Element Use and Acquisition Strategies in Biological Soil Crusts

by

Kathryn Alexander Noonan

A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Approved May 2012 by the
Graduate Supervisory Committee:

Hilairy Hartnett, Chair
Ferran Garcia-Pichel
Ariel Anbar
Thomas Sharp
Everett Shock

ARIZONA STATE UNIVERSITY

May 2012

ABSTRACT

Biological soil crusts (BSCs) are critical components of arid and semiarid environments and provide the primary sources of bioavailable macronutrients and increase micronutrient availability to their surrounding ecosystems. BSCs are composed of a variety of microorganisms that perform a wide range of physiological processes requiring a multitude of bioessential micronutrients, such as iron, copper, and molybdenum. This work investigated the effects of BSC activity on soil solution concentrations of bioessential elements and examined the microbial production of organic chelators, called siderophores. I found that aluminum, vanadium, copper, zinc, and molybdenum were solubilized in the action of crusts, while nickel, zinc, arsenic, and zirconium were immobilized by crust activity. Potassium and manganese displayed behavior consistent with biological removal and mobilization, whereas phosphorus and iron solubility were dominated by abiotic processes. The addition of bioavailable nitrogen altered the effects of BSCs on soil element mobilization. In addition, I found that the biogeochemical activites of BSCs were limited by molybdenum, a fact that likely contributes to co-limitation by nitrogen. I confirmed the presence of siderophore producing microbes in BSCs. Siderophores are low-molecular weight organic compounds that are released by bacteria to increase element solubility and facilitate element uptake; siderophore production is likely the mechanism by which BSCs affect the patterns I observed in soil solution

element concentrations. Siderophore producers were distributed across a range of bacterial groups and ecological niches within crusts, suggesting that siderophore production influences the availability of a variety of elements for use in many physiological processes. Four putative siderophore compounds were identified using electrospray ionization mass spectrometry; further attempts to characterize the compounds confirmed two true siderophores. Taken together, the results of my work provide information about micronutrient cycling within crusts that can be applied to BSC conservation and management. Fertilization with certain elements, particularly molybdenum, may prove to be a useful technique to promote BSC growth and development which would help prevent arid land degradation. Furthermore, understanding the effects of BSCs on soil element mobility could be used to develop useful biomarkers for the study of the existence and distribution of crust-like communities on ancient Earth, and perhaps other places, like Mars.

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ACKNOWLEDGEMENTS

I thank Thomas E. Alexander, Jayne Belnap, Jessica Block, Elizabeth Rampe, Miriam Riner, and Alka Tripathy for assistance with soil collection; Katie Bern, Greg Brennecka, Jesse Coe, Tom Colella, Gwyneth Gordon, Megan Kelly, Stan Klonowski, Cathy Kochert, Zureyma Martinez, Zachary Smith, Tyler Viliborghi, Laura Wayslenki, and Felisa Wolfe-Simon for support with laboratory analyses and data processing; Scott Bingham and the ASU DNA Laboratory for sequencing; George A. Hamilton, Jessica Martin, Patrick Noonan, Ruth Potrafka, Amisha Poret-Peterson, and Jessie Shipp for assistance with experimental design and manuscript preparation; my advisor, Hilairy Hartnett; and my committee, Ariel Anbar, Ferran Garcia-Pichel, Thomas Sharp, and Everett Shock.

This work was funded by NSF grant 0525569 to Hilairy Hartnett, Ariel Anbar, and Ferran Garcia-Pichel, NSF grant 0846188 to Hilairy Hartnett, a NASA Space Grant Fellowship to Kathryn Noonan, and the NASA Astrobiology Institute.

CHAPTER 1

OVERVIEW

The purpose of my work was to assess the factors that control biological soil crust (BSC) growth and development, and to elucidate the mechanisms by which BSCs acquire much needed elements to support their physiology. To these ends, I utilized an assortment of complimentary techniques from a variety of disciplines, including: geochemistry, biochemistry, and molecular biology and microbiology. I found that biological activity in BSCs influenced the concentrations of a range of elements, and that they do so through the production of chelating compounds that increase element solubility and facilitate element uptake.

Biological soil crusts are diverse microbial communities that colonize the soil surface in arid and semiarid environments. Crusts can include cyanobacteria, heterotrophic bacteria, archaea, algae, fungi, lichens, and mosses (Belnap et al., 2001); however, the BSCs used in my study were "dark" crusts dominated by cyanobacteria. Dark crusts represent one stage in crust succession and are characterized by an assortment of cyanobacteria, but are without substantial lichen or moss cover (Figure 1-1; Garcia-Pichel & Belnap, 1996; Garcia-Pichel et al., 2001). Dark crusts were selected because I was interested in the effects of free-living cyanobacteria on soil element mobility. Crusts were collected from two field sites (Figure 1-2) located on the Colorado Plateau, north of Moab,

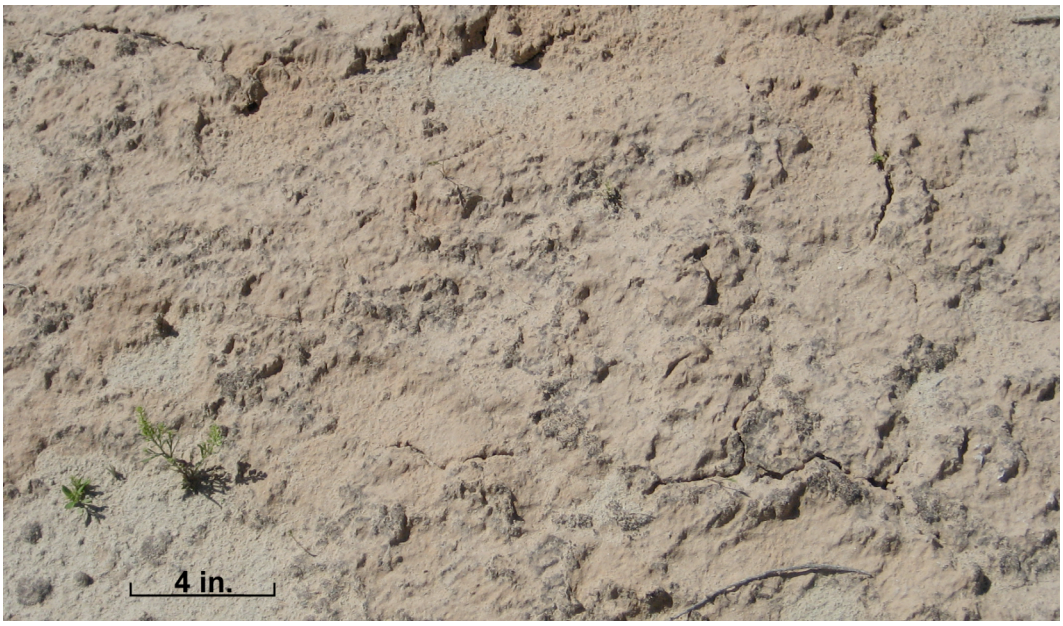

Figure 1-1. Dark, cyanobacteria-dominated crust in the field. Crusts are the dark grey to black material covering the soil.

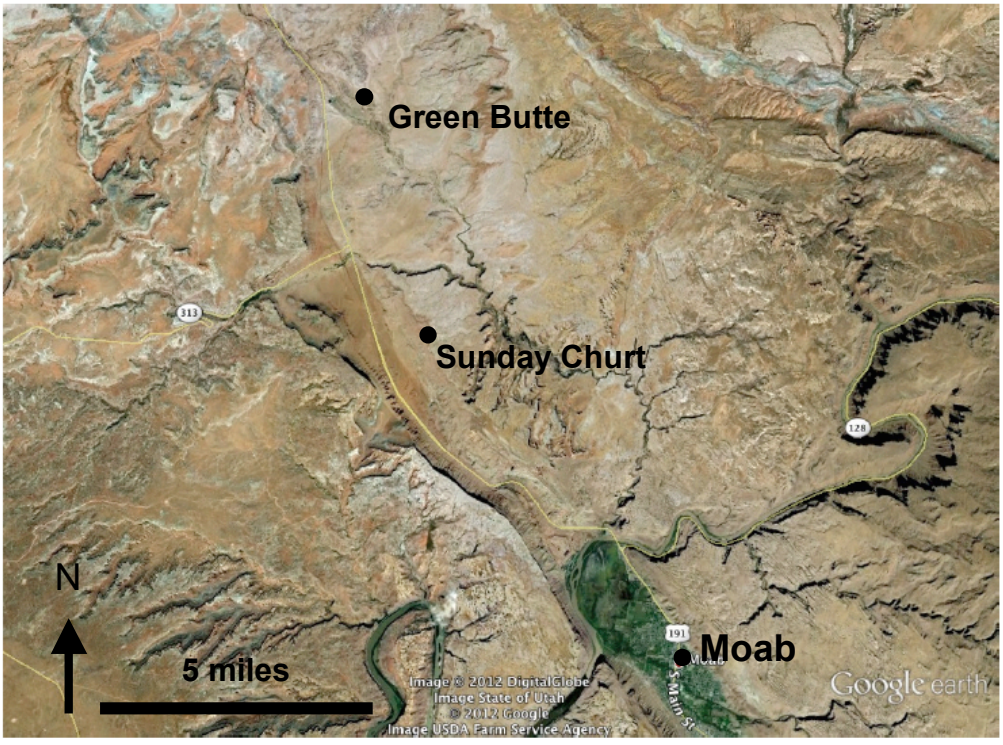

Figure 1-2. Google Earth image of the filed sites used in my research.

UT. Chapter 1 covers work conducted with dark crusts from the Sunday Churt site (Figure 1-2; N 38°39′22.3″, W 109°39′13.8″), and the remaining chapters address studies performed with dark crusts from the Green Butte site (Figure 1-2; N 38°42′56.2″, W 109°41′32.7″). These sites were chosen because a significant amount was already known about the microbial communities and active physiological processes in these areas. This existing body of knowledge allowed me to address new research questions and put them in the ecological and physiological context for the crust ecosystem.

It is important to understand the factors that contribute to BSC physiology because these factors promote BSC growth and development, which in turn positively influence the fertility of arid environments. Cyanobacteria are photosynthetic prokaryotes, some of which are also capable of converting atmospheric nitrogen gas (N_2) into bioavailable ammonium (NH_4^+), a process called nitrogen fixation. Cyanobacteria are one of the few microbes that fix N_2 aerobically. On the Colorado Plateau, cyanobacteria in BSCs are the main source of bioavailable carbon (Beymer & Klopatek, 1991; Evans & Lange, 2001; Garcia-Pichel, 2002) and nitrogen (Rychert & Skujins, 1974; Jeffries et al., 1992; Steppe et al., 1996; Belnap, 2002) to the immediate crust community, as well as to the surrounding ecosystem. Many cyanobacteria are filamentous and secrete exopolysaccharides, allowing them to stabilize the soil surface (Figure 1-3;

Campbell, 1979; Schulten, 1985; Danin & Ganor, 1991; Belnap & Garnder, 1993; Belnap, 2001; Belnap et al., 2001; Reynolds et al., 2006; Garcia-Pichel & Wojciechowski, 2009; Rossi et al., 2012). Taken together, the microbial activity of BSCs, particularly that of the cyanobacteria, serve to support arid land productivity and avert arid land degradation, or desertification. My research may provide useful crust conservation tools that could be used to combat desertification — a serious, and increasing, socioeconomical, hydrological, and ecological problem in the developed and developing world (Sheridan, 1981; Puigdefábregas, 1998; Arnalds & Archer, 2000; Pimentel, 2000; Weibe, 2003).

Figure 1-3. Scanning electron micrograph (x100) of a cyanobacterial sheath binding soil particles together (photo courtesy of soilcrust.org). This image highlights the role that cyanobacteria play in soil stabilization, and also displays the intimate association between crust microbes and the soil solid-phase.

Biological soil crusts on the Colorado Plateau are capable of enduring extreme environmental conditions, such as intense UV radiation, infrequent and brief periods of hydration, and temperature fluctuations from below zero to 50°C (Bowker et al., 2002). BSCs' ability to thrive under these intense conditions makes them excellent analogues for life in other extreme environments, and thus, this work has astrobiological relevance. The results of my work could be used to establish biomarkers for investigating the presence of crust-like communities that might have existed on ancient Earth or, perhaps, on a slightly wetter, earlier Mars.

Finally, BSCs differ from other soil microbial communities, such as those found in temperate forests or the rhizosphere. Crusts have different community compositions (Kuske et al., 2002) with lower species richness and diversity (Nakatsua et al., 2000), and they have a higher abundance of unique organisms, not previously identified or cultured (Garcia-Pichel et al., 2003). BSC's ability to thrive under extreme conditions also makes them likely to possess distinct adaptations for survival (Gundlapally & Garcia-Pichel, 2006).

Photosynthesis and nitrogen fixation provide BSCs with fixed carbon (C) and nitrogen (N), but in order to perform these vital physiological processes BSCs must have access to a range of other elements including, but not limited to, sodium, magnesium, phosphorus, potassium,

manganese, iron, nickel, copper, zinc, and molybdenum (Bertini et al., 2007). Because BSCs live in a water-limited environment, their only source of these bioessential elements is the soil solid-phase. These elements exist either within mineral structures or adsorbed to the surfaces and interlayers of soil minerals and organics (Brady & Weil, 2002). The conduit between the soil solid-phase and the crust microbes is the soil solution; the fluid that exists in the soil pore-spaces when the crusts are wet, which only occurs during, and briefly after, a rain or snow event. When the crust is wet, some of the bioessential elements will dissolve or desorb automatically, allowing microbes to take them up. However, other elements will remain bound to the solid-phase due to chemical conditions that limit their solubility and prevent microbial uptake. Crust microbes must, therefore, have effective strategies to acquire the less soluble bioessential elements if they are to survive. The purpose of my research was to determine if I could demonstrate the effects of microbial activity on soil solution element concentrations and reveal what element acquisition strategies BSCs use.

My first study focused on the elements required for biological nitrogen fixation (see Chapter 2). Nitrogen (N_2) fixation is performed by prokaryotes using the enzyme nitrogenase that contains iron (Fe), molybdenum (Mo), and, in some organisms vanadium (V); N_2 fixation is a critical process in BSCs. I expected the need for Fe, Mo, and V to be high in BSCs, and I

hypothesized that Fe, Mo, and V concentrations would be lower in crusted samples than in killed controls as the result of microbial uptake. I further hypothesized that if a bioavailable source of N were added to crusts, the soil solution concentrations of Fe, Mo, and V would be higher than they were in the absence of added N. I expected that N addition would cause N_2 fixation to stop, thereby reducing the microbes' need for Fe, Mo, and V.

I used crusts collected in the field to conduct simulated rainfall experiments in the laboratory to test my hypotheses (see Chapter 2). As I expected, Mo and V concentrations were lower in crusted samples than in killed controls, indicating active microbial uptake. However, over the last 24 h of the incubations, soil solution Mo concentrations increased, suggesting that microbial solubilization also played a role in Mo cycling within the experimental crusts. When soil solution concentrations of Mo and V were compared between crusts that received a N treatment (+N), and those that did not (–N), I found that V concentrations were higher in the +N group. This indicated that V need was higher when N_2 fixation was active, suggesting that the crusts may have used V for N_2 fixation. There was no difference between Mo concentrations in the +N and –N treatments, nor were there any differences in Fe concentrations between soil type or N treatment. The results of this work clearly show that BSCs actively change soil solution concentrations of Mo and V, and, in the case of V, the effect is dependent on N availability. The findings implied that Mo

and V may limit BSC N_2 fixation, and therefore, may serve as potential candidates for fertilizers to promote crust conservation. In addition, if the effect of crusts on Mo and V leave characteristic signatures in solid-phase distributions of Mo and V, these elements may serve as effective biosignatures of ancient crust communities.

In the next phase of my research, I expanded upon the work in Chapter 2, by investigating the effect of BSCs on soil solution concentrations of a larger suite of elements that included sodium, magnesium, aluminum, phosphorus, potassium, vanadium, manganese, iron, nickel, copper, zinc, molybdenum, arsenic, and zirconium (see Chapter 3). The initial goal was to see which elements changed concentration in ways consistent with biological processes, and which elements were dominated by abiotic processes. Aluminum (Al) and zirconium (Zr), were expected to demonstrate abiotic processes, such as adsorption or dissolution, while all the other elements investigated have multiple biological roles, and so I expected to see changes in concentrations that provide evidence for biological processes, such as uptake and microbial solubilization. The second goal of this work was to investigate the influence of N and molybdenum (Mo) addition on the soil solution concentrations of all elements. I hypothesized that magnesium (Mg), phosphorus (P), vanadium (V), manganese (Mn), nickel (Ni), and zinc (Zn) concentrations would increase in crusted samples when

bioavailable N was added. I further hypothesized that iron (Fe), copper (Cu), and Mo concentrations would decrease with N addition because N addition would stimulate microbial processes that require Fe, Cu, and Mo. Under Mo addition, I hypothesized that Mg, P, Mn, Fe, Ni, Cu, Zn, and Mo concentrations would decrease in crusted samples, and that V concentrations would stabilize. I did not expect any changes in sodium (Na), Al, potassium (K), arsenic (As), or zirconium (Zr) concentrations under either treatment (see Chapter 3).

To test these hypotheses, I performed simulated rainfall experiments with water, N, and Mo additions (see Chapter 3). I found that Ni and Zn concentrations decreased, probably as a result of microbial uptake, while As and Zr concentrations decreased, likely due to biosorption. Increasing Cu concentrations were interpreted to result from microbial solubilization, increasing Al was supposed to result from cellular export, and increasing V and Zn was thought to result either from solubilization or export. Potassium and Mn showed increasing and decreasing concentrations during different incubation time periods, likely reflecting a shift in the balance among uptake, export, and solubilization. Phosphorus showed evidence for microbial solubilization as well as for abiotic processes. Iron concentrations showed a clear signal of abiotic mineral precipitation or adsorption. I also found that N addition resulted in reduced microbial solubilization. I interpret this to mean that when N_2 fixation is active, BSC

need for other elements is high and so they actively solubilize elements form the solid-phase, probably through the production of organic chelators. When N is added, N_2 fixation stops, element need is reduced, so chelator production decreases, and solubilization is depressed (see Chapter 3). This work added to the list of elements that may serve as effective fertilizers to be used in crust conservation, and suggested additional elements that may prove to be useful biomarkers.

Next, I hypothesized that N_2 fixation in BSCs from my field site was limited with respect to Mo (see Chapter 4). Limitation with respect to Mo, can result in co-limitation with respect to N (Saito et al., 2008) because of Mo's widespread use in nitrogenase enzymes. I used the acetylene reduction assay, a proxy for N_2 fixation, to measure N_2 fixation rates in crusts that did and did not receive a Mo addition. I found that N_2 fixation rates increased significantly when Mo was added — strong evidence for Mo-limitation. Based on my results, Mo fertilization would likely be an excellent BSC conservation tool as it would allow crusts to increase N_2 fixation, increasing the fertility of the BSCs and, ultimately, of the surrounding ecosystem.

The work discussed above showed that biological processes in BSCs, such as microbial uptake and solubilization, alter the soil solution concentrations of many elements. I was then interested in the mechanism by which BSCs affect the observed soil solution concentration changes,

and I hypothesized that BSCs release siderophores to serve their element acquisition needs (see Chapter 5). Siderophores are low-molecular weight organic compounds that bind iron (Fe) with high affinity. They are released from bacterial cells to increase Fe solubility and Fe uptake. Though siderophores typically bind Fe, they are also capable of binding a range of other metals including, Mo (Litos et al., 2006; Bellenger et al., 2007; Monteiro et al., 2010), Mn (Saal & Duckworth, 2010; Szabó & Farkas, 2011), Ni (Dimkpa et al., 2008), Cu (McKnight & Morel, 1980; Kim et al., 2004; Bellenger et al., 2007), and Zn (Bellenger et al., 2007). Siderophore production is known to occur in soils (Powell et al., 1980; Akers, 1983; Holmström et al., 2004; Essén et al., 2006), but to date, it has not been investigated in BSCs.

To test the hypothesis that BSCs produce siderophores, I used the chrome azurol S overlay (O-CAS) assay to screen crust microbes for siderophore production (Pérez-Miranda et al., 2007). I also used the traditional liquid CAS assay (Schwyn & Neilands, 1987) to test for siderophore production in two essential cyanobacterial members of dark crusts. The results of the screening assays confirmed that siderophore producers do exist in BSCs. I used nearly full-length 16S rRNA gene sequencing to identify the organisms that tested positive for siderophore production. I found that siderophore producers isolated from BSCs spanned a range of bacterial classifications and crust ecological roles. The

important crust cyanobacteria, *Microcoleus vaginatus* and *Nostoc punctiforme*, known for their crust building and N_2 fixation capabilities, respectively, both produced siderophores in the liquid CAS assay. An additional 8 siderophore-producers were isolated from the O-CAS assay that, although not among the most abundant or most physiologically dominant groups, still play important roles in crust formation and function. These organisms included members of the Firmicutes, Alpha- and Betaproteobacteria, and cyanobacteria. Some of the siderophore-producers were closely related to known microbes; however, others likely represented novel organisms that were not already in culture. Furthermore, the majority of siderophore producers made hydroxamate siderophores, but some made catecholate siderophores as well. The presence of both siderophore types has interesting implications for what metals can be bound (see Chapter 5). This is the first time that siderophore producers have been isolated from BSCs, and the results demonstrated that siderophore production is at least one mechanism driving the soil solution concentration changes described in Chapters 2 and 3.

The final step was to begin characterizing the siderophore compounds (see Chapter 6). To do this, I grew a large volume batch culture of the same *Nostoc punctiforme* described in Chapter 5. Siderophores were detected in the growth media after 6 weeks using the CAS assay. Cells

were removed, and organic fractions in the culture media were separated by column chromatography. The liquid CAS assay (Schwyn & Neilands, 1987) was used to determine which eluant fractions contained siderophores, and those fractions were analyzed with and without Fe, using high performance liquid chromatography electrospray ionization mass spectrometry (HPLC-ESI-MS). ESI-MS revealed 4 putative siderophore peaks. The criteria for determining siderophores were peaks for which the unbound masses decreased in intensity, and the Fe-bound masses increased in intensity, with increasing Fe concentration. This is the typical first line of evidence for the siderophore-nature of a peak (Jessica Martin, personal communication).

In order to confirm that these peaks represented siderophores, I hypothesized that peak intensities in the growth media would decrease with increasing Fe concentration in subsequent experiments with *Nostoc punctiforme*. To test this, I grew *Nostoc* under three different Fe concentrations, and used ESI-MS to scan for the putative siderophore peaks identified previously. However, intensities of the putative siderophore peaks increased with increasing Fe concentration, contrary to my hypothesis. It is possible this result occurred because the lowest Fe concentrations were too low to allow survival and led to a concomitant N-limitation. I was also unable to measure the putatitive siderophore peaks in crust extracts from previous experiments. Therefore, although I have

determined the masses of four putative siderophores, efforts to confirm their siderophore-nature were inconclusive. Future work with modified experimental design and analytical methods could provide more definitive results (see Chapter 6).

This work represents the first effort to asses biological soil crusts from a geochemical perspective. Microbes in the BSC community live in intimate association with soil minerals, and my studies demonstrate that through active manipulation of their geochemical environment, BSCs can create habitable zones in extreme environments.

In summary, the results of my work demonstrate that crusts actively alter soil solution concentrations of bioessential elements. They do so through the production of siderophores by a variety of crust organisms that span a range of phylogenetic classifications and ecological roles. Four putative siderophore compounds were identified, but more work is needed to further confirm the siderophore-nature of these compounds. My results can be applied to the development of fertilization treatments to conserve and restore biological soil crust cover in order to prevent arid land degradation. Furthermore, my results provide insight into potential biomarkers for crusts that may be used to investigate the distribution and evolution of crust-like communities in extreme environments such as those that might have existed on ancient Earth and other places of astrobiological interest, such as Mars.

References

Akers, H. 1983. Multiple hydroxamic acid microbial iron chelators (siderophores) in soils. *Soil Science* 135, no. 3: 156-159.

Arnalds, O. and S. Archer. 2000. Introduction. In *Rangeland desertification*, ed. O. Arnalds and S. Archer. 1-4. Dordrecht: Kluwer Academic Publishers. 209.

Bellenger, J.P., F. Arnaud-Neu, Z. Asfari, S.C.B. Myneni, E.I. Stiefel, and A.M.L. Kraepiel. 2007. Complexation of oxoanions and cationic metals by the biscatecholate siderophore azotochelin. *Journal of Biological Inorganic Chemistry* 12: 367-376.

Belnap, J. 2001. Factors influencing nitrogen fixation and nitrogen release in biological soil crusts. In *Biological soil crusts: Structure, function, and management*, ed. J. Belnap and O.L. Lange. 241-261. Berlin Heidelberg New York: Springer. 503.

_____. 2002. Nitrogen fixation in biological soil crusts from southeast Utah, USA. *Biol Fertil Soils* 35: 128-135.

Belnap, J., B. Budel, and O. L. Lange. 2001. Biological soil crusts: Characteristics and distribution. In *Biological soil crusts: Structure, function, and management*, ed. J. Belnap and O. L. Lange. 3-30. Berlin: Springer. 503.

Belnap, J. and J.S. Gardner. 1993. Soil microstructure in soils of the colorado plateau: The role of the cyanobacterium *Microcoleus vaginatus*. *Great Basin Naturalist* 53: 40-47.

Bertini, I., H.B. Gray, E.I Stiefel, and J.S. Valentine. 2007. *Biological inorganic chemistry: Structure and reactivity*. Edited by I. Bertini, H.B. Gray, E.I. Stiefel and J.S. Valentine. Sausalito, California: University Science Books.

Beymer, R.J. and J.M. Klopatek. 1991. Potential contribution of carbon by microphytic crusts in pinyon-juniper woodlands. *Arid Soil Research and Rehabilitation* 5: 187-198.

Bowker, M.A., S.C. Reed, J. Belnap, and S.L. Phillips. 2002. Temporal variation in community composition, pigmentation, and Fv/Fm of desert cyanobacterial soil crusts. *Microbial Ecology* 43: 13-25.

Brady, N.C. and R.R. Weil. 2002. *The nature and properties of soils*. Upper Saddle River: Prentice Hall.

Campbell, S.E. 1979. Soil stabilization by a prokaryotic desert crust: Implications for precambrian land biota. *Origins of Life* 9: 335-348.

Danin, A. and E. Ganor. 1991. Trapping of airborne dust by mosses in the Negev desert, Israel. *Earth Surface Processes and Landforms* 16, no. 2: 153-162.

Dimkpa, C., A. Svato, D. Merten, G. Büchel, and E. Kothe. 2008. Hydroxamate siderophores produced by *streptomyces acidiscabies* e13 bind nickel and promote growth in cowpea (*vigna unguiculata* l.) under nickel stress. *Canadian Journal of Microbiology* 54: 163-172.

Essén, S.A., D. Bylund, S.J.M. Holmstrom, M. Moberg, and U.S. Lundstrom. 2006. Quantification of hydroxamate siderophores in soil solutions of podzolic soil profiles in Sweden. *BioMetals* 19: 269-282.

Evans, R.D. and O.L. Lange. 2001. Biological soil crusts and ecosystem nitrogen and carbon dynamics. In *Biological soil crusts: Structure, function, and management*, ed. J. Belnap and O.L. Lange:263-279. Berlin: Springer-Verlag.

Garcia-Pichel, F. 2002. Desert environments: Biological soil crusts. In *Encyclopedia of environmental microbiology*, ed. G. Bitton:1019-1023. New York: John Wiley.

Garcia-Pichel, F. and J. Belnap. 1996. Microenvironments and microscale productivity of cyanobacterial desert crusts. *Journal of Phycology* 32: 774-782.

Garcia-Pichel, F., S.L. Johnson, D. Youngkin, and J. Belnap. 2003. Small-scale vertical distribution of bacterial biomass and diversity in biological soil crusts from arid lands in the Colorado Plateau. *Microbial Ecology* 46: 312-321.

Garcia-Pichel, F., A. Lopéz-Cortés, and U. Nübel. 2001. Phylogenetic and morphological diversity of cyanobacteria in soil desert crusts from the Colorado Plateau. *Applied and Environmental Microbiology* 67, no. 4: 1902-1910.

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, no. 11: e7801.

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.

Holmström, S.J.M., U.S. Lundstrom, R.D. Finlay, and P.A.W. Van Hees. 2004. Siderophores in forest soil solution. *Biogeochemistry* 71: 247-258.

Jeffries, D.L., J.M. Klopatek, S.O. Link, and J. Bolton. 1992. Acetylene reduction by cryptogamic crusts from a blackbrush community as related to resaturation and dehydration. *Soil Biology and Biochemistry* 24: 1101-1105.

Kim, H.J., D.W. Graham, A.A. DiSpirito, M.A. Alterman, N. Galeva, C.K. Larive, D. Asunskis, and P.M.A. Sherwood. 2004. Methanobactin, a copper-acquisition compound from methane-oxidizing bacteria. *Science* 305, no. 5690: 1612-1615.

Kuske, C.R., L.O. Ticknor, M.E. Miller, J.M. Dunbar, J.A. Davis, S.M. Barns, and J. Belnap. 2002. Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. *Applied and Environmental Microbiology* 68, no. 4: 1854-1863.

Litos, C., A. Terzis, C. Raptopoulou, A. Rontoyianni, and A. Karaliota. 2006. Polynuclear oxomolybdenum(VI) complexes of dihydroxybenzoic acids: Synthesis, spectroscopic and structure characterization of a tetranuclear catecholato-type coordinated 2,3-dihydroxybenzoate and a novel tridentate salicylato-type coordinated 2,5-dihydroxybenzoate trinuclear complex. *Polyhedron* 25: 1337-1347.

McKnight, D.M. and F.M.M. Morel. 1980. Copper complexation by siderophores from filamentous blue-green algae. *Limnology and Oceanography* 25, no. 1: 62-71.

“Moab, Utah.” 38º38'41.72"N 109º37'39.39"W. Elevation 4605 feet. Google Earth. Image date: September 14, 2011. Access date: March 31, 2012.

Monteiro, B., L. Cunha-Silva, S. Gago, J. Klinowski, F.A.A. Paz, J. Rocha, I.S. Gonçalves, and M. Pillinger. 2010. Crystal and supramolecular structures of dioxomolybdenum(VI) and dioxotungsten(VI) complexes of dihydroxybenzoic acids. *Polyhedron* 29: 719-730.

Nakatsua, C.H., V. Torsvik, and L. Øvreås. 2000. Soil community analysis using DGGE of 16s rDNA polymerase chain reaction products. *Soil Science Society of America Journal* 64: 1382-1388.

Pérez-Miranda, S., N. Cabirol, R. George-Téllez, L.S. Zamudio-Rivera, and F.J. Fernández. 2007. O-CAS, a fast and universal method for siderophore detection. *Journal of Microbiological Methods* 70: 127-131.

Pimentel, D., 2000. Soil erosion and the threat to food security and the environment. *Ecosystem Health* 6, no. 4: 221-226.

Powell, P.E., G.R. Cline, C.P.P. Reid, and P.J. Szaniszlo. 1980. Occurrence of hydroxamate siderophore iron chelators in soils. *Nature* 287: 833-834.

Puigdefábregas, J. 1998. Ecological impacts of global change on drylands and their implications for desertification. *Land Degradation and Development* 9: 393-406.

Reynolds, R., J. Neff, M. Reheis, and P. Lamothe. 2006. Atmospheric dust in modern soil on aeolian sandstone, Colorado Plateau (USA): Variation with landscape position and contribution to potential plant nutrients. *Geoderma* 130: 108-123.

Rossi, F., R. Potrafka, F. Garcia-Pichel, and R. De Philippis. 2012. Role of the exo-polysaccharides in enhancing hydraulic conductivity of biological soil crusts. *Soil Biology and Biochemistry* 46: 33-40.

Rychert, R.C. and J. Skujins. 1974. Nitrogen fixation by blue-green algae-lichen crust in the great basin desert. *Soil Science Society of America Proceedings* 38: 768-771.

Saal, L.B. and O.W. Duckworth. 2010. Synergistic dissolution of manganese oxides as promoted by siderophores and small organic acids. *Soil Science Society of America Journal* 74, no. 6: 2021-2031.

Saito, M.A., T.J. Goepgert, and J.T. Ritt. 2008. Some thoughts on the concept of colimitation: Three definitions and the importance of bioavailability. *Limnology and Oceanography* 53: 276-290.

Schulten, J.A. 1985. Soil aggregation by cryptogams of a sand prairie. *American Journal of Botany* 72, no. 11: 1657-1661.

Schwyn, B. and J.B. Neilands. 1987. Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry* 160: 47-56.

Sheridan, D. 1981. *Desertification of the United States*. Council on environmental quality. Washington, D.C.: U.S. Government Printing Office.

soilcrust.org. Southwest Biological Science Center. USGS Canyonlands Research Station. Access date: April 18th, 2012.

Steppe, T.F., J.B. Olson, H.W. Paerl, R.W. Litaker, and J. Belnap. 1996. Consortia N_2 fixation: A strategy for meeting nitrogen requirements of marine and terrestrial cyanobacterial mats. *FEMS Microbiology Ecology* 21: 149-156.

Szabó, O. and E. Farkas. 2011. Characterization of Mn(II) and Mn(III) binding capability of natural siderophores desferrioxamine b and desferricoprogen as well as model hydroxamic acids. *Inorganic Chimica Acta* 376: 500-508.

Wiebe, K. 2003. *Linking land quality, agircultural productivity, and food security.* Washington, DC: USDA Economic Research Service, Agricultural Economic Report No. 823.

CHAPTER 2

EFFECT OF NITROGEN-FIXING BIOLOGICAL SOIL CRUSTS ON SOIL SOLUTION TRACE METAL CONCENTRATIONS

Abstract

I studied the real-time effect of microbial activity on metal concentrations in the soil solution of biological soil crusts (BSCs) and how metal utilization by crusts changed with N availability. My focus was on the metals relevant for biological nitrogen (N_2) fixation, i.e., iron (Fe), molybdenum (Mo), and vanadium (V). I hypothesized that biological utilization by soil microbes would decrease soil solution metal concentrations. Fe concentrations in soil solutions were not affected by biological activity nor did they change with N availability. In contrast, Mo concentrations in soil solutions were lower in crusted samples than in killed controls, regardless of N availability. This indicated that the biological Mo-requirement in BSCs is high and was consistent with the utilization of Mo in multiple metabolic processes, including: N_2 fixation, assimilatory nitrate reduction, and nitrification. Similarly, concentrations of V in soil solution were lower in living crusts than in killed controls, which we interpreted as a signature of biological utilization. Soil solution V concentrations increased when N_2 fixation was interrupted by the addition of fixed nitrogen. I, therefore, speculate that some BSCs, may have a requirement for V during N_2 fixation. The results of this study show that

crust organisms actively alter soil solution metal concentrations in order to meet the significant metal requirements of N_2 fixation. This alteration may reflect a signature of crust metabolism that could be relevant for studies of crust activity in ancient terrestrial environments and has implications for arid-land conservation.

Introduction

Biological soil crusts (BSCs) are diverse microbial communities that colonize soils in arid and semi-arid environments (Garcia-Pichel et al. 2001; 2003). They cover large expanses between plants, and may include bacteria, archaea, algae, fungi, lichens, and bryophytes (Belnap et al. 2001). These organisms live in a vertically-stratified structure (Garcia-Pichel et al., 2003) similar to that of microbial mats (Cohen & Rosenberg, 1989) and biofilms (Doyle, 1999). The community includes photosynthetic organisms and nitrogen fixers (Garcia-Pichel, 2002), and thus provides the primary source of carbon and nitrogen to the environment (Beymer & Klopatek, 1991; Evans & Ehleringer, 1993; Evans & Lange, 2001; Belnap, 2002). The organisms in BSCs are adapted to withstand infrequent and, often, brief intervals of precipitation (Ashcroft et al., 1992), as well as intense UV radiation (Garcia-Pichel & Castenholz, 1991; Garcia-Pichel et al., 1992). Due to their crust-forming character, BSCs also play a critical role in erosion prevention in arid environments (Campbell, 1979; Schulten, 1985; Belnap, 1993).

After water, nitrogen (N) is the second-most limiting resource to biological systems in arid environments (Schlesinger, 1996; Evans & Lange, 2001). Samples for this study were collected on the Colorado Plateau, where biological fixation of atmospheric nitrogen gas (N_2) to ammonium (NH_4^+) is the primary source of N (Rychert & Skujins, 1974; Jeffries et al., 1992; Steppe et al., 1996; Belnap, 2002). Wet and dry atmospheric N deposition have been shown to be minimal in this region (West & Skujins, 1977; Evans & Ehleringer, 1993). N_2-fixation occurs during pulses of precipitation because crust organisms are only physiologically active when wet (Garcia-Pichel & Belnap, 1996). Cyanobacteria are the dominant group of N_2-fixing organisms in the "dark crusts" used in the present study (Belnap et al., 2001; Yeager et al., 2007). These "dark crusts" represent one type of crust, and they were chosen because they are dominated by diverse cyanobacteria with little to no lichen or moss cover (Garcia-Pichel & Belnap, 1996; Garcia-Pichel et al., 2001).

Biological N_2 fixation is performed by prokaryotes using the enzyme nitrogenase that requires more than 30 atoms of iron (Fe) and 2 atoms of either molybdenum (Mo), vanadium (V), or Fe at the active site where N_2 is reduced to NH_4^+ (Burgess & Lowe, 1996; Eady, 1996; Howard & Rees, 1996; Berman-Frank et al., 2003). The metal required at the active site is specific to the type of nitrogenase produced by an organism, which

depends on the particular nitrogenase genes the organism possesses. Thus, the ability to fix N_2 imparts an absolute requirement for Fe, accompanied by a requirement for either Mo, V, or additional Fe, depending on the type of nitrogenase produced. To date, almost all cyanobacteria examined use the Mo-dependent nitrogenase; the only three cyanobacteria shown to produce V-dependent nitrogenase are freshwater strains of *Anabaena* (Kentemich et al., 1988; Thiel, 1993; Boison et al., 2006). No cyanobacteria have been found to produce Fe-only nitrogenase. Therefore, it is not surprising that Mo-nitrogenase is found in the cyanobacterial-dominated dark crusts (Yeager et al., 2004; 2007). Evidence does not yet exist for the presence of V- or Fe-nitrogenase in BSCs of the Colorado Plateau; however, it is possible that some crust microbes produce these enzymes. Assuming that crust microbes behave like other organisms with alternative nitrogenase genes, if any crust organisms have the genes to produce V- or Fe-nitrogenase in addition to Mo-nitrogenase, they will only express V-nitrogenase in the absence of Mo, and only if both Mo and V are limiting, will they utilize Fe-nitrogenase (Jacobson et al., 1986; Joerger & Bishop, 1988; Pau et al., 1989; Luque & Pau, 1991; Jacobitz & Bishop, 1992).

Because crusts live in a water-limited environment, the only source of trace metals is the soil solid phase, i.e., minerals and organics. Crusts must obtain trace metals from the soil solid phase, either by taking

advantage of equilibrium dissolution following a wetting event, or by direct solubilization through the production of organic acids and metal chelators (Drever & Stillings, 1997; Kalinowski et al., 2000; Kraemer, 2004; Liermann et al., 2000; 2005). While the Colorado Plateau soils are dominated by quartz (~85%), they contain minor amounts (~10%) of clays and oxide/hydroxide minerals, that can provide a source of major cations and trace metals (Noonan, unpublished data; Reynolds et al., 2001; Reynolds et al., 2006). Previous work by Beraldi-Campesi et al. (2009) showed that crusted soil was depleted in calcium, chromium, manganese, copper, zinc, arsenic, and zirconium relative to nearby uncrusted soils; they interpreted the depletions as a signature of biological activity that mobilized the metals and left them vulnerable to leaching down the soil profile. Metal depletions in crusts suggest that BSCs are actively dissolving and/or desorbing metals from the solid-phase. The Beraldi-Campesi et al. study was a survey of bulk soils collected from several different locations; it did not assess the behavior of metals in soil solution during active crust metabolism. The present study complements and expands upon Beraldi-Campesi et al. (2009) by experimentally manipulating BSCs and measuring how crust metabolism influences soil geochemistry when the organisms are physiologically active.

As the conduit between microbes and minerals, I contend that the logical place to monitor biological influences on metal dynamics is in the

soil solution. Microbes are more likely to actively solubilize metals from the soil solid phase, including minerals and organics, if the available supply in the soil solution is insufficient for growth. Although microbial uptake will indirectly promote mineral dissolution by reducing soil solution concentrations, I postulate that limitation could still occur if rates of abiotic, equilibrium solid-phase dissolution are slower than rates of microbial uptake, or if BSCs are not wet long enough to achieve equilibrium between the solid-phase and soil solution. Therefore, I maintain that BSCs are likely to utilize direct dissolution strategies to obtain trace metals from the solid-phase if those metals exist at limiting concentrations in the soil solution. Using published data on cyanobacterial cellular metal contents and measured soil solution metal concentrations, I calculated that soil solution concentrations of Fe, Mo, and V are orders of magnitude lower than would be needed for BSCs to double their biomass. Thus, it is reasonable to expect that Fe, Mo and V availability limit growth of microbial populations in BSCs.

I hypothesized that BSCs would alter the soil solution concentrations of limiting nutrients, particularly the trace metals Fe, Mo, and V. I predicted that concentrations of Fe, Mo, and V would be lower in the soil solution of crusted samples than in killed controls as a result of both microbial uptake in living crusts and metal release from biomass during sterilization of killed controls. Additionally, I hypothesized that the effect of BSCs on soil

solution concentrations of trace metals would depend on N availability, and I expected that concentrations of Fe, Mo, and V would be lower in the soil solution of crusts actively fixing N_2 than in those not fixing N_2.

Methods

Field Site and Sample Collection

Individual samples of dark crusts and nearby uncrusted soil were collected at the "Sunday Churt" site (Garcia-Pichel et al., 2003) northeast of Moab, Utah on the Colorado Plateau (N 38°39′22.3″, W 109°39′13.8″) during October, 2007. Dark crusts were visually defined as areas with dark microbial cover and flat surfaces; these crusts were specifically targeted in order to avoid crusts with a high density of lichen cover.

Sample collection and storage were modeled after Garcia-Pichel et al. (2003). Prior to collection, sampling areas were wetted lightly with distilled water to improve soil surface cohesion. Briefly, plastic Petri dish lids (50 mm x 13 mm) were pressed into the soil surface. A plastic spatula was used to remove and invert the sample, allowing the bottom of the Petri dish to be inserted. Samples were stored dry in their original orientation for up to 6 months in the dark at room temperature (20ºC) prior to experimental manipulation (Campbell et al., 2009).

Experimental Setup

Simulated rainfall time-course experiments were conducted in incubation dishes modified from those described by Johnson et al. (2005).

Dishes were constructed using two plastic Petri dishes (50 mm x 13 mm). Small holes were drilled into the base of one dish, and a sampling port was drilled into the side of the other dish. The perforated dish was placed on top of the other dish, and they were sealed with three wraps of black electrical tape (Scotch® 3M Super33+) at the seam between the dishes. A Whatman™ GF/F filter (55 mm diameter, nominal pore size 0.7 µm) was placed above the perforated surface and aligned so that the edges of the filter rose ~1 mm above the base of the dish (Figure 2-1). Soils were placed on top of the filter. Soil solution was collected through the sampling port with gentle vacuum suction applied with a 60 mL syringe.

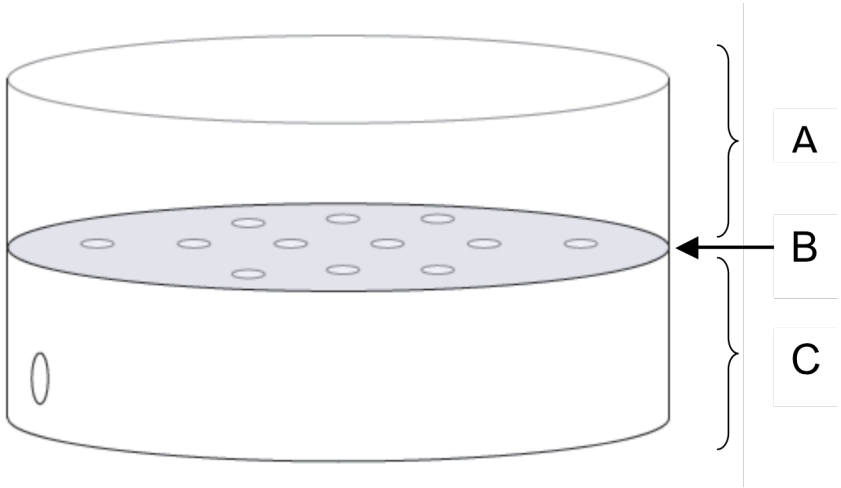

Figure 2-1. Schematic of incubation dishes constructed from two plastic Petri dishes. Soils were placed into upper dish (A). Perforations (B, arrow) in the base of Dish A covered by a GF/F filter allowed soil solution to pass from the soil into the collection chamber (C) with sampling port.

"Killed controls" were prepared by baking uncrusted soil at 500°C for 18 h in a muffle furnace. Because heating removed organic carbon and

nitrogen, it served to kill any soil microbes (Kang & Sajjapongse, 1980; Gustafsson et al., 1997; Acea & Carballas, 1999; Schumacher, 2002; Parlak, 2011). The predominant effect (if any) of heating on the mineral fraction of the soil should be to decrease the solubility of Fe (Kang & Sajjapongse, 1980; Quintana et al., 2007; Parlak, 2011), Mo, and V (Goldberg & Forster, 1998; Quintana et al., 2007). Cell lysis and the loss of biomass and soil organic carbon during heating, in contrast, are likely to increase the amount of soluble Fe, Mo, and V in killed controls by releasing metals present in cellular material and bound to organics. Therefore, I expected a modest increase in soluble metal concentration in the killed controls.

