundance (mean dissimilarity = 44.63)					
Taxon	Control	Small	Dissimilarity	Dissimilarity /SD	Contribution %	Cumulative %

Cyanobacteria	1,244,420,237	8,405,466,352	20.45	3.72	25.15	25.15
Proteobacteria	444,201,268	7,504,777,866	21.21	8	26.08	51.23
Bacteroidetes	0	1,174,638,792	3.77	3.99	4.63	55.86
Actinobacteria	333,117,286	4,310,599,083	12.01	7.57	14.77	70.63
Acidobacteria	19,996,204	2,496,427,108	7.85	7	9.65	80.28
Chloroflexi	42,598,323	2,205,647,691	6.63	9.01	8.15	88.43
Crenarchaeota	23,644,925	1,214,865,504	3.46	2.58	4.25	93

Table S6. Analyses of dispersion from centroid using permDISP. Significantly different populations are assigned different bold letters.

				2017		**2019**	
Bacteria/Archaeal Phyla			**Treatment**	Mean (+/- SE) (Dispersion from centroid)		Mean (+/- SE) (Dispersion from centroid)	
Pseudo-F	*df*	*P*	Control	9.60 +/- 1.9	**a**	25.5 +/- 4.8	**a**
4.07	5,24	**0.0388**	Large	8.40 +/- 3.3	**a**	24.1 +/- 4.8	**a**
			Small	13.1 +/- 5.2	**a**	23.5 +/- 4.8	**a**
Cyanobacterial genera/species				Mean (+/- SE) (Dispersion from centroid)		Mean (+/- SE) (Dispersion from centroid)	
Pseudo-F	*df*	*P*	Control	12.4 +/- 1.3	**a**	19.1 +/- 2.4	**a**
5.22	5,24	**0.0218**	Large	16.0 +/- 3.1	**a**	26.0 +/- 2.7	**a**
			Small	25.7 +/- 5.7	**b**	32.4 +/- 3.2	**a**

Table S7. SIMPER results showing, in ranked order, which cyanobacterial taxa contributed the most to the differences in community composition at the genus/species level between each pair of treatments in each year.

	2017					
	2017 mean abundance (mean dissimilarity = 32.18)					
Taxon	Control	Large	Dissimilarity	Dissimilarity /SD	Contribution %	Cumulative %
M. vaginatus	1,137,102,790	735,588,884	18.32	1.76	56.92	56.92
Ms PCC7113	74,912,334	51,679,878	2.95	0.95	9.18	66.1
Ms Pycnacronema	6,867,514	68,749,659	2.82	1.02	8.76	74.86
Other	12,591,785	43,526,866	1.48	1.5	4.6	79.46
Ms SON57	5,637,810	35,581,948	1.43	1.14	4.45	83.9
Leptolyngbya spp.	726,768	26,530,635	1.16	1.8	3.6	87.51
Scytonema spp.	0	23,761,227	1.07	1.78	3.33	90.84
	2017 mean abundance (mean dissimilarity = 53.47)					
Taxon	Control	Small	Dissimilarity	Dissimilarity /SD	Contribution %	Cumulative %
M. vaginatus	1,137,102,790	523,092,592	24.77	2.03	46.32	46.32
Scytonema spp.	0	122,065,757	4.41	1.09	8.26	54.57
Ms SON57	5,637,810	100,383,568	3.46	0.94	6.47	61.05
Other	12,591,785	98,690,421	3.29	2.37	6.15	67.2
Lyngbya spp.	213,715	84,641,404	3.01	0.79	5.62	72.82
Ms SEV30	623,958	77,580,741	2.86	2.05	5.35	78.17
Ms PCC7113	74,912,334	53,985,614	2.84	0.89	5.31	83.48
Leptolyngbya spp.	726,768	77,683,112	2.81	1.61	5.25	88.73
Ms HS024	59,347	75,384,380	2.68	0.88	5.02	93.75
	2017 mean abundance (mean dissimilarity = 36.39)					
Taxon	Large	Small	Dissimilarity	Dissimilarity /SD	Contribution %	Cumulative %
M. vaginatus	735,588,884	523,092,592	11.03	1.15	30.32	30.32
Scytonema spp.	23,761,227	122,065,757	3.93	0.94	10.79	41.12
Ms SON57	35,581,948	100,383,568	3.33	0.96	9.15	50.26
Lyngbya spp.	15,537,612	84,641,404	2.93	0.76	8.05	58.32
Ms HS024	10,614,121	75,384,380	2.72	0.9	7.48	65.79
Ms Pycnacronema	68,749,659	55,456,521	2.51	0.95	6.89	72.69
Ms SEV30	21,973,032	77,580,741	2.28	1.65	6.27	78.95
Leptolyngbya spp.	26,530,635	77,683,112	2.28	1.41	6.26	85.21
Other	43,526,866	98,690,421	2.27	1.59	6.23	91.44
	2019					
	2019 mean abundance (mean dissimilarity = 55.37)					
Taxon	Control	Large	Dissimilarity	Dissimilarity /SD	Contribution %	Cumulative %
M. vaginatus	3,205,465,447	926,547,426	36.52	1.98	65.95	65.95
Ms Undefined	38,389,493	212,636,206	3.52	1.53	6.36	72.31
Ms PCC7113	294,045,672	125,934,411	3.07	1.77	5.54	77.85
Ms Pycnacronema	119,126,260	213,159,742	2.92	1.44	5.28	83.13

Scytonema spp.	0	156,313,520	2.82	1.66	5.09	88.22
Ms SON57	50,521,483	97,167,363	1.85	0.84	3.35	91.57
	2019 mean abundance (mean dissimilarity = 66.07)					
Taxon	Control	Small	Dissimilarity	Dissimilarity /SD	Contribution %	Cumulative %
M. vaginatus	3,205,465,447	1,472,187,477	16.66	1.24	25.21	25.21
Ms Undefined	38,389,493	1,672,086,919	13.17	3.04	19.94	45.15
Scytonema spp.	0	1,274,559,035	8.99	1.72	13.61	58.76
Other	50,038,453	781,804,745	6.05	1.79	9.16	67.92
Ms Pycnacronema	119,126,260	861,600,116	5.34	1.28	8.08	76
Schizothrix sp. calcicola	23,713,216	681,495,826	5.17	0.53	7.83	83.83
Ms SON57	50,521,483	503,699,302	3	0.81	4.55	88.37
Ms PCC7113	294,045,672	437,448,777	2.27	1.29	3.44	91.82
	2019 mean abundance (mean dissimilarity = 61.23)					
Taxon	Large	Small	Dissimilarity	Dissimilarity /SD	Contribution %	Cumulative %
Ms Undefined	212,636,206	1,672,086,919	13.44	2.84	21.94	21.94
Scytonema spp.	156,313,520	1,274,559,035	8.65	1.41	14.13	36.07
M. vaginatus	926,547,426	1,472,187,477	8.3	1.69	13.56	49.63
Other	75,883,125	781,804,745	6.89	1.77	11.24	60.88
Schizothrix sp. calcicola	39,610,538	681,495,826	5.97	0.54	9.76	70.64
Ms Pycnacronema	213,159,742	861,600,116	4.91	1.05	8.01	78.65
Ms SON57	97,167,363	503,699,302	3.52	0.9	5.75	84.41
Ms PCC7113	125,934,411	437,448,777	3.25	1.18	5.31	89.72
Ms CYAN3	58,893,325	280,571,963	2.11	1.04	3.44	93.16

Table S8. Alpha-diversity indices (diversity, richness and evenness) for bacterial/archaeal communities assessed at the phylum level. Chi-squared was used to test the significance of Treatment or Treatment x Year effects. Below are the results of the decomposed differences in diversity within each treatment using planned contrasts of the model means. n.t. = not tested. Taxon richness was not tested, since all samples presented some abundance of all phyla.

	Shannon Diversity Index (*H'*)			**Taxon Richness**			**Shannon Evenness (J)**		
2017	Mean	SD		Mean			Mean	SD	
Control	1.37	0.08	**a**	10			0.60	0.08	**a**
Large	1.45	0.08	**ab**	10			0.63	0.08	**ab**
Small	1.53	0.09	**b**	10			0.67	0.08	**b**
2019									
Control	1.93	0.2	**a**	11			0.80	0.08	**a**
Large	1.90	0.2	**a**	11			0.79	0.08	**a**
Small	1.92	0.2	**a**	11			0.80	0.08	**a**
	X_2	*df*	*P*	X_2	*df*	*P*	X_2	*df*	*P*
Treatment	5.69	2	0.0581	*n.t.*	*n.t.*	*n.t.*	5.83	2	0.0543
Year	282.34	1	**<0.0001**	*n.t.*	*n.t.*	*n.t.*	202.63	1	**<0.0001**
Treatment x Year	5.85	2	0.0537	*n.t.*	*n.t.*	*n.t.*	5.99	2	0.0501

Table S9. Alpha-diversity indices (diversity, richness and evenness) for cyanobacterial communities assessed at the species/genius level. Chi-squared was

used to test the significance of Treatment or Treatment x Year effects. Below are the results of the decomposed differences in diversity within each treatment using planned contrasts of the model means.

	Shannon Diversity Index (*H'*)			**Taxon Richness**			**Shannon Evenness (J)**		
Treatment	Mean	SD		Mean	SD		Mean	SD	
Control	0.51	0.2	**a**	8.8	0.9	**a**	0.23	0.08	**a**
Large	1.45	0.2	**b**	11.4	0.8	**b**	0.60	0.08	**b**
Small	1.87	0.2	**c**	11.7	0.9	**c**	0.76	0.08	**c**
	X_2	*df*	*P*	X_2	*df*	*P*	X_2	*df*	*P*
Treatment	112.33	2	**<0.0001**	32.33	2	**<0.0001**	134.93	2	**<0.0001**
Year	19.28	1	**<0.0001**	8.60	1	**0.0034**	37.99	1	**<0.0001**
Treatment x Year	1.42	2	0.4924	8.99	2	**0.0112**	2.46	2	0.2928

Supporting Figures

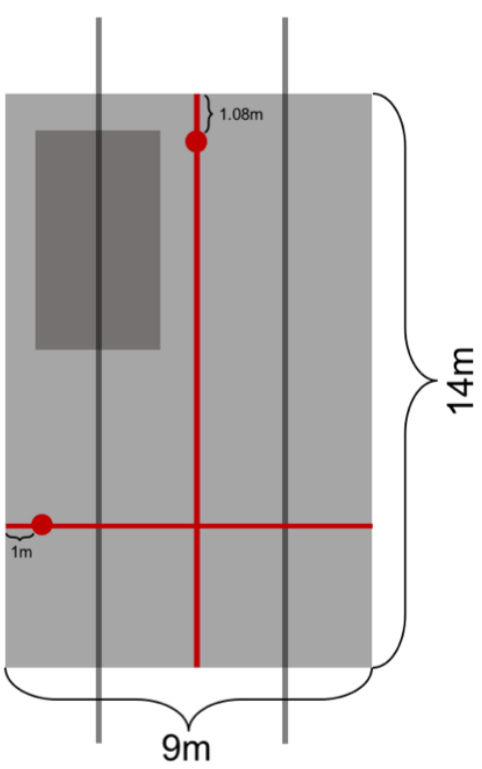

Figure S1. Schematic representation of transects used to sample the experiment plots. For each plot two transects were placed, one horizontally and another one vertically, with the purpose of capturing biocrust patchiness. 12 samples collected every 1.08m over the vertical transect and 8 samples collected every 1m over the horizontal transect, for a total of 20 samples per plot.

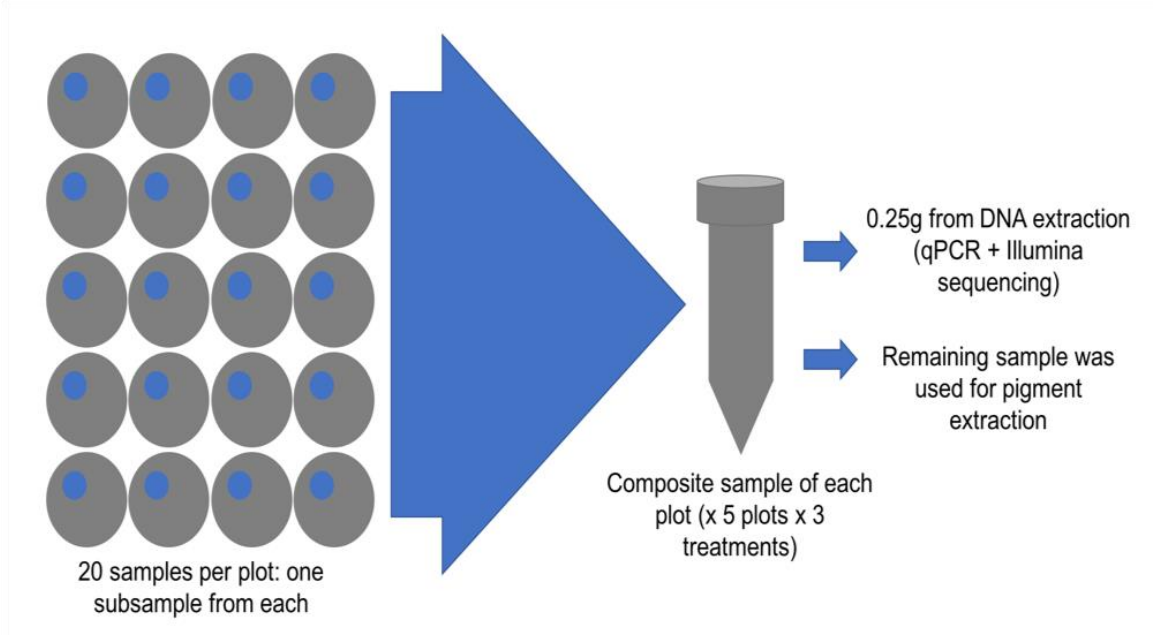

Figure S2. Schematic representation of pooling and subsampling of the 20 samples collected in each plot. One 1cm x 1cm core was taken from each Petri dish and pooled into a falcon tube. From that pooled sample, 0.25g were used for DNA extraction and the remaining sample was used for pigment extraction.

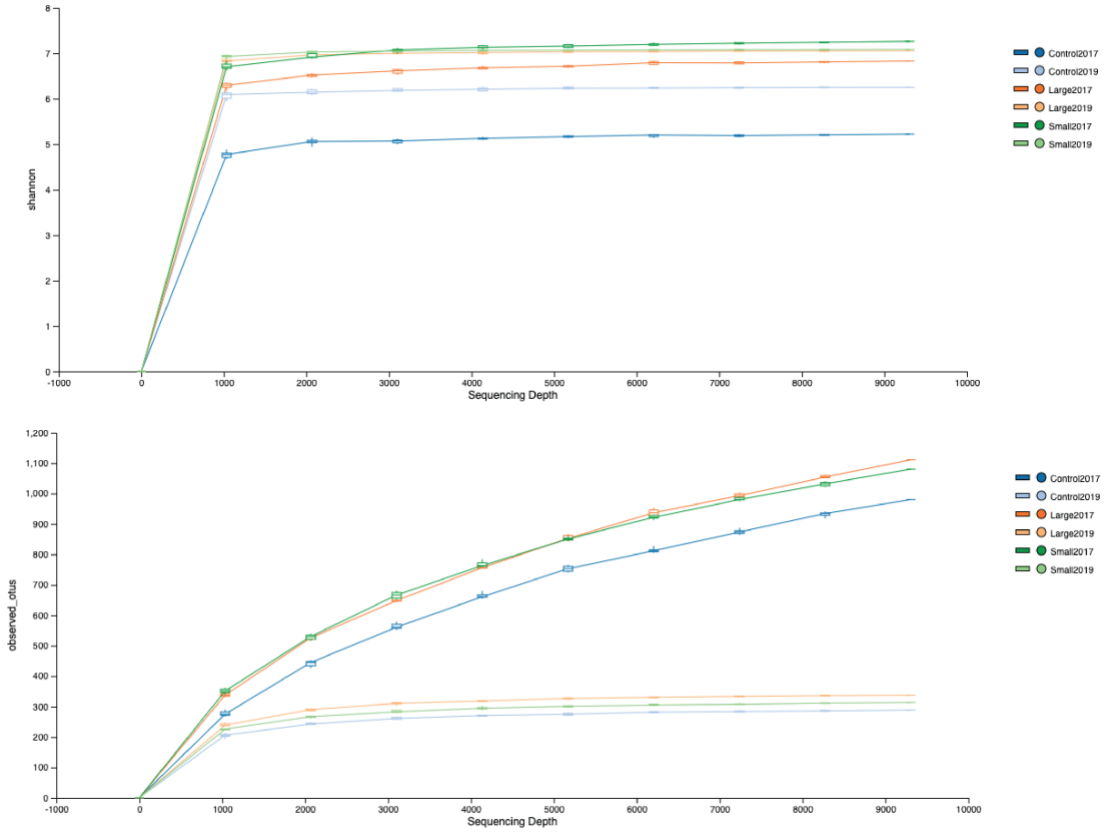

Figure S3. Alpha-rarefaction for Shannon diversity (top) and observed OTU's (bottom) after rarefaction of data from 2017 to match sequencing depth of 2019.

