Microbial Restoration Ecology of Biological Soil Crusts

by

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ABSTRACT

Biological soil crusts (biocrusts) are topsoil communities of organisms that contribute to soil fertility and erosion resistance in drylands. Anthropogenic disturbances can quickly damage these communities and their natural recovery can take decades. With the development of accelerated restoration strategies in mind, I studied physiological mechanisms controlling the establishment of cyanobacteria in biocrusts, since these photoautotrophs are not just the biocrust pioneer organisms, but also largely responsible for improving key soil attributes such as physical stability, nutrient content, water retention and albedo. I started by determining the cyanobacterial community composition of a variety of biocrust types from deserts in the Southwestern US. I then isolated a large number of cyanobacterial strains from these locations, pedigreed them based on their 16SrRNA gene sequences, and selective representatives that matched the most abundant cyanobacterial field populations. I then developed methodologies for large-scale growth of the selected isolates to produce location-specific and genetically autochthonous inoculum for restoration. I also developed and tested viable methodologies to physiologically harden this inoculum and improve its survival under harsh field conditions. My tests proved that in most cases good viability of the inoculum could be attained under field-like conditions. In parallel, I used molecular ecology approaches to show that the biocrust pioneer, *Microcoleus vaginatus*, shapes its surrounding heterotrophic microbiome, enriching for a compositionally-differentiated "cyanosphere" that concentrates the nitrogen-fixing function. I proposed that a mutualism based on carbon for nitrogen exchange between *M. vaginatus* and its cyanosphere creates a

consortium that constitutes the true pioneer community enabling the colonization of nitrogen-poor, bare soils. Using the right mixture of photosynthetic and diazotrophic cultures will thus likely help in soil restoration. Additionally, using physiological assays and molecular meta-analyses, I demonstrated that the largest contributors to N_2-fixation in late successional biocrusts (three genera of heterocystous cyanobacteria) partition their niche along temperature gradients, and that this can explain their geographic patterns of dominance within biocrusts worldwide. This finding can improve restoration strategies by incorporating climate-matched physiological types in inoculum formulations. In all, this dissertation resulted in the establishment of a comprehensive "cyanobacterial biocrust nursery", that includes a culture collection containing 101 strains, isolation and cultivation methods, inoculum design strategies as well as field conditioning protocols. It constitutes a new interdisciplinary application of microbiology in restoration ecology.

to Hernán, Lucía, María, Sachi, Eneko y Naia, my family

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1 – DISSERTATION INTRODUCTION

Theoretical background

Biocrust definition and ecosystem services

Plant interspaces in arid and semiarid lands (hereafter drylands) tend to be colonized by cryptic assemblages of organisms known as biological soil crust (biocrusts; see Garcia-Pichel 2003, for a primer, and Belnap et al. 2016, for a monograph). These topsoil microbial communities typically develop where plant growth is limited by water and nutrients. Because the geographical extent of drylands (nearly 45% of the total Earth continental area; Prăvălie 2016), and their predicted extension as aridity increases due to global warming (Seager and Vecchi 2010, Petrie et al. 2014, 2015), it has become rather clear that biocrusts matter not only locally, but also globally. They play an important role in the global biogeochemical cycles of carbon and nitrogen (Housman et al. 2006, Elbert et al. 2012). Their global carbon stock has been calculated to be about 54 x 10^{12} g (Garcia-Pichel et al. 2003), while the global carbon uptake of cryptogamic covers, much of which are biocrust, is thought to account for about 7% of the net primary production of terrestrial vegetation. Dinitrogen fixation, of these cryptogamic covers, has been estimated at 49 Tg/yr, nearly 50% of the biological nitrogen fixation on land (Elbert et al. 2012). Biocrusts influence soil fertility by secreting fixed atmospheric carbon and nitrogen (Thiet et al. 2005, Johnson et al. 2007, Thomazo et al. 2018), and by leaching micronutrients such as Mg, Al, Ca, V, Cr, Mn, Fe, As, Rb (Beraldi-Campesi et al. 2009) to underlying soils. Additionally, they may enrich the soil in nutrients such as P, Mg, Na,

K, Ca and Mo by trapping dust particles (Reynolds et al. 2001). Biocrusts also provide soil surface stabilization against wind (Belnap and Gillette 1997, Zhang et al. 2006), and water erosion (Gaskin and Gardner 2001); they can significantly warm the soil by reducing soil albedo (Couradeau et al. 2016), and modify soil water retention (Verrecchia et al. 1995, Rodríguez-Caballero et al. 2012, 2013, Faist et al. 2017), by influencing water infiltration and runoff in ways that are not yet fully understood. Biocrusts can have a positive (Defalco et al. 2001, Godínez-Alvarez et al. 2012), negative (Zaady et al. 1997) or no effect (Megill et al. 2011, Godínez-Alvarez et al. 2012) on plants, and their influences may be contingent upon biocrust community type, plant functional traits and disturbance (Havrilla et al. *in review* – Journal of Ecology).

Biocrust microbial community

Overall, biocrust microbial diversity increases as ecological succession proceeds (Couradeau et al. 2016). Different ecological succession stages are recognized in biocrust (Garcia-Pichel et al. 2001, Couradeau et al. 2016), with some organisms being generally considered common to all biocrust developmental stages (e.g. filamentous cyanobacteria, bacteria, and archaea), and others associated with a more advanced stage of development (e.g. N_2-fixing cyanobacteria, lichens and mosses). Cyanobacteria are key players to most biocrust developmental stages; they are not only considered the pioneer organisms (Garcia-Pichel and Wojciechowski 2009), but also represent the main source for atmospheric carbon input (Housman et al. 2006, Sancho et al. 2016) and an important source of the nitrogen (Housman et al. 2006, Yeager et al. 2007) available for the

community. Colonization of bare soil starts by motile filamentous cyanobacteria such as *Microcoleus vaginatus* and *Microcoleus steenstrupii*. These microbes synthesize a polysaccharide sheath that bundles many filaments together (Garcia-Pichel and Wojciechowski 2009), and that, in association with soil particles, form a crust that stabilizes loose soil. This succession stage is what is known as incipient or light crusts. By means of this soil stabilization, other bacteria (Garcia-Pichel et al. 2001, Nagy et al. 2005), archaea (Soule et al. 2009), and fungi (Bates and Garcia-Pichel 2009) settle, becoming part of the biocrust community. Biocrust's ecological succession can eventually lead to the establishment of dark pigmented sessile heterocystous cyanobacteria (Belnap 2001b, Garcia-Pichel et al. 2001, Ullmann and Büdel 2001) such as *Nostoc* sp., *Tolypothrix* sp. and *Scytonema* sp. (Yeager et al. 2007), in a stage known as dark crust. Mosses and/or lichens (Belnap 2001, Ullmann and Büdel 2001) may come in more mature crusts, if moisture and temperature conditions are favorable (Garcia-Pichel 2003). However, this general successional sequence can be altered by fire (REF), sand deposition (Bowker et al. 2004, Weber et al. 2016b), and favorable environments, including fog and dew deserts (Lalley and Viles 2008) and mesic climates (Read et al. 2011), where species traits play definitive roles in determining the starting community structure (Read et al. 2011, Concostrina-Zubiri et al. 2014, Weber et al. 2016b)

While the role of cyanobacteria in the biocrust community has been widely studied (Yeager et al. 2007, Bowker et al. 2008, Garcia-Pichel and Wojciechowski 2009, Büdel et al. 2016, Couradeau et al. 2016), not much attention has been given to other prokaryotic organisms and how their presence may influence the community. For example, it has been shown that ammonia oxidizing archaea (AOA) and bacteria (AOB),

which play a role in the transformation of crust ammonium, have a temperature-driven niche partitioning (Marusenko et al. 2013). AOA are more enriched in biocrusts from warmer deserts, while AOB are prevalent in colder locations. Additionally, after a wetting event, significant increases in population sizes of Firmicutes (Angel and Conrad 2013, Karaoz et al. 2018), Sphingobacteriales and Alphaproteobacteria (Angel and Conrad 2013) are indicative of a dynamic heterotrophic community that responds to resuscitation from dormancy. Atmospheric N_2-fixation is perhaps the most important input of this nutrient to the system. This role has been mostly attributed to heterocystous cyanobacteria (Yeager et al. 2007); however, these nitrogen fixers only come later in the ecological succession. This, along with the fact that *Microcoleus* spp. do not fix nitrogen (Starkenburg et al. 2011, Rajeev et al. 2013), leaves as an open question the origin of the initial nitrogen source to support *Microcoleus* spp. as they colonize bare soil. In light of this question, the presence of heterotrophic nitrogen-fixers had been predicted (Johnson et al. 2005) and indeed recently detected (Pepe-Ranney et al. 2015) in light crusts, but more research needs to be carried out to fully understand this matter.

Challenges to biocrust organisms and global warming

The challenges that biocrust organisms face due to extreme environmental conditions include but are not limited to extreme solar radiation, which in Southwestern United States ranges from 5.87 to 7.71 Kwh/m^2/day (Direct Normal Irradiance (DNI) scale: < 2.5-8.5; National Solar Radiation database); and extreme daily air temperature variations (from 0 to 35 °C; average annual temperature – Southwest United States, U.S. Climate database). Low precipitation regimes < 5 mm y^{-1}(Austin et al. 2004), characterized by

successive cycles of short periods of rains (Sala and Lauenroth 2014) and long periods of dryness (Knapp et al. 2008), pose additional challenges. Biocrust organisms can survive these long periods of dryness by entering into a dormant stage from which they will promptly resuscitate during short periods of hydration (Angel and Conrad 2013, Rajeev et al. 2013, Karaoz et al. 2018). In spite of the capacity of these organisms to thrive under extreme conditions, given the marked variations in temperature and precipitation, 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 (Reed et al. 2012, Johnson et al. 2012, Fernandes et al. 2018).

Drylands will likely become warmer and drier in response to global warming. In particular, the southwestern United States is predicted to experience an increase in temperature of about 1 °C per decade (Seager and Vecchi 2010), and alterations in precipitation frequency (Cable and Huxman 2004, Knapp et al. 2008, Sala and Lauenroth 2014). Recent studies in biocrust communities have shown that increased temperature and altered precipitation may impact species composition and their physiological function (Reed et al. 2012, Johnson et al. 2012, Ferrenberg et al. 2015, Fernandes et al. 2018). For example, long term surveys have demonstrated that warmer temperatures led to a dramatic decrease in relative cover of lichens (Ferrenberg et al. 2015), while alterations in the precipitation regime led to a decline in the cover of mosses (Reed et al. 2012, Ferrenberg et al. 2015). Changes in precipitation season, and increased drought resulted in a less diverse biocrust cyanobacterial community, with some cyanobacterial taxa (i.e. *M. steenstrupii*, *Scytonema* spp.) being more sensitive to such changes in precipitation regime (Fernandes et al. 2018). Alterations of both temperature and precipitation regimes

seem therefore to hinder mature biocrust and promote instead early successional community stages. Small rainfall events may also result in a scenario where sufficient moisture initiates cell respiration, but subsequent desiccation hinders the biocrust photosynthetic recovery before the system can achieve a net carbon balance (carbon compensation point), ultimately resulting in carbon starvation (Johnson et al. 2012). Increasing temperatures are also likely to promote an imbalance in biocrust's nitrogen cycle, resulting in further N-limitations for the biocrust communities and drylands (Zhou et al. 2016).

Biocrust organism's adaptation to extreme environments

Biocrust cyanobacteria possess a set of physiological adaptations key for their survival in these extreme environments. For example, i) *Nostoc* spp., *Tolypothrix* spp. and *Scytonema* spp., synthetize 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 (Garcia-Pichel and Castenholz 1991, Soule et al. 2009). Also, ii) *Microcoleus* spp. are quite sensitive to reduced water potential, suggesting that these organisms are not biologically active under dry conditions (Brock 1975), iii) motile filamentous cyanobacteria such as *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), increasing their overall survival, iv) the bundle forming behavior in *Microcoleus* spp. not only allows these cyanobacteria to colonize bare soils (Garcia-Pichel and Wojciechowski 2009), but it 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). Additionally, v) expression of glycogen debranching enzymes upon drying in the cyanobacterium *M. vaginatus* (Rajeev et al. 2013) has been linked to the conversion of cumulated glycogen into compatible solutes (Baran et al. 2017, Jose et al. 2018), which may be important for survival under variable light and water availability, and vi) 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 1996, Rajeev et al. 2013).

All of the above adaptations contribute to describe the mechanisms by which biocrust communities will respond to environmental conditions and survive. However, many aspects of these mechanisms remain unknown or poorly characterized. Most studies aiming to learn from biocrust dynamics have been conducted on the whole community (Reed et al. 2012, Rajeev et al. 2013, Ferrenberg et al. 2015), which makes it difficult to directly link a specific function to a single organism. Studies based on monospecific laboratory cultures successfully predicted the succession from *M. vaginatus* to *M. steenstrupii* in response to warmer temperatures (Garcia-Pichel et al. 2013), but studies of this type are scarce due to difficulties in isolating and growing these organisms in the laboratory (Belnap and Eldridge, 2001). Therefore, a better understanding of the factors that limit the species' fitness and distribution under current environmental conditions will help to improve our understanding of arid land systems, and our ability to predict future impacts of global warming on biocrust communities, as well as to develop remediation strategies to restore biocrust communities after large-scale disturbances.

Biocrust restoration

Compressional forces such those brought about by agriculture, grazing by livestock, construction, human foot impact, vehicular traffic, mining, and military training (Belnap and Eldridge 2001, Zaady et al. 2016) greatly impact biocrust communities. These disturbances can break cyanobacterial filaments, converting them to a non-functional state. Damaged biocrusts may quickly become a local source of loose soil that can be easily transported and deposited on healthy patches of biocrust, leaving the photosynthetic community (cyanobacteria, mosses and liches) in the dark. Biocrusts are also susceptible to natural disturbances such as fire (Bowker et al. 2004, Ford and Johnson 2006), sand deposition (Wang et al. 2007, Rao et al. 2012), and prolonged drought (Williams et al. 2008) which may cause alterations in functional properties, in community composition, and decreases in biomass. Global warming, which has been shown to affect biocrust diversity (Reed 2012, Fernandes et al. 2018) by shifting communities towards more immature stages of biocrust development (Reed 2012, Ferrenberg et al. 2015, Fernandes et al. 2018), poses an additional stress. Loss of these communities consequently results in losses of the ecosystem services they provide to drylands (Belnap and Eldridge 2001, Barger et al. 2006). Additionally, when disturbed, loose particles may become a significant source of atmospheric dust, impacting air quality (Griffin 2007), and traffic of nearby metropolitan areas (i.e. Phoenix, Las Cruces).

Natural recovery rates vary widely from few a years to centuries depending on factors such as climate, soil conditions, and severity of disturbance (Belnap 1993, Weber et al. 2016a), and that can be particularly lengthy if biocrust remnants that can serve as natural inoculum are scarce in the surrounding area. Assisted recovery by inoculation of

biocrusts organisms to enhance recovery rates of degraded biocrust communities (see review by Bowker (2007) arose as a way to preserve the functioning of local ecosystems. Multiple attempts using healthy biocrusts indicated that recovery of denuded soils was possible (St Clair et al. 1986, Maestre et al. 2006, Xiao et al. 2008), but harvesting of intact biocrust to recover damaged areas is an unsustainable practice than cannot be scaled-up. Therefore, a way to separately obtain inoculant emerged as a sustainable solution. Inoculum production has been attempted based on different biocrust organisms, and two strategies have been explored: mixed-community rearing and cultivation-based. Both approaches use small quantities of remnant biocrust as a seed to grow large amounts of inoculum in a greenhouse, and in a laboratory setting under conditions that are experimentally optimized to promote growth (Velasco Ayuso et al. 2016). Mixed-community biocrust rearing strategies have been developed for the production of inoculant, from field collected biomass, based on the cyanobacterial community as a whole (Velasco Ayuso et al. 2016), or based on either mosses and lichens, or both (Antoninka et al. 2016, 2018, Bowker and Antoninka 2016). A large proportion of the efforts have used cultivation-based production of cyanobacteria isolates to obtain inoculant (Wang et al. 2009, Zheng et al. 2010, Wu et al. 2013, Zhang et al. 2013, Lan et al. 2014, Chamizo et al. 2018, Román et al. 2018, Roncero-Ramos et al. 2019); however, the technical detail needed to grow large biomass quantities of the biocrust pioneer cyanobacteria (*M. vaginatus* and *M. steenstrupii*) under laboratory conditions has not been provided in any of these studies, leaving, in turn, unreproducible results. The studies that provided such technical details use as inoculant either individual or mixtures of N_2-fixing cyanobacteria such as *Nostoc* spp., *Tolypothrix* spp. and *Scytonema* spp. (Chamizo

et al. 2018, Román et al. 2018, Roncero-Ramos et al. 2019), for which growth in liquid can be achieved using traditional scaling-up techniques (Guedes et al. 2014, Takenaka and Yamguchi 2014). Next advances in biocrust restoration should include the development of techniques to speed up growth of the biocrust pioneer cyanobacteria *Microcoleus* spp., testing the fitness of the produced inoculum under field conditions and identify loss factors in order to mitigate mortality in the field. Investigating potential positive or negative influences of the heterotrophic bacterial community associated with phototrophs may be another venue to explore.

Dissertation research objective

My overarching objective was to investigate particular physiological mechanisms underlying the adaptation of biocrust microorganisms to extreme environments, as well as some of the microbial interactions (among microbes and between them and their local environment) driving community composition and structure, to further utilize these new findings to support biocrust restoration efforts through the establishment of a "cyanobacterial biocrust nursery".

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, water retention and albedo, iii) they affect the biogeochemical cycling of C (carbon) and N (nitrogen), and iv) they are therefore quite

relevant in the context of biocrust restoration. Military facilities along the Southwestern United States were chosen as a biocrust source and research site because they represent an interesting venue for biocrust restoration due to damage caused by military training and the US military's interest in maintaining as sustainable an operation as possible; this translated into an opportunity to fully fund the research.

My first task was to determine field cyanobacterial community structure to guide efforts in culturing the main biocrust cyanobacteria from each of the selected field locations. I then isolated multiple cyanobacterial strains from biocrust communities as a vehicle to gain new knowledge on the biology of microbial biocrust species. Cultures are advantageous in that they allow for the testing of ecophysiological hypotheses (Acharya et al. 2004, Garcia-Pichel et al. 2013), enabling a direct link to be made between a specific function or response and a single organism. Both field biocrust samples and cyanobacterial cultures were then subjected to physiological assays and genetic surveys in order to gain new understanding of the factors that drive cyanobacterial establishment and community structure in biocrusts. This new knowledge was also used to inform the production of cyanobacterial biocrust inoculum under laboratory conditions, and its subsequent scale-up to support biocrust restoration efforts directly in the field.

Dissertation structure

My dissertation document is comprised of one introductory chapter, followed by four data chapters that are structured as stand-alone 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 biocrust restoration.

Chapter 2. Nursing biocrusts: isolation, cultivation and fitness test of indigenous cyanobacteria (Published in Restoration Ecology, impact factor: 2.544)

This chapter presents in detail a multi-step protocol for large scale cultivation of biomass to restore cyanobacterial biocrusts. The protocol includes specific pipelines to isolate pedigreed strains of biocrust cyanobacteria, methods for scaling-up cyanobacterial biomass to produce inoculum for large scale soil restoration, and tests of the fitness of the inoculum on native soils under field-like conditions.

Chapter 3. Effect of preconditioning to the soil environment on the performance of 20 cyanobacterial cultured strains used as inoculum for biocrust restoration (*Submitted* to Restoration Ecology, impact factor: 2.544)

This chapter presents a series of experiments designed to assess the potential benefits of preconditioning treatments for cyanobacteria to grow on native soils under field-like conditions as a means to increase inoculum fitness.

Chapter 4. Spatial segregation of the biological soil crust microbiome around its foundational cyanobacterium, *Microcoleus vaginatus*, and the formation of a nitrogen-fixing cyanosphere. (Published in Microbiome, impact factor: 9.133)

This chapter presents data demonstrating that *M. vaginatus* acts as a significant spatial organizer of the biocrust microbiome. This cyanobacterium not only shapes the microbial populations of heterotrophs around it by forming a compositionally differentiated cyanosphere that concentrates the nitrogen fixing function, but it also segregates away from its vicinity a large number of biocrust community members, potentially through competition for light or CO_2, or because of a preference for oligotrophy.

Chapter 5. Niche partitioning with temperature among heterocystous cyanobacteria (*Scytonema* spp., *Nostoc* spp., and *Tolypothrix* spp.) from biological soil crusts (*In prep*)

This chapter presents data investigating the niche partitioning among the three most common heterocystous cyanobacteria from biocrusts using enrichment cultivation. *Scytonema* spp. were found to be the most thermotolerant, whereas *Tolypothrix* spp. were more psychrophilic. *Nostoc* spp. responded well at the intermediate temperatures. Heat sensitivity was also correlated in *Nostoc* spp. and *Tolypothrix* spp. strains with nitrogen fixation because the thermal range for growth could be increased under nitrogen replete conditions. This sensitivity could be traced to an inability to develop heterocysts at high temperatures. The relevance of this apparent niche partitioning was tested using a meta-analysis of a large set of molecular surveys of biocrust cyanobacteria, and it was

determined that the geographic distribution of the three taxa is clearly constrained by the mean temperature during the growth season in the sites of origin. Finally, by combining the physiological responses of the three taxa to temperatures with their observed geographic distributions, potential shifts in dominance in many locales as a result of global warming, was predicted.

Chapter 6. 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 restoration.

References

Acharya, K., M. Kyle, and J. J. Elser. 2004. Biological stoichiometry of Daphnia growth: An ecophysiological test of the growth rate hypothesis. Limnol. Oceanogr. 49:656–665.

Angel, R., and R. Conrad. 2013. Elucidating the microbial resuscitation cascade in biological soil crusts following a simulated rain event. Environmental Microbiology 15:2799–2815.

Antoninka, A., M. A. Bowker, P. Chuckran, N. N. Barger, S. Reed, and J. Belnap. 2018. Maximizing establishment and survivorship of field-collected and greenhouse-cultivated biocrusts in a semi-cold desert. Plant and Soil 429:213–225.

Antoninka, A., M. A. Bowker, S. C. Reed, and K. Doherty. 2016. Production of greenhouse-grown biocrust mosses and associated cyanobacteria to rehabilitate dryland soil function. Restoration Ecology 24.

Austin, A. T., L. Yahdjian, J. M. Stark, J. Belnap, A. Porporato, U. Norton, D. a. Ravetta, and S. M. Schaeffer. 2004. Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia 141:221–235.

Baran, R., R. Lau, B. P. Bowen, S. Diamond, N. Jose, F. Garcia-Pichel, and T. R. Northen. 2017. Extensive Turnover of Compatible Solutes in Cyanobacteria Revealed by Deuterium Oxide (D2O) Stable Isotope Probing. ACS Chemical Biology 12:674–681.

Barger, N. N., J. E. Herrick, J. Van Zee, and J. Belnap. 2006. Impacts of biological soil crust disturbance and composition on C and N loss from water erosion. Biogeochemistry 77:247–263.

Bates, S. T., and F. Garcia-Pichel. 2009. A culture-independent study of free-living fungi in biological soil crusts of the Colorado Plateau: Their diversity and relative contribution to microbial biomass. Environmental Microbiology 11:56–67.

Belnap, J. 1993. Recovery rates of cryptobiotic crusts: Inoculant use and assessment methods. The Great Basin Naturalist 53:89–95.

Belnap, J. 2001. Factors Influencing Nitrogen Fixation and Nitrogen Release in Biological Soil Crust. Pages 241–261 *in* J. Belnap and O. L. Lange, editors. Biology and Fertility of Soils. 1st edition. Springer-Verlag, Berlin.

Belnap, J., and D. Eldridge. 2001. Disturbance and Recovery of Biological Soil Crusts. Pages 363–383 *in* J. Belnap and O. Lange, editors. Biological soil cruts: structure, function, and management. Springer, Berlin.

Belnap, J., and D. A. Gillette. 1997. Disturbance of Biological Soil Crusts: Impacts on

Potential Wind Erodibility of Sandy Desert Soils in Southeastern Utah. Land Degrad. Develop 8:355–362.

Belnap, J., B. Weber, and B. Büdel. 2016. Biological Soil Crusts as an Organizing Principle in Drylands. Pages 3–14 Biological Soil Crusts: An Organizing Principle in Drylands. 2nd edition. Springer, Switzerland.

Beraldi-Campesi, H., H. E. Hartnett, A. Anbar, G. W. Gordon, and F. Garcia-Pichel. 2009. Effect of biological soil crusts on soil elemental concentrations: Implications for biogeochemistry and as traceable biosignatures of ancient life on land. Geobiology 7:348–359.

Bowker, M. A. 2007. Biological soil crust rehabilitation in Theory and Practice - an Underexploited Opportunity. Restoration Ecology 15:13–23.

Bowker, M. A., and A. J. Antoninka. 2016. Rapid ex situ culture of N-fixing soil lichens and biocrusts is enhanced by complementarity. Plant and Soil 408:415–428.

Bowker, M. a., J. Belnap, V. Bala Chaudhary, and N. C. Johnson. 2008. Revisiting classic water erosion models in drylands: The strong impact of biological soil crusts. Soil Biology and Biochemistry 40:2309–2316.

Bowker, M. A., J. Belnap, R. Rosentreter, and B. Graham. 2004. Wildfire-resistant biological soil crusts and fire-induced loss of soil stability in Palouse prairies, USA. Applied Soil Ecology 26:41–52.

Brock, T. D. 1975. Effect of water potential on a *Microcoleus* (cyanophyceae) from a desert crusts. Journal of phycology 11:316–320.

Büdel, B., T. Dulic, T. Daridenko, N. Rybalka, and T. Friedl. 2016. Cyanobacteria and Algae of Biological Soil Crusts. Pages 55–81 Biological Soil Crusts: An Organizing Principle in Drylands. 2nd edition. Springer, Switzerland.

Cable, J. M., and T. E. Huxman. 2004. Precipitation pulse size effects on Sonoran Desert soil microbial crusts. Oecologia 141:317–324.

Chamizo, S., G. Mugnai, F. Rossi, G. Certini, and R. De Philippis. 2018. Cyanobacteria Inoculation Improves Soil Stability and Fertility on Different Textured Soils: Gaining Insights for Applicability in Soil Restoration. Frontiers in Environmental Science 6.

Concostrina-Zubiri, L., E. Huber-Sannwald, I. Martínez, J. L. Flores Flores, J. A. Reyes-Agüero, A. Escudero, and J. Belnap. 2014. Biological soil crusts across disturbance-recovery scenarios: Effect of grazing regime on community dynamics. Ecological Applications 24:1863–1877.

Couradeau, E., U. Karaoz, H. C. Lim, U. Nunes da Rocha, T. Northen, E. Brodie, and F.

Garcia-Pichel. 2016. Bacteria increase arid-land soil surface temperature through the production of sunscreens. Nature Communications 7:10373.

Couradeau, E., V. J. M N L Felde, D. Parkinson, D. Uteau, A. Rochet, C. Cuellar, G. Winegar, S. Peth, T. R. Northen, and F. Garcia-Pichel. 2018. In Situ X-Ray Tomography Imaging of Soil Water and Cyanobacteria from Biological Soil Crusts Undergoing Desiccation. Frontiers in Environmental Science | www.frontiersin.org 1:65.

Defalco, L. A., J. K. Detling, C. R. Tracy, and S. D. Warren. 2001. Physiological variation among native and exotic winter annual plants associated with microbiotic crusts in the Mojave Desert. Plant and Soil 234:1–14.

Elbert, W., B. Weber, S. Burrows, J. Steinkamp, B. Büdel, M. O. Andreae, and U. Pöschl. 2012. Contribution of cryptogamic covers to the global cycles of carbon and nitrogen. Nature Geoscience 5:459–462.

Faist, A. M., J. E. Herrick, J. Belnap, J. W. V. Zee, and N. N. Barger. 2017. Biological soil crust and disturbance controls on surface hydrology in a semi-Arid ecosystem. Ecosphere 8.

Fernandes, V. M. C., N. M. Machado de Lima, D. Roush, J. Rudgers, S. L. Collins, and F. Garcia-Pichel. 2018. Exposure to predicted precipitation patterns decreases population size and alters community structure of cyanobacteria in biological soil crusts from the Chihuahuan Desert. Environmental Microbiology 20:259–269.

Ferrenberg, S., S. C. Reed, and J. Belnap. 2015. Climate change and physical disturbance cause similar community shifts in biological soil crusts. Proceedings of the National Academy of Sciences 112:12116–12121.

Ford, P. L., and G. V. Johnson. 2006. Effects of dormant- vs. growing-season fire in shortgrass steppe: Biological soil crust and perennial grass responses. Journal of Arid Environments 67:1–14.

Garcia-Pichel, F. 2003. Desert Environments: Biological Soil Crusts. Pages 1019–1023 *in* G. Bitton, editor. Encyclopedia of Environmental Microbiology 6 volume. Set. Wiley-Interscience, New York, NY.

Garcia-Pichel, F., and J. Belnap. 1996. Microenvironments and Microscale productivity of cyanobacteria desert crusts:774–783.

Garcia-Pichel, F., J. Belnap, S. Neuer, and F. Schanz. 2003. Estimates of global cyanobacterial biomass and its distribution. Algological Studies 109:213–227.

Garcia-Pichel, F., and R. W. Castenholz. 1991. Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. Journal of Phycology 409:395–409.

Garcia-Pichel, F., A. López-Cortés, and U. Nübel. 2001. Phylogenetic and Morphological Diversity of Cyanobacteria in Soil Desert Crusts from the Colorado Plateau. Appl. Environ. Microbiol. 67:1902–1910.

Garcia-Pichel, F., V. Loza, Y. Marusenko, P. Mateo, and R. M. Potrafka. 2013. Temperature Drives the Continental-Scale Distribution of Key Microbes in Topsoil Communities. Science 340:1574–1577.

Garcia-Pichel, F., and O. Pringault. 2001. Cyanobacteria track water in desert soils 413:380–381.

Garcia-Pichel, F., and M. F. Wojciechowski. 2009. The evolution of a capacity to build supra-cellular ropes enabled filamentous cyanobacteria to colonize highly erodible substrates. PloS ONE 4:7801.

Gaskin, S., and R. Gardner. 2001. The role of cryptogams in runoff and erosion control on Bariland in the Nepal middle hills of the Southern Himalaya. Earth Surface Processes and Landforms 26:1303–1315.

Godínez-Alvarez, H., C. Morín, and V. Rivera-Aguilar. 2012. Germination, survival and growth of three vascular plants on biological soil crusts from a Mexican tropical desert. Plant Biology 14:157–162.

Griffin, D. W. 2007. Atmospheric movement of microorganisms in clouds of desert dust and implications for human health. Clinical microbiology reviews 20:459–77, table of contents.

Guedes, C., N. G. Katkam, J. Varela, and F. X. Malcata. 2014. Photobioreactors for cyanobacterial culturing. Pages 271–292 *in* K. N. Sharma, K. R. Ashwani, and J. L. Stal, editors. Cyanobacteria: an economic perspective. First edit. John Wiley & Sons, Ltd., Oxford, UK.

Havrilla, A. C., V. Bala Chaudhary, S. Ferrenberg, A. J. Antoninka, B. Jayne, M. A. Bowker, D. J. Eldridge, A. M. Faist, E. Hubber-Sannwald, A. D. Leslie, E. Rodriguez-Caballero, Y. Zhang, and N. N. Barger. 2018. When communities collide: a meta-analysis of context- dependency in plant responses to biocrusts. Journal of Ecology.

Housman, D. C., H. H. Powers, a. D. Collins, and J. Belnap. 2006. Carbon and nitrogen fixation differ between successional stages of biological soil crusts in the Colorado Plateau and Chihuahuan Desert. Journal of Arid Environments 66:620–634.

Johnson, S. L., C. R. Budinoff, J. Belnap, and F. Garcia-Pichel. 2005. Relevance of ammonium oxidation within biological soil crust communities. Environmental Microbiology 7:1–12.

Johnson, S. L., C. R. Kuske, T. D. Carney, D. C. Housman, L. V. Gallegos-Graves, and J.

Belnap. 2012. Increased temperature and altered summer precipitation have differential effects on biological soil crusts in a dryland ecosystem. Global Change Biology 18:2583–2593.

Johnson, S. L., S. Neuer, and F. Garcia-Pichel. 2007. Export of nitrogenous compounds due to incomplete cycling within biological soil crusts of arid lands. Environmental microbiology 9:680–9.

Jose, N. A., R. Lau, T. L. Swenson, N. Klitgord, F. Garcia-Pichel, B. P. Bowen, R. Baran, and T. R. Northen. 2018. Flux balance modeling to predict bacterial survival during pulsed-activity events. Biogeosciences 15:2219–2229.

Karaoz, U., E. Couradeau, U. N. da Rocha, H.-C. Lim, T. Northen, F. Garcia-Pichel, and E. L. Brodie. 2018. Large Blooms of Bacillales (Firmicutes) Underlie the Response to Wetting of Cyanobacterial Biocrusts at Various Stages of Maturity. mBio 9:e01366-16.

Knapp, A. K., C. Beier, D. D. Briske, A. T. Classen, Y. Luo, M. Reichstein, M. D. Smith, S. D. Smith, J. E. Bell, P. a. Fay, J. L. Heisler, S. W. Leavitt, R. Sherry, B. Smith, and E. Weng. 2008. Consequences of More Extreme Precipitation Regimes for Terrestrial Ecosystems. BioScience 58:811.

Lalley, J. S., and H. A. Viles. 2008. Recovery of lichen-dominated soil crusts in a hyper-arid desert. Biodiversity and Conservation 17:1–20.

Lan, S., Q. Zhang, L. Wu, Y. Liu, D. Zhang, and C. Hu. 2014. Artificially accelerating the reversal of desertification: Cyanobacterial inoculation facilitates the succession of vegetation communities. Environmental Science and Technology 48:307–315.

Maestre, F. T., N. Martín, B. Díez, R. López-Poma, F. Santos, I. Luque, and J. Cortina. 2006. Watering, fertilization, and slurry inoculation promote recovery of biological crust function in degraded soils. Microbial ecology 52:365–377.

Marusenko, Y., S. T. Bates, I. Anderson, S. L. Johnson, T. Soule, and F. Garcia-Pichel. 2013. Ammonia-oxidizing archaea and bacteria are structured by geography in biological soil crusts across North American arid lands. Ecological Processes 2:9.

Megill, L., L. R. Walker, C. Vanier, and D. Johnson. 2011. Seed Bank Dynamics and Habitat Indicators of *Arctomecon californica*, a Rare Plant in a Fragmented Desert Environment. Western North American Naturalist 71:195–206.

Nagy, M. L., A. Pérez, and F. Garcia-Pichel. 2005. The prokaryotic diversity of biological soil crusts in the Sonoran Desert (Organ Pipe Cactus National Monument, AZ). FEMS Microbiology Ecology 54:233–245.

Pepe-Ranney, C., C. Koechli, R. Potrafka, C. Andam, E. Eggleston, F. Garcia-Pichel, and D. H. Buckley. 2015. Non-cyanobacterial diazotrophs dominate dinitrogen fixation

in biological soil crusts during early crust formation. The ISME Journal 10:287–298.

Petrie, M. D., S. L. Collins, D. S. Gutzler, and D. M. Moore. 2014. Regional trends and local variability in monsoon precipitation in the northern Chihuahuan Desert, USA. Journal of Arid Environments 103:63–70.

Petrie, M. D., S. L. Collins, and M. E. Litvak. 2015. The ecological role of small rainfall events in a desert grassland. Ecohydrology 8:1614-1622

Prăvălie, R. 2016. Drylands extent and environmental issues. A global approach 161:259–278.

Pringault, O., and F. Garcia-Pichel. 2004. Hydrotaxis of cyanobacteria in desert crusts. Microbial ecology 47:366–73.

Rajeev, L., U. N. Da Rocha, N. Klitgord, E. G. Luning, J. Fortney, S. D. Axen, P. M. Shih, N. J. Bouskill, B. P. Bowen, C. A. Kerfeld, F. Garcia-Pichel, E. L. Brodie, T. R. Northen, and A. Mukhopadhyay. 2013. Dynamic cyanobacterial response to hydration and dehydration in a desert biological soil crust. The ISME Journal:2178–2191.

Rao, B., Y. Liu, S. Lan, P. Wu, W. Wang, and D. Li. 2012. Effects of sand burial stress on the early developments of cyanobacterial crusts in the field. European Journal of Soil Biology 48:48–55.

Read, C. F., D. H. Duncan, P. A. Vesk, and J. Elith. 2011. Surprisingly fast recovery of biological soil crusts following livestock removal in southern Australia. Journal of Vegetation Science 22:905–916.

Reed, C. 2012. Tree Species Control Rates of Free-Living Nitrogen Fixation in a Tropical Rain Forest Author (s): Sasha C. Reed, Cory C. Cleveland and Alan R. Townsend Published by: Ecological Society of America content in a trusted digital archive. We use inform 89:2924–2934.

Reed, S. C., K. K. Coe, J. P. Sparks, D. C. Housman, T. J. Zelikova, and J. Belnap. 2012. Changes to dryland rainfall result in rapid moss mortality and altered soil fertility. Nature Climate Change 2:752–755.

Reynolds, R., J. Belnap, M. Reheis, P. Lamothe, and F. Luiszer. 2001. Aeolian dust in Colorado Plateau soils: nutrient inputs and recent change in source. Proceedings of the National Academy of Sciences of the United States of America 98:7123–7.

Rodríguez-Caballero, E., Y. Cantón, S. Chamizo, A. Afana, and A. Solé-Benet. 2012. Effects of biological soil crusts on surface roughness and implications for runoff and erosion. Geomorphology 145–146:81–89.

Rodríguez-Caballero, E., Y. Cantón, S. Chamizo, R. Lázaro, and A. Escudero. 2013. Soil

Loss and Runoff in Semiarid Ecosystems: A Complex Interaction Between Biological Soil Crusts, Micro-topography, and Hydrological Drivers. Ecosystems 16:529–546.

Román, J. R., B. Roncero-Ramos, S. Chamizo, E. Rodríguez-Caballero, and Y. Cantón. 2018. Restoring soil functions by means of cyanobacteria inoculation: Importance of soil conditions and species selection. Land Degradation and Development:3184–3193.

Roncero-Ramos, B., J. R. Román, C. Gómez-Serrano, Y. Cantón, and F. G. Acién. 2019. Production of a biocrust-cyanobacteria strain (Nostoc commune) for large-scale restoration of dryland soils. Journal of Applied Phycology:1–14.

Sala., O. E., and W. K. Lauenroth. 2014. Small Rainfall Events: An Ecological Role in Semiarid Regions. Oecologia 53:301–304.

Sancho, L. G., J. Belnap, C. Colesie, J. Raggio, and B. Weber. 2016. Carbon Budgets of Biological Soil Crusts at Micro-, Meso-, and Global Scales. Pages 287–304 Biological Soil Crusts: An Organizing Principle in Drylands. Second. Springer, Switzerland.

Seager, R., and G. A. Vecchi. 2010. Greenhouse warming and the 21st century hydroclimate of southwestern North America. Proceedings of the National Academy of Sciences of the United States of America 107:21277–21282.

Sherrod, S. K., J. Belnap, and M. E. Miller. 2002. Comparison of methods for nutrient measurement in calcareous soils: ion-exchange resin bag, capsule, membrane, and chemical extractions 167:666–679.

Soule, T., K. Palmer, Q. Gao, R. M. Potrafka, V. Stout, and F. Garcia-Pichel. 2009. A comparative genomics approach to understanding the biosynthesis of the sunscreen scytonemin in cyanobacteria. BMC genomics 10:336.

St Clair, L. L., J. R. Johansen, and B. L. Webb. 1986. Rapid stabilization of fire-disturbed sites using a soil crust slurry: Inoculation studies. Reclam.Reveg.Res. 4:261–269.

Starkenburg, S. R., K. G. Reitenga, T. Freitas, S. Johnson, P. S. G. Chain, F. Garcia-Pichel, and C. R. Kuske. 2011. Genome of the cyanobacterium Microcoleus vaginatus FGP-2, a photosynthetic ecosystem engineer of arid land soil biocrusts worldwide. Journal of bacteriology 193:4569–70.

Takenaka, H., and Y. Yamguchi. 2014. Commercial-scale culturing of cyanobacteria: an industrial experience. Pages 293–318 *in* K. N. Sharma, A. K. Rai, and J. L. Stal, editors. Cyanobacteria: An Economic Perspective. First edit. John Wiley & Sons, Ltd., Oxford, UK.

Thiet, R. K., R. E. J. Boerner, M. Nagy, and R. Jardine. 2005. The effect of biological

soil crusts on throughput of rainwater and N into Lake Michigan sand dune soils. Plant and Soil 278:235–251.

Thomazo, C., E. Couradeau, and F. Garcia-Pichel. 2018. Possible nitrogen fertilization of the early Earth Ocean by microbial continental ecosystems. Nature Communications 9.

Ullmann, L., and B. Büdel. 2001. Ecological Determinants of Species Composition of Biological Soil Cruts on a Landscape Scale.pdf. Pages 203–213 *in* J. Belnap and O. L. Lange, editors. Biological Soil Cruts: Structure, Function, and Management. 1st edition. Springer-Verlag, Berlin.

Verrecchia, E., A. Yair, G. J. Kidron, and K. Verrecchia. 1995. Physical properties of the psammophile cryptogamic crust and their consequences to the water regime of sandy softs, north-western Negev Desert, Israel. Journal of Arid Environments:427–437.

Wang, W., Y. Liu, D. Li, C. Hu, and B. Rao. 2009. Feasibility of cyanobacterial inoculation for biological soil crusts formation in desert area. Soil Biology and Biochemistry 41:926–929.

Wang, W., C. Yang, D. Tang, D. Li, Y. Liu, and C. Hu. 2007. Effects of sand burial on biomass, chlorophyll fluorescence and extracellular polysaccharides of man-made cyanobacterial crusts under experimental conditions. Science in China, Series C: Life Sciences 50:530–534.

Weber, B., M. M. Caldwell, B. Jayne, W. Bettina, B. Büdel, J. Belnap, B. Weber, and B. Büdel. 2016a. Biological Soil Crusts: An Organizing Principle in Drylands. Pages 3–14 Biological Soil Crusts: An Organizing Principle in Drylands. 2nd edition. Springer, Switzerland.

Weber, B., B. Matt, Z. Yuanming, and J. Belnap. 2016b. Natural Recovery of Biological Soil Crusts After Disturbance. Pages 479–498 *in* B. Weber, B. Burkhard, and J. Belnap, editors. Biological Soil Crusts: An Organizing Principle in Drylands. Springer.

Williams, W. J., D. J. Eldridge, and B. M. Alchin. 2008. Grazing and drought reduce cyanobacterial soil crusts in an Australian Acacia woodland. Journal of Arid Environments 72:1064–1075.

Wu, Y., B. Rao, P. Wu, Y. Liu, G. Li, and D. Li. 2013. Development of artificially induced biological soil crusts in fields and their effects on top soil. Plant and Soil 370:115–124.

Xiao, B., Z. Yunge, and S. Ming'An. 2008. Artificial cultivation of biological soil crust and its effects on soil and water conservation in water-wind erosion crisscross region of loess plateau, China.

Yeager, C. M., J. L. Kornosky, R. E. Morgan, E. C. Cain, F. Garcia-Pichel, D. C. Housman, J. Belnap, and C. R. Kuske. 2007. Three distinct clades of cultured heterocystous cyanobacteria constitute the dominant N2-fixing members of biological soil crusts of the Colorado Plateau, USA. FEMS Microbiology Ecology 60:85–97.

Zaady, E., D. J. Eldridge, and M. A. Bowker. 2016. Effects of Local-Scale Disturbance on Biocrusts. Pages 425–445 Biological Soil Crusts: An Organizing Principle in Drylands. 2dn edition. Springer, Switzerland.

Zaady, E., Y. Gutterman, and B. Boeken. 1997. The germination of mucilaginous seeds of Plantago coronopus, Reboudia pinnata, and Carrichtera annua on cyanobacterial soil crust from the Negev Desert. Plant and Soil 190:247–252.

Zhang, B., Y. Zhang, Y. Su, J. Wang, and J. Zhang. 2013. Responses of Microalgal-Microbial Biomass and Enzyme Activities of Biological Soil Crusts to Moisture and Inoculated Microcoleus vaginatus Gradients. Arid Land Research and Management 27:216–230.

Zhang, Y. M., H. L. Wang, X. Q. Wang, W. K. Yang, and D. Y. Zhang. 2006. The microstructure of microbiotic crust and its influence on wind erosion for a sandy soil surface in the Gurbantunggut Desert of Northwestern China. Geoderma 132:441–449.

Zheng, Y., M. Xu, J. Zhao, S. Bei, and L. Hao. 2010. Effects of inoculated Microcoleus vaginatus on the structure and function of biological soil crusts of desert. Biology and Fertility of Soils 47:473–480.

Zhou, X., H. Smith, A. Giraldo Silva, J. Belnap, and F. Garcia-Pichel. 2016. Differential Responses of Dinitrogen Fixation, Diazotrophic Cyanobacteria and Ammonia Oxidation Reveal a Potential Warming-Induced Imbalance of the N-Cycle in Biological Soil Crusts. PloS ONE 11:1–15.

2 – NURSING BIOCRUSTS: ISOLATION, CULTIVATION AND FITNESS TEST OF INDIGENOUS CYANOBACTERIA

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Abstract

Biological soil crusts (biocrust) are microbial communities that develop at the soil surface of drylands and play an important role in erosion control and fertility. Soil surface disturbance from a broad range of natural and human processes (e.g. fire, livestock grazing, off-road traffic) cause significant losses in biocrust cover and associated ecosystems services. Hence, biocrust restoration is emerging as an important intervention strategy to rehabilitate degraded dryland soils. In a multi-step process, we designed protocols for the establishment of "microbial biocrust nurseries" to produce photosynthetic cyanobacterial inoculum for biocrust seeding at scale. We first report on the strategy for isolation, directly from the target site, of a large culture collection of cyanobacteria that included multiple representatives of the five most common biocrust taxa. After genetic pedigreeing of these isolates, we could select those that best matched field populations genetically for scale-up cultivation. We then developed protocols for effective cyanobacterial biomass production to obtain sufficient inoculum. This was followed by conditioning treatments (hardening off) to pre-acclimate this inoculum to the stressful conditions expected in the field. Finally, we show that the inoculum obtained was fit to thrive in its original soil under natural outdoor conditions if sufficient water was available. We repeated this process successfully for four sites, two in the hot Chihuahuan Desert and two in the cooler Great Basin Desert, and on two textural types of soils in each. The cyanobacterial biocrust nursery approach, represents a versatile, viable and safe tool for the rehabilitation of dryland soils.

Key words: Biological soil crusts, Biocrusts, degraded drylands soils, erosion control, cultivation, cyanobacteria, soil microbiome, soil restoration.

Introduction

In drylands, the soil surface can be colonized by microorganisms that form a top crust, known as biological soil crust ('biocrust') (see Garcia-Pichel 2003, for a primer, and Belnap et al. (2016) for a monograph). This microbial mantle provides ecosystem services such as protection from wind (Belnap and Gillette 1997, Zhang et al. 2006) and water erosion (Gaskin and Gardner 2001). Biocrusts also contribute to soil fertility by fixing atmospheric carbon (Elbert et al. 2012; Sancho et al. 2016) and nitrogen (Barger et al. 2016), by exporting significant proportions of both C and N, but also other elements to the soils they cover (Thiet et al. 2005; Johnson et al. 2007; Thomazo et al. 2018; Beraldi-Campesi et al. 2009), and by trapping dust particles (Reynolds et al. 2001). Unfortunately, biocrusts are very susceptible to trampling associated with human activities (Belnap and Eldridge 2001, Zaady et al. 2016). Various forms of global environmental change impose additional stresses (Evans et al. 2001, Reed et al. 2012, Fernandes et al. 2018). Losses in biocrust soil cover due to those stressors logically result in concomitant losses of ecosystem services (Belnap and Eldridge 2001, Barger et al. 2006).

To reverse these deleterious effects, there is a clear need to develop biocrust restoration approaches (reviewed by (Bowker 2007)). The early approach of "transplanting" existing biocrusts to promote recovery of crust-less areas showed that the approach is possible (Belnap 1993, Buttars et al. 1998, Maestre et al. 2006). But harvesting of intact biocrusts to aid in the recovery of damaged crusts represented a conservationist's Ponzi scheme that cannot be scaled-up sustainably. Thus, a means to create inoculum was clearly needed. For this, two alternative approaches are being

explored: laboratory production of cultivated biocrust organisms, and mixed-community nursery-based biocrust rearing. Although significant advances have been achieved, a standard manual for the successful production of fit, high quality biocrust inoculum is a work in progress. Cultivation-based production has been reported mostly for cyanobacteria (Buttars et al. 1998, Chen et al. 2006, Wang et al. 2009, Zheng et al. 2010, Lan et al. 2013, Wu et al. 2013, Zhang et al. 2013, Román et al. 2018b), yet technical detail required to cultivate the dominant biocrust cyanobacteria species under laboratory conditions is still needed. Although laboratory production of cultivated biocrust organisms is work intensive, it enables a stringent control of the composition of the inoculum produced. As an alternative to cultivation, mixed-community biocrust rearing represents a fairly non-destructive, culture-independent approach that uses a small amount of remnant biocrust as a seed to grow large amounts of inoculum under greenhouse-based conditions that are experimentally optimized to promote growth (Ayuso et al. 2017); (Antoninka et al. 2016, 2018, Bowker and Antoninka 2016). At least in one occasion, attention was given to keeping microbial composition close to that of the field sites (Ayuso et al. 2017). Independent of the benefits and shortcomings of these two approaches to produce inoculum at a reasonable scale, a common problem is the potential lack of inoculum fitness.

Here, we present a multi-step protocol for large scale cultivation of biomass to restore cyanobacterial biocrusts which describes in detail: 1) methods to obtain pedigreed cultured isolates for five dominant cyanobacterial biocrust community members that match the genetic identity of the natural local populations while avoiding cultivation of non-native microbes, 2) methods for scaling biomass from cultured isolates to larger

amounts for larger scale soil rehabilitation, and 3) tests of the fitness of the inoculum to successfully grow on native soils under field-like conditions.

Methods

Biocrust sources and sampling

Biocrust were from two climatically distinct deserts in the Southwestern U.S, and from two textural types of soil in each (sandy and silty). Sandy (HSN; sandy clay loam, 41.104198°, -113.023194°) and silty (HS; clay loam 41.104211°, -113.008204°) cold sites were at Hill Air Force Base (Great Basin Desert). The sandy hot site (FB; loamy sandy 32.431069°, -105.984151°) was at Fort Bliss Military Base, and the silty hot site (JS; clay loam 32.545580°, -106.723240°) at the Jornada Experimental Range, both in the Chihuahuan Desert. Biocrust samples were collected in 1.5 x 9 cm diameter Petri dishes and kept dry in the dark until further processing. Bulk soils (0 -10 cm depth) were sampled from each site to use in the fitness test.

Microbial community structure

Microbial community structure was determined through next-generation sequencing of 16S rRNA genes. For DNA extraction, from each site three cores (1cm deep, 1 cm in diameter) were randomly taken from each Petri dish and mixed together. DNA was extracted using the PowerSoil® DNA isolation kit from 0.25 g of that mixture. General bacterial primers 515F/806R targeting the 16S rRNA gene V4 region were used for library preparation, where, PCR was performed in triplicate, products pooled, and PCR protocols performed according to Caporaso et al. (2011). 240 ng of PCR product per sample were pooled and cleaned using the QIA Quick PCR Purification kit. DNA library concentration was quantified by qPCR using the ABI Prism® kit, brought to final

concentration of 4 nM, denatured, and diluted again to a final concentration of 4 pM. 180 μL of PhiX (Illumina) at a concentration of 12 pM and 150 μL of buffer HT1 (Illumina) were mixed with 270 μL of the pooled library and loaded in the MiSeq Illumina sequencer, adding custom 16S rRNA sequencing primers (Caporaso et al. 2011) on a paired-ends sequencing flow cell 2 (2 X 150 bp). Paired-end reads obtained were assembled with PANDAseq (Masella et al. 2012). Sequences with a minimum average Phred score of 25 were assigned to the corresponding samples, and barcodes removed using QIIME (Caporaso et al. 2010). Operational taxonomic units (OTUs) were defined with a threshold of 97% similarity and clustered using UCLUST (Edgar 2010). 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. 2007a), and representative sequences were then aligned against the Greengenes database core reference alignment (McDonald et al. 2012). Cyanobacterial OTUs' taxonomic assignment at the genus and species level was further informed throughout phylogenetic placement in our cyanobacteria reference tree version-rc1(https://github.com/FGPLab/cydrasil). Query cyanobacterial sequences were aligned to the reference alignment with PaPaRa (Berger and Stamatakis 2011), placed into the reference tree using the RaxML8 Evolutionary Placement Algorithm (Berger et al. 2011), and visualized on the iTOL 3 server (Letunic and Bork 2016).

Isolation, identification and molecular pedigreeing of cyanobacteria

For the isolation of nitrogen-fixing, scytonemin producing cyanobacteria, small biocrust fragments were placed in minimal liquid medium ($BG11_0$; Allen & Stanier 1998) where atmospheric N_2 was the only nitrogen source available, incubated at either 4, 25 or 35 °C, and Illuminated with 150 – 200 μmol (photon) m^{-2} s^{-1}, in a 14 h photoperiod, for approximately a week. Using forceps and a dissection microscope (NIKON SMZ-U), biomass clumps were separated and subsequently streaked on 1.5 % (w/v) agar-solidified, $BG11_0$ Petri plates, which were then further incubated. Single colonies were re-streaked on fresh agar plates and the process repeated. Once second-streak colonies were large enough, samples were inspected under the compound microscope to establish that they were unialgal and to provide an initial identification based on morphotype. Only then, isolates were given a strain designation and transferred to 20 mL $BG11_0$ liquid culture vessels. For the isolation of motile, filamentous, bundle-forming, non-nitrogen fixing cyanobacteria, we first wetted the biocrust to allow these cyanobacteria to migrate towards soil surface (Pringault and Garcia-Pichel 2004). After 30 minutes, we picked bundles directly from the biocrust surface using fine forceps (TED PELLA, INC No. 5385-7SU) under the dissecting microscope. Bundles were dragged over 2 % (w/v) agar-solidified Jaworski's medium (JM; Schlösser 1982) to remove soil particles and attached bacteria (Garcia-Pichel et al. 2013a), and transferred into 96-well plates containing liquid JM, incubated at 25 ± 2 °C and Illuminated with 20 to 30 μmol (photon) m^{-2} s^{-1} under a 14 h photoperiod. Well plates were covered with Kimwipes to diminish light during the first 24 h of incubation. After a week, drops of fresh medium were added to those wells where growth was evident and eventually transferred to larger vessels (24-well plates). Once enough biomass was observed there, the unialgal status of the enrichment was

confirmed by microscopic inspection, and a preliminary taxonomic assignment was made on the basis of morphology (Table S1). Each compliant isolate received a strain identification code. All isolates with a strain ID were allowed to fully grow and were transferred to new medium for at least three consecutive batches, then examined for consistency and lack of morphologically visible contaminants, before their identity was established by DNA sequencing. In total, we obtained 101 isolates. All are cryopreserved in our laboratory culture collection and are available upon request.