Eighteen crusted samples and 18 killed controls were transferred to Petri dish chambers in their original orientation (crusts at the surface) and weighed to establish initial dry soil mass prior to incubation. Two different nitrogen treatments were applied (~12 mL): a no nitrogen, or water-only, addition (–N), and a water plus nitrogen addition (+N). In the –N incubations, 18 MΩ·cm carbon-free water was added (NANOpure® DIamond™ UV, Barnstead International, Dubuque, IA) to 9 crusted samples and 9 killed controls. In the +N incubations an aqueous solution of 0.8 mM ammonium nitrate (NH_4NO_3) in 18 MΩ·cm water was added to the remaining 9 crusted samples and 9 killed controls. This NH_4NO_3

concentration was chosen to raise the ambient NH_4^+ and NO_3^- concentrations by approximately a factor of four.

At each time point, soils were sacrificed for soil solution collection. Therefore, the time-course data do not represent sequential sampling of the same soils through time. Rather, the data are from a set of soils sacrificed at 0 h, a second set sacrificed at 24 h, and a final set sacrificed at 48 h. This method was used because the soils could not retain enough water to allow three sequential soil solution collections.

The initial (0 h) samples were collected within 30 min of treatment water addition; 3 crusted samples and 3 killed controls from each treatment were sacrificed for a total of twelve 0 h samples (e.g., 3 –N, crusted; 3 –N, killed; 3 +N, crusted; 3 +N, killed). The remaining crusted samples and killed controls were transferred to a controlled environmental incubator maintained at 32°C ± 1°C with an average irradiance, measured as photosynthetically active radiation (PAR), of 115 μE m^{-2} s^{-1}). This temperature is comparable to summertime temperatures at the collection site. The irradiance I used was likely lower than what would be measured in the field on a sunny day, but high enough to allow BSCs to photosynthesize (Garcia-Pichel & Belnap, 1996). Chambers were placed under inverted 250 mL glass beakers to minimize evaporative water loss while maintaining gas exchange. Irradiance and temperature measured under the beakers did not differ from the ambient incubator conditions.

Soil solutions were collected from 3 crusted samples and 3 killed controls sacrificed from each treatment at 24 h; the final set of soil solutions was collected from the last 3 crusted samples and 3 killed controls at 48 h.

Soil Solution Processing

After collection with the 60 mL syringe, the soil solution was transferred to an acid-washed (10% HCl) polypropylene centrifuge tube, that was weighed empty and weighed again after soil solution addition to determine the mass of water collected. Soil solution was filtered through a 0.45 µm polysulfone membrane filter (Supor®, PES, Pall Corporation, Port Washington, NY), and 0.25-0.5 mL were transferred into additional tubes for analysis of nitrate (NO_3^-), ammonium (NH_4^+), and dissolved metals. All tubes were pre-rinsed and leached for at least 24 h with 18 MΩ·cm carbon-free water prior to sample collection.

Soil solution samples for NO_3^- and NH_4^+ analyses were diluted by a factor of 20-40 in 18 MΩ·cm water. Nitrate and NH_4^+ samples were stored at 4°C and analyzed within fourteen days. Samples for dissolved metals were stored undiluted at room temperature and analyzed within thirty days.

Nitrate was determined by ion chromatography on a Dionex Dual ICS 2000/1500 Ion Chromatograph (column: 4 mm AS23; eluant: 4.05 mM sodium carbonate/0.72 mM sodium bicarbonate; supressor: ASRS 300; flow rate: 1 mL min^{-1}). Ammonium was determined spectrophotometrically

using the Bertholet reaction on a Lachat Quick Chem 8000 Flow Injection Analyzer (Quick Chem method: 10-107-06-1-J). No additional sample preparation was required prior to analysis of NO_3^- and NH_4^+. Errors for NO_3^- and NH_4^+ represent the precision of triplicate analyses of quality control samples. Aliquots of soil solution for metals analysis were digested in concentrated nitric acid (HNO_3) and hydrogen peroxide to dissolve precipitates and remove organic phases. Following digestion, the samples were heated to dryness and reconstituted in 2% HNO_3. Concentrations of a suite of major and trace elements in the soil solutions were determined using inductively coupled plasma mass spectrometry (ICP-MS; Thermo Scientific X Series). Calibration of the instrument was obtained from a multi-element ICP-MS standard mixture. A mixed internal standard (Ge, Y, and In) was used to correct for instrumental drift throughout the runs. Errors for major and trace elements represent the standard deviation of triplicate instrument injections for individual samples.

Acetylene Reduction Assays

Nitrogenase activity was measured using a modification of the standard acetylene reduction assay (Capone, 1993; Jeffries et al., 1992; Berman-Frank et al., 2007). Because the nitrogenase enzyme can reduce acetylene ($HC{\equiv}CH$) to ethylene ($H_2C{=}CH_2$), in addition to reducing N_2 ($N{\equiv}N$) to NH_4^+, if ethylene is observed during an incubation it is assumed

that nitrogenase is active. This time-series experiment monitored the reduction of acetylene to ethylene over 48 h.

The acetylene reduction method was modified to accommodate the crust incubation dishes used in the –N and +N incubations described above. Polycarbonate Nalgene jars (125 mL; Nalge Nunc International Corporation; Rochester, NY) with rubber septa were used as incubation vessels. –N treatment water was added to 3 crusted samples and 3 killed controls. These crusts were similar to, but separate from, those used for soil solution collections. Dishes were placed in the Nalgene jars, and the lids were sealed with Parafilm® (American National Can) between the lid and jar. Air was removed (33 mL) from the jars using a 60 mL syringe, and 33 mL of acetylene was added (~20% headspace volume; Berman-Frank et al., 2007). Headspace samples (1 mL) were taken immediately after addition of acetylene for an initial time point. After collection of the 0 hr headspace samples, jars were incubated at 115 μE m^{-2} s^{-1} PAR and 32°C ± 1°C.

Headspace samples (1 mL) were taken at regular intervals over a period of 48 h. After sample collection, gas concentrations were measured using a Hewlett-Packard 5890 Series II Gas Chromatograph (Injection temp: 80ºC) with a thermal ionization detector (200ºC) and a 6' x 1/8" SS Porapak N 80/100 column (Ohio Valley Specialty Chemical, Marietta, OH) with helium carrier gas (14 mL min^{-1}). Acetylene and

ethylene were well resolved with retention times of 3.8 and 2.4 min, respectively. Ethylene peak area was converted to moles using a five-point calibration curve and then normalized to soil surface area.

After 48 h of incubation, soils were removed from the Nalgene jars and allowed to dry. After drying, the same soils were rewetted with the +N treatment water. The acetylene reduction assay was repeated under the incubation conditions described above. Supplementary experiments showed that exposure to acetylene for 48 h did not measurably affect acetylene reduction rates (data not shown).

Data Processing and Presentation

Concentrations from all analyses, except the acetylene reduction assay, were measured as mass per unit volume (i.e., mg L^{-1}). These data were converted to total moles in the soil solution and normalized to the dry mass of the soil, in grams (i.e., gdw^{-1}). This normalization allows comparison of data from different time points without the influence of evaporative concentration changes.

For nutrients (NO_3^-, NH_4^+) and the acetylene reduction assay, the method detection limit (MDL) was calculated according to Standard Method 1030C, using multiple measurements of a low concentration standard (Standard Method 1030C; Clesceri et al., 1998), and the limit of quantitation (LOQ) was calculated as 10 times the standard deviation of the same standard solution used to compute the MDL (Table 2-1). For

metal analyses, the limit of detection (LOD) and limit of quantitation (LOQ) were calculated as 3 and 10 times the standard deviation of a low concentration standard, respectively (Table 2-2). Measured concentrations below the LOD or MDL were plotted at zero. The few measured concentrations above the LOD or MDL, but below the LOQ were set to the LOQ value and used thus in downstream conversions and calculations.

Table 2-1. Limits of detection and quantitation for the nitrate, ammonium and acetylene reduction assays.

	MDL[a]	LOQ[b]
Nitrate	0.11 μM	0.39 μM
Ammonium	0.17 μM	0.56 μM
Acetylene Reduction	3.5 nmoles	20 nmoles

[a]MDL (method detection limit) calculated from Standard Method 1030C (Clesceri et al., 1998).
[b]LOQ (limit of quantitation) calculated as 10 times the standard deviation of the low concentration standard used for the MDL calcuation.

Table 2-2. Limits of detection and quantitation for iron, molybdenum, and vanadium analyses; all concentrations are in nM.

	LOD[a]	LOQ[b]
Iron	0.644	2.145
Molybdenum	0.062	0.209
Vanadium	0.471	1.570

[a]LOD (limit of detection) calculated as 3 times the standard deviation of a low concentration standard.
[b]LOQ (limit of quantitation) calculated as 10 times the standard deviation of a low concentration standard.

Statistical analyses were performed using SigmaPlot v11.0 (Systat Software, Inc., Chicago, IL). Linear regression analyses were performed for each nutrient in each soil type for both treatments. Datasets were then compared using either a Student's t-test or a Mann-Whitney Rank Sum Test. If both datasets passed the Shapiro-Wilk Normality Test, an Equal Variance Test and a Student's t-test were performed (unpaired). If one or both datasets failed the Shapiro-Wilk Normality Test, a Mann-Whitney Rank Sum Test was performed. Concentrations of each nutrient in crusted samples were compared to concentrations of the same nutrient in killed controls within each treatment (i.e., –N crusted vs –N killed or +N crusted vs +N killed). Significant differences between crusted samples and killed controls were interpreted to mean that BSC activity influenced soil solution nutrient concentrations. Finally, concentrations of each nutrient in the –N crusted samples were compared to concentrations of the same nutrient in +N crusted samples (i.e., –N Mo vs +N Mo). Significant differences between –N and +N crusted samples were inferred to mean that the effect of BSCs on nutrient concentrations was N-dependent. Significance is assumed for $p < 0.10$; significant result are shown in bold.

Results

Nitrate

At 0 h, nitrate (NO_3^-) was measurable in crusted samples for both the –N and +N incubations, and in killed controls of the +N incubation only

(Figure 2-2a, b). Soil solution NO_3^- concentrations in crusted samples rapidly decreased to below detection during both the –N and +N incubations (p = 0.06 and p = 0.01, respectively). Soil solution NO_3^-

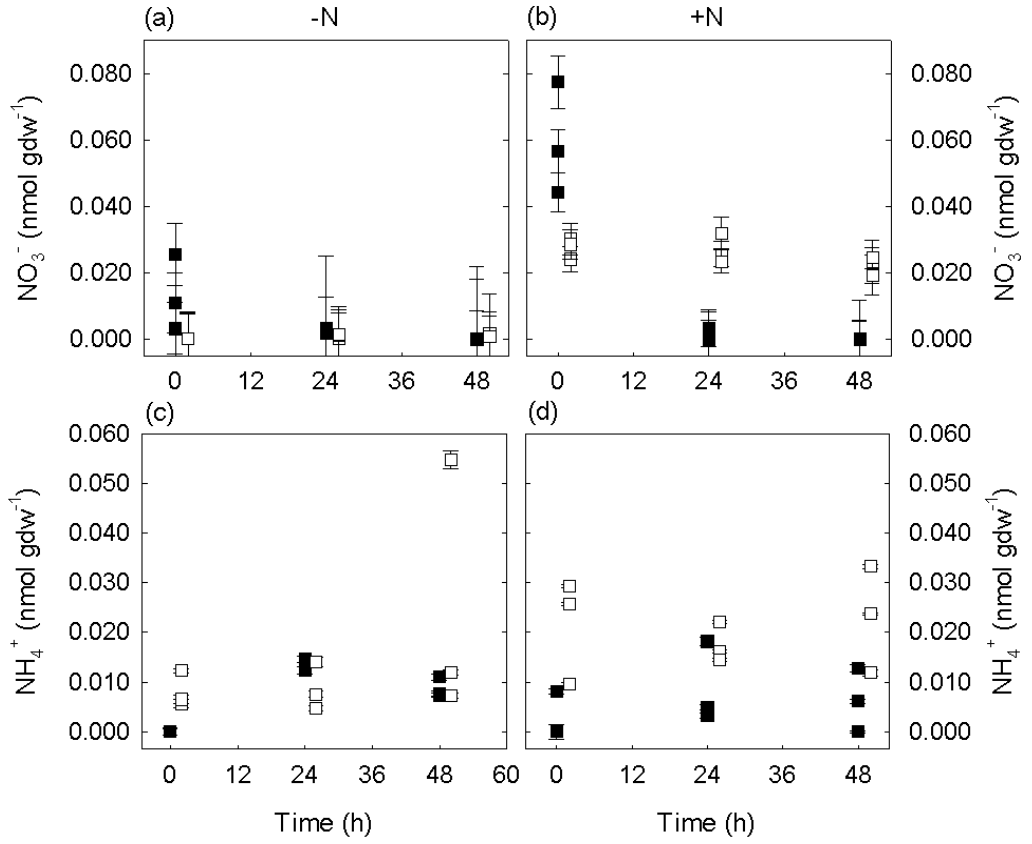

Figure 2-2. Normalized soil solution concentrations of nitrate (NO_3^-; panels (a) and (b)) and ammonium (NH_4^+; panels (c) and (d)). Crusted soils are solid squares (■) and killed controls are open squares (□); the open symbols are off-set by +2 h for clarity. Left-hand panels are from the –N incubation, right-hand panels are from the +N incubation. Error bars represent propagated error based on the analytical precision and the dry-weight normalization calculations. Data plotted at zero were below the LOD. Each data point represents an individual sacrificed soil sample. Concentrations of NO_3^- decreased rapidly in crusted samples, remained constant in +N killed controls, and increased very slightly in –N killed controls. Concentrations of NH_4^+ in crusted samples increased significantly over the duration of the –N incubation (p = 0.07), while NH_4^+ concentrations in crusted samples of the +N incubation and in killed controls of both incubations did not change significantly.

concentrations in –N killed controls increased slightly, but significantly (p = 0.01; Table 2-3), however concentrations were below the LOQ in all but one 48 h control, and below the LOD in half of the remaining soils. Soil solution NO_3^- concentrations in +N killed controls were generally higher than in crusted samples (Figure 2-2b) and constant during the experiment (Table 2-3). No statistical comparison could be made between crusted samples and killed controls in either incubation because NO_3^- decreased significantly in the crusted samples (Table 2-3, 2-4). For the same reason, NO_3^- concentrations could not be compared between treatments (Table 2-3, 2-5); but in both cases they decreased to near zero by 24 h.

Ammonium

Soil solution ammonium (NH_4^+) concentrations in crusted samples increased significantly over the course of the –N incubation (p = 0.07; Figure 2-2c, Table 2-3). Concentrations of soil solution NH_4^+ in killed controls of the –N incubation did not show a significant change over time, nor did those of the crusted samples or killed controls from the +N incubation. In general, soil solution concentrations of NH_4^+ were lower in crusted samples than in killed controls (Figure 2-2c, d), however, this difference was only significant in the +N incubation (p = 0.07; Table 2-4). Ammonium concentrations could not be statistically compared between treatments because NH_4^+ increased significantly in the –N incubation (Tables 2-3, 2-5), but they are generally somewhat higher (~2x).

Table 2-3. Results of linear regression analysis of concentration versus time for each nutrient, treatment, and soil type.

Nutrient	Treatment	Soil Type	Shapiro-Wilk Normality Test	Constant Variance Test	Slope	R^2	*p*-value[a]
NO_3^-	–N	Crusted	Passed	Failed	-0.0003	0.48	**0.06**
	+N	Killed	Passed	Passed	0.00002	0.63	**0.01**
	–N	Crusted	Passed	Passed	-0.0012	0.71	**0.01**
	+N	Killed	Passed	Passed	-0.0001	0.34	**0.10**
NO_4^+	–N	Crusted	Failed	Passed	0.0002	0.46	**0.07**
	+N	Killed	Failed	Failed	0.0003	0.21	0.22
	–N	Crusted	Passed	Passed	0.00003	0.01	0.77
	+N	Killed	Passed	Passed	0.00003	0.01	0.84
Fe	–N	Crusted	Passed	Passed	0.0006	0.01	0.77
	+N	Killed	Failed	Failed	-0.0569	0.16	0.33
	–N	Crusted	Failed	Passed	0.00005	0.00	0.96
	+N	Killed	Passed	Failed	-0.0094	0.16	0.32
Mo	–N	Crusted	Passed	Passed	0.0010	0.70	**0.01**
	+N	Killed	Passed	Passed	-0.0027	0.18	0.30
	–N	Crusted	Passed	Failed	0.00006	0.46	**0.05**
	+N	Killed	Passed	Passed	0.0009	0.13	0.39
V	–N	Crusted	Passed	Failed	0.0003	0.17	0.27
	+N	Killed	Passed	Failed	-0.0836	0.24	0.22
	–N	Crusted	Passed	Passed	0.00003	0.01	0.77
	+N	Killed	Passed	Passed	0.0024	0.00	0.88

[a]Significant values ($p < 0.10$) are shown in **bold**.

Table 2-4. Statistical comparison of nutrient concentrations in crusted soils and killed controls from the same treatment.

Treatment	Nutrient	Shapiro-Wilk Normality Test	Equal Variance	Comparison Performed	*p*-value	Soil with Higher Concentrations
–N	NO_3^-	NC[a]	NC	NC	NC	NC
	NH_4^+	NC	NC	NC	NC	NC
	Fe	Failed	NA[b]	Mann-Whitney	0.36	ND[c]
	Mo	NC	NC	NC	NC	NC
	V	Failed	NA	Mann-Whitney	**<0.001**[d]	Killed
+N	NO_3^-	NC	NC	NC	NC	NC
	NH_4^+	Passed	Passed	Student's unpaired t-test	**<0.001**	Killed
	Fe	Failed	NA	Mann-Whitney	0.16	ND
	Mo	Passed	NC	NC	NC	NC
	V	Failed	NA	Mann-Whitney	**<0.001**	Killed

[a]NC indicates the statistical comparison was not calculated because one or both datasets changed significantly over time.
[b]NA indicates a particular test did not apply to the given comparison.
[c]ND indicates no difference between soil types.
[d]Significant values ($p < 0.10$) are shown in **bold**.

Table 2-5. Statistical comparison of nutrient concentrations in crusted soils from the –N treatment with crusted soils from the +N treatment.

Nutrient	Shapiro-Wilk Normality Test	Equal Variance	Comparison Performed	*p*-value	Soil with Higher Concentrations
NO_3^-	NC[a]	NC	NC	NC	NC
NH_4^+	NC	NC	NC	NC	NC
Fe	Passed	Passed	Student's unpaired t-test	0.26	ND[b]
Mo	NC	NC	NC	NC	NC
V	Failed	NA[c]	Mann-Whitney	**0.01**[d]	+N

[a]NC indicates the statistical comparison was not calculated because one or both datasets changed significantly over time.
[b]ND indicates no difference between soil types.
[c]NA indicates a particular test did not apply to the given comparison.
[d]Significant values ($p < 0.10$) are shown in **bold.**

Iron

There were no significant changes in iron (Fe) concentration with time for either the –N or the +N incubation, nor were there any significant differences between soil solution iron (Fe) concentrations in crusted samples or killed controls (Figure 2-3a, b, Tables 3-5) or between treatments (Table 2-5).

Molybdenum

Concentrations of Mo in crusted samples increased significantly over the duration of both the –N and +N incubations (p = 0.01 and p = 0.05, respectively; Figure 2-3c, d, Table 2-3). There was no trend in Mo concentrations over time in the killed controls. Molybdenum concentrations in killed controls were generally two orders of magnitude higher than in crusted samples, however, p-values could not be calculated because Mo concentrations in crusted samples showed a significant increase with time (Table 2-3). Molybdenum concentrations in crusted samples did not vary between the –N and +N treatments (Figure 2-4a).

Vanadium

There were no significant changes in soil solution vanadium (V) concentration for any soil type over the duration of either the –N or the +N incubation (Figure 2-3e, f, Table 2-3). Vanadium concentrations were statistically higher in soil solutions of killed controls than in those of crusted samples (p < 0.001; Table 2-4). Soil solution V concentrations in

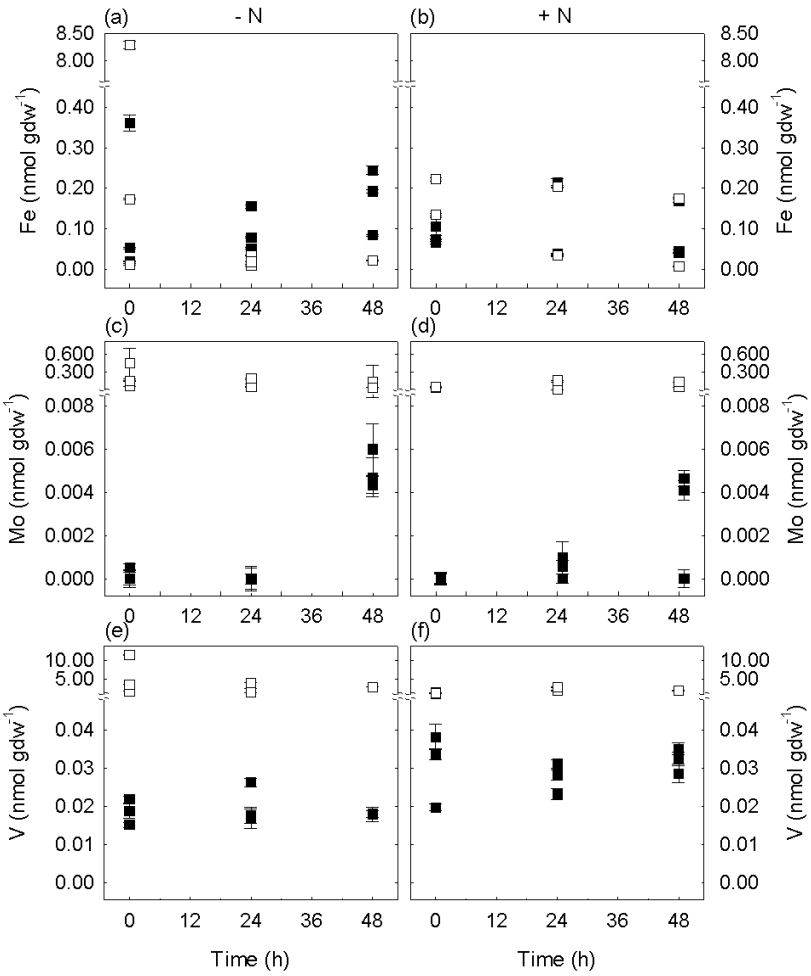

Figure 2-3. Soil solution metal concentrations normalized to soil dry-weight; panels (a) and (b) are Fe, (c) and (d) are Mo, and (e) and (f) are V. Crusted samples are solid squares (■) and killed controls are open squares (□). Left-hand panels are for the –N incubation, right-hand panels are for the +N incubation. Error bars represent propagated error based on the analytical precision and dry-weight normalization calculations. Each data point represents an individual sacrificed soil. The purpose of this figure is to highlight the differences between crusted samples and killed controls. Mo and V contents in killed controls were higher than those in crusted soils for both the –N and +N treatments. Results were significant for V ($p < 0.001$ for –N and +N). A statistical comparison between crusted samples and killed controls could not be performed for the Mo data because the crusted samples showed a significant increase with time. There was no statistical difference in Fe content between sample type or treatment.

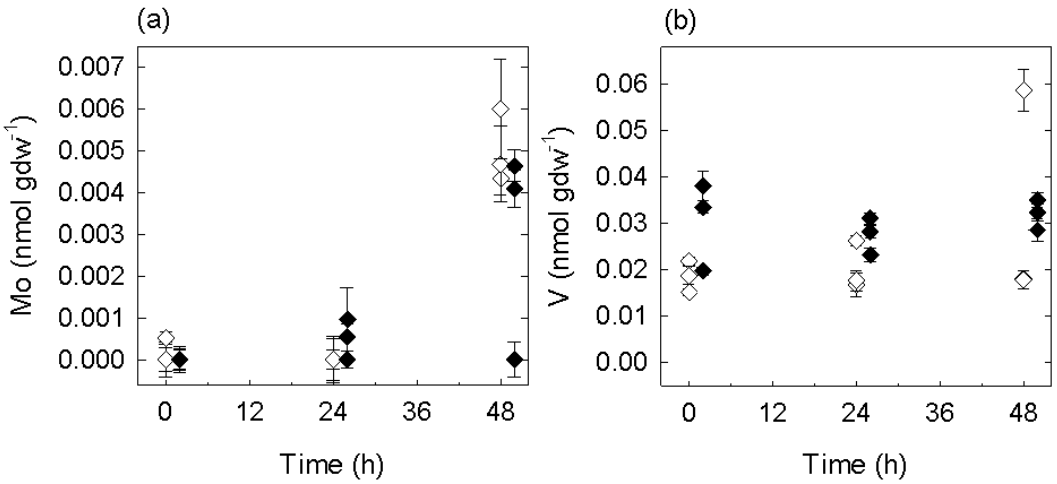

Figure 2-4. Soil solution metal concentrations normalized to soil dry weight for crusted soils in –N (open diamond, ◇) and +N (solid diamond, ◆) treatments; (a) Mo, (b) V. The solid symbols are off-set by +2 h for clarity. Error bars represent propagated error based on the analytical precision and the dry-weight normalization calculations. Data plotted at zero were below the LOD. Mo contents in both treatments were vanishingly low over the first 24 h and increased significantly at 48 h, but they cannot be statistically compared because both show significant increases over time (Table 2-3). V concentrations were significantly higher in the +N incubation (p = 0.01).

crusted samples differed by N treatment and were higher for the +N samples (p = 0.01; Figure 2-4b, Table 2-5).

Acetylene Reduction

In the –N treatment of crusted samples, ethylene was detectable after 22 h (Figure 2-5). Ethylene (C_2H_4) production rates ranged from 2.07 to 9.74 nmol C_2H_4 cm^{-2} h^{-1} over the course of the incubation; the rates were essentially constant through time and were statistically different from zero (p < 0.01) for all crusted soils. In contrast, ethylene was never detected in killed controls (data not shown).

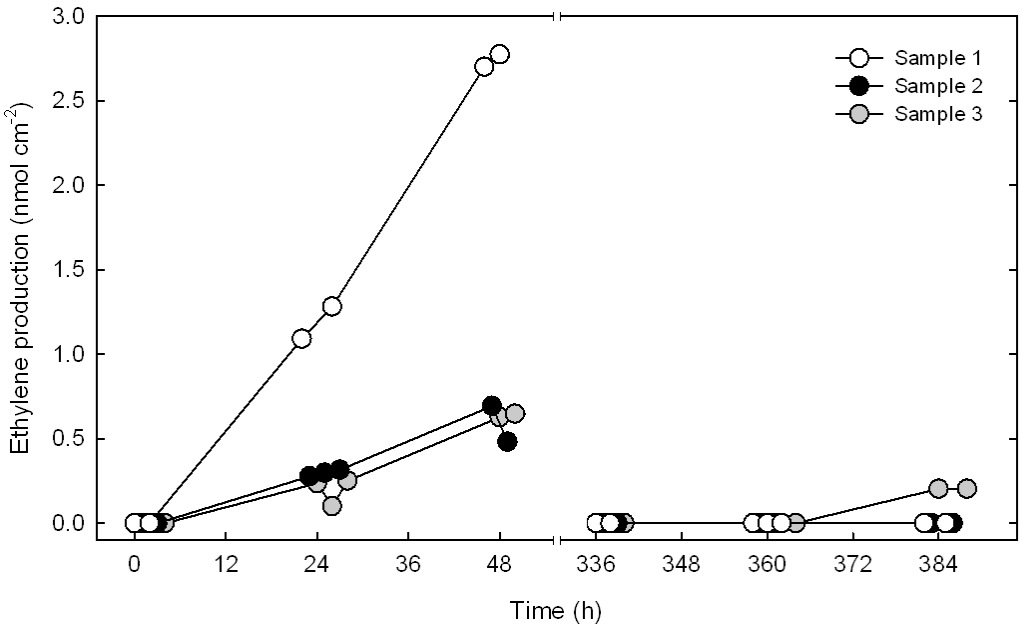

Figure 2-5. Ethylene production (nmoles cm^{-2}) in 3 crusted soils, as a function of time for –N (0 to 48 h) and +N (340 to 390 h) incubations. Each color represents a single soil followed during the first incubation (–N), and again during the second incubation (+N). Samples on the right are the same samples from the left. The x-axis break represents the drying period between the two incubations. All crusted samples produced ethylene under –N conditions; after drying, no crusts displayed ethylene production until 360 h, when just one of the three samples produced a small amount of ethylene.

After the –N acetylene reduction incubation, soils were allowed to dry, kept desiccated for 2 weeks in the dark, rehydrated with 0.8 mM aqueous NH_4NO_3, and used for the +N acetylene reduction incubation. All crusted samples produced ethylene in the –N incubation. In contrast, only one crusted sample produced a very small amount of ethylene toward the end of the +N incubation (Figure 2-5). This corresponds to a decrease in the average ethylene production rate from 2.26 nmol C_2H_4 cm^{-2} h^{-1} (–N) to

0.72 nmol C_2H_4 cm^{-2} h^{-1} (+N). No ethylene was detected in any of the other crusted samples or killed controls over the duration of the +N incubation.

Discussion

Effect of BSCs on Soil Solution Nutrient Concentrations

In order to determine whether BSC activity altered concentrations of the nutrients NO_3^-, Fe, Mo, and V, I compared their concentrations in the soil solutions of live crusted samples to concentrations in those of killed controls. The differences observed between the two soil types were interpreted to result from microbial activity. For example, using nitrate (NO_3^-) as a model nutrient, I found that, in general, soil solution NO_3^- concentrations were lower in crusted samples than in killed controls (Figure 2-2b). In addition, soil solution NO_3^- concentrations in crusted samples were measurable at 0 h, but decreased significantly (Table 2-3) to below detection limits (0.11 μM) after 48 h in both the –N and +N incubations (Figure 2-2a, b), and I interpret the swift removal of NO_3^- from the soil solution of live crusts to indicate microbial uptake.

Because crusted soil NO_3^- concentrations decreased significantly over the course of the incubation (Table 2-3), I could not statistically compare NO_3^- concentrations between crusted samples and killed controls. However, lack of any change in soil solution NO_3^- concentrations in the killed controls supports the conclusion that decreasing NO_3^- in crusted samples is the result of microbial uptake. Soil solution NO_3^- concentrations

in +N killed controls were above detection and stable over the course of the experiment (Figure 2-2a, b, Table 2-3). At the beginning of the +N incubation, the dry weight-normalized NO_3^- concentrations in the killed controls were lower than those of the crusted samples even though the same amount of N was added to all soils. This is most likely due to differences in soil mass (killed controls happened to have slightly higher initial weights, so their normalized concentrations were lower), as well as the fact that killed controls started out with less NO_3^- due to losses upon heating. In the –N killed controls, soil solution NO_3^- concentrations were low throughout the incubation, which I expected since N was was removed from the soil by our high-temperature sterilization technique (Kang & Sajjapongse, 1980; Giovannini et al., 1990; Parlak, 2011). Concentrations of NO_3^- in soil solutions of –N killed controls increased slightly over the course of the incubation, possibly as a result of desorption (Brady & Weil, 2002). But, although the NO_3^- concentrations in –N killed controls changed significantly over time, the direction of the change was opposite that of the crusted samples. Therefore, taken together, the invariant NO_3^- concentrations in +N killed controls and the increasing NO_3^- concentrations in –N killed controls are consistent with our interpretation that uptake by live crusts causes a decrease in soil solution NO_3^- concentrations.

The fact that soil solution NO_3^- concentrations were typically lower in crusted samples than in killed controls confirmed that BSC activity alters soil solution concentrations of limiting nutrients, causing nutrient concentrations to be lower in the soil solution of live crusts. The overall lower soil solution NO_3^- concentrations in crusted samples of the +N treatment, coupled with the rapid decrease in soil solution NO_3^- concentrations of crusted samples, were most likely caused by cellular uptake and assimilation under N-limiting conditions, rather than NO_3^- being used for denitrification or anaerobic ammonia oxidation (anammox), as these two dissimilatory processes have been shown to be very low in comparison to N_2 fixation in crusts (Johnson et al., 2007; Strauss et al., 2012). I thus conclude that: (1) Mo-requiring assimilatory nitrate reductases were active under my experimental conditions, and (2) the behavior of NO_3^- represented that of a limiting nutrient in the crust system. I expected that if crust organisms were also limited with respect to Fe, Mo, and/or V I would detect similar patterns in their concentrations over the course of the incubations.

The expectation that Fe, Mo, and V would behave like NO_3^- was only reasonable if the crust organisms were in need of these metals. I compared the amount of Fe, Mo and V theoretically present in the crust biomass (Madigan et al., 2003; Tuit et al., 2004; Tovar-Sanchez et al., 2006; White et al., 2006; Beraldi-Campesi et al., 2009; Glass et al., 2009)

with the amount of each metal measured in the soil solution (see Table 2-6 for an outline of this calculation and the input parameters used). The amount of metal in the soil solution was stoichiometrically 1-3 orders of magnitude lower than the amount calculated for the biomass. As discussed previously, removal of trace metals from the soil solution by BSCs will result in increased dissolution of metals from the solid-phase if the system is at equilibrium. However, I contend that it is possible for BSCs to be limited with respect to metals if dissolution rates are slower than microbial uptake rates or if the system is not active long enough to reach equilibrium. Therefore, I conclude it was reasonable to assume crusts were limited with respect to Fe, Mo, and V (Table 2-6); since the soils did not have enough dissolved Fe, Mo, or V in solution for crusts to double their biomass, I expected to see effects of microbial activity on soil solution metal concentrations.

Similar to the pattern observed for NO_3^-, soil solution concentrations of Mo and V were lower in crusted samples than in killed controls (Figure 2-3c-f, Table 2-4). The results of this comparison were significant for V in both incubations ($p < 0.001$). While a statistical comparison between crusted soils and killed controls could not be performed for the Mo data because the crusted samples showed a significant increase in dissolved Mo with time, there are clearly observable differences between crusted samples and killed controls. I interpret lower metal concentrations in

Table 2-6. Comparison of metal availability in the soil solution to estimated metal contents in crust biomass derived from a range of cyanobacteria cellular metal contents.

Cyanobacterium	Metal	Metal:Carbon Ratios Max.	Min.	Avg.	Calculated Biomass Content (μmol)[d]	Measured Soil Solution Content (μmol)[5]
Trichodesmium spp.[1,2]	Fe	17[a]	134	**75.5**[b]	0.91	0.002-0.003
	Mo	2	5	**3.5**	0.04	<0.001
	V	9	22	**15.5**	0.19	0.001
Nostoc 7120[3]	Mo	10	100	**55**	0.66	<0.001
Nostoc spp. CCMP2511[3]	Mo	1	6	**3.5**	0.04	<0.001
Crocosphaera spp.[4]	Fe			*15.8*[c]	0.19	0.002-0.003
	Mo			*0.70*	0.01	<0.001

[1]Tovar-Sanchez et al. (2006), [2]White et al. (2006), [3]Glass et al. (2009), [4]Tuit et al. (2004), [5]current study

[a]Metal:Carbon ratios for *Trichodesmium* spp. were calculated from metal-to-phosphorus ratios from Tovar-Sanchez et al. (2006) and carbon-to-phosphorus ratios from natural *Trichodesmium* populations reported by White et al. (2006).

[b]**Bold** indicates averages calculated from the reported minimum and maximum values

[c] *Italics* indicate values reported as averages in the literature

[d]Outline of the calculation sequence to determine metal content in crust biomass:
(1) Use total organic carbon (TOC) in crusted soils (0.65 wt%; Beraldi-Campesi et al., 2009); (2) Assume microbial biomass is ~2x TOC (Madigan et al., 2003); (3) calculate weight percent biomass; 2 x 0.65 = 1.3 wt % biomass; (4) Use average crust mass (23 g); (5) calculate g biomass; 1.3% x 23 g = 0.30 g biomass; (6) Assume microbial biomass is ~50% C by weight, calculate biomass carbon; 0.5 x 0.30 g = 0.15 g biomass C = 0.012 moles biomass C; (7) Use metal:carbon ratios of cyanobacteria from literature (Columns 3-5) and moles biomass C from step 4, calculate biomass metal; metal:carbon ratio x moles biomass C = μmol metal in crust (Column 6).

crusted samples as evidence for Mo and V uptake by BSCs. Higher Mo and V in the soil solutions of killed controls was likely due to release of these metals from biomass during heating. The difference in Mo and V concentrations between crusted samples and killed controls is consistent with the prediction that crusts were limited with respect to Mo and V, and the hypothesis that BSCs alter soil solution concentrations of limiting nutrients.

In contrast to the patterns described for NO_3^-, Mo, and V, soil solution Fe concentrations in crusted samples and killed controls were not statistically different from one another, nor did soil solution Fe concentrations in crusted samples change significantly over the duration of the experiment (Figure 2-3a, b, Tables 2-3, 2-4, 2-5,). This result demonstrates that I cannot observe the effects of microbial activity on soil solution Fe concentrations under our experimental conditions. Soil solution Fe concentrations were generally high, and it is possible biologically-mediated changes in soil solution Fe content were too small for my methods to detect, or that changes in Fe concentrations in BSCs are dominated by abiotic processes, such as adsorption/desorption reactions (Brady & Weil, 2002).

Ammonium (NH_4^+) did not behave like a typical limiting nutrient, most likely because multiple processes, both biotic and abiotic, influence NH_4^+ dynamics in soil (Brady & Weil, 2002; Johnson et al., 2005; Strauss et al.,

2012). However, I did observe a significant increase in soil solution NH_4^+ concentrations in crusted samples over the course of the –N incubation (p = 0.07; Figure 2-2c, Table 2-3) that most likely reflects active N_2-fixation. The acetylene reduction rate data can be used to estimate N_2-fixation rates and, thus, how much NH_4^+ could presumably be produced. The ethylene-to-equivalent-NH_4^+ ratios used to estimate N_2 fixation vary greatly (~2 to ~56; reviewed in Belnap, 2001); but regardless of the ratio used, all the measured acetylene reduction rates were sufficient to account for the observed increase in NH_4^+ in –N crusted soils (calculation not shown). And, of course, NH_4^+ oxidation would happen concurrently.

Influence of N Availability on Soil Solution Trace Metal Concentrations

From the previous analysis of crusted samples versus killed controls I can confidently say that BSC activity influences soil solution concentrations of NO_3^-, Mo, and V. Next, I wanted to know if the effect of BSC metabolism on Mo and V was affected by N availability. In order to do this I compared soil solution concentrations of Mo and V between –N and +N treatments of crusted samples (Figure 2-4a, b, Table 2-5). I predicted that Mo and V concentrations would be lower in –N soils because the microbes would need more Mo and V for N_2 fixation. When N was added (+N) I expected that Mo and V soil solution concentrations would be higher due to reduced metal requirements when N_2-fixation was not active. The acetylene reduction assay, showed that N_2 fixation was

inactive in the +N crusts and active in the –N crusts; i.e., BSCs fixed N_2 in their natural N-limited state (–N), and N_2-fixation ceased when N was supplied to N_2-fixing crusts (+N; Figure 2-5). The absolute rates of ethylene production in my crusts were similar to other published values (Table 2-7; Belnap, 2002; Johnson et al., 2005; Housman et al., 2006; Strauss et al., 2012), including rates measured at the same "Sunday Churt" site (Table 2-7; Strauss et al., 2012). Additionally, crusts given no

Table 2-7. Ethylene production rates (nmol C_2H_4 cm^{-2} h^{-1}) in biological soil crusts. Values from Strauss et al. (2012) are from the same Sunday Churt site sampled for the current study.

Ethylene Production Rate	Reference
0-5	Belnap, 2002
4.8	Johnson et al., 2005
0.2-1.2	Housman et al., 2006
2-4	Strauss et al., 2012
2.07-9.74	Current study

additional nitrogen in week 2 of the acetylene reduction assay continued to fix N_2 at rates comparable to those of week 1 (data not shown). I, therefore, conclude that prior and prolonged exposure to acetylene did not inhibit N_2-fixation in crusts that received N in the second week of the experiment. Furthermore, I conclude that the absence of N_2-fixation in +N crusted samples is indeed caused by alleviating N limitation. That organisms cease N_2-fixation when N is available is a well-known physiological response in N_2-fixing microorganisms, including cyanobacteria, where the activity and expression of nitrogenase are

repressed by bioavailable forms of N, like NH_4^+ and NO_3^- (Cejudo & Paneque, 1986; Kennedy & Toukdarian, 1987; Sanz et al., 1995; Sroga, 1997; Flores & Herrero, 2005). Importantly for my study, this phenomenon provides an opportunity to experimentally contrast the geochemical consequences of N_2-fixation, in that I can turn the process off while still maintaining an active microbial community.

Regardless of N status, soil solution Mo concentrations in crusted samples were low for the first 24 h of incubation, and increased significantly after 48 h (p = 0.01 for –N and p = 0.05 for +N; Figure 2-4a, Table 2-3). Molybdenum dynamics were, therefore, independent of N addition, suggesting N_2-fixation was not the only process driving Mo requirements in these soils. This is not too surprising in that I have evidence for the concurrent presence of assimilatory nitrate reduction (see above) that also uses a Mo-enzyme (Solomonson et al., 1984; Hille, 1996; Rubio et al., 1999; Rubio et al., 2002; Butler & Richardson, 2005) and for additional biological processes that require Mo as well (reviewed in McMaster et al., 2007).

Increasing dissolved Mo concentrations, suggests microbial Mo uptake was not the only biological process that influenced Mo concentrations in crusted samples. Since N_2 fixation rates and NO_3^- dynamics indicated an active microbial community in the crusts with increased Mo concentrations, it is unlikely that the increase was caused by Mo release

from cells after lysis. When bulk pH was monitored over the incubation time no significant change was observed that could account for an increase in Mo solubility (data not shown). Additionally, killed controls exhibited no change in soil solution Mo concentrations over the course of the experiment (Figure 2-3c, d, Table 2-3). Therefore, I interpret the increase in Mo concentration in crusted samples to result from direct solubilization of solid-phase Mo by metabolically active crust microbes.

Vanadium concentrations (in contrast to Mo) exhibited a response to N addition (Figure 2-4b, Table 2-5). In general, I observed that V concentrations in +N crusts were statistically higher than in –N crusts (p = 0.01). This is consistent with the hypothesis that microbial V uptake would decrease with N addition, resulting in increased soil solution metal concentrations. Vanadium requirements are relatively specific to N_2 fixation, but are not universal. Vanadium use for N_2-fixation depends entirely on whether or not the organisms fixing N_2 in BSCs have the genes to produce V-nitrogenase, which we do not know at this time. Vanadium use in nitrogenase also depends on whether or not organisms have sufficient Mo, since microbes will only produce V-nitrogenase under Mo-deficiency (Jacobson et al., 1986; Joerger & Bishop, 1988; Pau et al., 1989; Luque & Pau, 1991; Jacobitz & Bishop, 1992). In my case, Mo was below detection in all but the last hours of the incubations; concentrations were, therefore, low enough to expect the expression of V-nitrogenase to

be possible if the organisms have the capability. Thus, I interpret the lower soil solution V concentrations in the –N incubation as potential evidence that V may be taken up to supply nitrogenase when crusts are fixing N_2. As a cautionary note one must recognize, that in principle, BSC organisms could use V in haloperoxidases, a group of enzymes that catalyze the halogenation of organic compounds in the presence of peroxides (Butler, 1998; Littlechild, 1999). However, there is no logical reason to expect that haloperoxidase production would be dependent on N availability.

Implications

My results show that live crusts alter soil solution Mo and V concentrations, and that N cycling, in particular, may be linked to V availability in BSCs. Therefore, metal dynamics have the potential to provide clues about active biological processes and nitrogen availability in BSCs. Additionally, I provide experimental evidence to support the theory that metal depletions in soils may be a biosignature of BSC activity, as put forward by Beraldi-Campesi et al. (2009), and could be used to indicate the presence of crusts in terrestrial environments on the early Earth and in other places. However, the potential for a metal to be diagnostic of a particular biological process, or to serve as a biosignature, is entirely metal-specific and depends on both the abundance of the metal in the soil and the metabolic pathways that require the metal.

The results of this study may also prove useful for rangeland management on the Colorado Plateau and elsewhere. BSCs are crucial to ecosystem function in arid lands throughout the world, and understanding what controls their success is paramount for land conservation and restoration. Davidson et al. (2002) asserted that BSC management requires an understanding of the factors that limit BSC colonization, establishment, and growth rates. Similarly, Bowker et al. (2005) stressed the value in determining which micronutrients limit BSCs in order to develop BSC restoration techniques to combat desertification; they went on to propose that fertilization-based restoration methods would be appropriate to reduce soil fertility losses in arid environments throughout the world. My results show that Mo is actively manipulated by BSC community metabolism and that V may be a heretofore neglected nutrient that could be important for the ecology of BSCs. It remains to be determined if Mo and V additions increase the productivity and N_2 fixation capacity of BSC communities *in situ*, before concluding whether or not Mo and V are good candidates for crust fertilization efforts.

References

Acea, M.J. and T. Carballas. 1999. Microbial fluctuations after soil heating and organic amendment. *Bioresource Technology* 67: 65-71.

Ashcroft, G.L., D.T. Jensen, and J.L. Brown. 1992. *Utah climate*. Logan, UT: Utah State University.

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

_____. 2001. Factors influencing nitrogen fixation and nitrogen release in biological soil crusts. In *Biological soil crusts: Structure, function, and management*, ed. J. Belnap and O.L. Lange: 241-261. Berlin Heidelberg New York: Springer.

_____. 2002. Nitrogen fixation in biological soil crusts from southeast Utah, USA. *Biological Fertility of Soils* 35: 128-135.

Belnap, J., B. Budel, and O.L. Lange 2001. Biological soil crusts: Characteristics and distribution. In *Biological soil crusts: Structure, function, and management*, ed. Jayne Belnap and Otto L. Lange: 503. Heidelberg: Springer.