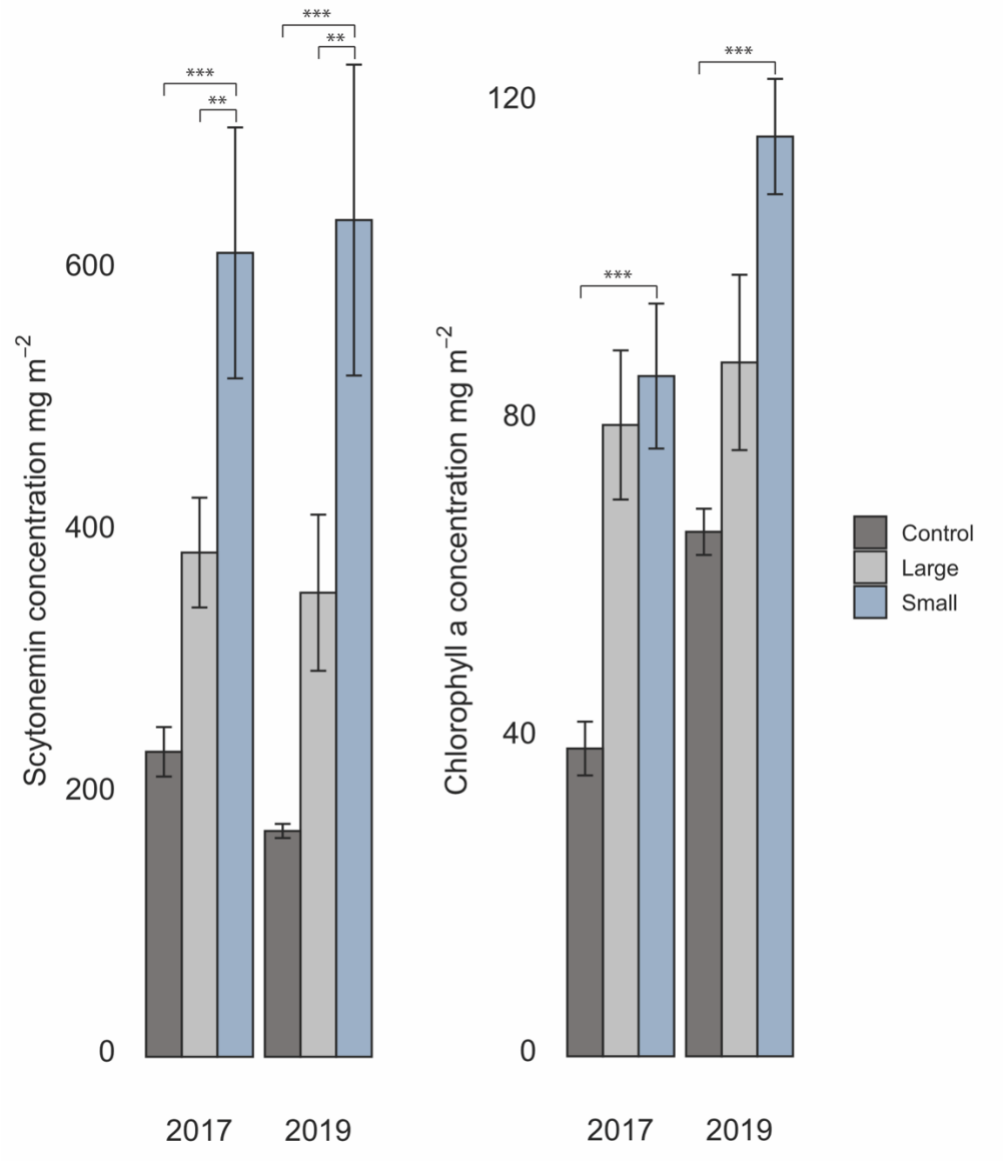

Figure S4. Areal concentrations of biomarker pigments Chl a and scytonemin in biological soil crusts for controls and treatments at both years. Error bars are ± s.d.(n = 5). Asterisks denote significant differences (**p < 0.05, ***p<0.001).

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4 – PORPHYROSIPHONACEAE, FAM. NOV., A MONOPHYLETIC HOME FOR THE "*MICROCOLEUS STEENSTRUPII*" COMPLEX AND OTHER DESICCATION-TOLERANT FILAMENTOUS CYANOBACTERIA

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Authors:

Vanessa M. C. Fernandes, Ana Giraldo-Silva, Daniel Roush and Ferran Garcia-Pichel

Abstract

The increase in 16S rRNA gene sequencing has revealed the need to re-evaluate multiple cyanobacterial taxa that were initially described based on morphology, leading to many phylogenetically and ecologically incoherent taxa. Cyanobacteria falling under the epithet *Microcoleus steenstrupii* J.B Petersen play a significant role as pioneers of biological soil crusts (biocrusts) but are also recognized as a very diverse complex. A meta-analysis of DNA biocrust sequencing analyses, showed that the complex encompasses a variety of well supported genus-level clades that respond differentially to environmental parameters, indicating significant diversification in their ecological roles. We present a polyphasic resolution of this complex group of taxa. Fifteen strains in the *Microcoleus steenstrupii* complex were selected as representative of naturally occurring clades in the complex and studied using 16S rRNA gene phylogeny, morphology and niche differentiation with respect to temperature and rainfall. They all fell within a monophyletic, family-level clade (91.4 % similarity) under Bayesian reconstructions in the context of the overall cyanobacterial phylogeny based on long 16S rRNA sequences, one that included some known genera of desiccation-resistant, largely terrestrial cyanobacteria. To accommodate this diversity, we describe the new family Porphyrosiphonaceae, composed of 11 genera, among which five are also described here (*Sociatus*, *Parifilum*, *Xeronema*, *Crassifilum* and *Crustifilum*) with corresponding type species, in addition to *Porphyrosiphon*, *Coleofasciculus*, *Pycnacronema*, *Potamolinea*, *Wilmottia* and *Allocoleopsis*. We also probed the differential global distribution of these genera within biocrusts using a metanalysis of molecular databases. Resolving the

systematics of the Porphyrosiphonaceae will improve understanding of the biology of biocrusts and terrestrial cyanobacteria in the future.

Key words: cyanobacteria, biocrusts, *Microcoleus steenstrupii*, morphology, phylogeny, taxonomy

Introduction

Achieving a coherent systematic treatment for cyanobacteria has been a centuries-long work in progress, but given the relevance of the subject organisms, remains a significant handicap for ecological studies. The Cyanobacteria are one of the most diverse and widely distributed phyla of bacteria, contributing greatly to global primary production. These microorganisms fix a substantial amount of biologically available carbon, especially in nutrient-limited environmental niches, such as biological soil crusts (biocrusts) (Garcia-Pichel et al. 2003a, Elbert et al. 2012a). Biocrusts are photosynthetically-driven microbial assemblages that develop in areas where light can penetrate directly to the soil surface without the limitation of plant coverage or litter (see review by Pointing and Belnap 2012). Estimated to cover ~12% of the global terrestrial surface, biocrusts are likely responsible for 15% of the net primary production and half of the overall N fixation of terrestrial surfaces around the globe (Elbert et al. 2012a, Rodriguez-Caballero et al. 2018). Additionally, these communities mitigate erosion of desert soils (Belnap et al. 2008). Cyanobacteria are the foundation of biocrusts, because crust formation is initiated by filamentous bundle-forming *Microcoleus vaginatus* and the entity currently known as *Microcoleus steenstrupii* (Garcia-Pichel and Wojciechowski 2009). These cyanobacteria colonize bare soils (Garcia-Pichel and Wojciechowski 2009) and are responsible for soil stabilization (Pointing and Belnap 2014) and soil nutrient enrichment due to carbon fixation.

According to molecular analyses, the genus *Microcoleus* Desmazières ex Gomont, defined as filamentous cyanobacteria with tapered end cells often developing distinct

calyptra, and the type species, *Microcoleus vaginatus* (Vaucher) Gomont ex Gomont (Gomont 1892), has been repeatedly found to polyphyletic and been the subject of criticisms and partial revision (Boyer et al. 2002, Siegesmund et al. 2008, Strunecký et al. 2011, 2013). *Microcoleus steenstrupii* was described originally as a species within the genus based largely on its ability to form bundles of filaments held by a common sheath (Petersen 1923), although other morphological characters were not concordant. More recent studies noted that the epithet "*Microcoleus steenstrupii*" was found likely a supra-generic entity instead of a single species based on ribosomal sequence divergence among isolates (Boyer et al. 2002), which included even non-variable regions of the 16S rRNA gene. This study also showed that *M. steenstrupii* strain showed deep genetic divergence from the type species. The case for a need of revision has been made by many other since (Strunecký et al. 2011, 2013, Komárek et al. 2014), but the needed work has not materialized. Further this genetic diversity translates clearly to a niche differentiation demonstrated by the fact that phylogenetic clades within *M. steenstrupii* respond differently to ecological factors such as drought intensity and timing (Fernandes et al. 2018).

By contrast, other cyanobacterial taxa originally with the genus, if much less internally diverse, were redescribed on the basis of their genetic distance to the type. A new generic entity *Coleofasciculus chthonoplastes* (Siegesmund et al. 2008), for example, was devised to accommodate the former *Microcoleus chthonoplastes*. Similarly, *M. sociatus* was transferred to *Trichocoleus*, as *T. sociatus* (Anagnostidis 2001). In a revision of the genus *Microcoleus* (Strunecký et al. 2013) reinforced the need to study and rename the

species within it that no longer belonged to their new description, such as *M. paludosus* and *M. steenstupii*, but took no action. The clear shortcomings notwithstanding and in the absence of taxonomic guidance, many ecological studies still classify this "*M. steenstrupii* complex" as a single species (Zhang et al. 2016a, Cano-Díaz et al. 2018, Becerra-Absalón et al. 2019).

And yet, biogeographic surveys across the western US showed that members of the complex dominate hotter locations (Garcia-Pichel et al. 2013) and together outnumber the single most abundant biocrust cyanobacterium *M. vaginatus*. This prompted us to clarify the systematics of this group using a polyphasic approach that includes assessing morphological, phylogenetic and ecological features. We aimed to establish a more complete, evolutionarily coherent, useful taxonomy to contribute to understanding the patterns of cyanobacteria distribution and abundance and their ecological consequences.

Methods

Meta-analyses of microbial diversity in the M. steesntrupii complex

In order to determine the interal extent of diversity within the *M. steenstrupii* complex that exist in the natural environment, we combined sequence data from previoulsy published studies (Couradeau et al. 2016, Velasco Ayuso et al. 2016, Fernandes et al. 2018, Muñoz-Martín et al. 2018, Machado-de-Lima et al. 2019). They were the result of molecular environmental surveys that used Illumina MiSeq 16S rRNA gene and were thus relatively short (~300bp), and included biocrust locations from Europe and the Americas. On those datasets, we imposed the following selection criteria to choose pertinent sequences. Sequences had to have > 96% identity by BLAST to NCBI sequences of cultivated strains assigned to *Microcoleus steenstrupii*, *Microcoleus* sp. or *M. paludosus*. Additionaly we included longer sequences (600 bp) of strains assigned to *M. steenstrupii* isolated from biocrusts in our laboratory (Garcia-Pichel et al. 2013, Giraldo-Silva et al. 2018). All 354 sequences were aligned using MAFFT (Katoh and Standley 2013) and Guidance2 (Sela et al. 2015) and used to build a maximum likelihood tree, on the CIPRES high performance computing cluster (Miller et al. 2010) using the RAxML-HPC2 (Stamatakis 2014) workflow with the ML+Thorough bootstrap (1000 bootstraps) method and the GTRGAMMA model.

In addition, and with the goal to place the diversity contained with the complex in the contect of the entire phylum, as well as to test for cases of paraphyly, we conducted phylogenetic placements of the same set of sequences in the comprehensive cyanobacteria reference database and phylogenetic tree (Cydrasil -

https://github.com/FGPLab/cydrasil/tree), using its query placement capabilities, using the RaxML8 Evolutionary Placement Algorithm (Berger et al. 2011).

Isolation and selection of cutured representatives

Based on the results of the previous meta-analyses, we distinguished 18 different, naturally occurring clades within the complex. Six corresponded to taxa previously described (*Coleofasciculus chthonoplastes, Potamolinea* sp., *Pycnacronema* sp., *Wilmottia* sp., *Allocoleopsis franciscanus* and *Porphyrosiphon notarisii*). For five clades, we had representatives in cultures collections, including ours. We set out a cultivation effort to obtained new isolates for the remaining 7 clades, using a modification of the isolation protocols used in Giraldo-Silva et al. (2018) and Garcia-Pichel et al (2013) that used varying concentrations of the isolation medium. Briefly, Petri dishes containing field biocrusts were wetted to saturation and placed under light for about 6 hours after which bundles were picked under a dissecting scope and transferred to agar plates containing Jaworski's medium (JM) (Schlösser 1984) diluted to 50%, 25% and 10% of its initial concentration. Plates were incubated at 25 ± 2°C and illuminated at 20-30 μmol (photon) m^{-2} s^{-1} under 14-hour photoperiod until growth was observed, typically for one to two weeks. Biomass was then transferred to fresh JM liquid medium under gentle shaking for about three weeks. Strain identity was first assessed by microscopy, and then confirmed by PCR amplification of the V4 region of the 16S rRNA gene using cyanobacteria specific primers CYA106F/CYA781R (Nübel et al. 1997; PCR protocol therein), resulting in partial sequences approximately 660 bp in length. PCR products

were Sanger sequenced; resulting sequences were blasted against the NCBI database and our own *M. steenstrupii* database for fit to orphan clades. Strains of interest grown in liquid as above in preparation for long-read sequencing and physiological assays.

Long-read sequencing 16S rRNA and bioinformatic analysis

Our previous steps resulted in the selection of 15 cyanobacterial strains (HSN013, HSN023, BN28, SON57, FB15, HS024, HS003, HS035, HS041, CYAN3, SEV30, SON62, BN27, SON60 and DW001) of interest. Their full 16S rRNA gene (~ 1500 bp) of these 15 strains was obtained using the LoopSeq 16S Long Read Kit from Loop Genomics (https://www.loopgenomics.com/16s). DNA from cyanobacterial cultures was extracted using the Qiagen DNeasy PowerSoil Kit, reference number 12888-100, following manufacturer's instructions. Library preparation and quality checks were performed using manufacturer's protocol. The Universal full-length primer set 27F/1492R was used to amplify the 16S rRNA gene in 20 cycles of PCR according to (Callahan et al., 2019). PCR products were sequenced on an Illumina's NovaSeq 6000 System SP2 2x150 flowcell at the Genomics and Microarray Core, University of Colorado - Anschutz Medical Campus. 16S rRNA gene assembly was performed on the Loop Genomics platform (https://www.loopgenomics.com) using the PacBio SMRT Link (Pacific Biosciences) (Schloss et al. 2016) software package to construct circular consensus sequence reads and demultiplex raw sequencing data. DADA2 R (Callahan et al. 2016, 2017) was used to remove primers, filter and trim consensus sequences before an error model was built and chimeras removed (Callahan et al. 2019). Resulting feature

tables, with full length 16S rRNA genes, were used to assign taxonomy to each of the 16S rRNA sequences obtained for each of the used strains. Because cyanobacterial cultures were not axenic, full 16S rRNA genes of the most abundant sequences from each strain were blasted using the NCBI database (Clark et al. 2016). After confirming their cyanobacterial identity, sequences were placed on Cydrasil version 2 to establish their phylogeny. Accession numbers for the isolate's 16S rRNA gene sequences can be found in Table S10.

Delineation of the naturally occuring Microcoleus steenstrupii complex clades

In order to delineate the different clades of the M. steenstrupii complex at a higher confidence than the one offered by a maximum-likelihood approach, we reconstructed its phylogeny using a Bayesian phylogenetic reconstruction method. This time, instead of using short 16S RNA sequences from biocrusts surveys (~ 300 bp), and its NCBI Blast, we used all 16S rRNA partial sequences (1100+ bp) of M. steenstrupii complex, including previously described ones, from the Cydrasil package (the package only contains sequences from an isolated strain according to NCBI or a single-cell genome according to JGI-IMG/M), for more information see: https://github.com/FGPLab/cydrasil (Roush et al. 2018), Additionally, short 16S rRNA sequences (~600 bp) from M. steesntrupii cultures were replaced by their full-length16S rRNA sequences, obtained for all 15 isolates as described above (see methods: Long read sequencing of the 16S rRNA and bioinformatic analysis). The two nearest neighbor non-M. steenstrupii complex clades in Cydrasil (Chroocosdiopsis and Lynbya) were included as well in the evaluation

to act as root on the phylogenetic reconstruction of the complex. The resulting 95 sequences were then aligned using SSU-Align 0.1.1 (Nawrocki 2009) with default parameters. Nucleotides aligned with low confidence (posterior probability) were masked using the ssu-mask option of SSU-Align with confidence values calculated from the alignment itself (default option). The resulting alignment was then used as input for a Bayesian phylogenetic reconstruction using BEAST 2.6.1 (Bouckaert et al. 2019). The parameters for the BEAST run were as follows: bModelTest (Bouckaert and Drummond 2017) (BMT) was used to average site models, the clock model was Strict with a rate of 1.0, and the priors used a Yule Model with default BMT options. The Markov chain Monte Carlo (MCMC) was constructed as a single chain and ran for 100 million iterations. The trace was sampled every 10,000 iterations and the tree every 20,000 iterations. The first 10% of samples were removed as burn-in and the posterior probability distributions and ESS values were assessed manually using Tracer 1.7.1 (Rambaut et al. 2018). Maximum clade credibility phylogenies were summarized using TreeAnnotator 2.6.2 (BEAST package), with a 10% burn-in and node heights set to mean height.