DNA was extracted from isolates using the Soil DNA extraction kit (MoBio), according to the manufacturer's instructions. The V4 region of the 16S rRNA gene was amplified using cyanobacteria-specific primers CYA359F/CYA781R (Nübel et al. 1997), using the PCR protocol provided therein. PCR products were sequenced commercially using Sanger sequencing. Forward and reverse sequences were aligned on Geneious version 8.0 (Kearse et al. 2012), and consensus sequences blasted against GenBank using BLASTN (Zhang et al. 2000) to obtain a taxonomic ID. Isolate sequences are available under the GenBank submission number SUB4485019.

Standard scale-up cultivation

Standard scale-up techniques in liquid medium (Guedes et al. 2014; Takenaka &Yamguchi 2014) were found effective to produce large biomass quantities of all selected isolates of the non-motile, N_2-fixing cyanobacteria. Briefly, stock cultures grown in 300 mL of $BG11_0$ in Erlenmeyer-flasks at 25 ± 2 °C, under a 14 h photoperiod regime, Illuminated at 100 - 180 μmol m^{-2} s^{-1} under agitation at 140 rpm to a biomass of 2 - 4 mg

Chl *a*/L, were moved to a greenhouse, allowed to acclimate for 48 h, and transferred to previously autoclaved 20 L carboys (transparent glass), filled with 14 L of $BG11_0$, at a ratio of 1/20 v/v. Carboys were bubbled with filter-sterilized air and incubated under natural daily variation of light and temperature. Carboys were placed in cooling basins in which water was circulated from a central, shaded reservoir, which helped maintain temperature between 22 and 28 °C. After 30 days, aeration was stopped, biomass settled, and was harvested by vacuuming into a sterile container. Harvested biomass was distributed onto sterile cellulose tissue (Kimwipes), air-dried for 48 h in a laminar flow hood and stored in the dark, at room temperature, under low humidity (15% < RH).

Floating cellulose tissue scale-up

Traditional scale-up techniques (Guedes et al. 2014, Takenaka and Yamguchi 2014), like the ones used to scaled-up N_2-fixing cyanobacteria, resulted in poor or no growth for all of the 33 isolates of the bundle-forming filamentous cyanobacterial cultures (*Microcoleus* spp.). Therefore, we developed an alternative method. Stock cultures were grown and maintained in 1 L Erlenmeyer-flasks containing 200 mL of JM, at 25 ± 2 °C, under a 14 h photoperiod, Illuminated at 100-180 μmol m^{-2} s^{-1}, and agitated at 100 rpm. They were periodically homogenized by repeatedly forcing biomass through a 60 mL sterile syringe (without needle). To scale-up, we inoculated sterile cellulose tissue with stock biomass and incubated it floating on liquid medium inside of large Petri dishes. Working in a laminar flow hood, 14-cm diameter Petri dishes were filled with 60 mL of medium. Small volumes of syringe-homogenized stock cultures were evenly distributed on tissue pieces pre-cut to size, using a cell spreader, and carefully placed in the dish, so that they

remained floating. Dishes were incubated at 25 ± 2 °C, under a 14 h photoperiod, and a light intensity of 20 - 30 µmol m^{-2} s^{-1} for 8 to 14 days, depending on strain. Plates were covered with Kimwipes to dampen light during the first 24 h of growth. To harvest, opened plates were allowed to slowly dry out under the laminar flow hood. Tissue containing the biomass was shredded using an office micro-shredder (previously sterilized with 70% ethanol and 30 minutes of exposure to a germicidal UV lamp). Shredded biomass was then stored at room temperature in the dark and low humidity (15% < RH).

Isolate conditioning (hardening off)

We inoculated open pots with cyanobacterial biomass of five species from each location and soil type, for a total of 20 strains. Pots were filled to 3 cm of sterilized native soil, offering a growth surface of approximately 0.3 m^2 each. Biomass for each isolate was added to the surface of pots containing their respective soil of origin so as to attain a concentration in the range of 0.13 to 8.81 µg Chl *a* per g of soil, most typically around 3 µg Chl *a* g^{-1}, and submitted to a 12-day long series of short-term incubations intended to promote the progressive acclimation of the isolates to the stressful environmental conditions encountered in the field. It included 12 recurrent dry-wet cycles, with the inoculum progressively exposed to increasing light intensity, from 20 to 100 % of full spectrum light, first in a culture room (25 ± 2 °C), second in a greenhouse (mean temperature range: 18-29 °C), and then outdoors (mean temperature range: 13-34 °C). Uninoculated pots filled with autoclaved native soil (from all locations) were used as controls. After this treatment, the conditioned biomass was allowed to completely dry,

and immediately after, it was used in the fitness test.

Fitness test of isolates in outdoor, native soils

Pots containing conditioned biomass were incubated outdoors for 45 days. A first run was performed in May/June 2016 (Spring), and a second on November/December 2017 (Fall). The pots were watered to field capacity with distilled water by wicking from an external container, following Doherty et al. (2015), every three days, which is the average frequency of rain events in the field during the growth season (Sorochkina et al. 2018). Triplicate pots per strain and controls were harvested to determine biomass (as Chl *a*) at each time period (0, 16, 31 and 45). Random microscopic inspection checks were carried out to ensure that growth was not attributable to aeolian contamination (see Sorochkina et al. 2018). The average maximum temperature during the incubation period was 41 and 24 °C, for Spring and Fall, respectively.

Chlorophyll *a* determinations

Chl *a* was measured as a proxy for biomass in all culture experiments, as well as a proxy for phototrophic biomass in natural biocrust and outdoor soil incubations. For natural biocrust and all culture experiments, Chl *a* was extracted according to Castle et al. (2011). For outdoor incubations Chl *a* extracts were obtained after grinding each sample in 90% acetone with mortar a pestle for 3 min, and then transferred to a Falcon tube where the volume was adjusted to 10 mL with 90% acetone and extracted for 24h at 4 °C. Initially we used Castle et al. (2011), but later found grinding to be more powerful for Chl *a* extraction. Extract absorbance spectra were recorded on a Shimadzu UV-1601

spectrophotometer. Interference from scytonemin and carotenoids was discounted using the trichromatic equations of Garcia-Pichel & Castenholz (1991). All determinations were done at least in triplicate, and for tissue cultures nine replicates were used.

Results

A comprehensive oversight flow chart is presented in Figure 1 to help the reader follow the results and discussion sections.

Bacterial community structure in source biocrusts

As typical for biocrusts, the bacterial phyla Cyanobacteria, Proteobacteria, Bacteroidetes, Actinobacteria and Acidobacteria accounted for the majority of the community members (Gundlapally & Garcia-Pichel 2006; Fernandes et al. 2018), with cyanobacteria being the dominant phototrophs (Figure S1). The cyanobacterial community structure (Figure 2) followed the expected, typical composition reported previously for the Southwestern US (Garcia-Pichel et al. 2013a, Fernandes et al. 2018). Bundle forming, non-heterocystous *Microcoleus vaginatus* and *M. steenstrupii* together accounted for more than 60% of the cyanobacterial reads, with *M. vaginatus* being the most abundant cyanobacterium in cold desert locations, while *M. steenstrupii* was dominant in hot desert locations (Garcia-Pichel et al. 2013). The three most typical heterocystous, N_2-fixing cyanobacteria in biocrusts (Yeager et al. 2007) were present in all field sites. *Tolypothrix* spp. was more abundant in the Great Basin locations, while *Scytonema* spp. was the most common in the Chihuahuan samples. The relative abundance of *Nostoc* spp. was similar among cold desert locations, and somewhat variable within hot desert locations.

Enrichment and isolation of cyanobacterial strains

Cyanobacterial community structure determined above (Figure 2) guided our isolation efforts, and we targeted the isolation of the non-motile, N_2-fixing cyanobacteria *Nostoc* spp., *Tolypothrix* spp. and *Scytonema* spp. (hereafter Nostocales) because of their known contribution to nitrogen input rates into the community (Johnson et al. 2005), and the production of the sunscreen-pigment scytonemin (Garcia-Pichel & Castenholz 1991). We also targeted the isolation of the bundle-forming, non-nitrogen fixing filamentous cyanobacteria *M. vaginatus* and *M. steenstrupii* (hereafter *Microcoleus* spp.) as the biocrust pioneer phototrophs (Garcia-Pichel & Wojciechowski 2009) of the studied communities. For the Nostocales, enrichment cultures of biocrust fragments in nitrogen free medium ($BG11_0$), followed by streaking, proved successful. Incubations at different temperatures could be successfully used to enrich differentially for the different genera, since they have different temperature optima for growth (Garcia-Pichel et al. 2013a, Zhou et al. 2016, Muñoz-Martín et al. 2018). This approach yielded cultures invariably at every trial, in a relatively short time (3 to 4 months). We established 16 strains of *Nostoc* spp., 8 of *Tolypothrix* spp. and 14 of *Scytonema* spp. (Fig. 3 C, D and E). For the *Microcoleus* spp. manually picking bundles was the most successful approach. Enrichment cultures, either on agar plates, in liquid culture, or using dilution series, invariably resulted in preferential growth of "weedy" filamentous cyanobacteria (*Trichocoleus* spp.-like, *Leptolyngby*a spp., and *Lyngbya* spp.) not present in large numbers in the communities of origin, so this approach is discouraged. Bundle picking as a source of inoculum, however had 1-3 % success rate only, so it requires a large number of initial trials to guarantee the isolations within a reasonably short time (~ four months). A total of 19 strains of *M. vaginatus*, and 13 of *M. steenstrupii* (Figure 3 A and B) were established. As a result of

our prospecting, we isolated and built what we believe is the first significant biocrust cyanobacteria culture collection, with 101 strains from both cold and hot deserts of the US Southwest (Northern Utah, Southern New Mexico and West Texas). Table S2 provides an overview of cyanobacterial cultures available.

Selection of isolates for scaling-up

We pedigreed our cultures in order to select the most representative isolate of field populations, defined as that most similar to the most abundant cyanobacterial 16S rRNA gene sequence group (or OTU, for operational taxonomic unit) in the site of origin. We used a phylogenetic placement approach as a tool for selection. Figure 4 shows just one example of the phylogenetic placement of field OTUs and laboratory culture sequences for *M. vaginatus* in the sandy soil site of the cold desert. The same procedures were carried out to select one isolate of each of the major cyanobacterial groups *(Microcoleus* spp. and Nostocales) for each location and soil type.

Cyanobacterial biomass production

The next step was to develop feasible approaches to produce enough biomass. All isolates belonging to *Nostoc* spp., *Tolypothrix* spp. and *Scytonema* spp. (non-motile, N_2-fixing cyanobacteria) could be easily scaled-up with standard liquid cultures, in batches of up to 15 L. All of the 38 isolates exhibited robust growth in liquid cultures in standard incubation chambers. Twelve out of 12 strains that were tested in a greenhouse also showed robust growth. For *Nostoc* spp. strains, doubling time (obtained from growth curves based on Chl *a*) ranged from 6 to 11 days, for *Tolypothrix* spp. from 8 to 15 days,

and for *Scytonema* spp. from 8 to 18 days. The final yield of these scaled-up cultures was in the range 0.8 to 1.2 mg Chl *a* L^{-1}, so that principally 1 L of scaled-up inoculum would suffice to inoculate 5-50 m^2 of soil at 5% of the biomass typically found in the biocrusts of origin.

In contrast, isolates of *Microcoleus* spp. submitted to a liquid-culture based scale-up approach, invariably had low yields or no growth at all, even if we used variations in incubation conditions that included light exposure, temperature, nutrient concentration, shaking intensity, or adding glass beads. This was surprising because mass growth of *Microcoleus vaginatus* has been reported in a greenhouse setting (Wang et al. 2009, Zheng et al. 2010), and in open-raceway facilities (Chen et al. 2006, Xie et al. 2007, Wu et al. 2013, Lan et al. 2014, 2015), although no details on cultivation were given, nor any QC of the final product usually reported (with the exception of Lan et al. 2015), but also no reports on cultivation difficulties. In our experiment, all 32 *Microcoleus* spp. isolates tended to rapidly clump together into an irregular mass that ceased to grow. In most cases, clumps remained viable for months but exhibited no further growth. Because of this, we developed fundamentally different approaches for *Microcoleus* strains. Among those, we found that evenly inoculating an artificially homogenized stock culture on cellulose tissue support followed by incubation floating on the medium resulted in fastest growth (see Figure 5 A and B). Similarly, positive results were obtained with *Microcoleus* strains from all locations. Under these conditions, for example, *M. vaginatus* HSN003 grew at exponential rates of 0.47 d^{-1} (Figure 5 C), and *M. vaginatus* FB020 at 0.85 d^{-1}. In the same line, *M. steenstrupii* HS024 grew at exponential rates of 0.31 d^{-1} (Figure 5 D), and *M. steenstrupii* JS010 and 0.73 d^{-1}. More importantly however, the

yield was high, with biomass fully covering the tissue surface within 8-14 days. However, the population would conspicuously turn yellow and crash rather quickly if it was not harvested after the maximum (8-14 days; strain dependent). Typical maximal yields were in the range of 0.20 to 0.64 mg Chl *a* per Petri dish. At this yield, a single plate would suffice to inoculate between 0.2 to 3.3 m^2 of soil (strain dependent) at 5% Chl *a* concentrations of those typical for biocrusts in the field.

Inoculum fitness in native soils outdoors

Single strain biomass mixed with their original soil was used in our fitness test incubated outdoors. The net growth of the inoculum is reported in Table 1. In the Spring run (hotter, drier conditions: May-June), all of the cyanobacterial isolates either died or did not grow, while in the Fall run (mild conditions; November-December), none of the cyanobacterial isolates suffered any significant losses in population size, and most strains showed several doublings in biomass. No photosynthetic biomass growth was observed in control plots. Microscopic observations did not reveal cross contamination among experimental microcosms.

Discussion

We developed and tested protocols to obtain large quantities of biomass of pedigreed cyanobacterial strains for use in the restoration of biocrusts cover in dryland soils and the rehabilitation of its ecosystem services. We succeeded in obtaining isolates that were representative of field populations, in growing and scaling-up the biomass, and in reproducing this approach for different climatic and edaphic settings. Finally, we show that, with a high degree of reproducibility, the inoculum obtained was fit to thrive in its original soil under natural outdoor settings if water was made available and moderate temperatures prevented fast evaporation. All of this supports the notion that quality-controlled laboratory production of cultivated biocrust organisms is a feasible approach to soil crust restoration. However, among the different approaches to produce field inoculant, the laboratory methods detailed here present both advantages and shortcomings.

One advantage of this laboratory-based method is that very little source material was needed relative to approaches based on inoculating with field collected crusts (Belnap 1993, Maestre et al. 2006, Xiao et al. 2008), and thus has negligible impact on existing communities. In this sense, the impact is even less than that of the production of nursery-grown biocrusts out of small quantities of field remnants from the sites to restore (Velasco Ayuso et al. 2017). Developing inoculum under laboratory control until the final phase of hardening ensures that it will match what is found in the field and adventitious microbes are not part of the inoculum formulation. The microbial composition cannot be fully guaranteed using greenhouse-reared mixed biocrusts (i.e. Velasco Ayuso et al. 2016; Sorochkina et al. 2018), or open cultivation systems for either cyanobacteria (i.e.

Chen et al. 2006; Xie et al. 2007; Lan et al. 2014, 2015) or mosses (Antoninka et al. 2016). The tight control on the product, its known genetic pedigree, coupled to the strictly local isolation of cyanobacterial strains ensures that only local genetic stock is introduced to soils. This may in fact represent an important aspect that makes the present approach attractive under stringent regulatory settings (Wozniak et al. 2012). More pragmatically, the traceability of the inoculum to a local source makes it probable that it will be genetically pre-adapted to the conditions of the site, increasing the odds of success. Finally, the fact that different community members are cultivated separately, makes it possible to formulate mixed inoculants to match the rough composition of the communities of origin, or even to use cultivated inoculum to "fortify" greenhouse reared biocrust with respect to important biocrust components.

However, the approach is effort-intensive, at least initially, requiring specialized equipment, techniques and growth facilities. In particular, the establishment of a culture collection from the local site can take significant expertise and time investment. In our case, the complete culture collection used to select the isolates that best matched the field populations took approximately 12 months to complete. Additional expert investment comes from the need to genetically describe the local communities in order to guide cultivation efforts. The need to establish multiple isolates from which to select the most appropriate ones adds a layer of complexity to the process. Of course, once this isolate collection is available, it can principally serve as an established resource for application to the same or neighboring areas; in a way, a culture repository represents an investment in future efforts. These authors realize that the need to implement techniques that are not mainstream in ecological restoration may hamper its widespread application. However,

the risks, foreseeable and unforeseen, posed by proceeding in dryland soil restoration without using stringent and rigorous microbial inoculum quality controls, such as those described here, are simply not to be ignored in good practice.

Another time-consuming step is that of isolate conditioning. This step seems advisable because the harsh conditions expected in the field differ from the much more benign cultivation settings needed to optimize cyanobacterial scale-up. However, hardening treatments seem not to have contributed significantly to field establishment of mosses (Antoninka et al. 2018). Experiments showing with certainty that this additional effort pays off have yet to be formally carried out with cyanobacteria. In any event, the overwhelmingly positive fitness test results (In the Fall, 15 out of 20 strains showed significant growth, none showed no growth), leads us to recommend the procedure, at least until such formal comparisons are available. Differences in inoculation density between Spring and Fall runs (see Table 1) potentially could have played a role in the observed growth of the cyanobacterial isolates during the fitness test at these two inoculation seasons. However, we believe, instead, that the results from this effort also provide confirmation that the season used in inoculation matters (see also: Sorochkina et al. 2018), in that the rather extreme Phoenix heat resulted in population stasis of net population losses in all 20 tested strains in the Spring. Therefore, field inoculations are likely to be most successful at a time when temperatures are moderate, and water is more likely to be available for longer periods upon wetting.

Inoculating solely biocrust pioneers vs. blended biomass mixtures

Biocrusts establishment on bare soils is typically pioneered by motile filamentous cyanobacteria such as *M. vaginatus* or *M. steenstrupii* (Garcia-Pichel and Wojciechowski 2009) in the US Southwest. Other sessile cyanobacteria (Garcia-Pichel et al. 2001, Ullmann and Büdel 2001) will soon follow in their footsteps. Heterotrophic bacteria (Garcia-Pichel et al. 2001, Nagy et al. 2005), archaea (Soule et al. 2009b), and fungi (Bates and Garcia-Pichel 2009) will develop on the cyanobacterial leaked organics (Baran et al. 2015). Mosses and/or lichens (Belnap 2001, Ullmann and Büdel 2001) may eventually colonize some locations. Hence, in order to promote biocrust restoration one should make sure that pioneer organisms are included in the inoculum formulation, and if only one type is included, it should be this type. This idea has driven previous rehabilitation efforts in China (Chen et al. 2006, Wang et al. 2009, Zheng et al. 2010, Zhang et al. 2013) and we also initiated the work with a focus on obtaining isolates of those bundle-forming filamentous forms that were dominant at our sites. The unexpected difficulties we encountered to isolate and scale-up the production of these organisms likely speak to a high degree of biological specialization to the habitat that requires finely tuned regulation of desiccation resistance and damage repair (Rajeev et al. 2013), behavioral motility responses to the pulsed nature of water availability (Pringault and Garcia-Pichel 2004), and the mysterious formation of supra-cellular rope-like dynamic structures that enable soil stabilization (Garcia-Pichel & Wojciechowski 2009). All of this speaks to an inability of soil *Microcoleus* spp. to grow well in liquid culture as a planktonic cyanobacterium would. In hindsight, one has to wonder if adventitious photosynthetic microbes are not what's behind the very high yields of the alleged *Microcoleus* spp. inoculum obtained in open-raceway liquid cultivation systems reported

in the literature (Chen et al. 2006, Xie et al. 2007, Wu et al. 2013, Lan et al. 2014). Whatever the case, establishing the proven presence of sufficient numbers of *Microcoleus* spp. in biocrust inoculum should likely become a primary goal of quality control procedures in the future.

The focus on pioneers should not, however, detract from the fact that blended species mixtures may in principle offer a more robust inoculum and promote faster succession dynamics. Although there is no evidence for it, it is a possibility that microbe-microbe interactions could play a role in the fitness of bacteria in crust. Addition of heterocystous cyanobacteria (*Nostoc, Scytonema, Tolypothrix*) will likely contribute to the rates of nitrogen input into the community (Johnson et al. 2005), to the temperature conditioning through albedo changes and to the protection of the entire community from UV radiation damage due to the synthesis of sunscreens (Couradeau et al. 2016). This comes with relatively little added effort, since biocrusts heterocystous cyanobacteria are easy to isolate and cultivate, at least in comparison to *Microcoleus* spp.

Tables

Table 1. Growth rate of biocrust cyanobacterial isolates incubated for 45 days in outdoor, native soils. Spring and Fall runs were conducted under severe (May-June, 2016; min. temperature: 15 °C, max temperature: 47 °C, mean temperature: 32 °C) and moderate (November-December, 2017; min temperature: 11 °C, max. temperature: 28 °C, mean temperature: 18 °C) environmental conditions, respectively. Strain denominations include coding for the site of origin (HS: cold desert clay loam soil; HSN: cold desert sandy clay loam soil; JS: warm desert clay loam soil; FB: warm desert loamy sandy soil). Doubling times were calculated from initial and final chlorophyll *a* levels under the assumption of exponential growth model. ∗ Denotes significant differences between initial and final biomass density (fold change). All data was assessed for normality and homogeneity of variance, and either a t-test or a Wilcox test was run accordingly.

		Spring (May-June)			**Fall (Nov-Dec)**		
Species	**Strain**	Inoculation density (mg Chl *a*/ m^2)	Stationary density (mg Chl *a*/ m^2)	**Fold-change**	Inoculation density (mg Chl *a*/ m^2)	Stationary density (mg Chl *a*/ m^2)	**Fold-change**
M. vaginatus	HSN003	1.7 ± 0.5	0.3 ± 0.1	0.17*	9.9 ± 1.9	29.6 ± 4.8	3.0*
	HSN015	0.5 ± 0.1	0.2 ± 0.2	0.33	16.7 ± 2.4	63.6 ± 16.8	3.8∗
	JS001	0.7 ± 0.2	1.01 ± 0.4	0.30	0.7 ± 0.2	1.0 ± 0.5	1.4
	FB020	0.1 ± 0.0	0.2 ± 0.1	1.70	3.6 ± 1.0	7.4 ± 2.7	2.0*
M. steenstrupii	HS024	0.3 ± 0.0	0.1 ± 0.0	0.24*	14.0 ± 1.9	59.1 ± 12.7	4.2*
	HSN002	0.4 ± 0.1	0.1 ± 0.1	0.4*	10.4 ± 2.0	40.8 ± 9.2	3.9*
	JS010	1.3 ± 0.3	0.2 ± 0.0	0.17*	1.0 ± 0.2	1.1 ± 0.3	1.1
	FB015	0.8 ± 0.1	0.1 ± 0.0	0.18*	3.9 ± 1.2	7.2 ± 1.7	1.8
Nostoc spp.	HS004	1.2 ± 0.3	0.2 ± 0.1	0.19*	14.6 ± 2.3	52 ± 5.5	3.5∗
	HSN008	2.1 ± 1.0	0.3 ± 0.1	0.14	5.8 ± 1.2	28.5 ± 11.5	4.9∗
	FB025	0.5 ± 0.0	0.1 ± 0.1	0.22*	2.2 ± 0.2	6.2 ± 1.7	2.8∗
	FB023	0.4 ± 0.2	0.0 ± 0.0	0.01	0.5 ± 0.1	3.5 ± 1.4	6.6∗
	HS042	1.7 ± 1.1	0.1 ± 0.0	0.05	11.3 ± 1.1	50.8 ± 4.8	4.5∗

Tolypothrix spp.	HSN030	0.5 ± 0.0	0.3 ± 0.3	0.65	8.2 ± 3.1	35.3 ± 20.6	4.3∗
	JS100	0.6 ± 0.1	0.2 ± 0.0	0.25*	1.6 ± 0.5	3.5 ± 1.7	2.2
	FB100	0.3 ± 0.0	0.1 ± 0.0	0.23*	1.4 ± 0.5	12.7 ± 2.6	9.2∗
Scytonema spp.	HS006	0.5 ± 0.0	0.0 ± 0.1	0.03*	12.2 ± 1.9	45.6 ± 4.8	3.8∗
	HSN006	0.5 ± 0.0	0.3 ± 0.1	0.74	6.3 ± 1.7	20.0 ± 2.1	3.2∗
	JS009	0.3 ± 0.0	0.3 ± 0.2	1.16	1.3 ± 0.8	6.6 ± 2.0	5.1∗
	FB005	0.4 ± 0.0	0.2 ± 0.1	0.44*	3.0 ± 0.3	6.9 ± 2.3	2.3

Figures

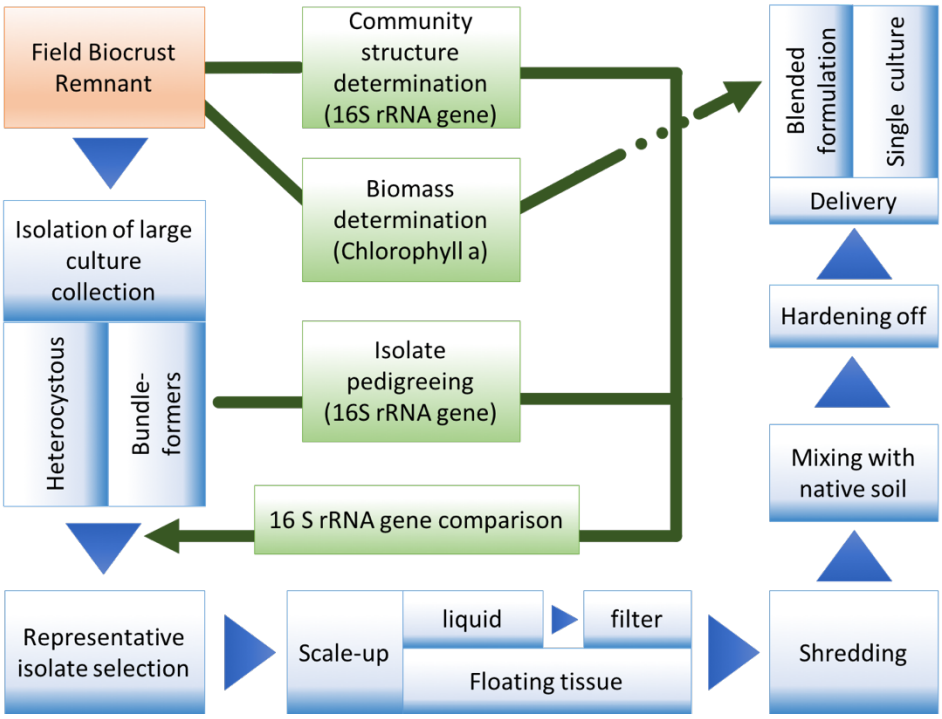

Figure 1. General protocol for the establishment of culture-based cyanobacterial biocrust nurseries. Blue arrows and boxes represent action flow. Green arrows and boxes represent information flow.

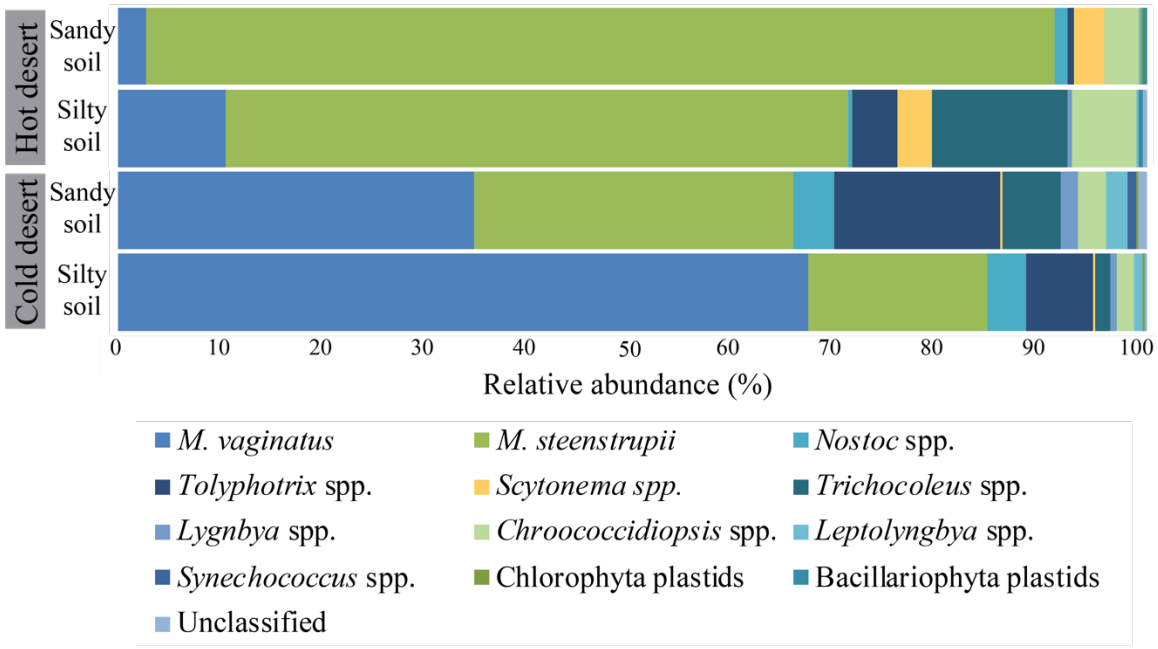

Figure 2. Cyanobacterial community structure for each of the four field locations as determined by amplicon sequencing and bioinformatic analyses of 16S rRNA gene of community DNA resolved to the genus/species level.

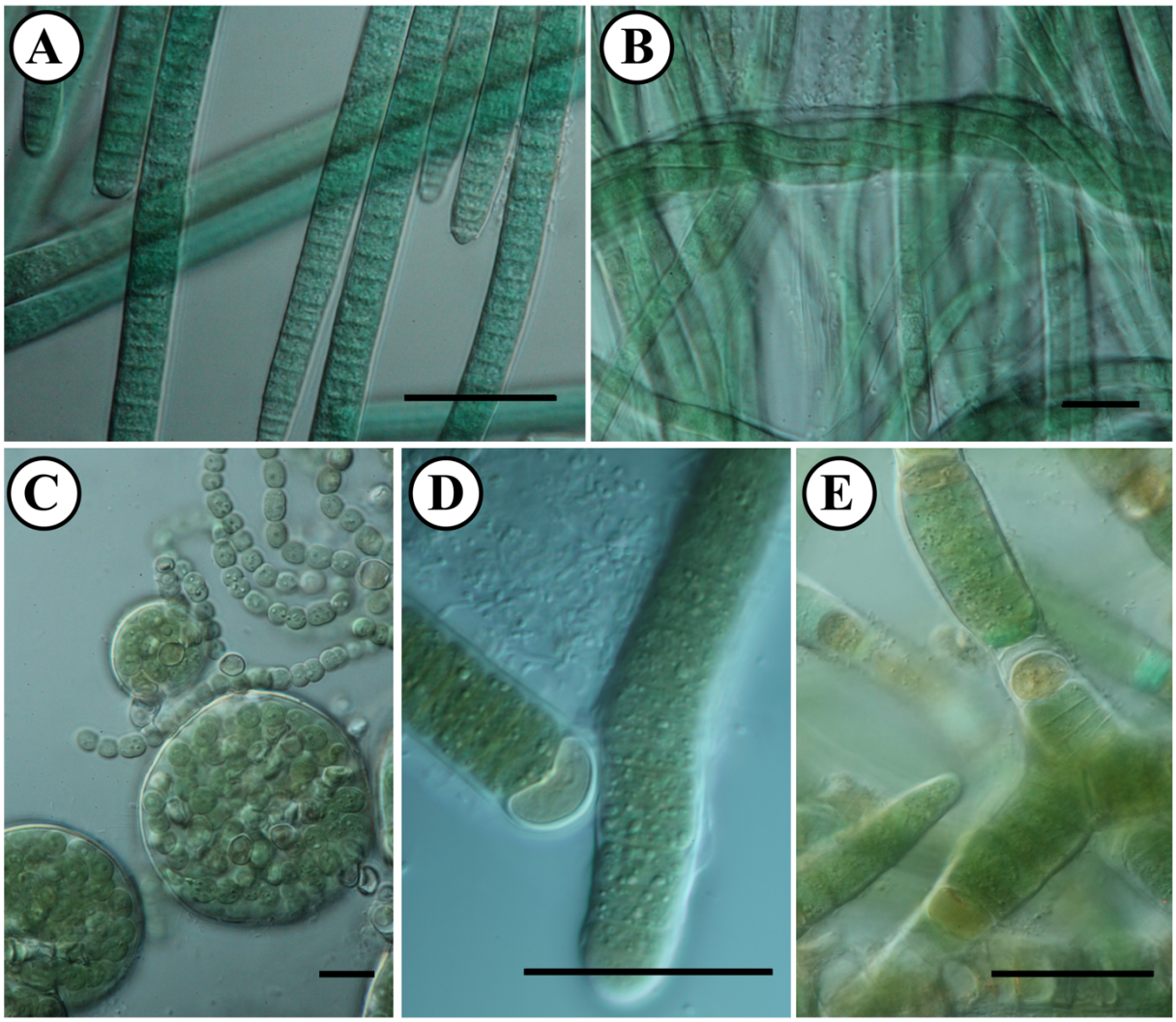

Figure 3. Typical photomicrographs of isolates of the five most common cyanobacteria in biocrust communities in the studied locations, showing their typical morphology that allows for initial preliminary classification. Bars are 20 μm. A: *Microcoleus vaginatus*, B: *Microcoleus steenstrupii*, C: *Nostoc* spp., D: *Tolypothrix* spp., and E: *Scytonema* spp.

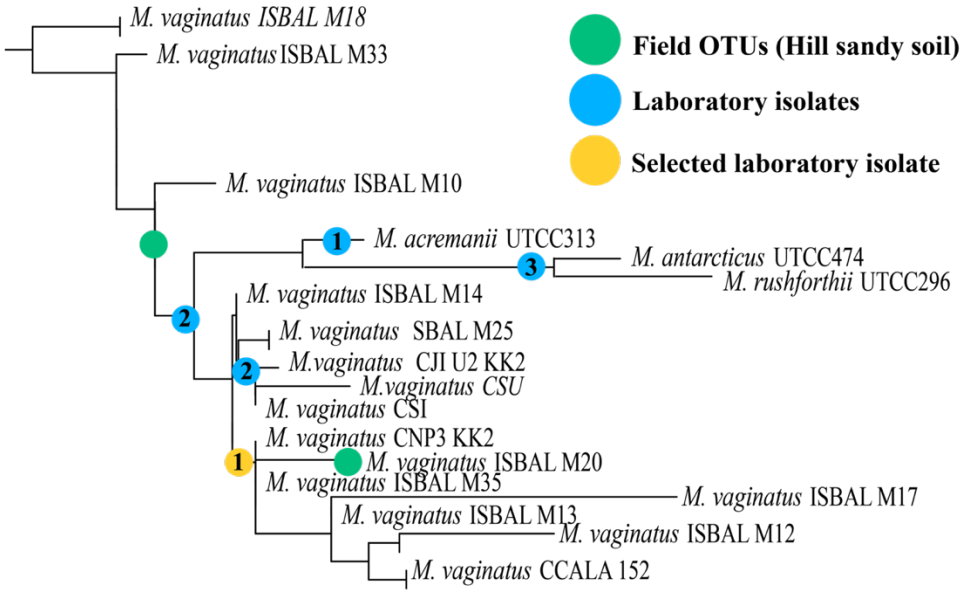

Figure 4. Phylogenetic placement on a reference cyanobacterial tree of field sequence groups (OTUs; operational taxonomic units) and pedigreed laboratory isolates of *M. vaginatus* from the sandy soil location in the cold desert. The tree was constructed with full sequences available in public databases. Figure displays a zoom-in of the *M. vaginatus* clade. On it, green circles represent algorithm placement of field OTUs, and blue circles the placement of laboratory isolate sequences. Digits inside circles indicate the number of isolates placed in a particular node. The yellow circle represents the isolate chosen for scale up and fitness tests. In this case, one of the nine available cultures of *M. vaginatus* isolated from this location, was clearly most representative and thus chosen to scale-up

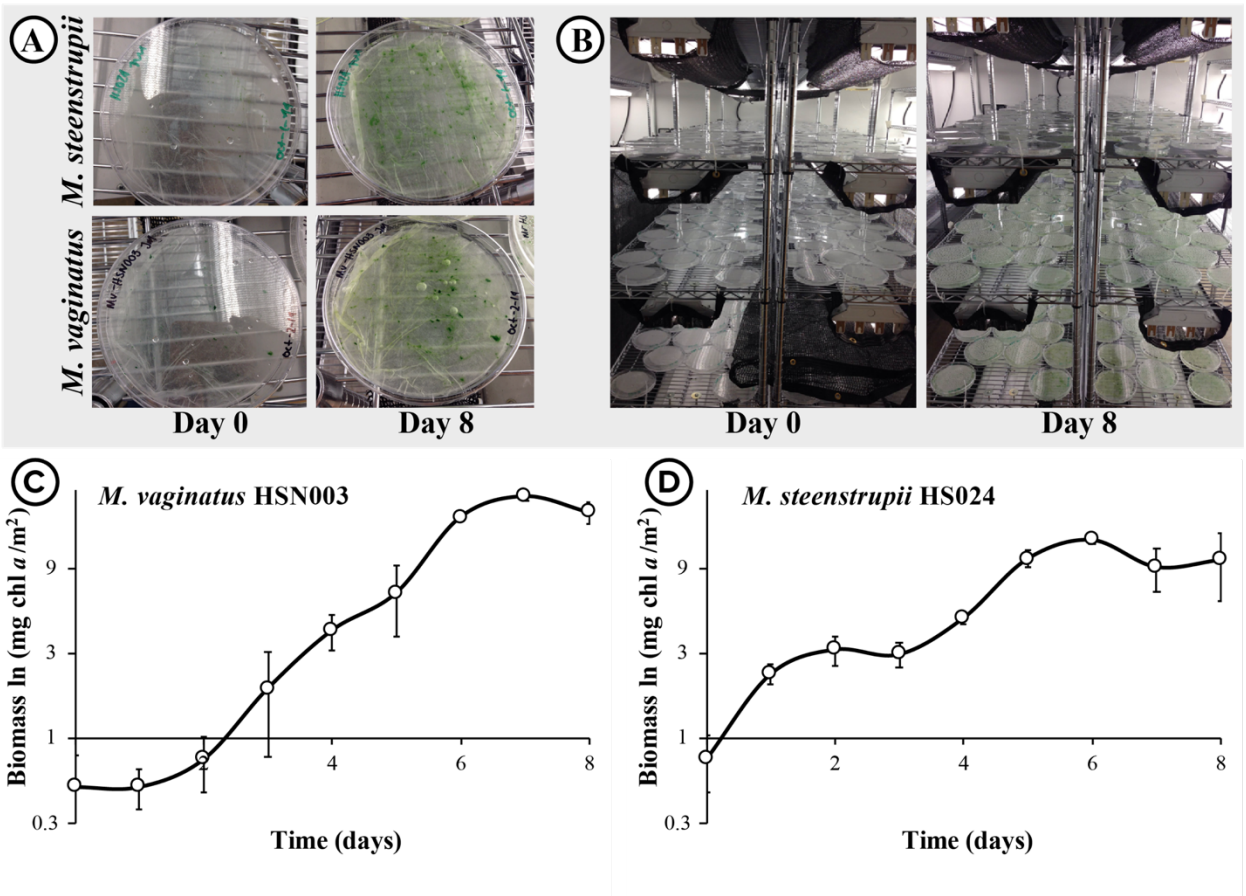

Figure 5. Growing *M. vaginatus* and *M. steenstrupii* with the floating cellulose tissue technique. A: visual aspects of set up and growth. B: scale-up. C and D: growth dynamics showing exponential growth and maximum yields, *M. vaginatus* HSN003 (C; isolated from the cold desert sandy soil) and *M. steenstrupii* HS024 (D; isolated from the cold desert silty soil). Error bars indicate ± 1 SE.

Supplementary information

Supporting Tables

Table S1. Morphological description of the five most common cyanobacteria of biological soil crust communities in the Southwestern US.

Species	Morphological description	Reference
Microcoleus vaginatus	Motile filaments, unconstricted at the cross-walls, with cells tapering towards the end, 4.0-6.0 μm wide. Cells shorter than wide, commonly 2-5 μm long. End cells can be rounded, conical, or with calyptra.	Boyer et al (2002)
Microcoleus steenstrupii	Motile filaments, constricted at the cross-walls, 4.0-5.5 μm wide. Cell size vary from 3.5-9 μm long. End cells commonly elongated, can also be rounded, without calyptra.	Boyer et al (2002)
Nostoc spp.	Untapered trichomes with conspicuous constrictions at cross-walls, 2-8 μm wide. Cells are cylindrical, spherical or ovoid. Heterocytes are intercalary, solitary. Common to observe as a confluent gel holding masses of trichomes together, often in the form of massive thallus which may be spherical, ovoid, or of a less discernable shape.	Bergey et al (1974)
Tolypothrix spp.	Trichomes are uniseriate with one or several basal heterocytes and free apical ends, sheathed, false branching, Cells slightly	Barrendero et al (2001)

	longer or shorter than wide (from 9-14 μm to 5-14 μm long).	
Scytonema spp.	Trichomes are uniseriate, sheathed, constricted at cross-walls, with false branches, 2-20 μm. Cells may be longer or shorter than wide. Heterocytes intercalary, solitary, cylindrical or barrel-shape.	Bergey et al (1974)

Table S2. Number of cyanobacterial isolates obtained from major biocrust cyanobacteria organized by site of origin.

Cyanobacterial strains	Cold desert Silty soil	Cold desert Sandy soil	Hot desert Silty soil	Hot desert Sandy soil
Microcoleus vaginatus	7	9	2	2
Microcoleus steenstrupii	4	3	3	3
Microcoleus sociatus	1	4		
Nostoc spp.	8	3	3	2
Tolypothrix spp.	2	2	2	2
Scytonema spp.	5	2	2	5
Pseudanabaena spp.	1			
Trichocoleus spp.	15			
Leptolyngbya spp.		2	6	
Lyngbya spp.			1	

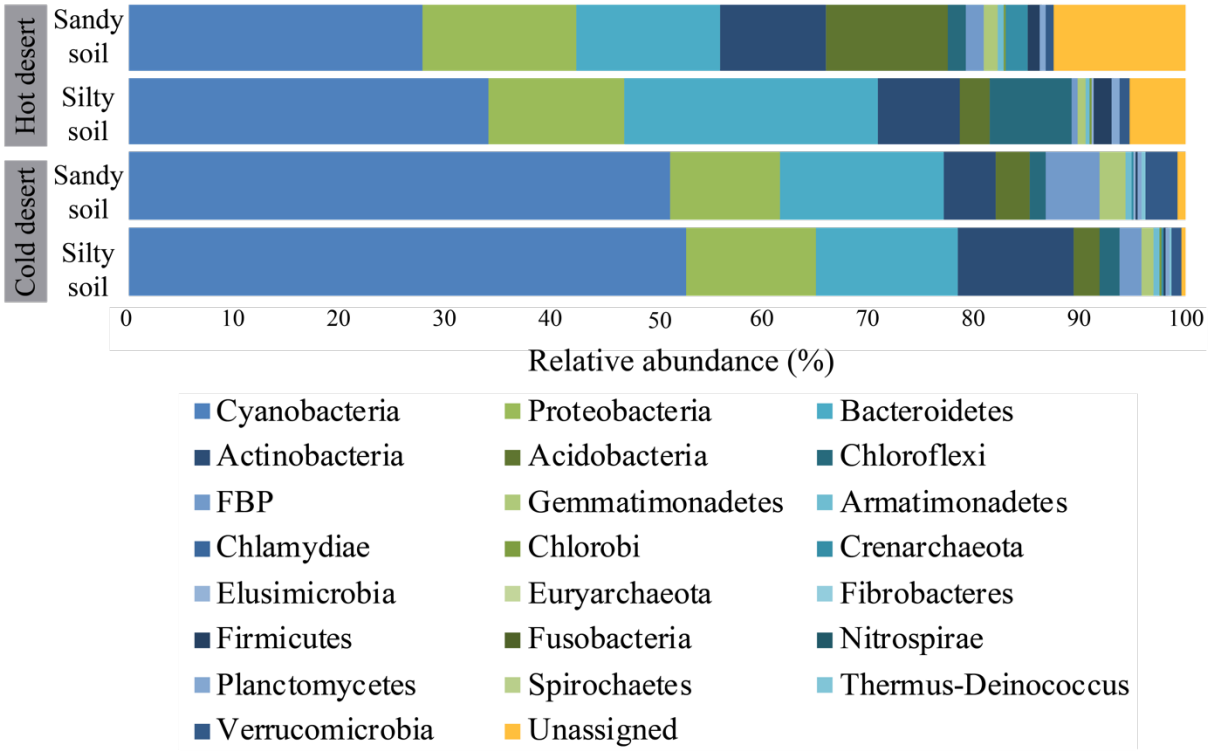

Figure S1. Bacterial community structure at the Phylum level for each of the four field locations as determined by amplicon sequencing and bioinformatic analyses of 16S rRNA gene of community DNA.

References

Allen, M. M., and R. Stanier. 1998. Growth and Division of Some Unicellular Blue-green Algae. J.gen.Microbiol. 51:1998–1998.

Antoninka, A., M. A. Bowker, P. Chuckran, N. N. Barger, S. Reed, and J. Belnap. 2018. Maximizing establishment and survivorship of field-collected and greenhouse-cultivated biocrusts in a semi-cold desert. Plant and Soil 429:213–225.

Antoninka, A., M. A. Bowker, S. C. Reed, and K. Doherty. 2016. Production of greenhouse-grown biocrust mosses and associated cyanobacteria to rehabilitate dryland soil function. Restoration Ecology 24.

Baran, R., E. L. Brodie, J. Mayberry-Lewis, E. Hummel, U. N. Da Rocha, R. Chakraborty, B. P. Bowen, U. Karaoz, H. Cadillo-Quiroz, F. Garcia-Pichel, and T. R. Northen. 2015. Exometabolite niche partitioning among sympatric soil bacteria. Nature Communications 6:8289.

Barger, N. N., J. E. Herrick, J. Van Zee, and J. Belnap. 2006. Impacts of biological soil crust disturbance and composition on C and N loss from water erosion. Biogeochemistry 77:247–263.

Barger, N. N., B. Webber, F. Garcia-Pichel, E. Zaady, and J. Belnap. 2016. Patterns and Controls on Nitrogen Cycling of Biological Soil Crusts. Pages 257–285 *in* B. Weber, M. M. Caldwell, B. Jayne, W. Bettina, B. Büdel, J. Belnap, B. Weber, and B. Büdel, editors. Biological Soil Crusts: An Organizing Principle in Drylands. 2nd edition. Switzerland.

Bates, S. T., and F. Garcia-Pichel. 2009. A culture-independent study of free-living fungi in biological soil crusts of the Colorado Plateau: Their diversity and relative contribution to microbial biomass. Environmental Microbiology 11:56–67.

Belnap, J. 1993. Recovery rates of cryptobiotic crusts: Inoculant use and assessment methods. The Great Basin Naturalist 53:89–95.

Belnap, J. 2001. Factors Influencing Nitrogen Fixation and Nitrogen Release in Biological Soil Crust. Pages 241–261 *in* J. Belnap and O. L. Lange, editors. Biology and Fertility of Soils. 1st edition. Springer-Verlag, Berlin.

Belnap, J., B. Büdel, and O. L. Lange. 2001. Biological Soil Crust: Characteristics and Distribution. Pages 3–30 Biological Soil Cruts: Structure, Function, and Management. Springer-Verlag, Berlin.

Belnap, J., and D. Eldridge. 2001. Disturbance and Recovery of Biological Soil Crusts. Pages 363–383 *in* J. Belnap and O. Lange, editors. Biological soil cruts: structure, function, and management. Springer, Berlin.

Belnap, J., and D. A. Gillette. 1997. Disturbance of Biological Soil Crusts: Impacts on Potential Wind Erodibility of Sandy Desert Soils in Southeastern Utah. Land Degrad. Develop 8:355–362.

Belnap, J., B. Weber, and B. Büdel. 2016. Biological Soil Crusts as an Organizing Principle in Drylands. Pages 3–14 Biological Soil Crusts: An Organizing Principle in Drylands. 2nd edition. Springer, Switzerland.

Berger, S. A., and A. Stamatakis. 2011. Aligning short reads to reference alignments and trees. Bioinformatics 27:2068–2075.

Bowker, M. A. 2007. Biological soil crust rehabilitation in Theory and Practice - an Underexploited Opportunity. Restoration Ecology 15:13–23.

Bowker, M. A., and A. J. Antoninka. 2016. Rapid ex situ culture of N-fixing soil lichens and biocrusts is enhanced by complementarity. Plant and Soil 408:415–428.

Buttars, S. M., L. L. St. Clair, J. R. Johansen, J. C. Sray, M. C. Payne, B. L. Webb, R. E. Terry, B. K. Pendlenton, and S. D. Warren. 1998. Pelletized cyanobacterial soil amendments: Laboratory testing for survival, escapability, and Nitrogen fixation. Arid Soil Research and Rehabilitation 12:165–178.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. Mcdonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight. 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7:335–336.

Castle, S. C., C. D. Morrison, and N. N. Barger. 2011. Extraction of chlorophyll a from biological soil crusts: A comparison of solvents for spectrophotometric determination. Soil Biology and Biochemistry 43:853–856.

Chen, L., Z. Xie, C. Hu, D. Li, G. Wang, and Y. Liu. 2006. Man-made desert algal crusts as affected by environmental factors in Inner Mongolia, China. Journal of Arid Environments 67:521–527.

Couradeau, E., U. Karaoz, H. C. Lim, U. Nunes da Rocha, T. Northen, E. Brodie, and F. Garcia-Pichel. 2016. Bacteria increase arid-land soil surface temperature through the production of sunscreens. Nature Communications 7:10373.

Doherty, K. D., A. J. Antoninka, M. A. Bowker, S. Velasco Ayuso, and N. C. Johnson. 2015. A Novel Approach to Cultivate Biocrusts for Restoration and Experimentation. Ecological Restoration 33:13–17.

Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461.

Elbert, W., B. Weber, S. Burrows, J. Steinkamp, B. Büdel, M. O. Andreae, and U. Pöschl. 2012. Contribution of cryptogamic covers to the global cycles of carbon and nitrogen. Nature Geoscience 5:459–462.

Evans, R. D., J. Belnap, F. Garcia-Pichel, and S. L. Phillips. 2001. Global Change and Future of Biological Soil Crusts. Pages 417–429 *in* J. Belnap and O. L. Lange, editors. Biological Soil Crusts: Structure, Function, and Management. Springer, Berlin, Heindelberg.

Fernandes, V. M. C., N. M. Machado de Lima, D. Roush, J. Rudgers, S. L. Collins, and F. Garcia-Pichel. 2018. Exposure to predicted precipitation patterns decreases population size and alters community structure of cyanobacteria in biological soil crusts from the Chihuahuan Desert. Environmental Microbiology 20:259–269.

Garcia-Pichel, F. 2003. Desert Environments: Biological Soil Crusts. Pages 1019–1023 *in* G. Bitton, editor. Encyclopedia of Environmental Microbiology 6 volume. Set. Wiley-Interscience, New York, NY.

Garcia-Pichel, F., and R. W. Castenholz. 1991. Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. Journal of Phycology 409:395–409.

Garcia-Pichel, F., A. López-Cortés, and U. Nübel. 2001. Phylogenetic and Morphological Diversity of Cyanobacteria in Soil Desert Crusts from the Colorado Plateau. Appl. Environ. Microbiol. 67:1902–1910.

Garcia-Pichel, F., V. Loza, Y. Marusenko, P. Mateo, and R. M. Potrafka. 2013. Temperature Drives the Continental-Scale Distribution of Key Microbes in Topsoil Communities. Science 340:1574–1577.

Garcia-Pichel, F., and M. F. Wojciechowski. 2009. The evolution of a capacity to build supra-cellular ropes enabled filamentous cyanobacteria to colonize highly erodible substrates. PloS ONE 4:7801.

Gaskin, S., and R. Gardner. 2001. The role of cryptogams in runoff and erosion control on Bariland in the Nepal middle hills of the Southern Himalaya. Earth Surface Processes and Landforms 26:1303–1315.

Guedes, C., N. G. Katkam, J. Varela, and F. X. Malcata. 2014. Photobioreactors for cyanobacterial culturing. Pages 271–292 *in* K. N. Sharma, K. R. Ashwani, and J. L. Stal, editors. Cyanobacteria: an economic perspective. First edit. John Wiley & Sons, Ltd., Oxford, UK.

Johnson, S. L., C. R. Budinoff, J. Belnap, and F. Garcia-Pichel. 2005. Relevance of ammonium oxidation within biological soil crust communities. Environmental Microbiology 7:1–12.

Johnson, S. L., S. Neuer, and F. Garcia-Pichel. 2007. Export of nitrogenous compounds due to incomplete cycling within biological soil crusts of arid lands. Environmental microbiology 9:680–9.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Meintjes, and A. Drummond. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649.

Lan, S., L. Wu, D. Zhang, and C. Hu. 2013. Assessing Level of Development and Successional Stages in Biological Soil Crusts with Biological Indicators. Microbial Ecology 66.

Lan, S., L. Wu, D. Zhang, and C. Hu. 2015. Effects of light and temperature on open cultivation of desert cyanobacterium Microcoleus vaginatus. Bioresource Technology 182:144–150.

Lan, S., Q. Zhang, L. Wu, Y. Liu, D. Zhang, and C. Hu. 2014. Artificially accelerating the reversal of desertification: Cyanobacterial inoculation facilitates the succession of vegetation communities. Environmental Science and Technology 48:307–315.

Letunic, I., and P. Bork. 2016. Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. Nucleic Acids Research 44:242–245.

Maestre, F. T., N. Martín, B. Díez, R. López-Poma, F. Santos, I. Luque, and J. Cortina. 2006. Watering, fertilization, and slurry inoculation promote recovery of biological crust function in degraded soils. Microbial ecology 52:365–377.

Masella, A. P., A. K. Bartram, J. M. Truszkowski, D. G. Brown, and J. D. Neufeld. 2012. PANDAseq: paired-end assembler for Illumina sequences. BMC bioinformatics 13:31.

McDonald, D., M. N. Price, J. Goodrich, E. P. Nawrocki, T. Z. DeSantis, A. Probst, G. L. Andersen, R. Knight, and P. Hugenholtz. 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. The ISME journal 6:610–8.

Muñoz-Martín, M. Á., I. Becerra-Absalón, E. Perona, L. Fernández-Valbuena, F. Garcia-Pichel, and P. Mateo. 2018. Cyanobacterial biocrust diversity in Mediterranean ecosystems along a latitudinal and climatic gradient. New Phytologist.

Nagy, M. L., A. Pérez, and F. Garcia-Pichel. 2005. The prokaryotic diversity of biological soil crusts in the Sonoran Desert (Organ Pipe Cactus National Monument, AZ). FEMS Microbiology Ecology 54:233–245.