Beraldi-Campesi, H., H. Hartnett, A. Anbar, G. 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, no. 3: 348-359.

Berman-Frank, I., P. Lundgren, and P.G. Falkowski. 2003. Nitrogen fixation and photosynthetic oxygen evolution in cyanobacteria. *Research in Microbiology* 154: 157-164.

Berman-Frank, I., A. Quigg, Z.V. Finkel, A.J. Irwin, and L. Haramaty. 2007. Nitrogen-fixation strategies and Fe requirements in cyanobacteria. *Limnology and Oceanography* 52, no. 5: 2260-2269.

Beymer, R.J. and J.M. Klopatek. 1991. Potential contribution of carbon by microphytic crusts in pinyon-juniper woodlands. *Arid Soil Research and Rehabilitation* 5: 187-198.

Boison, G., C. Steingen, L.J. Stal, and H. Bothe. 2006. The rice field cyanobacteria *Anabaena azotica* and *Anabaena* sp. Ch1 express vanadium-dependent nitrogenase. *Archives of Microbiology* 186: 367-376.

Bowker, M.A., J. Belnap, D.W. Davidson, and S.L. Phillips. 2005. Evidence for micronutrient limitation of biological soil crusts: Importance to arid-lands restoration. *Ecological Applications* 15, no. 6: 1941-1951.

Brady, N.C. and R.R. Weil. 2002. *The nature and properties of soils*. Upper Saddle River: Prentice Hall.

Burgess, B.K. and D.J. Lowe. 1996. Mechanism of molybdenum nitrogenase. *Chemical Reviews* 96: 2983-3011.

Butler, A. 1998. Vanadium haloperoxidases. *Current Opinion in Chemical Biology* 2: 279-285.

Butler, C.S. and D.J. Richardson. 2005. The emerging molecular structure of the nitrogen cycle: An introduction to the proceedings of the 10th annual N-cycle meeting. *Biochemical Society Transactions* 33, no. 1: 113-118.

Campbell, J.H., J.S. Clark, and J.C. Zak. 2009. PCR-DGGE comparison of bacterial community structure in fresh and archived soils sampled along a Chihuahuan desert elevational gradient. *Microbial Ecology* 57: 261-266.

Campbell, S.E. 1979. Soil stabilization by a prokaryotic desert crust: Implications for precambrian land biota. *Origins of Life* 9: 335-348.

Capone, D.G. 1993. Determination of nitrogenase activity in aquatic samples using the acetylene reduction procedure. In *Handbook of methods in aquatic microbial ecology*, ed. P.F. Kemp, B.F. Sherr, E.B. Sherr and J.J. Cole: 621-631. New York: CRC Press LLC.

Cejudo, F.J. and A. Paneque. 1986. Short-term nitrate (nitrite) inhibition of nitrogen fixation in *Azotobacter chroococcum*. *Journal of Bacteriology* 165, no. 1: 240-243.

Clesceri, L.S., A.E. Greenberg, and A.D. Eaton. 1998. *Standard methods for the examination of water and wastewater*. Baltimore, MD, USA: United Book Press, Inc.

Cohen, Y. and E. Rosenberg, eds. 1989. *Microbial mats: Physiological ecology of benthic microbial communities*. Washington, DC: American Society for Microbiology.

Davidson, D.W., M.A. Bowker, D. George, S.L. Phillips, and J. Belnap. 2002. Treatment effects on performance of N-fixing lichens in disturbed soil crusts of the Colorado Plateau. *Ecological Applications* 12, no. 5: 1391-1405.

Doyle, R.J., ed. 1999. *Biofilms*. San Diego: Academic Press.

Drever, J.I. and L.L. Stillings. 1997. The role of organic acids in mineral weathering. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 120: 167-181.

Eady, R.E. 1996. Structure-function relationships of the alternative nitrogenases. *Chemical Reviews* 96: 3013-3030.

Evans, R.D. and J.R. Ehleringer. 1993. A break in the nitrogen cycle in aridlands? Evidence from delta15N of soils. *Oecologia* 94: 314-317.

Evans, R.D. and O.L. Lange. 2001. Biological soil crusts and ecosystem nitrogen and carbon dynamics. In *Biological soil crusts: Structure, function, and management*, ed. J. Belnap and O.L. Lange: 263-279. Berlin: Springer-Verlag.

Flores, E. and A. Herrero. 2005. Nitrogen assimilation and nitrogen control in cyanobacteria. *Biochemical Society Transactions* 33, no. 1: 164-167.

Garcia-Pichel, F. 2002. Desert environments: Biological soil crusts. In *Encyclopedia of environmental microbiology*, ed. G. Bitton:1019-1023. New York: John Wiley.

Garcia-Pichel, F. and J. Belnap. 1996. Microenvironments and microscale productivity of cyanobacterial desert crusts. *Journal of Phycology* 32: 774-782.

Garcia-Pichel, F. and R.W. Castenholz. 1991. Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. *Journal of Phycology* 27, no. 3: 395-409.

Garcia-Pichel, F., S.L. Johnson, D. Youngkin, and J. Belnap. 2003. Small-scale vertical distribution of bacterial biomass and diversity in biological soil crusts from arid lands in the Colorado Plateau. *Microbial Ecology* 46: 312-321.

Garcia-Pichel, F., A. Lopez-Cortes, and U. Nubel. 2001. Phylogenetic and morphological diversity of cyanobacteria in soil desert crusts from the colorado plateau. *Applied and Environmental Microbiology* 67, no. 4: 1902-1910.

Garcia-Pichel, F., N.D. Sherry, and R.W. Castenholz. 1992. Evidence for an ultraviolet sunscreen role of the extracellular pigment scytonemin in the terrestrial cyanobacterium *Chlorogloeopsis sp*. *Photochemistry and Photobiology* 56, no. 1: 17-23.

Giovannini, G., S. Lucchesi, and M. Giachetti. 1990. Effects of heating on some chemical parameters related to soil fertility and plant growth. *Soil Science* 149, no. 6: 344-350.

Glass, J.B., F. Wolfe-Simon, and A. Anbar. 2009. Coevolution of metal availability and nitrogen assimilation in cyanobacteria and algae. *Geobiology* 7, no. 2: 100-123.

Goldberg, S. and H.S. Forster. 1998. Factors affecting molybdenum adsorption by soils and minerals. *Soil Science* 163, no. 2: 109-114.

Gustafsson, O., F. Haghseta, C. Chan, J. Macfarlane, and P.M. Gschwend. 1997. Quantification of the dilute sedimentary soot phase: Implications for PAH speciation and bioavailability. *Environmental Science and Technology* 31: 203-209.

Hille, R. 1996. The mononuclear molybdenum enzymes. *Chemical Reviews* 96: 2757-2816.

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.

Howard, J.B. and D.C. Rees. 1996. Structural basis of biological nitrogen fixation. *Chemical Reviews* 96: 2965-2982.

Jacobitz, S. and P.E. Bishop. 1992. Regulation of nitrogenase-2 in *azotobacter vinelandii* by ammonium, molybdenum, and vanadium. *Journal of Bacteriology* 174, no. 12: 3884-3888.

Jacobson, M.R., R. Premakumar, and P.E. Bishop. 1986. Transcriptional regulation of nitrogen fixation by molybdenum in *Azotobacter vinelandii*. *Journal of Bacteriology* 167, no. 2: 480-486.

Jeffries, D.L., J.M. Klopatek, S.O. Link, and J. Bolton. 1992. Acetylene reduction by cryptogamic crusts from a blackbrush community as related to resaturation and dehydration. *Soil Biology and Biochemistry* 24: 1101-1105.

Joerger, R.D. and P.E. Bishop. 1988. Bacterial alternative nitrogen fixation systems. *Critical Reviews in Microbiology* 16: 1-14.

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, no. 1: 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, no. 3: 680-689.

Kalinowski, B.E., L.J. Liermann, S. Givens, and S.L. Brantley. 2000. Rates of bacteria-promoted solubilization of Fe from minerals: A review of problems and approaches. *Chemical Geology* 169: 357-370.

Kang, B.T. and A. Sajjapongse. 1980. Effect of heating on properties of some soils from southern Nigeria and growth of rice. *Plant and Soil* 55: 85-95.

Kennedy, C. and A. Toukdarian. 1987. Genetics of azotobacters: Applications to nitrogen fixation and related aspects of metabolism. *Annual Review in Microbiology* 41: 227-258.

Kentemich, T., G. Danneberg, B. Hundeshagen, and H. Bothe. 1988. Evidence for the occurrence of the alternative, vanadium-containing nitrogenase in the cyanobacterium *Anabaena variabilis FEMS Microbiology Letters* 51: 19-24.

Kraemer, S.M. 2004. Iron oxide dissolution and solubility in the presence of siderophores. *Aquatic Science* 66: 3-18.

Liermann, L.J., R.L. Guynn, A. Anbar, and S.L. Brantley. 2005. Production of a molybdophore during metal-targeted dissolution of silicates by soil bacteria. *Chemical Geology* 220: 285-302.

Liermann, L.J., B.E. Kalinowski, S.L. Brantley, and J.G. Ferrey. 2000. Role of bacterial siderophores in dissolution of hornblende. *Geochimica et Cosmochimica Acta* 64, no. 4: 587-602.

Littlechild, J. 1999. Haloperoxidases and their role in biotransformation reactions. *Current Opinion in Chemical Biology* 3: 28-34.

Luque, F. and R.N. Pau. 1991. Transcriptional regulation by metals of structural genes for *Azotobacter vinelandii* nitrogenases. *Molecular and General Genetics* 227: 481-487.

Madigan, T.M., J.M. Martinko, and J. Parker. 2003. *Biology of microorganisms*. Edited by T.D. Brock. Upper Saddle River, NJ: Prentice Hall.

McMaster, J., C.D. Garner, and E.I. Stiefel. 2007. Molybdenum enzymes. In *Biological inorganic chemistry structure and reactivity*, ed. I. Bertini, H.B. Gray, E.I. Stiefel and J.S. Valentine: 518-544. Sausalito, California: University Science Books.

Parlak, M. 2011. Effect of heating on some physical, chemical and mineralogical aspects of forest soil. *Journal of the Bartin Faculty of Forestry* 13, no. 19: 143-152.

Pau, R.N., L.A. Mitchenall, and R.L. Robson. 1989. Genetic evidence for an *Azotobacter vinelandii* nitrogenase lacking molybdenum and vanadium. *Journal of Bacteriology* 171, no. 1: 124-129.

Quintana, J.R., V. Cala, A.M. Moreno, and J.G. Parra. 2007. Effect of heating on mineral components of the soil organic horizon from a spanish juniper (*Juniperus thurifera* l.) woodland. *Journal of Arid Environments* 71: 45-56.

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 Science of the United States of America* 98, no. 13: 7123-7127.

Reynolds, R., J. Neff, M. Reheis, and P. Lamothe. 2006. Atmospheric dust in modern soil on aeolian sandstone, Colorado Plateau (USA): Variation with landscape position and contribution to potential plant nutrients. *Geoderma* 130: 108-123.

Rubio, L.M., E. Flores, and A. Herrero. 1999. Molybdopterin guanine dinucleotide cofactor in *Synechococcus* sp. Nitrate reductase: Identification of *mobA* and isolation of a putative *moeB* gene. *FEBS Letters* 462: 358-362.

_____. 2002. Purification, cofactor analysis, and site-directed mutagenesis of *Synechococcus* ferredoxin-nitrate reductase. *Photosynthesis Research* 72: 13-26.

Rychert, R.C. and J. Skujins. 1974. Nitrogen fixation by blue-green algae-lichen crust in the great basin desert. *Soil Science Society of America Proceedings* 38: 768-771.

Sanz, A.P., C. Moreno-Vivián, J.M. Maldonado, A. González-Fontes. 1995. Effect of a constant supply of different nitrogen sources on protein and carbohydrate content and enzyme activities of *Anabaena variabilis* cells. *Physiologia Plantarum* 95: 39-44.

Schlesinger, W.H. 1996. *Biogeochemistry, an analysis of global change*. San Diego: Academic Press.

Schulten, J.A. 1985. Soil aggregation by cryptogams of a sand prairie. *American Journal of Botany* 72, no. 11: 1657-1661.

Schumacher, B.A. 2002. Methods for the determination of total organic carbon (TOC) in soils and sediments: U.S. Environmental Protection Agency, Washington, DC.

Solomonson, L.P., M.J. Barber, and W.D. Howard. 1984. Electron paramagnetic resonance studies of the molybdenum center of assimilatory NADH:Nitrate reductase from *Chlorella vulgaris*. *The Journal of Biological Chemistry* 259, no. 2: 849-853.

Sroga, G.E. 1997. Regulation of nitrogen fixation by different nitrogen sources in the filamentous non-heterocystous cyanobacterium *Microcoleus* sp. *FEMS Microbiology Letters* 153: 11-15.

Steppe, T.F., J.B. Olson, H.W. Paerl, R.W. Litaker, and J. Belnap. 1996. Consortia N2 fixation: A strategy for meeting nitrogen requirements of marine and terrestrial cyanobacterial mats. *FEMS Microbiology Ecology* 21: 149-156.

Strauss, S.L., T.A. Day, and F. Garcia-Pichel. 2012. Nitrogen cycling in desert biological soil crusts across biogeographic regions in the southwestern United States. *Biogeochemistry* 108: 171-182.

Thiel, T. 1993. Characterization of genes for an alternative nitrogenase in the cyanobacterium *Anabaena variabilis*. *Journal of Bacteriology* 175, no. 19: 6276-6286.

Tovar-Sanchez, A., S.A. Sanudo-Wilhelmy, A.B. Kustka, S. Agusti, J. Dachs, D.A. Hutchins, D.G. Capone, and C.M. Duarte. 2006. Effects of dust deposition and river discharges on trace metal composition of *Trichodesmium* spp. In the tropical and subtropical North Atlantic Ocean. *Limnology and Oceanography* 51, no. 4: 1755-1761.

Tuit, C., J. Waterbury, and G. Ravizza. 2004. Diel variation of molybdenum and iron in marine diazotrophic cyanobacteria. *Limnology and Oceanography* 49, no. 4: 978-990.

West, N.E. and J. Skujins. 1977. The nitrogen cycle in North American cold-winter semi-desert ecosystems. *Oecologia Plantarum* 12, no. 1: 45-53.

White, A.E., Y.H. Spitz, D.M. Karl, and R.M. Letelier. 2006. Flexible elemental stoichiometry in *Trichodesmium* spp. and its ecological implications. *Limnology and Oceanography* 51, no. 4: 1777-1790.

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, no. 2: 973-983.

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.

CHAPTER 3

EVIDENCE FOR MICROBIAL UPTAKE AND SOLUBILIZATION OF BIOESSENTIAL ELEMENTS IN BIOLOGICAL SOIL CRUSTS

Abstract

Biological soil crusts (BSCs) require a variety of elements to support a range of physiological processes, such as photosynthesis and nitrogen fixation, that contribute greatly to arid land fertility. Previous work has highlighted the effect of BSCs on soil solution concentrations of vanadium, iron, and molybdenum, and the current project was aimed at expanding the suite of elements investigated. The goal was to determine which elements in the soil solution were affected by biological processes such as microbial solubilization and uptake, and which elements were dominated by abiotic processes, like mineral precipitation or adsorption. I found that when crusts were metabolically active, nickel and zinc decreased, likely reflecting microbial uptake. Arsenic and zirconium also decreased, but probably as a result of biosorption. Aluminum, vanadium, copper, and zinc concentrations all increased when crusts were active. Increasing aluminum was interpreted to result from cellular export, perhaps in response to toxicity, while increasing copper was deemed the result of microbial solubilization. Increasing vanadium and zinc concentrations were more difficult to interpret as microbial solubilization and cellular export were both valid explanations. Potassium and manganese showed

changes in concentration that appeared to be caused by an alteration in the balance of biological processes, while phosphorus showed evidence for both biological and abiotic cycling. Iron showed a clear pattern that could be explained by abiotic mineral precipitation or adsorption. Comparison of concentrations in crusts that received water, molybdenum, and nitrogen additions showed that, in general, nitrogen addition reduced microbial solubilization of multiple elements. I conclude that this is the result of decreased element need that manifests in decreased production of metal chelators. This work supports previous findings that proposed manganese, copper, and zinc as potential biomarkers of past BSC communities, and further suggests that some elements may be useful fertilizers to sustain and promote BSCs in order to prevent arid land degradation.

Introduction

Biological soil crusts (BSCs) are microbial communities composed of bacteria, archaea, algae, fungi, lichens, and bryophytes (Belnap *et al.,* 2001) that are especially adapted to thrive in arid and semi-arid environments (Bowker et al., 2002; Gundlapally & Garcia-Pichel, 2006). BSCs perform a variety of valuable ecosystem services that include: input of fixed carbon (C) through photosynthesis (Beymer & Klopatek, 1991; Evans & Lange, 2001; Garcia-Pichel, 2002), input of bioavailable nitrogen (N) through N_2 fixation (Rychert & Skujins, 1974; Jeffries et al., 1992;

Steppe et al., 1996; Belnap, 2002), enhanced iron (Fe) availability through siderophore production (see Chapters 5 & 6), and erosion prevention (Campbell, 1979; Schulten, 1985; Belnap, 1993). All these contributions serve to increase arid land habitability and reduce arid land degradation (Isichei, 1990; Belnap et al., 1994; Belnap, 1995; Bowker et al., 2008a). Therefore, sustaining and increasing BSC cover is critical for protecting and enhancing arid lands, and requires knowledge of the factors that limit and promote BSC growth and development (Davidson et al., 2002; Bowker et al., 2005). In addition to C, N, and water, limiting factors for crust ecosystems may include major elements such as magnesium and/or minor elements such as manganese and molybdenum. The dynamics of these, and in fact most, elements in the BSC system are not well understood; however, their availability likely plays an important role in BSC function.

Living organisms require a suite of elements to build their biomass and perform physiological processes. The general categories of biologically-relevant elements are shown in Table 3-1; the major and minor elements are generally considered essential for all life, while the rare elements are necessary or only possibly required in just a few organisms (Bertini et al., 2007). The elements listed in Table 3-1 serve a range of functions in biological systems. Of the bulk elements, H, C, N, O, P, and S are the primary building blocks of biological macromolecules like proteins, lipids,

Table 3-1. Categories of bioessential elements. Major elements are required for all life, minor elements are generally considered necessary for all life, and rare elements are essential for a few organisms. Elements in bold are the focus of this study.

Category	Elements	
Major elements	Hydrogen (H)	**Phosphorus (P)**
	Carbon (C)	Sulfur (S)
	Nitrogen (N)	Chloride (Cl)
	Oxygen (O)	**Potassium (K)**
	Sodium (Na)	Calcium (Ca)
	Magnesium (Mg)	
Minor elements	Boron (B)	Cobalt (Co)
	Fluoride (F)	**Nickel (Ni)**
	Silicon (Si)	**Copper (Cu)**
	Vanadium (V)	**Zinc (Zn)**
	Chromium (Cr)	Selenium (Se)
	Manganese (Mn)	**Molybdenum (Mo)**
	Iron (Fe)	Iodine (I)
Rare elements	**Arsenic (As)**	Tin (Sn)
	Bromine (Br)	Barium (Ba)
	Strontium (Sr)	Tungsten (W)
	Cadmium (Cd)	

polysaccharides, and nucleic acids. Other elements support the cycling of H, C, N, O, P, and S within cells by contributing to cellular maintenance processes such as charge balance and electrical potential (Na, K), enzymatic processes like electron transfer (Fe, Cu, Mo) and catalysis (Mg, Ca, V, Mn, Fe, Co, Ni, Cu; Fraústo da Silva & Williams, 2001; Bertini et al., 2007). This list is not exhaustive, but should give the impression that a multitude of elements are critical to the functioning of biological systems.

In the current study, I was interested in how BSCs affected the soil

solution concentrations of bioessential elements as a result of microbial uptake and/or solubilization.

I focused on a group of elements from Table 3-1 (shown in bold) that are particularly relevant to prokaryotic C and N cycling within biological soil crusts, specifically: Mg, P, V, Mn, Fe, Ni, Cu, Zn, and Mo. Cyanobacteria dominate C and N cycling in BSCs because they perform photosynthesis and N_2 fixation, thus providing the primary sources of fixed C and N to the soil ecosystem (Rychert & Skujins, 1974; Beymer & Klopatek, 1991; Jeffries et al., 1992; Evans & Ehleringer, 1993; Steppe et al., 1996; Evans & Lange, 2001; Belnap, 2002). Photosynthesis converts inorganic carbon into organic carbon by coupling light capture to ATP synthesis using multiple reaction centers. Photosynthesis in cyanobacteria requires Mg in chlorophyll (Conant et al., 1931; Ferguson-Miller et al., 2007), Mn in the oxygen-evolving complex (Umena et al., 2011), Cu in various components of photosynthetic (and respiratory) electron transport (Lockau, 1981; Peschek et al., 2004; Bernroitner et al., 2008), Zn in carbonic anhydrase (Smith & Ferry, 2000), and Fe in a variety of capacities (Raven et al., 1999; Ferguson-Miller et al., 2007). Nitrogen fixation depends on the nitrogenase enzyme that converts atmospheric nitrogen gas (N_2) into bioavailable ammonium (NH_4^+). Nitrogenases typically incorporate Mo and Fe, but some cyanobacteria are also capable of producing a V-dependent enzyme (Burgess & Lowe, 1996; Eady, 1996;

Howard & Rees, 1996; Berman-Frank et al., 2003). We cannot neglect the fact that N_2 fixation is an energy-requiring process, and so also requires readily available Mg, P, and organic substrates to generate Mg-ATP; 16 molecules of Mg-ATP are required per molecular of N_2 reduced (Zhao et al., 2006). In addition, hydrogenase enzymes that require Fe and Ni, convert H_2 to $2H^+$and thus provide a source of reducing power for N_2 fixing organisms (Bothe et al., 2010).

Ammonium oxidation and N assimilation are N-cycling processes that are also active in BSCs. Ammonium oxidation (AO) is the first step in nitrification that converts ammonia (NH_3) into hydroxylamine (NH_2OH). Rates of AO in BSCs have been shown to be comparable to those of N_2 fixation (Johnson et al., 2005; Strauss et al., 2012), and so AO is a significant contributor to N cycling in crusts. Organisms that perform AO produce the enzyme ammonia monooxygenase that requires both Fe and Cu (Holmes et al., 1995; Zahn et al., 1996; Ferguson, 1998). Nitrogen assimilation is a two-step process that first reduces nitrate (NO_3^-) to nitrite (NO_2^-) and then further reduces NO_2^- to ammonia (NH_3). The enzymes used to perform these processes are assimilatory nitrate reductase and nitrite reductase, respectively (Guerrero et al., 1981). The former requires both Fe and Mo (Rubio et al., 1999; Rubio et al., 2002), while the latter contains both heme- and non-heme Fe (Murphy et al., 1974; Aparicio et al., 1975; Lancaster et al., 1979).

In addition to the elements required for the specific physiological processes described above, I focused on elements required for general cellular maintenance such as Na and K that are important for establishing charge balance and osmotic and electric potentials (Booth et al., 1999; Fraústo da Silva & Williams, 2001; Epstein, 2003). I also examined arsenic (As) in part because it can be used as an electron acceptor, but also because it is known to be detrimental to some organisms so might reflect active detoxicfication processes. Finally, I included two elements that are not known to be biologically relevant (Al and Zr) to gain information about the behavior of elements that reflect abiotic processes in the BSC system. While the elements listed above are used in additional enzymes and processes not listed here, for the sake of brevity, I have chosen to focus only on the elements relevant to processes known to be active in BSCs.

Previous studies have investigated the relationship between BSCs and soil elements. Rogers (1972) determined that lichen species distribution was somewhat dependent on Ca availability, which suggests that the BSCs used in that study were limited with respect to calcium. Another study conducted with crusts from the Colorado Plateau, demonstrated that manganese and zinc may limit the abundance of certain BSC lichens (Bowker et al., 2005), however, further investigations did not support the oridingal finding (Bowker et al., 2008b). Beraldi-Campesi et al. (2009)

showed that the soil contents of calcium, chromium, manganese, copper, zinc, arsenic, and zirconium were depleted in crusts when compared to neighboring uncrusted soils. The authors postulated the depletion was the result of microbial solubilization that made some elements susceptible to loss by leaching. To summarize, Rogers (1972) showed how soil nutrient content influenced BSC cover, while Beraldi-Campesi et al. (2009) looked at the effects of BSCs on soil elemental distributions. However, these studies were based on soil element contents, and, therefore, did not provide insight into the real-time influence of BSCs on soil element mobility. In an effort to fill this gap, I investigated elemental concentrations in the soil solution of live crusts from the Colorado Plateau to determine how physiologically active BSCs influence metal mobility (see Chapter 2). I determined that soil solution concentrations of V and Mo were lower in active crusts than in killed controls, which I interpreted to result from microbial uptake and subsequent loss from biomass during sterilization of the killed controls. I also observed that Mo concentrations increased in crusted samples over the duration of my experiment, suggesting Mo solubilization by the microbial community; the study focused only on the trace metals used in N_2 fixation, and , thus, it remains to be seen if BSCs actively alter soil solution concentrations of other elements.

BSCs are water-limited and so the soil solid-phase is their only source for bioessential elements, except C and N. The soils used in the current

study are dominated by quartz sands, but do contain ~10% clays and oxide/hydroxide minerals that could serve as a source of many elements (Noonan, unpublished data; Reynolds et al., 2001; Reynolds et al., 2006). Soil organics are also a potential source of bioessential elements (Brady & Weil, 2002). BSCs are only physiologically active when wet (Garcia-Pichel & Belnap, 1996), and so element acquisition has to occur during, or shortly after, rain or snow events. In order to obtain elements from the solid-phase, BSCs must either depend on abiotic dissolution, after which microbes can take up elements from the soil solution, or they must release organic acids and chelators into the soil solution to promote element solubility (Drever & Stillings, 1997; Kalinowski et al., 2000; Kraemer, 2004; Liermann et al., 2000; 2005). In both instances, the soil solution serves as the link between the solid-phase and the microbes, and so I proposed that monitoring soil solution concentrations of bioessential elements was an effective method to determine how BSCs influence element mobility in soils.

The first goal of this work was to determine which elements were influenced by BSC activity and what processes contributed to increasing or decreasing element concentrations in the soil solution. By comparing concentration changes in crusted samples and killed controls it was possible to determine what processes were active. For example, changing concentrations of an element in living crusts, but not in killed controls,

were likely indicative of microbial processes; in contrast, changing concentrations in killed controls were more likely representative of abiotic processes. Microbial processes include solubilization, cellular export, uptake, biosorption, and indirect effects through the alteration of pH and redox conditions. Abiotic processes include precipitation/dissolution and adsorption/desorption reactions.

The second goal was to establish how separate N and Mo additions influenced soil solution element concentrations. To achieve this goal, I added water, water plus N, and water plus Mo to different groups of crusts and compared trends and concentrations between and within the treatment groups. Different patterns in soil solution element concentrations in crusts that received different nutrient additions speaks to the influence of nutrient addition on biological processes that affect metals. I hypothesized that I would be able to see the effects of BSCs on bioessential element concentrations, and that the patterns in element concentrations would indicate whether biological removal (e.g., uptake) or addition (e.g., solubilization) processes were active.

With N addition, I hypothesized that concentrations of Mg, P, V, Mn, Ni, and Zn would increase in crusted samples (Table 3-2). Previous work has shown that N_2 fixation in BSCs ceases with the addition of bioavailable N (see Chapters 2 & 4), and I expected BSCs to need lower amounts of these elements when N_2 fixation was inactive, thus leading to higher soil

Table 3-2. Summary of hypotheses tested for different groups of elements under the N and Mo additions during week 2.

Condition	Elements	Hypothesis	Justification
Changes that result from N addition to crusted soils	Mg, P, V, Mn, Ni, Zn	Increase	Decreased uptake of elements that support N_2 fixation because need is lower when N_2 fixation is inactive
	Fe, Cu, Mo	Decrease	Increased uptake to support increased rates of nitrate assimilation and ammonium oxidation
	Na, K	No change	Need should not depend on N status
Changes that result from Mo addition to crusted soils	Mg, P, Mn, Fe, Ni, Cu, Zn, Mo	Decrease	Increased uptake to support increased N_2 fixation
	V	No change	Reduced need when sufficient Mo is available resulting in stabilized concentrations
	Na, K	No change	Need should not depend on Mo status
All crusted soils	Al, As, Zr	No change	No physiological requirement; Minimal changes expected for bulk pH and redox effects
All killed controls	All elements	No change	Minimal changes expected for bulk pH and redox effects

solution concentrations. Because rates of ammonia oxidation and N assimilation were anticipated to rise as a result of N addition, I expected concentrations of Fe, Cu, and Mo to decrease since these elements are required for AO and N assimilation. I predicted that increased need for these elements would result in decreased soil solution concentrations. I hypothesized that Mo addition would cause soil solution concentrations of Mg, P, Mn, Fe, Ni, Cu, Zn, and Mo to decrease in crusted samples (Table 3-2). When crusts receive Mo, their N_2 fixation rates increase (see Chapter 4), so, consequently, their need for Fe and Mo would also increase. Increased need would result in increased uptake and subsequent decreases in soil solution concentrations. In order to increase N_2 fixation, BSCs also need to increase other processes, such as photosynthesis, that require the elements Mg, P, Mn, Ni, Cu, and Zn. V concentrations were predicted to remain stable under Mo addition. If crusts have the genes for V-nitrogenase they will not express it when Mo is available (Jacobson et al., 1986; Joerger & Bishop, 1988; Luque & Pau, 1991; Jacobitz & Bishop, 1992). Therefore, I expected that V would not be taken up by cells using Mo-nitrogenase.

No changes were predicted for Na and K with either treatment because I did not expect their use in general cellular processes to change with N or Mo addition. I also did not anticipate changes in Al, As or Zr concentrations because these elements lack biological roles (Table 3-2).

Methods

Soil Collection

"Dark" crusts, a predominantly cyanobacterial community, were collected at the Green Butte site (Strauss et al., 2012) north of Moab, UT on the Colorado Plateau (N 38°42′56.2″, W 109°41′32.7″) during May, 2009. Dark crusts have minimal lichen and moss content (Garcia-Pichel & Belnap, 1996; Garcia-Pichel *et al.,* 2001); crusts were selected based on a visual assessment of surface topography and lichen cover in order to focus on processes driven by cyanobacteria. Soils were collected according to Garcia-Pichel et al. (2003). Briefly, the soil was lightly wetted with distilled water in order to increase surface soil coherence. Petri dish lids (50 mm x 13 mm) were placed into the soil and a plastic spatula was used to remove the lid and soil within. The soil was then inverted to allow the base of the dish to be inserted. Soils were stored dry and in the dark at room temperature (20ºC) until the experiment in October, 2009. Storage of this duration was expected to have little effect on BSC community composition (Campbell et al., 2009).

Experimental Design

The experiment was designed to simulate two sequential rainfall events. Incubation dishes were prepared using the method described in Chapter 2. Incubation dishes were constructed from the bottoms of 2 plastic Petri dishes (50 mm x 13 mm). Approximately 20-25 small holes

were drilled into the base of one dish to allow soil solution to move from the first dish that held the soil into the second dish that served as a collection chamber. A sampling port was drilled into the side of the collection chamber. The dish with the perforated base was sealed to the top of the dish with the sampling port using 3 complete wraps of black electrical tape (Scotch® 3M Super33^{+}). The sampling port was likewise sealed with electrical tape to prevent leaking. A Whatman™ GF/F filter (55 mm diameter, nominal pore size 0.7 μm) was placed over the perforated base to prevent soil particles from entering the collection chamber.

Twelve crusts were transferred to glass Petri dishes and autoclaved (121°C, 30 min) to serve as “killed controls”. Killed controls represent abiotic soils with mineralogy comparable to that of the crusted samples. I anticipated that autoclaving would cause cell lysis and release of intracellular material. As a result, I predicted that soil solution concentrations of biogenic elements would be higher in killed controls as compared to crusted samples.

Twenty-four crusts and 12 killed controls were transferred to the incubation dishes and wetted with 15 mL deionized carbon-free (DI) water (18.2 MΩ·cm; NANOpure® DIamond™ UV, Barnstead International, Dubuque, IA). This same deionized water was used for all water additions and dilutions. Immediately after wetting, day 1 soil solutions were collected

through the sampling port using gentle vacuum suction applied with a 60 mL syringe that drew the soil solution through the perforated surface and into the collection chamber. After collection, soils were moved to an environmental incubator maintained at 25°C and an irradiance (measured as photosynthetically active radiation) of 110 $\mu E\ m^{-2}\ s^{-1}$ under fluorescent lights. This temperature is within the typical temperature range where the crusts were collected, and this irradiance level is sufficient for BSC photosynthesis (Garcia-Pichel & Belnap, 1996). On days 3 and 5, 10 mL of DI water were added to the soils to provide sufficient fluid for collection, and soil solution was collected as before. The water addition is necessary because the crusts will dry to the point where sample collection is impossible over the multi-day incubation. The volume and any concentration addition from this water was accounted for in subsequent calculations of actual soil solution concentrations. Days 1-5 represent week 1 of the experiment. After the day 5 soil solution collection, soils were removed from the incubator and allowed to dry in the dark for 5 days. On day 10, soils were transferred to clean incubation dishes with fresh GF/F filters and rewetted with 15 mL of one of three treatment waters. Treatments were as follows: 12 crusted samples and 6 killed controls were rewetted with DI water (+H_2O soils), 6 crusted samples and 3 killed controls were rewetted with 5 mM ammonium nitrate (NH_4NO_3; i.e., +N soils), and 6 crusted samples and 3 killed controls were rewetted with

2 mM sodium molybdate ($Na_2MoO_4 \bullet 2H_2O$; i.e., +Mo soils). Immediately after rewetting, day 10 soil solutions were collected and the soils were placed in the incubator as before. On days 12 and 14, 10 mL of the same treatment water applied on day 10 were added to the soils and soil solutions were collected. Days 10-14 constitute week 2 of the experiment.

Two milliliters of soil solution collected from each soil at each time point were filtered through 0.45 µm polysulfone membrane filters (Supor®, PES, Pall Corporation, Port Washington, NY) into acid washed polypropylene tubes and trace metals were analyzed within 8 weeks. On each collection day “water blanks” that were treatment water with no alterations were collected. Water blanks were collected before soil solution collection and again after every set of 12 soils were processed. Water blanks were analyzed alongside soil solutions, and they were used to correct for any element additions that resulted from the daily water supplements.

Trace Metal Analysis

Soil solutions and blanks were digested in concentrated nitric acid (HNO_3) to dissolve precipitates and hydrogen peroxide to remove organics. After drying, samples were re-dissolved in 2% HNO_3 and concentrations of elements were quantified by inductively coupled plasma mass spectrometry (ICP-MS; Thermo Scientific X Series). The instrument was calibrated using a multi-element ICP-MS standard, and instrumental

drift was corrected with a mixed internal standard containing Ge, Y, and In. Errors were defined as the standard deviation of triplicate injections for each sample.

Data Processing

The limit of detection (LOD) and limit of quantitation (LOQ) were calculated as 3 and 10 times the standard deviation of a low concentration standard, respectively. Measured concentrations below the LOD were set to zero, and concentrations between the LOD and the LOQ were set to the LOQ value for the particular element. Initial concentrations were corrected for the dilution that resulted from ICP-MS sample preparation according to Equation 1, and concentrations were converted from ppb to nM:

Equation 1. $(V_{final} \times C_{final}) \div V_{initial} = C_{initial}$,

where V_{final} = volume after digestion (mL), C_{final} = measured concentration (ppb), V_{intial} = volume collected from experimental soils (mL), and C_{intial} = concentration in the original soil solution (ppb).

Any increase in concentration that resulted from the addition of treatment water at each collection time was accounted for by subtracting the quantity (nmol) of that element in the water blank from the quantity (nmol) in the sample or control. When soils started dry on days 1 and 10, the subtraction was done according to Equation 2. Here, the number of nanomoles of each element in the blank was calculated by multiplying the volume of water added by the concentration in the blank. This was then

subtracted from the number of nanomoles in the soil solution, calculated by multiplying the concentration measured in the soil solution by the volume of water added. In this case the volumes were identical since the soil started dry, but after being in contact with the soil, the concentration in the water changed. Therefore, it was possible to subtract what was in the water before addition to the soil. Finally, the nanomoles of a given element in the soil solution after blank subtraction were divided by the total volume to determine the concentration in the soil solution:

Equation 2. $$\frac{([\text{soil solution}]_m \times V_{blank}) - ([\text{blank}] \times V_{blank})}{V_{blank}} = [\text{soil solution}]_c$$

where [concentrations] are in nM, $[\text{soil solution}]_m$ is the concentration in the soil solution that was measured, V_{blank} is the volume of water, in L, that was originally added to the soil, and $[\text{soil solution}]_c$ is the concentration in the soil solution after subtraction of material in the added water.

When soils were already wet (days 3 and 5, and days 12 and 14) the blank subtraction was performed according to Equation 3:

Equation 3. $$\frac{([\text{soil solution}]_m \times V_3) - ([\text{blank}] \times V_2)}{V_1} = [\text{soil solution}]_c$$

where [concentrations] are in nM, $[\text{soil solution}]_m$ is the concentration measured in the soil solution, V_1 = L of water in soil solution from previous addition(s), V_2 = L of water added in current addition, V_3 = total volume (L) in the soil solution after water addition, including water remaining in the soil from previous additions, and $[\text{soil solution}]_c$ is the concentration in the

soil solution before water addition. If the concentration of a particular element in the water blank was below detection, no subtraction was performed. When the blank subtraction resulted in a negative number the resulting sample or control concentration was set to zero. Error was propagated for each calculation using standard methods (Bevington & Robinson, 2002). All data processing was conducted using Microsoft Excel (Microsoft Corporation, Redmond, WA).

Statistical Analysis

Statistics were performed in SigmaPlot v11.0 (Systat Software Inc., Chicago, IL). *P*-values <0.10 are considered statistically significant.

Linear regression analyses of concentration (nM) against time (h) were carried out for each element in each sample type for weeks 1 and 2, separately. For instance, Mg concentrations in crusted samples during week 1 were regressed separately from Mg concentrations in crusted samples during week 2.

Week 1 concentrations of each element were then compared to week 2 concentrations of the same element using a Wilcoxon Signed Rank Test. Comparisons were performed by soil type and treatment. For example, concentrations of Mg in crusted samples that received N in week 2 were compared to concentrations of Mg in the same group of crusted samples from week 1.

Results

Week 1 Trends

Week 1 linear regression analyses were performed based on the treatment groups in week 2, even though all soils received water only during week 1. This allowed comparison of absolute concentrations and concentration changes between weeks 1 and 2. Tables 3-3a and 3-3b contain data for the crusted samples and killed controls of the $+H_2O$ experiment, respectively. Tables 3-3c and 3-3d contain data for the crusted samples and killed controls of the +N experiment, respectively and Tables 3-3e and 3-3f contain data for the crusted samples and killed controls of the +Mo experiment, respectively.

Crusted Samples

During week 1, concentrations of P ($p < 0.001$), K ($p = 0.03$), and Fe ($p = 0.10$) decreased, and V concentrations increased ($p = 0.01$) in the crusted samples of the $+H_2O$ treatment group (Table 3-3a). Concentrations of Ni ($p = 0.01$), Zn ($p = 0.03$), and As ($p < 0.001$) decreased in crusted samples of the +N treatment group (Table 3-3c). Concentrations of P ($p = 0.02$), K ($p = 0.09$), Mn ($p = 0.06$), and Zr ($p = 0.07$) decreased in crusted samples of the +Mo treatment group (Table 3-3e). All unspecified elements did not change significantly during week 1.

Table 3-3a. Week 1 and week 2 linear regression results for concentration vs time in crusted samples of the +H_2O treatment group. *P*-values ≤ 0.10 are considered significant and shown in bold.

		Na	Mg	Al	P	K	V	Mn
Week 1	Slope (nM h^{-1})	190864	-3765	211	-3958	-16889	65	22
	Change	None	None	None	Decrease	Decrease	Increase	None
	R^2	< 0.01	0.01	0.02	0.41	0.15	0.20	0.04
	p-value	0.70	0.54	0.50	**< 0.001**	**0.03**	**0.01**	0.27
Week 2	Slope (nM h^{-1})	9282000	95452	41404	13684	307282	1221	531
	Change	None	Increase	Increase	Increase	Increase	None	Increase
	R^2	0.01	0.16	0.08	0.12	0.15	0.08	0.18
	p-value	0.64	**0.02**	0.11	**0.06**	0.**03**	0.12	**0.02**
		Fe	Ni	Cu	Zn	As	Zr	Mo
Week 1	Slope (nM h^{-1})	-71	5	-4	-236	0	10	16
	Change	Decrease	None	None	None	None	None	None
	R^2	0.08	0.01	< 0.01	0.08	< 0.01	0.02	0.06
	p-value	**0.10**	0.52	0.89	0.11	0.97	0.43	0.17
Week 2	Slope (nM h^{-1})	-560	15	316	1981	49	2	20
	Change	Decrease	None	Increase	None	None	None	None
	R^2	0.69	< 0.01	0.14	0.05	0.01	< 0.01	0.07
	p-value	**< 0.001**	0.86	**0.04**	0.20	0.64	0.75	0.13

Table 3-3b. Week 1 and week 2 linear regression results for concentration vs time in killed controls of the +H_2O treatment group. *P*-values ≤ 0.10 are considered significant and shown in bold.

		Na	Mg	Al	P	K	V	Mn
Week 1	Slope (nM h^{-1})	1881906	26940	53	-21495	40346	-5	63
	Change	Increase	None	None	Decrease	None	None	None
	R^2	0.24	0.08	< 0.01	0.55	0.10	< 0.01	< 0.01
	p-value	**0.05**	0.27	0.83	**< 0.001**	0.22	0.80	0.90
Week 2	Slope (nM h^{-1})	5581984	57656	96939	74816	226684	1037	1024
	Change	None	Increase	None	None	None	None	None
	R^2	0.11	0.18	0.10	0.12	0.14	0.11	0.13
	p-value	0.19	**0.09**	0.21	0.18	0.13	0.19	0.16
		Fe	Ni	Cu	Zn	As	Zr	Mo
Week 1	Slope (nM h^{-1})	692	58	-12	795	57	5	8
	Change	None	None	None	None	Increase	None	None
	R^2	0.12	0.07	< 0.01	0.06	0.25	0.01	0.02
	p-value	0.18	0.32	0.96	0.35	**0.04**	0.75	0.60
Week 2	Slope (nM h^{-1})	-579	39	855	1219	8	-2	91
	Change	Decrease	None	None	None	None	None	Increase
	R^2	0.61	0.04	0.10	0.06	< 0.01	0.01	0.17
	p-value	**< 0.001**	0.47	0.21	0.34	0.90	0.71	**0.10**

Table 3-3c. Week 1 and week 2 linear regression results for concentration vs time in crusted samples of the +N treatment group. *P*-values ≤ 0.10 are considered significant and shown in bold.

		Na	Mg	Al	P	K	V	Mn
Week 1	Slope ($nM\ h^{-1}$)	-571740	-6761	-1155	-3289	-13988	30	-314
	Change	None	None	None	None	None	None	None
	R^2	< 0.01	0.01	< 0.01	0.05	0.01	0.09	< 0.01
	p-value	0.78	0.66	0.95	0.41	0.74	0.23	0.95
Week 2	Slope ($nM\ h^{-1}$)	755569	0	-306	-1308	32680	-14	5
	Change	Increase	None	Decrease	None	None	None	None
	R^2	0.17	0.02	0.39	0.13	0.11	0.14	0.05
	p-value	**0.10**	0.56	**0.01**	0.15	0.20	0.14	0.38
		Fe	Ni	Cu	Zn	As	Zr	Mo
Week 1	Slope ($nM\ h^{-1}$)	-224	-7	-52	-325	-6	9	7
	Change	None	Decrease	None	Decrease	Decrease	None	None
	R^2	< 0.01	0.38	< 0.01	0.28	0.68	0.08	0.05
	p-value	0.93	**0.01**	0.79	**0.03**	**< 0.001**	0.28	0.39
Week 2	Slope ($nM\ h^{-1}$)	-85	-24	-183	758	-7	1	5
	Change	None	None	None	None	None	None	None
	R^2	0.15	0.08	0.18	0.03	0.05	< 0.01	0.04
	p-value	0.12	0.27	0.79	0.49	0.41	0.85	0.42

Table 3-3d. Week 1 and week 2 linear regression results for concentration vs time in killed controls of the +N treatment group. *P*-values ≤ 0.10 are considered significant and shown in bold.

		Na	Mg	Al	P	K	V	Mn
Week 1	Slope (nM h^{-1})	-46455	32915	-163	-803783	47519	-39	-40
	Change	None	None	None	None	None	None	None
	R^2	<0.01	0.04	0.03	0.20	0.09	0.16	0.01
	p-value	0.98	0.60	0.63	0.19	0.39	0.25	0.82
Week 2	Slope (nM h^{-1})	222765	65880	-76	-1511	58544	-12	105
	Change	None	None	None	None	None	None	None
	R^2	< 0.01	< 0.01	0.28	< 0.01	< 0.01	< 0.01	0.01
	p-value	0.99	0.96	0.14	0.96	0.98	0.98	0.79
		Fe	Ni	Cu	Zn	As	Zr	Mo
Week 1	Slope (nM h^{-1})	146	16	-57	-154	17	5	-7
	Change	None	None	None	None	None	None	None
	R^2	< 0.01	< 0.01	0.01	0.01	< 0.01	< 0.01	0.01
	p-value	0.89	0.91	0.83	0.77	0.81	0.85	0.77
Week 2	Slope (nM h^{-1})	0.347	-11	-534	-842	31	1	-2
	Change	None	None	None	None	None	None	None
	R^2	0.04	< 0.01	0.03	0.04	< 0.01	< 0.01	< 0.01
	p-value	0.62	0.94	0.65	0.62	0.96	0.89	0.99

Table 3-3e. Week 1 and week 2 linear regression results for concentration vs time in crusted samples of the +Mo treatment group. *P*-values ≤ 0.10 are considered significant and shown in bold.