Morphological and physiological characterization of cultivated representatives' organisms

Biomass from each culture was observed under a light microscope, and all visible morphological traits (i.e. cell size and width, presence/absence of sheath, apical cells shape, etc.) were recorded and used in formal descriptions of the genera and type strains

delineated above. Temperature range for growth and survival of isolates was described by using visual assessment along with a photographic record over the growing time, and by calculating biomass yield from initial and final chlorophyll *a* (Chl a) concentrations of the cultures (Giraldo-Silva et al. 2020). 12 strains (HSN013, HSN023, SON57, FB15, HS024, HS003, HS035, HS041, SEV30, SON62, SON60, DW001 and PCC7113), were chosen to be further characterized. Prior to inoculation, liquid cultures of each strain were homogenized by repeatedly forcing biomass through a 60 mL sterile syringe, and immediately washed with fresh sterile JM medium by five consecutive centrifugations (8 min, 8437 g, 25 °C) (Giraldo-Silva et al. 2019). Aliquots of this homogenized cultures served as inoculum (5% v/v) for experimental cultures, which were run on 50 mL cell culture flasks filled to the 10 mL mark. Each strain was incubated at 0, 4, 10, 15, 25, 30, 35, 40 and 45 °C in triplicate, exposed to a light intensity of 20-27 μmol (photon) m^{-2} s^{-1} provided by white fluorescent tubes, in a 12 h photoperiod regime for 15 days. Growth was estimated visually, and by photographic record, and reported either as positive, if there was an obvious increase in biomass at the end of the incubation period compared to initial inoculum, or negative if either no-growth (stasis) or patent death was observed. Assays assigned to "no-growth" looked healthy, with brightly pigmented cells, but did not show appreciable biomass increase during the incubation, whereas assays assigned to patent death exhibited a total loss of pigmentation (Giraldo-Silva et al. 2020). The whole experiment was replicated a second time in full, and growth in any of the trials was reported as positive. During the replication of the experiment, 100 μL aliquots were taken

at the beginning (Day 0) and at the end (Day 15) of the experiment for Chl *a* determination.

Pigment identification

The same 12 strains selected for temperature growth experiments were used . Cultures grown on 50mL culture flaks, filled to the 15 mL mark were grown for ten days (JM medium) at 25 ± 2 °C, under a 14 h photoperiod, illuminated at 20-27 μmol (photon) m^{-2} s^{-1} provided by white fluorescent tubes, yielding enough biomass to extract pigments (approximately 1 mL of dense culture with bright blue-green coloration). Lipid-soluble pigments were extracted in 90% acetone. Extracts were initially analyzed spectrophotometrically between 330 nm to 730 nm, and a strong absorption peak at 665 nm was used as an indicator of chlorophyll a extraction. Acetone extracts were then concentrated by evaporation in a chemical hood and 50 μl were then analyzed by high pressure liquid chromatography (HPLC) on a Waters e2695 equipped with a Supelco Discovery HS F5-5 column connected to a Waters 2998 PDA UV-Vis diode array detector using previously described conditions (Karsten and Garcia-Pichel 1996, Malla and Sommer 2014) with the following revisions (Malla and Sommer 2014): flow rate was 1.0 mL /min-1 with linear gradients of 10 mM ammonium formate buffer (pH 3 adjusted with hydrochloric acid) and acetonitrile, (0-1.5 minutes 0-25% acetonitrile, 1.5-7.5 minutes 25-100% acetonitrile, 7.5-12.5 minutes 100-100% acetonitrile, 12.5-27.5 minutes 100-25% acetonitrile). Carotenoids, chlorophyll a, chlorophyll d and chlorophyll f were monitored in the chromatograms at 450 nm, 665 nm, 695 nm and 705 nm, respectively.

Spectra were recorded continuously between 200-800 nm using the PDA detector. Individual compounds were identified by their characteristic absorption maxima and appropriate retention time.

Chlorophyll a determination as a proxy for biomass

Chlorophyll *a* was measured as a proxy for phototrophic biomass. Chl *a* was extracted in triplicate, in 90% acetone, according to (Castle et al. 2011) with the following changes: cell suspensions were bead beated three times, with 30 s intervals after addition of acetone, and incubated for 24h at 4 °C in the dark. Extracts were clarified by centrifugation (5 m at 8437 g). Absorbance spectra of the clarified extracts was recorded on a UV-visible Spectrophotometer (Shimadzu UV-1601) and chlorophyll *a* determinations were calculated using the equations from (Ritchie 2008).

Meta-analyses of distribution along environmental gradients

In an attempt to look for temperature and precipitation segregation patterns among the studied clades of the *M. steenstrupii* complex in the natural biocrust environment, we performed a meta-analysis of all bacterial 16S rRNA tallies available publicly up until December 2019 (Giraldo-Silva et al. 2018). After a literature search, we either downloaded from public databases or directly requested raw sequence data from multiple environmental biocrust surveys conducted at different locations around the world. This included locations in the USA (Garcia-Pichel et al. 2013a, Couradeau et al. 2016, Velasco Ayuso et al. 2017, Fernandes et al. 2018, Bethany et al. 2019), Mexico (Becerra-Absalón et al. 2019) and Australia (Moreira-Grez et al. 2019), from arid, semiarid and

alpine regions in Europe (Williams et al. 2016, Muñoz-Martín et al. 2018), from the arid Gurbantunggut desert in China (Zhang et al. 2016a), and from the Brazilian savanna (Machado-de-Lima et al. 2019). A complete list of the biocrust surveys with locations, environmental variables, and other relevant information can be found in Table S11.

Forward reads obtained with pyrosequencing (Zhang et al. 2016a) and paired-end reads obtained with Illumina were demultiplexed, and quality controlled using the DADA2 plugin (Callahan et al. 2015) available in QIIME 2018.6 (Caporaso et al. 2010a), creating a feature table containing representative sequences (features) and their frequency of occurrence. Highly variable positions were removed using MAFFT (Katoh and Standley 2013), and phylogenetic trees were generated using FastTree (Price et al. 2010). Preliminary taxonomic assignment was done using the Naïve Bayes classifier (Xu 2016) trained on the Greengenes 13.8 release database (McDonald et al. 2012). For the (Garcia-Pichel et al. 2013a) dataset, because quality files (.fastq) were not available, to control for sequence quality before preforming downstream analysis, raw sequences were first filtered using USEARCH 7 (Edgar 2010) to remove those sequences >210 bp (up to 5% of the sequences), and by trimming first and last 10 bp of each sequence using Fastx (http://hannonlab.cshl.edu/fastx_toolkit/). Quality controlled sequences were assigned to individual samples and barcodes were removed using Qiime 1.8 (Caporaso et al. 2010a) , using the *multiple_split_librairies_fastq.py* script. Operational taxonomic units (OTUs) were defined with a threshold of 97% similarity and clustered using UCLUST (Edgar 2010), using the *pick_open_reference_otus.py* script in Qiime. Potential chimeras, and singleton OTUs were removed from further consideration. Preliminary taxonomic

assignments were done with the RDP (Ribosomal Database Project) classifier (Wang et al. 2007), and representative sequences were then aligned against the Greengenes database core reference alignment (McDonald et al. 2012).

Cyanobacterial sequences (features) and OTUs were filtered out from the master file, and a more refined taxonomic assignment at the genus and species level was further informed throughout phylogenetic placements. Query cyanobacterial sequences (and OTUs) were phylogenetically placed in our cyanobacteria reference tree CYDRASIL version-2.0 (https://github.com/FGPLab/cydrasil/tree), following instructions provided and visualized in the iTOL4 server (Letunic and Bork 2016).

We expressed relative abundance at the genus level with respect to total reads of non-heterocystous reads. We included only locations where the relative abundance of genera of interest was > 20% of all reads to avoid noisy ratios due to low abundance. In all, 66 locations were included in the analysis. Metadata on mean annual temperature (MAT) and mean annual precipitation (MAP) were calculated from environmental variables of monthly climate data for minimum, mean, and maximum temperature and precipitation from 1970-2000 obtained from WorldClim - Global Climate Data -version 2 (http://www.worldclim.org) (Fick and Hijmans 2017). Linear regressions between the logit proportion of sequence reads of each taxon (among non-heterocystous cyanobacteria) and climatic parameters (MAT and MAP) were used to test significance of environmental patterns.

Results

Meta-analyses of microbial diversity in the M. steesntrupii complex & Isolation and selection of cultured representatives

The visualization of the naturally occurring diversity of the *M. steenstrupii* complex in biocrusts communities (maximum likelihood tree reconstruction using 16S rRNA sequences (~600bp) from isolated strains, and from Illumina sequencing (~300bp)) showed that the complex is a monophyletic group composed of 18 distinct clades (Figure 14). Strains previously isolated in our lab (Garcia-Pichel et al. 2013a, Giraldo-Silva et al. 2018) covered five clades (green squares on Fig. 14). Surprisingly, evolutionary placements of these same sequences on Cydrasil showed that another five clades described elsewhere are part of the complex (black stars on Figure 14). After our isolation efforts, we could add cultured strains to three more clades (red circles in Fig.14). Therefore, further characterization based on morphological traits, as well as ecological data of the clades they represent was performed on isolates from eight of the 18 clades in the *Microcoleus steenstrupii* complex. Because in some cases, more than one isolate was found to belong to a given clade, a total of 15 cultures were selected to obtain their full 16S rRNA sequences. Each of the strains selected are marked in their specific clade in Figure 14 and strains information such as isolator, accession number in NCBI database and origin location can be found in Table S10.

Long-read sequencing of the 16S rRNA gene of cultured representatives

We succeeded in obtaining the full 16S rRNA of 15 strains in the *M. steenstrupii* complex (Figure 14). Strains' GenBank accession numbers can be found in Table S1. According to our analysis, fourteen out of the 15 isolates were confirmed to be unicyanobacterial (a single cyanobacterial individual present in the culture), and one culture (BN28) had a minor contamination (10% of cyanobacterial reads) from an unidentified cyanobacteria (90% similarity to an *Oscillatoria* sp. strain - NCBI database - GenBank) (Clark et al. 2016). Despite the contaminant, our organism of interest accounted for the overwhelming majority of the reads (90%) in this culture, allowing us to filter out from the feature table the sequence of the full cyanobacterial 16S rRNA gene of interest to use in the phylogenetic reconstruction of the complex. The strain, however, was not used in temperature experiments to avoid misleading results. There is a current effort ongoing in the laboratory to clean this strain from the contaminant.

16S rRNA gene sequences similarity within and between clades was done using the BLAST tool in NCBI (Baumann et al. 1990). Similarity between the 15 studied strains ranged from 100% similar to 91.40%, showing that these strains encompass not only more than one species, but possibly many genera, according to the bacteriological criteria which states that similarities of less than 95% define a new genus (Wayne et al. 1987, Stackebrandt and Goebel 1994, Margheri et al. 2008).

Delineation of the Microcoleus steenstrupii complex clades – family Phormidiaceae

The full 16S rRNA gene sequences (~1500 bp) sequences from the 15 isolates, along with sequences from strains previously described, and that according to our analysis of the natural diversity of the complex belong to it (section *Molecular view of the Microcoleus steenstrupii complex diversity in field biocrust communities* above), and two outgroups (a clade of *Chrocoocidiopsis* and another of *Lyngbya* strains) were selected to build a new tree calculated applying the Bayesian method (Figure 2). This tree was then used to delineate the genera in this complex of taxa. Based on the similarity within and between clades, the bootstrap values and the overall topology of the tree presented in Figure 2, we would like to propose the *Microcoleus steenstrupii* complex to be redefined as a family, composed of many genera of mostly terrestrial (with the exception of *Coleofasciculus chthonoplastes*, a marine cyanobacterium that can also be found in intertidal zones), desiccation resistant cyanobacteria.

According to our analysis (Figure 15), the previously described genera *Pycnacronema* (Martins et al. 2019a), *Potamolinea* (Martins and Branco 2016), *Wilmottia* (Strunecký et al. 2011) and *Porphyrosiphon* Kützing ex Gomont 1892 (Gomont 1892) are also part of the complex. Our investigation also proposes the inclusion of other five genera described here, *Sociatus*, *Parifilum*, *Xeronema*, *Crassifilum* and *Crustifilum* (Figure 15) in the complex. We then propose to include all the genera inside the *Microcoleus steenstrupii* complex into a new family, Porphyrosiphonaceae (after *Porphyrosiphon*, the oldest described genus inside the complex (Gomont 1892)). Our results also supported the inclusion of *Allocoleopsis* genus (Walter et al. 2017) in the

Porphyronsiphonaceae family. Walter et al. described *Allocoleopsis* based exclusively in the whole genome phylogeny of one strain (PCC7113). We here propose a redescription of the genus *Allocoleopsis*, including the addition of new strains along with morphological and ecological features not included in the initial description of the genus.

Additionally, after delineating the clades that our strains belonged and assigning new genera to each of them, we also selected type strains. Strains designated as type species for the newly described genera (5) were sent to the UTEX culture collection. Additionally, all other strains are kept in our local culture collection and are available upon request. Information on the isolator, location of isolation, NCBI accession numbers for 16S rRNA gene sequences (both short and long) are also in Table S10.

With our delineation we were able to successfully include all strains currently available in public databases (with 16S rRNA gene sequences of 1100bp or more) described as *Microcoleus steentrupii* in our analysis and to divide them into phylogenetic coherent taxa, concluding our first step of the polyphasic approach. The previously bewildering *Microcoleus steenstrupii* complex is now a coherent cyanobacteria family (Porphyrosiphonaceae) consisting of 11 genera of desiccation tolerant cyanobacteria (*Porphyrosiphon*, *Coleofasciculus*, *Pycnacronema*, *Potamolinea*, *Wilmottia*, *Allocoleopsis*, *Sociatus*, *Parifilum*, *Xeronema*, *Crassifilum* and *Crustifilum*).

From the genera that are newly described (*Sociatus*, *Parifilum*, *Xeronema*, *Crassifilum* and *Crustifilum*) or redefined (*Allocoleopsis*) here, *Crassifilum* is composed of three strains (three – SON60, SON62 and BN27), all cultured strains previously isolated from biocrusts from the Southwestern US (Garcia-Pichel et al. 2013a, Giraldo-

Silva et al. 2018). Strains 16S rRNA gene similarity within *Crassifilum* ranged from 100% and 98.6%. *Crustiflum* is composed of one single strain isolated from the Chihuahuan Desert (SEV30) (this study). *Xeronema* is composed of six strains from biocrusts from the Southwestern US (HS041, HS003, HS035, HS024 and FB15) (Giraldo-Silva et al. 2018) and one isolate from biocrusts in Spain (CAU8) (Roncero-Ramos et al. 2019). Strains from *Xeronema* share 98.4 to 98%% similarity of the 16S rRNA gene. *Sociatus* is composed of 4 strains, two from biocrusts from biocrusts in the Southwestern US (HSN013 and HSN023) (Giraldo-Silva et al. 2019). The two other strains are a *Porphyrosiphon* sp. strain (ACKU Y DB1) from a study in Japan (now deleted from public databases) and *Trichocoleus sociatus* SAG2692 (West & G.S West) Anagnostidis 2001 (Anagnostidis 2001) (previously *Microcoleus sociatus* West & S.G West (West and West 1897)). *Sociatus* might contain two different species since the two strains isolated in our laboratory share 99% similarity but are only 96.4% similar to the other two strains inside the genus. *Parifilum* contains one strain previously isolated from the Sonoran Desert (SON57) (Garcia-Pichel et al. 2013a) and a strain previously described as *Microcoleus paludosus,* SAG1449, but this is not the only strain from the previously named *M. paludosus* inside the complex. The two strains inside *Parifilum* are 96% similar and might be different species, but more strains need be isolated to be sure. The strain Cf. Wilmottia sp. CAWBG522, although close to the *Parifilum* genus is only 93% similar to the other two strains of this genus and therefore, was not included in the genus. *Allocoleopsis* was previously described containing only *Microcoleus* sp. PCC7113 strain (Walter et al. 2017), but this study added four other strains to this genus. One strain

was isolated by Giraldo-Silva et al. 2019 (DW001) while the other three were in the NCBI database (*Microcoleus* sp. SAG2212, *M. steenstrupii* ATA12 3 DP02 and *Microcoleus* sp. HHT-U-KK5). The five strains from the *Allocoleopsis* genus are 99.8 – 99.2% similar.

Two other clades of the Porphyrosiphonaceae family now have cultured strains obtained by our isolation efforts (CYAN3 and BN28). These clades can be seen in both our initial field diversity trees (Figure 1), as well as in our more robust Bayesian tree (Figure 15), but due to inability to grow enough biomass to complete a polyphasic study of the clade (CYAN3), or the low contamination of other cyanobacterial species (BN28), we decided not to describe new genera for these clades in this here, leaving their phylogeny and physiology to be studied.

Morphology and pigment characterization of isolated strains from different genera in the Porphyrosiphonaceae family.