Nübel, U., G. Muyzer, F. Garcia-Pichel, and G. Muyzer. 1997. PCR primers to amplify 16S rRNA genes from cyanobacteria PCR Primers to Amplify 16S rRNA Genes from Cyanobacteria. Microbiology 63:3327–3332.

Pringault, O., and F. Garcia-Pichel. 2004. Hydrotaxis of cyanobacteria in desert crusts. Microbial ecology 47:366–73.

Rajeev, L., U. N. Da Rocha, N. Klitgord, E. G. Luning, J. Fortney, S. D. Axen, P. M. Shih, N. J. Bouskill, B. P. Bowen, C. A. Kerfeld, F. Garcia-Pichel, E. L. Brodie, T. R. Northen, and A. Mukhopadhyay. 2013. Dynamic cyanobacterial response to hydration and dehydration in a desert biological soil crust. The ISME Journal:2178–2191.

Reed, S. C., K. K. Coe, J. P. Sparks, D. C. Housman, T. J. Zelikova, and J. Belnap. 2012. Changes to dryland rainfall result in rapid moss mortality and altered soil fertility. Nature Climate Change 2:752–755.

Reynolds, R., J. Belnap, M. Reheis, P. Lamothe, and F. Luiszer. 2001. Aeolian dust in Colorado Plateau soils: nutrient inputs and recent change in source. Proceedings of the National Academy of Sciences of the United States of America 98:7123–7.

Román, J. R., B. Roncero-Ramos, S. Chamizo, E. Rodríguez-Caballero, and Y. Cantón. 2018. Restoring soil functions by means of cyanobacteria inoculation: Importance of soil conditions and species selection. Land Degradation and Development 29:3281–3193.

Sancho, L. G., J. Belnap, C. Colesie, J. Raggio, and B. Weber. 2016. Carbon Budgets of Biological Soil Crusts at Micro-, Meso-, and Global Scales. Pages 287–304 Biological Soil Crusts: An Organizing Principle in Drylands. Second. Springer, Switzerland.

Schlösser, U. G. 1982. Sammlung von algenkulturen. Ber Dtsch Bot Ges 95:181–276.

Sorochkina, K., S. Velasco Ayuso, and F. Garcia-Pichel. 2018. Establishing rates of lateral expansion of cyanobacterial biological soil crusts for optimal restoration. Plant and soil 429:199–211.

Soule, T., K. Palmer, Q. Gao, R. M. Potrafka, V. Stout, and F. Garcia-Pichel. 2009. A comparative genomics approach to understanding the biosynthesis of the sunscreen scytonemin in cyanobacteria. BMC genomics 10:336.

Takenaka, H., and Y. Yamguchi. 2014. Commercial-scale culturing of cyanobacteria: an industrial experience. Pages 293–318 *in* K. N. Sharma, A. K. Rai, and J. L. Stal, editors. Cyanobacteria: An Economic Perspective. First edit. John Wiley & Sons, Ltd., Oxford, UK.

Thiet, R. K., R. E. J. Boerner, M. Nagy, and R. Jardine. 2005. The effect of biological

soil crusts on throughput of rainwater and N into Lake Michigan sand dune soils. Plant and Soil 278:235–251.

Thomazo, C., E. Couradeau, and F. Garcia-Pichel. 2018. Possible nitrogen fertilization of the early Earth Ocean by microbial continental ecosystems. Nature Communications 9.

Ullmann, L., and B. Büdel. 2001. Ecological Determinants of Species Composition of Biological Soil Cruts on a Landscape Scale.pdf. Pages 203–213 *in* J. Belnap and O. L. Lange, editors. Biological Soil Cruts: Structure, Function, and Management. 1st edition. Springer-Verlag, Berlin.

Velasco Ayuso, S., A. Giraldo Silva, C. Nelson, N. N. Barger, and F. Garcia-Pichel. 2017. Microbial Nursery Production of High- Quality Biological Soil Crust Biomass for Restoration of Degraded Dryland Soils 83:1–16.

Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73:5261–5267.

Wang, W., Y. Liu, D. Li, C. Hu, and B. Rao. 2009. Feasibility of cyanobacterial inoculation for biological soil crusts formation in desert area. Soil Biology and Biochemistry 41:926–929.

Wozniak, C. A., G. McClung, J. Glagiardi, M. Segal, and K. Matthews. 2012. Regulation of Genetically Engineered Microorganisms Under FIFRA, FFDCA and TSCA. Pages 57–94 *in* C. A. Wozniak and A. McHughen, editors. Regulation of Agricultural Biotechnology: The United States and Canada. Springer, Berlin, Heindelberg, Heidelberg, Germany.

Wu, Y., B. Rao, P. Wu, Y. Liu, G. Li, and D. Li. 2013. Development of artificially induced biological soil crusts in fields and their effects on top soil. Plant and Soil 370:115–124.

Xiao, B., Z. Yunge, and S. Ming'An. 2008. Artificial cultivation of biological soil crust and its effects on soil and water conservation in water-wind erosion crisscross region of loess plateau, China.

Xie, Z., Y. Liu, C. Hu, L. Chen, and D. Li. 2007. Relationships between the biomass of algal crusts in fields and their compressive strength. Soil Biology and Biochemistry 39:567–572.

Yeager, C. M., J. L. Kornosky, R. E. Morgan, E. C. Cain, F. Garcia-Pichel, D. C. Housman, J. Belnap, and C. R. Kuske. 2007. Three distinct clades of cultured heterocystous cyanobacteria constitute the dominant N2-fixing members of biological soil crusts of the Colorado Plateau, USA. FEMS Microbiology Ecology 60:85–97.

Zaady, E., D. J. Eldridge, and M. A. Bowker. 2016. Effects of Local-Scale Disturbance on Biocrusts. Pages 425–445 Biological Soil Crusts: An Organizing Principle in Drylands. 2dn edition. Springer, Switzerland.

Zhang, B., Y. Zhang, Y. Su, J. Wang, and J. Zhang. 2013. Responses of Microalgal-Microbial Biomass and Enzyme Activities of Biological Soil Crusts to Moisture and Inoculated Microcoleus vaginatus Gradients. Arid Land Research and Management 27:216–230.

Zhang, Y. M., H. L. Wang, X. Q. Wang, W. K. Yang, and D. Y. Zhang. 2006. The microstructure of microbiotic crust and its influence on wind erosion for a sandy soil surface in the Gurbantunggut Desert of Northwestern China. Geoderma 132:441–449.

Zhang, Z., S. Schwartz, L. Wagner, and W. Miller. 2000. A Greedy Algorithm for Aligning DNA Sequences. Journal of Computational Biology 7:203–214.

Zheng, Y., M. Xu, J. Zhao, S. Bei, and L. Hao. 2010. Effects of inoculated Microcoleus vaginatus on the structure and function of biological soil crusts of desert. Biology and Fertility of Soils 47:473–480.

Zhou, X., H. Smith, A. Giraldo Silva, J. Belnap, and F. Garcia-Pichel. 2016. Differential Responses of Dinitrogen Fixation, Diazotrophic Cyanobacteria and Ammonia Oxidation Reveal a Potential Warming-Induced Imbalance of the N-Cycle in Biological Soil Crusts. PloS ONE 11:1–15.

3 - EFFECT OF PRECONDITIONING TO THE SOIL ENVIRONMENT ON THE PERFORMANCE OF 20 CYANOBACTERIAL CULTURED STRAINS USED AS INOCULUM FOR BIOCRUST RESTORATION

Submitted to Restoration Ecology

In review. Effect of preconditioning to the soil environment on the performance of 20 cyanobacterial cultured strains used as inoculum for biocrust restoration

Coauthors have acknowledged the use of this manuscript in my dissertation

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Abstract

Biological soil crusts are complex communities of organisms that develop on the top layer of dryland soils where they enhance important ecosystem services, including soil fertility and protection from erosion. Regrettably, a range of human activities such as cattle grazing, off-road driving, hiking, and global warming result in significant deterioration of biocrust cover and their associated services. This scenario has prompted efforts to develop effective biocrust restoration strategies, which often involve the production of biocrust inoculum, both in greenhouse and in laboratory settings. Oftentimes this inoculum is preconditioned in a process of "hardening" at considerable expense and effort in order to improve its fitness under harsh field conditions. But the positive effects of such hardening procedures have yet to be explicitly rigorously demonstrated. Here, we compared the growth performance of 20 cultured strains of biocrust cyanobacteria in outdoor tests on native soils as a function of preconditioning regimes consisting of increasingly high exposure to solar radiation, temperature and Illumination daily variability, and recurrent wet-dry cycles. Preconditioning improved performance in 13 out of 20 strains, particularly among pioneer crust-forming *Microcoleus* spp. (8 out of 8). Improvements were variable among heterocystous strains (3 out of 4 *Scytonema* spp., 2 of 4 *Tolypothrix* spp. and none of 4 *Nostoc* spp.). Based on these results, we recommend the inclusion of preconditioning treatments to increase inoculum survival rate and speed of cyanobacterial biocrust recovery in restoration of dryland soils.

Key words: Biocrusts, cyanobacterial biocrust inoculum, degraded drylands soils, ecological restoration, hardening, preconditioning.

Introduction

Biological soil crusts ('biocrusts') are communities of organisms that, in association with soil particles, develop on the upper layer of soils in arid and semi-arid lands (hereafter drylands; see Garcia-Pichel 2003) where they render important ecosystems services. They are crucial in the protection of soils against wind (Belnap and Gillette 1997, Zhang et al. 2006) and water erosion (Gaskin and Gardner 2001), and contribute to soil fertility by fixing carbon (Elbert et al. 2012, Sancho et al. 2016) and nitrogen (Barger et al. 2016) from the atmosphere. Similarly, they may enrich the soil in other nutrients by trapping dust particles (Reynolds et al. 2001) and lixiviating a large variety of elements down into the soil profile (Beraldi-Campesi et al. 2009). Their presence can also modify soil hydrological dynamics (Verrecchia et al. 1995, Rodríguez-Caballero et al. 2012, Faist et al. 2017), and soil surface temperature (Couradeau et al. 2016). Biocrust communities are vulnerable to natural disturbances such as fire (Bowker et al. 2004, Ford and Johnson 2006), sand deposition (Wang et al. 2007; Rao et al. 2012), and prolonged drought (Williams et al. 2008), and are also to a range of human impacts, especially to compressional forces caused by cattle grazing, construction, foot impact, vehicular traffic, mining, and military training (Belnap and Eldridge 2001, Zaady et al. 2016). Disturbance of these communities can result in a decline or loss of the ecosystem services they provide to drylands (Belnap and Eldridge 2001, Barger et al. 2006), potentially triggering a transition across structural and functional ecological thresholds (Bowker 2007). Soils denuded of biocrusts can become a significant source of atmospheric fugitive dust, which lowers air quality with consequences for public health (Griffin 2007).

Biocrust recovery can take up to hundreds of years in areas where disturbance is high (Belnap 1993; Weber et al. 2016), particularly if biocrust remnants that can serve as natural inoculum are scarce in the surrounding area. Hence, there has been an interest in developing methodologies to produce biocrust inoculum in aid of restoration efforts. These have focused on open-setting production of either lichens and mosses (Antoninka et al. 2016, 2018, Bowker and Antoninka 2016), or mixed cyanobacterial communities (Ayuso et al. 2017). Alternatively, laboratory cultivation of cyanobacterial strains as inoculum has also been pursued (Giraldo-Silva et al. 2019). The fact that biocrust inocula are grown under much milder conditions than those in the field, prompted the adoption of procedures to "harden" the inoculum with preconditioning treatments at the end of the cultivation phase (Antoninka et al. 2018, Giraldo-Silva et al. 2019) as a means to increase its fitness. But the effects, beneficial or not, of these treatments have not been tested rigorously. In fact, for biocrust mosses, such procedures may not necessarily be beneficial (Stark et al. 2012), and comparisons designed to test this explicitly (Antoninka et al. 2018) did not yield usable results because uninoculated controls grew as much as those with inoculum (preconditioned or not). While no comparisons are available for cyanobacteria, preconditioned cyanobacterial strains of the biocrust pioneers *Microcoleus vaginatus* and *M. steenstrupii* and the secondary colonizers *Nostoc* spp., *Tolypothrix* spp., and *Scytonema* spp. all showed robust growth when tested on native soils in outdoor conditions (Giraldo-Silva et al. 2019). Whether it is worth the effort and expense to carry out hardening preconditioning thus remains open. We present a series of experiments designed to assess the potential benefits of preconditioning treatments for cyanobacteria

to grow on native soils in outdoor conditions, and if any such effects are dependent on particular taxa or strains.

Methods

We used pedigreed cyanobacterial strains isolated from biocrusts; isolation techniques, pedigreeing protocols and sampling locations are described in detail in Giraldo-Silva et al. (2019). Briefly, biocrust communities and native soils were obtained from two texturally different soils in the cold Great Basin Desert and the hot Chihuahuan Desert (Great Basin: clay loam and sandy clay loam; Chihuahuan: clay loam and loamy sand). Strains of the bundle-forming, non-nitrogen fixing filamentous cyanobacteria *Microcoleus vaginatus* and *M. steenstrupii* (hereafter *Microcoleus* spp.) were isolated by picking bundles through micromanipulation, whereas isolates of the non-motile, N_2-fixing cyanobacteria *Nostoc* spp., *Tolypothrix* spp. and *Scytonema* spp. (hereafter "Nostocales") were obtained through enrichment in nitrogen free medium ($BG11_0$; Allen & Stanier 1998) followed by streaking. All strains are unicyanobacterial and are kept in our local culture collection and are available upon request.

Experimental organisms and growth conditions

We tested 20 strains, all of which were genetically representative of field populations existing in biocrust communities of the Southwestern US (Garcia-Pichel et al. 2013; Fernandes et al. 2018; Giraldo-Silva et al. 2019). This included four strains per taxonomic type and site of origin. A complete list is in Table S3. Strains were grown in 1 L Erlenmeyer flasks containing 200 mL of minimal medium: Jaworski's (Schlösser 1982) for *Microcoleus* spp. or $BG11_0$ (Allen & Stanier 1968) for Nostocales species. Cultures were maintained at 25 ± 2 °C, under a 14 h photoperiod, Illuminated at 100-200 μmol

photon m^{-2} s^{-1} provided by white fluorescent tubes, and agitated at 100 rpm. *Microcoleus* spp. cultures were periodically homogenized by repeatedly forcing biomass through a 60 mL sterile syringe to avoid biomass clumping, and inhibition of growth.

Inoculation of native soils

Native soils were autoclaved three times, mixing thoroughly between sterilizations, and used to fill open pots to a depth of 3 cm, each pot offering an area of 0.28 m^2. Pots were inoculated with cyanobacterial biomass so as to attain an initial areal concentration in the range of 0.24 to 13.61 mg Chl *a* m^{-2}, most typically around 5 mg Chl *a* m^{-2}. Each cyanobacterial strain was inoculated on its respective soil of origin. Pots constituted independent points and were harvested for analysis throughout the duration of the experiment.

We set-up 480 pots to account for four time points and 20 strains, sampled in triplicate, with 2 treatments (preconditioned cultures and nonconditioned; Figure S2. Additionally, we set up two pots per soil of origin and time point (a total of 32) that were left uninoculated, as controls for spurious growth stemming for aeolian contamination. Pots destined to measure fitness of nonconditioned biomass were inoculated, air-dried and stored dry, in the dark under low humidity (relative humidity < 15%) until use in the outdoor test. Pots destined to test fitness of preconditioned biomass, were inoculated, air dried and immediately subjected to preconditioning treatments.

Preconditioning treatment

Our preconditioning treatment involved the exposure of cyanobacterial biomass embedded in native soil to multiple wet-dry cycles, as well as step-wise increases in light intensity, and a shift from visible-only artificial light to full solar spectral radiation. The preconditioning treatment took place consecutively in three locations. Four dry-wet cycles were in a culture room (25 ± 2 °C, 14 h photoperiod), four in a greenhouse (mean temperature range: 18-29 °C), and four outdoors (mean temperature range: 13-34 °C). At each location, Illumination was progressively increased from 20, to 60 and 100 % of the maximum, which was 100-200 μmol m^{-2} s^{-1} in the culture room and full solar radiation in the greenhouse and outdoors. A detailed schedule for the preconditioning treatment is in Table S4. Pots were manually watered with deionized water to soil saturation every three days, which is close to the average frequency of rain events in the field during the growth season (Sorochkina et al. 2018), receiving additional water as need to ensure continuous hydration throughout the day (7AM to 5 PM), and then allowed to dry completely. Preconditioned biomass was then used immediately in the outdoor test.

Outdoor growth performance

Inoculated pots containing either preconditioned and nonconditioned biomass were incubated outdoors during November-December 2017 (average maximum temperature: 24 °C, typical intensity at solar noon 2500 μmol m^{-2} s^{-1}). Pots were randomly placed to avoid location and watering bias. Irrigation was carried out with a wicking system following Doherty et al. (2015) every three days for 15 cycles. The duration of the experiment was thus 45 days. Triplicate pots per strain and treatment (as well as eight uninoculated controls) were harvested at each of four sampling times (0, 16, 31 and 45

days, or 0, 5, 10 and 15 wet-dry cycles), and randomly inspected by microscopy to ensure that the observed growth was not attributable to aeolian contamination (see Sorochkina et al. 2018), but maintained the morphological characteristics of the strains that had been inoculated in each.

Chlorophyll *a* determination

Chl *a* was measured as a proxy for photosynthetic biomass. The top 3-5 mm of soil mix were collected and ground in 90% acetone with a mortar and pestle for 3 min. Soil-acetone slurries were then transferred to a Falcon tube, where the volume was adjusted to 10 mL with 90 % acetone, vortexed for 30 seconds, and then allowed to sit in the dark for 24h at 4 °C. Extracts were clarified by centrifugation (10 minutes at 8437 g, 15 °C). Absorbance spectra of clarified extracts were recorded on a UV-visible Spectrophotometer (Shimadzu UV-1601). Chlorophyll *a* concentrations were determined correcting for interference from scytonemin and carotenoids using the trichromatic equation of Garcia-Pichel & Castenholz (1991).

Results

In our outdoor test we surveyed the performance of five common and widespread biocrust cyanobacterial community members in the Southwestern US: the biocrust pioneers *M. vaginatus* and *M. steenstrupii* (Garcia-Pichel et al. 2013; Garcia-Pichel & Wojciechowski 2009), and the secondary colonizers *Nostoc* spp.*, Tolypothrix* spp., and *Scytonema* spp. (Yeager et al. 2007; Velasco Ayuso et al. 2016; Giraldo-Silva et sl. 2019). For each cyanobacterium we tested four strains isolated from two different climatic areas (hot and cold deserts) and from two different soil types in each area. Even though the tests were done in open containers, no photosynthetic biomass was detected in any of the uninoculated control plots, ensuring that none of the growth was attributable to allochthonous contamination. In every case, random microscopic examination corroborated that the biomass obtained was attributable to the inoculated strain, and no cross-contamination had occurred. Table 2 presents the short- and long-term growth performance, expressed as fold change in biomass since start of the incubation. Fold change at time *t* was calculated as the ratio of Chl *a* concentration at time *t* ($Chla_t$) to that at time zero ($Chla_0$). Under the assumption of an exponential growth model, fold change can be related directly to standard parameters in microbial growth theory such as generation time (t_D) or instantaneous growth rate (μ): $\log_2 (Chla_t / Chla_0) = t / t_D$, and $\mu = \ln 2 / t_D$.

The temporal dynamics of mean Chl *a* areal concentration (n=3) for each strain during the outdoor test are presented in Figure 6. Most of the cyanobacterial strains showed gains in population size regardless of preconditioning. However, a majority of the cyanobacterial strains displayed more robust growth having undergone the

preconditioning prior to outdoor growth (Table 2). For five out of eight *Microcoleus* spp., and for at least one *Tolypothrix* spp. and *Scytonema* spp. strains, these differences in growth rate were observable as early as 16 days in incubation (indicated as "short-term growth" in Table 2), which corresponds to 5 wetting events. For the sake of standardization of results, we grouped strains according to their responses to preconditioning treatment: positive, indifferent, and negative (Table 2). Any strain displaying increased growth with preconditioning, in either short- or long-term qualified as a positive. Thirteen out of the 20 strains (65% of the strains) responded positively to preconditioning treatments, four (20%) were indifferent, and only three (15%) showed a negative response. Importantly, all the tested *Microcoleus* spp. responded positively. For two strains, *M. vaginatus* JS001 and *M. steenstrupii* JS010, population size may have decreased in the short term, but only preconditioned biomass showed recovery during the long-term growth. The response of particular strains to preconditioned treatments was variable within Nostocales. No positive responses were observed among any of the tested *Nostoc* spp. strains; two of them (FB023 and FB025) responded negatively and the other two (HS004 and HSN008) showed no difference. Two out of four *Tolypothrix* spp. strains (HSN030 and JS100) showed no response, while the other two *Tolypothrix* spp. (HS042 and FB100) showed a positive response. Finally, three *Scytonema* spp. strains exhibited a positive response, and one responded negatively (FB005; Table 2).

Discussion

Cyanobacteria are the early colonizers and dominant organisms of many biological soil crust communities. They (and sometimes algae, mosses) are the main primary producers of the community (Garcia-Pichel et al. 2013; Velasco Ayuso et al. 2016; Fernandes et al. 2018), and are crucial to sustaining community function and therefore, the services biocrust provide to drylands. Our preconditioning of a representative set of biocrust cyanobacteria boosted growth in a majority (13 out of 20) of the strains when grown in their soil of origin under field-like conditions. Although we understand that adding preconditioning protocols to the production chain of cyanobacterial biocrust inoculum represents an additional time investment, our results support the notion that preconditioning will speed the establishment and recovery rates under field conditions. The logic behind the increase in fitness is that a step-wise acclimation of the organisms to the harsh conditions of UV and high visible light intensity exposure, fluctuating and often extreme temperatures, and frequent desiccation episodes will allow the expression of their genetic defense mechanisms in a much more effective manner. In contrast, a sudden, concurrent exposure to environmental stressors that were purposefully avoided during the cultivation period in order to boost biomass production would likely depress growth rates. The genetic responses to hydration and desiccation cycles, for example, are multiple in these organisms and only a few are well understood, but they all require sufficient time for full expression (Rajeev et al. 2013). Examples of such adaptations may be the creation of physical pathways for massive vertical migration in response to pulse hydration events, as is known to occur in some of the filamentous motile cyanobacteria (Pringault and Garcia-Pichel 2004), or the synthesis of UV sunscreen compounds like

scytonemin (Ferreira and Garcia-Pichel 2016) or mycosporine-like amino acids (Gao and Garcia-Pichel 2011), which are typical of the sessile heterocystous forms. The expression of dedicated biochemical pathways to turn polyglucose into compatible solutes, so that rapid osmotic homeostasis can be achieved as cells dry out, may be another important aspect of survival under pulsed activity regimes (Jose et al. 2018).

Interestingly, preconditioning was most effective among strains of the filamentous cyanobacteria, *Microcoleus vaginatus* and *Microcoleus steenstrupii,* that are important as well-known biocrust pioneers (Garcia-Pichel & Wojciechowski 2009). Their stabilization of bare soils allows for later settlement of other community members such as other cyanobacteria (Yeager et al. 2007), bacteria (Garcia-Pichel et al. 2001, Nagy et al. 2005a), archaea (Soule et al. 2009a), mosses and lichens (Ullmann and Büdel 2001). The advantage of preconditioning biomass of *Microcoleus* spp. species is very clear for the strains *M. vaginatus* JS001, and *M. steenstrupii* FB015 in which nonconditioned biomass never grew (Figure 6A and B). By contrast the effectiveness of preconditioning treatments for non-motile filamentous cyanobacteria *Nostoc* spp., *Tolypothrix* spp. and *Scytonema* spp., which are secondary colonizers in the natural ecological succession of biocrusts, was less universal. Among them however, 5 out of 12 strains benefited from preconditioning, but 3 did worse with preconditioning, which is rather perplexing.

It is worth noting however that growth of all cultures under our field-like conditions was very robust regardless of preconditioning. In fact, calculated generation times in the tests (Table 3) were generally much shorter than those obtained for the same isolates grown under purportedly optimal conditions in the laboratory. The clear corollary of this observation is that while preconditioning does help increase fitness, the lack of it

does not constitute a major impediment for an eventual adaptation to the harsher environment in the soil outdoors, with a couple of notable exceptions among *Microcoleus* strains. In this sense, one could argue that a preconditioning treatment may in most cases not be an absolute requirement. However, an ability to attain population growth as swiftly as possible may be of crucial fitness value under field conditions that include erosive forces, a factor that was not included in our tests. This caveat is particularly relevant because preconditioning benefits were so clear in biocrust-stabilizing *Microcoleus* strains. In other words, preconditioned cyanobacterial inoculant, especially those containing considerable amounts of *Microcoleus* spp. may require fewer rainfall events to stabilize the soils surface and hence stand a better change of withstanding major erosional events.

Tables

Table 2. Short- and long-term growth of biocrust-forming cyanobacterial strains expressed as fold change, over the 45 days of the outdoor test. ** Fold change either at day 31 or 45 (long term growth). Strain denominations include coding for the site of origin HS: Cold desert - silty soil, HSN: Cold desert – sandy soil, JS: hot desert – silty soil and FB: Hot desert – sandy soil. *Denotes significant differences in total biomass density between initials and either days 16, 31 or 45 (fold change). Data was assessed for normality and homogeneity of variance, and either a t-test or a Wilcox test was run accordingly.

Species	**Strain**	**Short term growth Fold change until day 16**		**Long term growth Fold change ****		**Strain response**
		Non conditioned	Pre conditioned	Non conditioned	Pre conditioned	Pre conditioned
M. vaginatus	HSN003	1.8	1.5	1.6	3.0*	Positive
	HSN015	0.7	2.6*	2.8	3.8	Positive
	JS001	0.8	0.7	0.5	1.4	Positive
	FB020	0.5	1.1	0.7	2.04*	Positive
M. steenstrupii	HS024	1.3	2.8*	2.7	4.2*	Positive
	HSN002	0.6	3.5*	2.5	3.9*	Positive
	JS010	0.4	0.5	0.1	1.1*	Positive
	FB015	0.2	1.6*	0.2	1.82*	Positive
Nostoc spp.	HS004	2.2	1.4	3.8	3.5	Indifferent
	HSN008	2.2	2.6	4.5	4.9	Indifferent
	FB025	22.5	1.3*	56.4	2.8*	Negative
	FB023	1.4	1.4	15.4	6.6*	Negative
Tolypothrix spp.	HS042	1.5	3.0*	3.8	4.5*	Positive
	HSN030	1.2	1.5	4.1	4.3	Indifferent

	JS100	0.8	0.9	1.7	2.2	Indifferent
	FB100	1.5	1.6	7.5	9.2	Positive
	HS006	2.3	3.4*	3.3	3.8	Positive
Scytonema	HSN006	2.0	2.0	2.2	3.2*	Positive
spp.	JS009	1.2	1.1	1.88	5.1*	Positive
	FB005	2.8	1.3	8.25	2.3*	Negative

Table 3. Generation (doubling) times of isolates in laboratory and in native soils outdoors. * Data are from Giraldo-Silva et al. (2019) with growth experiments lasting between 9 to 14 days. **Calculated from data in Table 1., counting only long-term under metabolically active time (1 out of every 3 days), for a total time of 10 to 15 days.

Species	Strain	Laboratory*	Outdoor**	
			Nonconditioned	Preconditioned
M. vaginatus	HSN003	2.12	0.6	0.3
	FB020	1.17	1.8	0.5
M. steenstrupii	HS024	3.22	0.3	0.3
	JS010	1.37	-1.0	14.8
Nostoc spp.	HS004	6.9	0.3	0.3
	HSN008	9.2	0.3	0.3
	FB025	6.8	0.4	0.6
	FB023	9.5	0.7	0.8
Tolypothrix spp.	HS042	12.8	0.3	0.3
	HSN030	11.1	0.3	0.3
	JS100	12.7	1.8	0.8
Scytonema spp.	HS006	18	0.3	0.3
	JS009	8.2	5.4	0.5
	FB005	9.1	0.6	0.5

Figures

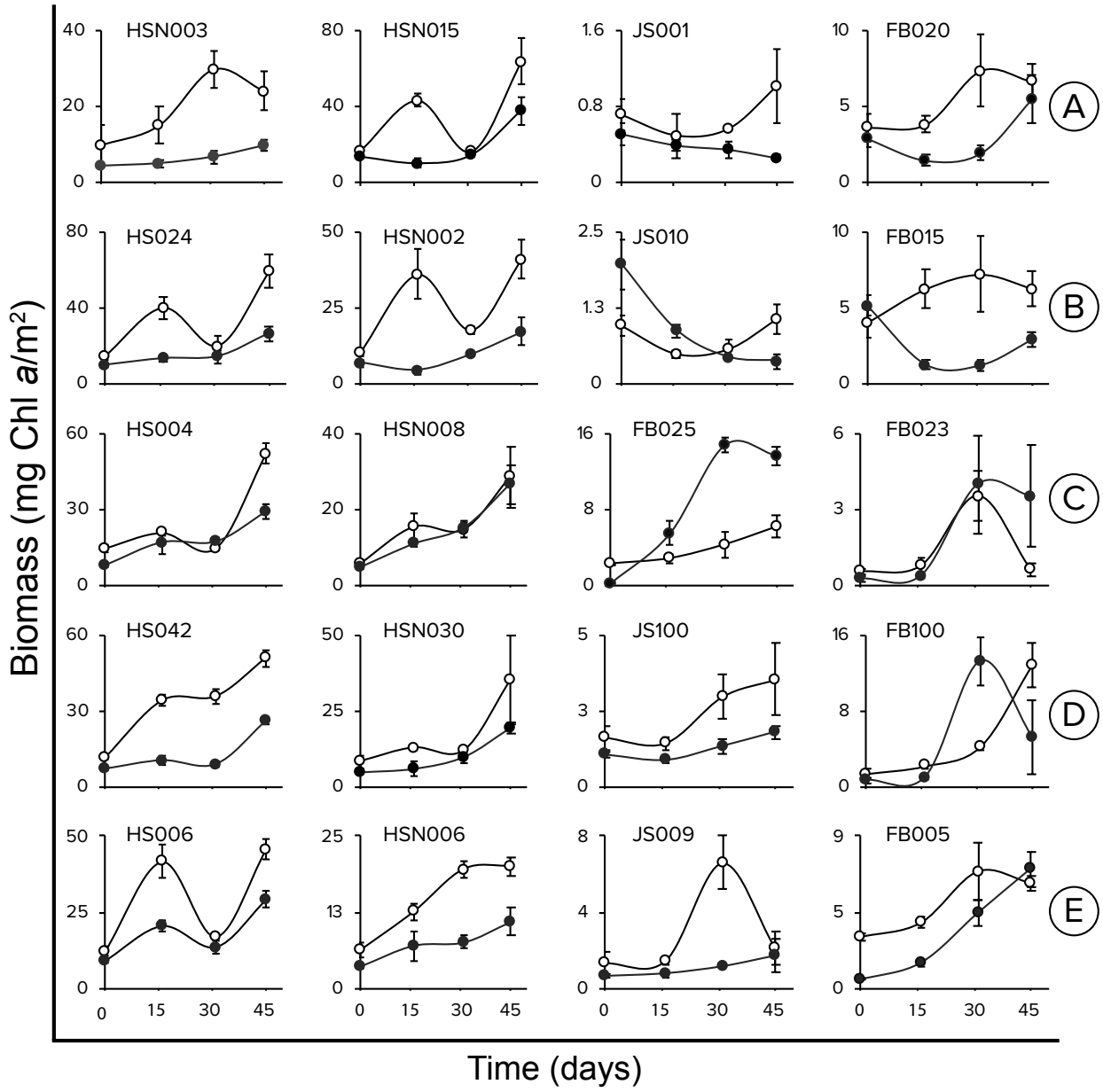

Figure 6. Population dynamics of the 20 tested cyanobacterial strains during the outdoor test. (O) Preconditioned biomass. (●) Nonconditioned biomass. A. *Microcoleus vaginatus,* B. *Microcoleus steenstrupii*, C. *Nostoc* spp. D. *Tolypothrix* spp. and E. *Scytonema* spp. Letters and numbers above each graph represent strain denominations according to the site of origin (see Table S3). Error bars indicate ± SD (n=3).

Supplementary information

Table S3. List of pedigreed cyanobacterial strains isolated from biocrusts used in outdoor test.

Species	Strain	Origin	
		Desert	Location -Soil texture
M. vaginatus	HSN003	Great Basin (cold)	Hill air force base - sandy clay loam
	HSN015	Great Basin (cold)	Hill air force base - clay loam
	JS001	Chihuahuan (hot)	Jornada experimental range – clay loam
	FB020	Chihuahuan (hot)	Fort Bliss military base – loamy sandy
M. steenstrupii	HS024	Great Basin (cold)	Hill air force base - sandy clay loam
	HSN002	Great Basin (cold)	Hill air force base - clay loam
	JS010	Chihuahuan (hot)	Jornada experimental range – clay loam
	FB015	Chihuahuan (hot)	Fort Bliss military base – loamy sandy
Nostoc spp.	HS004	Great Basin (cold)	Hill air force base - sandy clay loam
	HSN008	Great Basin (cold)	Hill air force base - clay loam
	FB025	Chihuahuan (hot)	Jornada experimental range – clay loam
	FB023	Chihuahuan (hot)	Fort Bliss military base – loamy sandy
Tolypothrix spp.	HS042	Great Basin (cold)	Hill air force base - sandy clay loam
	HSN030	Great Basin (cold)	Hill air force base - clay loam
	JS100	Chihuahuan (hot)	Jornada experimental range – clay loam
	FB100	Chihuahuan (hot)	Fort Bliss military base – loamy sandy
Scytonema spp.	HS006	Great Basin (cold)	Hill air force base - sandy clay loam
	HSN006	Great Basin (cold)	Hill air force base - clay loam
	JS009	Chihuahuan (hot)	Jornada experimental range – clay loam

	FB005	Chihuahuan (hot)	Fort Bliss military base – loamy sandy

Table S4. Complete schedule of our preconditioning treatment.

Dry-wet cycle	Location	Shade cover (%)
1	Culture room	80
2	Culture room	40
3	Culture room	0
4	Culture room	0
5	Greenhouse	80
6	Greenhouse	40
7	Greenhouse	0
8	Greenhouse	0
9	Outdoor	80
10	Outdoor	40
11	Outdoor	0
12	Outdoor	0

Supporting Figures

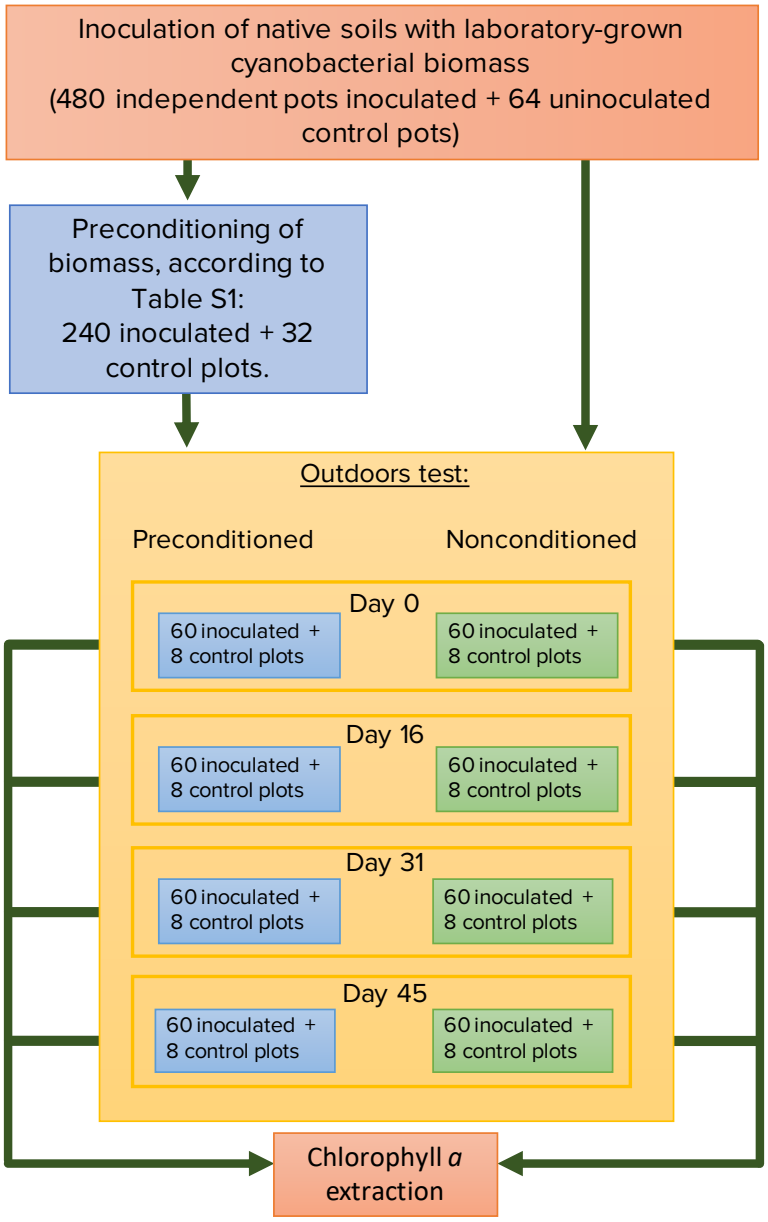

Figure S2. Biomass preconditioning experimental design

References

Allen, M. M., and R. Stanier. 1998. Growth and Division of Some Unicellular Blue-green Algae. J.gen.Microbiol. 51:1998–1998.

Antoninka, A., M. A. Bowker, P. Chuckran, N. N. Barger, S. Reed, and J. Belnap. 2018. Maximizing establishment and survivorship of field-collected and greenhouse-cultivated biocrusts in a semi-cold desert. Plant and Soil 429:213–225.

Antoninka, A., M. A. Bowker, S. C. Reed, and K. Doherty. 2016. Production of greenhouse-grown biocrust mosses and associated cyanobacteria to rehabilitate dryland soil function. Restoration Ecology 24.

Ayuso, S. V., A. G. Silva, C. Nelson, N. N. Barger, and F. Garcia-Pichel. 2017. Microbial Nursery Production of High- Quality Biological Soil Crust Biomass for Restoration of Degraded Dryland Soils 83:1–16.

Barger, N. N., J. E. Herrick, J. Van Zee, and J. Belnap. 2006. Impacts of biological soil crust disturbance and composition on C and N loss from water erosion. Biogeochemistry 77:247–263.

Barger, N. N., B. Webber, F. Garcia-Pichel, E. Zaady, and J. Belnap. 2016. Patterns and Controls on Nitrogen Cycling of Biological Soil Crusts. Pages 257–285 *in* B. Weber, M. M. Caldwell, B. Jayne, W. Bettina, B. Büdel, J. Belnap, B. Weber, and B. Büdel, editors. Biological Soil Crusts: An Organizing Principle in Drylands. 2nd edition. Switzerland.

Belnap, J. 1993. Recovery rates of cryptobiotic crusts: Inoculant use and assessment methods. The Great Basin Naturalist 53:89–95.

Belnap, J., and D. Eldridge. 2001. Disturbance and Recovery of Biological Soil Crusts. Pages 363–383 *in* J. Belnap and O. Lange, editors. Biological soil cruts: structure, function, and management. Springer, Berlin.

Belnap, J., and D. A. Gillette. 1997. Disturbance of Biological Soil Crusts: Impacts on Potential Wind Erodibility of Sandy Desert Soils in Southeastern Utah. Land Degrad. Develop 8:355–362.

Beraldi-Campesi, H., H. E. Hartnett, A. Anbar, G. W. Gordon, and F. Garcia-Pichel. 2009. Effect of biological soil crusts on soil elemental concentrations: Implications for biogeochemistry and as traceable biosignatures of ancient life on land. Geobiology 7:348–359.

Bowker, M. A. 2007. Biological soil crust rehabilitation in Theory and Practice - an Underexploited Opportunity. Restoration Ecology 15:13–23.

Bowker, M. A., and A. J. Antoninka. 2016. Rapid ex situ culture of N-fixing soil lichens

and biocrusts is enhanced by complementarity. Plant and Soil 408:415–428.

Bowker, M. A., J. Belnap, R. Rosentreter, and B. Graham. 2004. Wildfire-resistant biological soil crusts and fire-induced loss of soil stability in Palouse prairies, USA. Applied Soil Ecology 26:41–52.

Couradeau, E., U. Karaoz, H. C. Lim, U. Nunes da Rocha, T. Northen, E. Brodie, and F. Garcia-Pichel. 2016. Bacteria increase arid-land soil surface temperature through the production of sunscreens. Nature Communications 7:10373.

Doherty, K. D., A. J. Antoninka, M. A. Bowker, S. Velasco Ayuso, and N. C. Johnson. 2015. A Novel Approach to Cultivate Biocrusts for Restoration and Experimentation. Ecological Restoration 33:13–17.

Elbert, W., B. Weber, S. Burrows, J. Stinkdamp, B. Büdel, M. O. Andrea, and U. Pöschl. 2012. Contribution of cryptogamic covers to the global cycles of carbon and nitrogen. Nature Geoscience 5:459–462.

Faist, A. M., J. E. Herrick, J. Belnap, J. W. V. Zee, and N. N. Barger. 2017. Biological soil crust and disturbance controls on surface hydrology in a semi-Arid ecosystem. Ecosphere 8.

Fernandes, V. M. C., N. M. Machado de Lima, D. Roush, J. Rudgers, S. L. Collins, and F. Garcia-Pichel. 2018. Exposure to predicted precipitation patterns decreases population size and alters community structure of cyanobacteria in biological soil crusts from the Chihuahuan Desert. Environmental Microbiology 20:259–269.

Ferreira, D., and F. Garcia-Pichel. 2016. Mutational studies of putative biosynthetic genes for the cyanobacterial sunscreen scytonemin in Nostoc punctiforme ATCC 29133. Frontiers in Microbiology 7:1–10.

Ford, P. L., and G. V. Johnson. 2006. Effects of dormant- vs. growing-season fire in shortgrass steppe: Biological soil crust and perennial grass responses. Journal of Arid Environments 67:1–14.

Gao, Q., and F. Garcia-Pichel. 2011. An ATP-Grasp ligase involved in the last biosynthetic step of the iminomycosporine shinorine in Nostoc punctiforme ATCC 29133. Journal of Bacteriology 193:5923–5928.

Garcia-Pichel, F. 2003. Desert Environments: Biological Soil Crusts. Pages 1019–1023 *in* G. Bitton, editor. Encyclopedia of Environmental Microbiology 6 volume. Set. Wiley-Interscience, New York, NY.

Garcia-Pichel, F., and R. W. Castenholz. 1991. Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. Journal of Phycology 409:395–409.

Garcia-Pichel, F., A. López-Cortés, and U. Nübel. 2001. Phylogenetic and Morphological Diversity of Cyanobacteria in Soil Desert Crusts from the Colorado Plateau. Appl. Environ. Microbiol. 67:1902–1910.

Garcia-Pichel, F., V. Loza, Y. Marusenko, P. Mateo, and R. M. Potrafka. 2013a. Supp. material -Temperature drives the continental-scale distribution of key microbes in topsoil communities. Science 340:1574–1577.

Garcia-Pichel, F., V. Loza, Y. Marusenko, P. Mateo, and R. M. Potrafka. 2013b. Temperature Drives the Continental-Scale Distribution of Key Microbes in Topsoil Communities. Science 340:1574–1577.

Garcia-Pichel, F., and M. F. Wojciechowski. 2009. The evolution of a capacity to build supra-cellular ropes enabled filamentous cyanobacteria to colonize highly erodible substrates. PloS ONE 4:7801.

Gaskin, S., and R. Gardner. 2001. The role of cryptogams in runoff and erosion control on Bariland in the Nepal middle hills of the Southern Himalaya. Earth Surface Processes and Landforms 26:1303–1315.

Giraldo-Silva, A., C. Nelson, N. Barger, and F. Garcia-Pichel. 2019. Nursing biocrusts: isolation, cultivation and fitness test of indigenous cyanobacteria. Restoration Ecology:1–11.

Griffin, D. W. 2007. Atmospheric movement of microorganisms in clouds of desert dust and implications for human health. Clinical microbiology reviews 20:459–77, table of contents.

Jose, N. A., R. Lau, T. L. Swenson, N. Klitgord, F. Garcia-Pichel, B. P. Bowen, R. Baran, and T. R. Northen. 2018. Flux balance modeling to predict bacterial survival during pulsed-activity events. Biogeosciences 15:2219–2229.

Nagy, M. L., A. Pérez, and F. Garcia-Pichel. 2005. The prokaryotic diversity of biological soil crusts in the Sonoran Desert (Organ Pipe Cactus National Monument, AZ). FEMS Microbiology Ecology 54:233–245.

Pringault, O., and F. Garcia-Pichel. 2004. Hydrotaxis of cyanobacteria in desert crusts. Microbial ecology 47:366–73.

Rajeev, L., U. N. Da Rocha, N. Klitgord, E. G. Luning, J. Fortney, S. D. Axen, P. M. Shih, N. J. Bouskill, B. P. Bowen, C. A. Kerfeld, F. Garcia-Pichel, E. L. Brodie, T. R. Northen, and A. Mukhopadhyay. 2013. Dynamic cyanobacterial response to hydration and dehydration in a desert biological soil crust. The ISME Journal:2178–2191.

Rao, B., Y. Liu, S. Lan, P. Wu, W. Wang, and D. Li. 2012. Effects of sand burial stress on the early developments of cyanobacterial crusts in the field. European Journal of

Soil Biology 48:48–55.

Reynolds, R., J. Belnap, M. Reheis, P. Lamothe, and F. Luiszer. 2001. Aeolian dust in Colorado Plateau soils: nutrient inputs and recent change in source. Proceedings of the National Academy of Sciences of the United States of America 98:7123–7.

Rodríguez-Caballero, E., Y. Cantón, S. Chamizo, A. Afana, and A. Solé-Benet. 2012. Effects of biological soil crusts on surface roughness and implications for runoff and erosion. Geomorphology 145–146:81–89.

Sancho, L. G., J. Belnap, C. Colesie, J. Raggio, and B. Weber. 2016. Carbon Budgets of Biological Soil Crusts at Micro-, Meso-, and Global Scales. Pages 287–304 Biological Soil Crusts: An Organizing Principle in Drylands. Second. Springer, Switzerland.

Schlösser, U. G. 1982. Sammlung von algenkulturen. Ber Dtsch Bot Ges 95:181–276.

Sorochkina, K., S. Velasco Ayuso, and F. Garcia-Pichel. 2018. Establishing rates of lateral expansion of cyanobacterial biological soil crusts for optimal restoration. Plant and soil 429:199–211.

Soule, T., I. J. Anderson, S. L. Johnson, S. T. Bates, and F. Garcia-Pichel. 2009. Archaeal populations in biological soil crusts from arid lands in North America. Soil Biology and Biochemistry 41:2069–2074.

Stark, L. R., J. C. Brinda, D. Nicholas McLetchie, and M. J. Oliver. 2012. Extended Periods of Hydration Do Not Elicit Dehardening to Desiccation Tolerance in Regeneration Trials of the Moss *Syntrichia caninervis*. International Journal of Plant Sciences 173:333–343.

Ullmann, L., and B. Büdel. 2001. Ecological Determinants of Species Composition of Biological Soil Cruts on a Landscape Scale.pdf. Pages 203–213 *in* J. Belnap and O. L. Lange, editors. Biological Soil Cruts: Structure, Function, and Management. 1st edition. Springer-Verlag, Berlin.

Verrecchia, E., A. Yair, G. J. Kidron, and K. Verrecchia. 1995. Physical properties of the psammophile cryptogamic crust and their consequences to the water regime of sandy softs, north-western Negev Desert, Israel. Journal of Arid Environments:427–437.

Wang, W., C. Yang, D. Tang, D. Li, Y. Liu, and C. Hu. 2007. Effects of sand burial on biomass, chlorophyll fluorescence and extracellular polysaccharides of man-made cyanobacterial crusts under experimental conditions. Science in China, Series C: Life Sciences 50:530–534.

Williams, W. J., D. J. Eldridge, and B. M. Alchin. 2008. Grazing and drought reduce cyanobacterial soil crusts in an Australian Acacia woodland. Journal of Arid

Environments 72:1064–1075.

Yeager, C. M., J. L. Kornosky, R. E. Morgan, E. C. Cain, F. Garcia-Pichel, D. C. Housman, J. Belnap, and C. R. Kuske. 2007. Three distinct clades of cultured heterocystous cyanobacteria constitute the dominant N2-fixing members of biological soil crusts of the Colorado Plateau, USA. FEMS Microbiology Ecology 60:85–97.

Zaady, E., D. J. Eldridge, and M. A. Bowker. 2016. Effects of Local-Scale Disturbance on Biocrusts. Pages 425–445 Biological Soil Crusts: An Organizing Principle in Drylands. 2dn edition. Springer, Switzerland.

Zhang, Y. M., H. L. Wang, X. Q. Wang, W. K. Yang, and D. Y. Zhang. 2006. The microstructure of microbiotic crust and its influence on wind erosion for a sandy soil surface in the Gurbantunggut Desert of Northwestern China. Geoderma 132:441–449.

4 - SPATIAL SEGREGATION OF THE BIOLOGICAL SOIL CRUST MICROBIOME AROUND ITS FOUNDATIONAL CYANOBACTERIUM, *Microcoleus vaginatus*, AND THE FORMATION OF A NITROGEN-FIXING CYANOSPHERE.

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Abstract

Biological soil crusts (biocrusts) are a key component of arid land ecosystems, where they render critical services such as soil surface stabilization and nutrient fertilization. The bundle-forming, filamentous, non-nitrogen fixing cyanobacterium *Microcoleus vaginatus* is a pioneer primary producer, often the dominant member of the biocrust microbiome, and the main source of leaked organic carbon. We hypothesized that, by analogy to the rhizosphere of plant roots, *M. vaginatus* may shape the microbial populations of heterotrophs around it, forming a specialized cyanosphere. By physically isolating bundles of *M. vaginatus* from biocrusts we were able to study the composition of the microbial populations attached to it, in comparison to the bulk soil crust microbiome by means of high throughput 16S rRNA sequencing. We did this in two *M. vaginatus* dominated biocrust from distinct desert biomes. We found that a small, selected subset of OTUs, were significantly enriched in close proximity to *M. vaginatus.* Furthermore, we also found that a majority of bacteria (corresponding to some 2/3 of the reads) were significantly more abundant away from this cyanobacterium. Phylogenetic placements suggest that all typical members of the cyanosphere were copiotrophs, and that many were diazotrophs (Table S6 and S7). Nitrogen fixation genes were in fact orders of magnitude more abundant in this cyanosphere than in the bulk biocrust soil as assessed by qPCR. By contrary, competition for light, CO_2 and low organic carbon concentrations defined at least a part of the OTUs segregating from the cyanobacterium. We showed that *M. vaginatus* acts a significant spatial organizer of the biocrust microbiome. On the one hand, it possesses a compositionally differentiated cyanosphere that concentrates the nitrogen fixing function. We propose that a mutualism based on C for N exchange between *M. vaginatus* and

copiotrophic diazotrophs helps sustains this cyanosphere, and that this consortium constitutes the true pioneer community enabling the colonization of nitrogen-poor soils. On the other hand, a large number of biocrust community members segregate away from the vicinity of *M. vaginatus,* potentially through competition for light or CO_2, or because of a preference for oligotrophy.

Keywords: Biocrust, Cyanosphere, *Microcoleus vaginatus*, diazotrophs

Introduction

Biological soil crusts (biocrusts) are soil-surface microbial communities based on microbial or cryptogamic phototrophs that develop in areas where light can penetrate directly to the soil surface unimpeded by a layer of plant litter [see (Garcia-Pichel 2003b) for a primer, and, (Belnap et al. 2001b, 2016) for monographs]. They are prominent in arid-lands, where they contribute several important ecosystem properties, including the protection of soils against erosion and nutrient fertilization of the areas they cover.

Most studies on the biology and ecology of biocrust organisms have centered on the primary producers (largely cyanobacteria, but also sometimes microalgae, lichens and mosses), and much has been learned about their particular adaptations and ecology. And yet, biocrusts represent miniature ecosystems that are phylogenetically diverse, in which a variety of ecological functions are expressed. They constitute a particular type of soil microbiome, one in which the primary producers are an essential but certainly far from exclusive part (Kuske et al. 2012, Abed et al. 2012). Pioneering filamentous, bundle-forming cyanobacteria, such as *Microcoleus vaginatus* and *M. steenstrupii*, initiate biocrust formation by stabilizing the surface of loose soils (Garcia-Pichel and Wojciechowski 2009), allowing a succession that involves other cyanobacteria (Yeager et al. 2007), bacteria (Gundlapally and Garcia-Pichel 2006), archaea (Soule et al. 2009a), and fungi (Bates et al. 2012), as well as the lichens (Bates et al. 2010) and mosses (Antoninka et al. 2016) that are typical of the best developed crusts of milder environments. Most of the bacteria and archaea appear to be heterotrophs (Soule et al. 2009a, Nunes da Rocha et al. 2015), although crusts do contain significant populations of bacterial and archaeal chemolithotrophs that are crucial for nitrogen cycling (Johnson et

al. 2005, Marusenko et al. 2013). Under unusually long periods of wetness, spore-forming bacteria (Karaoz et al. 2018) or even methanogenic archaea (Angel et al. 2011) may develop sizeable biocrust populations. Microbial diversity and population density increase as succession proceeds (Couradeau et al. 2016). Even in successionally young biocrusts, biomass (estimated as total cell counts, or DNA content) is orders of magnitude larger than those typical of desert soils, and the microbial communities within them show evidence of vertical stratification similar to those of microbial mats or biofilms (Garcia-Pichel 2003b). At a larger, landscape scale, varying soil properties influence the biocrust microbiome composition (Nagy et al. 2005b), as do climatic variations at a continental scale (Garcia-Pichel et al. 2013a, Marusenko et al. 2013).

Biocrust microbes remain desiccated, and hence inactive, most of the time but, upon wetting, become quickly hydrated and active (Rajeev et al. 2013). During pulses of activity, high metabolic rates constrained within small spaces result in the rapid formation of steep chemical gradients and microenvironments, which include oxygen-supersaturated zones close to the surface and anoxic zones some 1– 3 mm deep (Garcia-Pichel and Belnap 2001). Biocrusts are not only locally, but also globally relevant. They cover some 12% of the Earth's continental area (Rodríguez-Caballero et al. 2018) and are major players in the global N cycle, as some ~31% of the biological nitrogen fixation on land can be attributed to their activity (Elbert et al. 2012, Barger et al. 2016). Their global standing stocks have been estimated to reach in the order of 54 x 10^{12} g C (Garcia-Pichel et al. 2006). The oldest fossil remains of biocrust communities data back to the Proterozoic (Beraldi-Campesi et al. 2014), and it is thought that these systems were

determinant for the global ecology of early continents before the advent of land plants (Thomazo et al. 2018b).