		Na	Mg	Al	P	K	V	Mn
Week 1	Slope ($nM\ h^{-1}$)	754793	-14630	220	-2440	-34458	21	-9
	Change	None	None	None	Decrease	Decrease	None	Decrease
	R^2	0.05	0.12	0.02	0.31	0.19	0.02	0.23
	p-value	0.39	0.19	0.64	**0.02**	**0.09**	0.55	**0.06**
Week 2	Slope ($nM\ h^{-1}$)	17623904	203636	193407	38776	782722	3919	1512
	Change	None	Increase	Increase	Increase	Increase	Increase	Increase
	R^2	0.02	0.41	0.21	0.35	0.47	0.24	0.33
	p-value	0.62	**0.01**	**0.07**	**0.01**	**0.002**	**0.05**	**0.02**
		Fe	Ni	Cu	Zn	As	Zr	Mo
Week 1	Slope ($nM\ h^{-1}$)	58	5	-16	-279	5	-1	-5
	Change	None	None	None	Decrease	None	Decrease	None
	R^2	0.03	0.09	0.02	0.09	0.15	0.21	0.02
	p-value	0.49	0.26	0.56	0.25	0.14	**0.07**	0.59
Week 2	Slope ($nM\ h^{-1}$)	NM[a]	-37	401	3706	NM	-1	-1869
	Change	NM	None	None	Increase	NM	Decrease	None
	R^2	NM	< 0.01	0.02	0.25	NM	0.23	< 0.01
	p-value	NM	0.84	0.60	**0.04**	NM	**0.05**	0.88

[a]Some values were not measured (NM) due to instrument malfunction and lack of extra soil solution for repeat analysis.

Table 3-3f. Week 1 and week 2 linear regression results for concentration vs time in killed controls of the +Mo treatment group. *P*-values ≤ 0.10 are considered significant and shown in bold.

		Na	Mg	Al	P	K	V	Mn
Week 1	Slope ($nM\ h^{-1}$)	1466015	22317	-627	-28625	25865	17	197
	Change	None	None	Decrease	Decrease	None	None	None
	R^2	0.26	0.11	0.54	0.51	0.06	< 0.01	0.04
	p-value	0.17	0.39	**0.03**	**0.03**	0.52	0.85	0.58
Week 2	Slope ($nM\ h^{-1}$)	29114529	331295	34995	248235	1264387	2063	4181
	Change	None	None	None	Increase	None	None	None
	R^2	0.03	0.11	0.18	0.44	0.21	0.22	0.20
	p-value	0.66	0.34	0.23	**0.04**	0.18	0.18	0.20
		Fe	Ni	Cu	Zn	As	Zr	Mo
Week 1	Slope ($nM\ h^{-1}$)	283	48	460	52	61	6	16
	Change	None	None	None	None	None	None	None
	R^2	0.02	0.04	0.03	< 0.01	0.10	0.02	0.02
	p-value	0.73	0.61	0.68	0.95	0.41	0.73	0.72
Week 2	Slope ($nM\ h^{-1}$)	NM[a]	644	3876	3336	NM	0	-63
	Change	NM	None	None	None	NM	None	Decrease
	R^2	NM	0.04	0.07	0.17	NM	< 0.01	0.46
	p-value	NM	0.58	0.45	0.23	N	1.00	**0.03**

[a]Some values were not measured (NM) due to instrument malfunction and lack of extra soil solution for repeat analysis.

Killed Controls

In general, very few elements in the killed controls showed a statistically significant change in concentration over week 1. In the killed controls of the +H_2O treatment group, P concentrations decreased ($p < 0.001$), and Na ($p = 0.05$) and As ($p = 0.04$) concentrations increased (Table 3-3b). No concentration changes were observed in killed controls of the +N treatment group (Table 3-3d). In the +Mo treatment group, only Al ($p = 0.03$) and P ($p = 0.03$) concentrations decreased and no elements increased (Table 3-3f).

Week 2 Trends

Crusted Samples

With a second water addition in week 2, concentrations of Mg ($p = 0.02$), P ($p = 0.06$), K ($p = 0.03$), Mn ($p = 0.02$), and Cu ($p = 0.04$) increased in crusted samples of the +H_2O treatment group (Table 3-3a). Concentrations of Fe decreased ($p < 0.001$) in the +H_2O treatment (Table 3-3a). In the +N treatment group, during week 2, only Na concentrations increased ($p = 0.10$) and only Al concentrations decreased ($p = 0.01$; Table 3-3c). In the +Mo treatment group concentrations of Mg ($p = 0.01$), Al ($p = 0.07$), P ($p = 0.01$), K ($p = 0.002$), V ($p = 0.05$), Mn ($p = 0.02$), and Zn ($p = 0.04$) increased in crusted samples, and no elements showed a decrease (Table 3-3e).

Killed Controls

As in the week 1 killed controls, very few elements in the week 2 killed controls showed statistically significant concentration changes. In the $+H_2O$ treatment group, Fe concentrations decreased ($p < 0.001$), and Mg ($p = 0.09$) and Mo ($p = 0.10$) concentrations increased (Table 3-3b). No elemental concentrations changed in killed controls of the +N treatment group during week 2 (Table 3-3d). In the +Mo treatment group, Mo concentrations decreased ($p = 0.03$), and P concentrations increased ($p = 0.04$; Table 3-3f).

Week 1 vs Week 2

Changes in element concentrations from week 1 to week 2 for each of the three treatments are presented in Tables 3-4a, b, and c.

Crusted Samples

For crusted samples in the $+H_2O$ treatment group, Mo concentrations were higher during week 1 ($p = 0.001$; Table 3-4a). Concentrations of Al ($p < 0.001$), K ($p = 0.01$), V ($p = 0.05$), Mn ($p = 0.02$), Fe ($p = 0.003$), Cu ($p = 0.07$), and As ($p < 0.001$) were higher during week 2 (Table 3-4a). In the +N treatment group, Na ($p = 0.02$) and V ($p = 0.03$) concentrations were higher during week 1, and As concentrations were higher during week 2 ($p = 0.01$; Table 3-4b). For the +Mo treatment group, concentrations of Na ($p = 0.01$), Mg ($p = 0.05$), Al ($p < 0.001$), K ($p = 0.04$), V ($p = 0.001$), Mn ($p < 0.001$), Fe ($p = 0.04$), Cu ($p = 0.002$), As ($p = 0.05$), and Mo ($p = 0.004$) were higher during week 2 (Table 3-4c).

Table 3-4a. Results of the Wilcoxon Rank Sum test comparing week 1 concentrations to week 2 concentrations in the $+H_2O$ treatment. Statistically significant *p*-values (< 0.10) are shown in bold. The week with the higher concentrations is indicated.

	Na	Mg	Al	P	K	V	Mn
Crusted Samples							
p-value	0.31	0.12	**<0.001**	0.20	**0.01**	**0.05**	**0.02**
Week with higher concentrations	2	2	2	2	2	2	2
Killed Controls							
p-value	0.15	**0.03**	**0.02**	0.86	0.64	**0.08**	0.76
Week with higher concentrations	2	1	2	2	2	2	2
	Fe	Ni	Cu	Zn	As	Zr	Mo
Crusted Samples							
p-value	**0.003**	0.13	**0.07**	0.23	**<0.001**	0.90	**0.001**
Week with higher concentrations	2	2	2	2	2	1	1
Killed Controls							
p-value	0.62	0.38	0.78	0.25	0.41	0.21	0.31
Week with higher concentrations	1	1	2	2	1	1	2

Killed Controls

As few elements exhibited statistically significant concentration changes in the killed controls, there were similarly few elements with statistically significant differences between week 1 and week 2. Mg concentrations were higher during week 1 in killed controls of the $+H_2O$ treatment group ($p = 0.03$; Table 3-4a), and concentrations of Al ($p = 0.02$) and V ($p = 0.08$) were higher during week 2 (Table 3-4a). For the +N treatment group, Al concentrations in killed controls were higher during

Table 3-4b. Results of the Wilcoxon Rank Sum test comparing week 1 concentrations to week 2 concentrations in the +N treatment. Statistically significant *p*-values (< 0.10) are shown in bold. The week with the higher concentrations is indicated.

	Na	Mg	Al	P	K	V	Mn
Crusted Samples							
p-value	**0.02**	0.82	0.42	0.15	**0.10**	**0.04**	0.56
Week with higher concentrations	1	2	1	1	2	1	1
Killed Controls							
p-value	0.16	0.25	**0.03**	0.22	0.13	0.30	0.94
Week with higher concentrations	2	2	1	1	2	2	2
	Fe	Ni	Cu	Zn	As	Zr	Mo
Crusted Samples							
p-value	0.38	0.31	0.13	0.21	**0.01**	0.91	0.68
Week with higher concentrations	1	2	1	2	2	1	2
Killed Controls							
p-value	1.00	0.69	0.57	0.38	0.36	0.22	0.13
Week with higher concentrations	1	1	2	2	2	1	2

week 1 (p = 0.01; Table 3-4b). In the +Mo treatment group, As concentrations were higher during week 2 (p = 0.03; Table 3-4c).

Discussion

Explanations for Changes in Concentrations During Week 1 and Week 2

Changes in soil solution element concentrations provide clues to the processes that influence element distributions in physiologically active BSCs. I hypothesized that a concentration change observed in crusted samples, but not in killed controls, is diagnostic of a biologically-mediated process. The biologically mediated processes that occur in this system are

Table 3-4c. Results of the Wilcoxon Rank Sum test comparing week 1 concentrations to week 2 concentrations in the +Mo treatment. Statistically significant *p*-values (< 0.10) are shown in bold. The week with the higher concentrations is indicated.

	Na	Mg	Al	P	K	V	Mn
Crusted Samples *p*-value	**0.01**	**0.05**	**<0.001**	0.63	**0.04**	**0.001**	**<0.001**
Week with higher concentrations	2	2	2	2	2	2	2
Killed Controls *p*-value	0.13	0.43	0.13	0.74	0.30	0.25	0.13
Week with higher concentrations	2	2	2	2	2	2	2
	Fe	Ni	Cu	Zn	As	Zr	Mo
Crusted Samples *p*-value	**0.04**	0.53	**0.002**	0.12	**0.05**	0.88	**0.004**
Week with higher concentrations	2	2	2	2	2	1	2
Killed Controls *p*-value	0.84	0.58	0.13	0.43	**0.03**	0.91	0.41
Week with higher concentrations	1	2	2	2	2	2	1

uptake, biosorption, and solubilization. For instance, microbial uptake and biosorption cause soil solution concentrations to decrease. On the other hand, solubilization of elements by microbes causes soil solution concentrations to increase. Regardless of whether solubilization is direct or indirect, it still reflects a biological effect. In contrast, if a change occurs in both crusted samples and killed controls, it is most likely the result of an abiotic process. Abiotic processes that are likely to occur in this system are precipitation, dissolution, desorption, and adsorption; these strictly geochemical processes are generally redox- and pH-dependent. Precipitation and adsorption reactions cause soil solution concentrations

to decrease, while dissolution and desorption cause soil solution concentrations to increase. To summarize, changes affected by abiotic processes are likely to be observed in killed controls as well as in live crusts, while changes under the control of biological processes only occur in live crusts. Therefore, by comparing concentration changes in crusted samples and killed controls, it is possible to deduce which elements are influenced by microbial activity. This section assesses the probable causes of observed concentration changes, without accounting for differences among treatments.

Evidence for Biological Processes

My data demonstrate that soil solution concentrations of Al, K, V, Mn, Ni, Cu, Zn, As, and Zr are influenced by microbial processes. Some of these elements are subject to biological removal (uptake or biosorption), while others appear to be affected by biological solubilization. Specifically, Ni, Zn, As, and Zr all decreased during week 1 in crusted samples; Zr also decreased during week 2 (Figure 3-1a-d). These decreases are not Concentrations of Ni, Zn, As, and Zr do not decrease in any of the killed controls during week 1 or week 2. Therefore, I conclude that decreasing concentrations of Ni, Zn, As, and Zr are biological and the result of either microbial uptake or biosorption. Microbial uptake is concluded for Ni because Ni is needed in hydrogenase enzymes that convert H_2 to $2H^+$

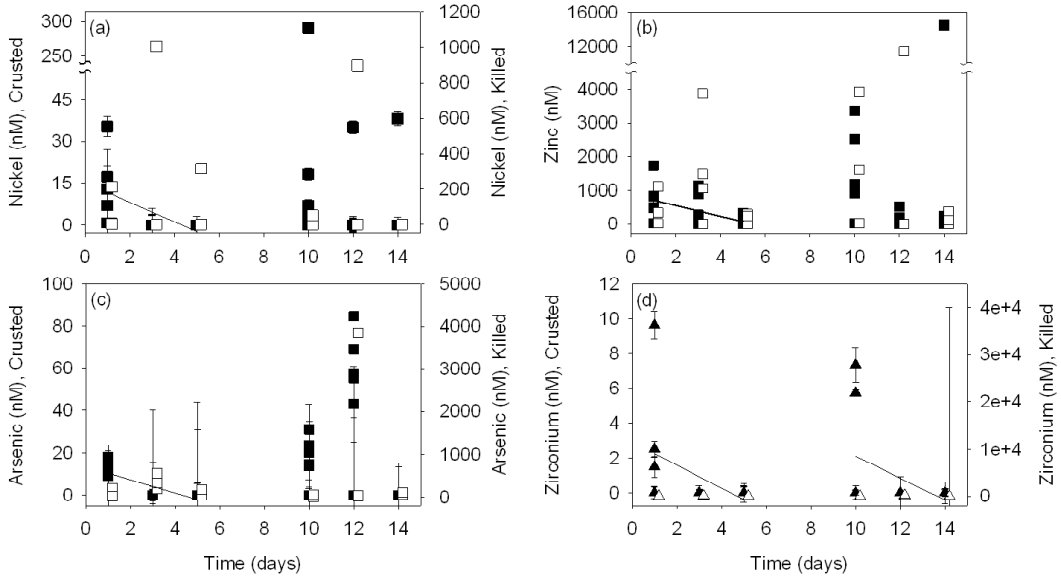

Figure 3-1. Elements with decreasing concentrations over time in soil crust incubations; the +N treatments are shown as squares (■, □), the +Mo treatments are shown as triangles (▲, △). Panels are: Ni (a), Zn (b), As (c) , and Zr (d). Concentrations decreased significantly in crusted samples (■, ▲), but not in killed controls (□, △). Decreasing Ni (p = 0.01) and Zn (p = 0.03) concentrations were interpreted to result from microbial uptake. Decreasing As (p < 0.001) and Zr (Wk 1 p < 0.001; Wk 2 p = 0.05) concentrations were assumed to be the result of biosorption. When trends were stastically significant the regression lines are plotted. Error bars are based on triplicate ICP-MS injections and propagated error. Where error bars are not visible they are smaller than the symbols.

during N_2 fixation in BSCs (Tamagnini et al., 2002; Bothe et al., 2010). Microbial uptake is also presumed for Zn which is used in a variety of physiological processes (Vallee & Auld, 1990; Coleman, 1998). To our knowledge, Zr does not play any known physiological role, and while a physiological relevance for As has recently been suggested (Wolfe-Simon et al., 2011), no evidence exists for a biological As requirement or tolerance in BSCs. Therefore, the removal of As and Zr in live crusts is probably the result of either biosorption (Garnham et al., 1993; Chowdhury

& Mulligan, 2011; Kumar & Oomen, 2012) or the indirect effect of pH and redox changes caused by microbial activity. BSC activity can drive pH values close to 10 and create anoxic microenvironments (Garcia-Pichel & Belnap, 1996). Under reducing conditions, As exists as As(III), the adsorption of which increases with increasing pH (Adriano, 2001), and thus, As concentrations should decrease in the presence of actively metabolizing crusts. Under a wide range of pH conditions, Zr exists as $Zr(OH)_2$ (Aja et al., 1995), and I expected Zr solubility, and therefore, soil solution concentrations, to decrease with increasing pH, similar to what occurs for Fe-hydroxide complexes (Brady & Weil, 2002).

In contrast, Al, V, Cu, and Zn concentrations increased in some crusted samples (+H_2O and +Mo; Figure 3-2a-d). That Zn concentrations increased in some samples (Figure 3-2d) and decreased in others (Figure 3-1b), speaks to the variability in soil and crusts within our field site. Increasing Al, V, Cu, and Zn concentrations were not observed in any killed controls, and so I ruled out abiotic dissolution and desorption. Decreased microbial uptake would not result in increasing concentrations; rather, decreased uptake would likely cause concentrations to stabilize. Therefore, there are two reasonable explanations for the observed increase in Al, V, Cu, and Zn concentrations. The first is solubilization promoted by microbial production of organic acids (Drever & Stillings, 1997; van Hees et al., 2000) and/or of siderophores (Kalinowski et al.,

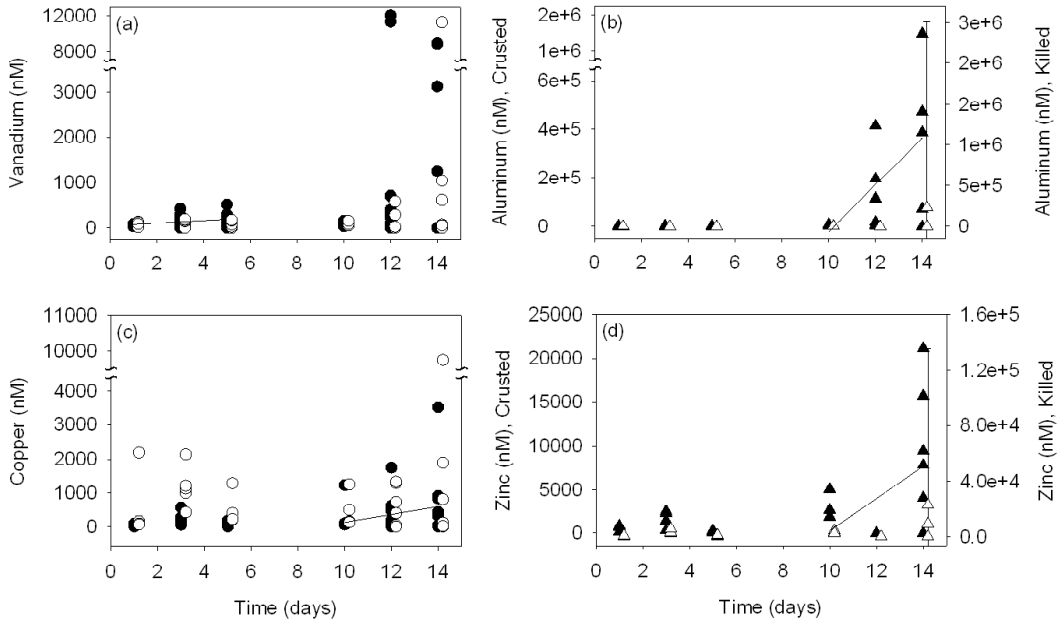

Figure 3-2. Elements with increasing concentrations over time in soil crust incubations; the +H_2O treatments are shown as circles (●,○), and the +Mo treatments are shown as triangles (▲, △). Al (a), V (b), Cu (c), and Zn (d) concentrations increased significantly in crusted samples (●, ▲), but not in killed controls (○, △). Increasing V and Zn concentrations (p = 0.01 & p = 0.04, respectively) are either the result of microbial solubilization or cellular export. Increasing Al concentrations (p = 0.04) are believed to result from cellular export. Increasing Cu concentrations (p = 0.04) are thought to be caused by microbial solubilization. Significant trends are shown by regression lines. Error bars are based on triplicate ICP-MS injections and propagated errors. Where error bars are not visible they are smaller than the symbols.observed in the +N (Ni, Zn, As) and +Mo (Zr) treatment groups.

2000; Liermann et al., 2000; 2005). Siderophores are low molecular weight organic compounds that bacteria produce to increase element solubility and facilitate element uptake (Lankford, 1973; Neilands, 1973). Though most siderophores typically bind Fe, some are known to bind Al (Roy & Chakrabartty, 2000), V (Bellenger et al., 2008), Cu (McKnight and Morel, 1980; Kim et al., 2004; Bellenger et al., 2007), and Zn (Bellenger et

al., 2007). Crusts contain organisms that produce siderophores (see Chapters 5 & 6), so it is possible that metallophore production is responsible for the increasing Al, V, Cu, and Zn concentrations. Cu is absolutely required by BSC microbes for photosynthetic and respiratory electron transport (Lockau, 1981; Peschek et al., 2004; Bernroitner et al., 2008) as well as for ammonium oxidation (Holmes et al., 1995; Zahn et al., 1996; Ferguson, 1998). The size, concentrated charge, and single oxidation state of Zn make it a valuable element in a variety of biological processes (Vallee & Auld, 1990; Coleman, 1998). Vanadium can be used in haloperoxidases (Butler, 1998; Littlechild, 1999) and in the alternative vanadium-dependent form of nitrogenase found in some bacteria (Eady, 1996); though we note, there is only indirect geochemical evidence that V is used in the latter within BSCs (see Chapter 2), and no evidence for the former. Although Al has no known physiological role, it could be solubilized serendipitously. It is logical, then, to assume the increases in soil solution Al, V, Cu, and Zn are due to active solubilization of Cu and Zn by siderophores, with the side-effect of increasing Al and V concentrations as well.

The second possible explanation for increasing Al, V, Cu, and Zn concentrations is export from biomass. Though I had no reason to expect cell death and lysis under my experimental conditions since previous experiments showed continual nitrate uptake and nitrogen fixation over the

timescale of our experiment (see Chapters 2 and 4), it is possible that Al, V, Cu, and Zn are actively pumped out of microbial cells because they had reached toxic levels or they are no longer required. Average Al concentrations in the soil solution of crusts are ~5 μM, but can reach 250 μM; this is well within range of toxic Al concentrations observed for N_2-fixing soil and root nodule bacteria (reviewed in Piña & Cervantes, 1996). Average soil solution V concentrations, on the other hand, were 0.1 μM, and never exceed 0.5 μM. This is much lower that the 10 μM concentrations reported to be toxic for *Azotobacter vinelandii* fixing N_2 with V-nitrogenase (Bellenger et al., 2011), although, this concentration does reach the 0.2-16.5 μM V level found to decrease photosynthesis in lakes (Nalewajko et al., 1995). Concentrations of Cu in the soil solution of crusted soils are generally less than ~0.5 μM, somewhat lower than the 1 μM toxic concentrations reported for cyanobacteria and algae (Chakraborty et al., 2010). Average soil solution Zn concentrations were 0.6 μM in crusts, which exceed the toxic levels (0.12 to 0.5 μM) reported by Paulsson et al. (2000) and Chakraborty et al. (2010). Cyanobacteria typically manage Zn toxicity by storing Zn in metallothioneins (Blindauer et al., 2002; Blindauer, 2008), but most bacteria deal with toxic levels of Zn levels by actively pumping Zn out of the cell (Nies, 2003). Therefore, increasing Zn concentrations may be the result of tightly regulated Zn export from bacterial cells (Hantke 2001; 2005).

I conclude that active export of Al resulting from toxicity is likely to be the primary cause of increasing Al concentrations, but that export of Cu is probably not the cause of its increased concentrations as Cu does not appear to exist at toxic concentrations within crusts. This further supports the interpretation that microbial solubilization is the cause of increasing Cu concentrations. The results are inconclusive for V and Zn as their concentrations just barely reach reported toxic levels, and so it is unclear whether solubilization or export is a more logical explanation for increasing V and Zn concentrations.

The concentrations of K and Mn decreased during week 1 and increased during week 2 in crusted samples (+Mo), but not in any of the killed controls (Figure 3-3a-b). The change in slope suggests the balance of microbial processes shifted during week 2 from uptake to solubilization. I postulate that decreasing concentrations are the result of microbial uptake and that increasing concentrations are the result of solubilization for Mn, and cellular release for K. Mn is used in a multitude of physiological processes (Fraústo da Silva & Williams, 2001), but probably the most relevant in BSCs is the use of Mn in the oxygen-evolving complex of photosynthetic cyanobacteria (Umena et al., 2011). Therefore, microbial uptake of Mn is a logical explanation for decreasing Mn concentrations in crusted samples. When Mn concentrations increase during week 2, I assumed it was the result of Mn solubilization by

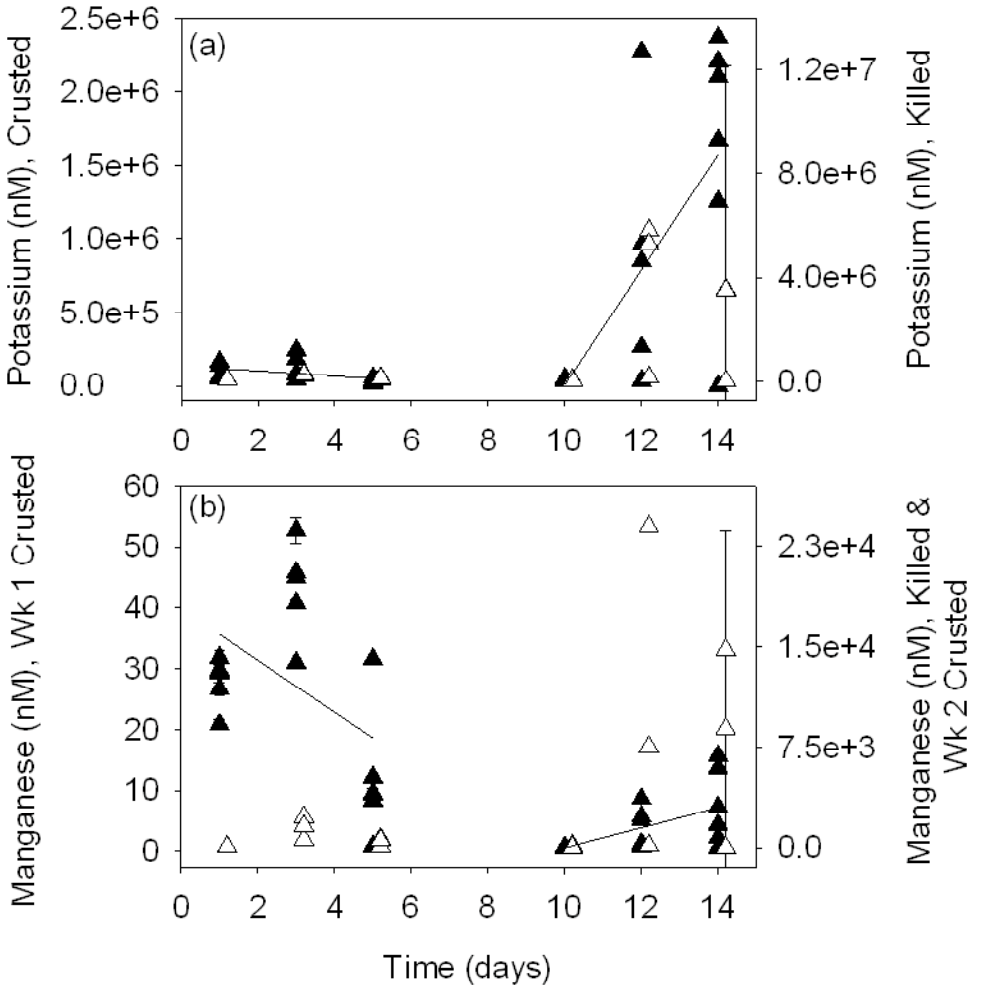

Figure 3-3. Potassium (a) and manganese (b) concentrations for crusted samples (▲) and killed controls (△) in the +Mo experiment. Both elements decreased during week 1 and increased during week 2. Decreasing K concentrations (p = 0.09) were interpreted as microbial uptake, and increasing K concentrations (p = 0.002) were thought to result from cellular export. Decreasing Mn concentrations (p = 0.06) were interpreted to result from microbial uptake, and increasing Mn concentrations were thought to come from microbial solubilization (p = 0.02). Significant trends are shown by regression lines. Error bars are based on triplicate ICP-MS injections and propagated errors. Where error bars are not visible they are smaller than the symbols.

chelators, such as siderophores that have been shown to bind Mn (Parker et al., 2004; Bellenger et al., 2007; Saal & Duckworth, 2010; Szabó & Farkas, 2011).

Bacteria use K for osmotic and pH balance as well as for regulation of enzymes and genes (Suelter, 1970; Booth, 1985; Epstein, 1986; Sutherland et al., 1986; Csonka & Hanson, 1991); cellular K concentrations are generally understood to be under the direct regulation of the bacterium (Epstein, 1986; Booth et al., 1999; Fraústo da Silva & Williams, 2001). Potassium binds weakly to organic compounds (Fraústo da Silva & Williams, 2001) so solubilization by chelation is not likely to be a dominant biological process influencing K mobility. Therefore, I believe that decreasing soil solution K concentrations is the result of microbial uptake, while increasing soil solution K concentrations are the result of active microbial export. Both processes are likely to occur as crusts engage in a range of metabolic processes.

Evidence for Abiotic Processes

When concentration changes were observed in killed controls I assumed that the changes were driven by abiotic processes. Increasing concentrations, such as those observed for Na, Mg, and P could be caused by dissolution and desorption reactions. Na and Mg are found in evaporite minerals such as salts and sulfates. With water addition, I expected these minerals to dissolve, resulting in increased soil solution Na and Mg concentrations, though this was not observed in all soils. In addition, water may compete with cations, like Na and Mg, for adsorption sites in clays, thus driving soil solution cation concentrations higher.

Phosphorus concentrations increased during week 2 in both the crusted samples and killed controls of the +Mo treatment group (Figure 3-4a-b). Because P concentrations increased in killed controls, I initially assumed that other increases in P concentration were abiotic as well. However, the increase in killed controls was observed only in soils that received Mo (Figure 3-4b). Under the pH conditions of our soils, Mo exists primarily as the molybdate ion (MoO_4^{2-}; Adriano, 2001), while P exists primarily as the very similar hydrogen phosphate ion (HPO_4^{2-}; Brady & Weil, 2002). Molybdate added at 22 μM is high enough to compete with HPO_4^{2-} for anion adsorption sites (Brady & Weil, 2002) and could result in increased P concentrations in the soil solution. If this is the case, increasing P concentrations in crusted samples that did not receive a Mo addition (Figure 3-4a) likely reflect biological solubilization as described above for other elements.

In the absence of Mo addition to killed controls, P concentrations decreased, demonstrating the typical behavior of P in soils (Figure 3-4) where phosphate tends to form insoluble precipitates with Al- and Fe-hydroxides, and can precipitate as insoluble Ca-phosphate minerals (Brady & Weil, 2002). This effect will be exacerbated with increasing pH (Brady & Weil, 2002), and pH has been shown to increase in photosynthetic crusts (Garcia-Pichel & Belnap, 1996); although, I did not observe changes in bulk pH during the incubations (data not shown).

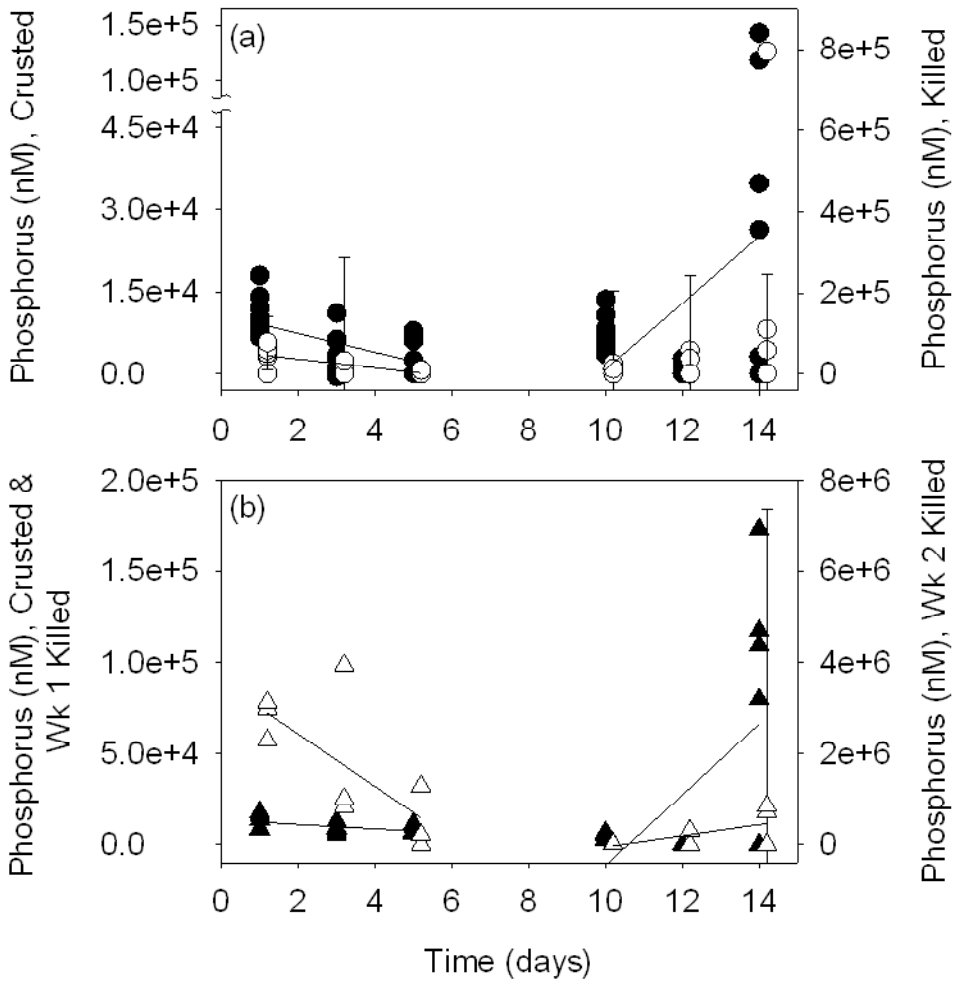

Figure 3-4. Phosphorus concentrations in crusted samples (●, ▲) and killed controls (○, △) of the +H_2O treatment (a) and the +Mo treatment (b). In the +H_2O treatment, P decreased significantly during week 1 for both crusted samples ($p < 0.001$) and killed controls ($p < 0.001$), but increased significantly during week 2 in the crusted samples ($p = 0.06$) only. In the +Mo treatment, P decreased significantly during week 1 in both crusted samples ($p = 0.02$) and killed controls ($p = 0.03$), and increases significantly during week 2 in both crusted samples ($p = 0.01$) and killed controls ($p = 0.04$). Increasing P concentrations in the +Mo treatment group are interpreted to result from competition with molybdate (MoO_4^{2-}) for adsorption sites. Since no Mo was added in the +H_2O treatment group, increasing P concentrations in those samples is assumed to be caused by microbial solubilization. Significant trends are shown by regression lines. Error bars are based on triplicate ICP-MS injections and propagated errors. Where error bars are not visible they are smaller than the symbols.

Decreasing concentrations, like those found for Fe (Figure 3-5), are most likely controlled by precipitation and adsorption reactions. Fe forms highly insoluble oxide and hydroxide minerals (Brady & Weil, 2002); therefore, it is not surprising that concentrations of Fe decreased in some soils. Similar to Fe, Al concentrations decreased in killed controls (data not shown), which is the opposite pattern from the increasing Al concentrations described above that were attributed to microbial solubilization. Al also forms insoluble oxide and hyroxide minerals, especially at elevated pH (Brady & Weil, 2002). It is possible that the dominant processes controlling Al solubility vary depending on what microbial processes are active and whether or not siderophores are produced.

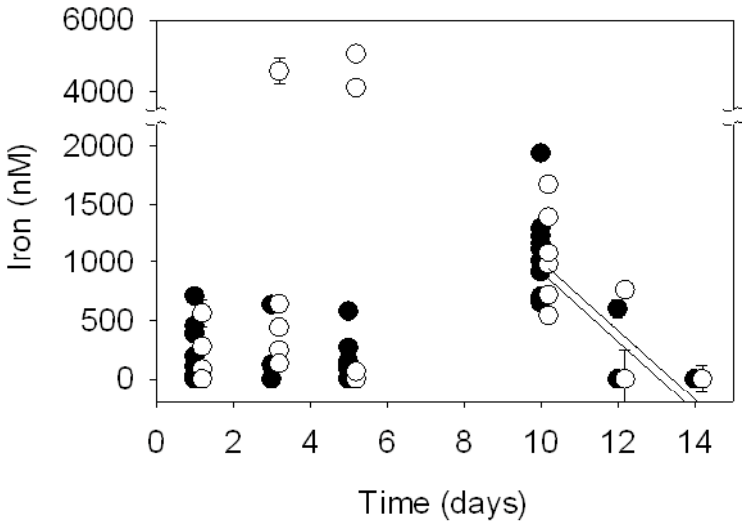

Figure 3-5. Iron concentrations in the $+H_2O$ treatment for crusted samples (●; $p < 0.001$) and killed controls (○; $p < 0.001$). Decreasing Fe concentrations were interpreted to result from abiotic precipitation and/or adsorption reactions. Significant trends are shown by regression lines. Error bars are based on triplicate ICP-MS injections and propagated errors. Where error bars are not visible they are smaller than the symbols.

Effect of Nitrogen and Molybdenum Addition to Crusted Samples

Not all of the concentration changes described above occurred in all treatment groups (i.e., +H_2O, +N, +Mo). Therefore, it is possible that some patterns are related to the treatment addition. Here I compare week 2 trends between treatment groups, and contrast pre- and post-treatment concentrations within treatment groups, to determine what elements were affected by N and Mo addition. All the concentration changes discussed below occurred in crusted samples during week 2, and are deemed to be biologically-mediated based on an evaluation of concentration changes in killed controls.

In general, I expected that N addition (which stops N_2-fixation) would cause increases in soil solution concentrations of those elements that are linked to N_2-fixation, but which do not participate in other N-cycle processes (Mg, P, V, Mn, Ni, Zn), because lower amounts of these elements would be needed when N_2-fixation was inactive. I demonstrated previously that addition of NH_4NO_3 decreased N_2-fixation rates to very near zero (see Chapters 2 & 4). I also predicted that concentrations of elements used in N assimilation and ammonium oxidation (Fe, Cu, Mo) would decrease when N was added because higher amounts of these elements would be needed in order to process the added NH_4NO_3 (Table 3-2).

With Mo addition, I anticipated decreases in concentrations of elements used directly for N_2-fixation (Fe, Mo) as well as in those elements that indirectly support N_2-fixation (Mg, P, Mn, Ni, Cu, Zn). Evidence exists for Mo-limitation of N_2 fixation in BSCs, and Mo addition has been show to result in increased N_2 fixation rates (see Chapter 4). With increased N_2-fixation rates, the microbial need for other metals involved in N_2-fixation would also increase, causing increased microbial uptake and subsequently, decreased soil solution concentrations. I expected that V would be an exception to this rule. Even if crust organisms possess the genes for V-nitrogenase, they will only express them in a situation of Mo-limitation (Jacobson et al., 1986; Joerger & Bishop, 1988). The addition of Mo would preclude the expression of V-nitrogenase by providing sufficient Mo to allow production of Mo-nitrogenase. Therefore, I expected soil solution V concentrations to stabilize (Table 3-2).

I did not anticipate concentration changes for Na and K with either treatment because I thought the need for these elements would be the same regardless of N or Mo availability, nor did I predict changes in Al, As, or Zr concentrations because they lack biological function in BSCs (Table 3-2).

Many elements showed significant trends in concentration in crusted samples over the duration of the incubations (Tables 3-3a, c, e).

Therefore, it is not absolutely valid to use statistical comparisons, such as the Wilcoxon Rank Sum test, to make inferences about long term effects of treatments. However, comparing week 1 and week 2 concentrations using the Wilcoxon test was useful in evaluating the influence of my treatments within the timeframe of the experiment.

Contrary to my hypothesis, Al concentrations increased when Mo was added (p = 0.07; Table 3-1e), but exhibited no change in concentration for the +H_2O treatment groups (Table 3-1a), and decreased in the +N treatment (p =0.01; Table 3-1c). When week 2 Al concentrations were compared to week 1 Al concentrations, I found that week 2 concentrations were higher in +H_2O and +Mo crusts (p < 0.001; Tables 3-4a-c, Figure 3-6a), and that week 1 concentrations were higher in the +N treatment group (p = 0.03; Figure 3-6a, Table 3-4b). As discussed above, it is possibile that Al is inadvertantly solubilized by microbial metallophores released into the soil solution to obtain other elements specifically needed for physiological processes (see Chapters 5 & 6). When N is added, N_2 fixation stops (see Chapters 2 & 4), thus lowering elemental requirements. Metallophores are typically produced under element-limitation, so, I expect that decreased element need would also result in decreased metallophore production. Therefore, Al concentrations increase when Mo is added and show higher concentrations in crusts that were fixing N_2 (+H_2O, +Mo) because higher metallophore production in these soils led to solubilization

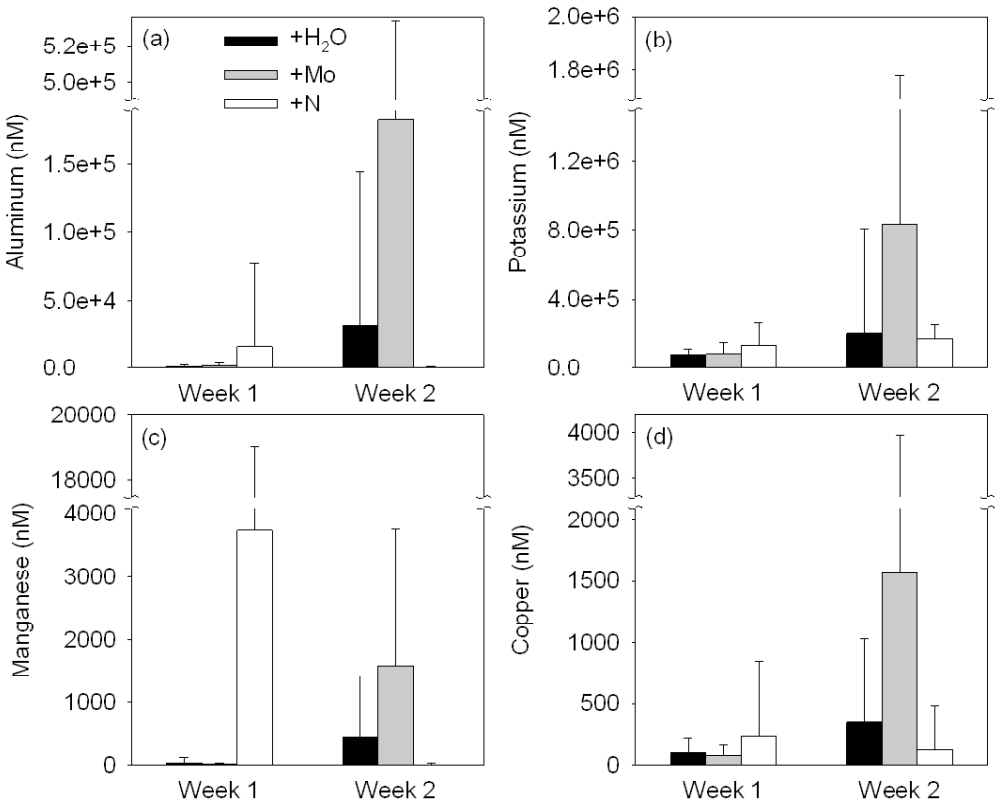

Figure 3-6. Average concentrations of Al (a), K (b), Mn (b) and Cu (d) in crusted samples. Colors denote treatments: +H_2O (black), +Mo (grey), and +N (white). K (p = 0.01, 0.04); Mn (p = 0.02, <0.001) and Cu (p = 0.07, 0.002) were all higher in week 2 of the +H_2O (black) and +Mo (grey) treatments, respectively. Al was higher in week 2 of the +H_2O treatment (p < 0.001). In the +N treatment, while Al concentrations were higher during week 1 (p = 0.03), K concentrations were higher during week 2 (p = 0.10), and there were no differences between week 1 and week 2 for Mn and Cu. Lack of higher week 2 concentrations for Al, Mn, and Cu with N addition is presumed to result from decreased metallophore production when N_2 fixation is inactive.

of Al. The Al concentrations were lower when N was added because metallophore production was depressed as a result of decreased element need, and Al solubility was then dominated by abiotic reactions.

K and Mn increased when H_2O (p = 0.01, 0.02) and Mo (p = 0.04, <0.001) were added, but showed no trend when N was added (Tables 3-

1a, c, e). Cu concentrations increased in +H_2O crusted samples only. Likewise, in week 2, K, Mn, and Cu concentrations were higher in the H_2O and +Mo treatments (Figure 3-6b-d). There was no difference between weeks 1 and 2 in the +N treatment, with the exception of K which had higher concentrations during week 2 (p = 0.10; Figure 3-6b). Higher K concentrations were interpreted to result from cellular export. It is possible that the addition of NH_4NO_3, altered the osmotic and/or charge balance within cells, thus changing the cycling of K. Increasing Mn and Cu concentrations were thought to be the consequence of solubilization, therefore, like Al, higher Mn and Cu concentrations in week 2 are consistent with persistent metallophore production when N_2 fixation is active (+H_2O, +Mo), and diminished metallophore production when N_2 fixation stops (+N).

V concentrations increased in crusts that received the Mo addition, but did not change significantly in +H_2O or +N crusts. Increasing V concentrations could be either the result of solubilization or cellular export. However, as described above, it is difficult to distinguish between the two processes based on the data available.

Zn concentrations also increased only in +Mo crusts; however, there were no differences in Zn concentrations between week 1 and week 2 for any treatment group.

Zr concentrations decreased during week 2 when Mo was added, but Zr also decreased during week 1. Therefore, I conclude that decreasing Zr concentrations are not related to Mo addition; they are likely the result of removal processes (i.e., biosorption) that are active in crusts of the +Mo treatment group and inactive in other treatment groups presumably due to the natural variability in crusts.