Morphology and pigment characterization of 12 strains (PCC7113, DW001, SON62, SON60, HS041, HS003, HS024, HS035, HSN013, HSN013, SEV30 and SON57) was done as part of the polyphasic approach. Morphology is similar within strains from the same genera; however, there were clear differences between strains from different genera (Figure 16). Overall, trichome width ranged from 3 µm to 8 µm, with strains from *Sociatus* spp. (Figure 16, N-P) and *Xeronema* spp. (Figure 16, Q-R) being the thinnest and *Crassifilum* spp. (Figure 16, A-C) being the thickest. Constriction and apical cell shape are also very divergent within genera. *Sociatus* spp. trichomes are very constricted

at cross walls and display pointed apical cells (Figure 16, N-P), while *Parifilum* spp. has trichomes with cross walls constriction patent every two cells and exhibits rounded apical cells (Figure 16, K-M). *Allocoleopsis and Crustifilum* show slight constriction of cross walls and rounded apical cells, with trichome widths of 5 µm, which fits more the classical morphological description of *Microcoleus steenstrupii*.

Our intent to characterize water soluble pigments (HPLC) confirmed, as expected, the presence of chlorophyll *a* (strong peak at 665 nm) in all the strains. Additionally, none of the strains was shown to produce alternative chlorophylls, such as chlorophyll *d* and *f*, under the tested conditions.

Temperature range for growth (or survival) of isolated strains from different genera in the Porphyrosiphonaceae family.

Following phylogenetical and morphological characterization, we also wanted to describe physiological characteristics of the strains; to do so, we studied differential responses of the genera to a range of temperature (0°C to 45°C). All 12 cyanobacterial strains studied (PCC7113, DW001, SON62, SON60, HS041, HS003, HS024, HS035, HSN013, HSN013, SEV30 and SON57) showed robust growth at 15°C - 30°C, while none grew at 45°C (Figure 17), the lower limit for moderate thermophilly (Berenguer 2011). Formally then, all strains were mesophiles with respect to temperature. At 0°C, all the strains appeared to be in stasis (they neither grew nor showed signs of cellular degradation). At 4°C, only strains from the genera *Crustifilum* (SEV30) and *Parifilum* (SON57) grew well, as well as one (HSN023) from the *Sociatus* genus, while all the others appeared to

be in stasis. At 40°C, all strains from the genus *Crassifilum* (SON60 and SON62) grew. The strains from the genera *Allocoleopsis* (PCC7113 and DW001) and *Sociatus* (HSN013 and HSN023) also grew or appeared to be in stasis at 40°C. Survival at 40°C at different time points can be found in Figure S5.

Biomass yield measured after 15 days of growth showed that while the majority of the genera grow better at higher temperatures (35°C – Figure 18), *Sociatus* strains and *Parifilum sonorensis* grow better at lower temperatures (10°C – Figure 518. Log yield difference (right side boxes on Figure 5) between growth in the upper (35°C) and lower (10°C) range of temperature show positive red bars for strains that grew better at higher temperatures (all strains from *Xeronema*, *Allocoleopsis*, *Crassifilum* and *Crustifilum*) and negative blue bars for the opposite trend (*Sociatus* and *Parifilum* strains).

Reponses of different genera in the Porphyrosiphonaceae family to climate through meta-analyses of molecular surveys

A total of 109 locations from eleven different biocrust surveys conducted in different arid and semiarid regions in North and South America, Europe, Australia, and China, and in the Brazilian Savanna (see Table S11), were initially collected in a meta-analysis to assess the relative contribution of the different genera from the Porphyrosiphonaceae family along climatic gradients. After analysis, locations where the complex relative abundance was 20% or more of the total cyanobacteria abundance were filtered from all future analysis, resulting in 66 locations. Figure 6 shows the relative abundance of the Porphyrosiphonaceae family genera across different climatic regions and domains (as

divided by Robert G. Bailey – (Bailey 2014)). The different genera are sorted, from top to bottom, from the most commonly represented to the least (*Parifilum* – *Porphyrosiphon*). *Parifilum* is the most cosmopolitan genera of the Porphyrosiphonaceae family, present in 87% of the samples in the survey, but it is clearly more abundant in colder climates, such as Marine and Mediterranean Regions and Mountains (Figure 19). Tallies were also ordered by aridity gradient – as shown by the arrow on the left side of the graph. The genus *Crustifilum*, present in 68% of the locations, was clearly more abundant in Dry Domain samples. The genus *Pycnacronema*, present in 66% of the locations, was more abundant in hotter climates, independently of the aridity gradient, as is abundant in both the humid Savanna Division, as well as in the most arid and hotter deserts (bottom of the graph). The genus *Porphyrosiphon* was the lest common, present in only 7 (10%) of the locations, 6 samples from the Brazilian Savanna (Machado-de-Lima et al. 2019) and one sample from the dry domain (from Mexico) (Becerra-Absalón et al. 2019). It is also important to point out the high relative abundance of undefined clades, especially in the Tropical/Subtropical Desert Division, which consist of arid and semiarid deserts in North America.

We did a logistic regression, by logit (applying log-linear models to sigmoidal curves – see (Walker 2003) for more details) transforming the proportion of each genus of the Porphyrosiphonaceae family against the mean annual temperature (MAT) and mean annual precipitation (MAP) of origin. Our proportions were calculated using the relative abundance of each genus in each location studied and dividing it by the relative abundance of all the non-heterocystous cyanobacteria. Biocrust cyanobacteria can be

divided in two groups that occupy somewhat distinct niches: 1) non-heterocystous pioneer taxa such as Microcoleus vaginatus and the *Microcoleus steenstrupii* complex, that typically inhabit the soil a few hundred microns below the surface, migrating to the surface during pulses of precipitation and 2) nitrogen-fixing heterocystous cyanobacteria that are sessile and inhabit the very surface of biocrusts and do not migrate. Because of that, we only used non-heterocystous cyanobacteria to calculate our proportions.

We used the previous reported negative responses of *M. vaginatus* to increased temperature (Garcia-Pichel et al. 2013b), and decrease in precipitation (drought; (Fernandes et al. 2018)), to test if this approach was accurate in predicting patterns of differential abundance of genera according to climatic variables (MAT, MAP). As expected, our correlations showed that *Microcoleus vaginatus* was highly and significantly negatively correlated with both MAT ($R2 = 0.45$, $p < 0.0001$) and MAP ($R2 = 0.29$, $p < 0.0001$), which validated our approach (Figure S6). When looking at all the strains in the Porphyrosiphonaceae family as one unit, there was not significant correlation found with MAT or MAP (Figure S6). However, when we separated the Porphyrosiphonaceae family into their genera, significant patterns emerged (Figure 20). *Xeronema* responded positively to MAT ($R2 = 0.23$, $p < 0.001$) and negatively to MAP ($R2 = 0.15$, $p < 0.001$). *Crustifilum* and *Crassifilum* both responded negatively and significantly to MAP ($R2 = 0.15$, $p < 0.01$ and $R2 = 0.28$, $p = 0.05$, respectively) (Figure 20). *Parifilum* responded negatively and significantly to MAT ($R2 = 0.16$, $p < 0.01$). *Sociatus* and *Allocoleopsis* did not correlate with the climatic variables presented here, although if samples were filtered to only include arid and semiarid locations both genera

correlated positively and significantly with MAP ($R2 = 0.26$, $p < 0.01$ and $R2 = 0.37$, $p = 0.0001$, respectively) (Figure S8). All correlation graphs are shown in Figure S7.

Discussion

In this study, we present a new structure for the *Microcoleus steenstrupii* complex based on the use of the polyphasic approach on 15 strains isolated from biological soil crusts, where this taxon is of extreme importance for initial colonization (Garcia-Pichel and Wojciechowski 2009) of bare soil, soil stabilization and fertilization (Belnap and Gardner 1993, Zheng et al. 2010). We define a new family of cyanobacteria, Porphyrosiphonaceae, composed, to this date, by 11 genera: *Porphyrosiphon*, *Coleofasciculus*, *Pycnacronema*, *Potamolinea*, *Wilmottia*, *Allocoleopsis*, *Sociatus*, *Parifilum*, *Xeronema*, *Crassifilum* and *Crustifilum*.

From the eleven genera included in the Porphyrosiphonaceae family, five were previously described (*Porphyrosiphon*, *Coleofasciculus*, *Pycnacronema*, *Potamolinea* and *Wilmottia*), with *Coleofasciculus* being the only genus that is not commonly found in biocrusts. *Allocoleopsis* was previously described based solely on one strain and without any morphological, physiological or ecological information. We here propose a re-description of the genus *Allocoleopsis* as well as the inclusion of 4 strains to it (DW001, SAG2212, HHT-U-KK5 and ATA12-3-DP02 – Table S12). In addition, this study proposes five new genera of cyanobacteria that belong to the Porphyrosiphonaceae family (*Sociatus*, *Parifilum*, *Xeronema*, *Crassifilum* and *Crustifilum*).

Recollection of the taxonomy of the genus Microcoleus

The genus *Microcoleus* was initially described in the early taxonomical system of Cyanobacteria, using the botanical code and proposed by Gomont in 1982 (Gomont

1892). The type species of the genus was *Microcoleus vaginatus* (Vaucher) Gomont ex Gomont. Later, in the work of J.B Petersen, a new *Microcoleus* species was described as *Microcoleus steenstrupii*, differing from *Microcoleus vaginatus* by the absence of calyptra and because *M. steenstrupii* presented cross walls constrictions not seen in *M. vaginatus*, as well as other morphological differences (Petersen 1923). Many systems of higher-level classification of Cyanobacteria were proposed later, all mainly based on morphology and dividing Cyanobacteria in orders or sections. From the system proposed by Geitler in 1925 (Geitler 1925) until the division proposed by Rippka et al. in 1979 (Rippka et al. 1979) the *Microcoleus* were grouped in the *Oscillatoriales* subsection. This system became the primary basis for the nomenclatural classification in Bergey's Manual of Systematic Bacteriology, which recognized five subsections for the Cyanobacteria (Castenholz 2001). With the advent of electron microscopy and molecular and genetic methods for characterization of cyanobacteria, many proposals on how to divide and describe cyanobacteria were made simultaneously, with *Cyanobacteria* being continually revised for the last fifty years.

In 2002, Boyer et. al. studied the genus *Microcoleus* using 16S rRNA gene based phylogenetic distances and noticed that *Microcoleus steenstrupii* was found phylogenetically distant from the type species for the genus *Microcoleus* (*Microcoleus vaginatus*) and was most likely composed of many different species and genera (Boyer et al. 2002). Based on this information, the marine species of the *Microcoleus* genus was re-described as *Coleofasciculus chthonoplastes* (Siegesmund et al. 2008) and put in the Phormidiaceae family, separate from *Microcoleus vaginatus,* that remained in the

Oscillatoriaceae family. Here, Siegsmund suggested that *Microcoleus* species should be divided into these two families (Phormidiaceae and Oscillatoriaceae) but did not assign the genera that should be placed in each family. In 2013, a study by Strunecký et al. revised and re-described the genus *Microcoleus* and described a new family, the family Microcoleaceae, excluding the phylogenetically distant species with constricted cross walls, such as *M. paludosus* and *M. steenstrupii* (Strunecký et al. 2013) from it. However, no new genus or family was proposed for these species.

In 2014, Komárek et. al proposed a new system of classification of Cyanobacteria, advocating for evolutionary history, monophyletic groups and the use of the polyphasic approach (Komárek et al. 2014). The proposed classification system, however, does not resolve the classification of the *Microcoleus steenstrupii* complex. Komarék proposed a new family of cyanobacteria called Coleofasciculaceae, that includes the well supported and described groups inside the *Microcoleus steenstrupii* complex, such as *Coleofasciculus* Siegsmund et al. 2008 (Siegesmund et al. 2008) and *Wilmottia* Strunecký et al 2011 (Strunecký et al. 2011), but does not include the not studied *Microcoleus steenstrupii* strains, since they still needed to be redefined. Later, *Pycnacronema* M.D. Martins et Branco and *Potamolinea* Martins et Branco are described and the authors add these genera to the Coleofasciculeceae family due to their similarity with other genera from this family.

In our efforts to study the responses of biocrust cyanobacteria to environmental changes, we started a manually curated cyanobacterial tree, Cydrasil, that now is a tool available for all cyanobacteria researchers (https://github.com/FGPLab/cydrasil). In order

to achieve a better phylogenetic reconstruction reference sequences for Cydrasil need to come from an isolated strain and the 16S rRNA sequence needs to have at least 1,100bp. The majority of available *Microcoleus steenstrupii* sequences on the NCBI database when we started this study did not meet Cydrasil criteria, which made it impossible for us to place sequences from this group in Cydrasil. Therefore, we decided to look at the natural diversity of the *Microcoleus steenstrupii* complex by constructing a maximum likelihood tree with short Illumina sequences (Figure 14) and noticed the presence of several clades. In parallel we noticed that different clades of the *Microcoleus steenstrupii* complex had differential responses to water availability and climate change factors (Fernandes et al. 2018). After our isolation and long-read sequencing efforts we successfully cultured 6 clades of the *Microcoleus steenstrupii* complex clades (marked with either green squares or red circles in Figure 14). With that, we were able to increase the number of sequences from the *Microcoleus steenstrupii* complex in Cydrasil. Surprisingly, we found that another 5 previously described genera were part of a monophyletic group comprising the *Microcoleus steenstrupii* complex. We found that it included many genera described as belonging to the Coleofasciculaceae family (*Coleofasciculus*, *Wilmottia*, *Pycnacronema* and *Potamolinea*). We also noticed that it included all the currently available strains from the genus *Porphyrosiphon*. Before this study, however, no molecular data was available on databases for *Porphyrosiphon* strains and 16S rRNA gene phylogeny was not possible. In our analysis we included sequences from recently isolated *Porphyrosiphon* strains from biocrusts in Mexico (Becerra-Absalón et al. 2019) as well as data from strains isolated from Brazilian biocrusts (not

publicly available) and previously used to confirm the presence of *Porphyrosiphon* in Brazilian biocrusts (Machado-de-Lima et al. 2019). With that, we were able to resolve the phylogeny of *Porphyrosiphon* and place it inside the *Microcoleus steenstrupii* complex and not in the Microcoleaceae family, where this genus was placed in the latest taxonomical classification of cyanobacteria (Komárek et al. 2014).

To further increase our phylogenetic reconstruction we built a Bayesian tree with all the genera included in the *Microcoleus steenstrupii* complex based on Cydrasil (https://github.com/FGPLab/cydrasil) and got the first comprehensive view of the complex (Figure 2). Therefore, more than a decade after *Microcoleus steenstrupii* being called a supra-genetic entity in need of reevaluation, we were able to build a comprehensive phylogenetic tree in a first attempt to separate the taxa present within the *Microcoleus steenstrupii* complex, that we now propose to be named a new cyanobacterial family, Porphyrosiphonaceae.

The Porphyrosiphonaceae family

Here we present the current state of the art for the proposed Porphyrosiphonaceae family. The family is presently composed of 11 genera, five of which were previously described using both morphological and phylogenetic characteristics (*Porphyrosiphon*, *Coleofasciculus*, *Wilmottia*, *Potamolinea* and *Pycnacronema*) and were classified into the Coleofasciculaceae family. One other genus, *Allocoleopsis*, was recently described using solely phylogenetic information (Walter et al. 2017) and using a single strain (*Microcoleus steenstrupii* PCC 7113) isolated from greenhouse soil. After our studies on

the morphology and physiology of this taxa, in this study we re-describe the genus *Allocoleopsis*. We also described five new genera inside the Porphyrosiphonaceae family: *Sociatus* (previously *Microcoleus sociatus* and later *Trichocoleus sociatus*), *Parifilum*, *Xeronema, Crustifilum* and *Crassifilum*.

Additionally, it is also important to point out that some of our strains fall within other clades of the family (BN28 and CYAN3 – Figure 2), but this study was not able of study those in depth. Besides these two clades, evolutionary placements on our maximum likelihood tree made shorth sequences from field biocrusts showed that, from 18 clades present naturally present inside the Porphyrosiphoneceae, only 11 are delineated here, showing the necessity of continued studies of biocrust cyanobacteria with the goal to cover this unknown diversity. This study provides a framework that future studies can use to describe the other clades present inside the family (Figure 14).

When looking at the similarities of the 16S rRNA gene, all the previously described genera and the ones proposed on this study share more phylogenetic similarity within their clade than with others. Similarity from strains within a single genus varies from 100% to 97% (with the exception of *Parifilum* where the two strains are only 94.88% similar to each other). Similarity from strains between different genera varies from 96% to 91.1%. *Crustifilum* strain, SEV30 showed low similarity to other strains inside the complex (<96%) and that was one of the reasons that we decided to describe this genus even though it only had one cultured strain. The other reason was the high representativity of *Crustifilum* in biocrusts (Figure 19).

Morphologically, all the strains described in our study (and the majority of strains inside the Porphyrosiphonaceae family) have constricted cross walls and no calyptra. Trichomes width vary from 3 – 8 µm.

To our surprise, the overwhelming majority of the strains of the Porphyrosiphonaceae family belong to terrestrial cyanobacteria and can be present in biological soil crusts, as shown by our meta-analysis of biocrusts surveys (Figure 19). The only exception is the marine cyanobacterium *Coleofasciculus chthonoplastes*. Previous studies on biological soil crusts reported the presence of this cyanobacterium (Machado-de-Lima et al. 2019), but after our isolation and sequencing effort in this study, no more sequences from our meta-analysis were placed within the *Coleofasciculus* clade using the Cydrasil placement tool. Although this group is not composed of terrestrial cyanobacteria, *Colefasciculus chthonoplastes* can be found in microbial mats in intertidal zones (Garcia-Pichel et al. 1996) that are dried frequently. Therefore, the Porphyrosiphonaceae family is a group of taxa composed of terrestrial or partially terrestrial cyanobacteria, which is not a common occurrence within cyanobacterial clades.