In a large proportion of biocrusts world-wide *M. vaginatus* plays a central role by being both a foundational species and a metabolic pivot to the biocrust community. Uniquely, *M. vaginatus* does not only fix carbon but excretes a large fraction of its photosynthate directly into the soil (Baran et al. 2013, 2015). In using a plant analogy, *M. vaginatus* would serve both as a leave and a root. However, *M. vaginatus* does not have the capacity to fix nitrogen (Starkenburg et al. 2011, Jose et al. 2018), so it remains somewhat surprising that a non-diazotroph be the main colonizer of such typically N-limited, bare arid soils. In mature crusts, most of the nitrogen fixation is attributed to heterocystous cyanobacteria (Yeager et al. 2007), and in early crusts that lack the latter, to the activity of heterotrophic diazotrophs (Pepe-Ranney et al. 2015).

We hypothesized that *M. vaginatus* may rely on the N_2-fixation of other bacteria for their nitrogen needs, and that such metabolic interaction may result in an enrichment of certain bacterial types in the proximity of its bundles within the biocrusts. By analogy to a plant rhizosphere (Sasse et al. 2018), this sphere of influence would be the basis of a spatial "cyanosphere" (contraction of the words "cyanobacterium" and "sphere") based on functional interactivity. We tested this hypothesis directly taking advantage of the large size of *M. vaginatus* bundles, which makes it possible to physically excise and isolate them from the rest of the biocrust community, enabling the characterization and comparative analyses of the microbial communities found close and away from its bundles.

Methods

Sample collection and bundle picking.

We studied biocrusts from 2 locations in the Southwestern US: Chihuahuan Desert (near El Paso, TX; 32.431069° -105.984151°), and the Great Basin Desert (near Salt Lake City, UT; 32.54558° -106.72324°). Biocrusts were wetted *in situ* with distilled water for sampling, then dried, and stored in dark and dry conditions until experimentation, when they were wetted for 24 h prior to sampling. Using forceps under a dissection scope, we picked *M. vaginatus* bundles from each site, which were then individually washed in autoclaved Milli-Q water and observed under the microscope to assign species. Five pieces of autoclaved sewing thread, used to mimic *M. vaginatus* bundles, were subjected to the same procedure and used as negative controls. For the respective bulk soil crusts, we sampled in triplicate (6 samples total) taking 0.5 cm deep and 1 cm (internal diameter) cores. Each bulk soil, bundle or control (sewing thread) was transferred to 2 mL tubes containing SDS, and DNA was extracted immediately.

DNA purification, 16S libraries preparation and sequencing.

DNA from all samples was isolated using a PowerSoil DNA isolation Kit (MoBio, Carlsbad CA), following the manufacturer's protocol. General prokaryotic primers targeting the 16S rRNA V4 region: 515F 5'-GTGCCAGCMGCCGCGGTAA-3' and , 806R 5'-GGACTACHVGGGTWTCTAAT-3'(Caporaso et al. 2011) were used for library preparation. PCR was performed in triplicate and products pooled for each sample, with an initial phase of denaturation at 94 °C for 3 min, followed by 35 cycles (denaturation 64 °C for 45 seconds, annealing 50 °C for 50 seconds, extension 72 °C for

90 seconds), followed by a final extension phase at 72 °C for 10 minutes. Determination of total DNA concentrations in PCR products was assessed by Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies, New York, USA) and pooled to a total concentration of 240 ng of DNA per sample in the library. DNA was cleaned using the QIA Quick PCR Purification kit (QIAGEN, Valencia, CA, USA). The library DNA concentration was quantified using the Kit ABI Prism® (Kapa Biosystems, Wilmington, MA, USA), following the manufacturer's instructions, diluted to a final concentration of 4 nM, then denatured and diluted to a final concentration of 4 pM, spiked with a 30% PhiX solution, then was loaded on the MiSeq Illumina Sequencer (Illumina, San Diego, CA, USA). The sequencing was performed in the Microbiome Analysis Laboratory at Arizona State University (Tempe, AZ, USA), using custom primers, paired ends sequencing, and default chemistry.

Quantitative PCR.

Real-time polymerase chain reaction (qPCR) was used to quantify gene copy numbers of 16S rRNA and *nifH* genes in bulk soil crust and *M. vaginatus* bundles, using appropriate standard primers (respectively: 338F 5′-ACTCCTACGGGAGGCAGCAG-3′ 518R 5'-GTATTACCGCGGCTGCTGG-3'(Nübel et al. 1997) and PolF 5'-TGCGAYCCSAARGCBGACTC-3' PolR 5'-ATSGCCATCATYTCRCCGGA- 3')(Poly et al. 2001). Two standard curves were made using gBlocks® Gene Fragments from Integrated DNA Technologies. The 16S rRNA gene standard curve used a dilution series from 10^7 to 10 gene copy numbers, while for the *nifH* gene the dilution series was from 10^4 to 1 copy. For both assays, the reactions were prepared in triplicate in a final volume

of 20 µl. Each reaction contained 5 µl of template DNA, 10 µl of Sybr Mix Green (TaqMan®), 0.4 µl of primers (500nM for each), and 4.6 µl of water. Two negative controls were used, one with no template and one with no primers. The samples were amplified and quantified using an ABI7900HT thermocycler. The protocol for the 16S rRNA PCR included an initial denaturation phase (98˚C for 2.00 minutes), followed by 40 cycles of a second phase (95˚C for 10 minutes and finally, 55˚C for 30 minutes), and then a dissociation stage (beginning at 55 ˚C and ending at 95˚C with a 2% ramp rate) (Couradeau et al. 2016). For the *nifH* gene assay(Ceja-Navarro et al. 2014), PCR involved an initial denaturation stage (95˚C for 3 minutes), followed by 45 cycles of 95˚C for 10 minutes, and 59˚C for 30 minutes, and then a dissociation stage beginning at 59 ˚C and ending at 95˚C with a 2% ramp rate. The *nifH*/16S rRNA genes ratio was calculated from values of copy number per ng of DNA. The final dataset was log transformed to comply to the normality (Shapiro-Wilk test) and variance homogeneity (Levene's Test) requirement of a One-Way ANOVA test. This test was run to test whether the bundles and soils groups from both FB and HSN location had different *nifH*/16S rRNA genes ratio.

Bioinformatics analyses.

The raw FastaQ file was multiplexed within the MiSeq Illumina workflow under default parameters. Retrieved sequences were paired using PANDAseq(Masella et al. 2012) with an alignment threshold score of 0.95. High quality sequences (length > 200 bp, minimum average Phred score 25) were further assigned to individual samples and barcodes were removed using the Qiime 1.8 (Caporaso et al 2010) *split_librairies.py* script. The master

file created was used to pick Operational Taxonomic Units (OTUs) using the *pick_open_reference_otus.py* pipeline in Qiime under default parameters. More specifically, we used the UCLUST algorithm (Edgar 2010) to pick OTUs at a 97% similarity threshold and assigned taxonomy using the rdp (Wang et al. 2007a) classifier against the Greengenes reference database release 13.5(McDonald et al. 2012) (Table S5). The OTU table produced was filtered to remove rare OTUs including potential chimeras, and only OTUs shared by at least 3 samples in the dataset were kept. Overall these steps filtered out 5% of the total sequence count and 70% of the OTU count. All sequences attributable to *Microcoleus vaginatus* (see below for assignments) were removed from the OTU table. The *M. vaginatus*-free table was Hellinger normalized using the decostand script of the R vegan package. Beta diversity Bray-Curtis pairwise distances were calculated on the Hellinger transformed matrix and further ordinated using NMDS in Qiime. The significance of differential OTU distribution between bundles *vs*. bulk soil crust was assessed using an Adonis test on the Bray Curtis distance matrix with the *compare_categories.py* Qiime script. We further determined which OTUs were differentially abundant in the bundles *vs.* total community using the DefSeq2 method (McMurdie and Holmes 2014). After checking the good agreement between the fit line and the shrinked data on the dispersion plot, a Wald test was applied to each OTU to reject the null hypothesis (p value<0.05) that the logarithmic fold change between communities (i.e. in our case bundle *vs.* bulk soil crust) for a given OTU is null. The 5 control samples (sewing thread) were analyzed the same way in an effort to account for any external contamination (i.e. operator or environmental source) in our bundle samples handling.

Phylogenetic analyses.

Phylogenetic placement of the 21 aggregating and 1160 segregating OTUs sequences was resolved by constructing 16 trees encompassing their phylogenetic diversity. For all but the Cyanobacteria tree, the dataset used was a combination of our sequences along with their first Blastn hit and the closest cultured relative downloaded from SILVA rRNA database project and the NCBI 16s ribosomal RNA sequences (see supplementary OTU_classifier.ipynb). Each phylum level dataset was then treated independently. Sequences were aligned with SSU-ALIGN(Nawrocki 2009), using a profile-based alignment strategy, in which each target sequence is aligned independently to a covariance model that uses the 16s rRNA gene secondary structure. Poorly aligned columns were removed from the alignment based on a 95% confidence profile calculated within SSU-ALIGN. The alignment was trimmed to coordinates on Geneious version 8.0 (Kearse et al. 2012b) so all sequences in the alignment will begin and end at the same positions. Tree topology was inferred on the CIPRES high performance computing cluster(Miller et al. 2010), using the RAxML-HPC2 (Stamatakis 2014) workflow on XSEDE with the ML + Thorough bootstrap (1000 bootstraps) method and the GTRGAMMA model. For the Cyanobacteria tree, all 16S rRNA genes sequences of at least 1100 bp were manually downloaded from NCBI (Bethesda (MD): National Library of Medicine (US) 1988). A reference alignment was built from these 1034 high quality sequences using SSU-ALIGN(Bethesda (MD): National Library of Medicine (US) 2004). The reference cyanobacteria tree (https://github.com/FGPLab/cydrasil/tree/0.22a) was constructed on the CIPRES high performance computing cluster(Miller et al. 2010),

using the RAxML-HPC2(Stamatakis 2014) workflow on XSEDE with the ML + Thorough bootstrap (1000 bootstraps, GTRGAMMA model). Cyanobacteria OTUs sequences were aligned to the reference alignment with PaPaRa (Berger and Stamatakis 2011) using a probabilistic gap model, and then placed into the reference tree using the RaxML8 Evolutionary Placement Algorithm (Berger et al. 2011). Additionally, the RaxML8 Evolutionary Placement Algorithm (Berger et al. 2011), was used for some of the previous constructed trees (Acidobacteria, Deinococcus-Thermus, Armatimonadetes, Chlorobi, Chloroflexi, Firmicutes, Planctomycetes and Verrucomicrobia) in an effort to taxonomically assign as many OTUs as possible. The resulting trees were imported into the iTOL 3 server (Letunic and Bork 2007), and can be visualized at http://itol.embl.de/shared/microbiomelandscaper; aggregating sequences are shown in red while segregating sequences are in blue.

Results

A cyanosphere composed of a selected subset of the biocrust microbiome exists around *M. vaginatus.*

We carried out our analyses in samples from two contrasted geographical locations, one from the warm Chihuahuan Desert (Fort Bliss, or FB) and one from the cold Great Basin Desert (Hill Sandy, or HSN) (Figure 7). The two sites, their soils and biocrusts are fully described elsewhere (Velasco Ayuso et al. 2017). After excising and isolating single bundles of *M. vaginatus* from the soil, we analyzed the microbiome tightly associated with them using high throughput 16S rRNA gene amplicon sequencing, and compared using bioinformatics the composition of the microbial community intimately associated with these bundles (n = 44) to the total biocrust community analyzed separately (n = 6) (Table S5), as the simplest assessment of spatial organization: close to and away from *M. vaginatus*. In a first check, we made sure that our original microscopic assignment of the bundles to *M. vaginatus* was correct, as other bundle forming cyanobacterial species populate biocrusts (Figure 8). This was indeed the case. We then compared the composition of the rest of the microbiomes (to the exclusion of all OTU's attributable to *M. vaginatus*). We found that overall the bundle OTU richness (average chao1 202 ± 97) was an order of magnitude lower than the richness of the total biocrust community (average chao1 2107 ± 320). While the OTU richness of bundles was not different between locations, the HSN site biocrust community was significantly more diverse (average chao1 2432 ± 56) than that of the FB site (average chao1 1801 ± 115) (Table S5).

An NMDS ordination of the beta-diversity Bray-Curtis metric on the Hellinger transformed OTU table (Figure 9A) revealed that the composition of the bundle communities was distinct from those of their respective biocrust soil community of origin (Adonis, F = 4.7, *p* value = 0.001), forming a compositional "cyanosphere" (by analogy to the plant rhizosphere). The cyanosphere composition was also differentiated according to the sampling location (Great Basin or Chihuahuan Desert).

In order to further probe the factors driving the differentiation between cyanosphere and biocrust microbiome, we calculated the ratio of abundance of each Operation Taxonomic Unit (OTU) in the bundles *vs.* the bulk soil, for those OTUs that were detected in both settings (669 shared OTUs at FB, and 2177 shared OTUs at HSN). The frequency distribution of these ratios was clearly skewed towards negative values (Figure 9B), implying that many more microbial types tended to segregate away from *M. vaginatus* than tended to aggregate within its cyanosphere. In order to identify the OTUs involved in this spatial organization we used the DESeq2 method (McMurdie and Holmes 2014), which computes statistical significance for differential distributions of OTUs between two possible outcomes. Twenty OTUs in the cold desert cyanospheres (HSN) and two OTUs in those from the hot desert (FB) could be classified with statistical confidence ($p < 0.05$; listed in Tables S6 and S7, respectively), as consistent *M. vaginatus* close neighbors across different bundles, while 758 OTUs (HSN), and 592 OTUs (FB) were statistically more abundant away from it (Figure 9C; listed in Table S8). This analysis confirmed that the significant difference between the cyanosphere and the total soil community is driven by a small number of bacteria associated with *M. vaginatus* bundles (aggregating OTUs), while there are large numbers of bacteria (segregating

OTUs) that were preferentially found away from them, as part of the bulk soil. Accounting for the relative contribution of each OTU, we could compute that altogether more than 2/3 of all the biocrust bacteria were significantly affected in their spatial distribution by the presence of *M. vaginatus* (Table 4, Figure 9C), the large majority segregating away from the cyanosphere.

From the 5 negative control samples (sterilized sewing cotton thread) that we analyzed in the same way in an effort to account for any external contamination (i.e. operator or environmental source) during our handling of bundle samples, we recovered a total of 92 OTUs, among which 4 matched (>99% sequence similarity) one of our aggregating OTUs (Table S9). A conservative take on this result, is that they are all contaminants. However, one out of these four OTUs has been detected by other methods as one of the most common heterotrophic nitrogen fixers in early biocrust stages (Pepe-Ranney et al. 2015). The same OTU matches (100%) a culture recently isolated from *M. vaginatus* bundles in nitrogen free media (Nelson et al., unpublished data). This suggests that we may not have the taxonomic resolution to resolve the true status of these OTUs, and therefore decided not to filter out these 4 OTUs, but rather to flag them in Table S9.

The *M. vaginatus* cyanosphere is enriched in nitrogen-fixing members

We further analyzed the identity of the 21 OTUs that were statistically *bona fide* cyanosphere members using a refined phylogenetic placement in search for functional inference (see Methods and Tables S6 and S7). We found that all taxonomically-assignable OTUs could be inferred to be from copiotrophic bacteria, which are rather uncommon in organic-poor desert soils and otherwise typical of organic-rich

rhizospheres, animal microbiomes or dung (among them several enterobacteria, pseudomonads, *Streptococcus*, *Bacteroides*, and *Myxobacteria*; Tables S6 and S7). We also found that at least 6 OTUs from those 21 could be inferred by phylogenetic placement to be likely members of N_2-fixing clades (Tables S6 and S7). Three of these OTUs (assigned to *Escherichia/Shigella*, *Acinetobacter,* and *Stenotrophomonas)* matched (>99%) 3 of the phylotypes identified elsewhere as important heterotrophic diazotrophs of biocrusts through ^{15}N- DNA SIP and genomic analyses (Pepe-Ranney et al. 2015). This suggests that diazotrophic capacity may be a common denominator of the cyanosphere community. In order to gauge the relative potential for N_2-fixation of the cyanosphere community more directly, we performed quantitative PCR to determine the ratio of *nifH* genes (coding for a nitrogenase subunit) to 16S rRNA copy numbers existing in the bundle cyanosphere *vs.* that in the bulk biocrust microbiome. We found that the *nifH* gene was some 100-fold more abundant in the cyanosphere of *M. vaginatus* bundles (Figure 10) than in the bulk soil crusts, regardless of geographic origin.

Oligotrophs, phototrophs and autotrophs members among those segregated from *M. vaginatus.*

We again used phylogenetic placement on the 1350 soil OTUs that were significantly more abundant away from *M. vaginatus* bundles, in an attempt to refine their potential function. Since most microbial taxonomic diversity is not well described functionally, we could not find relevant inferences for the majority of these OTUs, which prevented us from carrying out a fully quantitative estimation. Instead, we asked specific hypotheses based on logical predictions. A simple such prediction would be that competition for light

may drive other phototrophs away from the dominant *M. vaginatus.* Indeed, no other known phototrophs were found among aggregating bacteria and all *bona fide* phototrophs were among the segregating OTUs, including other cyanobacteria, proteobacterial purple non-sulfur phototrophs, and several *Chloroflexi*. In a similar manner, one could predict that competition for CO_2 would tend to segregate other autotrophs from *M. vaginatus,* which was again the case (including all other photoautotrophs like cyanobacteria, purple non-sulfurs, some Chloroflexales, as well as nitrifying chemolithoautotrophic Archaea and Bacteria, such as *Nitrososphaera* and *Nitrospira*). A final case could be made on the basis of the fact that bacteria in the cyanosphere tend to gather uncommon copiotrophs (see above), so it is possible that oligotrophs grow better away from the sources of leaking photosynthate that *M. vaginatus* represents. Our analysis revealed that members of well-known oligotrophic bacterial genera (*Caulobacter*, *Asticcacaulis*, *Brevundimonas* and *Sphingomonas* in the Proteobacteria, *Modestobacter, Blastococcus, Geodermatophilus*, *Nocardioides,* and *Arthrobacter* in the Actinobacteria, *Fimbriimonas, Chthonomonas,* and *Armatimonas* in the Armatimonadetes, and *Longimicrobium* in the Gemmatimonadetes) were preferentially represented among the segregating microbiome fraction, but absent from the cyanosphere (Table S6, S7 and S8).

Discussion

The cyanosphere as a differentiated compartment of the biocrust microbiome

We could show that the community closely associated to *M. vaginatus* bundles, while containing many of the same microbial OTUs found in the bulk biocrust soil, differs from it in that it attracts a specific set of bacteria that are otherwise quite rare. This phenomenon is not unlike microbial hotspots that are found around plant roots in the soil (Kuzyakov and Blagodatskaya 2015), and so we called this specialized community the *cyanosphere*. This is consistent with the developing notion of an evolutionarily deeply rooted continuum of specific interconnections between phototrophic and heterotrophic systems, from "algal spheres" to root microbiomes (Graham et al. 2018). Interestingly, all OTUs that define the *M. vaginatus* cyanospheres would belong to the "rare biosphere" (Lynch and Neufeld 2015) by virtue of their extremely low abundance in the biocrust microbiome (the median rank of aggregating OTUs in soils was 2549th), and yet they may be playing significant functional roles in biocrust systems.

The cyanosphere compartment possesses differential features that might explain why a specific set of bacteria thrive in it, compared to the rest of the biocrust soils. First it is an organic carbon hotspot based on the high concentration of the extracellular polymeric substances (EPS) that make up a bundle's sheath (Swenson et al. 2017, 2018) and by the dynamic excretion of a large variety of small molecular weight organics by *M. vaginatus* cells (Baran et al. 2013). The EPS sheath likely offers means for physical anchoring of bacteria and might help retain hydration water during desiccation (Couradeau et al. 2018). Altogether, the cyanosphere likely constitutes top real estate

within the biocrust where occupancy might be determined by microbe-microbe competition for this resource-rich hotspot (Coyte et al. 2015).

***M. vaginatus'* cyanosphere may be at least partly based on a mutualistic C for N exchange**

Clearly, the abundance of nitrogenase *nifH* gene in the cyanosphere is roughly 100-fold higher than that in the bulk crust soil, which strongly suggest that nitrogen fixation "concentrates" there, a fact supported by the high abundance of typical nitrogen fixing taxa among cyanosphere members. We therefore propose that there must exist an active mutualistic relationship established between the diazotrophic copiotrophic heterotrophs and *M. vaginatus* based on a C for N exchange. Proof of such a symbiotic relationship will necessitate the deployment of alternative approaches, which could include using $^{13}CO_2/^{15}N_2$ stable isotope tracers in combination with NanoSIMS imaging for direct visualization of a coupled exchange (Samo et al. 2018), or, even more directly, the reconstitution of the mutualistic relationship from representative isolates of each partner. Unfortunately, no cultured representatives are yet available of these heterotrophic diazotrophs. Chemical characterization of the C-compound used by the N-fixing heterotrophs and their consumption spectrum by other biocrust organisms (Swenson et al. 2018) would allow to determine how targeted and precisely controlled this C to N exchange might be.

In any event, the fact that nitrogen fixation rates do not differ significantly between early stage and mature biocrusts (Johnson et al. 2005), illustrate the critical role that this heterotrophic diazotrophs may play in the establishment and early development of biocrusts. That *M. vaginatus* carries its own built-in nitrogen fixation "microbiome

module" must offer it very significant fitness value as a colonizer of N-depleted soils. In a way, it is *M. vaginatus* plus its cyanosphere what constitutes the true pioneer of biocrust. As such, it should prove interesting to target the use of mixed cultures in current efforts for arid land soil rehabilitation in which inoculation and survival of *Microcoleus vaginatus* is key (Giraldo-Silva et al. 2019).

A spatially organized microbiome

It seems from our results that the powers for spatial organization of the biocrusts microbiome by *M. vaginatus* may not be relegated to the formation of a cyanosphere, but potentially extend to a significant proportion of the community that segregates from it. Our effort to interrogate the putative function of those segregating OTUs showed that competitors for light and for CO_2 predictably count among them, as did members of typically oligotrophic bacterial groups, as one would have expected. However, given that a large fraction of the segregating OTUs could not be confidently functionally assigned, it is premature to conclude that such distribution patterns based on competition could hold for all. Our knowledge of the principles of microbiome assembly has clearly lagged behind a bewildering advance of the technological ability to describe in detail their complex composition and potential capabilities through "omics" techniques (Parks et al. 2017). The use of network theory and analysis has been at the forefront of such efforts (Milo et al. 2002, Coyte et al. 2015, Guidi et al. 2015). At the base of network studies is the assumption that functional interactions among microbial types are the main drivers of spatial patterns of occurrence, such that detection of microbial co-occurrence can reveal essentially functional networks. This is of course true for cases of obligate, strong

interactions like symbioses, which tend to promote the formation of tight, microscale consortial aggregates (Hatzenpichler et al. 2016). Theoretical and experimental work points to subtler nutrient gradients as crucial to the maintenance of spatially structured microbiomes (Nadell et al. 2010, Mitri et al. 2015). If this were correct, one would expect that microbial species that are functionally central in a microbiome will play an inordinately large role on the spatial structuring of the rest of the components (i.e., they will effectively landscape the microbiome) through metabolic interactivity. This is precisely what our results seem imply. Our observations provide a first glimpse at the fact that spatial organization of microbiomes might further constrain and be constrained by metabolic interactivity.

Tables

Table 4. Bacterial population size (as % of total 16S rRNA gene reads) of bacteria that show spatial responsiveness to *M. vaginatus*, Aggregating and segregating OTUs were determined statistically as per Fig. 9C, each OTU was then weighed by its relative abundance, and all contributions added.

	FB Soils	**HSN Soils**
Aggregating	0.22	0.13
Segregating	52.55	69.97
Nonsignificant	47.23	29.89

Figures

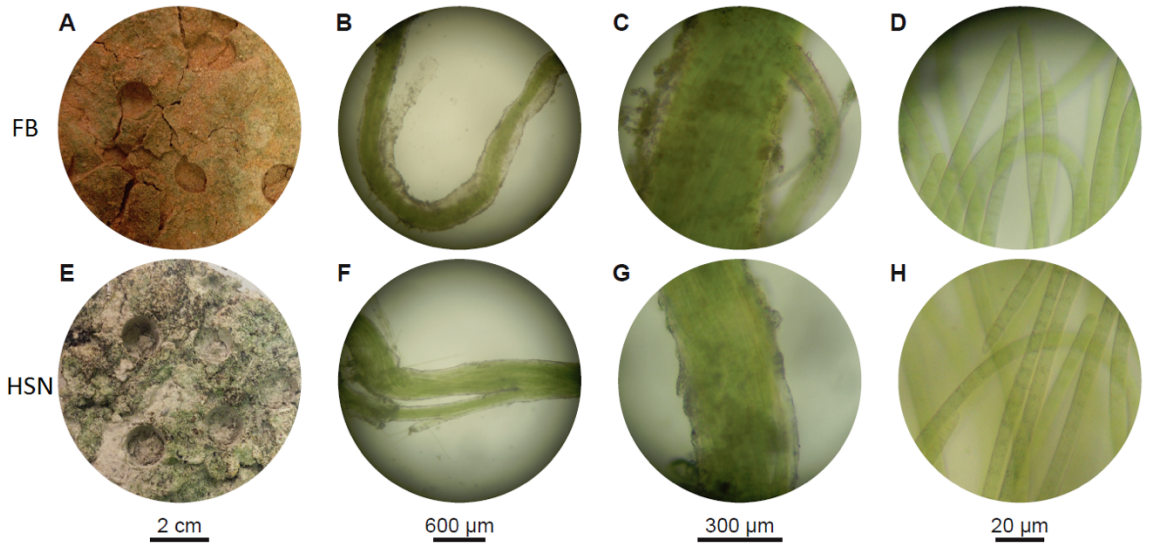

Figure 7. Biocrust samples from the Chihuahuan and the Great Basin Deserts. A and E are top views of Chihuahuan (A) and Great Basin (E) biocrusts before bundle picking. Depressions are from coring for the bulk soil samples. B and F are examples of cyanobacterium bundles picked from the biocrust. Each bundle comprised the cyanobacterium and the exopolysaccharide sheath that bundles the filaments together and hosts the cyanosphere community. C and G offer a closer look at the bundles. D and H show single *M. vaginatus* trichomes under the compound microscope (100x) for preliminary identification, before corroborating their identity by 16S rRNA gene typing. FB: Fort Bliss - hot desert, HSN: Hill Sandy Soil – cold desert

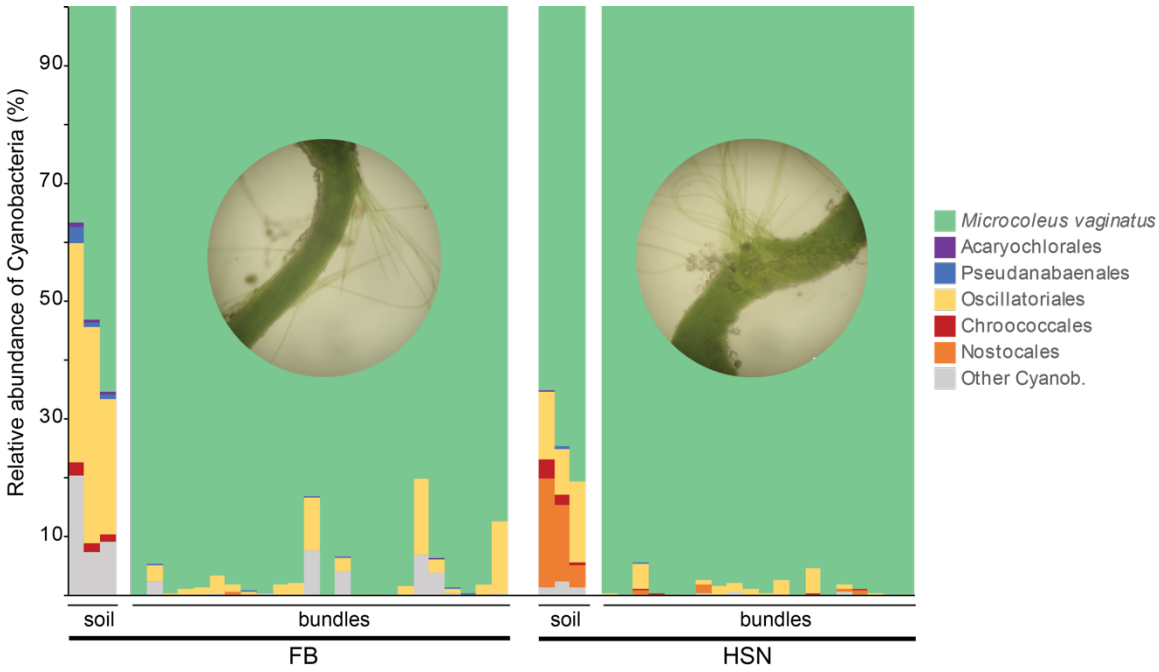

Figure 8. Cyanobacterial community structure and bundle identification. Relative abundance of cyanobacteria based on high-throughput sequence of 16S rRNA genes and bioinformatics analysis in *M. vaginatus* bundles and bulk biocrust soil from each location. Three OTUs belonging to *M. vaginatus* constituted the most abundant cyanobacterium in the community, and the overwhelming majority of the cyanobacteria in the excised bundles. FB: Fort Bliss - hot desert, HSN: Hill Sandy Soil – cold desert

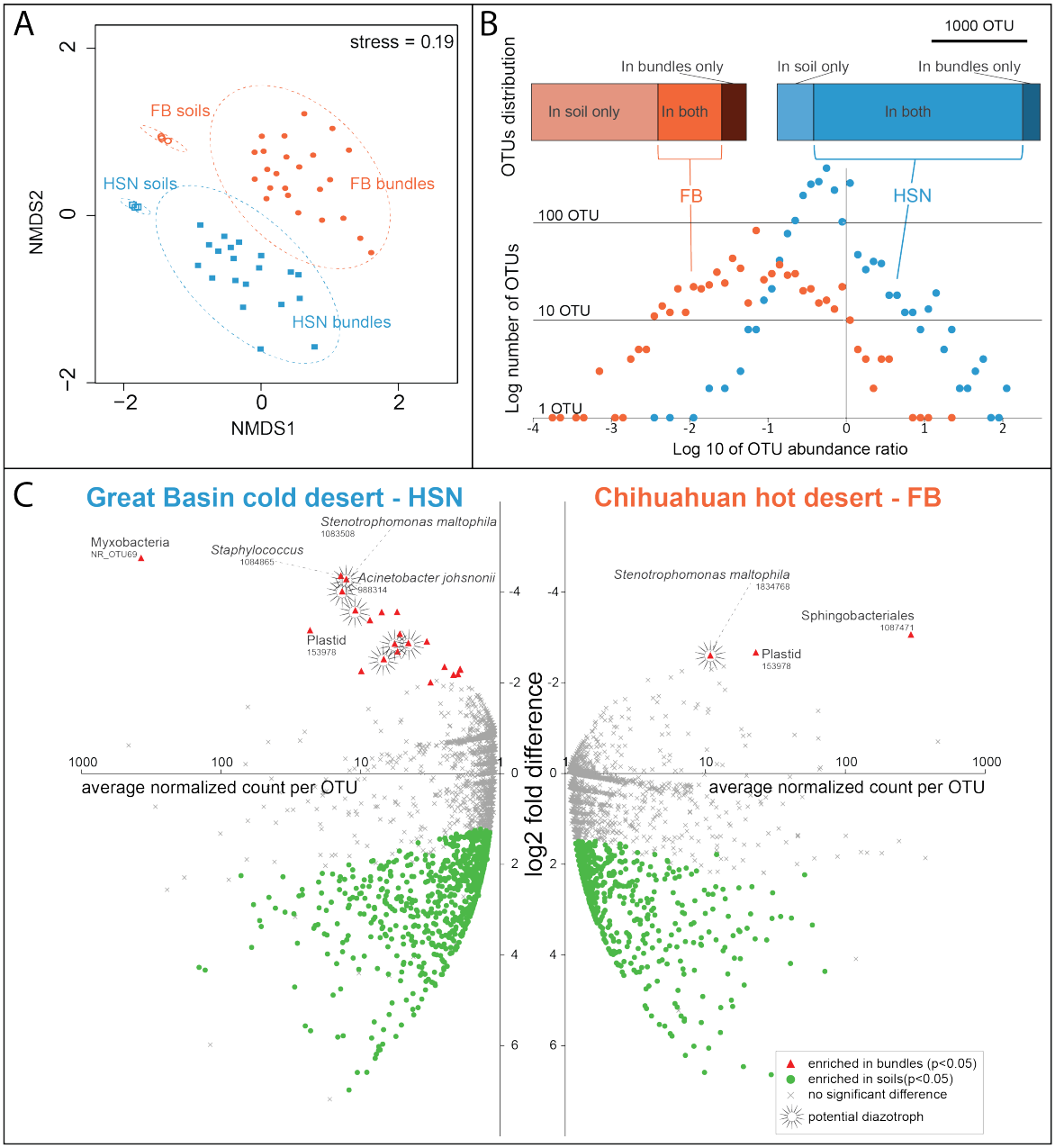

Figure 9. Spatial separation of microbial types close to, and away from, *M. vaginatus* in soil crusts. A: NMDS ordination of Bray-Curtis pairwise distance computed on the Hellinger-transformed OTU-composition in bulk soil or *M. vaginatus* cyanospheres (sans *M. vaginatus*), with 95% confidence ellipses drawn for each with a stress value of 0.19. In each setting, bulk soil communities differ in composition from their respective *M. vaginatus* cyanosphere (bundle communities). FB: Chihuahuan Desert (hot desert). HSN: Great Basin Desert (cold desert). B: Frequency distribution of the ratios in relative abundance for microbial OTUs that co-occurred in the cyanospheres of *M. vaginatus* and in the bulk soil crusts, showing a skewed distribution towards segregation. C: Differential abundance of microbial OTUs (sans *M. vaginatus*) in the cyanosphere vs. bulk soil crust

community assessed with the DESeq2 method for cold and hot desert locations. For each OTU, the average normalized counts are plotted against their differential abundance. OTUs that were differentially abundant ($p < 0.05$) are represented as solid triangles and circles, while cross symbols denote those with non-significant preference. Negative values indicate enrichment in the cyanosphere and positive in the bulk soil crust.

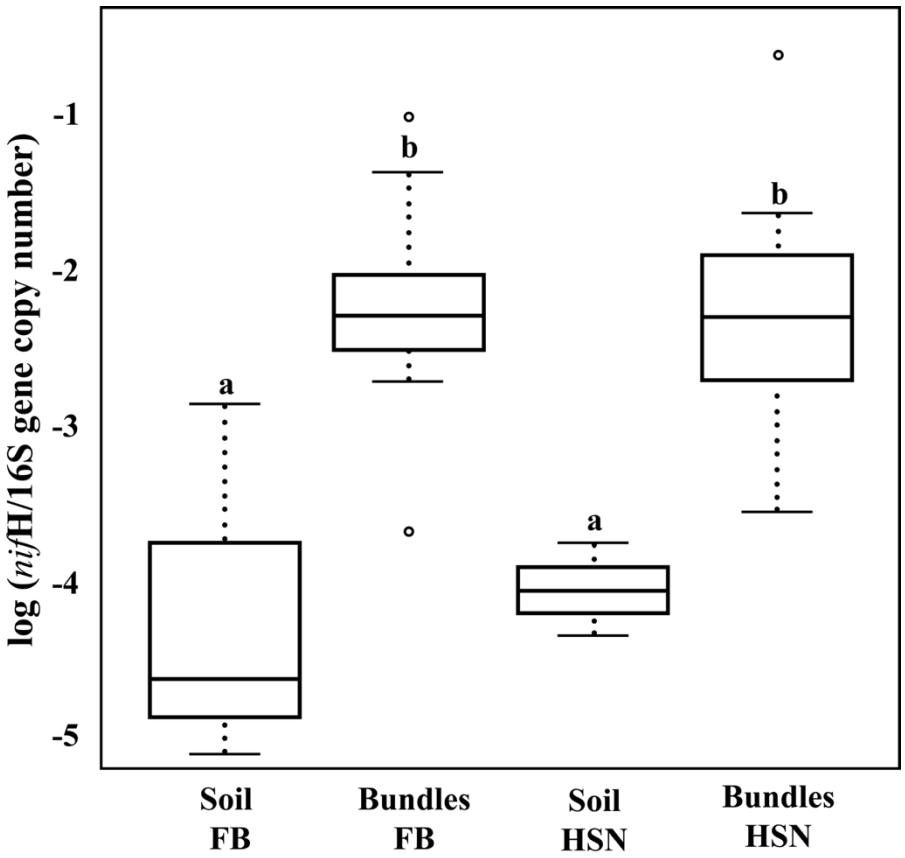

Figure 10. Ratio of the nifH to 16S rRNA gene copy number in bulk biocrust soil and *M. vaginatus* bundles (cyanosphere) communities. The nifH/16S rRNA ratio was obtained by quantitative PCR assays of each and was two to three orders of magnitude higher in the cyanosphere than in the bulk soil crust. A one-Way ANOVA test showed that differences between groups (*M. vaginatus* bundles vs. bulk biocrust soil) were significant (p value < 0.005).

Supplementary Information

Table S5. Summary of SSU rRNA gene libraries analyzed from HSN and FB sample set and associated coverage and α-diversity indices

			good's coverage	% of sequences kept through filtering	% of OTUs kept through filtering	total number of sequences analyzed	total number of OTUs	chao1
Chihuahuan Desert	Bundles	FBsample01	99.0	96.1	75.5	1873	77	101.4
		FBsample04	99.5	98.3	82.2	9366	208	253.0
		FBsample06	97.6	93.6	83.3	757	65	82.0
		FBsample07	99.3	84.3	83.3	1030	45	48.5
		FBsample08	98.0	95.2	83.3	1622	75	130.1
		FBsample09	99.3	99.2	88.7	4180	141	160.8
		FBsample11	99.4	77.7	67.6	1406	46	52.0
		FBsample14	99.2	85.3	72.4	2624	89	136.5
		FBsample15	99.3	98.3	90.8	4195	158	183.2
		FBsample17	98.3	96.7	85.5	2439	141	189.2
		FBsample19	99.4	97.5	87.8	1435	43	49.0
		FBsample2	98.3	99.3	90.8	5493	227	348.4
		FBsample22	97.6	92.5	81.5	545	53	66.0
		FBsample24	99.2	98.4	86.6	5686	181	234.7
		FBsample25	98.5	98.4	88.2	1054	82	89.5
		FBsample26	99.0	94.9	84.7	2408	105	120.8
		FBsample28	99.2	98.9	88.0	6268	228	266.6
		FBsample29	98.8	97.1	84.3	2516	107	158.7
		FBsample3	99.6	99.3	86.8	12995	203	269.3
		FBsample30	96.7	98.6	93.2	1825	178	222.6
		FBsample33	98.3	96.3	86.3	2085	158	183.2
		FBsample35	98.7	98.0	86.0	3543	191	219.3
		FBsample4	99.4	95.7	84.0	3028	89	106.1
		FBsample5	99.2	97.6	85.7	4788	150	191.6
	Soils	FBsampleA	98.4	95.2	68.5	25464	1293	1684.1
		FBsampleB	95.4	94.0	72.9	10800	1200	1760.3
		FBsampleC	98.0	89.3	56.1	23120	1616	1958.6
Great Basin Desert	Bundles	HSNsample01	98.9	99.3	88.5	6969	192	281.5
		HSNsample10	98.5	93.1	86.1	1744	118	147.5
		HSNsample11	98.6	98.7	87.9	5275	218	311.9
		HSNsample15	97.4	95.9	92.1	1361	128	206.8
		HSNsample18	97.2	99.0	93.7	2435	207	291.4
		HSNsample20	96.4	99.3	95.5	672	63	90.6
		HSNsample22	98.3	97.2	91.9	2399	147	192.6
		HSNsample23	98.1	98.6	92.3	3068	240	286.9
		HSNsample24	97.1	95.6	87.9	1387	131	209.0
		HSNsample25	99.3	91.0	73.2	4367	104	149.1
		HSNsample27	97.8	98.4	92.3	3251	203	291.1

		HSNsample28	99.4	97.7	75.6	9967	248	325.5
		HSNsample29	98.6	97.4	90.8	2841	108	154.3
		HSNsample34	99.1	98.6	89.8	6762	256	323.2
		HSNsample37	98.3	98.4	89.2	3811	223	290.3
		HSNsample42	99.4	98.8	88.0	17391	387	488.0
		HSNsample43	99.0	98.3	84.8	7662	291	343.4
		HSNsample44	99.3	86.4	72.1	10876	227	322.6
		HSNsample45	99.3	98.4	85.5	5408	141	187.3
		HSNsample46	99.6	99.4	90.3	6235	102	122.0
	Soils	HSNsampleA	99.1	94.8	57.8	51549	1985	2336.6
		HSNsampleB	99.2	94.3	55.9	58807	2113	2466.8
		HSNsampleC	99.0	94.1	57.6	51940	2038	2438.2

Table S6. Potential contaminants. Responding OTUs detected after amplification and sequencing of negative controls (n=5) without target *Microcoleus* bundles.

Phylum	Deepest Taxonomic Assignment	OTU ID	Presence in controls
Firmicutes	*Staphylococcus*	1084865	1 out of 5
Betaproteobacteria	*Pelomonas saccharophila*	1108275	3 out of 5
Gammaproteobacteria	*Moraxella*	990864	2 out of 5
Gammaproteobacteria	*Escherichia/Shigella*	1111294	2 out of 5

References

Abed, R. M. M., A. Ramette, V. Hübner, P. De Deckker, and D. de Beer. 2012. Microbial diversity of eolian dust sources from saline lake sediments and biological soil crusts in arid Southern Australia. FEMS Microbiology Ecology 80:294–304.

Angel, R., D. Matthies, and R. Conrad. 2011. Activation of methanogenesis in arid biological soil crusts despite the presence of oxygen. PloS ONE 6:1–8.

Antoninka, A., M. A. Bowker, S. C. Reed, and K. Doherty. 2016. Production of greenhouse-grown biocrust mosses and associated cyanobacteria to rehabilitate dryland soil function. Restoration Ecology 24.

Ayuso, S. V., A. G. Silva, C. Nelson, N. N. Barger, and F. Garcia-Pichel. 2017. Microbial Nursery Production of High- Quality Biological Soil Crust Biomass for Restoration of Degraded Dryland Soils 83:1–16.

Baran, R., E. L. Brodie, J. Mayberry-Lewis, E. Hummel, U. N. Da Rocha, R. Chakraborty, B. P. Bowen, U. Karaoz, H. Cadillo-Quiroz, F. Garcia-Pichel, and T. R. Northen. 2015. Exometabolite niche partitioning among sympatric soil bacteria. Nature Communications 6:8289.

Baran, R., N. N. Ivanova, N. Jose, F. Garcia-Pichel, N. C. Kyrpides, M. Gugger, and T. R. Northen. 2013. Functional genomics of novel secondary metabolites from diverse cyanobacteria using untargeted metabolomics. Marine Drugs 11:3617–3631.

Barger, N. N., B. Webber, F. Garcia-Pichel, E. Zaady, and J. Belnap. 2016. Patterns and Controls on Nitrogen Cycling of Biological Soil Crusts. Pages 257–285 *in* B. Weber, M. M. Caldwell, B. Jayne, W. Bettina, B. Büdel, J. Belnap, B. Weber, and B. Büdel, editors. Biological Soil Crusts: An Organizing Principle in Drylands. 2nd edition. Switzerland.

Bates, S. T., T. H. Nash, and F. Garcia-Pichel. 2012. Patterns of diversity for fungal assemblages of biological soil crusts from the southwestern United States. Mycologia 104:353–361.

Bates, S. T., T. H. Nash, K. G. Sweat, and F. Garcia-Pichel. 2010. Fungal communities of lichen-dominated biological soil crusts: Diversity, relative microbial biomass, and their relationship to disturbance and crust cover. Journal of Arid Environments 74:1192–1199.

Belnap, J., B. Büdel, and O. L. Lange. 2001. Biological Soil Crusts: Characteristics and Distribution. Pages 3–30 Biological Soil Crusts: Structure, Function, and Management. Springer.

Belnap, J., B. Weber, and B. Büdel. 2016. Biological Soil Crusts as an Organizing Principle in Drylands. Pages 3–14 Biological Soil Crusts: An Organizing Principle

in Drylands. 2nd edition. Springer, Switzerland.

Beraldi-Campesi, H., J. D. Farmer, and F. Garcia-Pichel. 2014. Modern Terrestrial Sedimentary Biostructures and Their Fossil Analogs in Mesoproterozoic Subaerial Deposits. Palaios 29:45–54.

Berger, S. A., D. Krompass, and A. Stamatakis. 2011. Performance, accuracy, and web server for evolutionary placement of short sequence reads under maximum likelihood. Systematic Biology 60:291–302.

Berger, S. A., and A. Stamatakis. 2011. Aligning short reads to reference alignments and trees. Bioinformatics 27:2068–2075.

Bethesda (MD): National Library of Medicine (US), N. C. for B. I. 1988. National Center for Biotechnology Information (NCBI).

Bethesda (MD): National Library of Medicine (US), N. C. for B. I. 2004. 16s ribosomal RNA sequences (bacteria/archaea) [Internet].

Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, C. A. Lozupone, P. J. Turnbaugh, N. Fierer, and R. Knight. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences 108:4516–4522.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, Mcdonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, K. R. 2010. QIIME allows analysis of high-thorughput community sequencing data. Nature Methods 7:335–336.

Ceja-Navarro, J. a, N. H. Nguyen, U. Karaoz, S. R. Gross, D. J. Herman, G. L. Andersen, T. D. Bruns, J. Pett-Ridge, M. Blackwell, and E. L. Brodie. 2014. Compartmentalized microbial composition, oxygen gradients and nitrogen fixation in the gut of Odontotaenius disjunctus. The ISME journal 8:6–18.

Couradeau, E., U. Karaoz, H. C. Lim, U. Nunes da Rocha, T. Northen, E. Brodie, and F. Garcia-Pichel. 2016. Bacteria increase arid-land soil surface temperature through the production of sunscreens. Nature Communications 7:10373.

Couradeau, E., V. J. M N L Felde, D. Parkinson, D. Uteau, A. Rochet, C. Cuellar, G. Winegar, S. Peth, T. R. Northen, and F. Garcia-Pichel. 2018. In Situ X-Ray Tomography Imaging of Soil Water and Cyanobacteria From Biological Soil Crusts Undergoing Desiccation. Frontiers in Environmental Science | www.frontiersin.org 1:65.

Coyte, K. Z., J. Schluter, and K. R. Foster. 2015. The ecology of the microbiome: Networks, competition, and stability. Science 350:663–666.

Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461.

Elbert, W., B. Weber, S. Burrows, J. Steinkamp, B. Büdel, M. O. Andreae, and U. Pöschl. 2012. Contribution of cryptogamic covers to the global cycles of carbon and nitrogen. Nature Geoscience 5:459–462.

Garcia-Pichel, F. 2003. Desert Environements: Biological Soil Crusts.

Garcia-Pichel, F., and J. Belnap. 2001. Small-scale environments and distribution of biological soil crusts. Pages 193–201 Biological soil crusts: Structure, function, and management. Springe.

Garcia-Pichel, F., J. Belnap, S. Neuer, and F. Schanz. 2006. Estimates of global cyanobacterial biomass and its distribution. Algological Studies 109:213–227.

Garcia-Pichel, F., V. Loza, Y. Marusenko, P. Mateo, and R. M. Potrafka. 2013. Temperature Drives the Continental-Scale Distribution of Key Microbes in Topsoil Communities. Science 340:1574–1577.

Garcia-Pichel, F., and M. F. Wojciechowski. 2009. The evolution of a capacity to build supra-cellular ropes enabled filamentous cyanobacteria to colonize highly erodible substrates. PloS ONE 4:7801.

Giraldo-Silva, A., C. Nelson, N. Barger, and F. Garcia-Pichel. 2019. Nursing biocrusts: isolation, cultivation and fitness test of indigenous cyanobacteria. Restoration Ecology:1–11.

Graham, L. E., J. M. Graham, L. W. Wilcox, M. E. Cook, P. Arancibia-Avila, and J. J. Knack. 2018. Evolutionary Roots of Plant Microbiomes and Biogeochemical Impacts of Nonvascular Autotroph-Microbiome Systems over Deep Time. International Journal of Plant Sciences 179:000–000.

Guidi, L., S. Chaffron, L. Bittner, D. Eveillard, A. Larhlimi, S. Roux, Y. Darzi, S. Audic, L. Berline, J. Brum, L. P. Coelho, J. C. I. Espinoza, S. Malviya, S. Sunagawa, C. Dimier, S. Kandels-Lewis, M. Picheral, J. Poulain, S. Searson, T. O. Coordinators, L. Stemmann, F. Not, P. Hingamp, S. Speich, M. Follows, L. Karp-Boss, E. Boss, H. Ogata, S. Pesant, J. Weissenbach, P. Wincker, S. G. Acinas, P. Bork, C. de Vargas, D. Iudicone, M. B. Sullivan, J. Raes, E. Karsenti, C. Bowler, and G. Gorsky. 2015. Plankton networks driving carbon export in the oligotrophic ocean. Nature 532:in review.

Gundlapally, S. R., and F. Garcia-Pichel. 2006. The community and phylogenetic diversity of biological soil crusts in the Colorado Plateau studied by molecular fingerprinting and intensive cultivation. Microbial Ecology 52:345–357.

Hatzenpichler, R., S. A. Connon, D. Goudeau, R. R. Malmstrom, T. Woyke, and V. J.

Orphan. 2016. Visualizing in situ translational activity for identifying and sorting slow-growing archaeal−bacterial consortia. Proceedings of the National Academy of Sciences 113:E4069–E4078.

Johnson, S. L., C. R. Budinoff, J. Belnap, and F. Garcia-Pichel. 2005. Relevance of ammonium oxidation within biological soil crust communities. Environmental Microbiology 7:1–12.

Jose, N. A., R. Lau, T. L. Swenson, N. Klitgord, F. Garcia-Pichel, B. P. Bowen, R. Baran, and T. R. Northen. 2018. Flux balance modeling to predict bacterial survival during pulsed-activity events. Biogeosciences 15:2219–2229.

Karaoz, U., E. Couradeau, U. N. da Rocha, H.-C. Lim, T. Northen, F. Garcia-Pichel, and E. L. Brodie. 2018. Large Blooms of Bacillales (Firmicutes) Underlie the Response to Wetting of Cyanobacterial Biocrusts at Various Stages of Maturity. mBio 9:e01366-16.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Meintjes, and A. Drummond. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data 28:1647–1649.

Kuske, C. R., C. M. Yeager, S. Johnson, L. O. Ticknor, and J. Belnap. 2012. Response and resilience of soil biocrust bacterial communities to chronic physical disturbance in arid shrublands. The ISME journal 6:886–897.

Kuzyakov, Y., and E. Blagodatskaya. 2015. Microbial hotspots and hot moments in soil: Concept & review. Soil Biology and Biochemistry 83:184–199.

Letunic, I., and P. Bork. 2007. Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinformatics 23:127–128.

Lynch, M. D. J., and J. D. Neufeld. 2015. Ecology and exploration of the rare biosphere. Nature Reviews Microbiology 13:217–229.

Marusenko, Y., S. T. Bates, I. Anderson, S. L. Johnson, T. Soule, and F. Garcia-Pichel. 2013. Ammonia-oxidizing archaea and bacteria are structured by geography in biological soil crusts across North American arid lands. Ecological Processes 2:9.

Masella, A. P., A. K. Bartram, J. M. Truszkowski, D. G. Brown, and J. D. Neufeld. 2012. PANDAseq: paired-end assembler for Illumina sequences. BMC bioinformatics 13:31.

McDonald, D., M. N. Price, J. Goodrich, E. P. Nawrocki, T. Z. DeSantis, A. Probst, G. L. Andersen, R. Knight, and P. Hugenholtz. 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. The ISME journal 6:610–8.

McMurdie, P. J., and S. Holmes. 2014. Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. PLoS Computational Biology 10.

Miller, M. A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees.

Milo, R., S. Shen-Orr, S. Itzkovitz, and N. Kashtan. 2002. Network Motif: Simple Building Blocks of Complex Networks. Science 298:298.

Mitri, S., E. Clarke, and K. R. Foster. 2015. Resource limitation drives spatial organization in microbial groups. The ISME Journal 10:1–12.

Nadell, C. D., K. R. Foster, and J. B. Xavier. 2010. Emergence of spatial structure in cell groups and the evolution of cooperation. PLoS Computational Biology 6.

Nagy, M. L., A. Pérez, and F. Garcia-Pichel. 2005. The prokaryotic diversity of biological soil crusts in the Sonoran Desert (Organ Pipe Cactus National Monument, AZ). FEMS Microbiology Ecology 54:233–245.

Nawrocki, E. P. 2009. Structural RNA homology search and alignment using covariance models. Washington University in St. Louis.

Nübel, U., G. Muyzer, F. Garcia-Pichel, and G. Muyzer. 1997. PCR primers to amplify 16S rRNA genes from cyanobacteria PCR Primers To Amplify 16S rRNA Genes from Cyanobacteria. Microbiology 63:3327–3332.

Nunes da Rocha, U., H. Cadillo-Quiroz, U. Karaoz, L. Rajeev, N. Klitgord, S. Dunn, V. Truong, M. Buenrostro, B. P. Bowen, F. Garcia-Pichel, A. Mukhopadhyay, T. R. Northen, and E. L. Brodie. 2015. Isolation of a significant fraction of non-phototroph diversity from a desert Biological Soil Crust. Frontiers in Microbiology 6:1–14.

Parks, D. H., C. Rinke, M. Chuvochina, P.-A. Chaumeil, B. J. Woodcroft, P. N. Evans, P. Hugenholtz, and G. W. Tyson. 2017. Recovery of nearly 8,000 metagenome-assembled genomes substantially expands the tree of life. Nature Microbiology 903:1–10.

Pepe-Ranney, C., C. Koechli, R. Potrafka, C. Andam, E. Eggleston, F. Garcia-Pichel, and D. H. Buckley. 2015. Non-cyanobacterial diazotrophs dominate dinitrogen fixation in biological soil crusts during early crust formation. The ISME Journal 10:287–298.

Poly, F., L. J. Monrozier, and R. Bally. 2001. Improvement in the RFLP procedure for studying the diversity of nifH genes in communities of nitrogen fixers in soil. Research in Microbiology 152:95–103.

Rajeev, L., U. N. Da Rocha, N. Klitgord, E. G. Luning, J. Fortney, S. D. Axen, P. M. Shih, N. J. Bouskill, B. P. Bowen, C. A. Kerfeld, F. Garcia-Pichel, E. L. Brodie, T.

R. Northen, and A. Mukhopadhyay. 2013. Dynamic cyanobacterial response to hydration and dehydration in a desert biological soil crust. The ISME Journal:2178–2191.

Rodríguez-Caballero, E., J. Belnap, B. Büdel, P. Crutzen, M. O. Andreae, U. Pöschl, and B. Weber. 2018. Dryland photoautotrophic soil surface communities endangered by global change. Nature Geoscience 11:185-189.

Samo, T. J., J. A. Kimbrel, D. J. Nilson, J. Pett-Ridge, P. K. Weber, and X. Mayali. 2018. Attachment between heterotrophic bacteria and microalgae influences symbiotic microscale interactions. Environmental Microbiology.