Summary & Implications

The results of this study provide real-time evidence for microbial solubilization of solid-phase elements in Colorado Plateau BSCs. This supports the conclusions of Beraldi-Campesi et al. (2009), who found that Mn, Cu, and Zn were depleted in the solid-phase of crusted soils relative to proximate uncrusted soils. They interpreted the depletions to be the result of microbial mobilization of Mn, Cu, and Zn that left them susceptible to leaching. The current study found that, at least in some crusts and during some point during the incubations, Mn, Cu, and Zn concentrations increased, and microbial solubilization was a logical explanation for the trend. Although Mn concentrations did decrease during week 1, prolonged microbial activity in the $+H_2O$ and +Mo treatments eventually led to increasing Mn concentrations. Therefore, I concur that Mn, Cu, and Zn depletions measured in crusted samples by Beraldi-Campesi et al. (2009) were the result of leaching after microbial activity mobilized these elements.

Beraldi-Campesi et al. (2009) also found that As and Zr were depleted in crusted soils, however, I did not observe evidence for microbial solubilization of As and Zr. Soil solution concentrations of As and Zr seem likely to have been influenced by biosorption over the course of our experiments; therefore our results for these elements do not reflect the patterns reported by Beraldi-Campesi et al. (2009). But, it is possible that over the longer timescales needed to influence the soil solid-phase these two elements could exhibit different patterns.

If prolonged solubilization consistently results in increasing Mn, Cu, and Zn mobility that lead to depletions of Mn, Cu, and Zn in the soil solid-phase, these depletions may serve as useful biomarkers of past BSC communities, as put forth by Beraldi-Campesi et al. (2009). Reliable biomarkers of soil microbial communities would be valuabe tools for studying the evolution of early terrestrial land colonization, and could also be used to evaluate the past existence of life elsewhere, such as on Mars.

Since BSCs are known to contain organisms that produce siderophores, low-molecular weight organic chelators that bind Fe and other metals with high affinity, we assume siderophore-production is the likely mechanism by which BSCs influence metal mobility (see Chapters 5 & 6). Soil organisms are also known to produce organic acids that increase the mobility of solid-phase elements (Drever & Stillings,

1997), and produce a characteristic pattern of dissolution that may be useful as signature of past biological activity (Neaman et al., 2005a, b).

Finally, Mn and Zn have been implicated previously as limiting factors for the development of lichenized crusts (Bowker et al., 2005), however, further studies did not support this original finding (Bowker et al., 2008b). My results suggest that Mn and Zn concentrations are manipulated by dark crusts dominated by cyanobacteria, that represent an earlier successional stage of crust development. Therefore, although Bowker et al. (2008b) did not find evidence for Mn and Zn limitation in the more developed, lichenized crusts, Mn and Zn may be important factors in earlier stages of crust succession, such as the dark cyanobacterial crusts used in the current study. For this reason, Mn and Zn may have potential utility as fertilizers that could promote the growth and development of dark crusts. These elements could prove useful for efforts to increase crust cover and thereby decrease arid land degradation.

References

Adriano, D.C. 2001. *Trace elements in terrestrial environments: Biogeochemistry, bioavailability, and risks of metals*. New York: Springer-Verlag.

Aja, S.U., S.A. Wood, and A.E. Williams-Jones. 1995. The aqueous geochemistry of Zr and the solubility of some Zr-bearing minerals. *Applied Geochemistry* 10: 603-620.

Aparicio, P.J., D.B. Knaff, and R. Malkn. 1975. The role of iron-sulfur center and siroheme in spinach nitrite reductase. *Archives of Biochemistry and Biophysics* 169: 102-107.

Bellenger, J.P., F. Arnaud-Neu, Z. Asfari, S.C.B. Myneni, E.I. Stiefel, and A.M.L. Kraepiel. 2007. Complexation of oxoanions and cationic metals by the biscatecholate siderophore azotochelin. *Journal of Biological Inorganic Chemistry* 12: 367-376.

Bellenger, J.P., T. Wichard, and A.M.L. Kraepiel. 2008. Vanadium requirements and uptake kinetics in the dinitrogen-fixing bacterium *azotobacter vinelandii*. *Applied and Environmental Microbiology* 74, no. 5: 1478-1484.

Bellenger, J.P., T. Wichard, Y. Xu, and A.M.L. Kraepiel. 2011. Essential metals for nitrogen fixation in a free-living N2-fixing bacterium: Chelation, homeostasis and high use efficiency. *Environmental Microbiology* 13, no. 6: 1395-1411.

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

Belnap, J. 1995. Surface disturbances: Their role in accelerating desertification. *Environmental Monitoring and Assessment* 37, no. 1-3: 39-57.

_____. 2002. Nitrogen fixation in biological soil crusts from southeast Utah, USA. *Biological Fertility of Soils* 35: 128-135.

Belnap, J., B. Budel, and O. L. Lange. 2001. Biological soil crusts: Characteristics and distribution. In *Biological soil crusts: Structure, function, and management*, ed. J. Belnap and O. L. Lange: 503. Berlin: Springer.

Belnap, J., K.T. Harper, and S.D. Warren. 1994. Surface disturbance of cryptobiotic soil crusts: Nitrogenase activity, chlorophyll content, and chlorophyll degradation. *Arid Soil Research and Rehabilitation* 8: 1-8.

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, no. 3: 348-359.

Berman-Frank, I., P. Lundgren, and P.G. Falkowski. 2003. Nitrogen fixation and photosynthetic oxygen evolution in cyanobacteria. *Research in Microbiology* 154: 157-164.

Bernroitner, M., M. Zamocky, M. Pairer, P.G. Furtmüller, G.A. Peschek, and C. Obinger. 2008. Heme-copper oxidases and their electron donors in cyanobacterial respiratory electron transport. *Chemistry and Biodiversity* 5, 1927-1961.

Bertini, I., H.B. Gray, E.I Stiefel, and J.S. Valentine. 2007. *Biological inorganic chemistry: Structure and reactivity*. Edited by I. Bertini, H.B. Gray, E.I. Stiefel and J.S. Valentine. Sausalito, California: University Science Books.

Bevington, P. and D.K. Robinson. 2002. *Data reduction and error analysis for the physical sciences*. New York: The McGraw Hill Companies, Inc.

Beymer, R.J. and J.M. Klopatek. 1991. Potential contribution of carbon by microphytic crusts in pinyon-juniper woodlands. *Arid Soil Research and Rehabilitation* 5: 187-198.

Blindauer, C.A. 2008. Zinc-handling in cyanobacteria: An update. *Chemistry and Biodiversity* 5: 1990-2013.

Blindauer, C.A., M.D. Harrison, A.K. Robinson, J.A. Parkinson, P.W. Bowness, P.J. Sadler, and N.J. Robinsong. 2002. Multiple bacteria encode metallothioneins and SMTA-like zinc fingers. *Molecular Microbiology* 45, no. 5: 1421-1432.

Booth, I.R. 1985. Regulation of cytoplasmic pH in bacteria. *Microbiological Reviews* 49, no. 4: 359-378.

Booth, I.R., T.A. Krulwich, E. Padan, J.B. Stock, G.M. Cook, V. Skulachev, G.N. Bennett, W. Epstein, J.L. Slonczewski, R.J. Rowbury, A. Martin, J.W. Foster, R.K. Poole, W.N. Konings, G. Schafer, and P. Dimroth. 1999. *The regulation of intracellular pH in bacteria*. ed. D. Chadwick and G. Cardew. Novartis foundation symposium. Chichester: John Wiley & Sons Ltd.

Bothe, H., O. Schmitz, M.G. Yates, and W.E. Newton. 2010. Nitrogen fixation and hydrogen metabolism in cyanobacteria. *Microbiology and Molecular Biology Reviews* 74, no. 4: 529-551.

Bowker, M.A., J. Belnap, V.B. Chaudhary, and N.C. Johnson. 2008a. 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, D.W. Davidson, and S.L. Phillips. 2005. Evidence for micronutrient limitation of biological soil crusts: Importance to arid-lands restoration. *Ecological Applications* 15, no. 6: 1941-1951.

Bowker, M.A., G.W. Koch, J. Belnap, and N.C. Johnson. 2008b. Nutrient availability affects pigment production but not growth in lichens of biological soil crusts. *Soil Biology and Biochemistry* 40: 2819-2826.

Bowker, M.A., S.C. Reed, J. Belnap, and S.L. Phillips. 2002. Temporal variation in community composition, pigmentation, and Fv/Fm of desert cyanobacterial soil crusts. *Microbial Ecology* 43: 13-25.

Brady, N.C. and R.R. Weil. 2002. *The nature and properties of soils*. Upper Saddle River: Prentice Hall.

Burgess, B.K. and D.J. Lowe. 1996. Mechanism of molybdenum nitrogenase. *Chemical Reviews* 96: 2983-3011.

Butler, A. 1998. Vanadium haloperoxidases. *Current Opinion in Chemical Biology* 2: 279-285.

Campbell, J.H., J.S. Clark, and J.C. Zak. 2009. PCR-DGGE comparison of bacterial community structure in fresh and archived soils sampled along a chihuahuan desert elevational gradient. *Microbial Ecology* 57: 261-266.

Campbell, S.E. 1979. Soil stabilization by a prokaryotic desert crust: Implications for precambrian land biota. *Origins of Life* 9: 335-348.

Chakraborty, P., P.V.R. Babu, T. Acharyya, and D. Bandyopadhyay. 2010. Stress and toxicity of biologically important transition metals (Co, Ni, Cu and Zn) on phytoplankton in a tropical freshwater system: An investigation with pigment analysis by HPLC. *Chemosphere* 80: 548-553.

Chowdhury, M.R.I. and C.N. Mulligan. 2011. Biosorption of arsenic from contaminated water by anaerobic biomass. *Journal of Hazardous Materials* 190: 486-492.

Coleman, J.E. 1998. Zinc enzymes. *Current Opinion in Chemical Biology* 2: 222-234.

Conant, J.B., E.M. Dietz, C.F. Bailey, and S.E. Kamerling. 1931. Studies in the chlorophyll series. V. The structure of chlorophyll a. *Journal of the American Chemical Society* 53, no. 6: 2382-2393.

Csonka, L.N. and A.D. Hanson. 1991. Prokaryotic osmoregulation: Genetics and physiology. *Annual Reviews in Microbiology* 45: 569-606.

Davidson, D.W., M.A. Bowker, D. George, S.L. Phillips, and J. Belnap. 2002. Treatment effects on performance of N-fixing lichens in disturbed soil crusts of the Colorado Plateau. *Ecological Applications* 12, no. 5: 1391-1405.

Drever, J.I. and L.L. Stillings. 1997. The role of organic acids in mineral weathering. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 120: 167-181.

Eady, R.E. 1996. Structure-function relationships of the alternative nitrogenases. *Chemical Reviews* 96: 3013-3030.

Epstein, W. 1986. Osmoregulation by potassium transport in *Escherichia coli*. *FEMS Microbiology Reviews* 39: 73-78.

_____. 2003. The roles and regulation of potassium in bacteria. *Progress in Nucleic Acid Research and Molecular Biology* 75: 293-320.

Evans, R.D. and J.R. Ehleringer. 1993. A break in the nitrogen cycle in aridlands? Evidence from delta15N of soils. *Oecologia* 94: 314-317.

Evans, R.D. and O.L. Lange. 2001. Biological soil crusts and ecosystem nitrogen and carbon dynamics. In *Biological soil crusts: Structure, function, and management*, ed. J. Belnap and O.L. Lange: 263-279. Berlin: Springer-Verlag.

Ferguson, S.J. 1998. Nitrogen cycle enzymology. *Current Opinion in Chemical Biology* 2: 182-193.

Ferguson-Miller, S., G.T. Babcock, and C. Yocum. 2007. Photosynthesis and respiration. In *Biological inorganic chemistry: Structure and reactivity*, ed. I. Bertini, H.B. Gray, E.I. Stiefel and J.S. Valentine. Sausalito, California: University Science Books.

Fraústo da Silva, J.J.R. and R.J.P. Williams. 2001. *The biological chemistry of the elements: The inorganic chemistry of life*. New York: Oxford University Press.

Garcia-Pichel, F. 2002. Desert environments: Biological soil crusts. In *Encyclopedia of environmental microbiology*, ed. G. Bitton: 1019-1023. New York: John Wiley.

Garcia-Pichel, F. and J. Belnap. 1996. Microenvironments and microscale productivity of cyanobacterial desert crusts. *Journal of Phycology* 32: 774-782.

Garcia-Pichel, F., S.L. Johnson, D. Youngkin, and J. Belnap. 2003. Small-scale vertical distribution of bacterial biomass and diversity in biological soil crusts from arid lands in the Colorado Plateau. *Microbial Ecology* 46: 312-321.

Garcia-Pichel, F., A. Lopez-Cortes, and U. Nubel. 2001. Phylogenetic and morphological diversity of cyanobacteria in soil desert crusts from the Colorado Plateau. *Applied and Environmental Microbiology* 67, no. 4: 1902-1910.

Garnham, G.W., G.A. Codd, and G.M. Gadd. 1993. Accumulation of zirconium by microalgae and cyanobacteria. *Applied Microbiology and Biotechnology* 39, no. 4-5: 666-672.

Guerrero, M.G., J.M. Vega, and M. Losada. 1981. The assimilatory nitrate-reducing system and its regulation. *Annual Reviews in Plant Physiology* 32: 169-204.

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.

Hantke, K. 2001. Bacterial zinc transporters and regulators. *BioMetals* 14: 239-249.

_____. 2005. Bacterial zinc uptake and regulators. *Current Opinion in Microbiology* 8: 196-202.

Holmes, A.J., A. Costello, M.E. Lidstrom, and J.C. Murrell. 1995. Evidence that particular methane monooxygenase and ammonia monooxygenase may be evolutionarily related. *FEMS Microbiology Letters* 132: 203-208.

Howard, J.B. and D.C. Rees. 1996. Structural basis of biological nitrogen fixation. *Chemical Reviews* 96: 2965-2982.

Isichei, A.O. 1990. The role of algae and cyanobacteria in arid lands. A review. *Arid Soil Research and Rehabilitation* 4: 1-17.

Jacobitz, S. and P.E. Bishop. 1992. Regulation of nitrogenase-2 in *Azotobacter vinelandii* by ammonium, molybdenum, and vanadium. *Journal of Bacteriology* 174, no. 12: 3884-3888.

Jacobson, M.R., R. Premakumar, and P.E. Bishop. 1986. Transcriptional regulation of nitrogen fixation by molybdenum in *Azotobacter vinelandii*. *Journal of Bacteriology* 167, no. 2: 480-486.

Jeffries, D.L., J.M. Klopatek, S.O. Link, and J. Bolton. 1992. Acetylene reduction by cryptogamic crusts from a blackbrush community as related to resaturation and dehydration. *Soil Biology and Biochemistry* 24: 1101-1105.

Joerger, R.D. and P.E. Bishop. 1988. Bacterial alternative nitrogen fixation systems. *Critical Reviews in Microbiology* 16: 1-14.

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, no. 1: 1-12.

Kalinowski, B.E., L.J. Liermann, S. Givens, and S.L. Brantley. 2000. Rates of bacteria-promoted solubilization of Fe from minerals: A review of problems and approaches. *Chemical Geology* 169: 357-370.

Kim, H.J., D.W. Graham, A.A. DiSpirito, M.A. Alterman, N. Galeva, C.K. Larive, D. Asunskis, and P.M.A. Sherwood. 2004. Methanobactin, a copper-acquisition compound from methane-oxidizing bacteria. *Science* 305, no. 5690: 1612-1615.

Kraemer, S.M. 2004. Iron oxide dissolution and solubility in the presence of siderophores. *Aquatic Science* 66: 3-18.

Kumar, J.I.N. and C. Oommen. 2012. Removal of heavy metals by biosorption using freshwater alga *Spirogyra hyalina*. *Journal of Environmental Biology* 33: 27-31.

Lancaster, J.R., J.M. Vega, H. Kamin, N.R. Orme-Johnson, W.H. Orme-Johnson, R.J. Krueger, and L.M. Siegel. 1979. Identification of the iron-sulfur center of spinach ferredoxin-nitrite reductase as a tetranuclear center, and preliminary EPR studies of mechanism. *The Journal of Biological Chemistry* 254, no. 4: 1268-1272.

Lankford, C.E. 1973. Bacterial assimilation of iron. *CRC Critical Reviews in Microbiology* 2: 273-331.

Liermann, L.J., R.L. Guynn, A. Anbar, and S.L. Brantley. 2005. Production of a molybdophore during metal-targeted dissolution of silicates by soil bacteria. *Chemical Geology* 220: 285-302.

Liermann, L.J., B.E. Kalinowski, S.L. Brantley, and J.G. Ferrey. 2000. Role of bacterial siderophores in dissolution of hornblende. *Geochimica et Cosmochimica Acta* 64, no. 4: 587-602.

Littlechild, J. 1999. Haloperoxidases and their role in biotransformation reactions. *Current Opinion in Chemical Biology* 3: 28-34.

Lockau, W. 1981. Evidence for a dual role of cytochrome c-553 and plastocyanin in photosynthesis and respiration of the cyanobacterium, *anabaena variabilis*. *Archives of Microbiology* 128, no. 3: 336-340.

Luque, F. and R.N. Pau. 1991. Transcriptional regulation by metals of structural genes for *azotobacter vinelandii* nitrogenases. *Molecular and General Genetics* 227: 481-487.

McKnight, D.M. and F.M.M. Morel. 1980. Copper complexation by siderophores from filamentous blue-green algae. *Limnology and Oceanography* 25, no. 1: 62-71.

Murphy, M.J., L.M. Siegel, S.R. Tove, and H. Kamin. 1974. A new prosthetic group participating in six-electron reduction reactions catalyzed by both sulfite and nitrite reductases. *Proceedings of the National Academy of Sciences of the United States of America* 71, no. 3: 612-616.

Nalewajko, C., K. Lee, and T.R. Jack. 1995. Effects of vanadium on freshwater phytoplankton photosynthesis. *Water, Air, and Soil Pollution* 81: 93-105.

Neaman, A., J. Chorover, and S.L. Brantley. 2005. Implications of the evolution of organic acid moieties for basalt weathering over geological time. *American Journal of Science* 305: 147-185.

_____. 2005. Element mobility patterns record organic ligands in soils on early earth. *Geology* 33, no. 2: 117-120.

Neilands, J.B. 1973. Microbial iron transport compounds (siderochromes). In *Inorganic Biochemistry*, ed. J.B. Neilands, 1:2-34. New York: Academic Press.

Nies, D.H. 2003. Efflux-mediated heavy metal resistance in prokaryotes. *FEMS Microbiology Reviews* 27: 313-339.

Parker, D.L., G. Sposito, and B.M. Tebo. 2004. Manganese(III) binding to a pyoverdine siderophore produced by a manganese(II)-oxidizing bacterium. *Geochimica et Cosmochimica Acta* 68, no. 23: 4809-4820.

Paulsson, M., B. Nyström, and H. Blanck. 2000. Long-tern toxicity of zinc to bacteria and algae in perophyton communities from the river Göta Älv, based on a microcosm study. *Aquatic Toxicology* 47: 243-257.

Peschek, G.A., C. Obinger, and M. Paumann. 2004. The respiratory chain of blue-green algae (cyanobacteria). *Physiologia Plantarum* 120: 358-369.

Piña, R.G. and C. Cervantes. 1996. Microbial interactions with aluminum. *BioMetals* 9: 311-316.

Raven, J.A., M.C.W. Evans, and R.E. Korb. 1999. The role of trace metals in photosynthetic electron transport in O2-evolving organisms. *Photosynthesis Research* 60: 111-149.

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 Science of the United States of America* 98, no. 13: 7123-7127.

Reynolds, R., J. Neff, M. Reheis, and P. Lamothe. 2006. Atmospheric dust in modern soil on aeolian sandstone, Colorado Plateau (USA): Variation with landscape position and contribution to potential plant nutrients. *Geoderma* 130: 108-123.

Rogers, R.W. 1972. Soil surface lichens in arid and subarid south-eastern Australia. *Australian Journal of Botany* 20: 301-316.

Roy, N. and P.K. Chakrabartty. 2000. Effect of aluminum on the production of siderophores by *Rhizobium* sp. (*Cicer arietinum*). *Current Microbiology* 41: 5-10.

Rubio, L.M., E. Flores, and A. Herrero. 1999. Molybdopterin guanine dinucleotide cofactor in *Synechococcus* sp. Nitrate reductase: Identification of *mobA* and isolation of a putative *moeB* gene. *FEBS Letters* 462: 358-362.

_____. 2002. Purification, cofactor analysis, and site-directed mutagenesis of *Synechococcus* ferredoxin-nitrate reductase. *Photosynthesis Research* 72: 13-26.

Rychert, R.C. and J. Skujins. 1974. Nitrogen fixation by blue-green algae-lichen crust in the great basin desert. *Soil Science Society of America Proceedings* 38: 768-771.

Saal, L.B. and O.W. Duckworth. 2010. Synergistic dissolution of manganese oxides as promoted by siderophores and small organic acids. *Soil Science Society of America Journal* 74, no. 6: 2021-2031.

Schulten, J.A. 1985. Soil aggregation by cryptogams of a sand prairie. *American Journal of Botany* 72, no. 11: 1657-1661.

Smith, K.S. and J.G. Ferry. 2000. Prokaryotic carbonic anhydrase. *FEMS Microbiology Reviews* 24: 335-366.

Steppe, T.F., J.B. Olson, H.W. Paerl, R.W. Litaker, and J. Belnap. 1996. Consortia N2 fixation: A strategy for meeting nitrogen requirements of marine and terrestrial cyanobacterial mats. *FEMS Microbiology Ecology* 21: 149-156.

Strauss, S.L., T.A. Day, and F. Garcia-Pichel. 2012. Nitrogen cycling in desert biological soil crusts across biogeographic regions in the southwestern United States. *Biogeochemistry* 108: 171-182.

Suelter, C.H. 1970. Enzymes activated by monovalent cations. *Science* 168, no. 3933: 789-795.

Sutherland, L., J. Cairney, M.J. Elmore, I.R. Booth, and C.F. Higgins. 1986. Osmotic regulation of transcription: Induction of the *proU* betaine transport gene is dependent on accumulation of intracellular potassium. *Journal of Bacteriology* 168, no. 2: 805-814.

Szabó, O. and E. Farkas. 2011. Characterization of Mn(II) and Mn(III) binding capability of natural siderophores desferrioxamine b and desferricoprogen as well as model hydroxamic acids. *Inorganic Chimica Acta* 376: 500-508.

Tamagnini, P., R. Axelsson, P. Lindberg, F. Oxelfelt, R. Wünschiers, and P. Lindblad. 2002. Hydrogenases and hydrogen metabolism of cyanobacteria. *Microbiology and Molecular Biology Reviews* 66, no. 1: 1-20.

Umena, Y., K. Kawakami, J.-R. Shen, and N. Kamiya. 2011. Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. *Nature* 473: 55-61.

Vallee, B.L. and D.S. Auld. 1990. Zinc coordination, function, and structure of zinc enzymes and other proteins. *Biochemistry* 29, no. 24: 5647-5659.

van Hees, P.A.W., U.S. Lundström, and R. Giesler. 2000. Low molecular weight organic acids and their Al-complexes in soil solution-composition, distribution and seasonal variation in three podzolized soils. *Geoderma* 94: 173-200.

Wolfe-Simon, F., J.S. Blum, T.R. Kulp, G.W. Gordon, S.E. Hoeft, J. Pett-Ridge, J.F. Stolz, S.M. Webb, P.K. Weber, P.C.W. Davies, A.D. Anbar, and R.S. Oremland. 2011. A bacterium that can grow by using arsenic instead of phosphorus. *Science* 332: 1163-1166.

Zahn, J.A., D.M. Arciero, A.B. Hooper, and A.A. DiSpirito. 1996. Evidence for an iron center in the ammonia monooxygenase from *Nitrosomas europaea*. *FEBS Letters* 397: 35-38.

Zhao, Y., S.-M. Bian, H.-N. Zhou, and J.-F. Huang. 2006. Diversity of nitrogenase systems in diazotrophs. *Journal of Integrative Plant Biology* 48, no. 7: 745-755.

CHAPTER 4
MOLYBDENUM LIMITS NITROGEN FIXATION IN BIOLOGICAL SOIL CRUSTS

Abstract

Biological soil crusts (BSCs) are critical components of many arid ecosystems. Not only do BSCs serve as ecosystem engineers providing carbon, nitrogen, and structure to soils, but they also play a vital role in arid land fertility even after the establishment of higher plants and animals. Nitrogen is often limiting in BSCs, despite the presence of cyanobacteria capable of nitrogen fixation. It has been shown in other ecosystems that nitrogen fixation is limited by additional nutrients such as phosphorus, iron, or molybdenum (Mo). In the current study, I found that Mo addition significantly increased nitrogen fixation rates, supporting my hypothesis that nitrogen fixation in BSCs of the Colorado Plateau is limited with respect to Mo. My results suggest that Mo fertilization of BSCs may be a viable conservation and restoration technique to facilitate the growth and development of BSCs and, thus, reduce the degradation of arid lands.

Introduction

Nitrogen (N) is a limiting factor for biological systems across multiple environments (Vitousek & Howarth, 1991; Vitousek et al., 2002; Galloway et al., 2004). While anthropogenic N is now a significant source for terrestrial N ecosystems, biological N_2 fixation (BNF) is still the dominant source of bioavailable N to most ecosystems (Galloway et al., 2004).

Globally, BNF contributes almost 200 Tg of fixed N per year (Cleveland et al., 1999). However, in many ecosystems, BNF is restricted or limited by other resources such as water and light (Jones, 1977; Coxson & Kershaw, 1983; Belnap, 2001), or phosphorus and trace metals like iron and molybdenum (Vitousek & Howarth, 1991; Berman-Frank et al., 2001; Sañudo-Wilhelmy et al., 2001; Mills et al., 2004; Zerkle et al., 2006; Glass et al., 2010).

In arid ecosystems, like the Colorado Plateau, N is the second-most limiting factor after water (Schlesinger, 1996; Evan & Lange, 2001). Because wet and dry N deposition rates are very low in this area (~4 kg h^{-1} yr^{-1}; West and Skujins, 1977), biological N_2 fixation is the primary source of bioavailable N to the ecosystem (Rychert & Skujins, 1974; Jeffries et al., 1992; Steppe et al., 1996; Belnap, 2002), providing up to 13-25 kg h^{-1} yr^{-1} (West & Skujins, 1977; Belnap, 2002). N_2 fixation can be performed by a diversity of prokaryotes that possess genes for the enzyme nitrogenase, which catalyzes the conversion of atmospheric nitrogen gas (N_2) to ammonium (NH_4^+). The dominant N_2-fixing organisms on the Colorado Plateau are cyanobacteria in biological soil crusts (BSCs; Belnap et al., 2001; Yeager et al., 2007), complex microbial consortia that construct vertically stratified biosedimentary structures in arid and semi-arid environments (Belnap et al., 2001; Garcia-Pichel et al., 2001; 2003). It is the N_2 fixation activity of cyanobacteria in BSCs, either as free-living

organisms or as symbionts within lichens, that provides bioavailable N and helps to creates a hospitable environment for life to thrive (Evans & Ehleringer, 1993; Belnap, 2002).

Limitation of N_2 fixation by other nutrients is common in many ecosystems (Vitousek & Howarth, 1991; Berman-Frank et al., 2001; Sañudo-Wilhelmy et al., 2001; Mills et al., 2004; Zerkle et al., 2006; Glass et al., 2010). This type of limitation is termed co-limitation, and occurs when the low availability of one nutrient provokes limitation with respect to another nutrient (Saito et al., 2008). Co-limitation of Mo and N has been observed in a variety of aquatic cyanobacteria, in which the activity of nitrogenase, N_2 fixation rates, and growth of the organisms were dependent on Mo concentration (Fay & de Vasconcelos, 1974; ter Steeg et al., 1986; Zerkle et al., 2006; Glass et al., 2010). In these cases, higher Mo availability reduced the effects of N limitation. Mo limitation has also been observed in forest soils where the addition of Mo enhanced N_2 fixation activity (Silvester, 1989; Barron et al., 2009), although I note, these studies were conducted with heterotrophic N_2-fixing bacteria and not with cyanobacterial soil crusts. To my knowledge, Mo-limitation in BSCs as a contributing factor to N-limitation has only been investigated in one study (Hartley & Schlesinger, 2002), and this work was conducted in the Chihuahuan Desert of New Mexico. In the current study I hypothesized that N_2 fixation in BSCs of the Colorado Plateau is limited by Mo, and I

present experimental results showing that N_2 fixation rates in BSCs increase with Mo addition. I chose to focus on Mo in this study because previous work showed that BSCs actively influence soil solution Mo concentrations (see Chapter 2); no such biologically-mediated changes in soil solution Fe concentrations were observed, so I did not investigate the presence of Fe-N co-limitation.

The most common and efficient form of the nitrogenase enzyme requires 2 atoms of molybdenum (Mo) at its active site and more than 30 atoms of iron (Fe) for electron transfer (Burgess & Lowe, 1996; Eady, 1996; Howard & Rees, 1996; Berman-Frank et al., 2003). Two Mo-independent versions of nitrogenase exist that use either vanadium (V) or additional iron (Fe) at the active site (Eady, 1996; Zhao et al., 2006); however, both forms are much less widespread than the Mo-dependent enzyme (Young, 1992; Zehr et al., 2003). All three versions of nitrogenase are produced by different, yet, closely related genes, and cyanobacteria typically possess only the genes for the Mo-nitrogenase (Young, 1992). The V-nitrogenase has been found in just three strains of freshwater *Anabaena* (Kentemich *et al.,* 1988; Thiel, 1993; Boison *et al.,* 2006); to date, no cyanobacteria have been discovered that have genes for the Fe-nitrogenase. Roughly 80-90% of the N_2 fixing community in biological soil crusts is made up of *Nostoc* species that have the Mo-nitrgoenase (Yeager et al., 2004; 2007). The putative existence of V-nitrogenase in

BSCs is based on indirect geochemical evidence (See Chapter 2); molecular biology techniques have been, as yet, unable to detect V-nitrogenase genes in BSCs (Noonan, unpublished data). Therefore, it can be assumed that the majority of the N_2 fixation that takes place in BSCs is dependent on the availability of Mo.

The bioavailability of Mo is determined by its speciation, which in turn is controlled by the redox conditions and pH of the environment, as well as the presence of other elements (Adriano, 2001). Microenvironments of pH and redox conditions exist in active BSCs, with pH ranging from 7-10 and redox conditions varying between oxic and anoxic (Garcia-Pichel & Belnap, 1996). In the oxic microenvironments at all pH values reported, Mo will exist primarily as the molybdate ion (MoO_4^{2-}). In the anoxic microenvironments, Mo will exist as molybdenum sulfides (MoS_2; Adriano, 2001). Mo is one of the least plentiful trace metals in soil (Wedepohl, 1995; Alloway, 1995). Its low abundance is often exacerbated by its high solubility under oxic conditions that leaves it vulnerable to leaching (Reddy & Gloss, 1993) as well as its tendency to adsorb strongly to soil components like oxide minerals (Adriano, 2001). Adsorption of Mo to exchange sites in clays (Adriano, 2001) and organics (Wichard et al., 2009) provides a mechanism of Mo retention that leaves it relatively bioavailable. Colorado Plateau soils are quite low in organics, with total organic carbon contents at less than 1% by weight (Beraldi-Campesi et

al., 2009). However, the soils do contain ~10% clay by weight (Noonan et al., unpublished data), so it is presumable that much of the bioavailable Mo in these soils is from the exchangeable pool. Crusts may access these exchangeable Mo stores through siderophore production.

Siderophores are low molecular weight organic compounds produced by microbes to increase metal solubility and facilitate metal uptake. Sideophores have been shown to promote the dissolution of clay minerals, thus liberating adsorbed metals (Rosenberg & Maurice, 2003), and although most siderophore typically bind Fe, some microbes produce siderophores capable of binding Mo as well (Liermann et al., 2005; Bellenger et al., 2007). Crust organisms have been shown to produce siderophores, and it is possible that some of them have Mo-binding capabilities (see Chapters 4 & 5). Therefore, siderophore production is one mechanism by which crust microbes might access clay- and organic-bound Mo. Abiotic processes in BSCs should also increase Mo availability because Mo adsorption to minerals and organics decreases with increasing pH (Goldberg & Forster, 1998; Adriano, 2001). Photosynthesis creates micro-zones of pH up to ~10 (Garcia-Pichel & Belnap, 1996), so Mo should be very soluble in these microenvironments. The average Mo concentration in the soil solution of BSCs is ~30 nM, reflecting its relative solubility and bioavailability in the crust system (See Chapter 2). Active N_2 fixation by BSCs has been measured in the field by multiple researchers

(Belnap, 2002; Johnson et al., 2005; Housman et al., 2006; Strauss et al., 2012), so 30 nM Mo is obviously sufficient for crust microbes to fix N_2. However, it is possible that with increased Mo availability, N_2 fixation rates in BSCs could be amplified.

Additional N fixation in BSCs could enhance the ability of crusts to provide other critical ecosystem services, such as fixed C input (Beymer & Klopatek, 1991; Evans & Lange, 2001; Garcia-Pichel, 2002) and erosion prevention (Campbell, 1979; Schulten, 1985; Belnap, 1993). Loss of BSC cover has been connected to arid land degradation (Isichei, 1990; Belnap et al., 1994; Belnap, 1995; Bowker et al., 2008a), a process that has serious ecological, political, and socioeconomical implications (Sheridan, 1981; Puigdefábregas, 1998; Arnalds & Archer, 2000; Pimentel, 2000; Weibe, 2003). Conserving existing BSCs and increasing BSC cover is, therefore, paramount to preserving arid land fertility. However, little is known about the factors that control BSC growth and development (Belnap et al., 1994; Bowker et al., 2005; 2008b). Determining whether or not the BSC N-cycle is limited by other resources, like Mo, could provide valuable information that will help develop tools for BSC conservation and management.

Methods

Crust Collection and Storage

Twelve crusts were collected in May, 2009 at the Green Butte site (Strauss et al., 2012) located north of Moab, UT on the Colorado Plateau (N 38°42′56.2″, W 109°41′32.7″). Crusts with minimal lichen and moss cover were selected based on a visual assessment in order to target "dark crusts" that were dominated by cyanobacteria (Garcia-Pichel & Belnap, 1996; Garcia-Pichel *et al.,* 2001). Sampling was based on methods outlined in Garcia-Pichel et al. (2003). The soil surface was sprayed lightly with distilled water, plastic Petri dish lids (50 mm x 13 mm) were pressed into the soil, and crusts were collected by inserting a plastic spatula under the dish. Inversion of the soil allowed the bottom of the dish to be inserted. Soils were allowed to dry and stored at room temperature in the dark until the current study. Soils can be stored without affecting community composition under these conditions for up to 24 months (Campbell et al., 2009).

Experimental Design

Crusts were fully wetted and allowed to dry once per week for the 3 weeks preceding the study to allow the crusts to come out of dormancy since they had been in storage for ~2 years.

At the beginning of the experiment, all crusts were wetted with 10 mL sterile, 18.2 MΩ·cm carbon-free water (NANOpure® DIamond™ UV,

Barnstead International, Dubuque, IA). Water was sterilized by autoclaving. Crusts were sealed in airtight containers constructed from polycarbonate Nalgene jars (125 mL) with rubber septa in the side and parafilm between the lid and the jar. Nitrogen fixation activity was measured with a modified acetylene reduction assay (Capone, 1993; Jeffries et al., 1992; Berman-Frank et al., 2007). Nitrogenase converts nitrogen gas (N≡N) to NH_4; the enzyme will also reduce the triple bond in acetylene (C≡C) to form ethylene ($H_2C=CH_2$). Therefore, monitoring ethylene production provides a proxy for N_2 fixation. Briefly, 33 mL of air was removed from the chamber with a 60 mL syringe, 33 mL of acetylene was added (~20% headspace volume; Berman-Frank et al., 2007), and ethylene production was monitored by gas chromatography. Immediately after acetylene addition, 1 mL of headspace gas was collected in a 3 mL syringe for a 0 h timepoint. Crusts were incubated at 32 ± 2°C under fluorescent lights (irradiance =120 $\mu E\ m^{-2}\ s^{-1}$). This temperature was similar to summertime temperatures observed at the field site and the luminosity is sufficient to allow crust cyanobacteria to photosynthesize (Garcia-Pichel & Belnap, 1996). Headspace aliquots (1 mL) were taken approximately 3 times a day for 2 days to establish pre-treatment ethylene production rates.

Once ethylene was detected at 4 or more time points, 5 mL of treatment water were added. There were two treatments: $+H_2O$ and +Mo.

The +H_2O treatment (sterile, 18 MΩ·cm water) was added to half the crusts (#1-6), and the +Mo treatment (sterile, 2000 nM $NaMoO_4 \cdot 2H_2O$ in 18 MΩ·cm water) was added to half the crusts (#7-12). The Mo concentration was chosen based on published non-limiting Mo concentrations previously used for N_2-fixing freshwater and coastal cyanobacteria (Glass et al., 2010).

After treatment addition, headspace samples were collected 3 times a day for 2 more days to determine post-treatment ethylene production rates.

Ethylene and acetylene were determined using a Hewlett-Packard 5890 Series II Gas Chromatograph (Injection temp: 80ºC) with a thermal ionization detector (200ºC) and a 6' x 1/8" SS Porapak N 80/100 column (Ohio Valley Specialty Chemical, Marietta, OH) with helium as a carrier gas (14 mL min^{-1}). Acetylene and ethylene were easily distinguished from one another with retention times of 3.8 and 2.4 min, respectively. The limit of detection (LOD) for ethylene was a peak area of 65, corresponding to 3 times the standard deviation of a low concentration standard. Values below the LOD were set to zero. Ethylene peak areas above the LOD were converted to nanomoles using an eight-point calibration curve. Final ethylene concentrations were normalized to soil surface area to yield final concentrations as nmol ethylene per square centimeter of crust (nmol cm^{-2}).

Statistical Analysis

Pre-treatment and post-treatment ethylene values (nmol cm^{-2}) were plotted against time (h) for individual crusts. A linear regression analysis was performed to obtain separate pre- and post-treatment slopes, R^2 values, and slope *p*-values for each individual crust. The slopes correspond to the pre- or post-treatment areal ethylene production rates (nmol cm^{-2} h^{-1}).

Pre-treatment rates from each treatment group were compared to one another using a Mann-Whitney Rank Sum Test to verify that all crusts had relatively similar rates before treatment water was added. Post-treatment rates from each treatment group (+H_2O, +Mo) were compared to pre-treatment rates for the same crusts using a Wilcoxon Signed Rank Test.

All statistical analyses were performed in SigmaPlot v11.0 (Systat Software Inc., Chicago, IL). Results are considered statistically significant for $p < 0.10$, and significant values are shown in bold in all tables and figures.

Results

Pre-Treatment Rate Comparison

There was no statistically significant difference between the pre-treatment ethylene production rates of the +H_2O and +Mo treatment groups ($p = 0.24$; Table 4-1).

Table 4-1. Results of comparison tests showing what comparison was done, the test used, the *p*-value, and interpretation. Significant values ($p < 0.10$) are highlighted in **bold**.

Comparison	Test Performed	*p*-value	Interpretation
+H_2O pre-treatment rates vs +Mo pre-treatment rates	Mann-Whitney	0.24	No statistical difference
+H_2O pre-treatment rates vs +H_2O post-treatment rates	Wilcoxon	0.69	No statistical difference
+Mo pre-treatment rates vs +Mo post-treatment rates	Wilcoxon	**0.03**	Post-treatment rates higher

Water Treatment

Pre-treatment rates for the +H_2O soils ranged from 1.08 to 3.34 nmol cm^{-2} h^{-1} and post-treatment rates ranged from 0.28 to 3.34 nmol cm^{-2} h^{-1} (Figure 4-1a, Table 4-2). Four of the six crusts had significant pre-treatment rates, and five of the six crusts had significant post-treatment rates ($p < 0.10$, Table 4-2). All but one pre-treatment and one post-treatment rate, had R^2 values greater than 0.75, indicating a strong correlation between time and ethylene production (Table 4-2).

When pre- and post-treatment rates for the water only treatment were compared, there was no significant difference ($p = 0.69$; Table 4-1).

Mo Treatment

For the +Mo soils, pre-treatment rates ranged from 0.28 to 3.07 nmol cm^{-2} h^{-1}, while post-treatment rates ranged from 1.48

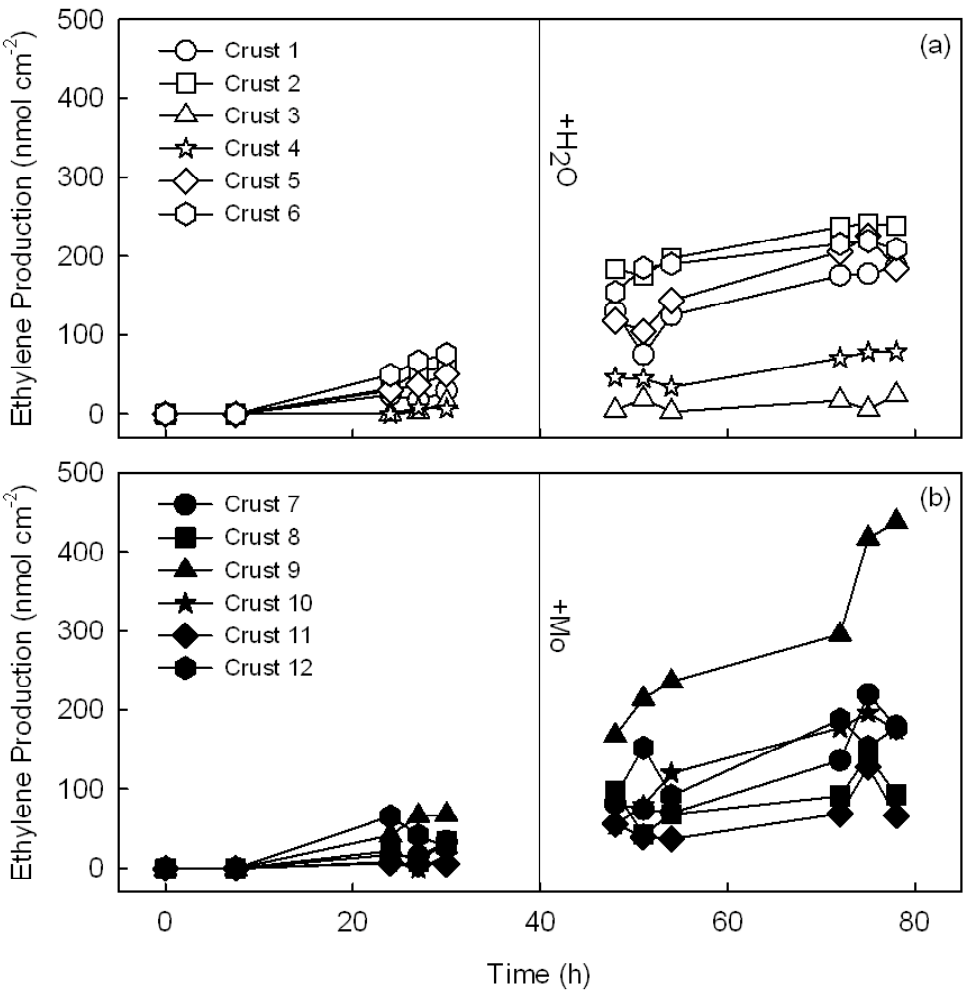

Figure 4-1. Area normalized ethylene production (nmol cm^{-2}) as a function of time. Panel (a) shows the results for the crusts that received the $+H_2O$ treatment (crusts 1-6, open symbols), and panel (b) shows the results for the +Mo treatment (crusts 7-12, solid symbols). Water was added to all crusts at 0 h, and ethylene production was monitored for 36 h (pre-treatment rates). Treatment water ($+H_2O$ or +Mo) was added at 40 h (indicated by the vertical lines), and ethylene production was measured for an additional 48 h (post-treatment rates). Ethylene production did not change after the $+H_2O$ treatment ($p = 0.69$), but ethylene production increased significantly after the Mo addition (***p* = 0.03**).

to 7.82 nmol cm^{-2} h^{-1} (Figure 4-1b, Table 4-1). Only two of the pre-treatment rates were significant (crusts #9 and #11); four post-treatment rates were significant ($p < 0.10$, Table 4-1). The R^2 values were in general,

greater than 0.80 except for two pre-treatment rates and three post-treatment rates. All R^2 values for the +Mo treatment were above 0.35, which still points to a strong correlation between time and ethylene production (Table 4-1). After Mo addition, post-treatment rates were significantly higher than pre-treatment rates (p = 0.03, Table 4-2).

Discussion

A process (in this case, N_2 fixation) is limited by a particular nutrient (in this case, Mo), if addition of that nutrient results in an increase in the process rate (Gibson, 1971). With the addition of Mo, ethylene production rates, and therefore, N_2 fixation rates, increased significantly at the 97% confidence level (p = 0.03, Table 4-2). Since addition of Mo resulted in increased N_2 fixation rates, we conclude that N_2 fixation in biological soil crusts from the Colorado Plateau is limited with respect to Mo.

It is difficult to use ethylene production rates to calculate exact N_2 fixation rates, because the ethylene-to-NH_4^+ conversion factor varies greatly among organisms and environments (see review in Belnap, 2001). However, it is valid to assume that increased ethylene production rates are caused by increased nitrogenase activity and, therefore reflect increased N_2 fixation rates.

The lack of statistically significant differences between pre-treatment rates of the two treatment groups (p = 0.24; Table 4-2) demonstrated that all crusts were in a similar physiological state prior to treatment water

Table 4-2. Results of linear regression ethylene production (nmol cm^{-2}) vs time for pre- and post-treatment incubations. Slopes and slope standard error, *p*-value, and R^2 are reported. *P*-values < 0.10 indicate slopes that are statistically different from zero and represent a significant change over time (**bold**). Slopes are ethylene production rates (nmol cm^{-2} h^{-1}). After 40 h of incubation, crusts 1-6 received the +H_2O treatment, and crusts 7-12 received the +Mo treatment. Significant results are highlighted in bold.