Previous studies reported that *Microcoleus vaginatus* was negatively correlated to temperature, while *Microcoleus steenstrupii* (here treated as single species) was positively correlated to the same variable (Garcia-Pichel et al. 2013a). Our study shows that, when temperature was the only variable studied (strains grown in different temperatures in the laboratory), *Crassifilum*, *Allocoleopsis*, *Crustifilum* and *Xeronema* all responded positively to temperature (Figures 16 and 17), while *Parifilum* and *Sociatus* responded negatively. When responses to temperature were observed using our meta-

analysis comparing relative proportions of each genus across a global survey, the responses were not always consistent with our laboratory-controlled conditions. The only genera that responded similarity were *Crassifilum* and *Xeronema*, that responded positively to temperature, and *Parifilum* with a negative correlation. All the other clades did not correlate with mean annual temperature (MAT – Figure 20). Differences in genera responses between laboratory-controlled experiments and the global survey show that strains might respond differentially when one variable is isolated from all the others. Responses to temperature, such as those of *Allocoleopsis* and *Crustifilum*, that only responded positively in laboratory-controlled conditions, highlights that cyanobacterial responses to environment are complex and involve many factors. Genera that responded either positively (*Crassifilum* and *Xeronema*) or negatively (*Parifilum*) to temperature in both laboratory conditions and global surveys, shows that for some genera, temperature might be the main factor influencing their abundance in the environment.

Previous studies also showed that members of the *Microcoleus steenstrupii* complex responded differentially to precipitation [28, 60] and in this study, we found that *Crassifilum*, *Crustifilum* and *Xeronema* responded negatively to mean annual precipitation (MAP), but no clades responded positively. Although, when samples were filtered and correlations were made within arid and semiarid regions (excluding the Savanna Region and a few samples from the Marine Regime Mountains), *Allocoleopsis*, *Parifilum* and *Sociatus* all responded positively and significantly to MAP (Figure S8). Here again, we see that the responses of cyanobacteria to environmental factors are not simple and that their relative abundance might be different if looking at just one single

factor or a combination of two or more factors. A good example of this type of differential response can be found in climate change studies done with biocrust cyanobacteria. When looking at just increased rainfall variability, cyanobacteria biomass increases (Steven et al. 2013a, Ferrenberg et al. 2015), selecting for specific taxa such as *Scytonema* spp. (Fernandes et al., *in prep*). But together with warming, increased rainfall was detrimental to biocrust cyanobacteria (Steven et al. 2015).

With this study we demonstrated the importance of the polyphasic taxonomy approach, integrating data and information from ecology, morphology, phylogeny and physiology, providing clarification and a meaningful division of cyanobacterial groups. Continuous use of the new taxonomy of the *Microcoleus steenstrupii* complex should inform on each genus main contribution to biocrusts, much as we already know about the other biocrust cyanobacteria taxa, such as *Microcoleus vaginatus*.

Formal descriptions

Porphyrosiphonaceae fam. nov.

Description: Desiccation resistant, filamentous cyanobacteria, mostly terrestrial, but also found in intertidal zones. Filaments are solitary or entangled and forming mats. Filaments without branching, heterocysts, necridia or akinetes. Trichomes are cylindrical, uniseriate, iso- or heteropolar, slight or very constricted to no constricted, with thin colorless to thick colored sheaths; trichomes enveloped by a common sheath are frequently found in the family. Apical cells are attenuated, elongated and conical or elongated and pointed, or rounded with no calyptra. Trichomes and cultures are blue green in color. Cells ± isodiametrical or slightly shorter or longer than wide, with fasciculated to radial, sometimes irregular thylakoids arrangement. Cell content is homogeneous to finely granulated, sometimes with scattered larger granules. Reproduction by disintegration of trichomes into hormogonia is present in the family. Sunscreens pigments such as mycosporine-like amino acids (MAAs) are present in at least one genus of the family as well as accessory pigments as Phycoerythrin. Gliding motility is present.

This family comprises all the organisms before recognized as *Micrcocoleus steenstrupii* J.B. Petersen 1923, as well as the previously described and well supported genera *Coleofasciculus* Siegesmund et. al. 2008, *Wilmottia* Strunecký et. al 2011, *Potamolinea* Martins & Branco 2016, *Pycnacronema* Martins et. al. 2019, and *Porphyrosiphon* Kützing ex Gomont 1892. On the bases of our phylogenetic analysis, the family also include newly genera that are described in here: *Sociatus*, *Parifilum*,

Xeronema, *Crassifilum* and *Crustifilum*, along with the redescribed genus *Allocoleopsis* Walter et. al. 2017. There are likely many species (and/or genera) that will be described and added to this family when the diversity of available strains along with their morphological and ecological characterizations increase.

Newly described genera, and type species, within the Porphyrosiphonaceae family:

***Allocoleopsis* comb. nov.**

Allocoleopsis Allo.cole.o'psis. Gr. adj. allos, other; N.L. n. coleus from Greek koleon, sheath; Gr. suffix –opsis, looking like. *Allocoleopsis*, looking like another coleus.

Description: Terrestrial. Trichomes are cylindrical, 5 – 7 µm wide, iso- or heteropolar, constricted at the cross walls, sometimes attenuated towards the apex, with facultative, thin to thick, colorless, hyaline sheaths that can be open at the end and extend outside the filament; multiple trichomes enveloped by a single sheath are common. Apical cells are conical and elongated or rounded. Cells are elongated to quadratic and granulated.

The genus currently has two strains that are 99.8 – 99.2% similar to each other and 96.5 – 92% similar to other genera in the family.

Type species: *Allocoleopsis franciscana* comb. nov.

***Allocoleopsis franciscana* comb. nov.**

Figure 15, D-J

Allocoleopsis franciscana fran.cis.ca.na L.suffix *-an* native of, relating to. *Franciscana*, referring to the isolation area, San Francisco.

Description: Solitary filaments or bundled into rope like structures. Trichomes are heteropolar, 5 - 6 µm wide, slightly constricted at cross walls (visible under light microscopy at 100x), with thin or diffluent and colorless sheaths that do not open at the end. Trichomes are olive green in culture. Cells are elongated to quadratic, 6 – 7.3 µm long and 5 – 5.8 µm wide. Apical cells are rounded to conical-rounded, not tapered. Terminal cells are rounded.

Mesophilic, grows at 10 °C – 35 °C and its sensitive to temperatures below 10°C. Growth is optimal at 35°C. Commonly found in biological soil crusts. Type strain is PCC7113, isolated from a greenhouse in San Francisco, CA, USA. Deposited in the Paris Culture Collection (PCC), Paris, France.

Synonyms *Allocoleopsis franciscanus* Walter JM et al.

***Sociatus* gen. nov.**

Sociatus So.ci.a'.tus. M.L.m. n., from L. a. perf. Infin. *sociatus*, united or associated.

Description: Terrestrial. Filaments are solitary or entangled. Trichomes are cylindrical, around or slightly less than 4 µm wide, very constricted, with thin, colorless sheaths that open at the end and extend outside the filament. Multiple trichomes

enveloped by a single sheath are common Apical cells are pointed, longer than wide, not tapered. Cells are quadratic and granulated.

The genus currently has two strains that are 99.1 – 97.6% similar to each other and 94.5 – 92.4% similar to other genera in the family.

Type species: *Sociatus tenuis* sp. nov.

***Sociatus tenuis* sp. nov.**

Figure 15, N-P

Sociatus tenuis te'.nu.is L.adj. *tenuis* slender.

Description: Solitary filaments or bundled into rope like structures. Trichomes are heteropolar, highly constricted at cross walls (visible under light microscopy at 400x and 1000x), with thin or diffluent and colorless sheaths that open at the end and extend outside the filament. Cells are quadratic to shorter than wide, 2.5 – 4 µm long, and finely granulated. Apical cells are pointed and elongated, not tapered.

Mesophilic, grows at 4°C – 35°C and its sensitive to temperatures below 4°C. Growth is optimal at 30°C. Commonly found in biological soil crusts. Type strain is HSN023, isolated from biological soil crusts from the Great Basin Desert, USA. Geographic location: 41°1' N, 113°0' W. Deposited in the University of Texas at Austin Culture Collection of Algae (UTEX). 16S rRNA NCBI accession number: MT667357.

Synonyms *Microcoleus sociatus* West & S.G West, *Trichocoleus sociatus* (West & G.S West) Anagnostidis

Description of *Crassifilum* gen. nov.

Crassifilum Cra.ssi.fi'.lum. L. *crassus*, thick or stout. *Crassifilum*, thick filament.

Description: Terrestrial. Solitary filaments or bundled into rope like structures. Trichomes are slightly constricted (visible under light microscopy at 1000x), cylindrical, iso- or heteropolar, 7 - 8.5 µm wide. Sheaths facultative, thin, colorless, hyaline and sometimes open at the end and extend outside the filament. Apical cells are rounded, sometimes elongated. Cells are quadratic to shorter.

The genus currently has three strains that are 100 – 98.6% similar to each other and 96.5 – 91.2% similar to other genera in the family.

Type species: *Crassifilum sonorensis* sp. nov.

***Crassifilum sonorensis* sp. nov.**

Figure 15, A-C

Crassifilum sonorensis so.no.re'n.sis L.denonymic a., originating from Sonoran Desert.

Description: Filaments are solitary or entangled. Trichomes are slightly constricted at cross walls (visible under light microscopy at 1000x), with thin, colorless sheaths, sometimes sheaths open at the end and extend outside the filament. Mostly one trichome per sheath, sometimes two. Cells are quadratic to shorter than wide, 6 – 7.5 µm long and 7.5 – 8.5 µm wide. Apical cells are rounded, not tapered, sometimes elongated.

Mesophilic, grows at 10°C – 40°C and its sensitive to temperatures below 10°C. Growth is optimal at 35°C. Type strain is SON62, isolated from biological soil crusts from the Sonoran Desert, USA. Geographic location: 32°8' N, 113°7' W. Deposited in

the University of Texas at Austin Culture Collection of Algae (UTEX). 16S rRNA NCBI accession number: MT664816.

***Xeronema* gen. nov.**

Xeronema Xe.ro.ne'ma. Gr.a. *xeros* dry; Gr.n.n. *nema* filament. *Xeronema* dry filament.

Description: Terrestrial. Filaments solitary or entangled. Trichomes are thin, cylindrical, around or slightly less than 4 µm wide, constricted to not constricted at cross walls. Sheaths facultative, thin to thick, colorless, hyaline, sometimes open at the end and extend outside the filament. Multiple trichomes enveloped by a single sheath are common, sometimes twist simulating a knot or a spiral. Apical cells are conical, but sometimes pointed, no calyptra. Cells are quadratic or slightly shorter or longer than wide, and finely granulated.

The genus currently has five strains that are 100 – 98% similar to each other and 96.5 – 91.4% similar to other genera in the family.

Type species: *Xeronema commune* sp. nov.

***Xeronema commune* sp. nov.**

Figure 15, Q-R

Xeronema commune com.mu'.ne L.n.a. *commune* common, universal.

Description: Filaments are solitary or form bundles. Trichomes are not constricted at cross walls. Filaments with thin or diffluent and colorless sheaths sometimes open at the end and extend outside the filament. Cells are quadratic to shorter

than wide, 2.8-3.8 µm long. Apical cells are pointed or conical, sometimes with bulge on either side, sometimes tapered.

Mesophilic, grows at 10°C – 35°C and is sensitive to temperatures below 10°C. Growth is optimal at 30°C. Type strain is HS024, isolated from biological soil crusts from the Great Basin Desert, USA. Geographic location: 41°1’ N, 113°0’ W. Deposited in the University of Texas at Austin Culture Collection of Algae (UTEX). 16S rRNA NCBI accession number: MT664815.

Description of *Crustifilum* gen. nov.

Crustifilum Crus.ti.fi’.lum. L.f.n. *crusta* crust. L.n.n. *fil*um, thread. *Crustifilum* thread of crust.

Description: Terrestrial. Filaments are solitary or tangled. Trichomes are cylindrical, around or slightly less than 5 µm wide, are slightly constricted at cross walls with facultative, thin, colorless, hyaline sheaths. Multiple trichomes enveloped by a single sheath are common. Apical cells are rounded, but sometimes can be conical. Cells are quadratic or longer than wide, granulated, and cell wall is well defined.

There’s currently only one strain genus, but the similarity with other genera in the Porphyrosiphonaceae family is low (96 – 92%) and they are very abundant in biological soil crusts, making it an important addition to the ecology of the ecosystem.

Type species: *Crustifilum hispaluculae* sp. nov.

***Crustifilum hispaluculae* sp. nov.**

Figure 15, S-U

Crustifilum hispaluculae his.pa.lu.cu.lae. L. n. *Hispalis,* the city of Seville; L. diminutive suffix, *-cula;* N.L. f.n., *hispaliculae,* of the small Seville, from the location of Sevilleta, New Mexico.

Description: Filaments are solitary or form bundles. Trichomes are slightly constricted at cross walls (visible under light microscopy at 1000x). Apical cells are elongated and conical or elongated and rounded, sometimes paler that the rest of the trichome. Cells are quadratic or longer than wider, 5 – 7 µm long, granulated with well-defined cell walls.

Mesophilic, grows at 4°C – 35°C and its sensitive to temperatures below 4°C. Growth is optimal at 25°C. Type strain is SEV30, isolated from biological soil crusts from the Chihuahuan Desert, USA. Geographic location: 34°3’ N, 106°7’ W. Deposited in the University of Texas at Austin Culture Collection of Algae (UTEX). 16S rRNA NCBI accession number: MT664819.

Description of *Parifilum* gen. nov.

Parifilum Pa.ri.fi.lum. L. n. n. par *doublet, set of two*; L. n. n *filum*, thread. *Parifilum,* thread made of doublets.

Description: Terrestrial. Filaments are solitary or bundled into rope like structures. Trichomes are cylindrical, around or slightly less than 5 µm wide, slightly constricted at cross walls. Sheaths facultative, thin, colorless, hyaline. Multiple trichomes

enveloped by a single sheath are common, sometimes twist simulating a knot or a spiral. Apical cells are always rounded. Cells are quadratic to longer than wider.

The genus currently has two strains that are 95% similar to each other and 94.5 – 91% similar to other genera in the family.

Type species: *Parifilum solicrustae* sp. nov.

Description of *Parifilum solicrustae* sp. nov.

Figure 15, K-M

Parifilum solicrustae L. n. n. *solum,* soil.; L. f. n. *crusta,* crust; N. L, solicrustae, of the soil crust.

Description: Filaments are solitary or entangled. Trichomes are slightly constricted at cross walls (visible under light microscopy at 1000x) and more constricted every two cells with facultative, thin or diffluent and colorless sheaths that do not open at the end. Cells are quadratic to elongated, 5 – 7 µm long. Apical cells are always rounded.

Mesophilic, grows at 10°C – 35°C and it's sensitive to temperatures below 10°C. Growth is optimal at 15°C. Type strain is SON57, isolated from biological soil crusts from the Sonoran Desert, USA. Geographic location: 32°8' N, 113°7' W. Deposited in the University of Texas at Austin Culture Collection of Algae (UTEX). 16S rRNA NCBI accession number: MT664820.