Sasse, J., E. Martinoia, and T. Northen. 2018. Feed Your Friends: Do Plant Exudates Shape the Root Microbiome? Trends in Plant Science 23:25–41.

Soule, T., I. J. Anderson, S. L. Johnson, S. T. Bates, and F. Garcia-Pichel. 2009. Archaeal populations in biological soil crusts from arid lands in North America. Soil Biology and Biochemistry 41:2069–2074.

Stamatakis, A. 2014. RAxML Version 8 : A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies:2010–2011.

Starkenburg, S. R., K. G. Reitenga, T. Freitas, S. Johnson, P. S. G. Chain, F. Garcia-Pichel, and C. R. Kuske. 2011. Genome of the cyanobacterium Microcoleus vaginatus FGP-2, a photosynthetic ecosystem engineer of arid land soil biocrusts worldwide. Journal of bacteriology 193:4569–70.

Swenson, T. L., E. Couradeau, B. P. Bowen, R. de Philippis, F. Rossi, G. Mugnai, and T. R. Northen. 2017. A novel method to evaluate nutrient retention by biological soil crust exopolymeric matrix. Plant and Soil.

Swenson, T. L., U. Karaoz, J. M. Swenson, B. P. Bowen, and T. R. Northen. 2018. Linking soil biology and chemistry in biological soil crust using isolate exometabolomics. Nature Communications 9.

Thomazo, C., E. Couradeau, and F. Garcia-Pichel. 2018. Possible nitrogen fertilization of the early Earth Ocean by microbial continental ecosystems. Nature Communications 9.

Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73:5261–5267.

Yeager, C. M., J. L. Kornosky, R. E. Morgan, E. C. Cain, F. Garcia-Pichel, D. C. Housman, J. Belnap, and C. R. Kuske. 2007. Three distinct clades of cultured heterocystous cyanobacteria constitute the dominant N2-fixing members of biological soil crusts of the Colorado Plateau, USA. FEMS Microbiology Ecology 60:85–97.

5 - NICHE PARTITIONING WITH TEMPERATURE AMONG HETEROCYSTOUS CYANOBACTERIA (*Scytonema* spp., *Nostoc* spp., AND *Tolypothrix* spp.) FROM BIOLOGICAL SOIL CRUSTS

Submitted

Niche partitioning with temperature among heterocystous cyanobacteria (*Scytonema* spp., *Nostoc* spp., and *Tolypothrix* spp.) from biological soil crusts

Coauthors has acknowledged the use of this manuscript in my dissertation

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Abstract

Nitrogen inputs from biological fixation are crucial for dryland ecology and heterocystous cyanobacteria are key players in this process. We studied the niche partitioning among the three most common heterocystous cyanobacteria from biocrusts using enrichment cultivation, determining the ranges for growth in a set of 30 isolates. *Scytonema* spp. were the most thermotolerant, typically growing up to 40 °C, whereas *Tolypothrix* spp. was the only group growing well at 4 °C. *Nostoc* spp. responded well at intermediate temperatures. We could also correlate the heat sensitivity in *Nostoc* spp. and *Tolypothrix* spp. strains with N_2-fixation because the thermal range for growth could be increased under nitrogen replete conditions. This sensitivity could be traceable to an inability to develop heterocysts (specialized nitrogen fixing cells) at high temperatures. We tested the relevance of this apparent niche partitioning using a meta-analysis of a large set of molecular surveys of biocrust cyanobacteria. In agreement with the physiological data, the geographic distribution of the three taxa is clearly constrained by the mean temperature during the growth season in the sites of origin. This allows us to predict a potential shift in dominance in many locales as a result of global warming, to the benefit of *Scytonema* spp. populations.

Introduction

In drylands, where plant growth is limited by water and nutrients, the soil surface can be occupied by communities of microorganisms known as biological soil crusts (biocrusts; see Garcia-Pichel 2003, for a primer, and Belnap et al. (2016) for a monograph). Biocrusts play crucial roles for the fertility and stability of drylands. Their presence enhances resistance to erosion caused by water (Gaskin and Gardner 2001) or wind (Belnap and Gillette 1997, Zhang et al. 2006), modifies soil surface temperature (Couradeau et al. 2016), and influences water retention and runoff (Verrecchia et al. 1995, Rodríguez-Caballero et al. 2012, Faist et al. 2017). Colonization of bare soils, typically pioneered by highly motile filamentous cyanobacteria like *Microcoleus vaginatus* and *Microcoleus steenstrupii* (Garcia-Pichel and Wojciechowski 2009) results in the formation of incipient communities (early successional biocrusts). Once the surface is stabilized, sessile, heterocystous cyanobacteria constitute secondary colonizers in the crust-forming succession. The community also host a variety of populations of heterotrophic bacteria (Nagy et al. 2005, Nunes da Rocha et al. 2015), archaea (Soule et al. 2009) and fungi (Bates et al . 2012). Once established, these heterocystous cyanobacteria are significant contributors to dinitrogen inputs in soils crusts (Johnson et al. 2005), taking over this role from heterotrophic diazotrophic bacteria (Pepe-Ranney et al. 2015) that enter in C for N symbioses with *Microcoleus vaginatus* in early succession stages (Couradeau et al. 2019). Three clades, *Scytonema* spp., *Nostoc* spp. and *Tolypothrix/Spirirestis* spp., have been identified as the most abundant diazotrophic cyanobacteria in biocrusts communities of the Southwestern US (Yeager et al. 2007). Soil crusts are typically in a perennial state of nitrogen deficiency because the internal nitrogen cycle is broken (Johnson et al. 2007,

Strauss et al. 2011). Biological fixation thus remains a necessity for continued growth. Fixed atmospheric carbon and nitrogen (Thiet et al. 2005, Johnson et al. 2007, Thomazo et al. 2018a), along with other elements (Beraldi-Campesi et al. 2009) can then be exported to underlying soils, improving landscape soil fertility. Because drylands cover nearly 45% of the total Earth continental area (Prăvălie 2016), and aridity is predicted to increased due to global warming (Seager and Vecchi 2010, Petrie et al. 2014, 2015), this N export activity of biocrusts matter not only locally, but also globally. In fact, the global dinitrogen fixation of cryptogamic covers, much of which are biocrusts, has been estimated at 49 Tg/yr, nearly 50% of the biological nitrogen fixation on land (Elbert et al. 2012).

US Southwest biocrust N_2-fixation activity has been determined experimentally to be optimal in the range of 15 - 30 °C regardless of the biocrusts origin or successional stage assayed (Barger et al. 2013, Zhou et al. 2016), with rates decreasing significantly between 30 and 35 °C (Zhou et al. 2016). This sensitivity has been ascribed to possible deleterious effects of temperature on N_2-fixing cyanobacteria (Zhou et al. 2016). Thermophysiological studies using laboratory isolates (Zhou et al. 2016, Muñoz-Martín et al. 2018) or geographical distribution in molecular tallies (Garcia-Pichel et al, 2013) have shown that the three main clades of biocrust cyanobacteria are characterized by different temperature range for growth: the *Scytonema* spp. clade tends to be more thermotolerant, whereas the *Tolypothrix* spp. clade shows psychrophilic preferences, and strains in the *Nostoc* spp. clade showed a preference for mild temperatures (15 to 30 °C). However, these results come from the evaluation of a restricted number of sites or strains, and the patterns are not always robust. Clearly, however, the results point to a potential

for differential sensitivity of these cyanobacteria to environmental warming, a future scenario with which biocrust will have to contend. Drylands at large will likely become warmer and drier in response to global warming. In particular, the southwestern United States is predicted to experience an increase in temperature of about 1 °C per decade (Seager and Vecchi 2010), accompanied by alterations in precipitation frequency (Cable and Huxman 2004, Knapp et al. 2008, Sala and Lauenroth 2014)

In this contribution we wanted to evaluate in detail the thermophysiology of biocrust heterocystous cyanobacteria using cultivated isolates, and to test if it is the sensitivity of N_2-fixation that determines their temperature niche differentiation. Finally, we wanted to test if the physiological data obtained from cultures, can explain the current biogeographic distribution of each clade, and hence potentially help us predict their fate in the face of global warming.

Methods

Enrichment cultures

Field biocrusts were collected from the cold Great Basin Desert (Utah-USA), and from the hot Chihuahuan desert (New Mexico-USA), and from two textural types in each (Great Basin: sandy clay loam and clay loam, and Chihuahuan: clay loam and loamy sand; locations and soil types details are given in Geraldo-Silva *et al*., (2019), using 1.5 X 9 cm diameter Petri plates. Three enrichment cultures (per tested temperature) were prepared from each site by randomly taking cores (0,5 cm deep, 1 cm in diameter) from each Petri plate. Cored biocrusts were crumbled and placed on 1.5 % (w/v) agar-solidified nitrogen-free Petri plates (BG11o; Allen and Stanier, 1998). Triplicates were incubated at 4, 25 and 30 °C, for 20 days at 20 to 27 $\mu mol.m^{-2}. s^{-1}$. After incubation, grown colonies were counted, sampled and observed under the compound microscope (NIKON labophot-2).

Differences in the relative proportions of the cyanobacterial colonies counted at different temperatures were assessed via permutational multivariate analysis of variance (PERMANOVA). PERMANOVAs were performed on the Bray-Curtis distance matrices of relative proportions derived from colonies counts and used 999 permutations. PERMANOVAS were run on PRIMER 6 (Clarke and Gorley, 2006).

Experimental organisms and growth conditions

Thirty cyanobacterial strains: 12 *Scytonema* spp., 10 *Nostoc* spp., and 8 *Tolypothrix* spp. previously isolated as a part of our "microbial biocrust nurseries" protocols (see Giraldo-Silva *et al.*, 2019), were used in our experiments. Briefly, strains were isolated by

enrichment cultures in agar-solidified BG11o Petri plates at different temperatures (4, 25 and 30 °C), followed by multiple streaking colonies on fresh agar plates. Strains 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 CYA359F/CYA781R (Nübel *et al.*, 1997; PCR protocol therein), blast comparisons, and by placing the sequences on the cyanobacterial tree Cydrasil. PCR products were sequenced using Sanger sequencing. All strains are unicyanobacterial and are kept in our local culture collection, and are available upon request. Strain accession numbers along with their denomination coding for site of origin can be found in Table S10.

Stock cultures were grown in 175 mL cell culture flasks containing 100 mL of minimal nitrogen free medium (BG11o). Cultures were maintained at 25 ± 2 °C, under a 14 h photoperiod, Illuminated at 20-27 μmol (photon) m^{-2} s^{-1} provided by white fluorescent tubes.

Delineation of temperature range for growth of isolates

Prior to inoculation, stock liquid cultures of each strain were homogenized by repeatedly forcing biomass through a 60 mL sterile syringe, and immediately washed with fresh BG11o medium by five consecutive centrifugations (8 min, 8437 g, 25 °C). 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 20 mL mark. Each strain was incubated at 4, 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. Growth

was estimated visually after 30 days as either positive for growth or negative for growth (either no growth or patent death). The whole experiment was replicated a second time in full, and growth in any of the trials was reported as positive.

Influence of diazotrophy on the upper temperature limit for growth

A homogenized, cleaned culture mix was prepared for each of the strains as detailed above, and inoculated (5% v/v) in 50 mL cell culture flasks containing either nitrogen-free medium ($BG11_o$) or nitrogen containing medium (BG11, Allen & Stanier 1968). Triplicate cultures were incubated at 35 and 40 °C, Illuminated with 20-27 μmol (photon) m^{-2} s^{-1} provided by white fluorescent tubes, in a 14 h photoperiod regime, for 30 days.

Heterocyst and vegetative cell counts

To determine the frequency of heterocysts we conducted microscopic cell counts on fresh wet mounts under bright field Illumination in a NIKON labophot-2 compound microscope. At least 200 cells were counted in each determination. To determine the effect of nitrogen source and incubation temperature on heterocysts frequency we examined triplicate cultures of each strain at 25, 35 and 40 °C, all at day 7 after inoculation. The full experiment was replicated for a total n = 6.

Chlorophyll *a* determination

Chlorophyll *a* (Chl *a*) was measured as a proxy for phototrophic biomass. Chl *a* was extracted in triplicate, in 90% acetone, according to Castle et al. (2011), vortexed for 30 s. and allowed to extract for 24 h 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). Interference from scytonemin and carotenoids was corrected using the trichromatic equation of Garcia-Pichel & Castenholz (1991).

Meta-analysis of temperature niches

In an attempt to look for a temperature segregation pattern among the studied taxa in the natural biocrust environment, we performed a meta-analysis of all bacterial 16S rRNA tallies available publicly. We either downloaded from public databases or directly requested raw sequence data from authors from multiple environmental biocrust surveys conducted at different locations around the world. We collected data from different arid and semiarid regions in USA (Garcia-Pichel *et al.*, 2013; Couradeau *et al.*, 2016; Velasco Ayuso *et al.*, 2016; Fernandes *et al.*, 2018; Bethany *et al*., submitted), 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. 2016), and from the Brazil savannah (Machado de Lima *et al*., *in prep*). A complete list of the biocrust surveys with locations, environmental variables, and other relevant information to perform this metanalysis can be found in Table S12.

For all but the dataset from Garcia-Pichel *et al.,* (2013), forward reads obtained with pyrosequencing (Zhang et al. 2016) 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. 2010), 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* (2013) dataset, because quality files (.fastq) were not available, and in an effort to control for sequence quality before preforming any downstream analysis, raw sequences were first filtered using USEARCH 7 (Edgar 2010) to remove all sequences with less than 210 bp. Overall this step filtered out up to 5% of the total sequences in some but not all samples. Additionally, the first and last 10 bp of each sequence were trimmed 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., 2010) 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. 2007a), 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-0.22a (https://github.com/FGPLab/cydrasil/tree/0.22a), by aligning sequences to the cyanobacterial tree alignment (reference alignment) using PaPaRa (Berger and Stamatakis 2011), and then placing them into the reference tree using the RaxML8 Evolutionary Placement Algorithm (Berger et al. 2011). The resulting trees were imported and visualized in the iTOL4 server (Letunic and Bork 2016).

The proportion of *Scytonema* spp., *Nostoc* spp. and *Tolypothrix* spp. within the cyanobacterial community was calculated by dividing the total number of reads of either *Scytonema* spp., *Nostoc* spp. or *Tolypothrix* spp., by the sum of the total number of reads of all heterocystous cyanobacteria found at each given location. Resulting proportions were plotted against the mean annual temperature (MAT) and the mean temperature of the wettest quarter of the year (growth season) in each location of origin. A total of 19 (out of 89) locations at which the total relative abundance of N_2-fixing cyanobacteria was lower than 0.5 % were excluded from final plots. Mean annual temperature and mean temperature of the wettest quarter of the year were calculated from environmental variables of monthly climate data for minimum, mean, and maximum temperature and for precipitation for 1970-2000. Data was downloaded from WorldClim -Global Climate Data -version 2 (http://www.worldclim.org; Fick and Hijmans, 2017).

Results

Enrichment cultivation

Enrichment cultures for diazotrophic photoautotrophs carried out at different temperatures using inoculum from four different biocrust was very revealing. Expectedly, only heterocystous cyanobacteria were enriched for under these conditions, and all 994 colonies examined belonged to one of the 3 major clades known from biocrusts: *Nostoc* spp., *Tolypothrix* spp., and *Scytonema* spp. (Yeager et al. 2007). The relative proportions obtained, however, were strongly dependent on the temperature of incubation (Figure 11). Counted colonies growing at 4 °C were significantly different from those growing at 25 °C (PERMANOVA pseudo-F: 6.22 df: 22 p = > 0.001) and 30 °C (PERMANOVA pseudo-F: 9.36 df: 22 p = > 0.001); the same was true for communities growing at 25 and 30 °C (PERMANOVA pseudo-F: 6.43 df: 22 p = >0.001). *Scytonema* spp. made up the majority of the colonies at 30 °C, whereas *Tolypothrix* spp. was preferentially selected for at 4°C. *Nostoc* spp. had a slight advantage at lower temperatures as well. This was so regardless of the origin of the crusts used for inoculation, in that there was no significant effect on outcomes by location (PERMANOVA, p = <0.2; full dataset presented in Table S10).

Temperature range for growth of isolated strains

All cyanobacterial strains (tested in nitrogen depleted media) showed robust growth at 15 and 25 °C, while none grew at 45 °C (Figure 12), the lower limit of moderate thermophilic organisms. Formally then, all these strains are mesophiles with respect to temperature. At 4 °C, all *Tolypothrix* spp. strains grew well, while only one *Scytonema* spp. strain did. At this

temperature, three *Nostoc* spp. strains did not grow, while five strains were in apparent stasis (they did not grow, but did not show signs of cellular degradation). At 30 °C four out of eight *Tolypothrix* spp., nine out of 10 *Nostoc* spp., and 11 out of 12 *Scytonema* spp. strains grew well. At 35 and 40 °C, no *Nostoc* spp. or *Tolypothrix* spp. strains grew, while 11 out of 12 *Scytonema* spp. did.

Upper temperature limit for growth for and N_2-fixation

We looked at growth responses in more detail as a function of nitrogen source (N_2-fixing vs. non N_2-fixing conditions) in the upper range of temperature (35 and 40 °C) to determine if N_2-fixation was the most sensitive cellular process determining the observed outcomes. Figure 13 shows the biomass yield of the 30 cyanobacterial strains after 30 days of growth cultivated in nitrogen-free and nitrogen replete media. It was obvious that providing a source of nitrogen expanded the range for growth in many of them to 35 °C (*Scytonema* spp. JS003; *Nostoc* spp. HSN008, HS002, HS094, HS096, HS013, FB25; *Tolypothrix* spp., HSN032, HSN033, HSN034) and in some cases to 40 °C (*Nostoc* spp. HSN008, HS020, HS002, HS096, FB23, FB26; *Tolypothrix* sp. HSN042). The last column in Figure 13. shows the biomass yield in nitrogen replete minus those attained in nitrogen free medium at 35 °C, indicating a generalized positive effect on growth under N_2-replete conditions. For 16 out 30 strains this difference in growth was significant. This supports the contention that the upper temperature for growth is often determined by the sensitivity of nitrogen fixation in *Nostoc* spp. and *Tolypothrix spp.*, whereas it is not nearly as determinant for *Scytonema* spp.

Heterocyst frequency

To determine if this effect of N_2-fixation was due to an inability to develop heterocysts (a developmentally specialized cell type dedicate to this process), we conducted microscopic counts of vegetative cells and heterocysts in strains incubated for seven days at different temperatures (Table 5). Counts were performed only on apparently healthy filaments, but at 35 and 40 °C, biomass from all replicates of *Nostoc* spp. HSN008 and *Tolypothrix* spp. HSN042 looked yellowish, and microscopy revealed high cell mortality as well. In fact, in one occasion, one set of replicates of *Nostoc* spp. HSN008 did not survive to day 7 (Table 5). All strains looked healthy were counts were performed at 25 °C. Those caveats aside, the frequency of heterocysts declined precipitously for *Nostoc* spp. strains above 35 degrees, and above 30 degrees for *Tolypothrix* spp. strains. In *Scytonema* spp., there were only slight decreases in this frequency in the temperature range tested. This is consistent with a cell developmental basis for the sensitivity of N_2-fixation with temperature in *Nostoc* spp. and *Tolypothrix* spp.

Thermal niche of biocrust heterocystous cyanobacteria through meta-analyses of molecular surveys

A total of 89 locations from nine different biocrust surveys conducted in different arid and semiarid regions in North and South America, Europe and China (see Table S12), were used in a meta-analysis to assess the relative contribution of the three main clades of heterocystous cyanobacteria along temperature related parameters. Figure 14 shows the relative proportion of *Scytonema* spp., *Nostoc* spp. and *Tolypothrix* spp. with respect to all other heterocystous

cyanobacteria, plotted against the mean annual temperature (MAT) of origin and the mean temperature during the wettest quarter of the year (MTempWetQ). MTempWetQ was used as a proxy for growth season since biocrust organisms are metabolically active only when water is available and are relatively insensitive to heat stress when dry. Using MAT as an explanatory variable, no trends were conspicuous. However, when MTempWetQ was used, patterns became evident. *Scytonema* spp. could attain dominance at warmer temperatures (Figure 14A), while at lower temperatures, *Tolypothrix* spp. (Figure 14C), followed by *Nostoc* spp. (Figure 14B) attain higher maximal relative abundances.

Discussion

The cyanobacteria *Scytonema* spp., *Nostoc* spp. and *Tolypothrix* spp. are secondary colonizers in the ecological succession of biocrust communities (Couradeau et al. 2016), where they are among the most common heterocystous organisms (Yeager et al. 2007, Couradeau et al. 2016, Williams et al. 2016, Muñoz-Martín et al. 2018, Giraldo-Silva et al. 2019), and contribute with much of the nitrogen inputs to the community at this stage of development (Yeager et al. 2004). Therefore, it is logical to assume that their presence and relative abundance have direct effects on the N_2-fixation capability of late successional biocrusts. Using quantitative enrichment cultures we could clearly demonstrate differential fitness in these cyanobacteria at different temperatures, in a pattern that confirms the preferences inferred in prior field studies (Garcia-Pichel *et al.*, 2013; Muñoz-Martín *et al.*, 2018).

Using a set of cultivated strains (12 *Scytonema* spp., 10 *Nostoc* spp. and 8 *Tolypothrix* spp.) isolated from cold and hot desert locations of the Southwestern US, the temperature range for growth revealed a pattern of niche differentiation according to temperature: *Tolypothrix* spp. strains having an advantage at the lower temperatures, and *Scytonema* spp. strains at higher temperatures. *Nostoc* spp. strains occupied only the mesic part of the range. This niche separation is similar to that found in non-heterocystous filamentous cyanobacteria of soil crusts (Garcia-Pichel *et al.*, 2013), and parallels the much more conspicuous niche differentiation of cyanobacteria known from hot springs at temperatures between 45-73 °C (Castenholz 1969). We could also show that that the upper temperature limit for N_2-fixing activity in the studied strains is more constrained than the temperature range at which they can grow under non N_2-fixing conditions (Figure 13), implicating nitrogen fixation as a determinant of the effective range in

nature. The observed thermophysiological responses of the tested strains at 35 °C, coincide with the more dramatic decreases in N_2-fixation rates (above 30 °C) from cold than from hot biocrusts locations shown by Zhou *et al.*, (2016), in that *Nostoc* spp. and particularly *Tolypothrix* spp. are more abundant in biocrusts from colder locations, while *Scytonema* spp. typically dominates warmer ones (Garcia-Pichel *et al*., 2013; Velasco Ayuso *et al.*, 2016; Giraldo-Silva *et al.*, 2019).

In an effort to better understand the basis for this effect on N_2-fixation we determined the ratio of heterocysts frequency at different temperatures in a selected set of strains, which were responsive to our experimental conditions (*Scytonema* spp. JS006, *Nostoc* spp. HSN008 and *Tolypothrix* spp. HSN042, Figure 13). The results suggest that in *Nostoc* spp. and *Tolypothrix* spp., the impossibility of these strains to grow under N_2-fixation conditions at temperatures above 30 °C may be determined by an inability to carry out the developmental cycle leading to the differentiation of heterocysts. While *Scytonema* spp. may have overcome such developmental problems (Table 5), nitrogenase degradation, which has been reported to happen at temperatures above 39 °C (Hennecke and Shanmugam 1979) could be the basis for the observed differences in *Scytonema* spp. strains' biomass yield at 35 °C (Figure 13). Nitrogen fixation and heterocyst differentiation at temperatures above 40 °C is not a problem in principle, in that the freshwater thermophilic cyanobacterium *Mastigocladus laminosus* performs N_2-fixation at 45 °C (Nierzwicki Bauer et al. 1984), and is able to grow at temperatures as high as 57 °C (Miller et al. 2007).

We tested the relevance of this temperature-based niche differentiation in nature by studying the distribution of the studied cyanobacterial types as a function of climate parameters in a meta-analysis of a large dataset of biocrust surveys. Indeed, we found that the maximal

proportion of *Scytonema* spp. among all heterocystous cyanobacteria increased along the temperature gradient with increasing temperatures (Figure 14A), when the average temperatures of the growth (wet) season was considered. It thus seems that the physiological niche differentiation found in culture experiments does translate to the distribution of these cyanobacteria in nature. Given the observed differential response of biocrust N_2-fixing cyanobacteria to temperature, and in agreement with (Muñoz-Martín *et al.*, (2018), it is reasonable to forecast that a microbial replacement within biocrust heterocystous cyanobacteria, may indeed be in store as a result of global warming. *Scytonema* spp. may replace more cold- and mesic-temperature adapted taxa such *Tolypothrix* spp. and *Nostoc* spp. In places such as the Colorado Plateau, the Mojave desert, the north part of the Chihuahuan Desert (Sevilleta LTER) in the USA, Alicante in Spain and the Brazilian Savanah, where the mean annual temperature during the growth season falls between the 17 and 23 °C range, this microbial replacement will likely happen faster than at those locations exhibiting mean average temperatures below 17 °C, that are not projected to reach sensitive temperature ranges for decades to centuries, or locations with average temperatures above 24 °C, which already exhibit a dominance of *Scytonema* spp. (Figure 14). This microbial replacement could have implications for drylands and biocrust nitrogen inputs beyond a mere compositional change. *Scytonema* spp. has been shown to be one of the most sensitive taxa in biocrust to changes in precipitation patterns (Fernandes et al. 2018). In this scenario, the N_2-fixing cyanobacteria taxa that seem to be better adapted to withstand increases in temperature, ironically, seem to be among the least adapted to withstand drought. Although it makes sense that cyanobacterial distribution patterns with increasing temperature only became apparent when mean temperature during the wettest quarter of the year was used as an explanatory variable, we were

surprise by the fact that plots using MAT did not show any patterns in the distribution of the cyanobacteria of interest (Figure 14). This highlights the need to take into account the ecophysiology of microorganisms when seeking to find important climatic drivers.

These results can also serve to improve strategies to restore biological soil crust communities by providing information that can be used to decide the best inoculation season. Considerations of current and predicted temperature changes of the desired restoration site in response to global warming could also be factored into the design of the most adequate microbial mixture for the restoration inoculum.

Tables

Table 5. Frequency of heterocysts (number of vegetative cells per heterocyst) in representative cyanobacterial strains after incubation at 30, 35 and 40 °C for 7 days. Averages of n=6 determinations, ± standard deviation are given.

Strain	Incubation Temperature (°C)		
	30	35	40
Nostoc spp. HSN008	8.5 ± 1.4	18 ± 4.8*	50 ± 4.9
Tolypothrix spp. HSN042	13.8 ± 1.0	115 ± 19	262 ± 103
Scytonema spp. JS006	19.2 ± 2.9	29.4 ± 5.4	28.8 ± 2.6

* n = 5

Figures

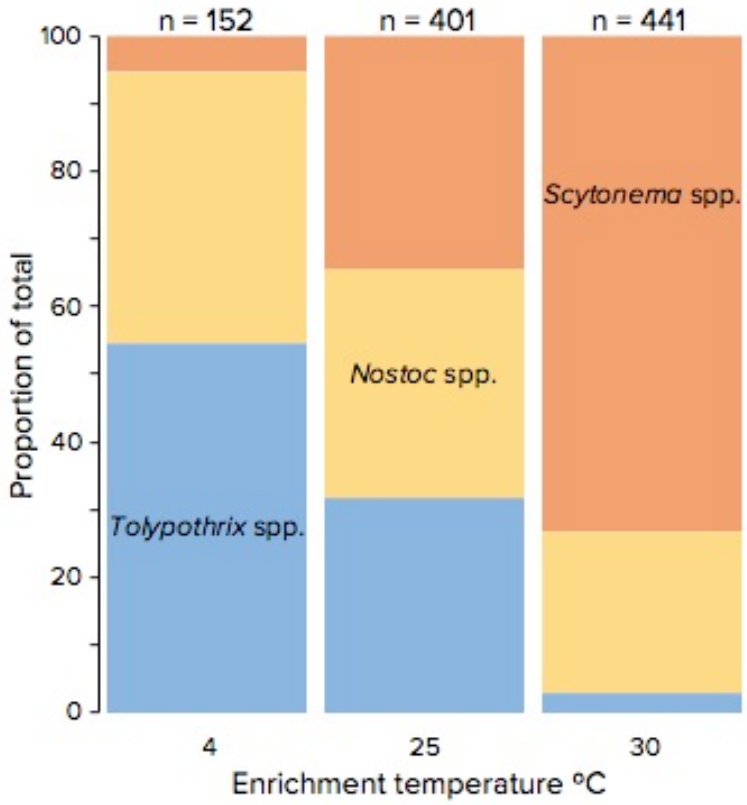

Figure 11. Relative proportion of colonies assignable to *Scytonema* spp., *Nostoc* spp., and *Tolypothrix* spp. in enriched cultures grown on nitrogen-free agar-solidified Petri plates for 20 days as a function of incubation temperature

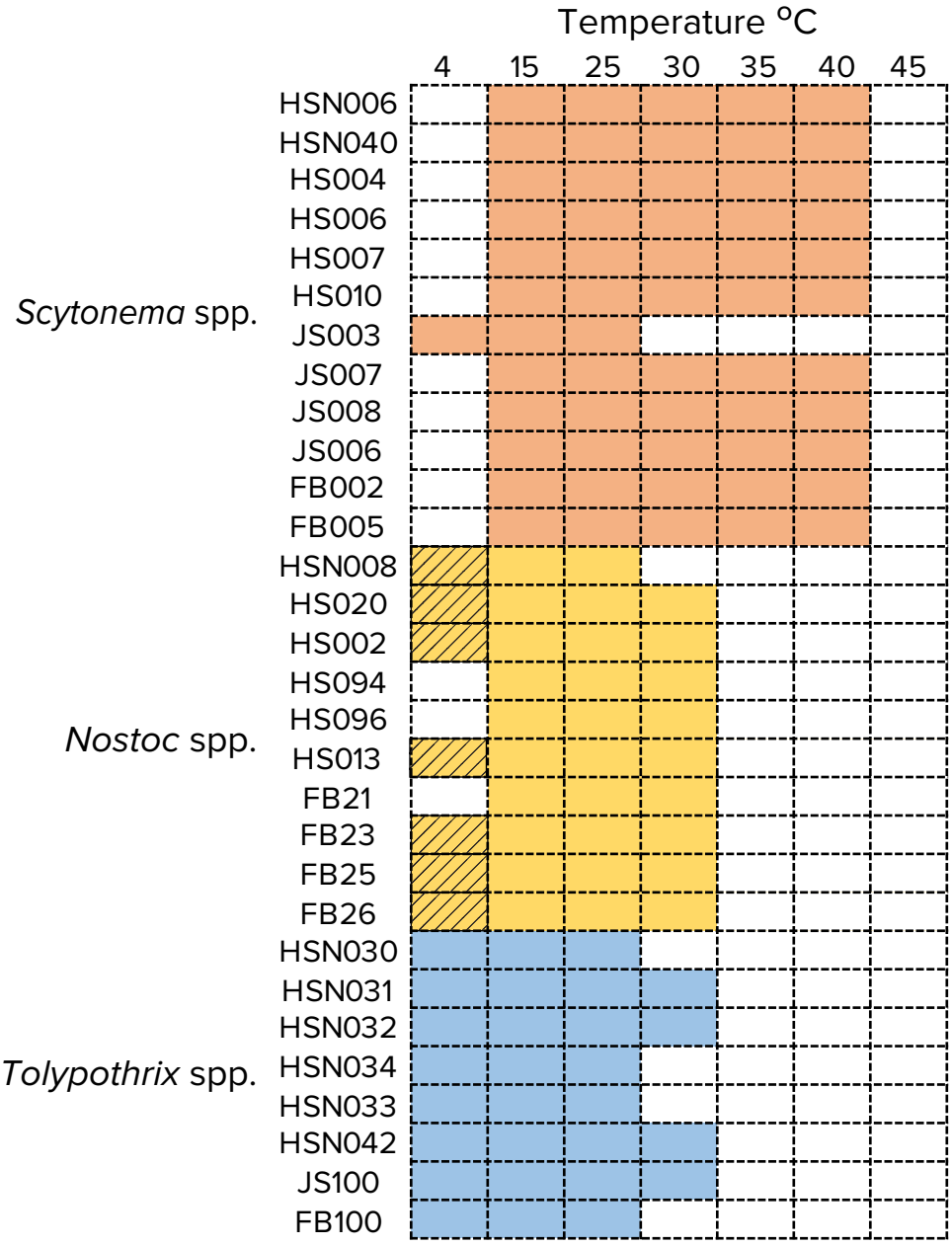

Figure 12. Temperature range for growth of cyanobacterial strains under diazotrophic growth. Colored rectangles indicate positive growth; hatched rectangles indicate stasis (no growth, but no obvious deterioration).

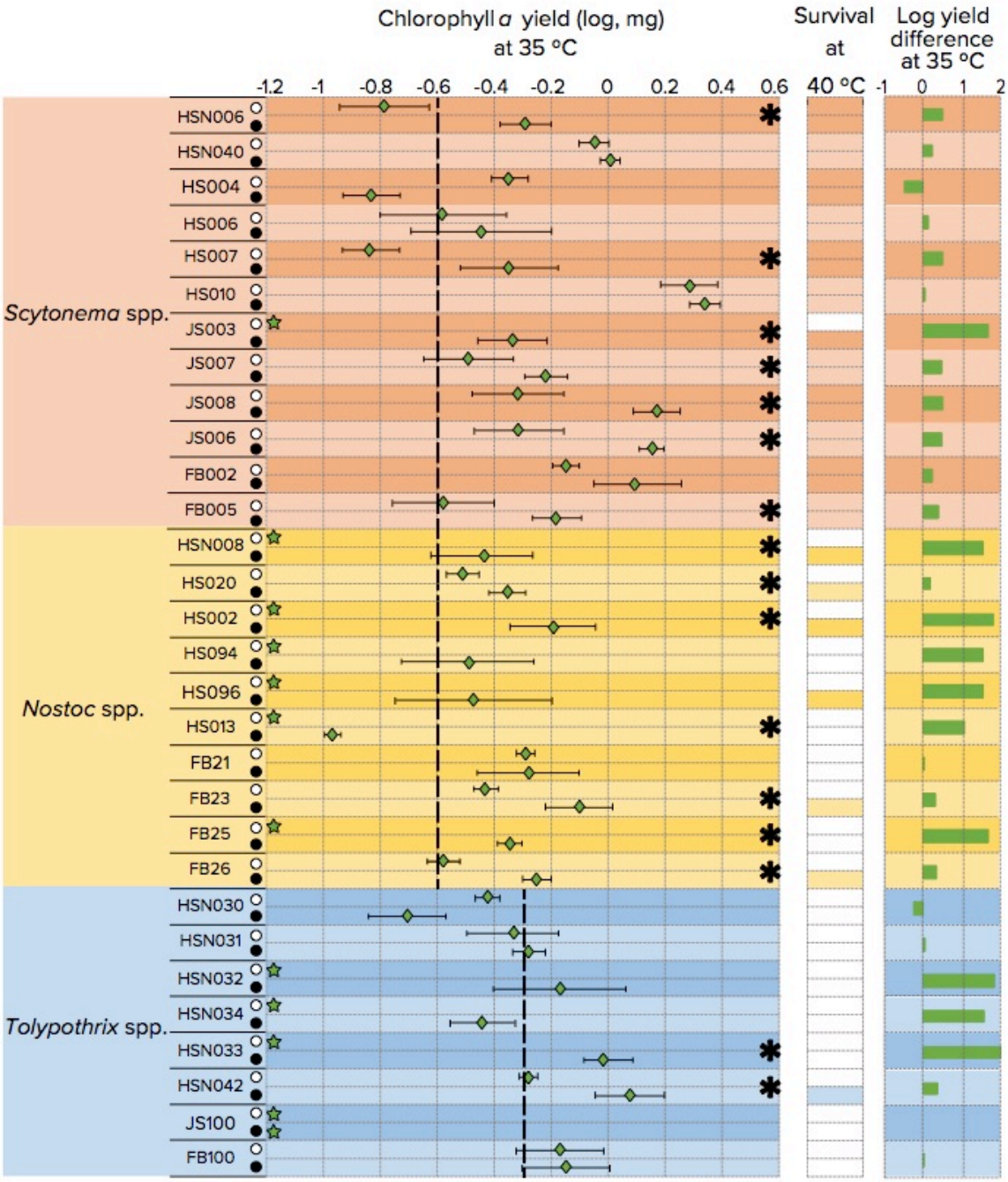

Figure 13. Growth yield of N_2-fixing cyanobacterial strains in the upper range of temperature for growth as a function of nitrogen source availability after 30 days of incubation. Nitrogen free ( ) and nitrogen replete media( ). Biomass yield as the difference between initial and final Chlorophyll *a* concentrations. Error bars indicate ± 1 SE, with n=3. Vertical dashed lines indicate the initial amount of inoculum provided. At 40 °C, only observational data were recorded:

colored rectangles indicate survival and white rectangles indicate death. *Denotes statistically significant differences between growing conditions according to Wilcox's test.

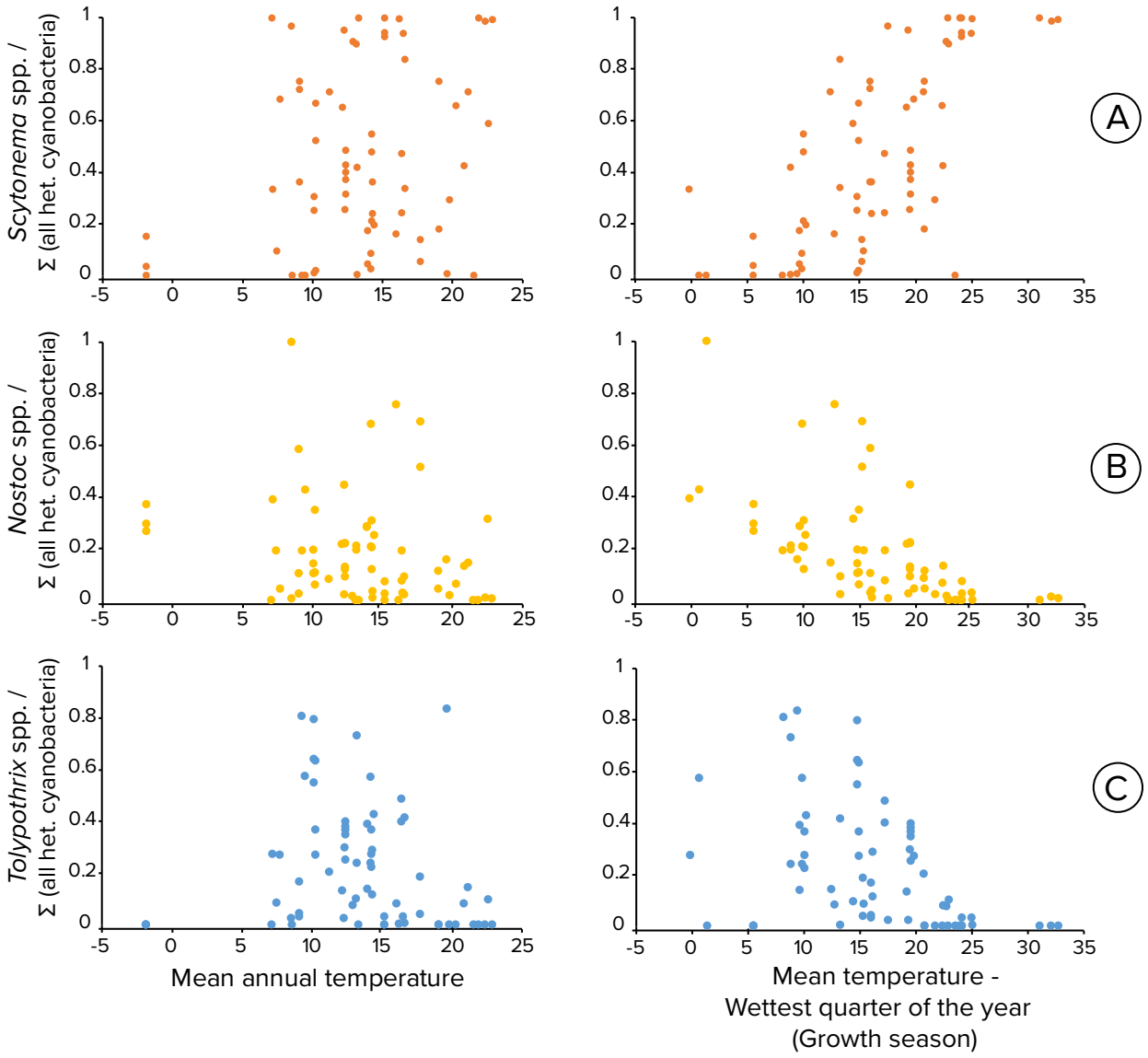

Figure 14. Proportion of sequence reads assignable to *Scytonema* spp. (orange), *Nostoc* spp. (yellow), and *Tolypothrix* spp. (blue) to those assignable to all heterocystous (Order Nostocales) cyanobacteria, in 16S rRNA molecular survey datasets, as a function of climate temperature indicators. Data are from biocrust communities surveyed at 70 locations around the world (see Table 12, Supplementary information). Each dot represents a different location.

Supplementary information

Table S10. Outcome of enrichment cultures for nitrogen-fixing photoautotrophs (nitrogen and organic carbon free medium, in the light) using variously sourced biocrusts as inoculum as a function of the incubation temperature. Given are the number of colonies containing each cyanobacterial taxa of interest, as identified morphologically by microscopy inspection. "S" stands for *Scytonema* spp., "N" for *Nostoc* spp., and "T" for *Tolypothrix* spp.

Inoculum origin	Replicate Enrichment	Incubation Temperature (°C)								
		4			25			30		
		S	N	T	S	N	T	S	N	T
Cold desert - sandy clay loam soil	1	0	7	9	14	15	10	32	25	0
	2	1	3	6	12	12	14	27	10	1
	3	0	4	12	11	18	9	33	27	3
Cold desert - clay loam soil	1	1	9	10	13	12	10	20	16	3
	2	2	6	7	15	9	12	17	0	0
	3	1	8	9	10	13	14	20	27	3
Hot desert - loamy sand soil	1	1	3	5	8	11	9	10	0	0
	2	0	3	6	11	10	10	40	0	0
	3	1	6	4	10	9	9	1	0	0
Hot desert - clay loam soil	1	0	2	7	15	6	12	40	0	0
	2	0	5	3	9	9	8	44	0	2
	3	1	5	5	10	12	10	39	1	0

Table S11. Cyanobacteria strains and their accession number in NCBI of their partial 16S rRNA sequence. Strain denominations include coding for the site of origin (HSN: cold desert sandy clay loam soil; HS: cold desert clay loam soil; FB: warm desert loamy sandy soil; JS: warm desert clay loam soil)

Cyanobacterial taxa	Strain	Accession number
***Scytonema* spp.**	HSN006	MK487668
	HSN040	MK487667
	HS004	MK487662
	HS006	MK487664
	HS007	MK487669
	HS010	MK487673
	JS003	MK487663
	JS007	MK487665
	JS008	MK487672
	JS006	MK487670
	FB002	MK487671
	FB005	MK487666
***Nostoc* spp.**	HSN008	MK487645
	HS020	MK487648
	HS002	MK487653
	HS094	MK487646
	HS096	MK487652
	FB21	MK487647
	FB23	MK487651
	FB25	MK487650
	FB26	MK487649
***Tolypothrix* spp.**	HSN30	MK487655
	HSN031	MK487654
	HSN032	MK487658
	HSN034	MK487657
	HSN33	MK487656
	HSN042	MK487661
	JS100	MK487660
	FB100	MK487659

Table S12. Environmental biocrust surveys conducted at different locations around the world used in the meta-analysis and corresponding climate data. Raw sequences were downloaded from bacterial 16S rRNA tallies available publicly (see references). Environmental data was downloaded from WorldClim. "MAT" stands for mean annual temperature and "MTemWetQ" for mean temperature during the wettest quarter of the year (growth season).

Original location Descriptor	Latitude	Longitude	MAT	MTemp WetQ	Sequencing Platform	Reference (s)
Murcia, Carrascoy (Dark and light)	37.8	-1.3	16.6	13.3	Illumina	(Muñoz-Martín et al. 2018)
Albacete, Barrax (Dark and light)	39.0	-2.2	14.2	9.9		
Madrid, Campo Real (Dark and light)	40.3	-3.4	14.3	10.0		
Almeria, Amoladeras (Dark and light)	36.8	-2.2	17.8	15.3		
Almeria, Amoladeras (Light)	36.8	-2.2	17.8	15.3		
Navarra, Bardenas Reales (Light)	42.1	-1.4	14.3	16.0		
Alicante, Relleu (Dark and light)	38.5	-0.3	16.4	17.3		
Guadalajara, Zorita (Dark and light)	40.3	-2.8	14.0	9.7		
Cuenca, Huelves (Dark and light)	40.0	-2.9	13.2	8.9		
Huesca, Monegros (Dark and light)	41.9	-0.2	14.3	16.1		
Madrid, Morata (Light)	40.2	-3.4	14.5	10.2		
Madrid, Campo Real (Dark)	4.3	-3.4	13.8	9.5		
site17-Chihuahuan-WilcoxPlya	32.1	-109.9	16.5	25.0	454 Pyrosequencing	(Garcia-Pichel et al. 2013b)
site8-NorthernGreatBasin-BlzdGap	42.1	-119.7	7.3	0.1		
site15-Sonoran-Chandler	33.3	-113.7	22.4	32.1		

site19-Mojave-CactusPln	34.1	-114.2	22.6	14.4		
site16-Sonoran-Dateland	32.8	-113.7	22.9	32.7		
site20-Mojave-SearlesLk	35.6	-117.4	19.7	9.5		
site13-Chihuahuan-FivePts	34.3	-106.8	13.2	23.0		
site22-Mojave-SodaLk	35	-111.8	8.6	17.5		
site11-NorthernGreatBasin-WhiteFlt	41.9	-118.9	9.5	0.7		
site18-Chihuahuan-Jornada	32.5	-106.7	15.2	24.1		
site14-Chihuahuan-SevilletaGyps	34.2	-106.8	13.4	22.9		
site10-NorthernGreatBasin-AlbertLk	42.1	-119.6	7.2	-0.1		
site21-Mojave-SodaLk	35.3	-116	21.2	12.4		
site5-ColoradoPlateau-Canyonlands	38.2	-109.7	12.2	19.2		
site3-ColoradoPlateau-GreenButte	38.7	-109.7	12.4	19.5		
site1-SonoranBatesW	32.2	-112.9	21.9	31.1		
site4-ColoradoPlateau-SundayChurt	38.6	109.6	7.7	19.9		
site2-ColoradoPlateau-SlickRock	38.6	-109.5	12.3	19.3		
site6-ColoradoPlateau-AcomaEx	35	-107.5	11.2	20.7		
site12-NorthernGreatBasin-CulverRd	44.5	-121.1	8.6	1.4		
site9-NorthernGreatBasin-AlvordHS	42.5	-118.5	9.3	8.2		

site7-ColoradoPlateau-ElMorro	35	-108.3	8.2	17.6		
Homburg, Goessenheim, Germany	50	9.8	9.1	16.0	Illumina	(Williams et al. 2016)
Tabernas, Almeria, Spain	37	-2.4	16.0	12.8		
Nat, Reserve Gynge Alvar, Sweden	56.5	16.4	7.5	15.3		
Hohe Tauern National Park, Austria	47	12.8	-1.8	5.5		
Cold Desert Silty - clay loam soil	41.1	-113.0	10.3	15.0	Illumina	(Velasco Ayuso et al. 2016; Bethany *et al*., *submitted*)
Cold Desert - sandy clay loam soil	41.1	-113.0	10.1	14.8		
Hot Desert Silty - clay loam soil	32.5	-106.7	15.2	24.2		
Hot Desert Sandy - loamy sand soil	32.4	-105.9	16.2	25.0		
Desert, early-developed biocrusts (China)	44.8	88.2	7.1	24.0	454 Pyrosequencing	(Zhang et al. 2016)
Moab, Green Butte site	38.7	-109.6	12.4	19.5	Illumina	(Couradeau et al. 2016)
Canastra National Park	-20.3	-46.6	19.8	21.7	Illumina	Machado de Lima *et al., in prep*
Capao National Park	-19.3	-43.5	19.1	20.8		
Cipo National Park	-19.3	-43.5	19.1	20.8		
Furnas National Park	-20.2	-47.4	20.9	22.5		
Vassununga National Park	-20.3	-46.3	20.3	22.4		
Zagaia National Park	-21.3	-47.6	21.6	23.5		
Blue gramma	34.3	-106.6	12.8	22.2	Illumina	(Fernandes et al. 2018)
Black gramma	34.3	-106.7	12.9	22.8		

References

Allen, M. M., and R. Stanier. 1998. Growth and Division of Some Unicellular Blue-green Algae. J.gen.Microbiol. 51:1998–1998.

Austin, A. T., L. Yahdjian, J. M. Stark, J. Belnap, A. Porporato, U. Norton, D. a. Ravetta, and S. M. Schaeffer. 2004. Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia 141:221–235.

Barger, N. N., S. C. Castle, and G. N. Dean. 2013. Denitrification from nitrogen-fixing biologically crusted soils in a cool desert environment, southeast Utah, USA. Ecological Processes 2:1.

Bates, S. T., Nash, T. H., and F. Garcia-Pichel. 2012. Patterns of diversity for fungal assemblages of bilogical soil crusts from the Southwestern United States. Mycologia 104: 353-361.

Belnap, J., B. Büdel, and O. L. Lange. 2001. Biological Soil Crust: Characteristics and Distribution. Pages 3–30 Biological Soil Cruts: Structure, Function, and Management. Springer-Verlag, Berlin.

Belnap, J., and D. A. Gillette. 1997. Disturbance of Biological Soil Crusts: Impacts on Potential Wind Erodibility of Sandy Desert Soils in Southeastern Utah. Land Degrad. Develop 8:355–362.

Belnap, J., B. Weber, and B. Büdel. 2016. Biological Soil Crusts as an Organizing Principle in Drylands. Pages 3–14 Biological Soil Crusts: An Organizing Principle in Drylands. 2nd edition. Springer, Switzerland.

Beraldi-Campesi, H., H. E. Hartnett, A. Anbar, G. W. Gordon, and F. Garcia-Pichel. 2009. Effect of biological soil crusts on soil elemental concentrations: Implications for biogeochemistry and as traceable biosignatures of ancient life on land. Geobiology 7:348–359.

Berger, S. A., D. Krompass, and A. Stamatakis. 2011. Performance, accuracy, and web server for evolutionary placement of short sequence reads under maximum likelihood. Systematic Biology 60:291–302.

Berger, S. A., and A. Stamatakis. 2011. Aligning short reads to reference alignments and trees. Bioinformatics 27:2068–2075.

Brock, T. D. 1975. Effect of water potential on a *Microcoleus* (cyanophyceae) from a desert crusts. Journal of phycology 11:316–320.

Cable, J. M., and T. E. Huxman. 2004. Precipitation pulse size effects on Sonoran Desert soil microbial crusts. Oecologia 141:317–324.

Callahan, B. J., P. J. Mcmurdie, M. J. Rosen, A. W. Han, A. J. Johnson, and S. P. Holmes. 2015. DADA2: High resolution sample inference from amplicon data. bioRxiv 13:0–14.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. Mcdonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight. 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7:335–336.

Castenholz, R. W. 1969. Thermophilic blue-green algae and the thermal environment. Bacteriological reviews 33:476–504.

Castle, S. C., C. D. Morrison, and N. N. Barger. 2011. Extraction of chlorophyll a from biological soil crusts: A comparison of solvents for spectrophotometric determination. Soil Biology and Biochemistry 43:853–856.

Clarke, K. R., Gorley, R. N. 2006. PRIMER v6: User Manual/Tutorial. Prim Plymouth UK 192 p.

Couradeau, E., A. Giraldo-Silva, F. De Martini, and F. Garcia-Pichel 2019. Spatial segregation of the biological soil crust microbiome around its foundational cyanobacterium, Microcoleus vaginatus, and the formation of a nitrogen-fixing cyanosphere. Microbiome 7:1–12.

Couradeau, E., U. Karaoz, H. C. Lim, U. Nunes da Rocha, T. Northen, E. Brodie, and F. Garcia-Pichel. 2016. Bacteria increase arid-land soil surface temperature through the production of sunscreens. Nature Communications 7:10373.

Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461.

Elbert, W., B. Weber, S. Burrows, J. Steinkamp, B. Büdel, M. O. Andreae, and U. Pöschl. 2012. Contribution of cryptogamic covers to the global cycles of carbon and nitrogen. Nature Geoscience 5:459–462.

Faist, A. M., J. E. Herrick, J. Belnap, J. W. V. Zee, and N. N. Barger. 2017. Biological soil crust and disturbance controls on surface hydrology in a semi-Arid ecosystem. Ecosphere 8.

Fernandes, V. M. C., N. M. Machado de Lima, D. Roush, J. Rudgers, S. L. Collins, and F. Garcia-Pichel. 2018. Exposure to predicted precipitation patterns decreases population size and alters community structure of cyanobacteria in biological soil crusts from the Chihuahuan Desert. Environmental Microbiology 20:259–269.

Ferreira, D., and F. Garcia-Pichel. 2016. Mutational studies of putative biosynthetic genes for the

cyanobacterial sunscreen scytonemin in Nostoc punctiforme ATCC 29133. Frontiers in Microbiology 7:1–10.

Fick, S. E., and R. J. Hijmans. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology 37:4302–4315.

Garcia-Pichel, F. 2003. Desert Environments: Biological Soil Crusts. Pages 1019–1023 *in* G. Bitton, editor. Encyclopedia of Environmental Microbiology 6 volume. Set. Wiley-Interscience, New York, NY.

Garcia-Pichel, F., and R. W. Castenholz. 1991. Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. Journal of Phycology 409:395–409.

Garcia-Pichel, F., A. López-Cortés, and U. Nübel. 2001. Phylogenetic and Morphological Diversity of Cyanobacteria in Soil Desert Crusts from the Colorado Plateau. Appl. Environ. Microbiol. 67:1902–1910.

Garcia-Pichel, F., V. Loza, Y. Marusenko, P. Mateo, and R. M. Potrafka. 2013a. Temperature Drives the Continental-Scale Distribution of Key Microbes in Topsoil Communities. Science 340:1574–1577.

Garcia-Pichel, F., V. Loza, Y. Marusenko, P. Mateo, and R. M. Potrafka. 2013b. Supp. material - Temperature drives the continental-scale distribution of key microbes in topsoil communities. Science 340:1574–1577.

Garcia-Pichel, F., and M. F. Wojciechowski. 2009. The evolution of a capacity to build supra-cellular ropes enabled filamentous cyanobacteria to colonize highly erodible substrates. PloS ONE 4:7801.

Gaskin, S., and R. Gardner. 2001. The role of cryptogams in runoff and erosion control on Bariland in the Nepal middle hills of the Southern Himalaya. Earth Surface Processes and Landforms 26:1303–1315.

Giraldo-Silva, A., C. Nelson, N. Barger, and F. Garcia-Pichel. 2019. Nursing biocrusts: isolation, cultivation and fitness test of indigenous cyanobacteria. Restoration Ecology:1–11.

Hennecke, H., and K. T Shanmugam. 1979. Temperature control of nitrogen fixation in Klebsiella pneumoniae. Page Archives of microbiology.