	Pre-treatment				Post-treatment			
Sample	Slope (nmol cm^{-2} h^{-1})	Std Err[a]	*p*-value	R^2	Slope (nmol cm^{-2} h^{-1})	Std Err	*p*-value	R^2
+H_2O								
1	1.17	0.32	**0.07**	0.87	2.84	0.76	**0.02**	0.78
2	2.59	0.44	**0.03**	0.94	2.20	0.26	**<0.001**	0.95
3	2.54	0.92	0.22	0.88	0.28	0.30	0.40	0.18
4	1.08	0.98	0.47	0.55	1.36	0.26	**0.01**	0.88
5	2.07	0.26	**0.02**	0.97	3.34	0.74	**0.01**	0.84
6	3.34	0.23	**0.005**	0.99	1.64	0.41	**0.02**	080
+Mo								
7	1.21	0.43	0.11	0.80	4.21	0.97	**0.01**	0.82
8	1.15	0.65	0.22	0.61	1.48	0.99	0.21	0.36
9	3.07	0.44	**0.02**	0.96	7.82	1.36	**0.005**	0.89
10	0.47	0.43	0.39	0.37	4.13	0.64	**0.003**	0.91
11	0.28	0.09	**0.09**	0.82	1.67	0.90	0.14	0.46
12	1.75	1.48	0.36	0.41	2.42	0.98	**0.07**	0.60

[a]The linear regression analysis reported the slope standard error.

addition. Ethylene production rates did not change in crusts that received the $+H_2O$ treatment (p = 0.69; Table 4-2), confirming that water addition alone was insufficient to influence nitrogenase activity in these crusts. It was important to verify this fact to show that changes resulting from Mo addition were triggered by the metal, and not by rewetting. Additionally, the results of the $+H_2O$ treatment established that prolonged exposure to acetylene did not have a detrimental effect on N_2 fixation rates as rates were constant over the 4 days of incubation. Finally, crusts are quite heterogeneous, and the lower N_2 fixation rates in some crusts likely reflect the variable amounts of nitrate, ammonium, and Mo present in individual crusts (see Chapters 2 & 3). The range in observed N_2 fixation rates are of course due, in part, to the ambient N and Mo concentrations in the soil.

Previous work has demonstrated Mo-limitation of N_2 fixation in aquatic cyanobacteria and in soil heterotrophic bacteria. In pure culture studies of cyanobacteria, Mo concentrations of 10 nM or less caused decreased nitrogenase activity, N_2 fixation rates, and growth (Fay & de Vasconcelos, 1974; ter Steeg et al., 1986; Zerkle et al., 2006; Glass et al., 2010). In both lab and field studies, increased Mo concentrations resulted in increased nitrogenase activity (Fay & de Vasconcelos, 1974; ter Steeg et al., 1986; Silvester, 1989; Zerkle et al., 2006; Barron et al., 2009; Glass et al., 2010). Mo limitation was also shown to result in symptoms of N-starvation (Fay &

de Vasconcelos, 1974), indicating that low Mo concentrations can result in co-limitation with respect to N (Glass et al., 2010).

The average crust Mo concentration was ~30 nM, however, the median concentration was only ~12 nM, and it was not uncommon to measure Mo concentrations below our detection limit (see Chapters 2 and 3). This reflects the patchiness in Mo availability created by differences in soil Mo contents, pH, redox potential, and the abundance of other elements over small spatial scales (Adriano, 2001). While the Mo concentration required to induce Mo and N co-limitation varies by organism, less than 10 nM Mo was shown to reduce nitrogenase activity in freshwater and coastal *Nostoc* species (Glass et al., 2010) that are closely related to the dominant crust N_2-fixers. Therefore, concentrations at or below 12 nM are definitely approaching the point at which they may limit crust N_2-fixation ability. Further studies with different Mo concentrations would help to elucidate how much Mo is required to achieve maximum fixation rates in crusts.

If, as this study shows, Mo limits N_2 fixation in BSCs, Mo may serve as an appropriate fertilizer to increase arid land fertility in some areas. Mo application has been shown to increase nitrogenase activity in temperate and tropical forest soils (Silvester, 1989; Barron et al., 2009), and I have shown that under laboratory conditions, supplemental Mo increases N_2 fixation rates in Colorado Plateau BSCs. Therefore, it is reasonable to

infer that Mo fertilization in the field could enhance N_2 fixation on the Colorado Plateau, and possibly elsewhere. Increasing bioavailable N could improve BSC growth and development, making it possible to expand BSC coverage. Furthermore, because BSCs are net N-exporters (Johnson et al., 2007), increasing BSC N_2 fixation would provide additional N to the ecosystem as a whole. This increased N availability would benefit soil ecosystems and higher plants. Together, increasing BSC and plant cover could help to mitigate arid land degradation (Evans & Ehleringer, 1993; Bowker et al., 2005; Byers et al., 2006; Bowker, 2007).

Using estimates of soil characteristics and published values of cellular Mo contents it is possible to perform a back-of-the-envelope calculation to determine how much Mo would be required to double crust cover at my field site. Using the density of quartz (2.65 g cm^{-2}), the depth of peak cyanobacterial abundance (2 mm; Garcia-Pichel et al., 2003), the weight, and total organic carbon (TOC) content of the soil (~0.65%), combined with the following assumptions: that microbial biomass is about 2 times TOC (Madigan et al., 2003), that *Nostoc* species, the dominant N_2 fixers, make up 0.8% of the community (Potrafka et al., unpublished data), and that Mo-to-C ratios in *Nostoc* ranged from 0.9-2.3 μmol Mo per mol C (Glass et al., 2010), I calculated there is approximately 0.02-0.05 μmol Mo in one square meter of 2 mm deep crust. Therefore, it would take ~20-50 mmol Mo to fertilize 1 km^2 of crust. I realize that this is a very rough

estimate, and it assumes that *Nostoc* species are the only organisms fixing N_2 in the crusts, which may not be the case. However, it does provide an idea of how much Mo would be needed to double *Nostoc* biomass which would serve to double crust cover or, perhaps, to develop an equivalent amount of new crust cover in a disturbed area.

Crusts are susceptible to a variety of disturbances, such as pollution and mechanical destruction, that reduce crust species diversity, biomass content, and extent of cover (Belnap & Eldridge, 2001). Many disturbances can lead to decreased N_2 fixation activity (Terry & Burns, 1987; Jeffries et al., 1992; Evans & Belnap, 1999), and this results in serious negative effects for ecosystems that rely on crusts for bioavailable N (Evans & Ehleringer, 1993). Recovery times are estimated to be anywhere from a few to a few thousand years and depend on a variety of factors, including the type and severity of the disturbance, the soil characteristics, and the colonizing organisms (Belnap & Eldridge, 2001). N_2-fixing cyanobacteria typically appear during the second phase of colonization after large filamentous cyanobacteria like *Microcoleus* species have stabilized the soil (Belnap & Gardner, 1993; Belnap, 2002; Redfield et al., 2002). Inoculation of disturbed surfaces with cyanobacteria and lichens has had some success in boosting recovery rates (St. Clair et al., 1986; Belnap, 1993; Buttars et al., 1998; Xiao et al., 2011), however the influence of fertilization on crust recovery and nitrogenase activity has

been inconsistent (Davidson et al., 2002; Hartley & Schlesinger, 2002; Maestre et al., 2006). Restoration efficacy depends strongly on specific site characteristics (Bowker, 2007; Bowker & Belnap 2008), and so it is vital to know whether or not Mo addition could increase BSC recovery in different locations.

Emphasis on BSC recovery is very important for the restoration of arid lands, especially because it may speed the recovery of the entire ecosystem (Bowker, 2007). Because BSCs serve as effective ecosystem engineers (Bowker et al., 2006; Byers et al., 2006; Bowker, 2007), they may facilitate restoration (Byers et al., 2006), and so it is important to know what factors influence their growth and development (Bowker et al., 2005). The results of the current study indicate that, in some areas at least, crust N_2 fixation is limited by Mo, which may lead to co-limitation with respect to N. Similar investigations in other sites are necessary to determine the extent of Mo-N co-limitation in crusts, and to establish where Mo fertilization may be a suitable restoration technique.

References

Adriano, D.C. 2001. *Trace elements in terrestrial environments: Biogeochemistry, bioavailability, and risks of metals*. New York: Springer-Verlag.

Alloway, B.J. 1995. *Heavy metals in soils.* London, UK: Blackie Academic & Professional.

Arnalds, O. and S. Archer. 2000. Introduction. In *Rangeland desertification*, ed. O. Arnalds and S. Archer:1-4. Dordrecht: Kluwer Academic Publishers.

Barron, A.R., N. Wurzburger, J.P. Bellenger, S.J. Wright, A.M.L. Kraepiel, and L.O. Hedin. 2009. Molybdenum limitation of asymbiotic nitrogen fixation in tropical forest soils. *Nature Geoscience* 2: 42-45.

Bellenger, J.P., F. Arnaud-Neu, Z. Asfari, S.C.B. Myneni, E.I. Stiefel, and A.M.L. Kraepiel. 2007. Complexation of oxoanions and cationic metals by the biscatecholate siderophore azotochelin. *Journal of Biological Inorganic Chemistry* 12: 367-376.

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

_____. 1995. Surface disturbances: Their role in accelerating desertification. *Environmental Monitoring and Assessment* 37, no. 1-3: 39-57.

_____. 2001. Factors influencing nitrogen fixation and nitrogen release in biological soil crusts. In *Biological soil crusts: Structure, function, and management*, ed. J. Belnap and O.L. Lange:241-261. Berlin Heidelberg New York: Springer.

_____. 2002. Nitrogen fixation in biological soil crusts from southeast Utah, USA. *Biol Fertil Soils* 35: 128-135.

Belnap, J. and D. Eldridge. 2001. Disturbance and recovery of biological soil crusts. In *Biological soil crusts: Structure, function, and management*, ed. J. Belnap and O.L. Lange: 503. Berlin: Springer.

Belnap, J. and J.S. Gardner. 1993. Soil microstructure in soils of the Colorado Plateau: The role of the cyanobacterium *Microcoleus vaginatus*. *Great Basin Naturalist* 53: 40-47.

Belnap, J., K.T. Harper, and S.D. Warren. 1994. Surface disturbance of cryptobiotic soil crusts: Nitrogenase activity, chlorophyll content, and chlorophyll degradation. *Arid Soil Research and Rehabilitation* 8: 1-8.

Belnap, J., J.H. Kaltenecker, R. Rosentreter, J. Williams, S. Leonard, and D. Eldridge. 2001. Biological soil crusts: Ecology and management, ed. United States Department of the Interior Bureau of Land Management, Technical Reference 1730-2.

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, no. 3: 348-359.

Berman-Frank, I., J.T. Cullen, Y. Shaked, R.M. Sherrell, and P.G. Falkowski. 2001. Iron availability, cellular iron quotas, and nitrogen fixation in trichodesmium. *Limnology and Oceanography* 46, no. 6: 1249-1260.

Berman-Frank, I., P. Lundgren, and P.G. Falkowski. 2003. Nitrogen fixation and photosynthetic oxygen evolution in cyanobacteria. *Research in Microbiology* 154: 157-164.

Berman-Frank, I., A. Quigg, Z.V. Finkel, A.J. Irwin, and L. Haramaty. 2007. Nitrogen-fixation strategies and Fe requirements in cyanobacteria. *Limnology and Oceanography* 52, no. 5: 2260-2269.

Beymer, R.J. and J.M. Klopatek. 1991. Potential contribution of carbon by microphytic crusts in pinyon-juniper woodlands. *Arid Soil Research and Rehabilitation* 5: 187-198.

Boison, G., C. Steingen, L.J. Stal, and H. Bothe. 2006. The rice field cyanobacteria *Anabaena azotica* and *Anabaena* sp. Ch1 express vanadium-dependent nitrogenase. *Archives of Microbiology* 186: 367-376.

Bowker, M.A. 2007. Biological soil crust rehabilitation in theory and practice: An underexploited opportunity. *Restoration Ecology* 15, no. 1: 13-23.

Bowker, M.A. and J. Belnap. 2008. A simple classification of soil types as habitats of biological soil crusts on the Colorado Plateau, USA. *Journal of Vegetation Science* 19, no. 6: 831-840.

Bowker, M.A., J. Belnap, V.B. Chaudhary, and N.C. Johnson. 2008a. 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, D.W. Davidson, and S.L. Phillips. 2005. Evidence for micronutrient limitation of biological soil crusts: Importance to arid-lands restoration. *Ecological Applications* 15, no. 6: 1941-1951.

Bowker, M.A., J. Belnap, and M.E. Miller. 2006. Spatial modeling of biological soil crusts to support rangeland assessment and monitoring. *Rangeland Ecology and Management* 59: 519-529.

Bowker, M.A., G.W. Koch, J. Belnap, and N.C. Johnson. 2008b. Nutrient availability affects pigment production but not growth in lichens of biological soil crusts. *Soil Biology and Biochemistry* 40: 2819-2826.

Burgess, B.K. and D.J. Lowe. 1996. Mechanism of molybdenum nitrogenase. *Chemical Reviews* 96: 2983-3011.

Buttars, S.M., L.L. St. Clair, J.R. Johansen, J.C. Sray, M.C. Payne, B.L. Webb, R.E. Terry, B.K. Pendleton, and S.D. Warren. 1998. Pelletized cyanobacterial soil amendments: Laboratory testing for survival, escapability, and nitrogen fixation. *Arid Soil Research and Rehabilitation* 12, no. 2: 165-178.

Byers, J.E., K. Cuddington, C.G. Jones, T.S. Talley, A. Hastings, J.G. Lambrinos, J.A. Crooks, and W.G. Wilson. 2006. Using ecosystem engineers to restore ecological systems. *Trends in Ecology and Evolution* 21, no. 9: 493-500.

Campbell, J.H., J.S. Clark, and J.C. Zak. 2009. PCR-DGGE comparison of bacterial community structure in fresh and archived soils sampled along a chihuahuan desert elevational gradient. *Microbial Ecology* 57: 261-266.

Campbell, S.E. 1979. Soil stabilization by a prokaryotic desert crust: Implications for precambrian land biota. *Origins of Life* 9: 335-348.

Capone, D.G. 1993. Determination of nitrogenase activity in aquatic samples using the acetylene reduction procedure. In *Handbook of methods in aquatic microbial ecology*, ed. P.F. Kemp, B.F. Sherr, E.B. Sherr and J.J. Cole: 621-631. New York: CRC Press LLC.

Cleveland, C.C., A.R. Townsend, D.S. Schimel, H. Fisher, R.W. Howarth, L.O. Hedin, S.S. Perakis, E.F. Latty, J.C. Von Fischer, A. Elseroad, and M.F. Wasson. 1999. Global patterns of terrestrial biological nitrogen (N2) fixation in natural ecosystems. *Global Biogeochemical Cycles* 13, no. 2: 623-645.

Coxson, D.S. and K.A. Kershaw. 1983. Rehydration response of nitrogenase activity and carbon fixation in terrestrial *nostoc communie* in *stipa-bouteloua* grassland. *Canadian Journal of Microbiology* 29: 938-944.

Davidson, D.W., M.A. Bowker, D. George, S.L. Phillips, and J. Belnap. 2002. Treatment effects on performance of N-fixing lichens in disturbed soil crusts of the Colorado Plateau. *Ecological Applications* 12, no. 5: 1391-1405.

Eady, R.E. 1996. Structure-function relationships of the alternative nitrogenases. *Chemical Reviews* 96: 3013-3030.

Evans, R.D. and J. Belnap. 1999. Long-term consequences of disturbance on nitrogen dynamics in an arid ecosystem. *Ecology* 80, no. 1: 150-160.

Evans, R.D. and J.R. Ehleringer. 1993. A break in the nitrogen cycle in aridlands? Evidence from delta15N of soils. *Oecologia* 94: 314-317.

Evans, R.D. and O.L. Lange. 2001. Biological soil crusts and ecosystem nitrogen and carbon dynamics. In *Biological soil crusts: Structure, function, and management*, ed. J. Belnap and O.L. Lange: 263-279. Berlin: Springer-Verlag.

Fay, P. and L. De Vasconcelos. 1974. Nitrogen metabolism and ultrastructure in *Anabaena cylindrica*. *Archives of Microbiology* 99: 221-230.

Galloway, J.N., F.J. Dentener, D.G. Capone, E.W. Boyer, R.W. Howarth, S.P. Seitzinger, G.P. Asner, C.C. Cleveland, P.A. Green, E.A. Holland, D.M. Karl, A.F. Michaels, J.H. Porter, A.R. Townsend, and C.J. Vorosmarty. 2004. Nitrogen cycles: Past, present, and future. *Biogeochemistry* 70: 153-226.

Garcia-Pichel, F. 2002. Desert environments: Biological soil crusts. In *Encyclopedia of environmental microbiology*, ed. G. Bitton:1019-1023. New York: John Wiley.

Garcia-Pichel, F. and J. Belnap. 1996. Microenvironments and microscale productivity of cyanobacterial desert crusts. *Journal of Phycology* 32: 774-782.

Garcia-Pichel, F., S.L. Johnson, D. Youngkin, and J. Belnap. 2003. Small-scale vertical distribution of bacterial biomass and diversity in biological soil crusts from arid lands in the Colorado Plateau. *Microbial Ecology* 46: 312-321.

Garcia-Pichel, F., A. Lopez-Cortes, and U. Nubel. 2001. Phylogenetic and morphological diversity of cyanobacteria in soil desert crusts from the Colorado Plateau. *Applied and Environmental Microbiology* 67, no. 4: 1902-1910.

Gibson, C.E. 1971. Nutrient limitation. *Water Pollution Control Federation* 43, no. 12: 2436-2440.

Glass, J.B., F. Wolfe-Simon, J.J. Elser, and A. Anbar. 2010. Molybdenum-nitrogen co-limitation in freshwater and coastal heterocystous cyanobacteria. *Limnology and Oceanography* 55, no. 2: 667-676.

Goldberg, S. and H.S. Forster. 1998. Factors affecting molybdenum adsorption by soils and minerals. *Soil Science* 163, no. 2: 109-114.

Hartley, A.E. and W.H. Schlesinger. 2002. Potential environmental controls on nitrogenase activity in biological crusts of the northern Chihuahuan desert. *Journal of Arid Environments* 52: 293-304.

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.

Howard, J.B. and D.C. Rees. 1996. Structural basis of biological nitrogen fixation. *Chemical Reviews* 96: 2965-2982.

Isichei, A.O. 1990. The role of algae and cyanobacteria in arid lands. A review. *Arid Soil Research and Rehabilitation* 4: 1-17.

Jeffries, D.L., J.M. Klopatek, S.O. Link, and J. Bolton. 1992. Acetylene reduction by cryptogamic crusts from a blackbrush community as related to resaturation and dehydration. *Soil Biology and Biochemistry* 24: 1101-1105.

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, no. 1: 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, no. 3: 680-689.

Jones, K. 1977. The effects of moisture on acetylene reduction by mats of blue-green algae in sub-tropical grassland. *Annals of Botany* 41: 801-806.

Kentemich, T., G. Danneberg, B. Hundeshagen, and H. Bothe. 1988. Evidence for the occurrence of the alternative, vanadium-containing nitrogenase in the cyanobacterium *Anabaena variabilis FEMS Microbiology Letters* 51: 19-24.

Liermann, L.J., R.L. Guynn, A. Anbar, and S.L. Brantley. 2005. Production of a molybdophore during metal-targeted dissolution of silicates by soil bacteria. *Chemical Geology* 220: 285-302.

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.

Mills, M.M., C. Ridame, M. Davey, J. La Roche, and R.J. Geider. 2004. Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. *Nature* 429: 292-294.

Pimentel, D. 2000. Soil erosion and the threat to food security and the environment. *Ecosystem Health* 6, no. 4: 221-226.

Puigdefábregas, J. 1998. Ecological impacts of global change on drylands and their implications for desertification. *Land Degradation and Development* 9: 393-406.

Reddy, K.J. and S.P. Gloss. 1993. Geochemical speciation as related to the mobility of F, Mo and Se in soil leachates. *Applied Geochemistry* Supplmentary Issue 2: 159-163.

Redfield, E., S.M. Barns, J. Belnap, L.L. Daane, and C.R. Kuske. 2002. Comparative diversity and composition of cyanobacteria in three predominant soil crusts of the Colorado Plateau. *FEMS Microbiology Ecology* 40: 55-63.

Rosenberg, D.R. and P.A. Maurice. 2003. Siderophore adsorption to and dissolution of kaolinite at pH 3 to 7 and 22°C. *Geochimica et Cosmochimica Acta* 67, no. 2: 223-229.

Rychert, R.C. and J. Skujins. 1974. Nitrogen fixation by blue-green algae-lichen crust in the great basin desert. *Soil Science Society of America Proceedings* 38: 768-771.

Saito, M.A., T.J. Goepgert, and J.T. Ritt. 2008. Some thoughts on the concept of colimitation: Three definitions and the importance of bioavailability. *Limnology and Oceanography* 53: 276-290.

Sañudo-Wilhelmy, S.A., A.B. Kustka, C.J. Gobler, D.A. Hutchins, M. Yang, K. Lwiza, J.A. Burns, D.G. Capone, J.A. Raven, and E.J. Carpenter. 2001. Phosphorus limitation of nitrogen fixation by *Trichodesmium* in the central atlantic ocean. *Nature* 411: 66-69.

Schlesinger, W.H. 1996. *Biogeochemistry, an analysis of global change*. San Diego: Academic Press.

Schulten, J.A. 1985. Soil aggregation by cryptogams of a sand prairie. *American Journal of Botany* 72, no. 11: 1657-1661.

Sheridan, D. 1981. *Desertification of the United States*. Council on environmental quality. Washington, D.C.: U.S. Government Printing Office.

Silvester, W.B. 1989. Molybdenum limitation of asymbiotic nitrogen fixation in forests of pacific northwest America. *Soil Biology and Biochemistry* 21, no. 2: 283-289.

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. *Reclamation and Revegetation Research* 4: 261-269.

Steppe, T.F., J.B. Olson, H.W. Paerl, R.W. Litaker, and J. Belnap. 1996. Consortia N2 fixation: A strategy for meeting nitrogen requirements of marine and terrestrial cyanobacterial mats. *FEMS Microbiology Ecology* 21: 149-156.

Strauss, S.L., T.A. Day, and F. Garcia-Pichel. 2012. Nitrogen cycling in desert biological soil crusts across biogeographic regions in the southwestern United States. *Biogeochemistry* 108: 171-182.

ter Steeg, P.F., P.J. Hanson, and H.W. Paerl. 1986. Growth-limiting quantities and accumulation of molybdenum in *Anabaena oscillarioides* (cyanobacteria). *Hydrobiologia* 140: 143-147.

Terry, R.E. and S.J. Burns. 1987. Nitrogen fixation in cryptogamic soil crusts as affected by disturbance. 369-372.

Thiel, T. 1993. Characterization of genes for an alternative nitrogenase in the cyanobacterium *Anabaena variabilis*. *Journal of Bacteriology* 175, no. 19: 6276-6286.

Vitousek, P.M., K. Cassman, C.C. Cleveland, T.E. Crews, C.B. Field, N.B. Grimm, R.W. Howarth, R. Marino, L. Martinelli, E.B. Rastetter, and J.I. Sprent. 2002. Towards an ecological understanding of biological nitrogen fixation. *Biogeochemistry* 57/58: 1-45.

Vitousek, P.M. and R.W. Howarth. 1991. Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry* 13: 87-115.

Wedepohl, K.H. 1995. The composition of the continental crust. *Geochimica et Cosmochimica Acta* 59, no. 7: 1217-1232.

West, N.E. and J. Skujins. 1977. The nitrogen cycle in North American cold-winter semi-desert ecosystems. *Oecologia Plantarum* 12, no. 1: 45-53.

Wichard, T., B. Mishra, S.C.B. Myneni, J.P. Bellenger, and A.M.L. Kraepiel. 2009. Storage and bioavailability of molybdenum in soils increased by organic matter complexation. *Nature Geoscience* 2: 625-629.

Wiebe, K. 2003. *Linking land quality, agircultural productivity, and food security.* Washington, DC: USDA Economic Research Service, Agricultural Economic Report No. 823.

Xiao, B., Q. Wang, Y. Zhao, and M. Shao. 2011. Artificial culture of biological soil crusts and its effects on overland flow and infiltration under simulated rainfall. *Applied Soil Ecology* 48: 11-17.

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, no. 2: 973-983.

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.

Young, J.P.W. 1992. Phylogenetic classification of nitogen-fixing organisms. In *Biological nitrogen fixation*, ed. G. Stacey, R.H. Burris and H.J. Evans. New York: Chapman and Hall, Inc.

Zehr, J.P., B.D. Jenkins, S.M. Short, and G.F. Steward. 2003. Nitrogenase gene diversity and microbial community structure: A cross-system comparison. *Environmental Microbiology* 5, no. 7: 539-554.

Zerkle, A.L., C.H. House, R.P. Cox, and D.E. Canfield. 2006. Metal limitation of cyanobacterial N2 fixation and implications for the precambrian nitrogen cycle. *Geobiology* 4: 285-297.

Zhao, Y., S.-M. Bian, H.-N. Zhou, and J.-F. Huang. 2006. Diversity of nitrogenase systems in diazotrophs. *Journal of Integrative Plant Biology* 48, no. 7: 745-755.

CHAPTER 5

DESERT BIOLOGICAL SOIL CRUSTS HOST NOVEL SIDEROPHORE-PRODUCING MICROORGANISMS

Abstract

Biological soil crusts (BSCs) are pioneering microbial communities that increase arid ecosystem habitability. BSCs are successful pioneers in part because they fix carbon and nitrogen. These and other physiological processes active in BSCs require an assortment of metals that are largely insoluble in soil environments; however, BSCs thrive despite this high potential for metal limitation. I hypothesized that BSC microbes produce siderophores to increase metal solubility and facilitate metal uptake. I confirmed siderophore production in two dominant crust cyanobacteria. Furthermore, I isolated 8 additional siderophore-producers from less dominant BSC groups. The 16S rRNA gene sequences of these isolates place them in the Firmicutes, Alpha- and Betaproteobacteria, and the cyanobacteria. The BSC siderophore-producing isolates span a range of phylogenetic groups and physiological capabilities. While some siderophore-producers are not among the abundant groups, they may play a previously unrecognized role in increasing metal availability. This is the first report of siderophore production in BSCs, and thus, it is an important step towards understanding biologically-mediated metal cycling in arid ecosystems. Studying siderophore production in BSCs provides insight

into how BSCs tackle the challenge of acquiring insoluble metals, and may help conservationists determine useful strategies for BSC growth by facilitating metal acquisition.

Introduction

Biological soil crusts (BSCs) are complex microbial communities that colonize plant interspaces in arid and semi-arid environments (Belnap et al., 2001; Garcia-Pichel et al., 2001; 2003). They contain organisms that develop a vertically stratified, cohesive structure (Garcia-Pichel et al., 2003) similar to that of microbial mats (Cohen & Rosenberg, 1989) and biofilms (Doyle, 1999), and therefore, play a vital role in erosion prevention in arid lands (Campbell, 1979; Schulten, 1985; Belnap, 1993). Crusts include a diverse array of microorganisms, including bacteria, archaea, algae, fungi, lichens, and bryophytes (Belnap et al., 2001). The community performs a variety of metabolic processes, most notably photosynthesis and nitrogen fixation that supply bioavailable carbon (C) and nitrogen (N) to the entire BSC ecosystem (Beymer & Klopatek, 1991; Evans & Ehleringer, 1993; Evans & Lange, 2001; Belnap, 2002).

Crusts require a range of metals for use in enzymes and complex metabolic machinery (e.g., Photosystem I). One would not, *a priori*, expect metal availability in soil to be a problem since microbes are surrounded by metal-bearing minerals. However, even though metals may be abundant, they are not always present in bioavailable forms. For example, iron (Fe),

a key micronutrient in many BSC processes, usually comprises 1-6% of soil by weight (Powell et al., 1980). But, Fe solubility is pH-dependent, therefore; under typical environmental conditions (pH 6-7), Fe concentrations in the soil solution rarely surpass 10^{-18} M, which is more than 10 orders of magnitude lower than the micromolar (10^{-6}) concentrations required by most bacteria (Lankford, 1973). Iron availability in BSCs is of specific concern because when BSCs are physiologically active, pH values can reach 10 or higher (Garcia-Pichel & Belnap, 1996), which renders Fe even less soluble (Brady & Weil, 2002). Furthermore, an average 80 cm^2 crust requires up to 1 μmol Fe if one assumes the entire community is composed of cyanobacteria (see Chapter 2). This value is one to two orders of magnitude greater than the amounts of Fe present in the soil solution (see Chapters 2 and 3). In order to meet their metal demands despite the unfavorable environmental conditions, BSCs must have effective strategies for obtaining adequate amounts of Fe from their environment.

Siderophore production is one strategy soil microorganisms use to cope with low metal availability (Powell et al., 1980; Akers, 1983; Holmström et al., 2004; Essén et al., 2006). Siderophores are defined as low molecular weight organic compounds that bind Fe with high affinity (Lankford, 1973; Neilands, 1973), though some siderophores are capable of binding additional bioessential metals, such as molybdenum (Mo; Litos

et al., 2006; Bellenger et al., 2007; Monteiro et al., 2010), manganese (Saal & Duckworth, 2010; Szabó & Farkas, 2011), nickel (Dimkpa et al., 2008), copper (McKnight & Morel, 1980; Kim et al., 2004; Bellenger et al., 2007), and zinc (Bellenger et al., 2007). Siderophores are known to increase mineral solubility (Kalinowski et al., 2000; Kraemer, 2004; Liermann et al., 2000; 2005), and to facilitate microbial metal uptake (Neilands, 1973; Bellenger et al., 2008). Liermann et al. (2000) and Kalinowski et al. (2000) showed that Fe release from hornblende was enhanced in the presence of siderophore-producing bacteria. In addition, Liermann et al. (2005) found that Mo was similarly released from silicates in the presence of an N_2-fixing microbe capable of siderophore production. Mo-release was not observed when N_2-fixation was inactive, nor was it detected in the presence of a microbe incapable of N_2-fixation. Liermann et al. (2005), therefore, clearly show that active N_2 fixation, metal need, and siderophore production are inextricably linked, and a similar situation may exist for BSCs.

Previous work suggests that BSCs directly influence metal distributions in the soil mineral phase (Beraldi-Campesi et al., 2009) and soil solution (see Chapter 2). When examining bulk soils collected in the field, Beraldi-Campesi et al. (2009) found that some elements were depleted in crusted soil relative to adjacent uncrusted soils. They postulated this was the result of biological activity that solubilized metals and left them vulnerable

to leaching down the soil profile (Beraldi-Campesi et al., 2009). In laboratory studies of soil solutions from physiologically active crust communities, I found evidence that concentrations of many metals were influenced by biological processes such as uptake and solubilization (see Chapters 2 & 3). My studies demonstrate that BSCs actively alter the soil solution concentrations of metals and suggest a mechanism for the solid-phase metal distributions observed by Beraldi-Campesi et al. (2009).

It is plausible that BSCs affect these changes in the soil solution and solid-phase through siderophore production. Siderophores have been found in soils (Powell et al., 1980; Akers, 1983; Holmström et al., 2004; Essén et al., 2006), but their presence has not been studied in BSCs. However, some of the organisms that have been identified previously in BSCs (Garcia-Pichel et al., 2001; Gundlapally & Garcia-Pichel, 2006) are known to produce siderophores in other environments (Ito and Neilands, 1958; Peters & Warren, 1968; Umamaheswari, 1997; Temirov et al., 2003; Silva-Stenico et al., 2005; Dertz et al., 2006; Simionato et al., 2006; Lacava et al., 2008; Beneduzi et al., 2010; Raza & Shen, 2010).

Following from the results of Beraldi-Campesi et al. (2009) and Chapters 2 & 3, I hypothesized that BSC microbes influence soil metal dynamics through the production of siderophores. I predicted that the siderophore producers would be dominant crust organisms that perform Fe-intensive metabolisms (i.e., photosynthesis and N_2 fixation). I

confirmed siderophore production in two previously isolated crust microbes (*Microcoleus vaginatus* and *Nostoc punctiforme*) that play important roles in the BSC community. I also isolated 8 additional siderophore-producing microorganisms from BSCs and characterized them by 16S rRNA gene sequencing. I present the phylogenetic affiliations of these siderophore-producers, followed by a discussion of the relevance of these organisms to BSC community structure and function. I also speculate as to the probable siderophore compounds produced by these microbes. Overall, it is clear from my results that siderophore production is important for BSC function, though it is not universal among BSC community members. Even so, siderophore production is likely to influence metal dynamics in the ecosystem as a whole.

Methods

Soil Collection

Soils with relatively flat surfaces and minimal lichen cover, as determined by a visual assessment, were sampled at the Green Butte site northeast of Moab, UT (Strauss et al., 2012; N38°42′56.2″, W109°41′32.7″) in 2009. I modeled soil collection procedures and storage after Garcia-Pichel et al. (2003) and Chapters 2 and 3. Briefly, sampling areas were lightly wetted with distilled water and collected in disposable Petri dishes (50 mm x 13 mm). Soils were dried and stored in the dark at

room temperature (Campbell et al., 2009) until biomass was removed for isolation and identification of siderophore-producers.

Culture Techniques and CAS Assay

Cultures were grown on modified BG-11 agar plates (Allen and Stanier, 1968). Unless otherwise indicated, the media contained (per liter) 149.6 g $NaNO_3$, 20 g glucose, 7.5 g $MgSO_3 \cdot 7H_2O$, 3.6 g $CaCl_2 \cdot 2H_2O$, 0.715 g H_3BO_3, 0.453 g $MnCl_2 \cdot 4H_2O$, 0.055 g $ZnSO_4 \cdot 7H_2O$, 0.098 g $Na_2MoO_4 \cdot 2H_2O$, 0.02 g $CuSO_4 \cdot 5H_2O$, 0.01 g $NiCl_2 \cdot 6H_2O$, 0.01 g $CoCl_2 \cdot 6H_2O$, 0.009 g Na_3VO_4, 0.243 g $FeCl_3$, 1 g Na_2CO_3, 1.53 g K_2HPO_4, 0.03 g citric acid, 0.093 g Na_2EDTA, 15 g Bacto™ Agar, and 0.3 g cyclohexamide. The siderophore production assays (O-CAS and CAS), utilized agar and liquid media that was prepared without metals and without Na_2EDTA. All media were prepared with 18 MΩ·cm carbon-free water (NANOpure® DIamond™ UV, Barnstead International, Dubuque, IA)

Crust biomass was transferred to trace metal-free agar plates in one of two ways. First, surface crust (~0.5 mm) and sterile deionized water were slurried at a 1:2 ratio; 100 μL of this slurry was pipetted and spread evenly over the agar. Second, biomass material was manually removed from the crust with flame-sterilized tweezers and transferred directly to the agar. Cultures of the crust organisms *Microcoleus vaginatus* and *Nostoc punctiforme*, previously isolated in the Garcia-Pichel lab, were grown in liquid media alongside the crust biomass plates. I used agar plates with

four different carbon (C) and nitrogen (N) combinations to select organisms with different physiological capabilities (Table 5-1). Plates without C or N (–C–N) were intended to select for photosynthetic organisms capable of fixing N_2. Plates without C, but with N (–C+N), were designed to select for phototrophic organisms that do not fix N_2. Plates with C and without N (+C–N) were used to select for heterotrophic N_2fixers. Finally, plates with C and N (+C+N) were intended to select for heterotrophic organisms not able to fix N_2 (Table 5-1). Each biomass source was transferred to one plate of each of the C/N conditions. Plates were incubated for 40 days at room temperature. The –C plates were incubated under ambient laboratory light conditions (10 μE $m^{-2}s^{-1}$ measured as photosynthetically active radiation); the +C plates were incubated in the dark. *M. vaginatus* and *N. puntiforme* were grown on –C+N agar plates under ambient light conditions.

After microbial growth was observed on a plate, 15 mL of CAS agar was overlain on the surface of that plate. CAS agar contained (per liter) 60 mg chrome azurol S, 1.6 mg $FeCl_3$, 3.6 mg HCl, 73 mg hexadecyltrimethylammonium bromide, and 9 g BactoAgar (Schwyn and Neilands, 1987; Perez-Miranda et al., 2007). The original color of the CAS agar and liquid media was dark blue. In the presence of siderophores, this color changed to yellow or purple constituting a positive CAS assay

response. A positive control was prepared with *Bacillus cereus* ATCC 13061. Agar without added biomass was used as a negative control.

Table 5-1. Results of the O-CAS screening assay of soil slurries and manually selected biomass showing the media used, expected metabolic capabilities for each media type, the total number and percentage of those plates that exhibited a color change, and which color change (type of siderophere) was observed.

Plate media	Total # plates	Heterotrophos (H) or autotrophs (A) expected	Selective for N_2 fixers?	# (%) of plates with color change	Yellow (hydroxamate)	Purple (catecholate)
-C-N	13	A	Y	6 (46%)	6	0
-C+N	12	A	N	10 (83%)	10	0
+C-N	11	H, A	Y	8 (73%)	4	4
+C+N	11	H, A	N	10 (91%)	10	0

Plates were incubated until a color change was detected (generally 3-14 days). Colonies that displayed a color change were transferred to agar plates with added metals and Na_2EDTA. Organisms were isolated by 3-5 successive single-colony streaks. After isolation, I confirmed which strains produced siderophores using the traditional liquid CAS assay (recipe as above without BactoAgar; Schwyn and Neilands, 1987). Siderophore production by *M. vaginatus* and *N. punctiforme* was evaluated using the traditional liquid CAS assay.

Siderophore-Producer Identification

DNA was purified from liquid-grown cultures with the FastDNA® Spin Kit (MP Biomedicals, Solon, OH) and the 16S rRNA gene was amplified using primers 8F and 1492R from Lane (1991). Amplified DNA was

extracted from a low-melt agar gel and sequenced directly via 8F and 1492R priming sites to obtain at least 1200 bp sequences at the DNA Laboratory at Arizona State University. Sequencing reactions were performed with a BigDye® v3.1 Sequencing Kit on a 3730 DNA Analyzer (Applied Biosystems) per the manufacturer's protocol. Raw sequences were edited in MEGA (Tamura et al., 2007), and alignments and phylogenetic analyses were performed on the Ribosomal Database Project (RDP) website (Cole et al., 2007; 2009). Most-likely taxonomical identifications were determined using RDP's Classifier (Wang et al., 2007) and SeqMatch.

Pyrosequencing

Microbial community composition was determined by 16S rRNA gene tagged pyrosequencing. DNA was extracted from BSC samples using the UltraClean Soil DNA Extraction Kit (MoBio Laboratories, Inc., Carlsbad, CA). Bar-coded bacterial primers with Roche 454 FLX adapters were used to amplify the V4 variable region of the 16S rDNA gene from template community DNA. These primers yielded an amplicon of approximately 240 bp in length. The forward primer, V4F, was 5'-AYTGGGYDTAAAGNG-3'. Equimolar mixtures of 4 reverse primers were used: V4R, 5'-TACCRGGGTHTCTAATCC-3'; V4R2, 5'- TACCAGAGTATCTAATTC-3'; V4R3, 5'- CTACDSRGGTMTCTAATC-3'; and V4R4, 5'-TACNVGGGTATCTAATCC-3'. Forward and reverse primers targeted

positions ~560 and ~800 by standard *Escherichia coli* numbering. The PCR conditions were as described by Bates and Garcia-Pichel (2009) with 50 pmol of each primer, 2.5 units ExTaq polymerase and 10 ng of community DNA template. After initial denaturing, 30 cycles were completed at 94°C for 1 min., 50°C for 1 min, and 72°C for 2 min, with a final extension at 72°C for 5 min. The PCR products were quantified and quality checked in a 1% agarose gel, then purified using the QiaQuick PCR purification kit (Qiagen Inc., Valencia, CA), and commercially pyrosequenced and parsed by sample according to the individual barcodes, to yield ~7,000-21,000 sequences per sample. We used mothur (Schloss et al, 2009) pyrosequencing analysis software to trim bad sequences from the sample files. The Pyrosequencing Pipeline Alignment and Classification tools at Michigan State University's Ribosomal Database Project (RDP) website, (Cole et al. 2009) were used to align sequences and make taxonomic assignments (RDP Naive Bayesian rDNA Classifier, version 2.2) based on current taxonomy in *Bergey's Manual of Systematic Bacteriology*.

Results

Culturing and O-CAS

BSC biomass was transferred to plates with different combinations of C and N (+C+N, +C-N, -C+N, and -C-N). All 47 plates exhibited growth after 2-6 weeks. After growth was detected, the chrome azurol sulfonate

overlay (O-CAS; Pérez-Miranda et al., 2007) was poured over the surface, and plates were incubated until a color change was observed in the agar (3-14 days). A subset of plates from all C/N combinations screened positive for siderophore production (Table 5-1; Figures 5-1a-d). Of the 47 plates, 34 exhibited a color change from the original blue color to either yellow or purple (Table 5-1; Figures 5-1a-d). The majority of O-CAS positive plates (30) showed a color change from blue to yellow (Figure 5-1a, b), indicating hydroxamate-type siderophores (Pérez-Miranda et al., 2007). The remaining 4 plates showed a color change from blue to purple (Figure 5-1c, d), indicating catechol-type siderophores (Pérez-Miranda et al., 2007). Cultures grown on–C–N, –C+N, and +C+N plates and 4 of the +C-N plates produced hydroxamate siderophores. The remaining four +C-N plates turned purple, indicating catecholate siderophores. There was no difference in O-CAS assay response between the methods used to transfer crust biomass to plates (i.e., slurries or manually-selected).

Axenic cultures of two dominant crust cyanobacteria, *Microcoleus vaginatus* and a *Nostoc punctiforme*, were grown alongside the cultures isolated from the O-CAS plates. Siderophore production was discovered for both *M. vaginatus* and *N. punctiforme* grown on –C+N media.

Siderophore production was confirmed for the positive control, *Bacillus cereus*, grown on BG-11 media. Uninoculated plates (negative control) did not display a color change.

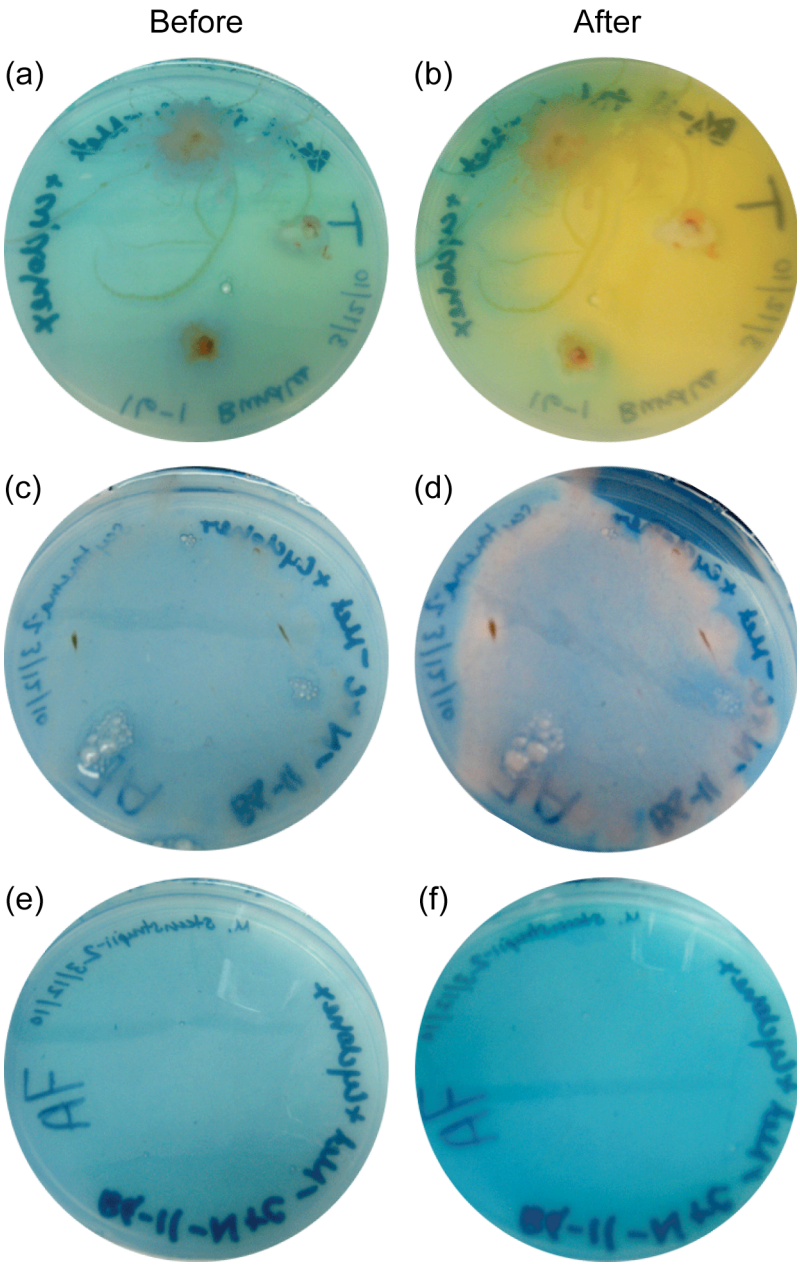

Figure 5-1. Before and after photos of O-CAS plates. Left-hand panels are photographs taken immediately after the addition of CAS agar. Right-hand panels are photographs taken after 3-14 days of growth under CAS agar. Images (a) and (b) show a color change from blue to yellow, indicative of hydroxamate siderophores. Images (c) and (d) show a color change from blue to purple, indicative of catecholate siderophores (Pérez-Miranda et al., 2007). Images (e) and (f) show a plate that was inoculated but did not produce a color change.