Figures

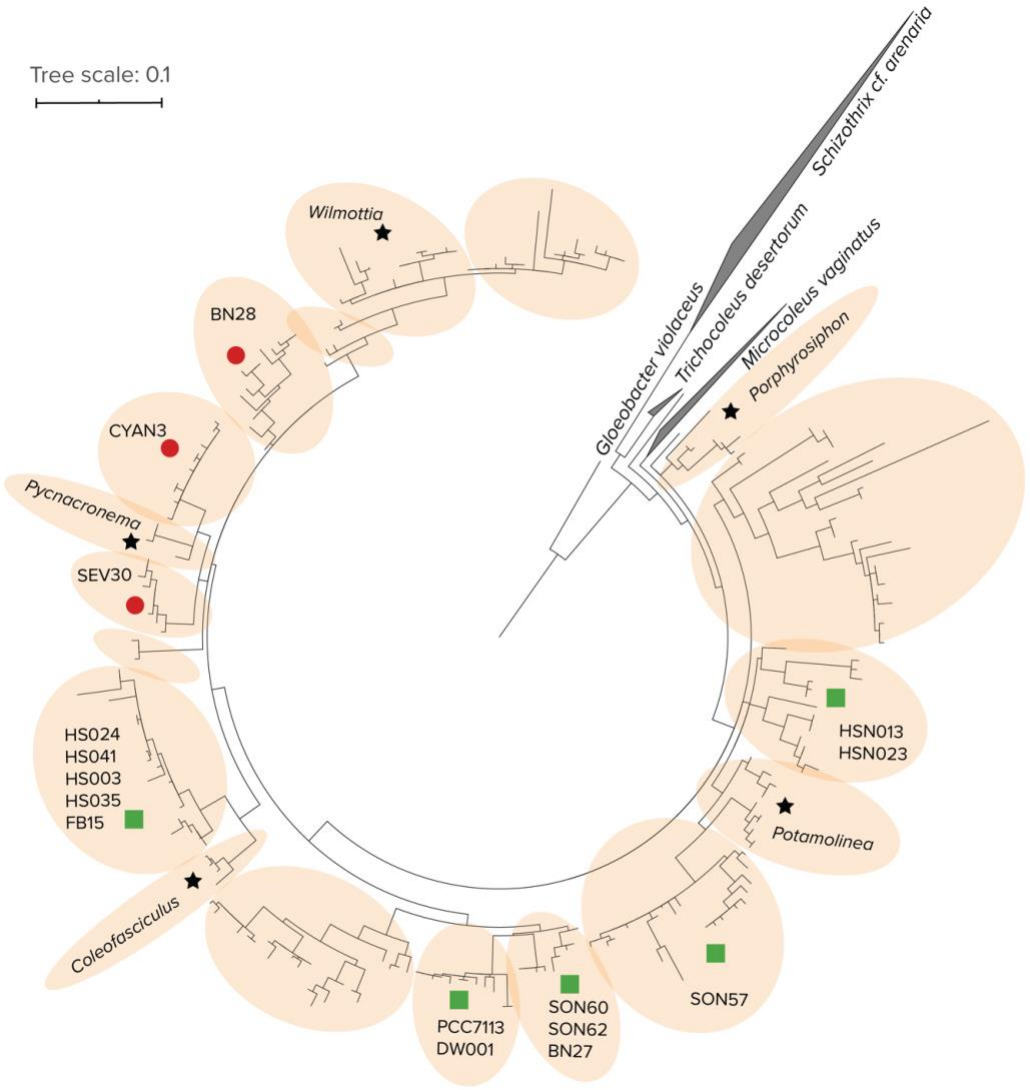

Figure 14. Maximum likelihood tree reconstruction of the natural occurring diversity of the *Microcoleus steenstrupii* complex based on short sequences (~ 300bp) of the 16S rRNA gene showing the diversity of clades present inside this group as seen in field studies. The tree was constructed using sequences assigned (using NCBI blast) to *Microcoleus steenstrupii*, *M. paludosus* or *Microcoleus* sp. (96% -100% identity) from Illumina MiSeq sequencing of biocrusts. *Microcoleus vaginatus*, *Trichocoleus desertorum* and *Schizothrix cf. arenaria* sequences were used as the outgroup. Bar equals 10% sequence divergence. Stars indicate clades from previously described genera (black star). Green squares indicate clades with cultivated isolates (strains ID inside each clade) from previous studies in our lab and described in this study. Red circles indicate isolated strains from this study (strains ID inside each clade).

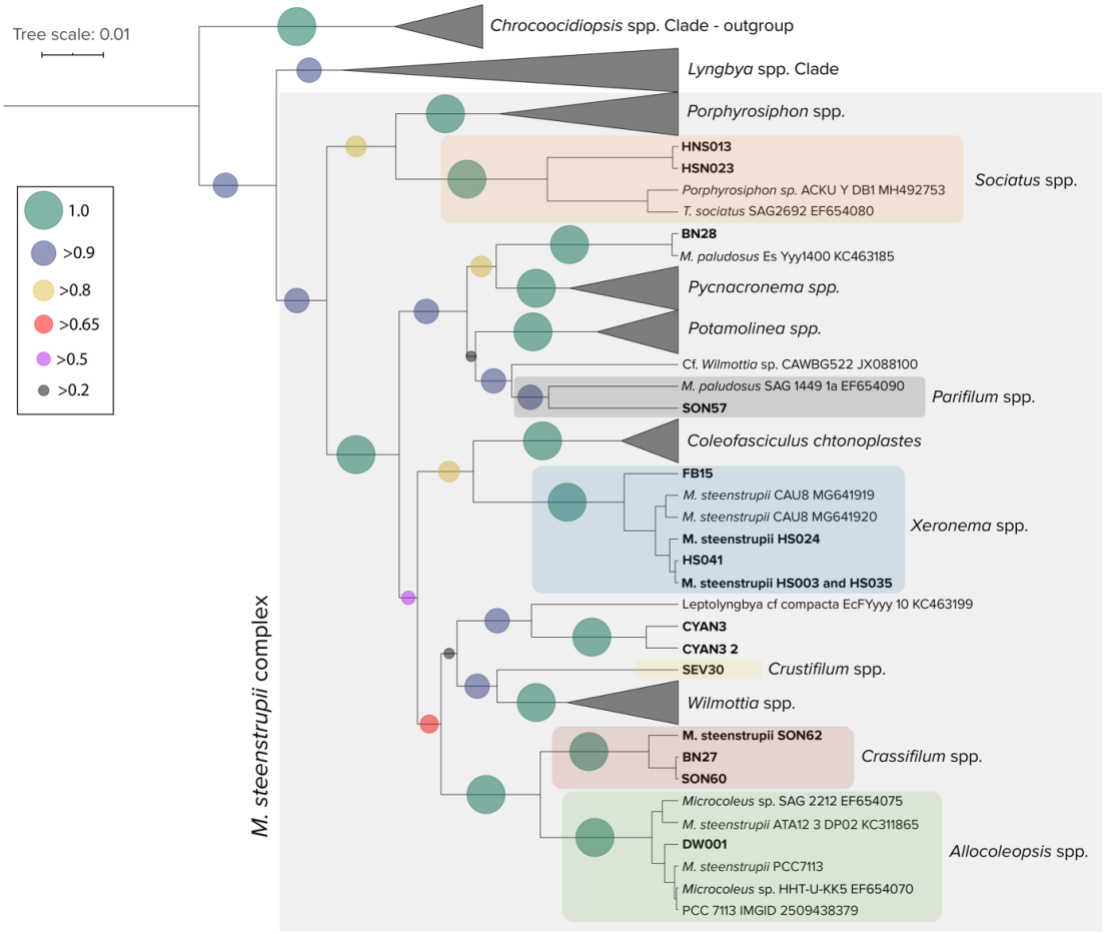

Figure 15. Bayesian phylogenetic reconstruction of the *Microcoleus steenstrupii* complex based on 16S rRNA gene sequences showing the affiliations of the 16S rRNA gene sequences obtained from the 15 investigated strains (in bold). The tree was constructed using all available 16S rRNA cyanobacterial complete sequences present currently in the complex (delineation of the complex done using a tree with all available 16S rRNA gene sequences from cyanobacteria – Cydrasil). The 16S rRNA gene sequences from clades closely related to the complex, but clearly non-members (*Chroococidipsis* spp. and *Lyngbya* spp.) were used as the outgroup. Bar equals 10% sequence divergence. Denomination of particular strains corresponds to those in the database and with no implication they are taxonomically correct. Bootstrap values from 1,000 trees are shown as circles and the legend is on the left.

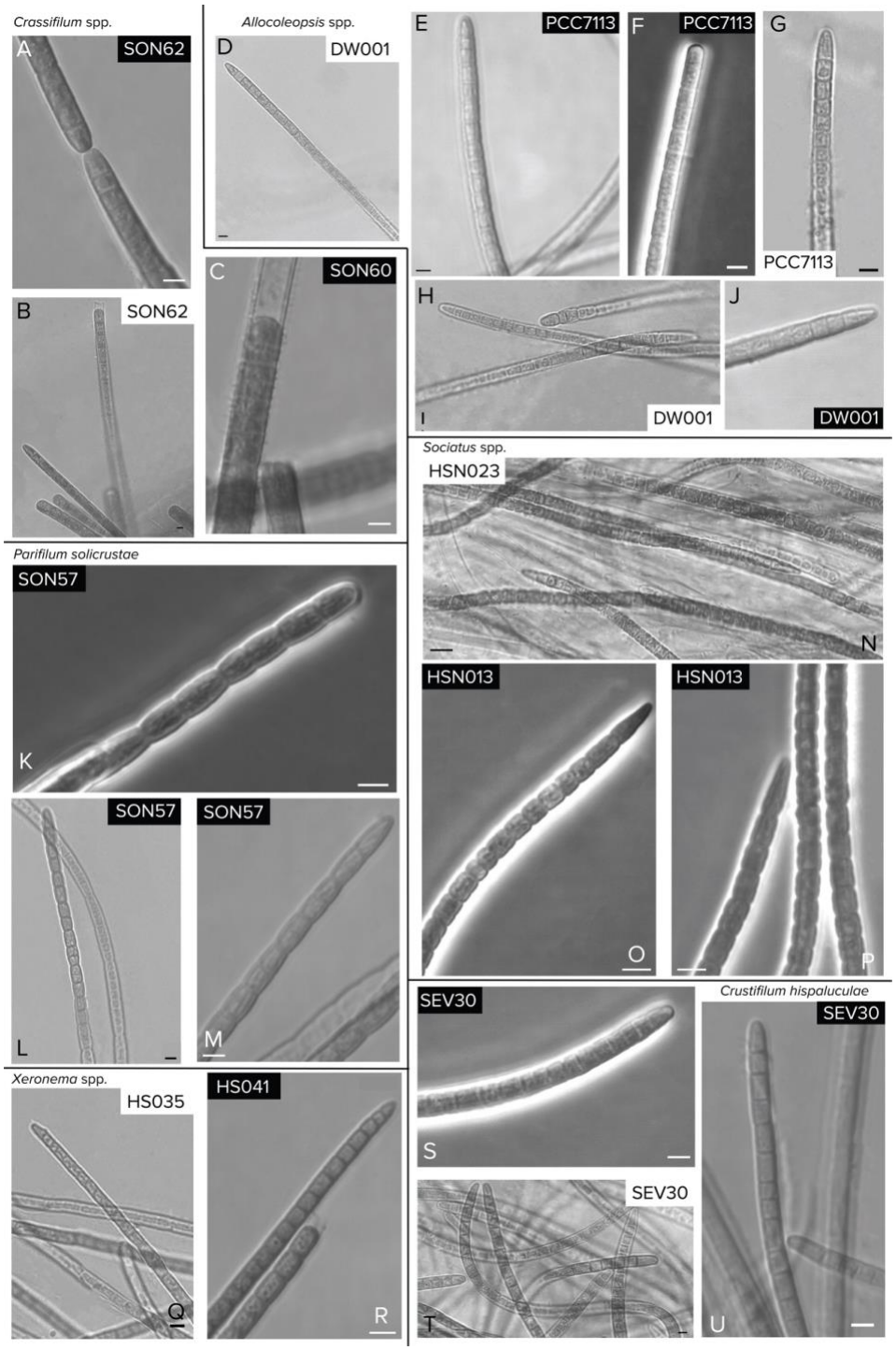

Figure 16. Photomicrographs of the filamentous, non-heterocystous cyanobacterial strains investigated grown in JM medium at 25°C. Strain denominations are indicated in each panel. Bar 5 µm.

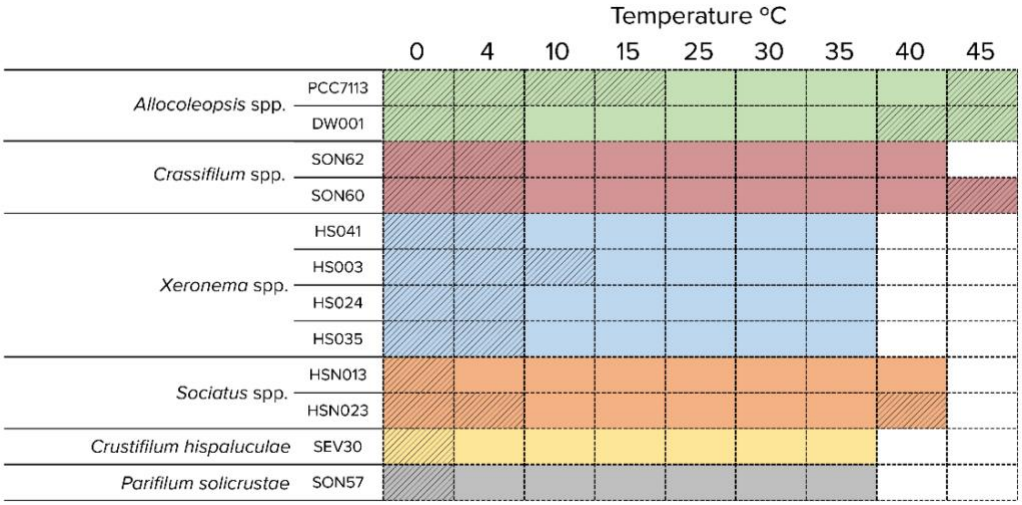

Figure 17. Temperature range at which the studied cyanobacterial strains can grow or survive. Colored rectangles indicate positive growth; hatched rectangles indicate stasis

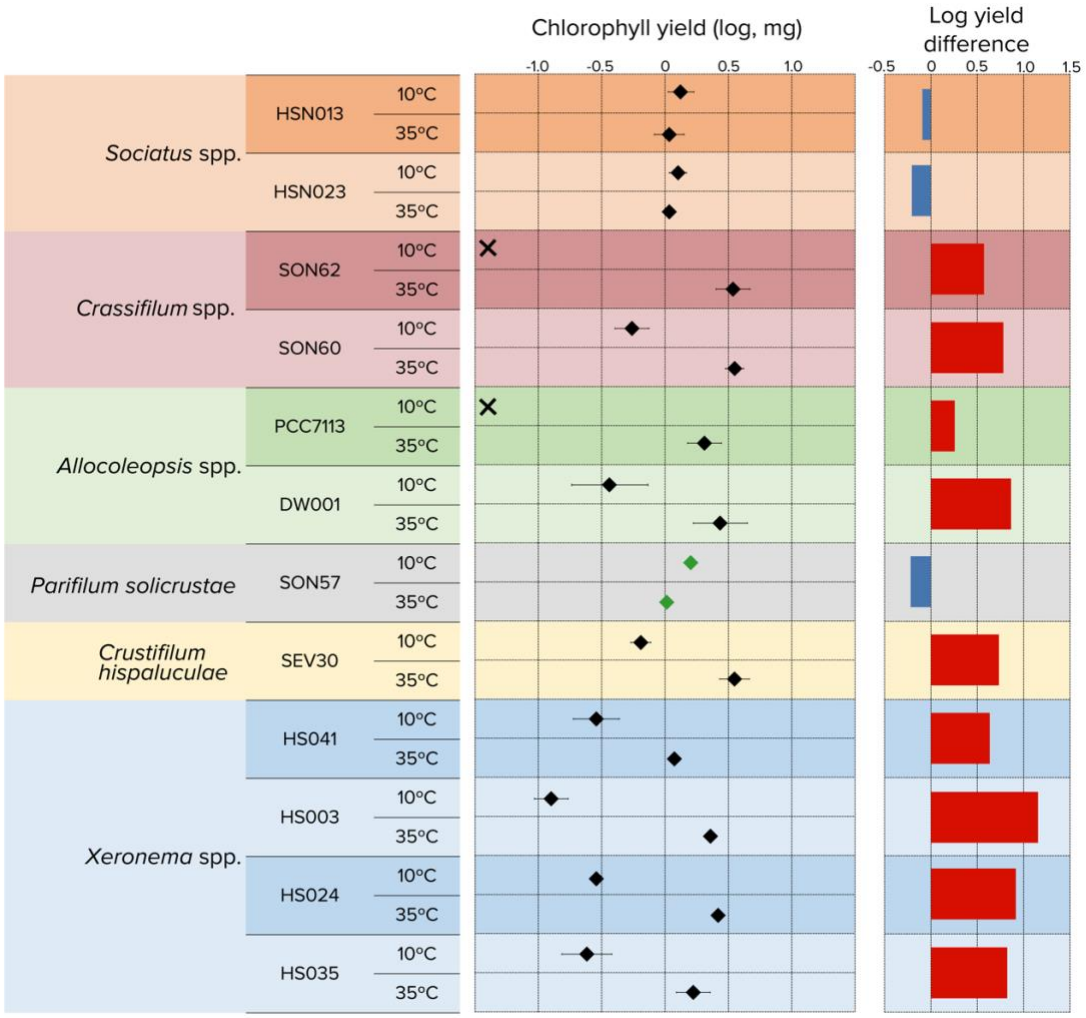

Figure 18. Growth yield of strains in the Porphyronsiphonaceae family (divided by genus) in the upper and lower ranges of temperature. Error bars indicate ± 1 SE, with n=5. Log yield difference on the left shows if strains grew better at higher (positive, red bars) or lower (negative, blue bars) temperatures, indicating that Porphyrosiphonaceae family genera have differential temperature preferences.