Johnson, S. L., C. R. Budinoff, J. Belnap, and F. Garcia-Pichel. 2005. Relevance of ammonium oxidation within biological soil crust communities. Environmental Microbiology 7:1–12.

Johnson, S. L., C. R. Kuske, T. D. Carney, D. C. Housman, L. V. Gallegos-Graves, and J. Belnap. 2012. Increased temperature and altered summer precipitation have differential effects on biological soil crusts in a dryland ecosystem. Global Change Biology 18:2583–

2593.

Johnson, S. L., S. Neuer, and F. Garcia-Pichel. 2007. Export of nitrogenous compounds due to incomplete cycling within biological soil crusts of arid lands. Environmental microbiology 9:680–9.

Katoh, K., and D. M. Standley. 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability Article Fast Track 30:772–780.

Knapp, A. K., C. Beier, D. D. Briske, A. T. Classen, Y. Luo, M. Reichstein, M. D. Smith, S. D. Smith, J. E. Bell, P. a. Fay, J. L. Heisler, S. W. Leavitt, R. Sherry, B. Smith, and E. Weng. 2008. Consequences of More Extreme Precipitation Regimes for Terrestrial Ecosystems. BioScience 58:811.

Lee, S. Y. 1996. Bacterial Polyhydroxyalkanoates 49:1–14.

Letunic, I., and P. Bork. 2016. Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. Nucleic Acids Research 44:242–245.

McDonald, D., M. N. Price, J. Goodrich, E. P. Nawrocki, T. Z. DeSantis, A. Probst, G. L. Andersen, R. Knight, and P. Hugenholtz. 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. The ISME journal 6:610–8.

Miller, S. R., R. W. Castenholz, and D. Pedersen. 2007. Phylogeography of the thermophilic cyanobacterium Mastigocladus laminosus. Applied and Environmental Microbiology 73:4751–4759.

Muñoz-Martín, M. Á., I. Becerra-Absalón, E. Perona, L. Fernández-Valbuena, F. Garcia-Pichel, and P. Mateo. 2018. Cyanobacterial biocrust diversity in Mediterranean ecosystems along a latitudinal and climatic gradient. New Phytologist.

Nagy, M. L., A. Pérez, and F. Garcia-Pichel. 2005. The prokaryotic diversity of biological soil crusts in the Sonoran Desert (Organ Pipe Cactus National Monument, AZ). FEMS Microbiology Ecology 54:233–245.

Nierzwicki Bauer, S. A., D. L. Balkwill, and S. E. Stevens. 1984. Heterocyst differentiation in the cyanobacterium Mastigocladus laminosus. Journal of Bacteriology 157:514–525.

Nübel, U., G. Muyzer, F. Garcia-Pichel, and G. Muyzer. 1997. PCR primers to amplify 16S rRNA genes from cyanobacteria PCR Primers to Amplify 16S rRNA Genes from Cyanobacteria. Microbiology 63:3327–3332.

Nunes da Rocha, U., H. Cadillo-Quiroz, U. Karaoz, L. Rajeev, N. Klitgord, S. Dunn, V. Truong, M. Buenrostro, B. P. Bowen, F. Garcia-Pichel, A. Mukhopadhyay, T. R. Northen, and E. L.

Brodie. 2015. Isolation of a significant fraction of non-phototroph diversity from a desert Biological Soil Crust. Frontiers in Microbiology 6:1–14.

Pepe-Ranney, C., C. Koechli, R. Potrafka, C. Andam, E. Eggleston, F. Garcia-Pichel, and D. H. Buckley. 2015. Non-cyanobacterial diazotrophs dominate dinitrogen fixation in biological soil crusts during early crust formation. The ISME Journal 10:287–298.

Petrie, M. D., S. L. Collins, D. S. Gutzler, and D. M. Moore. 2014. Regional trends and local variability in monsoon precipitation in the northern Chihuahuan Desert, USA. Journal of Arid Environments 103:63–70.

Petrie, M. D., S. L. Collins, and M. E. Litvak. 2015. The ecological role of small rainfall events in a desert grassland. Ecohydrology 8:1614-1622

Prăvălie, R. 2016. Drylands extent and environmental issues. A global approach 161:259–278.

Price, M. N., P. S. Dehal, and A. P. Arkin. 2010. FastTree 2 - Approximately maximum-likelihood trees for large alignments. PloS ONE 5.

Rajeev, L., U. N. Da Rocha, N. Klitgord, E. G. Luning, J. Fortney, S. D. Axen, P. M. Shih, N. J. Bouskill, B. P. Bowen, C. A. Kerfeld, F. Garcia-Pichel, E. L. Brodie, T. R. Northen, and A. Mukhopadhyay. 2013. Dynamic cyanobacterial response to hydration and dehydration in a desert biological soil crust. The ISME Journal:2178–2191.

Reed, S. C., K. K. Coe, J. P. Sparks, D. C. Housman, T. J. Zelikova, and J. Belnap. 2012. Changes to dryland rainfall result in rapid moss mortality and altered soil fertility. Nature Climate Change 2:752–755.

Rodríguez-Caballero, E., Y. Cantón, S. Chamizo, A. Afana, and A. Solé-Benet. 2012. Effects of biological soil crusts on surface roughness and implications for runoff and erosion. Geomorphology 145–146:81–89.

Sala., O. E., and W. K. Lauenroth. 2014. Small Rainfall Events: An Ecological Role in Semiarid Regions. Oecologia 53:301–304.

Seager, R., and G. A. Vecchi. 2010. Greenhouse warming and the 21st century hydroclimate of southwestern North America. Proceedings of the National Academy of Sciences of the United States of America 107:21277–21282.

Soule, T., I. J. Anderson, S. L. Johnson, S. T. Bates, and F. Garcia-Pichel. 2009. Archaeal populations in biological soil crusts from arid lands in North America. Soil Biology and Biochemistry 41:2069–2074.

Strauss, S. L., T. a. Day, and F. Garcia-Pichel. 2011. Nitrogen cycling in desert biological soil crusts across biogeographic regions in the Southwestern United States. Biogeochemistry

108:171–182.

Thiet, R. K., R. E. J. Boerner, M. Nagy, and R. Jardine. 2005. The effect of biological soil crusts on throughput of rainwater and N into Lake Michigan sand dune soils. Plant and Soil 278:235–251.

Thomazo, C., E. Couradeau, and F. Garcia-Pichel. 2018. Possible nitrogen fertilization of the early Earth Ocean by microbial continental ecosystems. Nature Communications 9.

Velasco Ayuso, S. V., A. Giraldo Silva, C. Nelson, N. N. Barger, and F. Garcia-Pichel. 2017. Microbial Nursery Production of High- Quality Biological Soil Crust Biomass for Restoration of Degraded Dryland Soils 83:1–16.

Verrecchia, E., A. Yair, G. J. Kidron, K. Verrecchia, R. Campus, I.- Jerusalem, and G. R. Campus. 1995. Physical properties of the Psammophile cryptogamic crust and their consequences to the water regime of sandy soils, north-western Nagev desert, Israel. Journal of Arid Environments 29:427–437.

Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73:5261–5267.

Williams, L., K. Loewen-Schneider, S. Maier, and B. Büdel. 2016. Cyanobacterial diversity of western European biological soil crusts along a latitudinal gradient. FEMS Microbiology Ecology 92:1–9.

Xu, S. 2016. Bayesian Naïve Bayes classifiers to text classification. Journal of Information Science 44:48–59.

Yeager, C. M., J. L. Kornosky, D. C. Housman, E. E. Grote, J. Belnap, and C. R. Kuske. 2004. Diazotrophic Community Structure and Function in Two Successional Stages of Biological Soil Crusts from the Colorado Plateau and Chihuahuan Desert. Applied and Environmental Microbiology 70.

Yeager, C. M., J. L. Kornosky, R. E. Morgan, E. C. Cain, F. Garcia-Pichel, D. C. Housman, J. Belnap, and C. R. Kuske. 2007. Three distinct clades of cultured heterocystous cyanobacteria constitute the dominant N2-fixing members of biological soil crusts of the Colorado Plateau, USA. FEMS Microbiology Ecology 60:85–97.

Zhang, B., W. Kong, W. Nan, and Y. Zhang. 2016. Bacterial diversity and community along the succession of biological soil crusts in the Gurbantunggut Desert, Northern China. Journal of Basic Microbiology 56:670–679.

Zhang, Y. M., H. L. Wang, X. Q. Wang, W. K. Yang, and D. Y. Zhang. 2006. The microstructure of microbiotic crust and its influence on wind erosion for a sandy soil

surface in the Gurbantunggut Desert of Northwestern China. Geoderma 132:441–449.

Zhou, X., H. Smith, A. Giraldo Silva, J. Belnap, and F. Garcia-Pichel. 2016. Differential Responses of Dinitrogen Fixation, Diazotrophic Cyanobacteria and Ammonia Oxidation Reveal a Potential Warming-Induced Imbalance of the N-Cycle in Biological Soil Crusts. PloS ONE 11:1–15.

6 – CONCLUSIONS

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

This dissertation investigates the adaptation mechanisms of biocrust cyanobacteria to extreme environments, including the factors driving community composition and structure, that will ultimately support biocrust restoration efforts.

In Chapters 2 and 3, we designed a multi-step approach to produce photosynthetic cyanobacterial inoculum to support large scale biocrust restoration efforts. Rather than consecutively harvesting natural biocrusts, this approach uses laboratory grown cultures isolated from native communities. The protocols were validated for a variety of climatic and edaphic factors. Protocols include the methods to isolate and select cyanobacterial strains that resemble the most abundant cyanobacterial population at each field location. The approach also includes methods for scaling-up biomass production from cultured isolates to larger volumes for restoration. It also incorporates the use of inoculum preconditioning treatments that increase exposure to solar radiation and temperature, and recurrent wet-dry cycles to pre-acclimate grown cyanobacterial isolates to the extreme conditions expected in the field. Preconditioning treatments were particularly beneficial for strains of the biocrust pioneer cyanobacteria *Microcoleus vaginatus* and *Microcoleus steenstrupii* (Giraldo-Silva et al. *submitted*). Finally, we showed that the inoculum obtained thrived in its original soil under natural outdoor conditions.

In chapter 4, using genetic surveys, we demonstrated that *M. vaginatus* possesses a compositionally differentiated cyanosphere that concentrates the nitrogen-fixing function. We propose that a mutualism based on C for N exchange between *M. vaginatus* and heterotrophic

diazotrophs helps sustains this cyanosphere, and that this consortium constitutes the true pioneer community enabling the colonization of nitrogen-poor soils (Couradeau et al. 2019). Consequently, the implementation of mixed cultures of *M. vaginatus* and representatives of these N_2-fixing heterotrophic diazotrophs should become a target in biocrust restoration since inoculation and survival of *M. vaginatus* are key for restoration success.

In chapter 5, we studied the niche partitioning among the three most common heterocystous cyanobacteria from biocrusts using enrichment cultivation, determining the temperature ranges for growth in a set of 30 isolates. We demonstrated that there is a pattern of niche differentiation, with *Scytonema* spp. being more thermophilic and *Tolypothrix* spp. more psychrophilic, while *Nostoc* spp. responded well to intermediate temperatures. We could also correlate the heat sensitivity in *Nostoc* spp. and *Tolypothrix* spp. strains with nitrogen fixation because the thermal range for growth could be increased under nitrogen replete conditions and were able to trace it to an inability to develop heterocysts (specialized nitrogen fixing cells) at high temperatures. In this chapter, we also showed that the physiological niche differentiation found in culture experiments does translate to the distribution of these cyanobacteria in nature. Based on this, we predicted a potential shift in dominance in many locales because of global warming, to the benefit of *Scytonema* spp. populations. These results provide insights into the factors limiting the species' fitness and distribution under current environmental conditions, and will therefore help to improve our understanding of drylands, and our ability to predict future impacts of global warming on biocrust communities. These results can also improve strategies to restore biological soil crust communities by providing information that can be used to decide the

best inoculation season and to design the most adequate microbial mixture inoculum for restoration.

Overall, this thesis constitutes an important contribution to the growing field of biocrust restoration that materializes in the establishment of the first cyanobacterial biocrust nursery (Giraldo-Silva et al. 2019), which includes a culture collection of 101 strains, isolation and cultivation methods, inoculum design strategies and conditioning protocols. The biocrust cyanobacteria culture collection includes strains from both cold and hot deserts of the Southwest of United states (Northern Utah, Southern New Mexico and West Texas). Those strains are publicly available, and they are currently used as reference strains by numerous laboratories. We demonstrated that the cultivation-based approach represents a feasible, non-destructive tool to produce quality-controlled seed to restore biological soil crust communities in degraded dryland soils at scale. The approach ensures stringent control of the composition of a microbial inoculum, through multiple quality controls, to avoid inoculation with adventitious, or non-native microbes. Additionally, the developed protocols, although derived for specific locales, are of wide geographical applicability, because they include cross-matching between cultures and local field populations.

References

Couradeau, E., A. Giraldo-Silva, F. De Martini, and F. Garcia-Pichel. Accepted. Spatial segregation of the biological soil crust microbiome around its foundational cyanobacterium, *Microcoleus vaginatus*, and the formation of a nitrogen-fixing cyanosphere. Microbiome 7:1-12

Giraldo-Silva, A., C. Nelson, N. N. Barger, and F. Garcia-Pichel. 2019. Nursing biocrusts: isolation, cultivation, and fitness test of indigenous cyanobacteria. Restoration Ecology. 1-11. DOI: 10.1111/rec.12920

Giraldo-Silva, A., C. Nelson, C. Penfold, N. N. Barger, and F. Garcia-Pichel. *In review*. Effect of preconditioning to the soil environment on the performance of 20 cyanobacterial cultured strains used as inoculum for biocrust restoration. Restoration Ecology

REFERENCES

Acharya, K., M. Kyle, and J. J. Elser. 2004. Biological stoichiometry of Daphnia growth: An ecophysiological test of the growth rate hypothesis. Limnol. Oceanogr. 49:656–665.

Allen, M. M., and R. Stanier. 1998. Growth and Division of Some Unicellular Blue-green Algae. J.gen.Microbiol. 51:1998–1998.

Angel, R., and R. Conrad. 2013. Elucidating the microbial resuscitation cascade in biological soil crusts following a simulated rain event. Environmental Microbiology 15:2799–2815.

Angel, R., D. Matthies, and R. Conrad. 2011. Activation of methanogenesis in arid biological soil crusts despite the presence of oxygen. PloS ONE 6:1–8.

Antoninka, A., M. A. Bowker, P. Chuckran, N. N. Barger, S. Reed, and J. Belnap. 2018. Maximizing establishment and survivorship of field-collected and greenhouse-cultivated biocrusts in a semi-cold desert. Plant and Soil 429:213–225.

Antoninka, A., M. A. Bowker, S. C. Reed, and K. Doherty. 2016. Production of greenhouse-grown biocrust mosses and associated cyanobacteria to rehabilitate dryland soil function. Restoration Ecology 24.

Austin, A. T., L. Yahdjian, J. M. Stark, J. Belnap, A. Porporato, U. Norton, D. a. Ravetta, and S. M. Schaeffer. 2004. Water pulses and biogeochemical cycles in arid and semiarid ecosystems. Oecologia 141:221–235.

Ayuso, S. V., A. G. Silva, C. Nelson, N. N. Barger, and F. Garcia-Pichel. 2017. Microbial Nursery Production of High- Quality Biological Soil Crust Biomass for Restoration of Degraded Dryland Soils 83:1–16.

Baran, R., E. L. Brodie, J. Mayberry-Lewis, E. Hummel, U. N. Da Rocha, R. Chakraborty, B. P. Bowen, U. Karaoz, H. Cadillo-Quiroz, F. Garcia-Pichel, and T. R. Northen. 2015. Exometabolite niche partitioning among sympatric soil bacteria. Nature Communications 6:8289.

Baran, R., N. N. Ivanova, N. Jose, F. Garcia-Pichel, N. C. Kyrpides, M. Gugger, and T. R. Northen. 2013. Functional genomics of novel secondary metabolites from diverse cyanobacteria using untargeted metabolomics. Marine Drugs 11:3617–3631.

Baran, R., R. Lau, B. P. Bowen, S. Diamond, N. Jose, F. Garcia-Pichel, and T. R. Northen. 2017. Extensive Turnover of Compatible Solutes in Cyanobacteria Revealed by Deuterium Oxide (D2O) Stable Isotope Probing. ACS Chemical Biology 12:674–681.

Barger, N. N., S. C. Castle, and G. N. Dean. 2013. Denitrification from nitrogen-fixing

biologically crusted soils in a cool desert environment, southeast Utah, USA. Ecological Processes 2:1.

Barger, N. N., J. E. Herrick, J. Van Zee, and J. Belnap. 2006. Impacts of biological soil crust disturbance and composition on C and N loss from water erosion. Biogeochemistry 77:247–263.

Barger, N. N., B. Webber, F. Garcia-Pichel, E. Zaady, and J. Belnap. 2016. Patterns and Controls on Nitrogen Cycling of Biological Soil Crusts. Pages 257–285 *in* B. Weber, M. M. Caldwell, B. Jayne, W. Bettina, B. Büdel, J. Belnap, B. Weber, and B. Büdel, editors. Biological Soil Crusts: An Organizing Principle in Drylands. 2nd edition. Switzerland.

Barraquio, W. L., B. C. P. JR, I. Watanabe, and R. Knowles. 1986. Nitrogen Fixation by Pseudomonas saccharophila Doudoroff ATCC 15946. Journal of General Microbiology 132:237–241.

Bates, S. T., and F. Garcia-Pichel. 2009. A culture-independent study of free-living fungi in biological soil crusts of the Colorado Plateau: Their diversity and relative contribution to microbial biomass. Environmental Microbiology 11:56–67.

Bates, S. T., T. H. Nash, and F. Garcia-Pichel. 2012. Patterns of diversity for fungal assemblages of biological soil crusts from the southwestern United States. Mycologia 104:353–361.

Bates, S. T., T. H. Nash, K. G. Sweat, and F. Garcia-Pichel. 2010. Fungal communities of lichen-dominated biological soil crusts: Diversity, relative microbial biomass, and their relationship to disturbance and crust cover. Journal of Arid Environments 74:1192–1199.

Belnap, J. 1993. Recovery rates of cryptobiotic crusts: Inoculant use and assessment methods. The Great Basin Naturalist 53:89–95.

Belnap, J. 2001. Factors Influencing Nitrogen Fixation and Nitrogen Release in Biological Soil Crust. Pages 241–261 *in* J. Belnap and O. L. Lange, editors. Biology and Fertility of Soils. 1st edition. Springer-Verlag, Berlin.

Belnap, J., B. Büdel, and O. L. Lange. 2001a. Biological Soil Crust: Characteristics and Distribution. Pages 3–30 Biological Soil Cruts: Structure, Function, and Management. Springer-Verlag, Berlin.

Belnap, J., B. Büdel, and O. L. Lange. 2001b. Biological Soil Crusts: Characteristics and Distribution. Pages 3–30 Biological Soil Crusts: Structure, Function, and Management. Springer.

Belnap, J., and D. Eldridge. 2001. Disturbance and Recovery of Biological Soil Crusts. Pages 363–383 *in* J. Belnap and O. Lange, editors. Biological soil cruts: structure,

function, and management. Springer, Berlin.

Belnap, J., and D. A. Gillette. 1997. Disturbance of Biological Soil Crusts: Impacts on Potential Wind Erodibility of Sandy Desert Soils in Southeastern Utah. Land Degrad. Develop 8:355–362.

Belnap, J., B. Weber, and B. Büdel. 2016. Biological Soil Crusts as an Organizing Principle in Drylands. Pages 3–14 Biological Soil Crusts: An Organizing Principle in Drylands. 2nd edition. Springer, Switzerland.

Beraldi-Campesi, H., J. D. Farmer, and F. Garcia-Pichel. 2014. Modern Terrestrial Sedimentary Biostructures and Their Fossil Analogs in Mesoproterozoic Subaerial Deposits. Palaios 29:45–54.

Beraldi-Campesi, H., H. E. Hartnett, A. Anbar, G. W. Gordon, and F. Garcia-Pichel. 2009. Effect of biological soil crusts on soil elemental concentrations: Implications for biogeochemistry and as traceable biosignatures of ancient life on land. Geobiology 7:348–359.

Berger, S. A., D. Krompass, and A. Stamatakis. 2011. Performance, accuracy, and web server for evolutionary placement of short sequence reads under maximum likelihood. Systematic Biology 60:291–302.

Berger, S. A., and A. Stamatakis. 2011. Aligning short reads to reference alignments and trees. Bioinformatics 27:2068–2075.

Bethesda (MD): National Library of Medicine (US), N. C. for B. I. 1988. National Center for Biotechnology Information (NCBI).

Bethesda (MD): National Library of Medicine (US), N. C. for B. I. 2004. 16s ribosomal RNA sequences (bacteria/archaea) [Internet].

Bowker, M. A. 2007. Biological soil crust rehabilitation in Theory and Practice - an Underexploited Opportunity. Restoration Ecology 15:13–23.

Bowker, M. A., and A. J. Antoninka. 2016. Rapid ex situ culture of N-fixing soil lichens and biocrusts is enhanced by complementarity. Plant and Soil 408:415–428.

Bowker, M. a., J. Belnap, V. Bala Chaudhary, and N. C. Johnson. 2008. Revisiting classic water erosion models in drylands: The strong impact of biological soil crusts. Soil Biology and Biochemistry 40:2309–2316.

Bowker, M. A., J. Belnap, R. Rosentreter, and B. Graham. 2004. Wildfire-resistant biological soil crusts and fire-induced loss of soil stability in Palouse prairies, USA. Applied Soil Ecology 26:41–52.

Brock, T. D. 1975. Effect of water potential on a *Microcoleus* (Cyanophyceae) from a

desert crusts. Journal of phycology 11:316–320.

Büdel, B., T. Dulic, T. Daridenko, N. Rybalka, and T. Friedl. 2016. Cyanobacteria and Algae of Biological Soil Crusts. Pages 55–81 Biological Soil Crusts: An Organizing Principle in Drylands. 2nd edition. Springer, Switzerland.

Buttars, S. M., L. L. St. Clair, J. R. Johansen, J. C. Sray, M. C. Payne, B. L. Webb, R. E. Terry, B. K. Pendlenton, and S. D. Warren. 1998. Pelletized cyanobacterial soil amendments: Laboratory testing for survival, escapability, and Nitrogen fixation. Arid Soil Research and Rehabilitation 12:165–178.

Cable, J. M., and T. E. Huxman. 2004. Precipitation pulse size effects on Sonoran Desert soil microbial crusts. Oecologia 141:317–324.

Callahan, B. J., P. J. Mcmurdie, M. J. Rosen, A. W. Han, A. J. Johnson, and S. P. Holmes. 2015. DADA2: High resolution sample inference from amplicon data. bioRxiv 13:0–14.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. Mcdonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight. 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7:335–336.

Caporaso, J. G., C. L. Lauber, W. A. Walters, D. Berg-Lyons, C. A. Lozupone, P. J. Turnbaugh, N. Fierer, and R. Knight. 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proceedings of the National Academy of Sciences 108:4516–4522.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, Mcdonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, K. R. 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7:335–336.

Castenholz, R. W. 1969. Thermophilic blue-green algae and the thermal environment. Bacteriological reviews 33:476–504.

Castle, S. C., C. D. Morrison, and N. N. Barger. 2011. Extraction of chlorophyll a from biological soil crusts: A comparison of solvents for spectrophotometric determination. Soil Biology and Biochemistry 43:853–856.

Ceja-Navarro, J. a, N. H. Nguyen, U. Karaoz, S. R. Gross, D. J. Herman, G. L. Andersen, T. D. Bruns, J. Pett-Ridge, M. Blackwell, and E. L. Brodie. 2014. Compartmentalized microbial composition, oxygen gradients and nitrogen fixation in the gut of Odontotaenius disjunctus. The ISME journal 8:6–18.

Chamizo, S., G. Mugnai, F. Rossi, G. Certini, and R. De Philippis. 2018. Cyanobacteria Inoculation Improves Soil Stability and Fertility on Different Textured Soils: Gaining Insights for Applicability in Soil Restoration. Frontiers in Environmental Science 6.

Chen, L., Z. Xie, C. Hu, D. Li, G. Wang, and Y. Liu. 2006. Man-made desert algal crusts as affected by environmental factors in Inner Mongolia, China. Journal of Arid Environments 67:521–527.

Christensen, H., P. Kuhnert, N. Norskov-Lauritsen, P. J. Planet, and M. Bisgaard. 2013. The Family Pasteurellaceae. Pages 535–556 *in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Gammaproteobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Couradeau, E., A. Giraldo-Silva, F. De Martini, and F. Garcia-Pichel. 2019. Spatial segregation of the biological soil crust microbiome around its foundational cyanobacterium, Microcoleus vaginatus, and the formation of a nitrogen-fixing cyanosphere. Microbiome 7: 1-12.

Couradeau, E., U. Karaoz, H. C. Lim, U. Nunes da Rocha, T. Northen, E. Brodie, and F. Garcia-Pichel. 2016. Bacteria increase arid-land soil surface temperature through the production of sunscreens. Nature Communications 7:10373.

Couradeau, E., V. J. M N L Felde, D. Parkinson, D. Uteau, A. Rochet, C. Cuellar, G. Winegar, S. Peth, T. R. Northen, and F. Garcia-Pichel. 2018. In Situ X-Ray Tomography Imaging of Soil Water and Cyanobacteria from Biological Soil Crusts Undergoing Desiccation. Frontiers in Environmental Science | www.frontiersin.org 1:65.

Couradeau, E., A. Giraldo-Silva, F. De Martini, and F. Garcia-Pichel. Accepted. Spatial segregation of the biological soil crust microbiome around its foundational cyanobacterium, *Microcoleus vaginatus*, and the formation of a nitrogen-fixing cyanosphere. Microbiome

Coyte, K. Z., J. Schluter, and K. R. Foster. 2015. The ecology of the microbiome: Networks, competition, and stability. Science 350:663–666.

Dawid, W. 2000. Biology and global distribution of myxobacteria in soils. FEMS Microbiology Reviews 24:403–427.

Defalco, L. A., J. K. Detling, C. R. Tracy, and S. D. Warren. 2001. Physiological variation among native and exotic winter annual plants associated with microbiotic crusts in the Mojave Desert. Plant and Soil 234:1–14.

Doherty, K. D., A. J. Antoninka, M. A. Bowker, S. Velasco Ayuso, and N. C. Johnson. 2015. A Novel Approach to Cultivate Biocrusts for Restoration and Experimentation. Ecological Restoration 33:13–17.

Edgar, R. C. 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26:2460–2461.

Elbert, W., B. Weber, S. Burrows, J. Steinkamp, B. Büdel, M. O. Andreae, and U. Pöschl. 2012. Contribution of cryptogamic covers to the global cycles of carbon and nitrogen. Nature Geoscience 5:459–462.

Evans, R. D., J. Belnap, F. Garcia-Pichel, and S. L. Phillips. 2001. Global Change and Future of Biological Soil Crusts. Pages 417–429 *in* J. Belnap and O. L. Lange, editors. Biological Soil Crusts: Structure, Function, and Management. Springer, Berlin, Heindelberg.

Faist, A. M., J. E. Herrick, J. Belnap, J. W. V. Zee, and N. N. Barger. 2017. Biological soil crust and disturbance controls on surface hydrology in a semi-Arid ecosystem. Ecosphere 8.

Fernandes, V. M. C., N. M. Machado de Lima, D. Roush, J. Rudgers, S. L. Collins, and F. Garcia-Pichel. 2018. Exposure to predicted precipitation patterns decreases population size and alters community structure of cyanobacteria in biological soil crusts from the Chihuahuan Desert. Environmental Microbiology 20:259–269.

Ferreira, D., and F. Garcia-Pichel. 2016. Mutational studies of putative biosynthetic genes for the cyanobacterial sunscreen scytonemin in Nostoc punctiforme ATCC 29133. Frontiers in Microbiology 7:1–10.

Ferrenberg, S., S. C. Reed, and J. Belnap. 2015. Climate change and physical disturbance cause similar community shifts in biological soil crusts. Proceedings of the National Academy of Sciences 112:12116–12121.

Fick, S. E., and R. J. Hijmans. 2017. WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology 37:4302–4315.

Ford, P. L., and G. V. Johnson. 2006. Effects of dormant- vs. growing-season fire in shortgrass steppe: Biological soil crust and perennial grass responses. Journal of Arid Environments 67:1–14.

Gao, Q., and F. Garcia-Pichel. 2011. An ATP-Grasp ligase involved in the last biosynthetic step of the iminomycosporine shinorine in Nostoc punctiforme ATCC 29133. Journal of Bacteriology 193:5923–5928.

Garcia-Pichel, F. 2003a. Desert Environments: Biological Soil Crusts. Pages 1019–1023 *in* G. Bitton, editor. Encyclopedia of Environmental Microbiology 6 volume. Set. Wiley-Interscience, New York, NY.

Garcia-Pichel, F. 2003b. Desert Environments: Biological Soil Crusts.

Garcia-Pichel, F., and J. Belnap. 1996. Microenvironments and Microscale productivity

of cyanobacteria desert crusts:774–783.

Garcia-Pichel, F., and J. Belnap. 2001. Small-scale environments and distribution of biological soil crusts. Pages 193–201 Biological soil crusts: Structure, function, and management. Springe.

Garcia-Pichel, F., J. Belnap, S. Neuer, and F. Schanz. 2003. Estimates of global cyanobacterial biomass and its distribution. Algological Studies 109:213–227.

Garcia-Pichel, F., J. Belnap, S. Neuer, and F. Schanz. 2006. Estimates of global cyanobacterial biomass and its distribution. Algological Studies 109:213–227.

Garcia-Pichel, F., and R. W. Castenholz. 1991. Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. Journal of Phycology 409:395–409.

Garcia-Pichel, F., A. López-Cortés, and U. Nübel. 2001. Phylogenetic and Morphological Diversity of Cyanobacteria in Soil Desert Crusts from the Colorado Plateau. Appl. Environ. Microbiol. 67:1902–1910.

Garcia-Pichel, F., V. Loza, Y. Marusenko, P. Mateo, and R. M. Potrafka. 2013a. Temperature Drives the Continental-Scale Distribution of Key Microbes in Topsoil Communities. Science 340:1574–1577.

Garcia-Pichel, F., V. Loza, Y. Marusenko, P. Mateo, and R. M. Potrafka. 2013b. Supp. material -Temperature drives the continental-scale distribution of key microbes in topsoil communities. Science 340:1574–1577.

Garcia-Pichel, F., and O. Pringault. 2001. Cyanobacteria track water in desert soils 413:380–381.

Garcia-Pichel, F., and M. F. Wojciechowski. 2009. The evolution of a capacity to build supra-cellular ropes enabled filamentous cyanobacteria to colonize highly erodible substrates. PloS ONE 4:7801.

Gaskin, S., and R. Gardner. 2001. The role of cryptogams in runoff and erosion control on Bariland in the Nepal middle hills of the Southern Himalaya. Earth Surface Processes and Landforms 26:1303–1315.

Giraldo-Silva, A., C. Nelson, N. Barger, and F. Garcia-Pichel. 2019. Nursing biocrusts: isolation, cultivation and fitness test of indigenous cyanobacteria. Restoration Ecology:1–11.

Godínez-Alvarez, H., C. Morín, and V. Rivera-Aguilar. 2012. Germination, survival and growth of three vascular plants on biological soil crusts from a Mexican tropical desert. Plant Biology 14:157–162.

Götz, F., T. Bannerman, and K.-H. Schleifer. 2006. The Genera Staphylococcus and Macrococcus. Pages 5–75 *in* M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, and E. Stackebrandt, editors. The Prokaryotes: Volume 4: Bacteria: Firmicutes, Cyanobacteria. Springer-Verlag Berlin Heidelberg, New York, NY.

Graham, L. E., J. M. Graham, L. W. Wilcox, M. E. Cook, P. Arancibia-Avila, and J. J. Knack. 2018. Evolutionary Roots of Plant Microbiomes and Biogeochemical Impacts of Nonvascular Autotroph-Microbiome Systems over Deep Time. International Journal of Plant Sciences 179:000–000.

Griffin, D. W. 2007. Atmospheric movement of microorganisms in clouds of desert dust and implications for human health. Clinical microbiology reviews 20:459–77, table of contents.

Guedes, C., N. G. Katkam, J. Varela, and F. X. Malcata. 2014. Photobioreactors for cyanobacterial culturing. Pages 271–292 *in* K. N. Sharma, K. R. Ashwani, and J. L. Stal, editors. Cyanobacteria: an economic perspective. First edit. John Wiley & Sons, Ltd., Oxford, UK.

Guidi, L., S. Chaffron, L. Bittner, D. Eveillard, A. Larhlimi, S. Roux, Y. Darzi, S. Audic, L. Berline, J. Brum, L. P. Coelho, J. C. I. Espinoza, S. Malviya, S. Sunagawa, C. Dimier, S. Kandels-Lewis, M. Picheral, J. Poulain, S. Searson, T. O. Coordinators, L. Stemmann, F. Not, P. Hingamp, S. Speich, M. Follows, L. Karp-Boss, E. Boss, H. Ogata, S. Pesant, J. Weissenbach, P. Wincker, S. G. Acinas, P. Bork, C. de Vargas, D. Iudicone, M. B. Sullivan, J. Raes, E. Karsenti, C. Bowler, and G. Gorsky. 2015. Plankton networks driving carbon export in the oligotrophic ocean. Nature 532:in review.

Gundlapally, S. R., and F. Garcia-Pichel. 2006. The community and phylogenetic diversity of biological soil crusts in the Colorado Plateau studied by molecular fingerprinting and intensive cultivation. Microbial Ecology 52:345–357.

Hatzenpichler, R., S. A. Connon, D. Goudeau, R. R. Malmstrom, T. Woyke, and V. J. Orphan. 2016. Visualizing in situ translational activity for identifying and sorting slow-growing archaeal−bacterial consortia. Proceedings of the National Academy of Sciences 113:E4069–E4078.

Havrilla, A. C., V. Bala Chaudhary, S. Ferrenberg, A. J. Antoninka, B. Jayne, M. A. Bowker, D. J. Eldridge, A. M. Faist, E. Hubber-Sannwald, A. D. Leslie, E. Rodriguez-Caballero, Y. Zhang, and N. N. Barger. 2018. When communities collide: a meta-analysis of context- dependency in plant responses to biocrusts. Journal of Ecology.

Hennecke, H., and K. T Shanmugam. 1979. Temperature control of nitrogen fixation in Klebsiella pneumoniae. Page Archives of microbiology.

Housman, D. C., H. H. Powers, a. D. Collins, and J. Belnap. 2006. Carbon and nitrogen

fixation differ between successional stages of biological soil crusts in the Colorado Plateau and Chihuahuan Desert. Journal of Arid Environments 66:620–634.

J. Towner, K. 2006. The Genus Acinetobacter. Pages 746–758 *in* M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, and E. Stackebrandt, editors. Prokaryotes: Volume 6: Proteobacteria: Gamma Subclass. Springer-Verlag Berlin Heidelberg, New York, NY.

Jeffrey Smith, C., E. R. Rocha, and B. Paster. 2006. The Medically Important Bacteroides spp. in Health and Disease. Pages 381–427 *in* M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, and E. Stackebrandt, editors. The Prokaryotes: Volume 7: Proteobacteria: delta, esilon subclass. Springer-Verlag Berlin Heidelberg, New York.

Johnson, S. L., C. R. Budinoff, J. Belnap, and F. Garcia-Pichel. 2005. Relevance of ammonium oxidation within biological soil crust communities. Environmental Microbiology 7:1–12.

Johnson, S. L., C. R. Kuske, T. D. Carney, D. C. Housman, L. V. Gallegos-Graves, and J. Belnap. 2012. Increased temperature and altered summer precipitation have differential effects on biological soil crusts in a dryland ecosystem. Global Change Biology 18:2583–2593.

Johnson, S. L., S. Neuer, and F. Garcia-Pichel. 2007. Export of nitrogenous compounds due to incomplete cycling within biological soil crusts of arid lands. Environmental microbiology 9:680–9.

Jose, N. A., R. Lau, T. L. Swenson, N. Klitgord, F. Garcia-Pichel, B. P. Bowen, R. Baran, and T. R. Northen. 2018. Flux balance modeling to predict bacterial survival during pulsed-activity events. Biogeosciences 15:2219–2229.

Jourand, P., E. Giraud, G. Béna, A. Sy, A. Willems, M. Gillis, B. Dreyfus, and P. de Lajudie. 2004. Methylobacterium nodulans sp. nov., for a group of aerobic, facultatively methylotrophic, legume root-nodule-forming and nitrogen-fixing bacteria. International Journal of Systematic and Evolutionary Microbiology 54:2269–2273.

Karaoz, U., E. Couradeau, U. N. da Rocha, H.-C. Lim, T. Northen, F. Garcia-Pichel, and E. L. Brodie. 2018. Large Blooms of Bacillales (Firmicutes) Underlie the Response to Wetting of Cyanobacterial Biocrusts at Various Stages of Maturity. mBio 9:e01366-16.

Katoh, K., and D. M. Standley. 2013. MAFFT Multiple Sequence Alignment Software Version 7 : Improvements in Performance and Usability Article Fast Track 30:772–780.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A.

Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Meintjes, and A. Drummond. 2012a. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649.

Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, C. Duran, T. Thierer, B. Ashton, P. Meintjes, and A. Drummond. 2012b. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data 28:1647–1649.

Knapp, A. K., C. Beier, D. D. Briske, A. T. Classen, Y. Luo, M. Reichstein, M. D. Smith, S. D. Smith, J. E. Bell, P. a. Fay, J. L. Heisler, S. W. Leavitt, R. Sherry, B. Smith, and E. Weng. 2008. Consequences of More Extreme Precipitation Regimes for Terrestrial Ecosystems. BioScience 58:811.

Kuske, C. R., C. M. Yeager, S. Johnson, L. O. Ticknor, and J. Belnap. 2012. Response and resilience of soil biocrust bacterial communities to chronic physical disturbance in arid shrublands. The ISME journal 6:886–897.

Kuzyakov, Y., and E. Blagodatskaya. 2015. Microbial hotspots and hot moments in soil: Concept & review. Soil Biology and Biochemistry 83:184–199.

Kwong, W. K., and N. A. Moran. 2013. Cultivation and characterization of the gut symbionts of honey bees and bumble bees: Description of Snodgrassella alvi gen. nov., sp. nov., a member of the family Neisseriaceae of the betaproteobacteria, and Gilliamella apicola gen. nov., sp. nov., a memb. International Journal of Systematic and Evolutionary Microbiology 63:2008–2018.

Lan, S., L. Wu, D. Zhang, and C. Hu. 2013. Assessing Level of Development and Successional Stages in Biological Soil Crusts with Biological Indicators. Microbial Ecology 66.

Lan, S., L. Wu, D. Zhang, and C. Hu. 2015. Effects of light and temperature on open cultivation of desert cyanobacterium Microcoleus vaginatus. Bioresource Technology 182:144–150.

Lan, S., Q. Zhang, L. Wu, Y. Liu, D. Zhang, and C. Hu. 2014. Artificially accelerating the reversal of desertification: Cyanobacterial inoculation facilitates the succession of vegetation communities. Environmental Science and Technology 48:307–315.

Letunic, I., and P. Bork. 2007. Interactive Tree of Life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinformatics 23:127–128.

Letunic, I., and P. Bork. 2016. Interactive tree of life (iTOL) v3 : an online tool for the display and annotation of phylogenetic and other trees. Nucleic Acids Research 44:242–245.

Liba, C. M., F. I. S. S. Ferrara, G. P. Manfio, F. Fantinatti-Garboggini, R. C. Albuquerque, C. Pavan, P. L. Ramos, C. A. Moreira-Filho, and H. R. Barbosa. 2006. Nitrogen-fixing chemo-organotrophic bacteria isolated from cyanobacteria-deprived lichens and their ability to solubilize phosphate and to release amino acids and phytohormones. Journal of Applied Microbiology 101:1076–1086.

Lynch, M. D. J., and J. D. Neufeld. 2015. Ecology and exploration of the rare biosphere. Nature Reviews Microbiology 13:217–229.

Maestre, F. T., N. Martín, B. Díez, R. López-Poma, F. Santos, I. Luque, and J. Cortina. 2006. Watering, fertilization, and slurry inoculation promote recovery of biological crust function in degraded soils. Microbial ecology 52:365–377.

Martinis Teiceira, L., and V. L. Carreira Merquior. 2014. The Family Moraxallaceae. Pages 443–476 *in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Gammaproteobacteria. London.

Marusenko, Y., S. T. Bates, I. Anderson, S. L. Johnson, T. Soule, and F. Garcia-Pichel. 2013. Ammonia-oxidizing archaea and bacteria are structured by geography in biological soil crusts across North American arid lands. Ecological Processes 2:9.

Masella, A. P., A. K. Bartram, J. M. Truszkowski, D. G. Brown, and J. D. Neufeld. 2012. PANDAseq: paired-end assembler for Illumina sequences. BMC bioinformatics 13:31.

Mcbride, M. J., W. Liu, L. Xuemei, Y. Zhu, W. Zhang, X. Lu, and W. Zhang. 2014. The Family Cytophagaceae. Pages 577–593 *in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Other Major Lineages of Bacteria and Archaea. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

McDonald, D., M. N. Price, J. Goodrich, E. P. Nawrocki, T. Z. DeSantis, A. Probst, G. L. Andersen, R. Knight, and P. Hugenholtz. 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. The ISME journal 6:610–8.

McMurdie, P. J., and S. Holmes. 2014. Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible. PLoS Computational Biology 10.

Megill, L., L. R. Walker, C. Vanier, and D. Johnson. 2011. Seed Bank Dynamics and Habitat Indicators of *Arctomecon californica*, a Rare Plant in a Fragmented Desert Environment. Western North American Naturalist 71:195–206.

Miller, M. A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES Science Gateway for Inference of Large Phylogenetic Trees.

Miller, S. R., R. W. Castenholz, and D. Pedersen. 2007. Phylogeography of the thermophilic cyanobacterium Mastigocladus laminosus. Applied and Environmental

Microbiology 73:4751–4759.

Milo, R., S. Shen-Orr, S. Itzkovitz, and N. Kashtan. 2002. Network Motif: Simple Building Blocks of Complex Networks. Science 298:298.

Mitri, S., E. Clarke, and K. R. Foster. 2015. Resource limitation drives spatial organization in microbial groups. The ISME Journal 10:1–12.

Muñoz-Martín, M. Á., I. Becerra-Absalón, E. Perona, L. Fernández-Valbuena, F. Garcia-Pichel, and P. Mateo. 2018. Cyanobacterial biocrust diversity in Mediterranean ecosystems along a latitudinal and climatic gradient. New Phytologist.

N. Green, P. 2006. Methylobacterium. Pages 257–265 *in* M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, and E. Stackebrandt, editors. Prokaryotes: Volume 5L Alpha and Beta Subclasses. Springer-Verlag Berlin Heidelberg, New York, NY.

Nadell, C. D., K. R. Foster, and J. B. Xavier. 2010. Emergence of spatial structure in cell groups and the evolution of cooperation. PLoS Computational Biology 6.

Nagy, M. L., A. Pérez, and F. Garcia-Pichel. 2005a. The prokaryotic diversity of biological soil crusts in the Sonoran Desert (Organ Pipe Cactus National Monument, AZ). FEMS Microbiology Ecology 54:233–245.

Nagy, M. L., A. Pérez, and F. Garcia-Pichel. 2005b. The prokaryotic diversity of biological soil crusts in the Sonoran Desert (Organ Pipe Cactus National Monument, AZ). FEMS Microbiology Ecology 54:233–245.

Nawrocki, E. P. 2009. Structural RNA homology search and alignment using covariance models. Washington University in St. Louis.

Nierzwicki Bauer, S. A., D. L. Balkwill, and S. E. Stevens. 1984. Heterocyst differentiation in the cyanobacterium Mastigocladus laminosus. Journal of Bacteriology 157:514–525.

Nübel, U., G. Muyzer, F. Garcia-Pichel, and G. Muyzer. 1997. PCR primers to amplify 16S rRNA genes from cyanobacteria PCR Primers to Amplify 16S rRNA Genes from Cyanobacteria. Microbiology 63:3327–3332.

Nunes da Rocha, U., H. Cadillo-Quiroz, U. Karaoz, L. Rajeev, N. Klitgord, S. Dunn, V. Truong, M. Buenrostro, B. P. Bowen, F. Garcia-Pichel, A. Mukhopadhyay, T. R. Northen, and E. L. Brodie. 2015. Isolation of a significant fraction of non-phototroph diversity from a desert Biological Soil Crust. Frontiers in Microbiology 6:1–14.

Octavia, S., and R. Lan. 2014. The Family Enterobacteraceae. Pages 226–273 *in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Gammaproteobacteria. Fourth edition. Springer-Verlag Berlin

Heidelberg, London.

Parks, D. H., C. Rinke, M. Chuvochina, P.-A. Chaumeil, B. J. Woodcroft, P. N. Evans, P. Hugenholtz, and G. W. Tyson. 2017. Recovery of nearly 8,000 metagenome-assembled genomes substantially expands the tree of life. Nature Microbiology 903:1–10.

Pepe-Ranney, C., C. Koechli, R. Potrafka, C. Andam, E. Eggleston, F. Garcia-Pichel, and D. H. Buckley. 2015. Non-cyanobacterial diazotrophs dominate dinitrogen fixation in biological soil crusts during early crust formation. The ISME Journal 10:287–298.

Petrie, M. D., S. L. Collins, D. S. Gutzler, and D. M. Moore. 2014. Regional trends and local variability in monsoon precipitation in the northern Chihuahuan Desert, USA. Journal of Arid Environments 103:63–70.

Petrie, M. D., S. L. Collins, and M. E. Litvak. 2015. The ecological role of small rainfall events in a desert grassland. Ecohydrology 8:1614-1622.

Poly, F., L. J. Monrozier, and R. Bally. 2001. Improvement in the RFLP procedure for studying the diversity of nifH genes in communities of nitrogen fixers in soil. Research in Microbiology 152:95–103.

Prăvălie, R. 2016. Drylands extent and environmental issues . A global approach 161:259–278.

Price, M. N., P. S. Dehal, and A. P. Arkin. 2010. FastTree 2 - Approximately maximum-likelihood trees for large alignments. PloS ONE 5.

Pringault, O., and F. Garcia-Pichel. 2004. Hydrotaxis of cyanobacteria in desert crusts. Microbial ecology 47:366–73.

Rajeev, L., U. N. Da Rocha, N. Klitgord, E. G. Luning, J. Fortney, S. D. Axen, P. M. Shih, N. J. Bouskill, B. P. Bowen, C. A. Kerfeld, F. Garcia-Pichel, E. L. Brodie, T. R. Northen, and A. Mukhopadhyay. 2013. Dynamic cyanobacterial response to hydration and dehydration in a desert biological soil crust. The ISME Journal:2178–2191.

Rao, B., Y. Liu, S. Lan, P. Wu, W. Wang, and D. Li. 2012. Effects of sand burial stress on the early developments of cyanobacterial crusts in the field. European Journal of Soil Biology 48:48–55.

Reed, C. 2012. Tree Species Control Rates of Free-Living Nitrogen Fixation in a Tropical Rain Forest Author (s): Sasha C . Reed , Cory C . Cleveland and Alan R . Townsend Published by : Ecological Society of America content in a trusted digital archive . We use inform 89:2924–2934.

Reed, S. C., K. K. Coe, J. P. Sparks, D. C. Housman, T. J. Zelikova, and J. Belnap. 2012.

Changes to dryland rainfall result in rapid moss mortality and altered soil fertility. Nature Climate Change 2:752–755.

Reynolds, R., J. Belnap, M. Reheis, P. Lamothe, and F. Luiszer. 2001. Aeolian dust in Colorado Plateau soils: nutrient inputs and recent change in source. Proceedings of the National Academy of Sciences of the United States of America 98:7123–7.

Rodríguez-Caballero, E., J. Belnap, B. Büdel, P. Crutzen, M. O. Andreae, U. Pöschl, and B. Weber. 2018. Dryland photoautotrophic soil surface communities endangered by global change. Nature Geoscience 11: 185-189.

Rodríguez-Caballero, E., Y. Cantón, S. Chamizo, A. Afana, and A. Solé-Benet. 2012. Effects of biological soil crusts on surface roughness and implications for runoff and erosion. Geomorphology 145–146:81–89.

Rodríguez-Caballero, E., Y. Cantón, S. Chamizo, R. Lázaro, and A. Escudero. 2013. Soil Loss and Runoff in Semiarid Ecosystems: A Complex Interaction Between Biological Soil Crusts, Micro-topography, and Hydrological Drivers. Ecosystems 16:529–546.

Román, J. R., B. Roncero-Ramos, S. Chamizo, E. Rodríguez-Caballero, and Y. Cantón. 2018a. Restoring soil functions by means of cyanobacteria inoculation: Importance of soil conditions and species selection. Land Degradation and Development:3184–3193.

Román, J. R., B. Roncero-Ramos, S. Chamizo, E. Rodríguez-Caballero, and Y. Cantón. 2018b. Restoring soil functions by means of cyanobacteria inoculation: Importance of soil conditions and species selection. Land Degradation and Development 29:3281–3193.

Roncero-Ramos, B., J. R. Román, C. Gómez-Serrano, Y. Cantón, and F. G. Acién. 2019. Production of a biocrust-cyanobacteria strain (Nostoc commune) for large-scale restoration of dryland soils. Journal of Applied Phycology:1–14.

Ryan, R. P., S. Monchy, M. Cardinale, S. Taghavi, L. Crossman, M. B. Avison, G. Berg, D. van der Lelie, and J. M. Dow. 2009. The versatility and adaptation of bacteria from the genus Stenotrophomonas. Nature Reviews Microbiology 7:514–525.

Sala., O. E., and W. K. Lauenroth. 2014. Small Rainfall Events : An Ecological Role in Semiarid Regions. Oecologia 53:301–304.

Samo, T. J., J. A. Kimbrel, D. J. Nilson, J. Pett-Ridge, P. K. Weber, and X. Mayali. 2018. Attachment between heterotrophic bacteria and microalgae influences symbiotic microscale interactions. Environmental Microbiology.

Sancho, L. G., J. Belnap, C. Colesie, J. Raggio, and B. Weber. 2016. Carbon Budgets of Biological Soil Crusts at Micro-, Meso-, and Global Scales. Pages 287–304

Biological Soil Crusts: An Organizing Principle in Drylands. Second. Springer, Switzerland.

Sasse, J., E. Martinoia, and T. Northen. 2018. Feed Your Friends: Do Plant Exudates Shape the Root Microbiome? Trends in Plant Science 23:25–41.

Schlösser, U. G. 1982. Sammlung von algenkulturen. Ber Dtsch Bot Ges 95:181–276.

Seager, R., and G. A. Vecchi. 2010. Greenhouse warming and the 21st century hydroclimate of southwestern North America. Proceedings of the National Academy of Sciences of the United States of America 107:21277–21282.

Sherrod, S. K., J. Belnap, and M. E. Miller. 2002. Comparison of methods for nutrient measurement in calcareous soils: ion-exchange resin bag, capsule, membrane, and chemical extractions 167:666–679.

Smith, D. L., J. R. Lamont, O. Wilkins, and M. Bywater-Ekegard. 2017. From yogurt to yield : Potential applications of lactic acid bacteria in plant production. Soil Biology & Biochemistry 111:1–9.

Sorochkina, K., S. Velasco Ayuso, and F. Garcia-Pichel. 2018. Establishing rates of lateral expansion of cyanobacterial biological soil crusts for optimal restoration. Plant and soil 429:199–211.

Soule, T., I. J. Anderson, S. L. Johnson, S. T. Bates, and F. Garcia-Pichel. 2009a. Archaeal populations in biological soil crusts from arid lands in North America. Soil Biology and Biochemistry 41:2069–2074.

Soule, T., K. Palmer, Q. Gao, R. M. Potrafka, V. Stout, and F. Garcia-Pichel. 2009b. A comparative genomics approach to understanding the biosynthesis of the sunscreen scytonemin in cyanobacteria. BMC genomics 10:336.

St Clair, L. L., J. R. Johansen, and B. L. Webb. 1986. Rapid stabilization of fire-disturbed sites using a soil crust slurry: Inoculation studies. Reclam.Reveg.Res. 4:261–269.

Stamatakis, A. 2014. RAxML Version 8 : A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies:2010–2011.

Stark, L. R., J. C. Brinda, D. Nicholas McLetchie, and M. J. Oliver. 2012. Extended Periods of Hydration Do Not Elicit Dehardening to Desiccation Tolerance in Regeneration Trials of the Moss *Syntrichia caninervis*. International Journal of Plant Sciences 173:333–343.

Starkenburg, S. R., K. G. Reitenga, T. Freitas, S. Johnson, P. S. G. Chain, F. Garcia-Pichel, and C. R. Kuske. 2011. Genome of the cyanobacterium Microcoleus vaginatus FGP-2, a photosynthetic ecosystem engineer of arid land soil biocrusts worldwide. Journal of bacteriology 193:4569–70.

Strauss, S. L., T. a. Day, and F. Garcia-Pichel. 2011. Nitrogen cycling in desert biological soil crusts across biogeographic regions in the Southwestern United States. Biogeochemistry 108:171–182.

Swenson, T. L., E. Couradeau, B. P. Bowen, R. de Philippis, F. Rossi, G. Mugnai, and T. R. Northen. 2017. A novel method to evaluate nutrient retention by biological soil crust exopolymeric matrix. Plant and Soil.

Swenson, T. L., U. Karaoz, J. M. Swenson, B. P. Bowen, and T. R. Northen. 2018. Linking soil biology and chemistry in biological soil crust using isolate exometabolomics. Nature Communications 9.

Takenaka, H., and Y. Yamguchi. 2014. Commercial-scale culturing of cyanobacteria: an industrial experience. Pages 293–318 *in* K. N. Sharma, A. K. Rai, and J. L. Stal, editors. Cyanobacteria: An Economic Perspective. First edit. John Wiley & Sons, Ltd., Oxford, UK.

Thiet, R. K., R. E. J. Boerner, M. Nagy, and R. Jardine. 2005. The effect of biological soil crusts on throughput of rainwater and N into Lake Michigan sand dune soils. Plant and Soil 278:235–251.

Thomazo, C., E. Couradeau, and F. Garcia-Pichel. 2018a. Possible nitrogen fertilization of the early Earth Ocean by microbial continental ecosystems. Nature Communications 9.

Thomazo, C., E. Couradeau, and F. Garcia-Pichel. 2018b. Possible nitrogen fertilization of the early Earth Ocean by microbial continental ecosystems. Nature Communications 9.

Ullmann, L., and B. Büdel. 2001. Ecological Determinants of Species Composition of Biological Soil Cruts on a Landscape Scale.pdf. Pages 203–213 *in* J. Belnap and O. L. Lange, editors. Biological Soil Cruts: Structure, Function, and Management. 1st edition. Springer-Verlag, Berlin.

Verrecchia, E., A. Yair, G. J. Kidron, and K. Verrecchia. 1995. Physical properties of the psammophile cryptogamic crust and their consequences to the water regime of sandy softs, north-western Negev Desert , Israel. Journal of Arid Environments:427–437.

Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007a. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73:5261–5267.

Wang, W., Y. Liu, D. Li, C. Hu, and B. Rao. 2009. Feasibility of cyanobacterial inoculation for biological soil crusts formation in desert area. Soil Biology and Biochemistry 41:926–929.

Wang, W., C. Yang, D. Tang, D. Li, Y. Liu, and C. Hu. 2007b. Effects of sand burial on biomass, chlorophyll fluorescence and extracellular polysaccharides of man-made cyanobacterial crusts under experimental conditions. Science in China, Series C: Life Sciences 50:530–534.

Weber, B., M. M. Caldwell, B. Jayne, W. Bettina, B. Büdel, J. Belnap, B. Weber, and B. Büdel. 2016. Biological Soil Crusts: An Organizing Principle in Drylands. Pages 3–14 Biological Soil Crusts: An Organizing Principle in Drylands. 2nd edition. Springer, Switzerland.

Williams, L., K. Loewen-Schneider, S. Maier, and B. Büdel. 2016. Cyanobacterial diversity of western European biological soil crusts along a latitudinal gradient. FEMS Microbiology Ecology 92:1–9.

Williams, W. J., D. J. Eldridge, and B. M. Alchin. 2008. Grazing and drought reduce cyanobacterial soil crusts in an Australian Acacia woodland. Journal of Arid Environments 72:1064–1075.

Wozniak, C. A., G. McClung, J. Glagiardi, M. Segal, and K. Matthews. 2012. Regulation of Genetically Engineered Microorganisms Under FIFRA, FFDCA and TSCA. Pages 57–94 *in* C. A. Wozniak and A. McHughen, editors. Regulation of Agricultural Biotechnology: The United States and Canada. Springer, Berlin, Heindelberg, Heidelberg, Germany.

Wu, Y., B. Rao, P. Wu, Y. Liu, G. Li, and D. Li. 2013. Development of artificially induced biological soil crusts in fields and their effects on top soil. Plant and Soil 370:115–124.

Xiao, B., Z. Yunge, and S. Ming'An. 2008. Artificial cultivation of biological soil crust and its effects on soil and water conservation in water-wind erosion crisscross region of loess plateau, China.

Xie, Z., Y. Liu, C. Hu, L. Chen, and D. Li. 2007. Relationships between the biomass of algal crusts in fields and their compressive strength. Soil Biology and Biochemistry 39:567–572.

Xu, S. 2016. Bayesian Naïve Bayes classifiers to text classification. Journal of Information Science 44:48–59.

Yeager, C. M., J. L. Kornosky, D. C. Housman, E. E. Grote, J. Belnap, and C. R. Kuske. 2004. Diazotrophic Community Structure and Function in Two Successional Stages of Biological Soil Crusts from the Colorado Plateau and Chihuahuan Desert. Applied and Environmental Microbiology 70.

Yeager, C. M., J. L. Kornosky, R. E. Morgan, E. C. Cain, F. Garcia-Pichel, D. C. Housman, J. Belnap, and C. R. Kuske. 2007. Three distinct clades of cultured heterocystous cyanobacteria constitute the dominant N2-fixing members of

biological soil crusts of the Colorado Plateau, USA. FEMS Microbiology Ecology 60:85–97.

Zaady, E., D. J. Eldridge, and M. A. Bowker. 2016. Effects of Local-Scale Disturbance on Biocrusts. Pages 425–445 Biological Soil Crusts: An Organizing Principle in Drylands. 2dn edition. Springer, Switzerland.

Zaady, E., Y. Gutterman, and B. Boeken. 1997. The germination of mucilaginous seeds of Plantago coronopus, Reboudia pinnata, and Carrichtera annua on cyanobacterial soil crust from the Negev Desert. Plant and Soil 190:247–252.

Zhang, B., W. Kong, W. Nan, and Y. Zhang. 2016. Bacterial diversity and community along the succession of biological soil crusts in the Gurbantunggut Desert, Northern China. Journal of Basic Microbiology 56:670–679.

Zhang, B., Y. Zhang, Y. Su, J. Wang, and J. Zhang. 2013. Responses of Microalgal-Microbial Biomass and Enzyme Activities of Biological Soil Crusts to Moisture and Inoculated Microcoleus vaginatus Gradients. Arid Land Research and Management 27:216–230.

Zhang, Y. M., H. L. Wang, X. Q. Wang, W. K. Yang, and D. Y. Zhang. 2006. The microstructure of microbiotic crust and its influence on wind erosion for a sandy soil surface in the Gurbantunggut Desert of Northwestern China. Geoderma 132:441–449.

Zhang, Z., S. Schwartz, L. Wagner, and W. Miller. 2000. A Greedy Algorithm for Aligning DNA Sequences. Journal of Computational Biology 7:203–214.

Zheng, Y., M. Xu, J. Zhao, S. Bei, and L. Hao. 2010. Effects of inoculated Microcoleus vaginatus on the structure and function of biological soil crusts of desert. Biology and Fertility of Soils 47:473–480.

Zhou, X., H. Smith, A. Giraldo Silva, J. Belnap, and F. Garcia-Pichel. 2016. Differential Responses of Dinitrogen Fixation, Diazotrophic Cyanobacteria and Ammonia Oxidation Reveal a Potential Warming-Induced Imbalance of the N-Cycle in Biological Soil Crusts. PloS ONE 11:1–15.

APPENDIX A.

TAXONOMIC ASSIGNMENTS AND FUNCTIONAL INFERENCE BASED ON PHYLOGENETIC PLACEMENT FOR AGGREGATING (CYANOSPHERE) OTUS IN THE COLD DESERT (HSN).

TAXONOMIC ASSIGNMENTS AND FUNCTIONAL INFERENCE BASED ON PHYLOGENETIC PLACEMENT FOR AGGREGATING (CYANOSPHERE) OTUS IN THE HOT DESERT (FB).

A. TAXONOMIC ASSIGNMENTS AND FUNCTIONAL INFERENCE BASED ON PHYLOGENETIC PLACEMENT FOR AGGREGATING (CYANOSPHERE) OTUS IN THE COLD DESERT (HSN).

Phylum	Deepest Taxonomic Assignment	OTU ID	Nutritional type / typical habitat	Reference	Diazotroph within genus	Reference
Firmicutes	*Staphylococcus*	1084865	Copiotrophs/ saprophytic fermentative	(Götz et al. 2006)	no	
Firmicutes	*Streptoccoccus gordonii*	1083194	Copiotrophs/ saprophytic fermentative (largely animal, but also rhizospheres, manure)	(Smith et al. 2017)	no	
Alphaproteobacteria	*Methylobacterium aerolaum*	4323871	Strict aerobe/ mostly copiotrophs and C1 facultative	(N. Green 2006)	yes	(Jourand et al. 2004)
Betaproteobacteria	*Pelomonas saccharophila*	1108275	Copiotrophs/ saprophytic, aerobic, facultative lithoautotroph	(Barraquio et al. 1986)	yes	(Barraquio et al. 1986)
Betaproteobacteria	*Snodgrassella*	933546	Copiotrophs/ saprophytic microaerophilli c	(Kwong and Moran 2013)	no	genome
Deltaproteobacteria	Myxobacteria (Chondromyces)	565046	Aerobic copiotrophs/ saprophytic/ dung	(Dawid 2000)	no	Kegg Pathways
Deltaproteobacteria	Myxobacteria	New.ReferenceOTU69	Aerobic copiotrophs/ saprophytic/ dung			
Deltaproteobacteria	Myxobacteria	New.CleanUp.ReferenceOTU14037	Aerobic copiotrophs/ saprophytic/ dung			
Gammaproteobacteria	*Escherichia/Shigella*	1111294	Copiotrophs/ saprophytic facultative anaerobe	(Octavia and Lan 2014)	yes	15
Gammaproteobacteria	*Haemophilus parinfluenzae*	865469	Aerobic copiotrophs/animal commensal	(Christensen et al. 2013)	no	KEGG Pathways
Gammaproteobacteria	*Acinetobacter johsnonii*	988314	Copiotrophs/ saprophytic obligate aerobe	(J. Towner 2006)	yes	(Liba et al. 2006)

Gammaproteobacteria	*Stenotrophomonas maltophila*	1083508	Copiotrophs/ saprophytic/nosocomial	(Ryan et al. 2009)	yes	(Liba et al. 2006)
Gammaproteobacteria	*Stenotrophomonas maltophila*	1834768	Copiotrophs/ saprophytic/nosocomial		yes	
Gammaproteobacteria	*Moraxella*	990864	Aerobic copiotrophs/animal commensal	(Martinis Teiceira and Carreira Merquior 2014)		
Bacteriodetes	*Bacteriodes vulgatus*	589277	Copiotrophs/ saprophytic/anaerobes, rare in soil	(Jeffrey Smith et al. 2006)	no	KEGG Pathways
Bacteriodetes	Cytophagaceae	New.CleanUp.ReferenceOTU1843	Found in terrestrial, marine and freshwater environments	(Mcbride et al. 2014)		
Bacteriodetes	Cytophagaceae	New.CleanUp.ReferenceOTU7233				
Actinobacteria	Actinomycetales	937735				
(Cyanobacteria)	higher plant plastid	153978	NA (from plant roots or pollen)			
(Cyanobacteria)	higher plant plastid	1126072	NA (from plant roots or pollen)			
Unassigned		New.ReferenceOTU31				
Unassigned		New.CleanUp.ReferenceOTU8675				

TAXONOMIC ASSIGNMENTS AND FUNCTIONAL INFERENCE BASED ON PHYLOGENETIC PLACEMENT FOR AGGREGATING (CYANOSPHERE) OTUS IN THE HOT DESERT (FB).

Phylum	**Deepest Taxonomic Assignment**	**OTU ID**	**Nutritional type/typical habitat**	**Reference**	**Diazotroph within genus**	**Reference**
Gammaproteobacteria	*Stenotrophomonas maltophila*	1834768	copiotrophs/ saprophytic/nosocomial	(Ryan et al. 2009)	yes	(Liba et al. 2006)
Bacteriodetes	Sphingobacteriales	1087471				
(Cyanobacteria)	higher plant plastid	153978	NA (from plant roots or pollen)			

References

Barraquio, W. L., B. C. P. JR, I. Watanabe, and R. Knowles. 1986. Nitrogen Fixation by Pseudomonas saccharophila Doudoroff ATCC 15946. Journal of General Microbiology 132:237–241.

Christensen, H., P. Kuhnert, N. Norskov-Lauritsen, P. J. Planet, and M. Bisgaard. 2013. The Family Pasteurellaceae. Pages 535–556 *in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Gammaproteobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Dawid, W. 2000. Biology and global distribution of myxobacteria in soils. FEMS Microbiology Reviews 24:403–427.

Götz, F., T. Bannerman, and K.-H. Schleifer. 2006. The Genera Staphylococcus and Macrococcus. Pages 5–75 *in* M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, and E. Stackebrandt, editors. The Prokaryotes: Volume 4: Bacteria: Firmicutes, Cyanobacteria. Springer-Verlag Berlin Heidelberg, New York, NY.

J. Towner, K. 2006. The Genus Acinetobacter. Pages 746–758 *in* M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, and E. Stackebrandt, editors. Prokaryotes: Volume 6: Proteobacteria: Gamma Subclass. Springer-Verlag Berlin Heidelberg, New York, NY.

Jeffrey Smith, C., E. R. Rocha, and B. Paster. 2006. The Medically Important Bacteroides spp. in Health and Disease. Pages 381–427 *in* M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, and E. Stackebrandt, editors. The Prokaryotes: Volume 7: Proteobacteria: delta, esilon subclass. Springer-Verlag Berlin Heidelberg, New York.

Jourand, P., E. Giraud, G. Béna, A. Sy, A. Willems, M. Gillis, B. Dreyfus, and P. de Lajudie. 2004. Methylobacterium nodulans sp. nov., for a group of aerobic, facultatively methylotrophic, legume root-nodule-forming and nitrogen-fixing bacteria. International Journal of Systematic and Evolutionary Microbiology 54:2269–2273.

Kwong, W. K., and N. A. Moran. 2013. Cultivation and characterization of the gut symbionts of honey bees and bumble bees: Description of Snodgrassella alvi gen. nov., sp. nov., a member of the family Neisseriaceae of the betaproteobacteria, and Gilliamella apicola gen. nov., sp. nov., a memb. International Journal of Systematic and Evolutionary Microbiology 63:2008–2018.

Liba, C. M., F. I. S. S. Ferrara, G. P. Manfio, F. Fantinatti-Garboggini, R. C. Albuquerque, C. Pavan, P. L. Ramos, C. A. Moreira-Filho, and H. R. Barbosa. 2006. Nitrogen-fixing chemo-organotrophic bacteria isolated from cyanobacteria-deprived lichens and their ability to solubilize phosphate and to release amino acids and phytohormones. Journal of Applied Microbiology 101:1076–1086.

Martinis Teiceira, L., and V. L. Carreira Merquior. 2014. The Family Moraxallaceae. Pages 443–

476 *in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Gammaproteobacteria. London.

Mcbride, M. J., W. Liu, L. Xuemei, Y. Zhu, W. Zhang, X. Lu, and W. Zhang. 2014. The Family Cytophagaceae. Pages 577–593 *in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Other Major Lineages of Bacteria and Archaea. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

N. Green, P. 2006. Methylobacterium. Pages 257–265 *in* M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer, and E. Stackebrandt, editors. Prokaryotes: Volume 5L Alpha and Beta Subclasses. Springer-Verlag Berlin Heidelberg, New York, NY.

Octavia, S., and R. Lan. 2014. The Family Enterobacteraceae. Pages 226–273 *in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Gammaproteobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Ryan, R. P., S. Monchy, M. Cardinale, S. Taghavi, L. Crossman, M. B. Avison, G. Berg, D. van der Lelie, and J. M. Dow. 2009. The versatility and adaptation of bacteria from the genus Stenotrophomonas. Nature Reviews Microbiology 7:514–525.

Smith, D. L., J. R. Lamont, O. Wilkins, and M. Bywater-Ekegard. 2017. From yogurt to yield : Potential applications of lactic acid bacteria in plant production. Soil Biology & Biochemistry 111:1–9.

APPENDIX B.

TAXONOMIC ASSIGNMENT AND FUNCTIONAL INFERENCES BASED ON PHYLOGENETIC PLACEMENT FOR SEGREGATING OTUS FOR BOTH BULK SOILS (COLD AND HOT DESERTS).

B. TAXONOMIC ASSIGNMENT AND FUNCTIONAL INFERENCES BASED ON PHYLOGENETIC PLACEMENT FOR SEGREGATING OTUS FOR BOTH BULK SOILS (COLD AND HOT DESERTS). ROWS COLORED IN YELLOW CORRESPOND TO THOSE OTUS FOR WHICH INFERRED FUNCTION WAS CONSISTENT WITH SEGREGATION FROM *M. VAGINATUS*.

Phylum	Deepest Taxonomic Assignment	OTU ID	Nutritional type/typical habitat	Reference
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	4359078	Chemoorganoheterotrophs/Abundant in soils	(Pascual et al. 2015)
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	830338		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	New.CleanUp.ReferenceOTU1120		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	New.CleanUp.ReferenceOTU23458		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	809387		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	4321498		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	New.CleanUp.ReferenceOTU9855		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	New.CleanUp.ReferenceOTU6851		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	279384		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	612580		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	4297666		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	New.CleanUp.ReferenceOTU23705		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	New.CleanUp.ReferenceOTU583		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	New.CleanUp.ReferenceOTU14239		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	1120231		

Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	447341		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	353816		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	New.CleanUp.ReferenceOTU549		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	687206		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	4399397		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	New.CleanUp.ReferenceOTU16177		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	New.CleanUp.ReferenceOTU13917		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	726866		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	4451552		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	1125708		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	New.CleanUp.ReferenceOTU6201		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	New.CleanUp.ReferenceOTU1640		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	New.CleanUp.ReferenceOTU4511		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	New.CleanUp.ReferenceOTU1380		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	551480		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	New.CleanUp.ReferenceOTU14817		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	171397		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	86097		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	627902		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	141861		

Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	213767		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	4297673		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	512304		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	113607		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	New.ReferenceOTU67		
Acidobacteria	In the Blastocatellaceae clade (Aridibacter/Blastocatella/Stenotrophobacter)	211578		
Acidobacteria	In the Blastocatellaceae clade (Aridibacter/Blastocatella/Stenotrophobacter)	151008		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	279436		
Acidobacteria	In the Blastocatellaceae clade (*Aridibacter*/*Blastocatella*/*Stenotrophobacter*)	212764		
Acidobacteria	*Vicinamibacter silvestris*	811187	Chemoorganoheterothroph/Abundant in soil	(Huber et al. 2016)
Acidobacteria	Sister clade to *Holophaga*	New.CleanUp.ReferenceOTU9452	Anaerobes/Found in fresh water, sediments and soils	(Fukunaga and Ichikawa 2014)
Acidobacteria	Sister clade to Solibacteraceae	806959		
Acidobacteria	Sister clade to Solibacteraceae	New.CleanUp.ReferenceOTU24928		
Acidobacteria	Sister clade to Solibacteraceae	New.CleanUp.ReferenceOTU20608		
Acidobacteria	Sister clade to Solibacteraceae	New.CleanUp.ReferenceOTU616		
Acidobacteria	Sister clade to Solibacteraceae	728545		
Acidobacteria	Sister clade to Solibacteraceae	4339765		
Acidobacteria	Sister clade to Solibacteraceae	New.CleanUp.ReferenceOTU15359		
Deinococcus-Thermus	*Deinococcus navajonensis*	1133399	Aerobes/Radioresistant	(Rosenberg 2014a)
Deinococcus-Thermus	Deinococcaceae	1018538		
Deinococcus-Thermus	Sister clade to *Truepera* sp.	2248445	Aerobes, Chemoorganotrophs/Radioresistant	(Rosenberg 2014a)
Deinococcus-Thermus	Sister clade to *Truepera* sp.	266995		
Deinococcus-Thermus	Sister clade to *Truepera* sp.	86556		

Deinococcus-Thermus	Sister clade to *Truepera* sp.	4024547		
Deinococcus-Thermus	Sister clade to *Truepera* sp.	274011		
Actinobacteria	Sister clade to Acidimicrobiales	1639776	Obligate acidophilic, oxidize ferrous iron or reduce ferric iron	(Stackebrandt 2014)
Actinobacteria	Sister clade to Acidimicrobiales	New.CleanUp.ReferenceOTU22804		
Actinobacteria	Sister clade to Acidimicrobiales	New.CleanUp.ReferenceOTU15026		
Actinobacteria	Sister clade to Acidimicrobiales	2345835		
Actinobacteria	Sister clade to Acidimicrobiales	New.CleanUp.ReferenceOTU13169		
Actinobacteria	Sister clade to Acidimicrobiales	New.CleanUp.ReferenceOTU18563		
Actinobacteria	Sister clade to Acidimicrobiales	830889		
Actinobacteria	Sister clade to Acidimicrobiales	223441		
Actinobacteria	Sister clade to Acidimicrobiales	4313541		
Actinobacteria	Sister clade to Acidimicrobiales	790420		
Actinobacteria	*Angustibacter*	New.CleanUp.ReferenceOTU17686	Facultative anaerobes/Gram-positive	(Tamura et al. 2010)
Actinobacteria	In the Kineosporiaceae (*Angustibacter*)	153548		
Actinobacteria	*Angustibacter*	726955		
Actinobacteria	*Saccharothrix*	4417388	Anaerobes/Gram-positive	(Labeda and Testa 1984)
Actinobacteria	*Cellulomonas*	788268	Anaerobes/Abundant in soils/Degrade cellulose/symbiosis with *Azotobacter*	(Stackebrandt and Schumann 2014)
Actinobacteria	*Pseudonocardia*	829373	Some species of *Pseudonocardia* are facultative autotrophs/ Common in soils, sediments and plant roots	(Franco and Labeda 2014)
Actinobacteria	In the Pseudonocardiaceae (*Pseudonocardia, Actinokineospora*)	501584		
Actinobacteria	*Pseudonocardia*	327290		
Actinobacteria	In the Pseudonocardiaceae (*Pseudonocardia, Actinokineospora*)	918840		
Actinobacteria	In the Pseudonocardiaceae (*Pseudonocardia, Actinokineospora*)	1079481		
Actinobacteria	In the Pseudonocardiaceae	805717		
Actinobacteria	In the Pseudonocardiaceae	823816		
Actinobacteria	In the Pseudonocardiaceae	1039041		
Actinobacteria	In the Pseudonocardiaceae	4332665		
Actinobacteria	In the Pseudonocardiaceae	869089		
Actinobacteria	*Geodermatophilus*	818388	Oligotrophs/Found mainly in soils, water and stone surfaces	(Normand et al. 2014)
Actinobacteria	*Geodermatophilus*	704830		

Actinobacteria	*Geodermatophilus*	156477		
Actinobacteria	*Modestobacter*	510174		
Actinobacteria	In the Geodermatophilaceae (*Blastococcus/Modestobacter*)	202381		
Actinobacteria	In the Geodermatophilaceae (*Blastococcus/Modestobacter*)	966091		
Actinobacteria	In the Geodermatophilaceae (*Blastococcus/Modestobacter*)	New.CleanUp.ReferenceOTU5044		
Actinobacteria	In the Geodermatophilaceae (*Blastococcus/Modestobacter*)	2855257		
Actinobacteria	In the Geodermatophilaceae (*Blastococcus/Geodermatophilus*)	11439		
Actinobacteria	In the Geodermatophilaceae (*Blastococcus/Geodermatophilus*)	4299608		
Actinobacteria	In the Geodermatophilaceae (*Geodermatophilus*)	11428		
Actinobacteria	*Oryzihumus*	538111	Abundant in soil and marine environments	(Stackebrandt et al. 2014)
Actinobacteria	In the Kineosporiaceae (*Kineosporia*)	New.CleanUp.ReferenceOTU17035	Form spores/ Found in soil, desert sands, plant litter, stems of plants	(Tamura and Suzuki 2014)
Actinobacteria	In the Kineosporiaceae (*Kineosporia*)	New.CleanUp.ReferenceOTU10380		
Actinobacteria	In the Kineosporiaceae (*Kineosporia*)	New.CleanUp.ReferenceOTU16749		
Actinobacteria	In the Kineosporiaceae (*Kineosporia*)	250148		
Actinobacteria	In the Kineosporiaceae (*Kineosporia*)	New.CleanUp.ReferenceOTU19427		
Actinobacteria	In the Kineosporiaceae (*Kineosporia*)	New.CleanUp.ReferenceOTU10429		
Actinobacteria	*Arthrobacter*	1081815	Oligotrophs/Ubiquitous/ Found in soils, predominant in non-rhizosphere samples	(Busse and Wieser 2014)
Actinobacteria	*Arthrobacter*	929901		
Actinobacteria	*Arthrobacter*	1101451		
Actinobacteria	In the Micromonosporaceae (*Asanoa*)	248468	Aerobic/ Widely distributed in soils and aquatic environments	(Trujillo et al. 2014)
Actinobacteria	In the Micromonosporaceae (*Dactylosporangium*)	New.CleanUp.ReferenceOTU19623		
Actinobacteria	In the Micromonosporaceae (*Dactylosporangium*)	408093		
Actinobacteria	In the Micromonosporaceae (*Actinoplanes, Spirilliplanes*)	580850		
Actinobacteria	In the Micromonosporaceae (*Actinoplanes,Couchioplanes*)	688259		

Actinobacteria	In the Micromonosporaceae (*Actinoplanes,Couchioplanes*)	357423		
Actinobacteria	In the Micromonosporaceae (*Actinoplanes,Couchioplanes*)	249571		
Actinobacteria	In the Micromonosporaceae (*Actinoplanes, Micromonospora*)	250572		
Actinobacteria	In the Micromonosporaceae (*Actinoplanes, Micromonospora*)	265094		
Actinobacteria	In the Micromonosporaceae (*Actinoplanes, Micromonospora*)	582813		
Actinobacteria	*Nocardioides*	1126182	Chemoorganotrophs/Aerobes/ They may adapt to oligotrophic conditions/ Found in soils and aquatic environments	(Tohn and Borsodi 2014)
Actinobacteria	*Nocardioides*	919487		
Actinobacteria	In the Nocardioidaceae	996116	Chemoorganotrophs/Aerobes/ Found in soils and aquatic environments	
Actinobacteria	In the Nocardioidaceae	902698		
Actinobacteria	In the Nocardioidaceae	954340		
Actinobacteria	In the Nocardioidaceae	1142263		
Actinobacteria	In the Nocardioidaceae	New.CleanUp.ReferenceOTU16525		
Actinobacteria	In the Nocardioidaceae	558911		
Actinobacteria	*Virgisporangium*	564093	Aerobes/Nitrate is reduced by all species/Found in soils and aquatic environments	(Trujillo et al. 2014)
Actinobacteria	In the Nakamurellaceae (*Nakamurella*)	New.CleanUp.ReferenceOTU4744	Aerobes/non-spore forming/ Unclear niche occupation: possible soil or water origin organism	(Kim and Lee 2014)
Actinobacteria	Frankiales	254635		
Actinobacteria	Frankiales	146122		
Actinobacteria	In the Euebyaceae	2219500	Aerobes/ Chemoorganotrophs	(Stackebrandt and Otten 2014)
Actinobacteria	In the Euebyaceae	4357799		
Actinobacteria	In the Solirubrobacteriales (*Solirubrobacter, Patulibacter, Conexibacter)*	New.CleanUp.ReferenceOTU10190	Gram-positive/Mesophilic/Found in soils with generally low temperature and neutral pH	(Albuquerque and da Costa 2014a)
Actinobacteria	In the Solirubrobacteriales (*Solirubrobacter, Patulibacter, Conexibacter)*	371783		
Actinobacteria	In the Solirubrobacteriales (*Solirubrobacter, Patulibacter, Conexibacter)*	New.CleanUp.ReferenceOTU20163		
Actinobacteria	In the Solirubrobacteriales	873887		
Actinobacteria	In the Solirubrobacteriales	New.CleanUp.ReferenceOTU20865		
Actinobacteria	In the Solirubrobacteriales	New.CleanUp.ReferenceOTU16389		
Actinobacteria	In the Solirubrobacteriales	243579		
Actinobacteria	In the Solirubrobacteriales	205267		

Actinobacteria	In the Solirubrobacteriales	217548		
Actinobacteria	In the Solirubrobacteriales	4327844		
Actinobacteria	In the Solirubrobacteriales	New.ReferenceOTU32		
Actinobacteria	In the Solirubrobacteriales	589372		
Actinobacteria	In the Solirubrobacteriales	235943		
Actinobacteria	In the Solirubrobacteriales	219818		
Actinobacteria	In the Solirubrobacteriales	1132235		
Actinobacteria	In the Solirubrobacteriales	217448		
Actinobacteria	In the Solirubrobacteriales	946860		
Actinobacteria	In the Solirubrobacteriales	2025460		
Actinobacteria	In the Solirubrobacteriales	New.CleanUp.ReferenceOTU13206		
Actinobacteria	In the Solirubrobacteriales	925200		
Actinobacteria	In the Solirubrobacteriales	111050		
Actinobacteria	In the Solirubrobacteriales	824845		
Actinobacteria	In the Solirubrobacteriales	1110625		
Actinobacteria	In the Solirubrobacteriales	957362		
Actinobacteria	In the Solirubrobacteriales	1044581		
Actinobacteria	In the Solirubrobacteriales	927367		
Actinobacteria	In the Solirubrobacteriales	New.CleanUp.ReferenceOTU4154		
Actinobacteria	In the Solirubrobacteriales	New.CleanUp.ReferenceOTU13003		
Actinobacteria	In the Solirubrobacteriales	864395		
Actinobacteria	In the Solirubrobacteriales	799959		
Actinobacteria	In the Solirubrobacteriales	864304		
Actinobacteria	In the Solirubrobacteriales	New.CleanUp.ReferenceOTU17467		
Actinobacteria	In the Solirubrobacteriales	New.CleanUp.ReferenceOTU9506		
Actinobacteria	In the Solirubrobacteriales	203418		
Actinobacteria	In the Solirubrobacteriales	837092		
Actinobacteria	In the Rubrobacterales	653788	*Rubrobacter* species tolerate high levels of ionizing radiation/moderately thermophilic or thermophilic/Halotolerant/ Found in hot springs, arid soils	(Albuquerque and da Costa 2014b)
Actinobacteria	In the Rubrobacterales	814924		
Actinobacteria	In the Rubrobacteriales	1032653		
Actinobacteria	In the Rubrobacterales	4466061		
Actinobacteria	In the Rubrobacterales	New.CleanUp.ReferenceOTU4244		
Actinobacteria	In the Rubrobacterales	1110235		
Actinobacteria	In the Rubrobacterales	546371		
Actinobacteria	In the Rubrobacterales	New.CleanUp.ReferenceOTU11707		
Actinobacteria	In the Rubrobacterales	562741		
Actinobacteria	In the Rubrobacterales	151012		

Actinobacteria	In the Rubrobacterales	257807		
Actinobacteria	In the Rubrobacterales (*Rubrobacter*)	New.CleanUp.ReferenceOTU6243		
Actinobacteria	In the Rubrobacterales (*Rubrobacter*)	New.CleanUp.ReferenceOTU19685		
Actinobacteria	In the Rubrobacterales (*Rubrobacter*)	New.CleanUp.ReferenceOTU10069		
Actinobacteria	In the Rubrobacterales (*Rubrobacter*)	238700		
Actinobacteria	In the Rubrobacterales (*Rubrobacter*)	1115272		
Actinobacteria	In the Rubrobacterales (*Rubrobacter*)	256163		
Actinobacteria	In the Rubrobacterales (*Rubrobacter*)	New.CleanUp.ReferenceOTU1552		
Actinobacteria	In the Rubrobacterales (*Rubrobacter*)	511366		
Actinobacteria	In the Rubrobacterales (*Rubrobacter*)	New.CleanUp.ReferenceOTU1105		
Actinobacteria	In the Rubrobacterales (*Rubrobacter*)	1107601		
Actinobacteria	In the Rubrobacterales (*Rubrobacter*)	673883		
Actinobacteria	In the Rubrobacterales (*Rubrobacter*)	166076		
Actinobacteria	In the Rubrobacterales (*Rubrobacter*)	825937		
Actinobacteria	In the Rubrobacterales (*Rubrobacter*)	1117022		
Actinobacteria	In the Rubrobacterales (*Rubrobacter*)	833324		
Actinobacteria	In the Rubrobacterales (*Rubrobacter*)	511572		
Actinobacteria	In the Rubrobacterales (*Rubrobacter*)	255018		
Actinobacteria	In the Rubrobacterales (*Rubrobacter*)	587534		
Actinobacteria	Sister clade to *Gaiella occulta*	3334373	Strictly aerobes/Isolated from a very poor in mineral ions environment/Identified in soil, water distributions systems and shallow lakes	(Albuquerque and da Costa 2014c)
Actinobacteria	Sister clade to *Gaiella occulta*	3334374		
Actinobacteria	Unassigned	New.CleanUp.ReferenceOTU15155		
Actinobacteria	Unassigned	New.CleanUp.ReferenceOTU20273		
Actinobacteria	Unassigned	New.CleanUp.ReferenceOTU23979		
Actinobacteria	Unassigned	939546		

Armatimonadetes	*Fimbriimonas ginsengisoli*	New.CleanUp.ReferenceOTU21444	Aerobic oligotrophs/Found in soils	(Im et al. 2012, Lee et al. 2014)
Armatimonadetes	*Fimbriimonas ginsengisoli*	New.CleanUp.ReferenceOTU21405		
Armatimonadetes	Sister clade to *Chthonomonas calidirosea*	New.CleanUp.ReferenceOTU11267	Aerobic oligotrophs/Found in soils	[22]
Armatimonadetes	Sister clade to *Chthonomonas calidirosea*	New.CleanUp.ReferenceOTU5198		
Armatimonadetes	In the Armatimonadaceae clade (*Armatimonas rosea*)	New.CleanUp.ReferenceOTU10796	Aerobic oligotrophs/ Found in soils in close association with plant roots	[22]
Armatimonadetes	In the Armatimonadaceae clade (*Armatimonas rosea*)	1112858		
Armatimonadetes	In the Armatimonadaceae clade (*Armatimonas rosea*)	1113667		
Armatimonadetes	In the Armatimonadaceae clade (*Armatimonas rosea*)	80475		
Armatimonadetes	In the Armatimonadaceae clade (*Armatimonas rosea*)	New.CleanUp.ReferenceOTU21874		
Armatimonadetes	In the Armatimonadaceae clade (*Armatimonas rosea*)	New.CleanUp.ReferenceOTU8905		
Armatimonadetes-Candidate class division FPB		New.CleanUp.ReferenceOTU13058		
Armatimonadetes-Candidate class division FPB		New.CleanUp.ReferenceOTU14215		
Armatimonadetes-Candidate class division FPB		New.CleanUp.ReferenceOTU17500		
Armatimonadetes-Candidate class division FPB		New.CleanUp.ReferenceOTU23745		
Armatimonadetes-Candidate class division FPB		New.CleanUp.ReferenceOTU6425		
Armatimonadetes-Candidate class division FPB		New.CleanUp.ReferenceOTU5536		
Armatimonadetes-Candidate class division FPB		143458		
Armatimonadetes-Candidate class division FPB		New.CleanUp.ReferenceOTU11136		
Armatimonadetes-Candidate class division FPB		1061059		

Armatimonadetes-Candidate class division FPB		New.CleanUp.ReferenceOTU4208		
Armatimonadetes-Candidate class division FPB		3091900		
Armatimonadetes-Candidate class division FPB		New.CleanUp.ReferenceOTU8394		
Armatimonadetes-Candidate class division FPB		New.CleanUp.ReferenceOTU8264		
Armatimonadetes-Candidate class division FPB		New.CleanUp.ReferenceOTU5944		
Armatimonadetes-Candidate class division FPB		866043		
Armatimonadetes-Candidate class division FPB		New.CleanUp.ReferenceOTU3853		
Armatimonadetes-Candidate class division FPB		934094		
Armatimonadetes-Candidate class division FPB		New.CleanUp.ReferenceOTU12573		
Armatimonadetes-Candidate class division FPB		New.CleanUp.ReferenceOTU14484		
Armatimonadetes-Candidate class division FPB		1067515		
Armatimonadetes-Candidate class division FPB		New.CleanUp.ReferenceOTU13707		
Armatimonadetes-Candidate class division FPB		New.CleanUp.ReferenceOTU17107		
Armatimonadetes-Candidate class division FPB		4359064		
Armatimonadetes-Candidate class division FPB		979102		
Armatimonadetes-Candidate class division FPB		3489297		
Armatimonadetes-Candidate class division FPB		New.CleanUp.ReferenceOTU23794		
Armatimonadetes-Candidate class division FPB		New.CleanUp.ReferenceOTU6076		
Armatimonadetes-Candidate class division FPB		1098195		
Armatimonadetes-Candidate class division FPB		940662		

Armatimonadetes-Candidate class division FPB		New.CleanUp.ReferenceOTU22922		
Armatimonadetes-Candidate class division FPB		4483288		
Armatimonadetes-Candidate class division FPB		512884		
Armatimonadetes-Candidate class division FPB		65686		
Armatimonadetes-Candidate class division FPB		New.CleanUp.ReferenceOTU20799		
Armatimonadetes-Candidate class division FPB		New.CleanUp.ReferenceOTU4261		
Armatimonadetes-Candidate class division FPB		1061713		
Bacteroidetes	*Flavia esturariibacter*	894024	Non-motile/Form multicellular filaments/Isolated from an estuary	(Kang et al. 2015)
Bacteroidetes	Sister clade to *Flavia esturariibacter*	New.CleanUp.ReferenceOTU19104		
Bacteroidetes	In the Chitinophagaceae (*Cnuella, Chitinophaga, Flavihumibacter*)	New.CleanUp.ReferenceOTU3022	Aerobes or facultative aerobes/Hydrolysis of cellulose is known in some species	(Rosenberg 2014b)
Bacteroidetes	In the Chitinophagaceae (*Cnuella, Chitinophaga, Flavihumibacter*)	725882		
Bacteroidetes	In the Chitinophagaceae (*Cnuella, Chitinophaga, Flavihumibacter*)	1002658		
Bacteroidetes	In the Chitinophagaceae (*Cnuella, Chitinophaga, Flavihumibacter*)	New.CleanUp.ReferenceOTU4576		
Bacteroidetes	In the Chitinophagaceae (*Flavitalea*)	4335431		
Bacteroidetes	In the Chitinophagaceae (*Flavitalea*)	255448		
Bacteroidetes	*Flavisolibacter*	New.CleanUp.ReferenceOTU236		
Bacteroidetes	*Flavisolibacter*	New.CleanUp.ReferenceOTU1178		
Bacteroidetes	In the Chitinophagaceae (*Flavisolibacter*)	4417921		
Bacteroidetes	In the Chitinophagaceae (*Flavisolibacter*)	243118		
Bacteroidetes	In the Chitinophagaceae (*Flavisolibacter*)	New.CleanUp.ReferenceOTU24933		
Bacteroidetes	In the Chitinophagaceae (*Flavisolibacter*)	311656		
Bacteroidetes	In the Chitinophagaceae (*Flavisolibacter*)	141786		

Bacteroidetes	In the Chitinophagaceae (*Flavisolibacter*)	New.CleanUp.ReferenceOTU1999		
Bacteroidetes	In the Chitinophagaceae (*Flavisolibacter*)	New.CleanUp.ReferenceOTU10349		
Bacteroidetes	In the Chitinophagaceae (*Flavisolibacter*)	New.CleanUp.ReferenceOTU16973		
Bacteroidetes	In the Chitinophagaceae (*Flavisolibacter*)	2525736		
Bacteroidetes	In the Chitinophagaceae (*Flavisolibacter*)	4418133		
Bacteroidetes	In the Chitinophagaceae (*Flavisolibacter*)	532743		
Bacteroidetes	In the Chitinophagaceae (*Flavisolibacter*)	594040		
Bacteroidetes	In the Chitinophagaceae (*Flavisolibacter*)	1071316		
Bacteroidetes	In the Chitinophagaceae (*Flavisolibacter*)	New.CleanUp.ReferenceOTU15377		
Bacteroidetes	In the Chitinophagaceae (*Flavisolibacter*)	945733		
Bacteroidetes	In the Chitinophagaceae (*Flavisolibacter*)	545436		
Bacteroidetes	In the Chitinophagaceae (*Flavisolibacter*)	4411669		
Bacteroidetes	In the Chitinophagaceae (*Flavisolibacter*)	1037111		
Bacteroidetes	In the Chitinophagaceae (*Flavisolibacter*)	997544		
Bacteroidetes	In the Chitinophagaceae (*Flavisolibacter*)	New.CleanUp.ReferenceOTU21126		
Bacteroidetes	In the Chitinophagaceae (*Flavisolibacter*)	1084705		
Bacteroidetes	In the Chitinophagaceae (*Flavisolibacter*)	813272		
Bacteroidetes	In the Chitinophagaceae (*Segetibacter*)	New.CleanUp.ReferenceOTU1852		
Bacteroidetes	In the Chitinophagaceae (*Segetibacter*)	New.CleanUp.ReferenceOTU6786		
Bacteroidetes	In the Chitinophagaceae (*Segetibacter*)	570693		
Bacteroidetes	In the Chitinophagaceae (*Segetibacter*)	New.CleanUp.ReferenceOTU10051		
Bacteroidetes	In the Chitinophagaceae (*Segetibacter*)	New.CleanUp.ReferenceOTU12383		
Bacteroidetes	In the Chitinophagaceae (*Segetibacter*)	324629		
Bacteroidetes	In the Chitinophagaceae (*Segetibacter*)	New.CleanUp.ReferenceOTU10088		
Bacteroidetes	In the Chitinophagaceae (*Segetibacter*)	702181		

Bacteroidetes	In the Chitinophagaceae	New.CleanUp.ReferenceOTU19093		
Bacteroidetes	In the Chitinophagaceae	New.ReferenceOTU34		
Bacteroidetes	In the Chitinophagaceae	1038987		
Bacteroidetes	In the Chitinophagaceae	4323607		
Bacteroidetes	In the Chitinophagaceae	New.CleanUp.ReferenceOTU24747		
Bacteroidetes	In the Chitinophagaceae	4301516		
Bacteroidetes	In the Chitinophagaceae	620656		
Bacteroidetes	In the Chitinophagaceae	New.CleanUp.ReferenceOTU11923		
Bacteroidetes	In the Chitinophagaceae	4297733		
Bacteroidetes	In the Chitinophagaceae	New.CleanUp.ReferenceOTU8880		
Bacteroidetes	In the Chitinophagaceae	1102554		
Bacteroidetes	In the Chitinophagaceae	1104847		
Bacteroidetes	In the Chitinophagaceae	4333673		
Bacteroidetes	In the Chitinophagaceae	513398		
Bacteroidetes	In the Chitinophagaceae	803240		
Bacteroidetes	In the Chitinophagaceae	1091321		
Bacteroidetes	In the Chitinophagaceae	1012195		
Bacteroidetes	In the Chitinophagaceae	1110139		
Bacteroidetes	In the Chitinophagaceae	1020262		
Bacteroidetes	In the Chitinophagaceae	4298761		
Bacteroidetes	In the Chitinophagaceae	1141864		
Bacteroidetes	In the Chitinophagaceae	New.CleanUp.ReferenceOTU4239		
Bacteroidetes	In the Chitinophagaceae	4436960		
Bacteroidetes	In the Chitinophagaceae	32581		
Bacteroidetes	In the Chitinophagaceae	New.CleanUp.ReferenceOTU22650		
Bacteroidetes	In the Chitinophagaceae	4044060		
Bacteroidetes	In the Chitinophagaceae	New.CleanUp.ReferenceOTU9971		
Bacteroidetes	In the Chitinophagaceae	912669		
Bacteroidetes	In the Chitinophagaceae	824675		
Bacteroidetes	In the Chitinophagaceae	933150		
Bacteroidetes	In the Chitinophagaceae	220305		
Bacteroidetes	In the Chitinophagaceae	New.CleanUp.ReferenceOTU16006		
Bacteroidetes	In the Chitinophagaceae	New.CleanUp.ReferenceOTU23695		

Bacteroidetes	In the Chitinophagaceae	New.CleanUp.ReferenceOTU331		
Bacteroidetes	In the Chitinophagaceae	New.CleanUp.ReferenceOTU14542		
Bacteroidetes	In the Chitinophagaceae	958571		
Bacteroidetes	In the Chitinophagaceae	New.CleanUp.ReferenceOTU5485		
Bacteroidetes	In the Chitinophagaceae	1118654		
Bacteroidetes	In the Chitinophagaceae	New.CleanUp.ReferenceOTU15598		
Bacteroidetes	In the Chitinophagaceae	1052435		
Bacteroidetes	In the Chitinophagaceae	New.ReferenceOTU24		
Bacteroidetes	In the Chitinophagaceae	New.CleanUp.ReferenceOTU1681		
Bacteroidetes	In the Chitinophagaceae	3549384		
Bacteroidetes	In the Chitinophagaceae	4323887		
Bacteroidetes	In the Chitinophagaceae	New.CleanUp.ReferenceOTU14507		
Bacteroidetes	In the Chitinophagaceae	New.ReferenceOTU91		
Bacteroidetes	In the Chitinophagaceae	4301518		
Bacteroidetes	*Rufibacter*	267557		
Bacteroidetes	*Rufibacter*	New.CleanUp.ReferenceOTU1645		
Bacteroidetes	*Rufibacter*	1068698		
Bacteroidetes	*Rufibacter*	4397932		
Bacteroidetes	*Flexibacter flexilis*	4370712		
Bacteroidetes	sister clade to Ohtaekwangia kribbensis	1135504		
Bacteroidetes	sister clade to Ohtaekwangia kribbensis	4318357		
Bacteroidetes	sister clade to Ohtaekwangia kribbensis	3511168		
Bacteroidetes	sister clade to Ohtaekwangia kribbensis	New.CleanUp.ReferenceOTU9084		
Bacteroidetes	*In the Cytophagaceae (Pontibacter)*	154032		
Bacteroidetes	*In the Cytophagaceae (Pontibacter)*	4364575		
Bacteroidetes	*In the Cytophagaceae (Adhaeribacter)*	356181		
Bacteroidetes	*In the Cytophagaceae (Adhaeribacter)*	4424717		
Bacteroidetes	*In the Cytophagaceae (Adhaeribacter)*	816789		
Bacteroidetes	*In the Cytophagaceae (Adhaeribacter)*	3230031		

Bacteroidetes	*In the Cytophagaceae (Adhaeribacter)*	1113105		
Bacteroidetes	*In the Cytophagaceae (Adhaeribacter)*	764312		
Bacteroidetes	*In the Cytophagaceae (Adhaeribacter)*	New.CleanUp.ReferenceOTU2522		
Bacteroidetes	*In the Cytophagaceae (Adhaeribacter)*	New.CleanUp.ReferenceOTU7834		
Bacteroidetes	*In the Cytophagaceae (Adhaeribacter)*	New.CleanUp.ReferenceOTU5735		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	New.CleanUp.ReferenceOTU6368		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	New.CleanUp.ReferenceOTU15399		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	811954		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	New.ReferenceOTU68		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	3040675		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	New.CleanUp.ReferenceOTU15483		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	811673		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	770226		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	New.CleanUp.ReferenceOTU13678		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	New.CleanUp.ReferenceOTU16410		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	New.CleanUp.ReferenceOTU22908		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	New.ReferenceOTU3		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	New.CleanUp.ReferenceOTU13091		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	New.CleanUp.ReferenceOTU23363		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	1124709		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	New.CleanUp.ReferenceOTU3757		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	New.CleanUp.ReferenceOTU7142		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	New.CleanUp.ReferenceOTU6972		

Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	New.CleanUp.ReferenceOTU24893		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	277776		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	4326799		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	New.CleanUp.ReferenceOTU14510		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	4379834		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	1106318		
Bacteroidetes	In the Cytophagaceae (*Rhodocytophaga*)	New.ReferenceOTU50		
Bacteroidetes	In the Cytophagaceae (*Cytophaga*)	4457944		
Bacteroidetes	In the Cytophagaceae	1131830		
Bacteroidetes	In the Cytophagaceae	1148341		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU5360		
Bacteroidetes	In the Cytophagaceae	New.ReferenceOTU65		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU8571		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU23386		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU14110		
Bacteroidetes	In the Cytophagaceae	821788		
Bacteroidetes	In the Cytophagaceae	317511		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU23235		
Bacteroidetes	In the Cytophagaceae	138309		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU7889		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU6899		
Bacteroidetes	In the Cytophagaceae	New.ReferenceOTU54		
Bacteroidetes	In the Cytophagaceae	947849		
Bacteroidetes	In the Cytophagaceae	697457		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU23781		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU10318		

Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU19865		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU581		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU16741		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU14621		
Bacteroidetes	In the Cytophagaceae	New.ReferenceOTU51		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU337		
Bacteroidetes	In the Cytophagaceae	New.ReferenceOTU55		
Bacteroidetes	In the Cytophagaceae	367415		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU4268		
Bacteroidetes	In the Cytophagaceae	357873		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU5617		
Bacteroidetes	In the Cytophagaceae	725240		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU1773		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU21344		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU7241		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU8828		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU16481		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU3410		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU8918		
Bacteroidetes	In the Cytophagaceae	1111968		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU21370		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU3951		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU5449		

Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU6082		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU10775		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU23785		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU1376		
Bacteroidetes	In the Cytophagaceae	175203		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU12840		
Bacteroidetes	In the Cytophagaceae	New.CleanUp.ReferenceOTU5299		
Bacteroidetes	In the Cytophagaceae	1103871		
Bacteroidetes	In the Cytophagaceae	1138934		
Bacteroidetes	In the Cytophagaceae	4256699		
Bacteroidetes	In the Cytophagaceae	4480958		
Bacteroidetes	In the Cytophagaceae	985339		
Bacteroidetes	In the Cytophagaceae	249391		
Bacteroidetes	*Adhaeribacter*	1069076	Heterotrophs, aerobes or facultative aerobes non-spore forming rods/Cytophaga-like bacteria are known to lyse cyanobacteria/Found in marine actinians, seawater, desert soils, dust particles, and forest soil	(Rashidan and Bird 2001, Mcbride et al. 2014)
Bacteroidetes	*Hymenobacter*	824700	Non-motile, Gram-negative/Isolated from sandstone, soil, irradiated pork, uranium mine wastes, freshwater, and air	[26]
Bacteroidetes	*Hymenobacter*	New.CleanUp.ReferenceOTU20920		
Bacteroidetes	*Hymenobacter*	New.CleanUp.ReferenceOTU14811		
Bacteroidetes	*Hymenobacter*	687649		
Bacteroidetes	*Hymenobacter*	541746		
Bacteroidetes	*Hymenobacter*	1090273		
Bacteroidetes	*Hymenobacter*	150955		
Bacteroidetes	*Hymenobacter*	789806		
Bacteroidetes	*Hymenobacter*	4404498		
Bacteroidetes	*Hymenobacter*	2621271		
Bacteroidetes	*Hymenobacter*	1981833		
Bacteroidetes	*Hymenobacter*	1090978		
Bacteroidetes	*Hymenobacter*	927623		
Bacteroidetes	*Hymenobacter*	New.ReferenceOTU105		
Bacteroidetes	*Hymenobacter*	3406670		

Bacteroidetes	In the Flammeovirgaceae	New.CleanUp.ReferenceOTU20855	Gram-negative/Found in soils and marine environments	(Yoon et al. 2011)
Bacteroidetes	In the Flammeovirgaceae	1105341		
Bacteroidetes	*Flavobacterium*	New.CleanUp.ReferenceOTU24487	Chemoorganotrophs/Found in freshwater and in soil	(McBride 2014)
Bacteroidetes	*Flavobacterium*	1055322		
Bacteroidetes	In the Sphingobacteriales	New.CleanUp.ReferenceOTU11436	Gram-negative, non-spore forming bacilli/Isolated from soils and compost	(Lambiase 2014)
Bacteroidetes	In the Sphingobacteriales	New.CleanUp.ReferenceOTU22392		
Bacteroidetes	In the Sphingobacteriaceae	New.CleanUp.ReferenceOTU14970		
Bacteroidetes	In the Sphingobacteriaceae	New.CleanUp.ReferenceOTU14605		
Bacteroidetes	In the Sphingobacteriaceae	New.CleanUp.ReferenceOTU24872		
Bacteroidetes	In the Sphingobacteriaceae	New.CleanUp.ReferenceOTU2937		
Bacteroidetes	In the Sphingobacteriaceae	586829		
Bacteroidetes	*Pedobacter*	New.CleanUp.ReferenceOTU2182		
Bacteroidetes	*Pedobacter*	New.CleanUp.ReferenceOTU23158		
Bacteroidetes	*Pedobacter*	1088120		
Bacteroidetes	*Pedobacter*	1098634		
Bacteroidetes	*Pedobacter*	575305		
Bacteroidetes	*Pedobacter*	718367		
Bacteroidetes	*Pedobacter*	810109		
Bacteroidetes	*Pedobacter*	635391		
Bacteroidetes	*Pedobacter*	New.CleanUp.ReferenceOTU24930		
Bacteroidetes	*Pedobacter*	987696		
Bacteroidetes	Unassigned	New.CleanUp.ReferenceOTU8662		
Bacteroidetes	Unassigned	235423		
Bacteroidetes	Unassigned	4366956		
Bacteroidetes	Unassigned	New.CleanUp.ReferenceOTU7193		
Bacteroidetes	Unassigned	4342317		
Bacteroidetes	Unassigned	New.CleanUp.ReferenceOTU21070		

Bacteroidetes	Unassigned	New.CleanUp.ReferenceOTU21661		
Bacteroidetes	Unassigned	4471717		
Bacteroidetes	Unassigned	New.CleanUp.ReferenceOTU13943		
Bacteroidetes	Unassigned	New.CleanUp.ReferenceOTU9825		
BCR	Unassigned – closest culture *Aciditerrimonas ferrireducens*	New.CleanUp.ReferenceOTU6554		
BCR	Unassigned – closest culture *Aciditerrimonas ferrireducens*	New.CleanUp.ReferenceOTU12288		
Chlorobi	Sister clade with Ignavibacteriales	New.CleanUp.ReferenceOTU16337	Facultative anaerobes/obligated heterotrophic bacteria/Found in terrestrial habitats	(Iino 2014)
Chlorobi	Sister clade with Ignavibacteriales	New.CleanUp.ReferenceOTU11621		
Chlorobi	Sister clade with Ignavibacteriales	107418		
Chloroflexi	Sister clade to Chloroflexaceae (*Chloroflexus auranticus/aggregans*)	554361	Anoxygenic phototrophic bacteria	(Hanada 2014)
Chloroflexi	In the Thermomicrobia	1110592	Thermophilic green non-sulfur bacteria	
Chloroflexi	In the Thermomicrobia	112867		
Chloroflexi	In the Thermomicrobia	217746		
Chloroflexi	In the Thermomicrobia	New.CleanUp.ReferenceOTU3941		
Chloroflexi	In the Thermomicrobia	New.CleanUp.ReferenceOTU21477		
Chloroflexi	Sister clade to Thermomicrobia	New.ReferenceOTU100		
Chloroflexi	*Herpetosiphon*	New.CleanUp.ReferenceOTU10489	Non-phototrophic bacteria/Isolated from slimy coated springs	
Chloroflexi	In the Kallotenuaceae (*Kallotenue*)	New.CleanUp.ReferenceOTU21147	Non-phototrophic bacteria/multicellular filaments	
Chloroflexi	In the Kallotenuaceae (*Kallotenue*)	New.CleanUp.ReferenceOTU17768		
Chloroflexi	In the Caldilineaceae (*Caldilinea, Litorilinea*)	New.CleanUp.ReferenceOTU3507		
Chloroflexi	In the Caldilineaceae (*Caldilinea, Litorilinea*)	3897233		
Chloroflexi	In the Caldilineaceae (*Caldilinea, Litorilinea*)	New.CleanUp.ReferenceOTU9864		
Chloroflexi	Unassigned Chloroflexi (*Kouleothrix*)	New.ReferenceOTU58		

Chloroflexi	Unassigned Chloroflexi (*Kouleothrix*)	New.CleanUp.ReferenceOTU21814		
Chloroflexi	Unassigned	New.CleanUp.ReferenceOTU4022		
Chloroflexi	Unassigned	4328659		
Chloroflexi	Unassigned	New.CleanUp.ReferenceOTU18331		
Chloroflexi	Unassigned	New.CleanUp.ReferenceOTU7328		
Chloroflexi	Unassigned	New.CleanUp.ReferenceOTU2212		
Chloroflexi	Unassigned	New.CleanUp.ReferenceOTU23974		
Chloroflexi	Unassigned	New.CleanUp.ReferenceOTU24897		
Chloroflexi	Unassigned	New.CleanUp.ReferenceOTU14864		
Chloroflexi	Unassigned	New.CleanUp.ReferenceOTU17904		
Chloroflexi	Unassigned	New.CleanUp.ReferenceOTU20667		
Chloroflexi	Unassigned	New.CleanUp.ReferenceOTU6887		
Chloroflexi	Unassigned	831877		
Chloroflexi	Unassigned	185950		
Chloroflexi	Unassigned	New.CleanUp.ReferenceOTU2534		
Chloroflexi	Unassigned	New.CleanUp.ReferenceOTU2928		
Chloroflexi	Unassigned	549954		
Chloroflexi	Unassigned	247875		
Chloroflexi	Unassigned	4482713		
Chloroflexi	Unassigned	New.CleanUp.ReferenceOTU2593		
Chloroflexi	Unassigned	New.CleanUp.ReferenceOTU11129		
Chloroflexi	Unassigned	52036		
Chloroflexi	Unassigned	New.CleanUp.ReferenceOTU15163		
Chloroflexi	Unassigned	1143895		