Biomass from plates that exhibited a color change was re-streaked onto fresh agar, and individual organisms were isolated after 3-5 successive single-colony streaks. 190 organisms from the O-CAS positive plates were randomly selected for growth in liquid media. Siderophore production was confirmed in 43 of these 190 organisms. Of the 43 siderophore-producing cultures, 17 were selected at random for identification.

Siderophore-Producer Identification

Sequencing based on nearly complete 16S rRNA genes of the 17 isolated siderophore-producing microbes revealed 8 distinct organisms. (The remaining 9 sequenced organisms were duplicates.) Sequences were compared to published sequences using the Ribosomal Database Project's (RDP) Classifier (Wang et al., 2007) and SeqMatch. When possible, I made an effort to identify cultures at the species level (Table 5-2). Failing that, I discuss them at the genus level.

Of the 8 distinct microbes, 5 had sequence similarities less than 98% suggesting they are novel organisms (Table 5-2). The remaining 3 cultures closely matched sequences of known organisms. Here I present the most closely matched sequence and the percentage similarity. Cultures 39 and 244 are Alphaproteobacteria; culture 39 is an uncultured member of the Methylobacteriaceae (92.2%), and culture 244 closely matches *Methylobacterium radiotolerans* (99.8%). Culture 265 is a

Table 5-2. Results of RDP Classifier and SeqMatch based on nearly full-length 16S rRNA sequences showing the similarity between bacteria isolated in this study and previously sequenced organisms from the RDP database. Bold indicates organisms that are most likely novel and not currently in culture.

Isolate	Closest Sequence	% Similarity
Cyanobacteria		
203	***Leptolyngbya* sp.**	**96.5**
259	***Leptolyngbya* sp.**	**92.4**
Firmicutes		
128	*Bacillus subtilis*, *B. vallismortis*	99.3
150	*B. licheniformis*, *B. mojavensis*, *B. subtilis*	99.1
247	***Paenibacillus polymyxa***	**90.8**
α-Proteobacteria		
39	**uncultured Methylobacteriaceae**	**92.2**
244	*Methylobacterium radiotolerans*	99.8
β-Proteobacteria		
265	***Janthinobacterium* sp.**	**95.9**

Betaproteobacterium most closely related to a *Janthinobacterium* sp. (95.9%). Cultures 128, 154, and 247 are Firmicutes (Table 5-2); cultures 128 and 150 closely match several *Bacillus* spp. at >99%, and culture 247 is most closely related to *Paenibacillus polymyxa* (90.8%). The six heterotrophs were all cultured on +C+N media. Cultures 203 and 259 are cyanobacteria belonging to the *Leptolyngbya* (96.5 and 92.4%, respectively; Table 5-2). Culture 203 was isolated on –C+N media, and culture 259 was isolated on –C–N media.

Pyrosequencing

Figure 5-2a shows that the Cyanobacteria, Bacteroidetes, Acidobacteria, and a group of unclassified organisms make up the majority (~77%) of the crust microbial community, followed by the AlphaProteobacteria and Verrucomicrobia (~13%); the remaining microbial groups each comprise less than 5% of the community (Potrafka et al., unpublished data). The 8 siderophore producers isolated in this study, and the 2 previously isolated BSC cyanobacteria, are distributed between dominant and less-dominant microbial groups. The heterotrophic isolates (2 Alphaproteobacteria, 1 Betaproteobacterium, and 3 Firmicutes) are from groups that comprise less than 10% of the total microbial population of BSCs at this site. Of the cyanobacteria found to produce siderophores, *Microcoleus* belongs in the dominant Family XIII, and the remaining 4 belong to the less abundant Families I and IV (Figure 5-2b).

Discussion

Studying the factors that contribute to BSC physiological function is valuable because BSCs are such vital components of arid ecosystems. Crusts influence soil fertility by providing the primary sources of bioavailable carbon (C) and nitrogen (N) to their soils (Beymer & Klopatek, 1991; Evans & Ehleringer, 1993; Evans & Lange, 2001; Belnap, 2002). The release of siderophores observed in this study combined with the mobilization of metals detected in previous work (Beraldi-Campesi et al.,

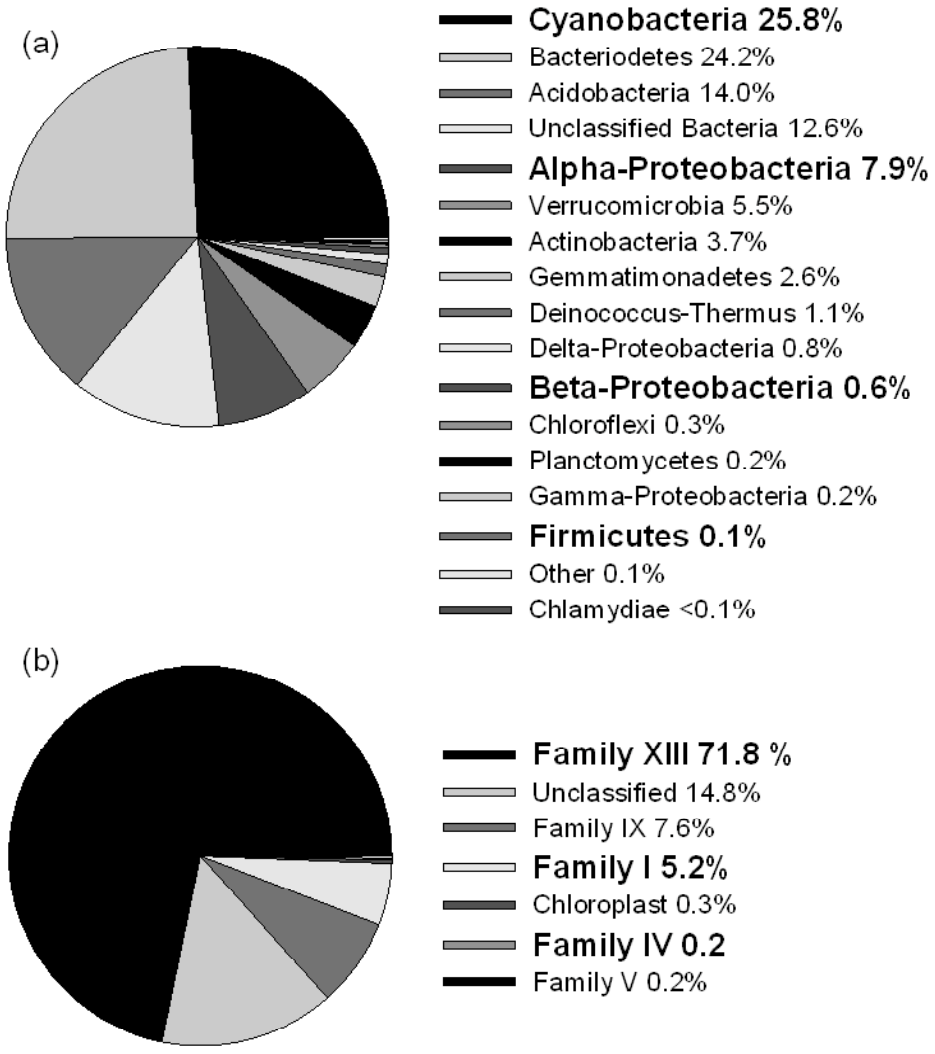

Figure 5-2. Pie diagrams summarizing pyrosequencing data of a 240 base pair fragment of the 16S rRNA gene from Green Butte crusts (reproduced with permission from Potrafka, unpublished data). (a) phylogenetic distribution of all microbes in the dark crusts from Green Butte (Potrafka, unpublished data). Siderophore-producers identified in this study, as well as the two previously isolated organisms, belong in the groups shown in bold: cyanobacteria, Alphaproteobacteria, Betaproteobacteria, and Firmicutes. (b) further delineation of the cyanobacteria (Potrafka, unpublished data). Cyanobacterial siderophore-producers include *Microcoleus vaginatus* in Family XIII, *Nostoc punctiforme* in Family I, and two species of *Leptolygbya* that belong in Family IV. These groups are highlighted in bold.

2009; Chapters 2 & 3) provide another way by which BSCs contribute to overall soil fertility. Together, these processes sustain arid and semiarid ecosystems and prevent desertification (Isichei, 1990; Belnap et al., 1994; Belnap, 1995; Bowker et al., 2008a), which has become a serious concern in many parts of the world (Sheridan, 1981; Puigdefábregas, 1998; Arnalds & Archer, 2000; Pimentel, 2000; Weibe, 2003).

BSCs are also quite distinct from soil communities in temperate environments and in the rhizosphere. Crusts contain lower species richness and diversity (Nakatsua et al., 2000), they have different community composition (Kuske et al., 2002), and they have a higher abundance of novel organisms (Garcia-Pichel et al., 2003). The Colorado Plateau BSCs are particularly interesting because they are able to withstand extreme environmental conditions (Bowker et al., 2002), making them likely to exhibit unique survival adaptations (Gundlapally & Garcia-Pichel, 2006).

Given that siderophore production is a common strategy soil microbes use to extract metals from their environments, the primary goal of this study was to confirm that siderophore-producers exist in BSCs. A secondary goal was to identify the siderophore-producing organisms in order to assess their ecological significance. While many studies have shown that soil communities contain siderophore-producers (Powell et al., 1980; Akers, 1983; Holmström et al., 2004; Essén et al., 2006), this is the

first to show that they exist specifically in BSCs and provide evidence for how crust microbes acquire trace metals like Fe. Understanding how BSCs obtain the micronutrients they need to grow and metabolize is important because of the key role BSCs play in arid lands.

Crusts are complex consortia of autotrophic and heterotrophic organisms that all contribute to the development and maintenance of the crust as a whole (Garcia-Pichel & Belnap, 1996; Garcia-Pichel et al., 2003; Gundlapally & Garcia-Pichel, 2006). The dark crusts used in the current study were dominated by cyanobacteria (~26% of species; Figure 5-2a), and *Microcoleus* spp. are the most abundant (Bowker et al., 2002; Garcia-Pichel et al., 2001; Yeager et al., 2004; Gundlapally & Garcia-Pichel, 2006). Cyanobacteria are the primary producers of bioavailable C and N in the soil system; their filamentous nature and excretion of exopolysaccharides (EPS) make them excellent crust engineers (Danin & Ganor, 1991; Belnap, 2001; Belnap et al., 2001; Reynolds et al., 2006; Garcia-Pichel & Wojciechowski, 2009; Rossi et al., 2012). The primary N_2-fixers in BSCs are the heterocystous cyanobacteria, and *Nostoc* spp. alone comprise 80-90% of the diazotrophic community (Yeager et al., 2004; 2007). As photosynthesizers, and the most important diazotrophs in BSCs, cyanobacteria may require 15-75 μmol Fe per mol C (Tuit et al., 2004; Tovar-Sanchez et al., 2006; White et al., 2006) and 3-55 μmol Mo per mol C (Glass et al., 2009). Producing siderophores is an obvious

mechanism that could help crust organisms obtain these critical micronutrients. It is not surprising, then, that the two previously isolated cyanobacteria, *M. vaginatus* and *Nostoc punctiforme*, produce siderophores. However, the *Leptolyngbya* spp. isolated in this study (cultures 203 and 259) are Family IV cyanobacteria and make up <1% of the microbial community (Figure 5-2b), indicating that non-dominant organisms may also play a critical role in increasing metal availability in BSCs.

Although the heterotrophic bacteria are not the foremost photosynthesizers or N_2-fixers in the dark BSCs used in this study, they collectively account for ~75% of the BSC community at the Green Butte site, and they play a significant role in crust formation and maintenance (Garcia-Pichel & Belnap, 1996; Garcia-Pichel et al., 2003; Gundlapally & Garcia-Pichel, 2006). The bacterial isolates from the current study belong to the less abundant Alpha- and Betaproteobacteria, and Firmicutes classes; these groups comprise 7.9, 0.6, and 0.1% of crust species at our field site, respectively. Members of these groups perform a variety of physiological processes (Garrity et al., 2005; Gillis & Logan, 2005; Green, 2005; Logan & De Vos, 2009; Priest, 2009), and many bacteria identified in similar environments are mycelial or mucoid and thought to contribute to crust structure (Gundlapally & Garcia-Pichel; 2006).

A BLAST analysis places isolates 39 and 244 in the Rhizobiales. Isolate 244 is closely matched to *Methylobacterium radiotolerans*, and isolate 39 does not match closely with any published sequences, likely making it a novel organism in the Methylobacteriaceae (Table 5-2). I was able to amplify *mxaF*, one of the genes that codes for methanol dehydrogenase (McDonald & Murrell, 1997), from isolates 39 and 244, indicating that these organisms are capable of growth on methanol. Therefore, these bacteria will require iron (Fe) and calcium (Ca) for methanol dehydrogenase (White et al., 1993; Anthony et al., 1994). However, I was unable to amplify *nifH*, one of the genes for nitrogenase, from these isolates, so I conclude at this point that isolates 39 and 244 are incapable of N_2 fixation.

Isolate 265 belongs in the heterotrophic Oxalobacteraceae, and it is likely an uncultured organism belonging in the Janthinobacteria (Table 5-2). The remaining cultures are Bacilli, two of which (128, and 150) are >99% similar to various species of *Bacillus* or *Brevibacterium*. Some Bacilli are capable of denitrification (Logan & De Vos, 2009); however, I was unable to amplify any of the denitrification genes (*nosZ*, *norB*, *nirK*, *nirS*, or *narG*; Philippot, 2002) from isolate 128 or 150. Therefore, I deduce that my isolates are not denitrifiers. This is not too surprising given that denitrification has been shown to be negligible in crusts (Johnson et al., 2007; Strauss et al., 2012). The final isolate, 247, is 90.8% similar to

Paenibacillus polymyxa, likely representing a novel *Paenibacillus* sp. I was able to amplify *nifH* from isolate 247, indicating that it has the capacity for N_2-fixation, a trait that is common to *Paenibacilli* (De Vos et al., 2009; Priest, 2009). This organism will, therefore, require Mo for use in N_2 fixation (Burgess & Lowe, 1996)

All of the organisms found in this study are related to previously identified BSC microbes (Garcia-Pichel et al., 2001; Gundlapally & Garcia-Pichel, 2006), but, aside from the dominant crust former (*Microcoleus*) and the dominant N_2 fixer (*Nostoc*), the isolates belong to minor groups of crust organisms (Figure 5-2). Even so, they require a variety of metals and most are known to produce siderophores in other environments (Ito & Neilands, 1958; Peters & Warren, 1968; Temirov et al., 2003; Silva-Stenico et al., 2005; Dertz et al., 2006; Simionato et al., 2006; Lacava et al., 2008; Beneduzi et al., 2010; Raza & Shen, 2010).

Siderophore production may be one way that crust heterotrophs contribute to overall BSC function; they produce siderophores that increase metal availability to the entire community. There are siderophore producers among the cyanobacteria, but making siderophores is energetically costly and nutrient-intensive. Many bacteria can take up siderophores produced by other organisms; presumably, in order to save themselves the metabolic cost (Crowley et al., 1991; Jurkevitch et al., 1992; Joshi et al., 2006; Khan et al., 2006; Joshi et al., 2008). It is possible

that some BSC organisms utilize this strategy – either because they are unable to produce siderophores themselves, or in an effort to conserve resources. Studies of the specific microbes that make siderophores within intact, physiologically active crusts would determine if this is the case and shed more light on siderophore ecology in BSCs.

My discussion of siderophore ecology cannot be complete without recognizing that the Acidobacteria and Bacteroidetes each represent >10% of BSC species at our field site (Figure 5-2a); however, no siderophore-producers were isolated from these bacterial groups in the present study. This could be because: (1) members of these divisions do not produce siderophores, (2) the cultivation methods used selected against members of these divisions, or (3) by chance, no members of these divisions were selected for 16S sequencing. There is evidence for siderophore production by some members of both the Acidobacteria and Bacteroidetes (Idris et al., 2004; Marques et al., 2010), and members of these groups have been identified in BSCs (Gundlapally & Garcia-Pichel, 2006). The compounds produced by these organisms bind Fe, and so should have been detected by the CAS assay if they were present. The lack of Acidobacteria and Bacteroidetes identified in the present study is, therefore, most likely attributed to exclusion by culture methods or by chance. Siderophore-production by these bacterial groups is, no doubt, important for metal cycling within BSCs, and additional studies in intact

crusts or using media more suitable to these organisms could help elucidate the role played by Acidobacteria and Bacteroidetes in crust siderophore ecology.

At this point, we can only speculate as to which specific siderophore compounds BSCs may produce. Isolates 128 and 150 closely match sequences of *Bacillus subtilis* and *Bacillus licheniformis* who both produce siderophores that have been identified and characterized (Ito & Neilands, 1958; Peters & Warren, 1968; Temirov et al., 2003; Dertz et al., 2006). The siderophores they produce include the hexadentate catechol Bacillibactin (Dertz et al., 2006), and a variety of bidentate catecholate dihydroxybenzoic acids (Ito and Neilands, 1958; Peters and Warren, 1968; Temirov et al., 2003). Interestingly, dihydroxybenzoic acids can bind Mo in addition to Fe (Litos et al., 2006; Monteiro et al., 2010). Bacillibactin has not been shown to bind Mo, but other catecholate siderophores do (Bellenger et al., 2007; 2008). Thus, it is possible that Bacillibactin has Mo-binding capacity as well, though experiments are needed to verify this assumption. Mo is used in nitrate assimilation (Rubio et al., 1999; Rubio et al., 2002) and N_2 fixation (Burgess & Lowe, 1996), processes that are both active in crusts. Therefore, production of these putative “molybdophores” by *Bacillus* spp. could also provide a crucial source of Mo for crusts, and could explain the increased Mo concentrations observed in the soil solution of live crusts (see Chapter 2).

Isolate 244 is most likely related to *Methylobacterium radiotolerans,* which has been shown to produce siderophores that have not yet been characterized (Lacava et al., 2008). Isolate 247 is likely a novel *Paenibacillus* sp. The siderophores produced by *Paenibacillus* spp. are also uncharacterized (Raza and Shen, 2010). What is known, is that both *Methylobacteria* and *Paenibacilli* produce hydroxamate siderophores (Silva-Stenico et al., 2005; Simionato et al., 2006; Beneduzi et al., 2008; Lacava et al., 2008; Beneduzi et al., 2010; Raza & Shen, 2010). Both hydroxamate and catecholate siderophores are capable of binding other metals in addition to Fe and Mo, such as manganese (Saal and Duckworth, 2010; Szabó and Farkas, 2011), nickel (Dimkpa et al., 2008), copper (McKnight & Morel, 1980; Kim et al., 2004; Bellenger et al., 2007), and zinc (Bellenger et al., 2007). Siderophores, therefore, may provide a source for many different metals.

This is the first report of siderophore production by *Nostoc punctiforme* and *Microcoleus vaginatus*. Other members of the *Nostoc* genus were shown to make a siderophore, that was putatively identified as the hydroxamate, schizokinen, based on infrared spectra (Umamaheswari et al., 1997). There are currently no reports in the literature of siderophore production by the remaining isolates. Evidence exists for siderophore production by members of the same order as isolate 39, which belongs in the Rhizobiales and is likely a member of the Methylobacteriaceae (Silva-

Stenico et al., 2005; Simionato et al., 2006; Lacava et al., 2008; Palaniappan et al., 2010). There is also evidence of siderophore production in the same order as isolate 265, which is a member of the Oxalobacteraceae and is most likely a Janthinobacterium (Rashedul et al., 2009). However, there is no evidence for siderophore production by close relatives of the final cyanobacterial isolates (203 and 259).

This work suggests that siderophore production may play a critical role in metal availability within BSCs. The organisms that produce siderophores under my experimental conditions are distributed across various phylogenetic groups that have assorted physiological capabilities and, therefore, diverse metal requirements. In addition, siderophore producers represent both dominant microbial groups, such as the cyanobacteria, and less abundant groups, like the Firmicutes (Figure 5-2a). The fact that BSC siderophore producers span such a range in physiology and abundance indicates that siderophore ecology in BSCs may be complex. Studies of siderophore production in intact BSCs would illuminate the roles that individual microbial groups play, and extraction of metal-bound siderophores from native soils would indicate whether or not metals other than Fe are affected.

It is exciting that some of the siderophore producers are novel organisms. Although BSC organisms may produce known siderophores, it is also possible that they synthesize novel compounds with unique metal-

biding capabilities. Future work is aimed at discovering the details of the siderophores' structure and function and how they fit into the overall story of metal acquisition in BSCs.

Finally, because BSCs provide crucial ecosystem services to their environments, promoting BSC establishment and growth is vital for preventing the degradation of arid lands. Determining strategies to sustain BSCs hinges on recognizing what nutrients and conditions promote BSC productivity (Davidson et al., 2002). Previous work showed that BSCs are likely limited with respect to metals such as Fe and Mo (see Chapters 2 & 4). Trace nutrient addition has been proposed to increase BSC productivity (Bowker et al., 2005), but fertilization with siderophores may serve the same purpose. If metals are present in the soil, but limiting because of low bioavailability, siderophore additions may help to alleviate metal limitation by increasing metal solubility. Furthermore, addition of trace metals bound to BSC-accessible siderophores would likely increase the efficacy of fertilization treatments. Exploration of siderophore fertilization as a viable BSC management practice requires a full understanding of the siderophores produced and the metals they bind, as well as how these siderophores are used within the BSC community.

References

Akers, H. 1983. Multiple hydroxamic acid microbial iron chelators (siderophores) in soils. *Soil Science* 135, no. 3: 156-159.

Allen, M.M. and J. Stanier. 1968. Selective isolation of blue-green algae from water and soil. *Journal of General Microbiology* 51: 203-209.

Anthony, C., M. Ghosh, and C. Blake. 1994. The structure and function of methanol dehydrogenase and related quinoproteins containing pyrrolo-quinoline quinone. *Biochemical Journal* 304: 665-674.

Arnalds, O. and S. Archer. 2000. Introduction. In *Rangeland desertification*, ed. O. Arnalds and S. Archer. 1-4. Dordrecht: Kluwer Academic Publishers. 209.

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, no. 1: 56-67.

Bellenger, J.P., F. Arnaud-Neu, Z. Asfari, S.C.B. Myneni, E.I. Stiefel, and A.M.L. Kraepiel. 2007. Complexation of oxoanions and cationic metals by the biscatecholate siderophore azotochelin. *Journal of Biological Inorganic Chemistry* 12: 367-376.

Bellenger, J.P., T. Wichard, A.B. Kustka, and A.M.L. Kraepiel. 2008. Uptake of molybdenum and vanadium by a nitrogen-fixing soil bacterium using siderophores. *Nature Geoscience* 1: 243-246.

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

_____. 1995. Surface disturbances: Their role in accelerating desertification. *Environmental Monitoring and Assessment* 37, no. 1-3: 39-57.

_____. 2001. Factors influencing nitrogen fixation and nitrogen release in biological soil crusts. In *Biological soil crusts: Structure, function, and management*, ed. J. Belnap and O.L. Lange:241-261. Berlin Heidelberg New York: Springer.

_____. 2002. Nitrogen fixation in biological soil crusts from southeast Utah, USA. *Biological Fertility of Soils* 35: 128-135.

Belnap, J., K.T. Harper, and S.D. Warren. 1994. Surface disturbance of cryptobiotic soil crusts: Nitrogenase activity, chlorophyll content, and chlorophyll degradation. *Arid Soil Research and Rehabilitation* 8: 1-8.

Belnap, J., R. Rosentreter, S. Leonard, J.H. Kaltenecker, J. Williams, and D. Eldridge. 2001. *Biological soil crusts: Ecology and management*, ed. U.S. Department of the Interior, Bureau of Land Management and U.S. Geological Survey, Technical References 1730-2: U.S. Department of the Interior Bureau of Land Management National Science and Technology Center Information and Communications Group.

Beneduzi, A., P.B. Costa, M. Parma, I.S. Melo, M.H. Bodanese-Zanettini, and L.M.P. Passaglia. 2010. *Paenibacillus riograndensis* sp. Nov., a nitrogen-fixing species isolated from the rhizosphere of *Triticum aestivum*. *International Journal of Systematic and Evolutionary Microbiology* 60: 128-133.

Beneduzi, A., D. Peres, L.K. Vargas, M.H. Bodanese-Zanettini, and L.M.P. Passaglia. 2008. Evaluation of genetic diversity and plant growth-promoting activities of nitrogen-fixing Bacilli isolated from rice fields of south Brazil. *Applied Soil Ecology* 39: 311-320.

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, no. 3: 348-359.

Beymer, R.J. and J.M. Klopatek. 1991. Potential contribution of carbon by microphytic crusts in pinyon-juniper woodlands. *Arid Soil Research and Rehabilitation* 5: 187-198.

Bowker, M.A., J. Belnap, D.W. Davidson, and S.L. Phillips. 2005. Evidence for micronutrient limitation of biological soil crusts: Importance to arid-lands restoration. *Ecological Applications* 15, no. 6: 1941-1951.

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

Brady, N.C. and R.R. Weil. 2002. *The nature and properties of soils*. Upper Saddle River: Prentice Hall.

Burgess, B.K. and D.J. Lowe. 1996. Mechanism of molybdenum nitrogenase. *Chemical Reviews* 96: 2983-3011.

Campbell, J.H., J.S. Clark, and J.C. Zak. 2009. PCR-DGGE comparison of bacterial community structure in fresh and archived soils sampled along a Chihuahuan desert elevational gradient. *Microbial Ecology* 57: 261-266.

Campbell, S.E. 1979. Soil stabilization by a prokaryotic desert crust: Implications for precambrian land biota. *Origins of Life* 9: 335-348.

Cohen, Y. and E. Rosenberg, eds. 1989. *Microbial mats: Physiological ecology of benthic microbial communities*. Washington, DC: American Society for Microbiology.

Cole, J.R., B. Chai, R.J. Farris, Q. Wang, A.S. Kulam-Syed-Mohideen, D.M. McGarrell, A.M. Bandela, E. Cardenas, G.M. Garrity, and J.M. Tiedje. 2007. The ribosomal database project (RDP-II): Introducing *myRDP* space and quality controlled public data. *Nucleic Acids Research* 35. Database issue: D169-172. doi: 10.1093/nar/gkl889 [PMID: 17090583].

Cole, J.R., Q. Wang, E. Cardenas, J. Fish, B. Chai, R.J. Farris, A.S. Kulam-Syed-Mohideen, D.M. McGarrell, T. Marsh, G.M. Garrity, and J.M. Tiedje. 2009. The ribosomal database project: Improved alignments and new tools for rRNA analysis. *Nucleic Acids Research* 37. (Database issue): D141-145; doi: 10.1093/nar/gkn879] [PMID: 19004872].

Crowley, D.E., Y.C. Wang, C.P.P. Reid, and P.J. Szaniszlo. 1991. Mechanisms of iron acquisition from siderophores by microorganisms and plants. *Plant and Soil* 130: 179-198.

Danin, A. and E. Ganor. 1991. Trapping of airborne dust by mosses in the Negev Desert, Israel. *Earth Surface Processes and Landforms* 16, no. 2: 153-162.

Davidson, D.W., M.A. Bowker, D. George, S.L. Phillips, and J. Belnap. 2002. Treatment effects on performance of N-fixing lichens in disturbed soil crusts of the Colorado Plateau. *Ecological Applications* 12, no. 5: 1391-1405.

De Vos, P., W. Ludwig, K.-H. Schleifer, and W.B. Whitman. 2009. Family iv. Paenibacillaceae. In *The Firmicutes*, ed. P. De Vos, G.M. Garrity, D. Jones, N.R. Krieg, W. Ludwig, F.A. Rainey, K.-H. Schleifer and W.B. Whitman, 3: 269. New York: Springer. 1450.

Dertz, E.A., A. Stintzi, and K.N. Raymond. 2006. Siderophore-mediated iron transport in *Bacillus subtilis* and *Corynebacterium glutamicum*. *Journal of Biological Inorganic Chemistry* 11: 1087-1097.

Dimkpa, C., A. Svato, D. Merten, G. Büchel, and E. Kothe. 2008. Hydroxamate siderophores produced by *Streptomyces acidiscabies* e13 bind nickel and promote growth in cowpea (*Vigna unguiculata* l.) under nickel stress. *Canadian Journal of Microbiology* 54: 163-172.

Doyle, R.J., ed. 1999. *Biofilms*. San Diego: Academic Press.

Essén, S.A., D. Bylund, S.J.M. Holmstrom, M. Moberg, and U.S. Lundstrom. 2006. Quantification of hydroxamate siderophores in soil solutions of podzolic soil profiles in Sweden. *BioMetals* 19: 269-282.

Evans, R.D. and J.R. Ehleringer. 1993. A break in the nitrogen cycle in aridlands? Evidence from delta15N of soils. *Oecologia* 94: 314-317.

Evans, R.D. and O.L. Lange. 2001. Biological soil crusts and ecosystem nitrogen and carbon dynamics. In *Biological soil crusts: Structure, function, and management*, ed. J. Belnap and O.L. Lange. 263-279. Berlin: Springer-Verlag.

Garcia-Pichel, F. and J. Belnap. 1996. Microenvironments and microscale productivity of cyanobacterial desert crusts. *Journal of Phycology* 32: 774-782.

Garcia-Pichel, F., S.L. Johnson, D. Youngkin, and J. Belnap. 2003. Small-scale vertical distribution of bacterial biomass and diversity in biological soil crusts from arid lands in the Colorado Plateau. *Microbial Ecology* 46: 312-321.

Garcia-Pichel, F., A. Lopez-Cortes, and U. Nubel. 2001. Phylogenetic and morphological diversity of cyanobacteria in soil desert crusts from the Colorado Plateau. *Applied and Environmental Microbiology* 67, no. 4: 1902-1910.

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, no. 11: e7801.

Garrity, G.M., J.A. Bell, and T. Lilburn. 2005. Family vii. Bradyrhizobiaceae *fam. Nov.* In *The proteobacteria*, ed. G.M. Garrity, D.J. Brenner, N.R. Krieg and J.T. Staley, 2: 438-443. New York: Springer.

Gillis, M. and N.A. Logan. 2005. Genus iv. Janthinobacterium. In *The proteobacteria*, ed. G.M. Garrity, D.J. Brenner, N.R. Krieg and J.T. Staley, 2: 636-641. New York: Springer.

Glass, J.B., F. Wolfe-Simon, and A. Anbar. 2009. Coevolution of metal availability and nitrogen assimilation in cyanobacteria and algae. *Geobiology* 7, no. 2: 100-123.

Green, P.N. 2005. Genus i. Methylobacterium. In *The proteobacteria*, ed. G.M. Garrity, D.J. Brenner, N.R. Krieg and J.T. Staley, 2: 567-570. New York: Springer.

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.

Holmström, S.J.M., U.S. Lundstrom, R.D. Finlay, and P.A.W. Van Hees. 2004. Siderophores in forest soil solution. *Biogeochemistry* 71: 247-258.

Idris, R., R. Trifonova, M. Puschenreiter, W.W. Wenzel, and A. Sessitsch. 2004. Bacterial communities associated with flowering plants of the Ni hyperaccumulator *Thlaspi goesingense*. *Applied and Environmental Microbiology* 70, no. 5: 2667-2677.

Isichei, A.O. 1990. The role of algae and cyanobacteria in arid lands. A review. *Arid Soil Research and Rehabilitation* 4: 1-17.

Ito, T. and J.B. Neilands. 1958. Products of "Low-iron fermentation" with *Bacillus subtilis*: Isolation, characterization and synthesis of 2,3-dihydroxybenzoylglycine. *Journal of the American Chemical Society* 80, no. 17: 4645-4647.

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, no. 3: 680-689.

Joshi, F.R., G. Archana, and A.J. Desai. 2006. Siderophore cross-utilization amongst rhizospheric bacteria and the role of their differential affinities for Fe3+ on growth stimulation under iron-limited conditions. *Current Microbiology* 53: 141-147.

Joshi, F.R., S.P. Kholiya, G. Archana, and A.J. Desai. 2008. Siderophore cross-utilization amongst nodule isolates of the cowpea miscellany group and its effect on plant growth in the presence of antagonistic organisms. *Microbiological Research* 163, no. 5: 564-570.

Jurkevitch, E., Y. Hadar, and Y. Chen. 1992. Differential siderophore utilization and iron uptake by soil and rhizosphere bacteria. *Applied and Environmental Microbiology* 58, no. 1: 119-124.

Kalinowski, B.E., L.J. Liermann, S. Givens, and S.L. Brantley. 2000. Rates of bacteria-promoted solubilization of Fe from minerals: A review of problems and approaches. *Chemical Geology* 169: 357-370.

Khan, A., R. Geetha, A. Akolkar, A. Pandya, G. Archana, and A.J. Desai. 2006. Differential cross-utilization of heterologous siderophores by nodule bacteria of *Cajanus cajan* and its possible role in growth under iron-limited conditions. *Applied Soil Ecology* 34: 19-26.

Kim, H.J., D.W. Graham, A.A. DiSpirito, M.A. Alterman, N. Galeva, C.K. Larive, D. Asunskis, and P.M.A. Sherwood. 2004. Methanobactin, a copper-acquisition compound from methane-oxidizing bacteria. *Science* 305, no. 5690: 1612-1615.

Kraemer, S.M. 2004. Iron oxide dissolution and solubility in the presence of siderophores. *Aquatic Science* 66: 3-18.

Kuske, C.R., L.O. Ticknor, M.E. Miller, J.M. Dunbar, J.A. Davis, S.M. Barns, and J. Belnap. 2002. Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. *Applied and Environmental Microbiology* 68, no. 4: 1854-1863.

Lacava, P.T., M.E. Silva-Stenico, W.L. Araújo, A.V.C. Simionato, E. Carrilho, S.M. Tsai, and J.L. Azevedo. 2008. Detection of siderophores in endophytic bacteria *Methylobacterium* spp. associated with *Xylella fastidiosa* subsp. *Pauca Pesquisa Agropecuária Brasileira* 43, no. 4: 521-528.

Lane, D.J. 1991. 16s/23s rRNA sequencing. In *Nucleic acid techniques in bacterial systematics*, ed. E. Stackebrandt and M. Goodfellow:115-175. New York, NY: John Wiley and Sons.

Lankford, C.E. 1973. Bacterial assimilation of iron. *CRC Critical Reviews in Microbiology* 2: 273-331.

Liermann, L.J., R.L. Guynn, A. Anbar, and S.L. Brantley. 2005. Production of a molybdophore during metal-targeted dissolution of silicates by soil bacteria. *Chemical Geology* 220: 285-302.

Liermann, L.J., B.E. Kalinowski, S.L. Brantley, and J.G. Ferrey. 2000. Role of bacterial siderophores in dissolution of hornblende. *Geochimica et Cosmochimica Acta* 64, no. 4: 587-602.

Litos, C., A. Terzis, C. Raptopoulou, A. Rontoyianni, and A. Karaliota. 2006. Polynuclear oxomolybdenum(VI) complexes of dihydroxybenzoic acids: Synthesis, spectroscopic and structure characterization of a tetranuclear catecholato-type coordinated 2,3-dihydroxybenzoate and a novel tridentate salicylato-type coordinated 2,5-dihydroxybenzoate trinuclear complex. *Polyhedron* 25: 1337-1347.

Logan, N.A. and P. De Vos. 2009. Genus i. Bacillus. In *The firmicutes*, ed. P. De Vos, G.M. Garrity, D. Jones, N.R. Krieg, W. Ludwig, F.A. Rainey, K.-H. Schleifer and W.B. Whitman, 3:21. New York: Springer.

Marques, A., C. Pires, H. Moreira, A. Rangel, and P. Castro. 2010. Assessment of the plant growth promotion abilities of six bacterial isolates using *Zea mays* as an indicator plant. *Soil Biology and Biochemistry* 42: 1229-1235.

McDonald, I.R. and J.C. Murrell. 1997. The methanol dehydrogenase structural gene *mxaF* and its use as a functional gene probe for methanotrophs and methylotrophs. *Applied and Environmental Microbiology* 63, no. 8: 3218-3224.

McKnight, D.M. and F.M.M. Morel. 1980. Copper complexation by siderophores from filamentous blue-green algae. *Limnology and Oceanography* 25, no. 1: 62-71.

Monteiro, B., L. Cunha-Silva, S. Gago, J. Klinowski, F.A.A. Paz, J. Rocha, I.S. Gonçalves, and M. Pillinger. 2010. Crystal and supramolecular structures of dioxomolybdenum(VI) and dioxotungsten(VI) complexes of dihydroxybenzoic acids. *Polyhedron* 29: 719-730.

Nakatsua, C.H., V. Torsvik, and L. Øvreås. 2000. Soil community analysis using dgge of 16s rDNA polymerase chain reaction products. *Soil Science Society of America Journal* 64: 1382-1388.

Neilands, J.B. 1973. Microbial iron transport compounds (siderochromes). In *Inorganic Biochemistry*, ed. J.B. Neilands, 1:2-34. New York: Academic Press.

Palaniappan, P., P.S. Chauhan, V.S. Saravanan, R. Anandham, and T. Sa. 2010. Isolation and characterization of plant growth promoting endophytic bacterial isolates from root nodule of *Lespedeza* sp. *Biology and Fertility of Soils* 46: 807-816.

Pérez-Miranda, S., N. Cabirol, R. George-Téllez, L.S. Zamudio-Rivera, and F.J. Fernández. 2007. O-CAS, a fast and universal method for siderophore detection. *Journal of Microbiological Methods* 70: 127-131.

Peters, W.J. and R.A.J. Warren. 1968. Itoic acid synthesis in *Bacillus subtilis*. *Journal of Bacteriology* 95, no. 2: 360-366.

Philippot, L. 2002. Denitrifying genes in bacterial and archaeal genomes. *Biochimica et Biophysica Acta* 1577: 355-376.

Pimentel, D. 2000. Soil erosion and the threat to food security and the environment. *Ecosystem Health* 6, no. 4: 221-226.

Powell, P.E., G.R. Cline, C.P.P. Reid, and P.J. Szaniszlo. 1980. Occurrence of hydroxamate siderophore iron chelators in soils. *Nature* 287: 833-834.

Priest, F.G. 2009. Genus i. Paenibacilus. In *The firmicutes*, ed. P. De Vos, G.M. Garrity, D. Jones, N.R. Krieg, W. Ludwig, F.A. Rainey, K.-H. Schleifer and W.B. Whitman, 3: 269-279. New York: Springer.

Puigdefábregas, J. 1998. Ecological impacts of global change on drylands and their implications for desertification. *Land Degradation and Development* 9: 393-406.

Rashedul, I.M., M. Madhaiyan, H.P.D. Boruah, W. Yim, G. Lee, V.S. Saravanan, Q. Fu, H. Hu, and T. Sa. 2009. Characterization of plant growth-promoting traits of free-living diazotrophic bacteria and their inoculation effects on growth and nitrogen uptake of crop plants. *Journal of Microbiology and Biotechnology* 19, no. 10: 1213-1222.

Raza, W. and Q. Shen. 2010. Growth, Fe3+ reductase activity, and siderophore production by *Paenibacillus polymyxa* sqr-21 under differential iron conditions. *Current Microbiology* 61: 390-395.

Reynolds, R., J. Neff, M. Reheis, and P. Lamothe. 2006. Atmospheric dust in modern soil on aeolian sandstone, Colorado Plateau (USA): Variation with landscape position and contribution to potential plant nutrients. *Geoderma* 130: 108-123.

Rossi, F., R. Potrafka, F. Garcia-Pichel, and R. De Philippis. 2012. Role of the exo-polysaccharides in enhancing hydraulic conductivity of biological soil crusts. *Soil Biology and Biochemistry* 46: 33-40.

Saal, L.B. and O.W. Duckworth. 2010. Synergistic dissolution of manganese oxides as promoted by siderophores and small organic acids. *Soil Science Society of America Journal* 74, no. 6: 2021-2031.

Schloss, P.D., S.L. Westcott, T. Ryabin, J.R. Hall, M. hartmann, E.B. Hollister, R.A. Lesniewski, B.B. Oakley, D.H. Parks, C.J. Robsincon, J.W. Sahl, B. Stres, G.G. Thallinger, D.J. Van Horn, and C.F. Weber. 2009. Introducing mothurs: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* 75, no. 23: 7537-7541.

Schulten, J.A. 1985. Soil aggregation by cryptogams of a sand prairie. *American Journal of Botany* 72, no. 11: 1657-1661.

Schwyn, B. and J.B. Neilands. 1987. Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry* 160: 47-56.

Sheridan, D. 1981. *Desertification of the United States*. Council on environmental quality. Washington, D.C.: U.S. Government Printing Office.

Silva-Stenico, M.E., F.T.H. Pacheco, J.L.M. Rodrigues, E. Carrilho, and S.M. Tsai. 2005. Growth and siderophore production of *Xylella fastidiosa* under iron-limited conditions. *Microbiological Research* 160: 429-436.

Simionato, A.V.C., C. Simo, A. Cifuentes, P.T. Lacava, W.L. Araujo, J.L. Azevedo, and E. Carrilho. 2006. Capillary electrophoresis-mass spectrometry of citrus endophytic bacteria siderophores. *Electrophoresis* 27: 2567-2574.

Strauss, S.L., T.A. Day, and F. Garcia-Pichel. 2012. Nitrogen cycling in desert biological soil crusts across biogeographic regions in the southwestern United States. *Biogeochemistry* 108: 171-182.

Szabó, O. and E. Farkas. 2011. Characterization of Mn(II) and Mn(III) binding capability of natural siderophores desferrioxamine b and desferricoprogen as well as model hydroxamic acids. *Inorganic Chimica Acta* 376: 500-508.

Tamura, K., J. Dudley, M. Nei, and S. Kumar. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24: 1596-1599.

Temirov, Y.V., T.Z. Esikova, I.A. Kashparov, T.A. Balashova, L.M. Vinokurov, and Y.B. Alakhov. 2003. A catecholic siderophore produced by the thermoresistant *Bacillus licheniformis* vk21 strain. *Russian Journal of Bioorganic Chemistry* 29, no. 6: 542-549.

Tovar-Sanchez, A., S.A. Sanudo-Wilhelmy, A.B. Kustka, S. Agusti, J. Dachs, D.A. Hutchins, D.G. Capone, and C.M. Duarte. 2006. Effects of dust deposition and river discharges on trace metal composition of *Trichodesmium* spp. In the tropical and subtropical North Atlantic Ocean. *Limnology and Oceanography* 51, no. 4: 1755-1761.

Tuit, C., J. Waterbury, and G. Ravizza. 2004. Diel variation of molybdenum and iron in marine diazotrophic cyanobacteria. *Limnology and Oceanography* 49, no. 4: 978-990.

Umamaheswari, A., D.R. Madhavi, and K. Venkateswarlu. 1997. Siderophore production in two species of *Nostoc* as influenced by the toxicity of nitrophenolics. *Bulletin of Environmental Contamination and Toxicology* 59: 306-312.

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, no. 16: 5261-5267.

White, A.E., Y.H. Spitz, D.M. Karl, and R.M. Letelier. 2006. Flexible elemental stoichiometry in *Trichodesmium* spp. and its ecological implications. *Limnology and Oceanography* 51, no. 4: 1777-1790.

White, S., G. Boyd, F.S. Mathews, Z. Xia, W. Dai, Y. Zhang, and V.L. Davidson. 1993. The active site structure of the calcium-containing quinoprotein methanol dehydrogenase. *Biochemistry* 32: 12955-12958.

Wiebe, K. 2003. *Linking land quality, agircultural productivity, and food security.* Washington, DC: USDA Economic Research Service, Agricultural Economic Report No. 823.

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, no. 2: 973-983.

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.

CHAPTER 6

IDENTIFICATION OF SIDEROPHORES IN BIOLOGICAL SOIL CRUSTS USING ELECTROSPRAY IONIZATION MASS SPECTROMETRY

Abstract

Biological soil crusts are known to influence soil solution concentrations of bioessential elements that are required for their physiology, and previous work identified siderophore-producing organisms in biological soil crusts (BSCs). Siderophores are low-molecular weight organic compounds that bind a variety of elements and serve to increase element solubility and facilitate element uptake. Therefore, it was presumed that siderophore production was responsible for the soil solution concentration changes observed in live crusts. The purpose of the present study was to determine the molecular weight of siderophores produced by crust microbes using electrospray ionization mass spectrometry (ESI-MS). Four putative siderophores were identified with masses of 169, 348, 398, and 403 amu. These masses do not match those of any known siderophores produced by crust microbes. ESI-MS peak intensities of the unbound masses decreased with increasing iron, and intensities of the iron-bound masses increased with increasing iron concentration, suggesting that these peaks represent siderophores. Further work to confirm the siderophore-nature of the compounds demonstrated that compounds with masses of 169 and 398 amu are likely true siderophores.

Introduction

Biological soil crusts (BSCs) are diverse microbial consortia that provide a plethora of valuable ecosystem services to arid environments. BSCs supply the primary sources of bioavailable carbon (Beymer & Klopatek, 1991; Evans & Lange, 2001; Garcia-Pichel, 2002) and nitrogen (Rychert & Skujins, 1974; Jeffries et al., 1992; Steppe et al., 1996; Belnap, 2002) and solubilize other bioessential elements, such as iron and molybdenum (Beraldi-Campesi et al., 2009; Chapters 2 & 3). Composed of a mix of phototrophic and heterotrophic bacteria, archaea, fungi, algae, lichens, and mosses, BSCs form vertically stratified biosedimentary structures at the soil surface that serve to increase soil stabilization (Campbell, 1979; Schulten, 1985; Belnap, 1993; Garcia-Pichel & Wojciechowski, 2009; Rossi et al., 2012). Together, increasing the availability of bioessential elements and preventing erosion, the activities of BSCs work to sustain arid land fertility and prevent degradation (Isichei, 1990; Belnap et al., 1994; Belnap, 1995; Bowker et al., 2008a). BSCs are slow-growing and extremely susceptible to disturbance; their recovery can take millennia (Belnap & Eldridge, 2001). Therefore, it is critical to understand what factors control BSC growth and development in order to promote arid land conservation and restoration (Belnap et al., 1994; Bowker et al., 2005; 2008c).