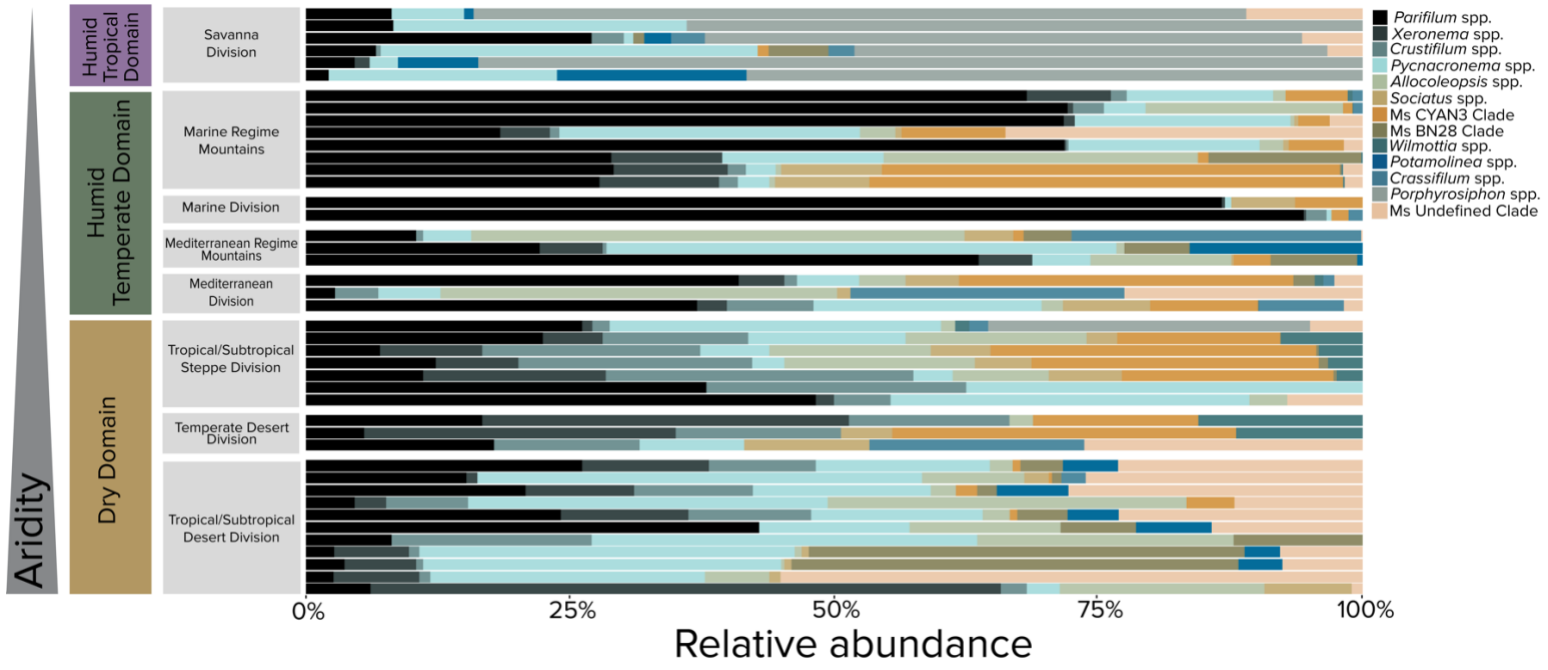

Figure 19. Shifts in community composition along a climatic gradient. Relative distribution of genera from the Porphyronsiphonaceae family defined by evolutionary placements done using an evolutionary placement tool (epa-ng) on Cydrasil. Some patterns are clear as some genera increases or decrease with aridity.

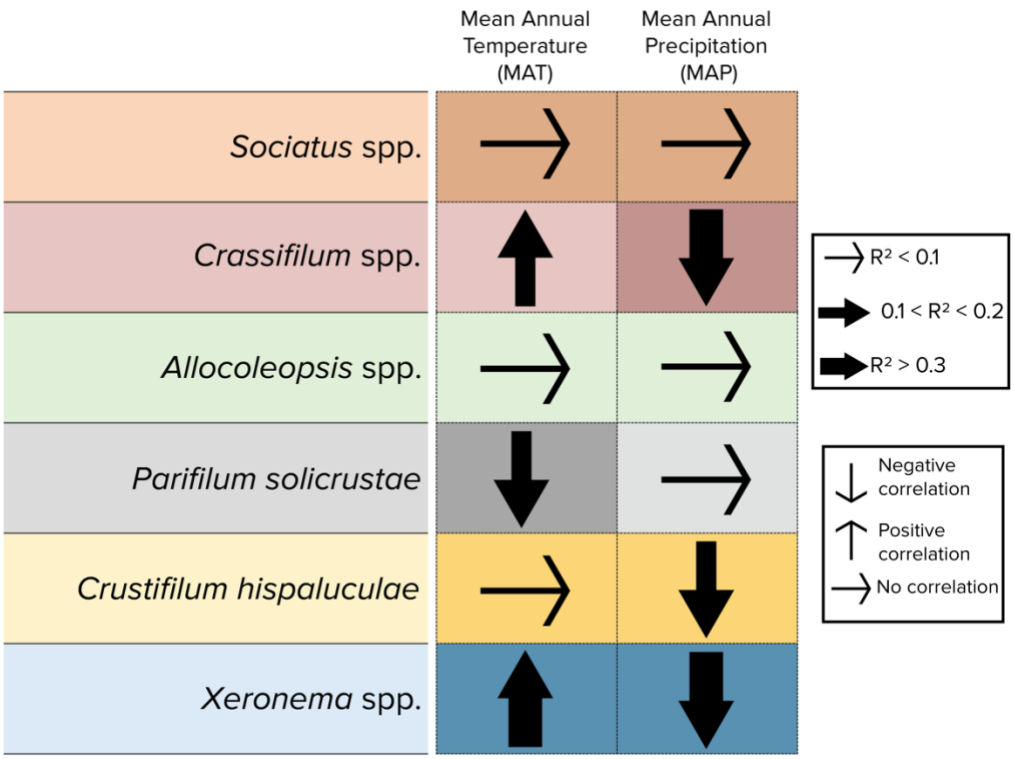

Figure 20. Correlations between the logit transformed relative proportion of Porphyronsiphonaceae family genera and two climatic factors: mean annual temperature (MAT) and mean annual precipitation (MAP). Arrows represent correlation direction (as show in the bottom right). Arrows' size represent R_2 values for the regressions of correlations (top right legend). Significant regressions are represented by darkened boxes. Correlations are shown in Figures S2 and S3.

Supplementary Information

Supplementary Tables

Table S10. Cyanobacterial cultures used in the delineation of the Porphyrosiphoneceae family. All the described genera within the family along with corresponding investigated strains are listed. ***** Indicates species type for a given genus. Isolation origin as well as isolator are given for each strain. 16Sr RNA sanger sequencing (~ 600 pb) accession numbers correspond to a NCBI submission associated to a given strain before this study. 16Sr RNA long read sequencing (1500 bp) accession numbers correspond to sequencing effort from this study in order to obtain the complete 16Sr RNA sequence for each strain. Note: the complete 16Sr RNA sequence for PCC7113 was available in (Shih et al. 2013).

Cyanobacterial taxon	**Strain**			**16Sr RNA Sanger Sequencing (~ 600 bp)**		**16Sr RNA Long Read Sequencing (1500 bp; Loop Genomics technology)**	
	ID	**Origin**	**Isolator**	**Accession number**	**Reference**	**Accession number**	**Reference**
***Allocoleopsis* spp.**	PCC7113*****	Orchid house in California	Cheryl A. Kerfeld	IMGID 2509438379	N/A	IMGID 2509438379	(Shih et al. 2013)
	DW001	Great Basin Desert	Ana Giraldo-Silva	MK487683	(Giraldo-Silva et al. 2019)	MT664811	This study
***Crassifilum* spp.**	SON62*****	Sonoran Desert	Pilar Mateo	KC999630	(Garcia-Pichel et al. 2013a)	MT664816	This study
	SON60	Sonoran Desert	Pilar Mateo	KC999632	Garcia-Pichel et al. 2013)	MT664821	This study
	BN27	Sonoran Desert	Ana Giraldo-Silva	N/A	N/A	MT664818	This study

***Parifilum* spp.**	SON57*****	Sonoran Desert	Pilar Mateo	KC999639	(Garcia-Pichel et al. 2013a)	MT664820	This study
***Xeronema* spp.**	HS024*****	Great Basin Desert	Ana Giraldo-Silva	N/A	N/A	MT664815	This study
	HS041	Great Basin Desert	Ana Giraldo-Silva	N/A	N/A	MT667354	This study
	HS003	Great Basin Desert	Ana Giraldo-Silva	MK487679	Giraldo-Silva et al. 2019)	MT664813	This study
	HS035	Great Basin Desert	Ana Giraldo-Silva	MK487680	Giraldo-Silva et al. 2019)	MT664814	This study
	FB15	Chihuahuan Desert	Ana Giraldo-Silva	MK487675	Giraldo-Silva et al. 2019)	MT664812	This study
***Crustifilum* spp.**	SEV30*****	Chihuahuan Desert	Vanessa Fernandes	N/A	N/A	MT664819	This study
***Sociatus* spp.**	HSN023*****	Great Basin Desert	Ana Giraldo-Silva	MK487625	Giraldo-Silva et al. 2019)	MT667357	This study
	HSN013	Great Basin Desert	Ana Giraldo-Silva	MK487623	Giraldo-Silva et al. 2019)	MT667355	This study
CYAN3 Clade – not described	CYAN3	Phoenix Metropolitan Area	Corey Nelson	N/A	N/A	MT667356	This study
BN28 Clade – not described	BN28	Sonoran Desert	Ana Giraldo-Silva	N/A	N/A	MT664817	This study

Table S11. Environmental biocrust surveys conducted at different locations around the world used in the meta-analysis and the corresponding climate data. Raw sequences were downloaded from bacterial 16S rRNA tallies available publicly (see references). Environmental data was downloaded from WorldClim. "MAT" is mean annual temperature and "MAP" is mean annual precipitation.

Original location Descriptor	Latitude	Longitude	MAT	MAP	Sequencing Platform	Reference (s)
Murcia, Carrascoy (Dark)	37.8	-1.3	16.6	320	Illumina	(Muñoz-Martín et al. 2018)
Murcia, Carrascoy (Light)	37.8	-1.3	16.6	320		
Albacete, Barrax Barrax (Light)	39.05	-2.23	14.2	430		
Albacete, Barrax Barrax (Dark)	39.05	-2.23	14.2	430		
Madrid, Campo Real (Light)	4.32	-3.43	14.3	424		
Madrid, Campo Real (Dark)	4.32	-3.43	14.3	424		
Almeria, Amoladeras (Dark)	36.83	-2.26	17.8	213		
Almeria, Amoladeras (Light)	36.83	-2.26	17.8	213		
Navarra, Bardenas Reales (Light)	42.17	-1.44	14.3	463		
Navarra, Bardenas Reales (Light)	42.17	-1.44	14.3	463		
Alicante, Relleu (Dark)	38.54	-0.3	16.4	347		
Guadalajara, Zorita (Light)	40.36	-2.88	14.0	418		
Guadalajara, Zorita (Dark)	40.36	-2.88	14.0	418		
Cuenca, Huelves (Light)	40.08	-2.9	13.2	460		
Cuenca, Huelves (Dark)	40.08	-2.9	13.2	460		
Huesca, Monegros (Dark)	41.91	-0.25	14.3	472		
Huesca, Monegros (Dark)	41.91	-0.25	14.3	472		
Huesca, Monegros (Light)	41.91	-0.25	14.3	472		
Madrid, Morata (Light)	40.21	-3.43	14.5	410		

Madrid, Campo Real (Dark)	4.32	-3.43	14.3	424		
Alicante, Relleu (light)	38.54	-0.3	16.4	347		
Murcia, Carrascoy (Dark)	37.8	-1.3	16.6	320		
Murcia, Carrascoy (Light)	37.8	-1.3	16.6	320		
Albacete, Barrax Barrax (Light)	39.05	-2.23	14.2	430		
site17-Chihuahuan-WilcoxPlya	32.1	-109.9	16.5	331	454 Pyrosequencing	(Garcia-Pichel et al. 2013a)
site15-Sonoran-Chandler	33.3	-113.7	22.4	172		
site19-Mojave-CactusPln	34.1	-114.2	22.6	136		
site16-Sonoran-Dateland	32.8	-113.7	22.9	120		
site20-Mojave-SearlesLk	35.6	-117.4	19.7	84		
site13-Chihuahuan-FivePts	34.3	-106.8	13.2	240		
site22-Mojave-SodaLk	35	-111.8	8.6	591		
site11-NorthernGreatBasin-WhiteFlt	41.9	-118.9	9.5	198		
site18-Chihuahuan-Jornada	32.5	-106.7	15.2	273		
site14-Chihuahuan-SevilletaGyps	34.2	-106.8	13.4	246		
site10-NorthernGreatBasin-AlbertLk	42.1	-119.6	7.2	311		
site21-Mojave-SodaLk	35.3	-116	21.2	87		
site5-ColoradoPlateau-Canyonlands	38.2	-109.7	12.2	244		
site3-ColoradoPlateau-GreenButte	38.7	-109.7	12.4	224		
site1-SonoranBatesW	32.2	-112.9	21.9	187		
site4-ColoradoPlateau-SundayChurt	38.6	109.6	7.7	378		
site2-ColoradoPlateau-SlickRock	38.6	-109.5	12.3	252		
site6-ColoradoPlateau-AcomaEx	35	-107.5	11.2	253		
site9-NorthernGreatBasin-AlvordHS	42.5	-118.5	9.3	224		

site7-ColoradoPlateau-ElMorro	35	-108.3	8.2	372		
Homburg, Goessenheim, Germany	50.0	9.8	9.1	677	Illumina	(Williams et al. 2016)
Homburg, Goessenheim, Germany	50.0	9.8	9.1	677		
Tabernas, Almeria, Spain	37.0	-2.4	16.0	305		
Tabernas, Almeria, Spain	37.0	-2.4	16.0	305		
Tabernas, Almeria, Spain	37.0	-2.4	16.0	305		
Nat. Reserve Gynge Alvar, Sweden	56.5	16.5	7.5	503		
Nat. Reserve Gynge Alvar, Sweden	56.5	16.5	7.5	503		
Homburg, Goessenheim, Germany	50.0	9.8	9.1	677		
Nat. Reserve Gynge Alvar, Sweden	56.5	16.5	7.5	503		
Hohe Tauern National Park, Austria	47.1	12.9	-1.8	1611		
Hohe Tauern National Park, Austria	47.1	12.9	-1.8	1611		
Hohe Tauern National Park, Austria	47.1	12.9	-1.8	1611		
Cold Desert Silty - clay loam soil	41.1	-113.0	10.3	246	Illumina	(Velasco Ayuso et al. 2016, Bethany et al. 2019)
Cold Desert - sandy clay loam soil	41.1	-113.0	10.1	247		
Hot Desert Silty - clay loam soil	32.5	-106.7	15.2	273		
Hot Desert Sandy - loamy sand soil	32.4	-105.9	16.2	273		
Desert, early-developed biocrusts (China)	44.8	88.2	7.1	169	454 Pyrosequencing	(Zhang et al. 2016a)
Desert, later-developed biocrusts (China)	44.8	88.2	7.1	169		
Moab, Green Butte site	38.7	-109.6	12.4	221	Illumina	(Couradeau et al. 2016)
Canastra National Park	-20.3	-46.6	19.8	1577	Illumina	(Machado-de-Lima et al. 2019)
Capao National Park	-19.3	-43.5	19.1	1482		
Cipo National Park	-19.3	-43.5	19.1	1482		

Furnas National Park	-20.2	-47.4	20.9	1536		
Vassununga National Park	-20.3	-46.3	20.3	1574		
Zagaia National Park	-21.3	-47.6	21.6	1472		
Blue gramma	34.3	-106.6	12.8	277	Illumina	(Fernandes et al. 2018)
Black gramma	34.3	-106.7	12.9	252		
MRME	34.3	-106.7	12.9	252		
Actopan	20.3	-98.92	16.8	470	Illumina	(Becerra-Absalón et al. 2019)
Atexcac	19.3	97.3	24.7	325		
Western Australia - ERR2940139	-29.2	116.7	20.0	330	Illumina Ion Torrent PGM	(Moreira-Grez et al. 2019)
Western Australia - ERR2940142	-29.2	116.7	20.1	322		
Western Australia - ERR2940151	-29.2	116.7	20.0	330		
Western Australia - ERR2940153	-29.2	116.7	20.0	330		
Western Australia - ERR2940159	-29.2	116.7	20.0	330		
Western Australia - ERR2940166	-29.2	116.7	20.0	330		
Western Australia - ERR2940172	-29.2	116.7	20.2	321		
Western Australia - ERR2940180	-29.2	116.7	20.0	330		

Supplementary Figures

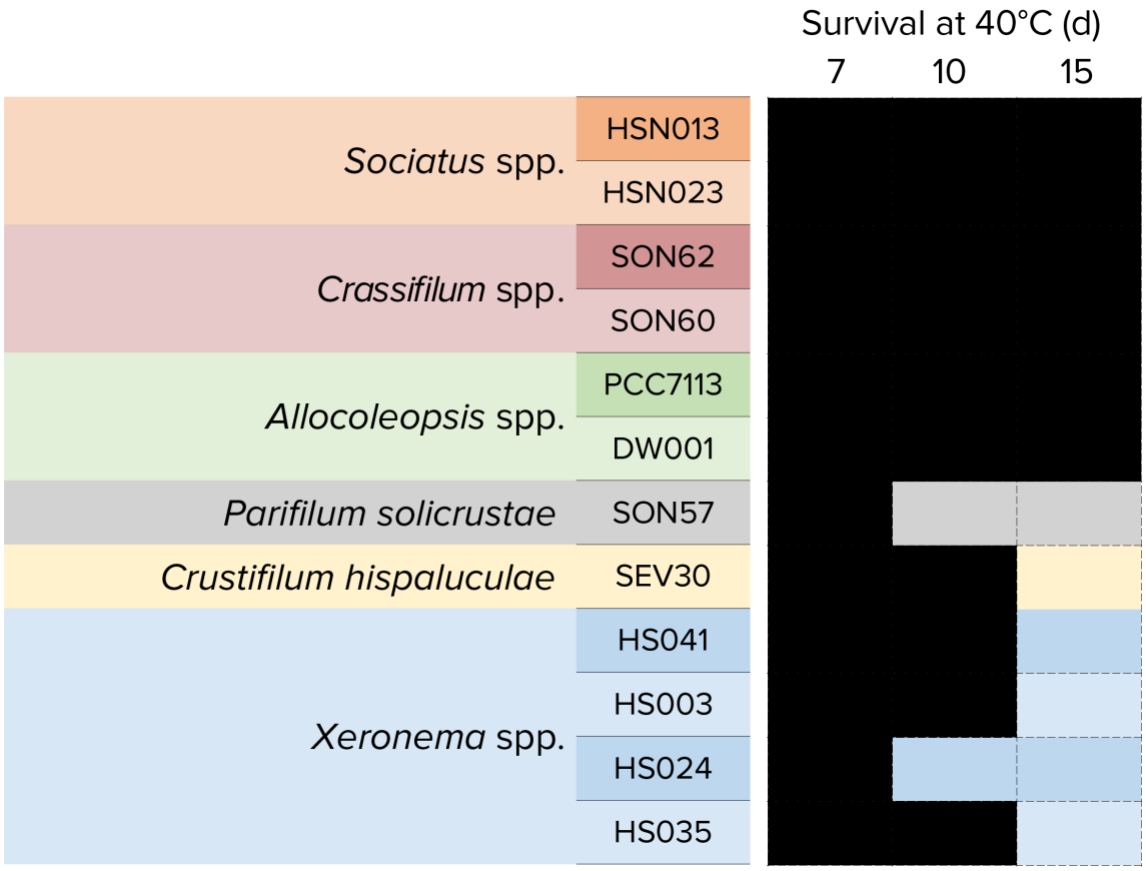

Figure S5. Survival of strains at 40 °C at 7, 10 and 15 days of incubation. Only observational data were recorded; black rectangles indicate survival and colored rectangles indicate death.