Chloroflexi	Unassigned	New.ReferenceOTU86		
Chloroflexi	Unassigned	New.CleanUp.ReferenceOTU16261		
Chloroflexi	Unassigned	New.CleanUp.ReferenceOTU18762		
Archaea	*Nitrososphaera*	720511	Autotrophic o mixotrophic, ammonia oxidizing organisms/Found in soils, marine environments, and hot springs	(Stieglmeier et al. 2014)
Archaea	*Nitrososphaera*	748601		
Archaea	*Nitrososphaera*	107234		
Cyanobacteria	*Oscillatoria*	New.CleanUp.ReferenceOTU21607	Photoautotrophs/ubiquitous	(Raven 2012)
Cyanobacteria	*Gleiterinema*	New.CleanUp.ReferenceOTU16228		
Cyanobacteria	*Lyngbya*	819703		
Cyanobacteria	*Lyngbya*	223377		
Cyanobacteria	*Lyngbya*	278544		
Cyanobacteria	*Lyngbya*	New.CleanUp.ReferenceOTU15447		
Cyanobacteria	*Lyngbya*	New.CleanUp.ReferenceOTU2491		
Cyanobacteria	*Lyngbya*	4342315		
Cyanobacteria	*Leptolyngybia*	361839		
Cyanobacteria	*Leptolyngybia*	575555		
Cyanobacteria	*Leptolyngybia*	New.CleanUp.ReferenceOTU12471		
Cyanobacteria	*Leptolyngybia*	New.CleanUp.ReferenceOTU14157		
Cyanobacteria	*Leptolyngybia*	1552835		
Cyanobacteria	*Leptolyngybia*	818188		
Cyanobacteria	*Leptolyngybia*	New.CleanUp.ReferenceOTU23046		
Cyanobacteria	*Leptolyngybia*	New.CleanUp.ReferenceOTU11872		
Cyanobacteria	*Leptolyngybia*	New.CleanUp.ReferenceOTU16449		
Cyanobacteria	*Leptolyngybia*	New.CleanUp.ReferenceOTU15934		
Cyanobacteria	*Leptolyngybia*	New.CleanUp.ReferenceOTU23168		
Cyanobacteria	*Leptolyngybia*	New.CleanUp.ReferenceOTU18462		

Cyanobacteria	*Leptolyngybia*	New.CleanUp.ReferenceOTU16266		
Cyanobacteria	*Leptolyngybia*	153279		
Cyanobacteria	*Leptolyngybia*	4432360		
Cyanobacteria	*Leptolyngybia*	273195		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU6677		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU18702		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU15091		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU18757		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU6947		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU9250		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU4651		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU23807		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU20390		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU20883		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU4505		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU3491		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU3019		
Cyanobacteria	*M. steenstrupii*	278371		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU1908		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU18710		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU10091		
Cyanobacteria	*M. steenstrupii*	820606		

Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU24007		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU23087		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU22975		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU20444		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU18539		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU17896		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU22902		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU9950		
Cyanobacteria	*M. steenstrupii*	649198		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU14148		
Cyanobacteria	*M. steenstrupii*	181039		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU13162		
Cyanobacteria	*M. steenstrupii*	New.ReferenceOTU70		
Cyanobacteria	*M. steenstrupii*	New.ReferenceOTU81		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU22202		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU6847		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU16628		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU999		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU4627		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU22529		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU21079		

Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU21091		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU20219		
Cyanobacteria	*M. steenstrupii*	New.CleanUp.ReferenceOTU1991		
Cyanobacteria	*M. chthonoplastes*	New.CleanUp.ReferenceOTU3218		
Cyanobacteria	In the Oscillatoriales (*Lyngbya/M. chthonoplastes)*	New.CleanUp.ReferenceOTU2541		
Cyanobacteria	In the Oscillatoriales (*Lyngbya/Oscillatoria*)	New.ReferenceOTU64		
Cyanobacteria	In the Oscillatoriales	4322506		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU17032		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU7367		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU15551		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU19772		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU14156		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU12647		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU10687		
Cyanobacteria	In the Oscillatoriales	769222		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU8630		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU17523		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU15920		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU24665		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU13676		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU3319		

Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU3322		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU3813		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU4436		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU3834		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU9960		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU20819		
Cyanobacteria	In the Oscillatoriales	New.ReferenceOTU76		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU17603		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU20406		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU2934		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU11834		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU12774		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU14426		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU1759		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU20821		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU19525		
Cyanobacteria	In the Oscillatoriales	3544		
Cyanobacteria	In the Oscillatoriales	New.ReferenceOTU17		
Cyanobacteria	In the Oscillatoriales	New.CleanUp.ReferenceOTU16459		
Cyanobacteria	In the Oscillatoriales	4466028		
Cyanobacteria	*Aphanocapsa*	702683		
Cyanobacteria	*Aphanocapsa*	New.CleanUp.ReferenceOTU23642		

Cyanobacteria	*Chlorogloea*	New.CleanUp.ReferenceOTU5391		
Cyanobacteria	*Chlorogloea*	203466		
Cyanobacteria	*Chlorogloea*	New.CleanUp.ReferenceOTU19232		
Cyanobacteria	*Chroococcidiopsis*	4466932		
Cyanobacteria	*Chroococcidiopsis*	649507		
Cyanobacteria	*Chroococcidiopsis*	New.CleanUp.ReferenceOTU19838		
Cyanobacteria	*Chroococcidiopsis*	New.ReferenceOTU75		
Cyanobacteria	*Chroococcidiopsis*	New.CleanUp.ReferenceOTU6003		
Cyanobacteria	*Chroococcidiopsis*	New.CleanUp.ReferenceOTU19966		
Cyanobacteria	*Chroococcidiopsis*	505954		
Cyanobacteria	*Chroococcidiopsis*	396285		
Cyanobacteria	*Chroococcidiopsis*	71326		
Cyanobacteria	*Chroococcidiopsis*	224486		
Cyanobacteria	*Chroococcidiopsis*	New.CleanUp.ReferenceOTU24557		
Cyanobacteria	*Chroococcidiopsis*	New.CleanUp.ReferenceOTU24021		
Cyanobacteria	*Chroococcidiopsis*	New.ReferenceOTU74		
Cyanobacteria	*Chroococcidiopsis*	810188		
Cyanobacteria	*Chroococcidiopsis*	818439		
Cyanobacteria	*Chroococcidiopsis*	813107		
Cyanobacteria	*Chroococcidiopsis*	New.CleanUp.ReferenceOTU13892		
Cyanobacteria	In the Chroococcales	New.CleanUp.ReferenceOTU10402		
Cyanobacteria	In the Chroococcales	New.CleanUp.ReferenceOTU20772		
Cyanobacteria	In the Chroococcales	New.ReferenceOTU18		
Cyanobacteria	In the Chroococcales	808252		
Cyanobacteria	In the Chroococcales	New.CleanUp.ReferenceOTU6679		
Cyanobacteria	In the Chroococcales	New.CleanUp.ReferenceOTU14429		
Cyanobacteria	*Loriellopsis*	129048	Photoautotrophs/Nitrogen fixers/ubiquitous	
Cyanobacteria	*Loriellopsis*	35330		

Cyanobacteria	*Loriellopsis*	2307137		(Raven 2012, Komárek et al. 2014)
Cyanobacteria	*Nostoc*	221674		
Cyanobacteria	*Nostoc*	312035		
Cyanobacteria	*Nostoc*	99364		
Cyanobacteria	*Tolypothrix*	198952		
Cyanobacteria	*Tolypothrix*	New.CleanUp.ReferenceOTU16262		
Cyanobacteria	*Tolypothrix*	178178		
Cyanobacteria	*Tolypothrix*	New.CleanUp.ReferenceOTU10337		
Cyanobacteria	*Tolypothrix*	New.CleanUp.ReferenceOTU12634		
Cyanobacteria	*Tolypothrix*	106317		
Cyanobacteria	*Tolypothrix*	221130		
Cyanobacteria	*Tolypothrix*	New.CleanUp.ReferenceOTU18974		
Cyanobacteria	*Tolypothrix*	New.CleanUp.ReferenceOTU20972		
Cyanobacteria	*Tolypothrix*	813352		
Cyanobacteria	*Scytonema*	716611		
Cyanobacteria	*Scytonema*	New.CleanUp.ReferenceOTU5012		
Cyanobacteria	*Scytonema*	277671		
Cyanobacteria	*Fischerella*	New.CleanUp.ReferenceOTU20746		
Cyanobacteria	*Fischerella*	New.ReferenceOTU85		
Cyanobacteria	In the Nostocales	New.CleanUp.ReferenceOTU17179		
Cyanobacteria	In the Nostocales	New.CleanUp.ReferenceOTU19428		
Cyanobacteria	In the Nostocales	New.CleanUp.ReferenceOTU11205		
Cyanobacteria	In the Nostocales	New.CleanUp.ReferenceOTU9276		
Cyanobacteria	Unassigned	New.CleanUp.ReferenceOTU9410	Photoautotrophs/ubiquitous	[33]
Cyanobacteria	Unassigned	New.CleanUp.ReferenceOTU9898		
Cyanobacteria	Unassigned	New.CleanUp.ReferenceOTU22839		

Cyanobacteria	Unassigned	New.CleanUp.ReferenceOTU20384		
Cyanobacteria	Unassigned	New.CleanUp.ReferenceOTU12747		
Cyanobacteria	Unassigned	New.CleanUp.ReferenceOTU21785		
Cyanobacteria	Unassigned	New.CleanUp.ReferenceOTU83		
Cyanobacteria	Unassigned	New.CleanUp.ReferenceOTU13613		
Cyanobacteria	Unassigned	327421		
Cyanobacteria	Unassigned	New.CleanUp.ReferenceOTU4762		
Cyanobacteria	Unassigned	New.CleanUp.ReferenceOTU10299		
Cyanobacteria	Unassigned	New.CleanUp.ReferenceOTU16982		
Cyanobacteria	Unassigned	808657		
Cyanobacteria	Unassigned	New.CleanUp.ReferenceOTU24793		
Cyanobacteria	Unassigned	New.CleanUp.ReferenceOTU15657		
Cyanobacteria	Unassigned	New.CleanUp.ReferenceOTU5708		
Cyanobacteria	Unassigned	New.CleanUp.ReferenceOTU9358		
Firmicutes	*Bacillus*	319982	Endospore forming bacteria/Resistant to desiccation/can survive extreme conditions/Found in terrestrial and aquatic environments	(Ludwing et al. 2009)
Firmicutes	*Bacillus*	New.ReferenceOTU63		
Firmicutes	In the Bacillaceae (*Bacillus*)	1078248		
Firmicutes	In the Bacillaceae (*Bacillus*)	823024		
Firmicutes	In the Bacillaceae (*Bacillus*)	New.CleanUp.ReferenceOTU1624		
Firmicutes	In the Bacillaceae (*Bacillus*)	827089		
Firmicutes	In the Bacillaceae (*Bacillus*)	954381		
Firmicutes	In the Bacillaceae (*Bacillus*)	854050		
Firmicutes	In the Bacilli (*Bacillus, Sporosarcina*)	1051517		
Firmicutes	In the Bacilli (*Bacillus, Sporosarcina*)	833645		
Firmicutes	In the Bacilli (*Bacillus, Sporosarcina*)	833317		
Firmicutes	In the Bacilli (*Planococcus, Planomicrobium*)	540737		

Firmicutes	*Brevibacillus*	640652		
Firmicutes	*Brevibacillus*	New.CleanUp.ReferenceOTU4908		
Firmicutes	*Brevibacillus*	New.CleanUp.ReferenceOTU17284		
Firmicutes	*Brevibacillus*	95847		
Firmicutes	*Brevibacillus*	New.CleanUp.ReferenceOTU9143		
Firmicutes	*Brevibacillus*	307934		
Firmicutes	*Paenibacillus*	240501		
Firmicutes	*Paenibacillus*	809744		
Firmicutes	*Paenibacillus*	4339146		
Firmicutes	*Paenibacillus*	4310348		
Firmicutes	*Paenibacillus*	New.CleanUp.ReferenceOTU13214		
Firmicutes	*Paenibacillus*	589407		
Firmicutes	*Paenibacillus*	553697		
Firmicutes	*Paenibacillus*	New.CleanUp.ReferenceOTU23826		
Firmicutes	*Paenibacillus*	New.CleanUp.ReferenceOTU467		
Firmicutes	*Paenibacillus*	583979		
Firmicutes	*Paenibacillus*	592043		
Firmicutes	*Sporacetigenium*	New.CleanUp.ReferenceOTU16354		
Firmicutes	*Sporacetigenium*	4336569		
Firmicutes	*Anaerosolibacter*	170026		
Firmicutes	*Clostridium*	New.CleanUp.ReferenceOTU18018		
Firmicutes	*Clostridium*	New.CleanUp.ReferenceOTU21937		
Firmicutes	*Clostridium*	70947		
Firmicutes	*Clostridium*	580518		
Firmicutes	*Clostridium*	587789		
Firmicutes	*Clostridium*	591223		
Firmicutes	*Clostridium*	4483035		
Firmicutes	In the Clostridiaceae (*Clostridium, Fervidicella*)	4322535		
Firmicutes	In the Clostridiaceae (*Anaerosolibacter, Thermotalea*)	New.CleanUp.ReferenceOTU15058		
Firmicutes	In the Clostridiaceae (*Anaerosolibacter, Thermotalea*)	918577		

Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	New.CleanUp.ReferenceOTU9456	Aerobic chemoorganoheterotrophs/ Slowly growing bacteria able to grow under low nutrient concentrations	(Pascual et al. 2016)
Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	New.CleanUp.ReferenceOTU371		
Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	New.CleanUp.ReferenceOTU16764		
Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	New.CleanUp.ReferenceOTU6912		
Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	New.CleanUp.ReferenceOTU5046		
Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	1111500		
Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	804877		
Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	1108030		
Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	New.CleanUp.ReferenceOTU3097		
Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	254895		
Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	New.CleanUp.ReferenceOTU23585		
Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	New.CleanUp.ReferenceOTU24127		
Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	4335435		
Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	New.CleanUp.ReferenceOTU2153		
Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	New.ReferenceOTU101		
Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	New.CleanUp.ReferenceOTU11011		
Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	3312248		
Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	993930		
Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	New.CleanUp.ReferenceOTU14916		
Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	257737		
Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	1007278		

Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	4461505		
Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	1075351		
Gemmatimonadetes	In the Longimicrobiaceae clade (*Longimicrobium terrae)*	New.CleanUp.ReferenceOTU8742		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	4427616		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	2738701		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	New.CleanUp.ReferenceOTU23863	Found in a variety of arid soils/Due to their biogeography and seasonal quantification in soils, an adaptation to low soil moisture has been suggested	(DeBruyn et al. 2011)
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	1104970		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	806026		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	4393102		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	New.CleanUp.ReferenceOTU6151		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	New.CleanUp.ReferenceOTU11566		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	New.CleanUp.ReferenceOTU9835		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	4112169		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	New.CleanUp.ReferenceOTU14650		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	New.CleanUp.ReferenceOTU3345		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	909173		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	New.CleanUp.ReferenceOTU16974		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	1103604		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	New.CleanUp.ReferenceOTU8226		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	New.CleanUp.ReferenceOTU18198		

Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	379634		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	New.CleanUp.ReferenceOTU19383		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	New.ReferenceOTU102		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	512952		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	New.CleanUp.ReferenceOTU15842		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	959195		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	557467		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	1044938		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	New.CleanUp.ReferenceOTU23369		
Gemmatimonadetes	In the Gemmatimonadaceae clade (*Gemmatimonas phototrophica/G. Aurantica)*	855996		
Gemmatimonadetes	Unclassified	New.CleanUp.ReferenceOTU19441		
Nitrospirae	*Nitrospira*	264343	Chemolithoautotrophic nitrite-oxidizing bacteria/ *Nitrospira*-like bacteria take up inorganic carbon (HCO_3^- and CO_2) as well as pyruvate under aerobic conditions.	(Daims et al. 2001)
Planctomycetes	In the Phycisphaeraceae clade (*Algisphaera*/*Phycisphaera*)	New.CleanUp.ReferenceOTU17353	Strictly aerobes, heterotrophs/Found in freshwater ponds and lakes, marine habitats, soils, wetlands	(Youssef and Elshahed 2014)
Planctomycetes	In the Phycisphaeraceae clade (*Algisphaera*/*Phycisphaera*)	New.CleanUp.ReferenceOTU977		
Planctomycetes	In the Phycisphaeraceae clade (*Algisphaera*/*Phycisphaera*)	2649117		
Planctomycetes	In the Phycisphaeraceae clade (*Algisphaera*/*Phycisphaera*)	New.CleanUp.ReferenceOTU3936		
Planctomycetes	In the Phycisphaeraceae clade (*Algisphaera*/*Phycisphaera*)	857776		
Planctomycetes	In the Phycisphaeraceae clade (*Algisphaera*/*Phycisphaera*)	New.CleanUp.ReferenceOTU11270		
Planctomycetes	In the Phycisphaeraceae clade (*Algisphaera*/*Phycisphaera*)	New.CleanUp.ReferenceOTU17520		

Planctomycetes	In the Phycisphaeraceae clade (*Algisphaera*/*Phycisphaera*)	223655		
Planctomycetes	In the Phycisphaeraceae clade (*Algisphaera*/*Phycisphaera*)	862250		
Planctomycetes	In the Phycisphaeraceae clade (*Algisphaera*/*Phycisphaera*)	New.CleanUp.ReferenceOTU907		
Planctomycetes	In the Phycisphaeraceae clade (*Algisphaera*/*Phycisphaera*)	New.CleanUp.ReferenceOTU20730		
Planctomycetes	In the Phycisphaeraceae clade (*Algisphaera*/*Phycisphaera*)	New.ReferenceOTU78		
Planctomycetes	In the Phycisphaeraceae clade (*Algisphaera*/*Phycisphaera*)	4128584		
Planctomycetes	In the *Aquisphaera* clade	New.CleanUp.ReferenceOTU3721		
Planctomycetes	In the *Aquisphaera* clade	801268		
Planctomycetes	*Pirellula staleyi*	900959		
Proteobacteria	In the Alphaproteobacteria	New.CleanUp.ReferenceOTU15423		
Proteobacteria	In the Alphaproteobacteria	151914		
Proteobacteria	In the Alphaproteobacteria	806201		
Proteobacteria	In the Alphaproteobacteria	332714		
Proteobacteria	In the Alphaproteobacteria	4360812		
Proteobacteria	In the Alphaproteobacteria	New.CleanUp.ReferenceOTU16760		
Proteobacteria	In the Alphaproteobacteria	985216		
Proteobacteria	In the Alphaproteobacteria	New.CleanUp.ReferenceOTU8617		
Proteobacteria	In the Alphaproteobacteria	1109385		
Proteobacteria	In the Alphaproteobacteria	3077248		
Proteobacteria	In the Alphaproteobacteria	New.CleanUp.ReferenceOTU20718		
Proteobacteria	In the Alphaproteobacteria	813522		
Proteobacteria	In the Alphaproteobacteria	308836		
Proteobacteria	In the Alphaproteobacteria	223020		
Proteobacteria	In the Alphaproteobacteria	New.CleanUp.ReferenceOTU23837		
Proteobacteria	In the Alphaproteobacteria	New.ReferenceOTU90		
Proteobacteria	Geminicoccus	New.CleanUp.ReferenceOTU18861	Aerobes/heterotrophic phototroph/Marine environments	(Foesel et al. 2007)
Proteobacteria	Geminicoccus	253754		
Proteobacteria	Geminicoccus	154063		

Proteobacteria	Asticcacaulis	1089812	Aerobes or facultative anaerobes/Adapted to oligotrophic habitats	(Abraham et al. 2012)
Proteobacteria	In the Caulobacteraceae	817706		
Proteobacteria	In the Caulobacteraceae	171288		
Proteobacteria	In the Caulobacteraceae	New.CleanUp.ReferenceOTU18760		
Proteobacteria	In the Caulobacteraceae	560770		
Proteobacteria	In the Caulobacteraceae	New.CleanUp.ReferenceOTU13556		
Proteobacteria	*Microvirga*	New.CleanUp.ReferenceOTU16834	Strictly aerobes/Reduces nitrate to nitrite/Found in soils, hot springs, and N_2-fixing nodules of *Listia* and *Lupinus*	(Bailey et al. 2014, Kelly et al. 2014)
Proteobacteria	In the Rhizobiales (*Microvirga, Bosea*)	New.CleanUp.ReferenceOTU22677	*Microvirga*: Strictly aerobes/Reduce nitrate to nitrite/Found in soils, hot springs, and N_2-fixing nodules of *Listia* and *Lupinus*. *Bosea*: Isolated from agricultural soils, also found as a commensal inhabitants of legume nodules	(Kelly et al. 2014, Marin and Ruiz Arahal 2014)
Proteobacteria	In the Rhizobiales (*Microvirga, Bosea*)	New.CleanUp.ReferenceOTU22810		
Proteobacteria	In the Rhizobiales (*Microvirga, Bosea*)	New.CleanUp.ReferenceOTU7503		
Proteobacteria	In the Rhizobiales (*Microvirga, Bosea*)	238412		
Proteobacteria	In the Rhizobiales (*Microvirga, Bosea*)	New.CleanUp.ReferenceOTU15783		
Proteobacteria	In the Rhizobiales (*Microvirga, Bosea*)	New.CleanUp.ReferenceOTU19694		
Proteobacteria	*Nitrobacter*	107036	*Nitrobacter f*ixes carbon dioxide via Calvin Cycle for their carbon requirements	(Marcondes de Souza et al. 2014)
Proteobacteria	In the Rhizobiales	1090290		
Proteobacteria	In the Rhizobiales	1977617		
Proteobacteria	In the Rhizobiales	804156		
Proteobacteria	In the Rhizobiales	137916		
Proteobacteria	In the Rhizobiales	2984012		
Proteobacteria	In the Rhizobiales	274150		
Proteobacteria	In the Rhizobiales	1111551		
Proteobacteria	In the Rhizobiales	New.CleanUp.ReferenceOTU17090		
Proteobacteria	In the Rhizobiales	New.CleanUp.ReferenceOTU3195		
Proteobacteria	In the Rhizobiales	New.CleanUp.ReferenceOTU1010		
Proteobacteria	In the Rhizobiales	247879		
Proteobacteria	In the Rhizobiales	4371349		

Proteobacteria	In the Rhizobiales	226516		
Proteobacteria	In the Rhizobiales	764838		
Proteobacteria	In the Rhizobiales	434250		
Proteobacteria	In the Rhizobiales	2545365		
Proteobacteria	In the Rhizobiales	362293		
Proteobacteria	In the Rhizobiales	567776		
Proteobacteria	In the Rhizobiales	681987		
Proteobacteria	In the Rhizobiales	New.ReferenceOTU98		
Proteobacteria	In the Rhizobiales	New.ReferenceOTU82		
Proteobacteria	In the Rhizobiales	New.CleanUp.ReferenceOTU6332		
Proteobacteria	In the Rhizobiales	827636		
Proteobacteria	In the Rhizobiales	New.CleanUp.ReferenceOTU22633		
Proteobacteria	In the Rhizobiales	New.CleanUp.ReferenceOTU1020		
Proteobacteria	In the Rhizobiales	589975		
Proteobacteria	In the Rhizobiales	835594		
Proteobacteria	In the Rhizobiales	New.ReferenceOTU15		
Proteobacteria	In the Rhizobiales	831289		
Proteobacteria	In the Rhizobiales	New.CleanUp.ReferenceOTU5261		
Proteobacteria	In the Rhizobiales	142261		
Proteobacteria	*Rubellimicrobium*	New.ReferenceOTU25	Strict aerobes, chemoorganotrophs/Found non-agricultural soils, air samples	(Pujalte et al. 2014)
Proteobacteria	*Rubellimicrobium*	952388		
Proteobacteria	In the Rhodobacteraceae (*Rubellimicrobium*)	New.CleanUp.ReferenceOTU2092		
Proteobacteria	In the Rhodobacteraceae (*Rubellimicrobium*)	New.CleanUp.ReferenceOTU18421		
Proteobacteria	In the Rhodobacteraceae (Rubellimicrobium)	New.CleanUp.ReferenceOTU22269		
Proteobacteria	In the Rhodobacteraceae (Rubellimicrobium)	New.CleanUp.ReferenceOTU910		
Proteobacteria	In the Rhodobacteraceae (Rubellimicrobium)	New.CleanUp.ReferenceOTU17869		
Proteobacteria	In the Rhodobacteraceae (Rubellimicrobium)	165827		
Proteobacteria	In the Rhodobacteraceae (Rubellimicrobium)	151172		
Proteobacteria	In the Rhodobacteraceae (Rubellimicrobium)	4348101		

Proteobacteria	In the Rhodobacteraceae (Rubellimicrobium)	688714		
Proteobacteria	In the Rhodobacteraceae (*Rhodobacter*)	1109246	Purple non-sulfur photosynthetic bacteria/Found in soils and freshwater environments	(Pujalte et al. 2014)
Proteobacteria	In the Rhodobacteraceae (*Amaricoccus, Oceanicella*)	New.CleanUp.ReferenceOTU23462	Aerobic chemoheterotrophs/ Found in soils and freshwater environments	(Pujalte et al. 2014)
Proteobacteria	In the Rhodobacteraceae (*Amaricoccus, Oceanicella*)	New.CleanUp.ReferenceOTU9107		
Proteobacteria	In the Rhodobacteraceae (*Amaricoccus, Oceanicella*)	New.CleanUp.ReferenceOTU12259		
Proteobacteria	In the Rhodospirillales (*Azospirillum, Skermanella*)	241204		
Proteobacteria	In the Rhodospirillales (*Azospirillum, Skermanella*)	828320		
Proteobacteria	In the Rhodospirillales (*Azospirillum, Skermanella*)	906820		
Proteobacteria	In the Rhodospirillales (*Azospirillum, Skermanella*)	4322410		
Proteobacteria	In the Rhodospirillales (*Azospirillum, Skermanella*)	622731		
Proteobacteria	In the Rhodospirillales	194558		
Proteobacteria	In the Rhodospirillales	246217		
Proteobacteria	In the Rhodospirillales	New.CleanUp.ReferenceOTU6809		
Proteobacteria	In the Rhodospirillales	New.CleanUp.ReferenceOTU302		
Proteobacteria	In the Rhodospirillales	New.CleanUp.ReferenceOTU17584		
Proteobacteria	In the Rhodospirillales	221365		
Proteobacteria	In the Rhodospirillales	88754		
Proteobacteria	In the Rhodospirillales	New.CleanUp.ReferenceOTU24763		
Proteobacteria	In the Rhodospirillales	New.CleanUp.ReferenceOTU10460		
Proteobacteria	In the Rhodospirillales	2693227		
Proteobacteria	In the Rhodospirillales	882616		
Proteobacteria	In the Rhodospirillales	New.ReferenceOTU83		
Proteobacteria	In the Rhodospirillales	370301		
Proteobacteria	In the Rhodospirillales	348570		
Proteobacteria	In the Rhodospirillales	701738		
Proteobacteria	In the Rhodospirillales	197174		

Proteobacteria	In the Rhodospirillales	New.CleanUp.ReferenceOTU246		
Proteobacteria	In the Rhodospirillales	New.CleanUp.ReferenceOTU16878		
Proteobacteria	In the Rhodospirillales	169755		
Proteobacteria	In the Rhodospirillales	909097		
Proteobacteria	In the Rhodospirillales	New.CleanUp.ReferenceOTU19233		
Proteobacteria	In the Rhodospirillales	1107148		
Proteobacteria	In the Rhodospirillales	677964		
Proteobacteria	In the Rhodospirillales	4562		
Proteobacteria	In the Rhodospirillales	New.CleanUp.ReferenceOTU21956		
Proteobacteria	In the Rhodospirillales	New.CleanUp.ReferenceOTU19807		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	New.CleanUp.ReferenceOTU17671	Chemoorganotrophs/ Its widespread distribution in the environment is due to its ability to utilize a wide range of organic compounds and to grow and survive under low nutrient conditions	(Glaeser and Kampfer 2014)
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	New.ReferenceOTU6		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	674742		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	2324042		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	559317		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	New.CleanUp.ReferenceOTU17989		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	329512		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	New.CleanUp.ReferenceOTU17772		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	1003206		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	New.CleanUp.ReferenceOTU12602		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	New.CleanUp.ReferenceOTU3922		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	240087		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	New.CleanUp.ReferenceOTU4783		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	699318		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	1052559		

Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	3723650		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	137881		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	113180		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	143392		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	343503		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	989109		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	New.CleanUp.ReferenceOTU8851		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	New.CleanUp.ReferenceOTU15767		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	222183		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	364155		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	494339		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	822489		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	810096		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	878663		
Proteobacteria	In the Sphingomonadaceae (*Sphingomonas*)	552687		
Proteobacteria	In the Sphingomonadaceae (E*rythrobacter*)	112754		
Proteobacteria	In the Betaproteobacteria	4301666		
Proteobacteria	In the Betaproteobacteria	New.CleanUp.ReferenceOTU24192		
Proteobacteria	In the Betaproteobacteria	567333		
Proteobacteria	In the Betaproteobacteria	558494		
Proteobacteria	In the Betaproteobacteria	New.CleanUp.ReferenceOTU1622		
Proteobacteria	In the Oxalobacteaceae (*Herbaspirillum, Niviherbaspirillum*)	New.CleanUp.ReferenceOTU20068	Aerobes, microaerobes to facultative anaerobes/ Found in soil, water and associated with plants	(Baldani et al. 2014)
Proteobacteria	In the Oxalobacteaceae (*Herbaspirillum, Niviherbaspirillum*)	111868		
Proteobacteria	In the Oxalobacteaceae (*Herbaspirillum, Niviherbaspirillum*)	334185		
Proteobacteria	In the Oxalobacteaceae (*Herbaspirillum, Niviherbaspirillum*)	553957		
Proteobacteria	In the Oxalobacteaceae (*Herbaspirillum, Niviherbaspirillum*)	573270		

Proteobacteria	In the Oxalobacteaceae (*Herbaspirillum, Niviherbaspirillum*)	New.CleanUp.ReferenceOTU4490		
Proteobacteria	In the Oxalobacteaceae (*Herbaspirillum, Niviherbaspirillum*)	533198		
Proteobacteria	In the Oxalobacteaceae (*Herbaspirillum, Niviherbaspirillum*)	7346		
Proteobacteria	In the Oxalobacteaceae (*Herbaspirillum, Niviherbaspirillum*)	759916		
Proteobacteria	In the Oxalobacteaceae (*Herbaspirillum, Niviherbaspirillum*)	566578		
Proteobacteria	In the Oxalobacteaceae (*Herbaspirillum, Niviherbaspirillum*)	256121		
Proteobacteria	In the Oxalobacteaceae (*Herbaspirillum, Niviherbaspirillum*)	792868		
Proteobacteria	In the Oxalobacteaceae (*Herbaspirillum, Niviherbaspirillum*)	New.CleanUp.ReferenceOTU11316		
Proteobacteria	In the Oxalobacteaceae (*Herbaspirillum, Niviherbaspirillum*)	586230		
Proteobacteria	In the Oxalobacteraceae (*Massilia*)	1105574		
Proteobacteria	In the Oxalobacteraceae (*Massilia*)	589123		
Proteobacteria	In the Oxalobacteraceae (*Massilia*)	New.ReferenceOTU60		
Proteobacteria	In the Oxalobacteraceae (*Massilia*)	539915		
Proteobacteria	In the Oxalobacteraceae (*Massilia*)	New.CleanUp.ReferenceOTU1626		
Proteobacteria	In the Oxalobacteraceae (*Massilia*)	849156		
Proteobacteria	In the Oxalobacteraceae (*Massilia*)	941487		
Proteobacteria	In the Oxalobacteraceae (*Massilia*)	210201		
Proteobacteria	In the Oxalobacteraceae (*Massilia*)	822419		
Proteobacteria	In the Oxalobacteraceae (*Massilia, Janthinobacterium*)	1033018		
Proteobacteria	In the Oxalobacteraceae (*Massilia, Janthinobacterium*)	510182		
Proteobacteria	In the Comamonadaceae	New.CleanUp.ReferenceOTU5566	Found in soil and water habitats	(Willems 2014)
Proteobacteria	*Caenimonas*	895220	Chemoorganotrophs, strictly aerobes/Found in soils and sludge	(Willems 2014)
Proteobacteria	*Piscinibacter*	810167	Chemoorganotrophs and facultative aerobes	(Stackebrandt et al. 2009)

Proteobacteria	In the Bdellovibrionales (*Bdellovibrio, Peredibacter, Bacteriovoraz*)	New.CleanUp.ReferenceOTU24305	Gram-negative obligate predator of other gram-negative bacteria	(Rotem et al. 2014)
Proteobacteria	In the Bdellovibrionales (*Bdellovibrio, Peredibacter, Bacteriovoraz*)	New.CleanUp.ReferenceOTU20697		
Proteobacteria	In the Bdellovibrionales (*Bdellovibrio, Peredibacter, Bacteriovoraz*)	4455981		
Proteobacteria	In the Bdellovibrionales	New.CleanUp.ReferenceOTU22326		
Proteobacteria	In the Bdellovibrionales	New.CleanUp.ReferenceOTU14933		
Proteobacteria	In the Bdellovibrionales	New.CleanUp.ReferenceOTU12590		
Proteobacteria	In the Bdellovibrionales	554390		
Proteobacteria	In the Bdellovibrionales	New.CleanUp.ReferenceOTU1597		
Proteobacteria	In the Bdellovibrionales	185100		
Proteobacteria	In the Deltaproteobacteria	New.CleanUp.ReferenceOTU24860		
Proteobacteria	In the Deltaproteobacteria	New.CleanUp.ReferenceOTU19985		
Proteobacteria	In the Deltaproteobacteria	New.CleanUp.ReferenceOTU8091		
Proteobacteria	In the Deltaproteobacteria	New.CleanUp.ReferenceOTU882		
Proteobacteria	In the Deltaproteobacteria	New.CleanUp.ReferenceOTU10609		
Proteobacteria	In the Deltaproteobacteria	541209		
Proteobacteria	In the Deltaproteobacteria	New.CleanUp.ReferenceOTU376		
Proteobacteria	In the Deltaproteobacteria	817141		
Proteobacteria	In the Deltaproteobacteria	New.CleanUp.ReferenceOTU13553		
Proteobacteria	In the Deltaproteobacteria	New.CleanUp.ReferenceOTU14472		
Proteobacteria	In the Deltaproteobacteria	New.CleanUp.ReferenceOTU18196		
Proteobacteria	In the Deltaproteobacteria	958375		
Proteobacteria	*Oligoflexus*	852722	Aerobes, non-motile and non-spore forming	(Nakai et al. 2014)

Proteobacteria	Sister clade to *Oligoflexus*	New.CleanUp.ReferenceOTU9482		
Proteobacteria	In the Oligoflexales	New.ReferenceOTU28		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU15168		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU8231		
Proteobacteria	In the Myxococcales	841077		
Proteobacteria	In the Myxococcales	1107143		
Proteobacteria	In the Myxococcales	336745		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU18084		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU15594		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU19587		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU2758		
Proteobacteria	In the Myxococcales	4353063		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU2732		
Proteobacteria	In the Myxococcales	993373		
Proteobacteria	In the Myxococcales	2963709		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU5897		
Proteobacteria	In the Myxococcales	4366579		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU17390		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU21396		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU20960		
Proteobacteria	In the Myxococcales	113261		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU23678		
Proteobacteria	In the Myxococcales	New.ReferenceOTU10		
Proteobacteria	In the Myxococcales	1131498		
Proteobacteria	In the Myxococcales	4432545		

Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU24375		
Proteobacteria	In the Myxococcales	237206		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU16687		
Proteobacteria	In the Myxococcales	254949		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU4810		
Proteobacteria	In the Myxococcales	4302753		
Proteobacteria	In the Myxococcales	501684		
Proteobacteria	In the Myxococcales	1021984		
Proteobacteria	In the Myxococcales	808319		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU8715		
Proteobacteria	In the Myxococcales	240506		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU16645		
Proteobacteria	In the Myxococcales	279206		
Proteobacteria	In the Myxococcales	313833		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU23156		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU11165		
Proteobacteria	In the Myxococcales	4299497		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU3193		
Proteobacteria	In the Myxococcales	1023267		
Proteobacteria	In the Myxococcales	1017063		
Proteobacteria	In the Myxococcales	4461509		
Proteobacteria	In the Myxococcales	2441354		
Proteobacteria	In the Myxococcales	824043		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU16846		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU5142		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU1612		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU3192		
Proteobacteria	In the Myxococcales	259044		

Proteobacteria	In the Myxococcales	803166		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU8504		
Proteobacteria	In the Myxococcales	559177		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU2855		
Proteobacteria	In the Myxococcales	New.CleanUp.ReferenceOTU1041		
Proteobacteria	In the Gammaproteobacteria	New.CleanUp.ReferenceOTU13139		
Proteobacteria	In the Gammaproteobacteria	1118948		
Proteobacteria	Sister clade to *Haliea/Halioglobus*	3038080		
Proteobacteria	Sister clade to *Haliea/Halioglobus*	931708		
Proteobacteria	*Lysobacter*	751138		
Proteobacteria	Unassigned	253724		
Proteobacteria	Unassigned	369436		
Proteobacteria	Unassigned	865778		
Proteobacteria	Unassigned	1524233		
Proteobacteria	Unassigned	256515		
Proteobacteria	Unassigned	New.CleanUp.ReferenceOTU1861		
Proteobacteria	Unassigned	New.CleanUp.ReferenceOTU16225		
Proteobacteria	Unassigned	New.CleanUp.ReferenceOTU19976		
Proteobacteria	Unassigned	New.CleanUp.ReferenceOTU23963		
Proteobacteria	Unassigned	830015		
Proteobacteria	Unassigned	New.CleanUp.ReferenceOTU12552		
Proteobacteria	Unassigned	New.CleanUp.ReferenceOTU481		
Verrucomicrobia	In the Chthoniobacterales clade	New.CleanUp.ReferenceOTU7500	Aerobes, non-motile	(Sangwan et al. 2004)
Verrucomicrobia	In the Chthoniobacterales clade	New.CleanUp.ReferenceOTU14345		
Verrucomicrobia	In the Chthoniobacterales clade	New.CleanUp.ReferenceOTU20422		

Verrucomicrobia	In the Chthoniobacterales clade	New.CleanUp.ReferenceOTU4737		
Verrucomicrobia	In the Chthoniobacterales clade	219498		
Verrucomicrobia	In the Chthoniobacterales clade	142335		
Verrucomicrobia	In the Chthoniobacterales clade	New.CleanUp.ReferenceOTU7883		
Verrucomicrobia	In the Chthoniobacterales clade	New.CleanUp.ReferenceOTU4765		
Verrucomicrobia	In the Chthoniobacterales clade	624312		
Verrucomicrobia	In the Chthoniobacterales clade	538238		
Verrucomicrobia	In the Chthoniobacterales clade	New.CleanUp.ReferenceOTU21867		
Verrucomicrobia	In the Chthoniobacterales clade	553562		
Verrucomicrobia	In the Chthoniobacterales clade	New.CleanUp.ReferenceOTU17962		
Verrucomicrobia	In the Chthoniobacterales clade	922698		
Verrucomicrobia	In the Chthoniobacterales clade	New.ReferenceOTU13		
Verrucomicrobia	In the Chthoniobacterales clade	New.CleanUp.ReferenceOTU543		
Verrucomicrobia	In the Chthoniobacterales clade	251499		
Verrucomicrobia	In the Chthoniobacterales clade	1049393		
Verrucomicrobia	In the Chthoniobacterales clade	New.CleanUp.ReferenceOTU9264		
Verrucomicrobia	In the Chthoniobacterales clade	544067		
Verrucomicrobia	In the Chthoniobacterales clade	352632		
Verrucomicrobia	In the Chthoniobacterales clade	564262		
Verrucomicrobia	In the Chthoniobacterales clade	New.ReferenceOTU16		
Verrucomicrobia	In the Chthoniobacterales clade	547960		
Verrucomicrobia	In the Chthoniobacterales clade	1108624		
Verrucomicrobia	In the Chthoniobacterales clade	559200		
Verrucomicrobia	In the Chthoniobacterales clade	New.CleanUp.ReferenceOTU8439		
Verrucomicrobia	In the Chthoniobacterales clade	New.CleanUp.ReferenceOTU8199		
Verrucomicrobia	In the Chthoniobacterales clade	1078065		
Verrucomicrobia	In the Chthoniobacterales clade	New.CleanUp.ReferenceOTU10655		
Verrucomicrobia	In the Chthoniobacterales clade	1028297		
Verrucomicrobia	In the Chthoniobacterales clade	New.CleanUp.ReferenceOTU14191		
Verrucomicrobia	In the Chthoniobacterales clade	4480292		
Verrucomicrobia	In the Chthoniobacterales clade	971170		

Verrucomicrobia	In the Chthoniobacterales clade	807473		
Verrucomicrobia	In the Chthoniobacterales clade	New.ReferenceOTU57		
Verrucomicrobia	In the Chthoniobacterales clade	586320		
Verrucomicrobia	*Opitutus*	142010	Obligate anaerobes/Found in soil	(Chin et al. 2001)
Verrucomicrobia	In the Verrucomicrobiaceae clade	3426090		
Verrucomicrobia	In the Verrucomicrobiaceae clade (*Luteolibacter*)	540464		
Unassigned	Unassigned	New.CleanUp.ReferenceOTU13671		
Unassigned	Unassigned	NewReferenceOTU97		
Unassigned	Unassigned	542433		
Unassigned	Unassigned	509980		
Unassigned	Unassigned	586275		
Unassigned	Unassigned	1128021		
Unassigned	Unassigned	274632		
Unassigned	Unassigned	509899		
Unassigned	Unassigned	2834426		
Unassigned	Unassigned	New.CleanUp.ReferenceOTU20553		
Unassigned	Unassigned	New.CleanUp.ReferenceOTU22663		
Unassigned	Unassigned	New.CleanUp.ReferenceOTU7002		
Unassigned	Unassigned	New.CleanUp.ReferenceOTU9775		
Unassigned	Unassigned	New.CleanUp.ReferenceOTU10725		
Unassigned	Unassigned	New.CleanUp.ReferenceOTU17710		
Unassigned	Unassigned	New.CleanUp.ReferenceOTU24680		
Unassigned	Unassigned	New.CleanUp.ReferenceOTU8452		
Unassigned	Unassigned	New.CleanUp.ReferenceOTU14422		
Unassigned	Unassigned	205635		
Unassigned	Unassigned	205900		
Unassigned	Unassigned	356083		
Unassigned	Unassigned	587047		

Unassigned	Unassigned	819659		
Unassigned	Unassigned	New.CleanUp.ReferenceOTU13513		
Unassigned	Unassigned	New.CleanUp.ReferenceOTU22946		
Unassigned	Unassigned	New.CleanUp.ReferenceOTU2691		
Unassigned	Unassigned	New.CleanUp.ReferenceOTU7950		
Unassigned	Unassigned	New.CleanUp.ReferenceOTU9079		
Unassigned	Unassigned	New.CleanUp.ReferenceOTU16975		
Unassigned	Unassigned	344495		
Unassigned	Unassigned	4311457		
Unassigned	Unassigned	New.CleanUp.ReferenceOTU23333		
Unassigned	Unassigned	547148		
Unassigned	Unassigned	New.CleanUp.ReferenceOTU7999		
Unassigned	Unassigned	New.CleanUp.ReferenceOTU5586		

References

Abraham, W.-R., M. Rohde, and A. Bennasar. 2012. The Family Caulobacteraceae. Pages 179–200*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Alphaproteobacteria and Betaproteobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Albuquerque, L., and M. S. da Costa. 2014a. The Families Conexibacteraceae, Patulibacteraceae and Solirubrobacteraceae. Pages 185–199*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Actinobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Albuquerque, L., and M. S. da Costa. 2014b. The Family Rubrobacteraceae. Pages 861–865*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Actinobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Albuquerque, L., and M. S. da Costa. 2014c. The Family Gaiellaceae. Pages 357–360*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Actinobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Bailey, A. C., M. Kellom, A. T. Poret-Peterson, K. Noonan, H. E. Hartnett, and J. Raymond. 2014. Draft Genome Sequence of Microvirga sp. Strain BSC265, Isolated from Biological Soil Crust of Moab, Utah. Genome Announcements 2:e01199-14-e01199-14.

Baldani, J. I., L. Rouws, L. Magalhaes Cruz, and F. Lopes Olivares. 2014. The Family Oxalobacteraceae. Pages 920–968*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Alphaproteobacteria and Betaproteobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Busse, H.-J., and M. Wieser. 2014. The Genus Arthrobacter. Pages 105–126*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Actinobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Chin, K., W. Liesack, and P. H. Janssen. 2001. Opitutus terrae gen. nov., sp. nov., to accommodate novel strains of the division " Verrucomicrobia " isolated from rice paddy soil. International Journal of Systematic and Evolutionary Microbiology 51:1965–1968.

Daims, H., J. L. Nielsen, P. H. E. R. H. Nielsen, M. Wagner, K. Schleifer, P. H. E. R. H. Nielsen, and K. Schleifer. 2001. In Situ Characterization of Nitrospira -Like Nitrite-Oxidizing Bacteria Active in Wastewater Treatment Plants. Applied and Environmental Microbiology 67:5273–5284.

DeBruyn, J. M., L. T. Nixon, M. N. Fawaz, A. M. Johnson, and M. Radosevich. 2011. Global biogeography and quantitative seasonal dynamics of Gemmatimonadetes in soil. Applied and Environmental Microbiology 77:6295–6300.

Foesel, B. U., A. S. Gößner, H. L. Drake, and A. Schramm. 2007. Geminicoccus roseus gen. nov., sp. nov., an aerobic phototrophic Alphaproteobacterium isolated from a marine aquaculture biofilter. Systematic and Applied Microbiology 30:581–586.

Franco, M. M. C., and D. P. Labeda. 2014. The Order Pseudonocardiales. Pages 745–850*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Actinobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Fukunaga, Y., and N. Ichikawa. 2014. The Class Holophagaceae. Pages 683–688*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Other Major Lineages of Bacteria and Archaea. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Glaeser, S., and P. Kampfer. 2014. The Family Sphingomonadaceae. Pages 642–693*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Alphaproteobacteria and Betaproteobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Hanada, S. 2014. The Phylum Chloroflexi, the Family Chloroflexaceae, and the Related Phototrophic Families Oscillochloridaceae and Roseiflexaceae. Pages 515–529*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Other Major Lineages of Bacteria and Archaea. Fourth. Springer-Verlag Berlin Heidelberg, London.

Huber, K. J., A. M. Geppert, and G. Wanner. 2016. The first representative of the globally widespread subdivision 6 Acidobacteria , Vicinamibacter silvestris gen . nov., sp. nov., isolated from subtropical savannah soil:2971–2979.

Iino, T. 2014. The Family Ignavibacteriaceae. Pages 701–702*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Other Major Lineages of Bacteria and Archaea. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Im, W.-T., Z.-Y. Hu, K.-H. Kim, S.-K. Rhee, H. Meng, S.-T. Lee, and Z.-X. Quan. 2012. Description of Fimbriimonas ginsengisoli gen. nov., sp. nov. within the Fimbriimonadia class nov., of the phylum Armatimonadetes. Antonie van Leeuwenhoek 102:307–317.

Kang, J. Y., J. Chun, J. W. Seo, C. H. Kim, and K. Y. Jahng. 2015. Flaviaesturariibacter amylovorans gen. nov., sp. nov., a starch-hydrolysing bacterium, isolated from estuarine water. International Journal of Systematic and Evolutionary Microbiology 65:2209–2214.

Kelly, D. P., I. R. McDonald, and A. P. Wood. 2014. The Family Methylobacteriaceae. Pages 313–333*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Alphaproteobacteria and Betaproteobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Kim, K. K., and J.-S. Lee. 2014. The Family Nakamurellaceae. Pages 577–584*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes:

Actinobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Komárek, J., J. Kaštovský, J. Mareš, and J. R. Johansen. 2014. Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. Preslia 86:295–335.

Labeda, D., and R. Testa. 1984. Saccharothrix: a new genus of the Actinomycetales related to Nocardiopsis. International journal of Systematic Bacteriology 34:426–431.

Lambiase, A. 2014. The Family Sphingobacteriaceae. Pages 907–912*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Other Major Lineages of Bacteria and Archaea. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Lee, K. C. Y., P. F. Dunfield, and S. M. B. 2014. The Phylum Armatimonadetes. Pages 447–457*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Other Major Lineages of Bacteria and Archaea. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Ludwing, W., K.-H. Schleifer, and W. B. Whitman. 2009. Volume 3: Firmicutes. Pages 1–13*in* P. Vos, G. Garrity, D. Jones, N. R. Krieg, W. Ludwing, R. F.A., K.-H. Schleifer, and W. Whitman, editors. Bergey's Manual of Systematic Bacteriology: Volume 3: Firmicutes. Springer, New York.

Marcondes de Souza, J. A., L. M. Carareto Alves, A. de Mello Varani, and E. G. de Macedo Lemos. 2014. the Family Bradyrhizobiaceae. Pages 135–150*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Alphaproteobacteria and Betaproteobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Marin, I., and D. Ruiz Arahal. 2014. The Family Beijerinckiaceae. Pages 114–129*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Alphaproteobacteria and Betaproteobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

McBride, M. J. 2014. The Family Flavobacteriaceae. Pages 643–677*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Other Major Lineages of Bacteria and Archaea. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Mcbride, M. J., W. Liu, L. Xuemei, Y. Zhu, W. Zhang, X. Lu, and W. Zhang. 2014. The Family Cytophagaceae. Pages 577–593*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Other Major Lineages of Bacteria and Archaea. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Nakai, R., M. Nishijima, N. Tazato, Y. Handa, F. Karray, S. Sayadi, H. Isoda, and T. Naganuma. 2014. Oligoflexus tunisiensis gen. nov., sp. nov., a Gram-negative, aerobic, filamentous bacterium of a novel proteobacterial lineage, and description of Oligoflexaceae fam. nov.,

Oligoflexales ord. nov. and Oligoflexia classis nov. International journal of systematic and evolutionary microbiology 64:3353–9.

Normand, P., D. Daffonchio, and M. Gtari. 2014. The Family Geodermatophilaceae. Pages 361–377*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Actinobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Pascual, J., M. García-López, G. F. Bills, and O. Encloud. 2016. Longimicrobium terrae gen. Nov., sp. Nov., an oligotrophic bacterium of the under-represented phylum Gemmatimonadetes isolated through a system of miniaturized diffusion chambers. International Journal of Systematic and Evolutionary Microbiology 66:1976–1985.

Pascual, J., P. K. Wüst, A. Geppert, B. U. Foesel, and K. J. Huber. 2015. Novel isolates double the number of chemotrophic species and allow the first description of higher taxa in Acidobacteria subdivision 4 38:534–544.

Pujalte, M., T. Lucena, M. Ruvira, D. Ruiz Arahal, and M. C. Macian. 2014. The Family Rhodobacteraceae. Pages 439–512*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Alphaproteobacteria and Betaproteobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Rashidan, K. K., and D. F. Bird. 2001. Role of predatory bacteria in the termination of a cyanobacterial bloom. Microbial Ecology 41:97–105.

Raven, J. A. 2012. Carbon. Pages 443–460*in* B. A. Whitton, editor. Ecology of Cyanobacteria II. Springer-Verlag Berlin Heidelberg, London.

Rosenberg, E. 2014a. The family Deinococcaceae. Pages 613–616*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Other Major Lineages of Bacteria and Archaea. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Rosenberg, E. 2014b. The Family Chitinophagaceae. Pages 493–495*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Other Major Lineages of Bacteria and Archaea. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Rotem, O., Z. Pasternak, and E. Jurkevitch. 2014. Bdellovibrio and Like Organisms. Pages 3–13*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Deltaproteobacteria and Epsilonproteobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Sangwan, P., X. Chen, P. Hugenholtz, and P. H. Janssen. 2004. Chthoniobacter flavus gen. nov., sp. nov., the first pure-culture representative of subdivision two, Spartobacteria classis nov., of the phylum Verrucomicrobia. Applied and Environmental Microbiology 70:5875–5881.

Stackebrandt, E. 2014. The Family Acidimicrobiaceae. Pages 5–12*in* E. Rosenberg, E. F.

DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Actinobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Stackebrandt, E., and L. G. Otten. 2014. The Class Nitriliruptoria. Pages 587–593*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Actinobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Stackebrandt, E., C. Scheuner, M. Goker, and P. Schumann. 2014. The Family Intraspoangiaceae. Pages 397–420*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Actinobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Stackebrandt, E., and P. Schumann. 2014. The Family Cellulomonadaceae. Pages 163–180*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Actinobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Stackebrandt, E., S. Verbarg, A. Frühling, H. J. Busse, and B. J. Tindall. 2009. Dissection of the genus Methylibium: Reclassification of Methylibium fulvum as Rhizobacter fulvus comb. nov., Methylibium aquaticum as Piscinibacter aquaticus gen. nov., comb. nov. and Methylibium subsaxonicum as Rivibacter subsaxonicus gen. nov., comb. n. International Journal of Systematic and Evolutionary Microbiology 59:2552–2560.

Stieglmeier, M., R. J. E. Alves, and C. Schleper. 2014. The Phylum Thaumarchaeota. Pages 347–358*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Other Major Lineages of Bacteria and Archaea. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Tamura, T., Y. Ishida, M. Otoguro, H. Yamamura, M. Hayakawa, and K. I. Suzuki. 2010. Angustibacter luteus gen. nov., sp. nov., isolated from subarctic forest soil. International Journal of Systematic and Evolutionary Microbiology 60:2441–2445.

Tamura, T., and K.-I. Suzuki. 2014. The Suborder Kineosporiineae. Pages 443–452*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Actinobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Tohn, E. M., and A. Borsodi. 2014. The Family Nocardioidaceae. Pages 651–690*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Actinobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Trujillo, M. E., K. Hong, and O. Genilloud. 2014. The Family Micromonosporaceae. Pages 500–561*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Actinobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Willems, A. 2014. The Family Comamonadaceae. Pages 777–845*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Alphaproteobacteria and Betaproteobacteria. Fourth edition. Springer-Verlag Berlin Heidelberg, London.

Yoon, J., K. Adachi, S. Park, H. Kasai, and A. Yokota. 2011. Aureibacter tunicatorum gen. nov., sp. nov., a marine bacterium isolated from a coral reef sea squirt, and description of flammeovirgaceae fam. nov. International Journal of Systematic and Evolutionary Microbiology 61:2342–2347.

Youssef, N. H., and M. S. Elshahed. 2014. The Phylum Planctomycetes. Pages 759–804*in* E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt, and F. Thompson, editors. The Prokaryotes: Other Major Lineages of Bacteria and Archaea. Fourth edition. Springer-Verlag Berlin Heidelberg, London.