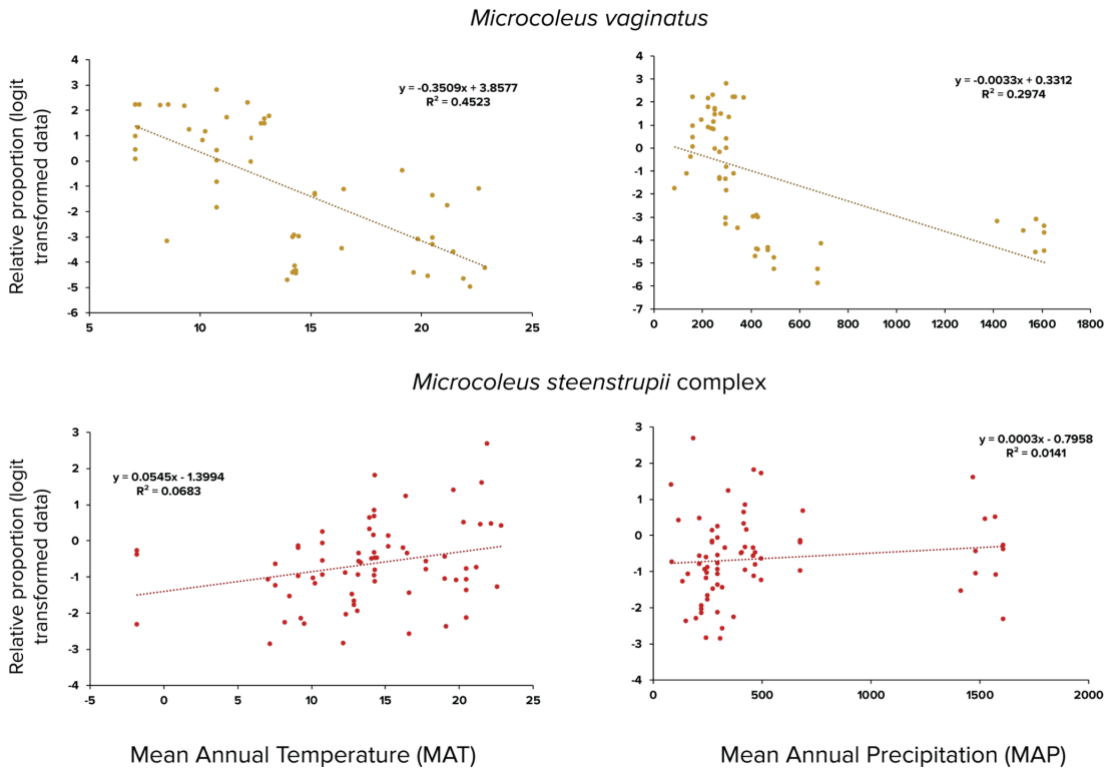

Figure S6. Linear regression between the proportion of sequence reads (logit transformed) of *Microcoleus vaginatus* and the *Microcoleus steenstrup*ii complex and climatic parameters (MAT and MAP). MAT: Mean annual temperature, MAP: Mean temperature annual precipitation.

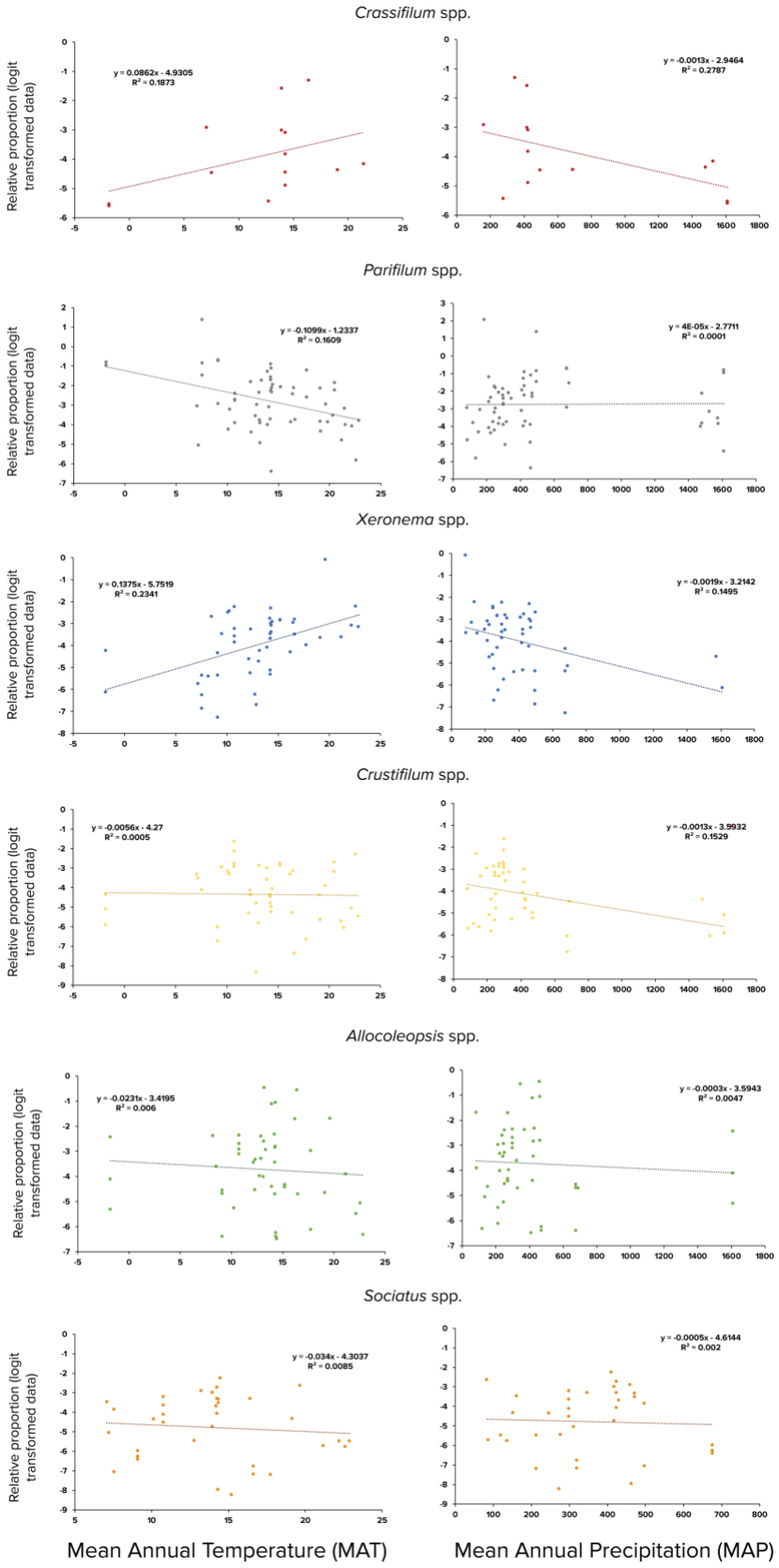

Figure S7. Linear regression between the proportion of sequence reads (logit transformed) the genera described in this study and climatic parameters (MAT and MAP). MAT: Mean annual temperature, MAP: Mean temperature annual precipitation.

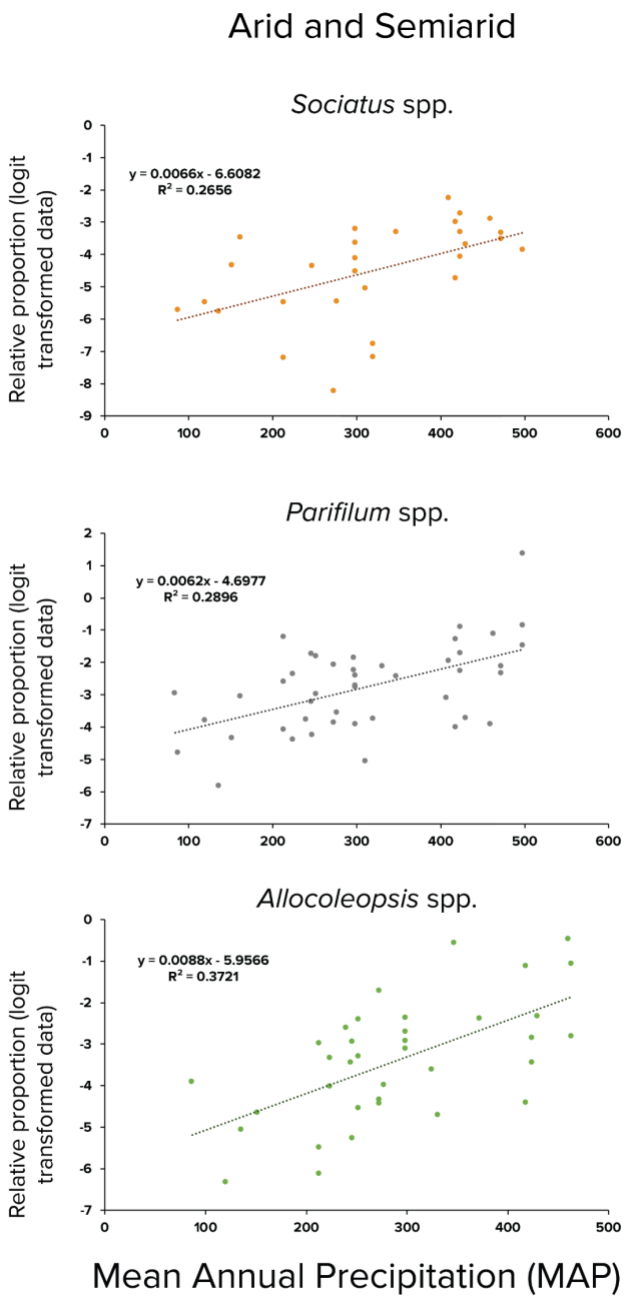

Figure S8. Linear regression between the proportion of sequence reads (logit transformed) of *Sociatus* spp., *Parifilum* spp. *and Allocoleopsis* spp. and parameters MAP. MAP: Mean temperature annual precipitation.

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5 – CONCLUSIONS

Main point (s) from each chapter and dissertation contribution (s)

This dissertation investigates the responses of biocrust cyanobacteria to predicted climate change alterations in precipitation patterns and the consequences of changes in the diversity and community structure of cyanobacteria to desert ecosystems.

In chapter 2, we studied biocrusts microorganisms' responses to chronic drought and a delayed monsoon season, two predicted changes to precipitation in the Southwestern U.S. that were never studied before for biocrust communities. Rather than exclusively analyzing changes to relative abundance of cyanobacteria, we coupled Illumina sequencing with qPCR of the 16S rRNA gene, allowing us to report changes in cyanobacterial population sizes. Surprisingly, both drought and a delay in the monsoon season, showed similar effects on the cyanobacterial community structure and population sizes, but the effects were dependent of biocrust successional stages. Incipient biocrusts, composed mostly of *Microcoleus vaginatus* and members of the *Microcoleus steenstrupii* complex were less affected by treatments and only suffered minor changes to their community structure and population size. Mature biocrusts that are characterized by the presence of nitrogen-fixing cyanobacteria, such as *Scytonema* spp., were much more affected, suffering tremendous loses of total cyanobacteria population. *Microcoleus vaginatus* showed to be more resistant to drought than other cyanobacteria (Fernandes et al. 2018). These results predict that a drought or a delayed monsoon season can prevent biocrusts from reaching maturity, decreasing their overall contribution, such as nitrogen inputs, to desert ecosystems.

In chapter 3, using the same approach of chapter 2 and to expand our knowledge on how cyanobacteria respond to changes in precipitation patterns, we studied how cyanobacteria are going to fare under smaller, more frequent rainfall events. Because the experimental design involved adding water beyond the ambient rainfall in either smaller more frequent events or larger, less frequent ones, this study also provided us with answers on water limitation in biocrust cyanobacteria. Surprisingly, water additions were not always beneficial to cyanobacteria since, when compared to controls that only received ambient rainfall, large less frequent rainfall events not always increased total biomass of cyanobacteria (measured by total 16S rRNA gene copies per cm_2). Smaller, more frequent rainfall events increased cyanobacteria diversity and total population when compared to large, less frequent ones. Smaller rainfall events benefited members of the *Microcoleus steenstrupii* complex, as well as heterocystous *Scytonema* spp., while *Microcoleus vaginatus* lost its dominance (Fernandes et al., *In prep*). This study shows that smaller, more frequent rainfall events predicted to happen in future climate would be beneficial to biocrust cyanobacteria diversity and biomass, pointing to higher nitrogen inputs judging by the positive effects it had on nitrogen-fixing cyanobacteria.

In chapter 4, after noticing that clades from the *Microcoleus steenstrupii* complex had differential responses to altered precipitation and water variability, we decided to study the natural diversity of this group and disentangle the taxonomy of the group into coherent taxa that could be used in ecological studies. We first showed that, in natural samples, the entity *Microcoleus steenstrupii* is actually composed of a diversity of clades and that our efforts to isolate members of this group only covered a portion of that

diversity. After isolation efforts to cover more clades within this group, we studied the phylogeny, as well as morphological and physiological characteristics of our strains. Surprisingly, after obtaining full 16S rRNA gene sequences from our strains and building a comprehensive phylogenetic tree of *Microcoleus steentrupii* complex, we found that the complex is a monophyletic group composed of at least 11 genera of terrestrial, desiccation tolerant cyanobacteria. We then proposed a new cyanobacteria family, Porphyrosiphonaceae, accommodating all the genera included in this monophyletic group, including five new genera composed mainly or exclusively by our cultivated strains. This is the first attempt to resolve this diverse and ecologically important complex (Fernandes et al., *In prep*). The genera described in this study showed differential responses to temperature in laboratory studies, contrary to the common knowledge that "*Microcoleus steenstrupii"* is more adapted to higher temperatures. In this chapter, we also showed that the differential responses of the genera inside the now called Porphyrosiphonaceae family could be translated to the distribution of these cyanobacteria in biocrusts worldwide (Fernandes et al., *In prep*). These results provide a new taxonomy and structure of the *Microcoleus steens*trupii complex, highlighting the differential responses to precipitation and temperature within the distinct genera of this group. The use of the framework we provided will aid future studies aiming to describe other clades present inside the Porphyrosiphonaceae family and the continued use of the taxonomy here presented will increase our knowledge of the responses of each genus to their surrounding environment, eventually providing biocrusts researchers with a clear

understanding of the importance of these changes to biocrust ecology and desert ecosystems.

Overall, this thesis constitutes an important contribution to the field of biocrust ecology, specially to the study of climate change effects on biocrust cyanobacteria and its consequences to deserts ecosystems. For the first time we opened the 'black box' of cyanobacterial responses to precipitation and water availability and showed how important it is to know how different cyanobacterial taxa will respond to altered precipitation. Understanding how these changes might affect cyanobacteria in biocrusts is important for both management strategies and restoration efforts, since knowing which microbe is more susceptible or resistant to certain environmental variable/condition will certainly help counteracting those effects or selecting the right inoculum to restore an area. To further increase our ability to predict how cyanobacteria are going to respond to environmental changes we also contributed with the first comprehensive study on the taxonomy of the *Microcoleus steenstrupii* complex. This new taxonomy based on the polyphasic approach provided the scientific community with coherent taxa and their distinct responses to climatic variables. Continuous use of the new taxonomy of the *Microcoleus steenstrupii* complex should inform on each genus main contribution to biocrusts, much as we already know about the other biocrust cyanobacteria taxa, such as *Microcoleus vaginatus*. Additionally, although our study focused on one system, the methodology used here can be transferred to other microbial ecology studies.

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