Crust microbes perform a variety of physiological processes that require trace metals for electron transfer and catalysis. For example, iron (Fe) and copper (Cu) are used in multiple components of microbial respiratory and photosynthetic electron transport (Lockau, 1981; Raven et al., 1999; Peschek et al., 2004; Ferguson-Miller et al., 2007; Bernroitner et al., 2008) and in ammonium oxidation (Murphy et al., 1974; Aparicio et al., 1975; Lancaster et al., 1979; Guerrero et al., 1981; Rubio et al., 1999; Rubio et al., 2002), Fe and molybdenum (Mo) are used in biological nitrogen fixation (Burgess & Lowe, 1996; Howard & Rees, 1996), manganese (Mn) plays a key role in photosynthesis (Umena et al., 2011), zinc (Zn) is used in carbonic anhydrase (Smith & Ferry, 2000), and nickel (Ni) is needed for hydrogenase enzymes (Bothe et al., 2010). While this list is not exhaustive, it emphasizes the point that many trace metals are required for mandatory physiological processes. Therefore, obtaining these trace metals must be a high priority for BSCs.

One might imagine that microbes living in intimate association with soil would be practically bathing in trace metals, however, this is not necessarily the case. Although trace metals may exist in high abundance either within mineral matrices or adsorbed to mineral surfaces and soil organics, the bioavailability of these trace elements is not guaranteed. The solubility of trace metals is pH and redox-dependent, and so certain conditions may render trace metals inaccessible to soil microbes. For

instance, under oxidizing conditions, Fe exists in the +3 oxidation state, which is less soluble than the +2 oxidation state (Brady & Weil, 2002). Exacerbating the low solubility of Fe^{3+} in most surface environments, is the decreased solubility of Fe with increasing pH. Therefore, at physiological pH values under oxidizing conditions, Fe becomes virtually unavailable to microbes, and the same is true of many other trace metals such as Mn and Cu (Brady & Weil, 2002). But, trace elements are essential components of many biological processes, and so soil microbes much have strategies to access them.

One such mechanism by which soil organisms obtain much needed trace metals is the production of metal chelators. The most common of these are the siderophores, which are low molecular weight organic compounds that bind Fe with high affinity (Neilands, 1981). Some siderophores are also capable of binding other trace elements such as Mn (Saal & Duckworth, 2010; Szabó & Farkas, 2011), Cu (McKnight & Morel, 1980; Kim et al., 2004; Bellenger et al., 2007), and Mo (Litos et al., 2006; Bellenger et al., 2007; Monteiro et al., 2010). When released from microbial cells, siderophores serve to increase metal solubility (Kalinowski et al., 2000; Kraemer, 2004; Liermann et al., 2000; 2005) and facilitate metal uptake (Neilands, 1973; Bellenger et al., 2008). The presence of siderophores in soils has been known for many years (Powell et al., 1980; Akers, 1983; Holmström et al., 2004; Essén et al., 2006), however,

siderophore production has never been explicitly studied in biological soil crusts. My previous work identified 10 siderophore-producing microbes in BSCs that belong to a range of bacterial lineages, perform multiple physiological processes, and play different ecological roles in crusts (see Chapter 5). The purpose of the current study was to investigate the siderophore compounds themselves; to attempt to determine their molecular weights.

To do this I utilized electrospray ionization mass spectrometry (ESI-MS), a technique that has proven effective for the identification and characterization of siderophores (Gledhill, 2001; Martinez et al., 2001; McCormack et al., 2003; Ross et al., 2003; Essen et al., 2006). ESI-MS is a soft ionization approach that allows the monitoring of intact molecules that become ionized through the loss or gain of one or more hydrogen ions. One benefit of soft ionization, is that it allowed me to observe siderophores in both their unbound and Fe-bound states. Another advantage of ESI-MS is that peak intensity is directly related to concentration and so I can infer relative abundances of ions in solution based on their relative peak intensities. Together, these qualities of ESI-MS allowed me to investigate the nature of the siderophores produced in BSCs.

Methods

Experiment 1 - Identification of Putative Siderophore Peaks

An axenic culture of *Nostoc punctiforme* isolated from the crusts was grown in 2.5 L of modified BG-11 media (Allen & Stanier, 1968) in the absence of trace metals. The following components were used to make the media (per liter): 7.5 g $MgSO_3 \cdot 7H_2O$, 3.6 g $CaCl_2 \cdot 2H_2O$, 0.715 g H_3BO_3, 1 g Na_2CO_3, 1.53 g K_2HPO_4, and 0.03 g citric acid. The culture was incubated at 25°C and an irradiance (measured as photosynthetically active radiation) of 132 ± 3 $\mu E\ m^{-2}\ s^{-1}$ for 6 weeks. This temperature is similar to temperatures recorded at the field site and the irradiance is sufficient for crusts to photosynthesize (Garcia-Pichel & Belnap, 1996). The media contained no fixed N so the *Nostoc* was actively fixing N_2 as well. The presence of siderophores in the culture media was monitored weekly using the liquid chrome azurol S (CAS) assay, which contained (per liter): 60 mg chrome azurol S, 1.6 mg $FeCl_3$, 3.6 mg HCl, and 73 mg hexadecyltrimethylammonium bromide (Schwyn & Neilands, 1987). Once a week, 1 mL of culture media was filtered through a 0.45 µm polysulfone membrane filter (Supor®, PES, Pall Corporation, Port Washington, NY) and mixed with 1 mL of CAS solution. The absorbance (A) of the mixture was measured at 630 nm on a Shimadzu UVmini-1240 spectrophotometer. The CAS dye changes color and absorbance in the presence of siderophores that bind Fe. The absorbance in the *Nostoc*

culture media was compared to the absorbance of a reference (A_{ref}) prepared by mixing 1 mL of uninnoculated media with 1 mL CAS solution. Lower absorbance observed in the inoculated media mixtures ($A/A_{ref} < 1$) indicated the presence of siderophores.

Once siderophores were detected, the culture media was centrifuged for 10 min at 4000 rpm, to separate cells from the media, and then filtered through a Whatman™ GF/F filter (55 mm diameter, nominal pore size 0.7 µm). Column chromatography was applied to separate the media into fractions and the CAS assay was used to determine which fractions contained siderophores. Cell-free culture supernatant was stirred for 3 h with Amberlite XAD-2 resin (100 mL; Supelco, SIGMA-ALDRICH, St. Louis, MO). Resin was prepared by rinsing 3 times with 250 mL methanol (Macron Chemicals, UntimAR®) and 5 times with deionized water (DI; 18.2 MΩ·cm; NANOpure® DIamond™ UV, Barnstead International, Dubuque, IA). After 3 h, the majority of the fluid was removed, leaving enough liquid to form a slurry and allow the resin to be loaded onto the column (GE Healthcare, C26/70). The column itself was previously acid-washed with 10% hydrochloric acid (HCl; EMD; ACS grade) and then rinsed with 3 volumes of 18.2 MΩ·cm water. After the resin was poured into the column, the remaining supernatant was collected in acid-washed (20% HCl) polypropylene tubes. The column was eluted with 200 mL each of 100% 18 MΩ·cm carbon-free water (NANOpure® DIamond™ UV, Barnstead

International, Dubuque, IA), 50/50 18 MΩ·cm water/HPLC grade methanol (Macron Chemicals, UntimAR®), and 100% HPLC grade methanol. Eluant fractions of 10 mL were collected sequentially in acid-washed (20% HCl) polypropylene tubes. Following collection, 50 μL of each fraction were transferred to a plastic 96-well plate, and 50 μL CAS solution was added. Negative controls were prepared from 50 μL of each eluant before it passed through the resin, and positive controls were prepared from 50 μL of 200 μM desferrioxamine B (DFAM). The 96-well plate was photographed immediately following CAS solution addition, after 1 h, and again after 24 h. Fractions that changed color from blue to yellow, orange, or purple were saved for further siderophore analysis.

Twenty eluted fractions changed color in the presence of the CAS dye (Figure 6-1). These fractions were pooled in groups of 5 and prepared for electrospray ionization mass spectrometry (ESI-MS). For each pooled sample, 3 subsamples of 0.5 mL were mixed with 0.5 mL of each of the following: water (–Fe), 90 μM Fe (low Fe), and 180 μM Fe (high Fe). Final Fe concentrations in the low and high Fe additions were 45 and 90 μM, respectively.

Samples were analyzed in positive ion mode on a triple quadropole electrospray ionization mass spectrometer (Finnigan™ TSQ® Quantum Discovery MAX™). Triplicate 25 μL injections of each sample were collected from vials with an autosampler (Finnigan™ Surveyor) and

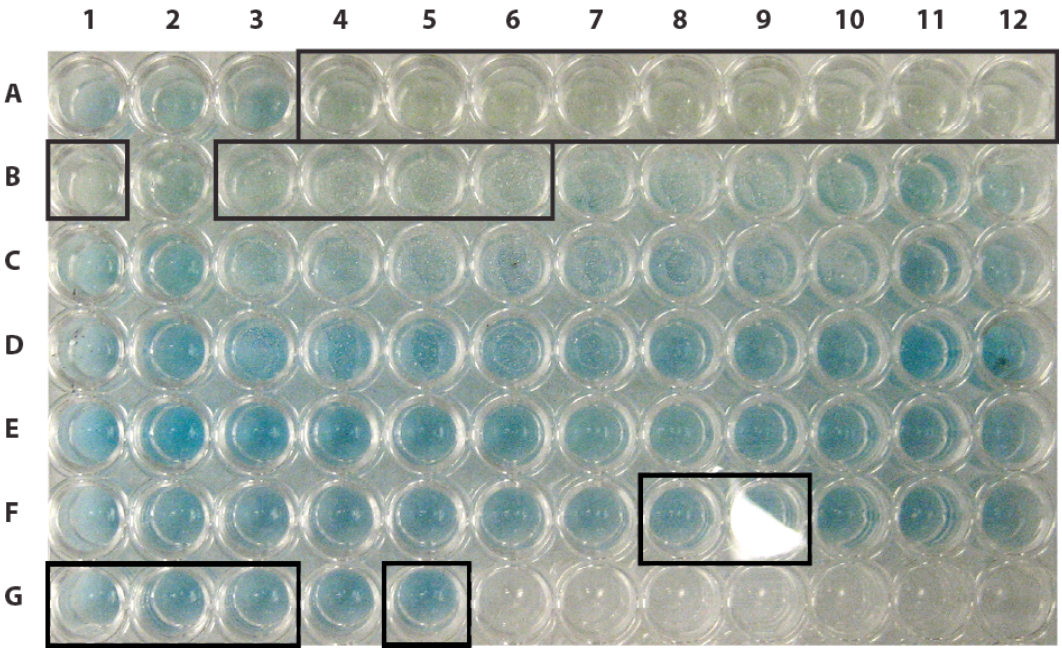

Figure 6-1. Photograph of the 96-well plate showing positive CAS assay results for the XAD column eluant fractions. Fractions that changed color from blue to purple or yellow were pooled and analyzed by HPLC-ESI-MS. The wells outlined in solid boxes turned a light green to yellow color, and the wells outlined in dashed boxes turned a dark blue to purple color.

passed through a C-18 high performance liquid chromatography (HPLC) column (Thermo Scientific®, Aquasil) and then into the ESI source. The mobile phase was 50/50 (vol/vol) 18.2 MΩ·cm water/HPLC grade methanol with 0.05% formic acid added as an ionization aid. The flow rate was 0.2 mL/min, the spray voltage was 4000 volts, and the capillary temperature was 300°C. The scanned mass range was 30-1500. Mass spectra were collected for 10 min with Xcalibur software (v2.0, Thermo Scientific®) to ensure that all material was eluted from the column before injecting the next sample. Water blanks were run every 5 samples.

Analysis of the total ion chromatograms revealed a well-defined peak over the 1.75-2.15 min retention time range in all samples (Figure 6-2). Therefore, mass spectra for blanks and samples were only integrated over this time range. Average blank and sample peak intensities were calculated from the triplicate injections for each mass. The average blank peak intensity was subtracted from the average sample peak intensity, and error was propagated according to Bevington & Robinson (2002). All negative values that resulted from the blank subtraction were set to zero.

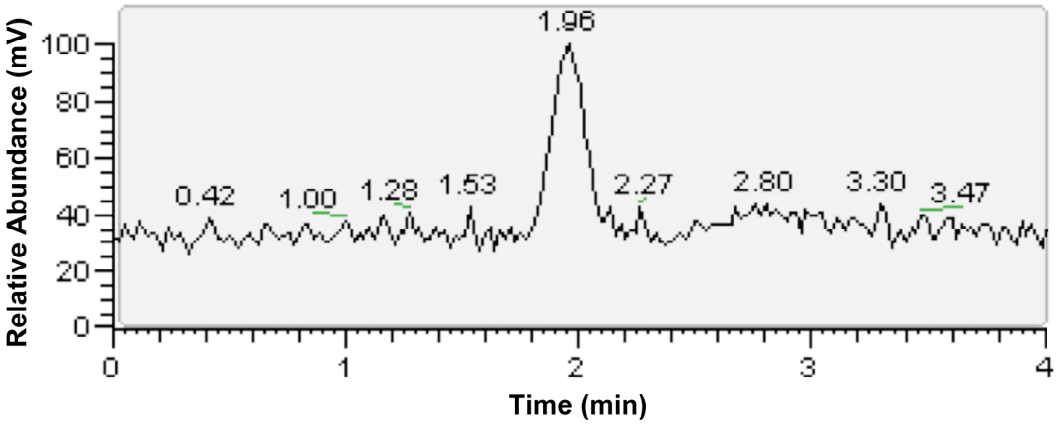

Figure 6-2. Representative Total Ion Chromatogram (TIC) of one of the CAS-positive XAD fractions from the 2.5 L culture of *Nostoc punctiforme*. The peak appeared between 1.75 and 2.15 min retention time; therefore, all ESI-MS spectra of culture media at various Fe concentrations and of soil solutions from crust experiments were integrated over this time period.

Data were grouped so that peak intensities for –Fe, low Fe, and high Fe solutions for each pooled sample formed an array, and a Linest function (Microsoft Excel) was used to determine which masses showed increasing and decreasing intensities with Fe concentration. For each mass-to-charge ratio, the mass-to-charge ratio for the matching doubly-

charged ion was calcualted, as well as the singly- and doubly-charged Fe-bound ions. These calculations generated peak groups with four ions in each group. Peak groups for which the unbound peak intensities decreased with increasing Fe concentration and the Fe-bound peak intensities increased with increasing Fe concentration were identified as putative siderophores. Linear regression analyses were performed in Excel for all peaks in each group that met the above criteria. Those groups that had 3 or more peaks that changed significantly with Fe concentration were selected for further analysis (Table 6-1), and peak intensities of those peaks were analyzed in the remaining experiments.

An aqueous solution of 200 μM desferrioxamine B (DFAM) with and without added Fe, was analyzed with ESI-MS, as described above, to determine what ions are observed in the mass spectrum of a typical siderophore in both the bound and unbound forms (Figure 6-3).

Experiment 2 - Confirmation of Siderophore Peaks

The same culture of *Nostoc punctiforme* was grown under 3 Fe concentrations (no added Fe, 5 μM Fe, and 10 μM Fe). Triplicate bottles of each Fe condition were prepared with 1 L volumes of BG-11 media with the following concentrations: 7.5 g $MgSO_3 \cdot 7H_2O$, 3.6 g $CaCl_2 \cdot 2H_2O$, 0.715 g H_3BO_3, 1 g Na_2CO_3, 1.53 g K_2HPO_4, 0.03 g citric acid, 0.453 g $MnCl_2 \cdot 4H_2O$, 0.055 g $ZnSO_4 \cdot 7H_2O$, 0.098 g $Na_2MoO_4 \cdot 2H_2O$, 0.02 g $CuSO_4 \cdot 5H_2O$, 0.01 g $NiCl_2 \cdot 6H_2O$, 0.01 g $CoCl_2 \cdot 6H_2O$, and 0.009 g

Table 6-1. Formulas and masses of the known siderophore desferrioxamine (DFAM) and 4 putative siderophores (PS1-4). Actual mass (M) is the mass of the uncharged, unbound compound. The ions observed in the ESI-MS spectra are displayed as functions of the actual mass. All masses are in atomic mass units (amu).

	Actual Mass	Ions Observed in ESI-MS Spectra			
	Uncharged Unbound	Singly-charged Unbound ion	Doubly-charged Unbound ion	Singly-charged Fe-bound ion	Doubly-charged Fe-bound ion
	M	$\frac{M+1}{1}$	$\frac{M+1}{2}$	$\frac{M+53+1}{1}$	$\frac{M+53+1}{2}$
DFAM	561	562	281	615	308
PS1	169	170	85	223	112
PS2	348	349	175	402	201
PS4	398	399	200	452	226
PS3	403	404	202	457	229

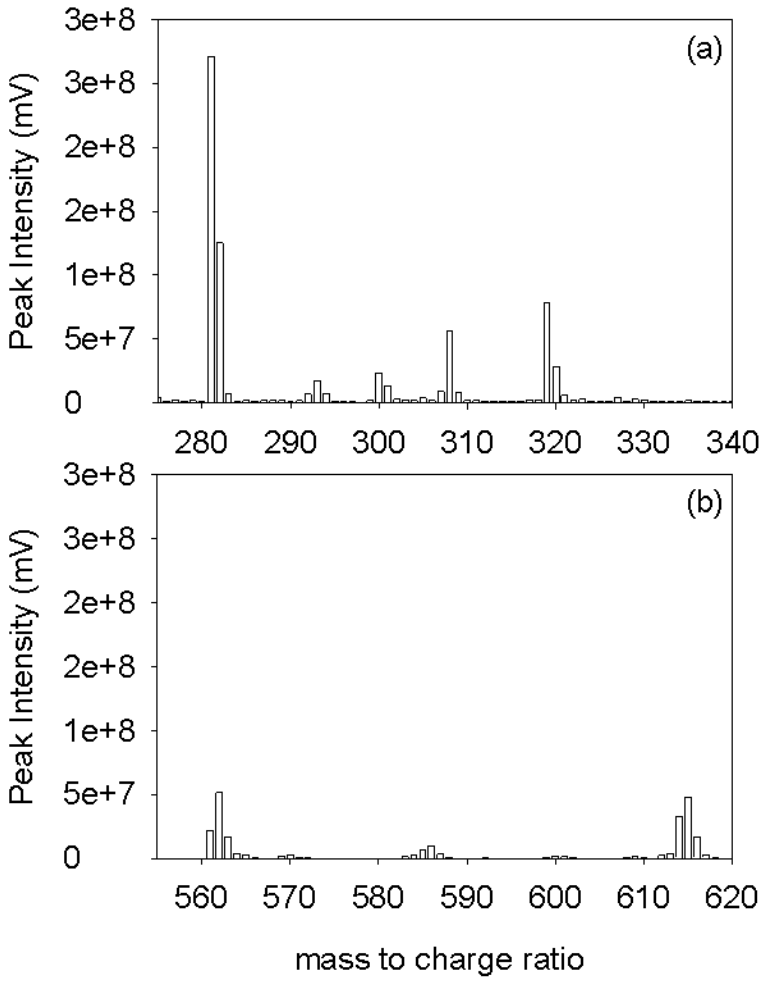

Figure 6-3. Mass spectra for desferrioxamine B (DFAM). Panel (a) shows mass-to-charge ratios from 175 to 340 amu, with DFAM peaks at 281 and 308, corresponding to the doubly-charged, unbound and doubly-charged, Fe-bound ions, respectively. Panel (b) shows mass-to-charge ratios from 555 to 620 amu and highlights peaks at 562 and 615 amu corresponding to the singly-charged, unbound DFAM and singly charged, Fe-bound DFAM, respectively. Formulas for calculating the mass to charge ratios of the four ions for putative siderophores were based on the ions observed in this spectrum.

Na_3VO_4. After mixing, the media was sterilized by autoclaving. Bottle caps were fitted with a long tygon tube that reached to the bottom of the bottle that allowed aeration and media sample collection, and 2 short tygon tubes were placed at the top for aeration and sampling. Tubes and caps

were sterilized by microwaving in 3 inches of water 4 times at 3, 2, 3, and 2 minute intervals (Felisa Wolfe-Simon, personal communication). Filters (0.8/0.2 μm, Pall Corporation, Acrodisc®, Supor®) were placed on the 2 short tubes to prevent contamination, and caps were placed on the 1 L bottles. All glass- and plasticware was soaked in clean 20% HCl for 48 h. Bottles were inoculated with ~20-25 mL *Nostoc punctiforme* culture and transferred to the incubator at 25°C and 132 ± 3 μE m^{-2} s^{-1}.

At the end of the experiment, 1 mL of culture media was collected for HPLC-ESI-MS analysis. Media was removed from each bottle using a sterile syringe and filtered through a 0.45 μm filter into 2 mL acid-washed (20% HCl) polypropylene tubes. Methanol (1 mL Macron Chemicals, UltimAR®) was added to each tube. Tubes were shaken to mix, wrapped with parafilm to minimize evaporative loss, and stored at -20 ºC for 1 to 3 weeks. Samples were analyzed with HPLC-ESI-MS as above. Putative siderophore peaks were identified in the previous experiment, so I focued on analyzing the intensities of those peaks in the mass spectra from Experiment 2.

Additionally, at the end of the experiment, three 100 mL volumes of culture media were vacuum filtered onto preweighed GF/F filters. Filters were allowed to dry and then reweighed to determine the dry weight biomass per 100 mL culture. Peak intensities were divided by the average dry weight biomass in order to normalize peak intensity to culture density.

Experiment 3 - Siderophore Production in Intact Crusts

Soil solutions from the +H_2O treatment described in Chapter 3 were used for ESI-MS analysis in the current study. Briefly, dark crust samples were collected at the Green Butte site (Strauss et al., 2012) north of Moab, UT (N 38°42′56.2″, W 109°41′32.7″) in May, 2009. Dark crusts were visually selected because they are dominated by a diverse cyanobacterial assemblage and have minimal lichen and moss cover (Garcia-Pichel & Belnap, 1996; Garcia-Pichel et al., 2001). Soils were collected by inserting the lid of a Petri dish (50 mm x 13 mm) into the soil and using a plastic spatula to remove the lid and soil within. Inversion of the soil allowed the bottom of the dish to be inserted, and crusts were then transported and stored in their original orientation (Garcia-Pichel et al., 2003). Soils were stored at 20ºC until October, 2009. Crusts may be stored for up to 24 months without significant changes to microbial community composition (Campbell et al., 2009). Killed control were prepared by autoclaving crusted soil.

Six crusts and 6 killed controls were placed into incubation dishes constructed by stacking the bases of two Petri dishes (50 mm x 13 mm) and sealing the seam between the dishes with black electrical tape. Small holes were melted into the base of the top dish to allow water to move into the bottom dish. One small hole was melted into the side of the bottom dish to serve as a sampling port for water collection. Whatman™ GF/F

filters (55 mm diameter, nominal pore size 0.7 μm) were placed over the perforated surface in the top dish, and the soils were transferred into the top dish in their original orientation.

The 12 soils were wetted with 15 mL DI water and transferred to an incubator maintained at 25°C and 110 $\mu E\ m^{-2}\ s^{-1}$. Soil solution was collected on Days 1, 3, and 5 by applying gentle vacuum suction through the sampling port with a 60 mL syringe. On Day 5, soils were removed from the incubator and allowed to dry for 5 days. On Day 10, soils were rewetted with 15 mL DI water, and soil solution was collected on Days 10, 12, and 14. Soil solution (1 mL) was filtered through 0.45 μm polysulfone membrane filters (Supor®, PES, Pall Corporation, Port Washington, NY) into acid washed polypropylene tubes. Methanol (1 mL) was added to each tube, solutions were mixed, tubes were wrapped in parafilm to avoid evaporative sample loss, and solutions were stored at -20°C. Solutions were analyzed by HPLC-ESI-MS according to the methods described above, with specific attention payed to the intensities of the putative siderophore peaks identified in Experiment 1.

Linear regression analyses of putative siderophore peak intensities against time (day) were run in SigmaPlot v11.0 (Systat Software Inc., Chicago, IL). Peak intensities in crusted samples were compared to peak intensities in killed controls using a Mann-Whitney Rank Sum test in SigmaPlot. *P*-values < 0.10 were considered statistically significant.

Results

Identification of Putative Siderophore Peaks

Twenty XAD column fractions tested positive for siderophores (Figure 6-1). When pooled XAD column eluant fractions were analyzed with HPLC-ESI-MS a well-defined peak was observed over the period of 1.75-2.15 min (Figure 6-2). All samples and blanks were integrated over this time period.

The DFAM siderophore reference spectrum revealed 4 dominant peaks (Figure 6-3). Peaks are characterized by mass and charge. For example, a peak with a mass of 100 atomic mass units (amu) and a single charge would appear at a mass-to-charge ratio (m/z) of 100 amu. If that same peak was doubly-charged it would appear at a m/z of 100/2 or mass-to-charge = 50 amu. The peaks in the DFAM reference spectrum included a singly-charged unbound ion [(M+1)/1], a doubly-charged unbound ion [(M+1)/2], the singly-charged, Fe-bound ion [(M+53+1)/1], and the doubly-charged Fe-bound ion [(M+53+1)/2] (Figure 6-3a-b, Table 6-1). For DFAM, with a molecular mass of 561 amu, the ESI-MS peaks correspond to mass-to-charge ratios of 562, 281, 615, and 308 amu, respectively. My studies looked for compounds that bound Fe in a similar manner as DFAM. The peaks representing these compounds, therefore, displayed identical mass-to-charge and iron-binding relationships to one another as the DFAM peaks.

Four peak groups from *Nostoc punctiforme* culture media met the criteria for putative siderophores. In these groups, the cumulative intensity of the peaks corresponding to the singly- and doubly-charged unbound ions decreased with increasing Fe concentration, while the cumulative intensity of the peaks corresponding to the Fe-bound ions increased with increasing Fe concentrations (Figure 6-4a-d). The actual masses of the putative siderophores are 169, 348, 398, and 403. The mass-to-charge ratios of the unbound and Fe-bound peaks are summarized in Table 6-1.

Confirmation of Siderophore Peaks

Biomass normalized cumulative intensities of several putative siderophore peaks from the batch culture studies of *Nostoc puntiforme* grown under variable Fe concentrations (Experiment 2) changed significantly with changing Fe concentration (Figure 6-5a-d, Table 6-2). This cumulative intensity assumes the presence of the siderophore in any form (bound or unbound, singly- or doubly-charged) and is sufficient to establish it's presence. I am essentially estimating the total amount of siderophore present as a function of the iron concentration of the *Nostoc* culture media. For the compound with mass 169 amu, peak intensities were lower under 10 μM Fe than 5 μM Fe. However, concentrations were also lower under 0 μM Fe than 5 μM Fe. Peak intensities increased significantly with Fe concentration for the compound with a mass of 348 amu (p = 0.10), and peak intensities decreased significantly with Fe

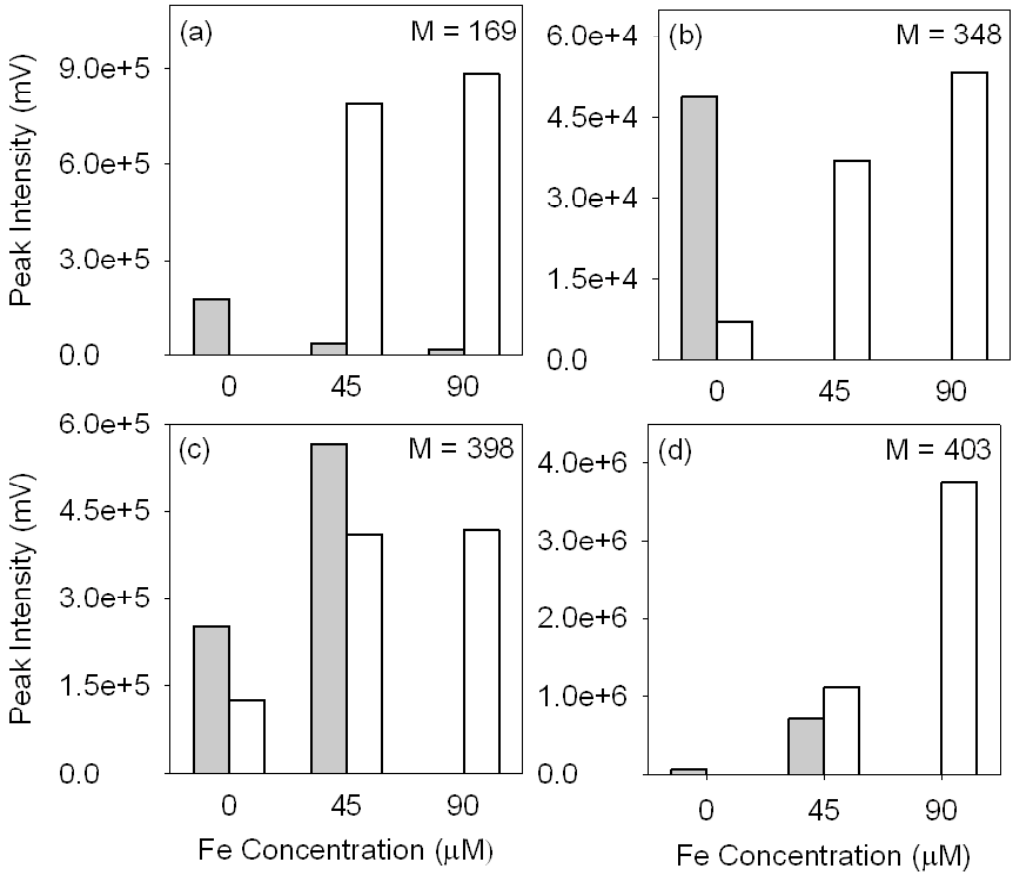

Figure 6-4. Cumulative peak intensity vs. iron treatment for four different peaks that correspond to putative siderophores; a) Mass, M = 168 amu, b) M = 348 amu, c) M = 398 amu, and d) M = 403 amu. Putative siderophores were identified as peaks for which the intensities of the unbound peaks decreased significantly with increasing Fe concentration and the intensities of the matching Fe-bound peaks increased significantly with increasing Fe concentration. Peak intensities of singly- and doubly-charged ions were summed for unbound ions (grey bars) and bound ions (white bars). The mass of the uncharged, unbound compound is shown on each panel, and the masses of the ions in each group are displayed in Table 6-1. The intensities of bars that represent the unbound peaks (grey) are highest for the 0 μM Fe treatment and decrease to below detection in the 90 μM Fe treatment. The bars that represent the Fe-bound peaks (white) have lower intensities for the 0 μM Fe treatment and are highest in the 90 μM Fe treatment. Peak intensities are generally intermediate under the 45 μM Fe treatment.

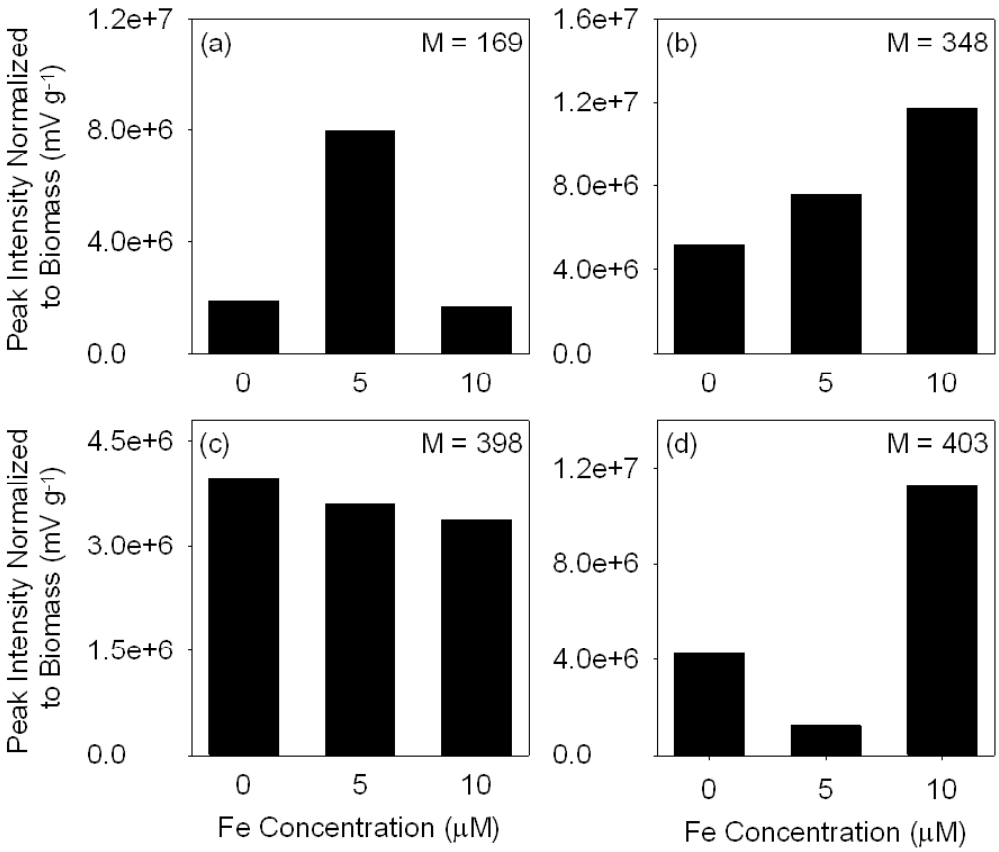

Figure 6-5. Cumulative peaks intensity normalized to biomass for the four putative siderophores under each Fe treatment in Experiment 2; a) Mass, M = 168 amu, b) M = 348 amu, c) M = 398 amu, and d) M = 403 amu. Peak intensity was highest under 5 μM Fe for the compound with mass 169 amu. Peak intensity increased significantly with increasing Fe concentration for the compound with mass of 348 amu (p = 0.10). Peak intensity decreased significantly with increasing Fe for the compound with mass of 398 amu (p = 0.09). Peak intensity did not change significantly with Fe concentration for the compound of mass 403 amu. Based on these results, compounds with masses 169 and 398 are most likely to be true siderophores.

concentration for the compound with a mass of 398 amu (p = 0.09; Table

6-2). Peak intensities did not change significantly with Fe concentration for

the compound with a mass of 403 amu (Table 6-2).

Table 6-2. Results of linear regression of cumulative biomass-normalized peak intensity and Fe concentration in Experiment 2.

Compound Mass (M)	R^2	*p*-value	Direction of change with increasing iron
169	<0.01	0.52	No change
348	0.98	**0.10**	**Increase**
398	0.98	**0.09**	**Decrease**
403	0.47	0.52	No change

Siderophore Production in Intact Crusts

Putative siderophore peaks were identified in the soil solutions of crusted samples; however, peak intensities did not change significantly with time in crusted samples, nor were there any significant differences between peak intensities in crusted samples and killed controls.

Discussion

Putative siderophore were identified as those for which the unbound masses decreased and the Fe-bound masses increased with increasing Fe concentration. This can be seen very clearly in Figure 6-4 where the cumulative peak intensities of the unbound masses (grey bars) are highest under 0 μM Fe conditions and disappear under 90 μM Fe conditions. In contrast, the peak intensities of the Fe-bound peaks (white bars) are lowest under the 0 μM Fe, and increase under 45 and 90 μM Fe. The increase in peak intensity of Fe-bound peaks with increasing Fe concentration was significant at better than the 90% confidence level for

the majority of individual peaks (singly- and doubly-charged, Fe-bound peaks) before peak intensities were added (data not shown). This pattern is typical for hydroxamate siderophores, such as desferrioxamine B (DFAM) shown in Figure 6-6a (Kiss & Farkas, 1998); DFAM loses 3 hydrogen atoms (mass = 1) when bound to Fe (mass = 56) (Jessica Martin, personal communication). Therefore, the net mass gain is 53 (i.e., 56-3), which allowed me to easily calculate the mass-to-charge ratios of the singly- and doubly-charged Fe-bound compounds associated with any given mass. If the peak intensities of the Fe-bound masses increased when Fe concentration increased, I could infer that those peaks represented a putative siderophore.

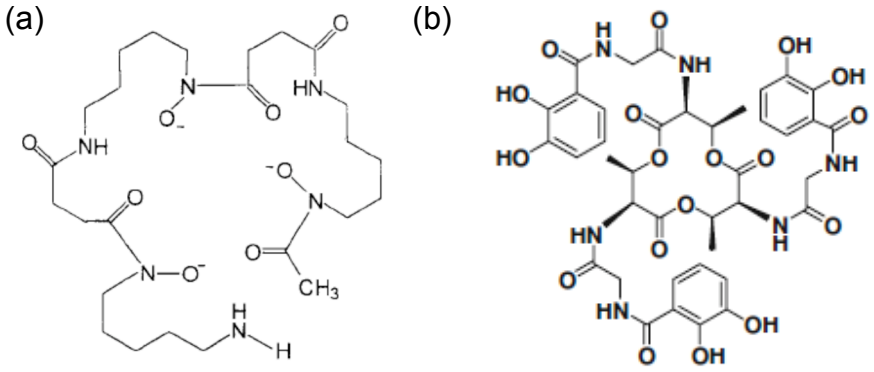

Figure 6-6. Structures of known siderophores; (a) is the hydroxamate siderophore desferrioxamine B (Kiss & Farkas, 1998), (b) is the catecholate siderophore Bacillibactin (Dertz et al., 2006).

Not only did my peaks show the correct behavior with increasing Fe concentration, as described above, but they were also within the right size range for siderophores. Siderophores are low-molecular weight organic

compounds that generally do not exceed 500-1000 amu (Neilands 1981); my compounds range in mass from 169-403 amu. Therefore, I conclude that compounds with masses 169, 348, 398, and 403 (Table 6-1, Figure 6-4), are excellent siderophore candidates.

When the peak intensities for all 4 peaks of each group are summed, they do not equal the same total intensity under each Fe treatment (Table 6-3). The 0 μM Fe, 45 μM Fe, and 90 μM Fe solutions were partitioned from the same pooled sample, so one might anticipate they should have the same concentrations of each compound. Since ESI-MS peak intensity is correlated with concentration, one might expect the sum of all peaks that represent a single compound to equal the same total intensity in each Fe condition. However, this is not always the case; for example, one can see that the sum of peak intensities for mass 169 under –Fe is 175,223 (Table 6-3). However, the sum of all peak intensities under low and high Fe is 800,000-900,000. This does not necessarily mean these peaks do not represent a siderophore, as peak intensities are not

Table 6-3. Sum of all peak intensities for a given putative siderophore mass.

	Total Peak Intensity (mV)		
Mass	–Fe	low Fe	high Fe
169	175,223	823,986	901,318
348	55,482	36,894	53,336
398	377,611	973,962	417,389
403	60,029	1,815,349	3,751,841

entirely quantitative. It is possible that I am not including some peaks in the spectrum, such as sodium-adducts or other metal complexes. If the putative siderophore is bound to a metal other than Fe in the native sample we would not detect it at M+53+1; upon addition of Fe, the siderophore might preferentially bind iron and we would see a disproportionate increase in the Fe-bound peak. It is also possible that the iron added to the sample during analysis altered the response factor for the siderophore peaks or caused ion suppression or enhancement. But, the fact that peaks representing unbound and Fe-bound compounds change intensities appropriately with increasing Fe concentrations is enough at this stage to call these compounds putative siderophores.

Siderophore-producers are known to exist in biological soil crusts (BSCs; see Chapter 5), however, the majority of the siderophores produced by organisms in crusts are as yet, uncharacterized. The exceptions are the catecholates produced by *Bacillus* spp. that include hexadentate Bacillibactin (Figure 6-6b; Dertz et al., 2006) and an assortment of bidentate dihydroxybenzoic acids (Ito and Neilands, 1958; Peters and Warren, 1968; Temirov et al., 2003). *Bacillus* spp. have been found in BSCs (see Chapter 5; Gundlapally & Garcia-Pichel, 2006), but, unfortunately, catecholates do not behave as predictably as hydroxamate sideropheres in the ESI-MS, nor do they follow the simple Mass+53 rule, that hydroxamate siderophores bound to Fe exhibit (Jessica Martin,

personal communication). However, the masses of Bacillibactin (~850 amu) and the other catechols produced by *Bacillus* spp. (~210 amu) are not close to the putative siderophore masses I observed, and so I can safely rule them out. *Nostoc* spp. comprise 80-90% of the nitrogen fixing community in the crusts used in the current study (Yeager et al., 2004; 2007), and members of the *Nostoc* genus have been shown to make a hydroxamate siderophore that was preliminarily identified as schizokinen (Umamaheswari et al., 1997). However, schizokinen has a mass of ~420 amu. Therefore, I conclude that my putative siderophores are not a known compound produced by either *Bacillus* or *Nostoc* spp., and are, therefore, likely to be novel compounds.

The ESI results of culture media from *Nostoc punctiforme* grown under variable Fe concentrations (Experiment 2) demonstrated that compounds with masses 169 and 398 amu are most likely true siderophores. Siderophores are typically produced under Fe-limitation, so the less bioavailable Fe that exists, the higher siderophore production should be. This was the case for the compound with mass of 398 amu for which the cumulative intensity of siderophore peaks decreased significantly with increasing Fe concentration (p = 0.09; Figure 6-5, Table 6-2). Therefore, I conclude that the compound with a mass of 398 amu is an uncharacterized siderophore.

For the compound with a mass of 169 amu, peak intensities decreased from 5 μM Fe to 10 μM Fe as expected, but peak intensities were lower under 0 μM Fe than 5 μM Fe, contrary to my expectations. Visual observations of the 0 μM Fe cultures in Experiment 2 showed very low cell density, and in fact, neither chlorophyll nor optical density was measureable in aliquots from the 0 μM Fe cultures. In addition, *Nostoc* biomass, which is normally a deep green color, was yellow to brown in the 0 μM Fe bottles, indicating severe chlorosis, a loss of photosynthetic pigment that can occur under Fe-limitation (Rueter et al., 1990; Wilhelm & Trick, 1995). Lack of biomass and discoloration suggest that the bacteria were so Fe-starved they were struggling to survive, and therefore would have had few resources to dedicate to siderophore production. This was likely exacerbated by the fact that no bioavailable nitrogen source was added to the culture media (since *Nostoc* is a known nitrogen fixer). Therefore, Fe-limitation would have resulted in co-limitation with respect to nitrogen (Vitousek & Howarth, 1991; Berman-Frank et al., 2001; Mills et al., 2004; Zerkle et al., 2006), making it even more difficult for the cyanobacteria to fabricate siderophores. Biomass content in 5 μM Fe and 10 μM Fe bottles increased slightly with Fe concentration and discoloration of the biomass was less apparent with increasing Fe concentrations (data not shown), although chlorophyll and optical density were similarly unmeasureable in these bottles. Therefore, I conclude that

the compound with a mass of 169 amu is a siderophore, and I infer that the 0 μM Fe condition was so iron-limited as to cause cell death, 5 μM Fe was low enough to induce Fe-limitation and siderophore production, but high enough to permit culture survival, and that finally, under 10 μM Fe, there was enough Fe to inhibit siderophore production. In addition, it is important to note that soil crust isolates do not grow particularly well in liquid media because they are accustomed to growing on a solid substrate. Thus, it is possible that a different experimental design would better facilitate their growth at low Fe concentrations.

I did not see any evidence for siderophore production in the soil solutions of intact crusts. One explanation is that Fe was not limiting in the particular crusts we used, and so siderophores were not produced. Bacterial Fe requirements are typically in the micromolar range (Lankford, 1973), and average Fe concentrations in the soil solution of crusts ranged from below detection to 34 μM (see Chapter 3). Therefore, it is probable that some crusts experienced Fe-limitation, while others did not. It is also possible that we did not detect siderophores because they were simply below detection in the soil solutions. (Typical detection limits for DFAM were around 2 μM.) This could be because collection techniques diluted siderophores, or because there was not enough biomass in the crust to produce siderophores in sufficient quantities. Different collection methods, coupled with pre-concentration techniques, might maintain siderophores at

detectable concentrations and allow further investigation of siderophore production in actively metabolizing soil crusts. Another potential explanation for the lack of siderophore detection in crust extracts is the storage time of soil solutions between collection and ESI-MS analysis. Although every effort was made to preserve soil solution organic matter by adding methanol and storing solutions at -20ºC, siderophores may have degraded during storage. Finally, it is possible that our experiments were too short to detect siderophore production given the limitations of our collection techniques.

Summary and Implications

Overall, I know that siderophore-producers exist in BSCs (see Chapter 5), and of the four putative siderophores I identified with ESI-MS, two compounds are true, novel siderophores. The ability to utilize siderophore-bound metals requires that an organism possess receptors to recognize the siderophore. Siderophore recognition imparts an ecological advantage on organisms because it provides them with a source of a potentially limiting micronutrient. Many organisms are able to recognize and utilize siderophores produced by other species, thus decreasing the competitive advantage of the siderophore producer. Therefore, the likely evolutionary drive is to produce novel compounds for which one's competitors do not have receptors. The fact that the siderophores produced by crust

microbes are novel, speaks to the ecologically competitive nature of crusts that must survive in extreme environments.

Future directions for this work could include the identification of additional siderophores produced by other crust organisms and elucidation of siderophore structures. Once siderophores are purified, it would also be possible to investigate the unique metal-binding capabilities of each compound. It would be interesting to measure siderophore production by isolated crust microbes grown on a solid substrate, such as sterile quartz sand, in order to more effectively simulate the natural conditions of BSCs. And, finally, additional studies of field crusts would provide information about siderophore production *in situ*. Any future work with intact crusts should use pre-concentration techniques prior to analysis and perform ESI-MS analyses within hours to days of soil solution collection.

References

Akers, H. 1983. Multiple hydroxamic acid microbial iron chelators (siderophores) in soils. *Soil Science* 135, no. 3: 156-159.

Allen, M.M. and J. Stanier. 1968. Selective isolation of blue-green algae from water and soil. *Journal of General Microbiology* 51: 203-209.

Aparicio, P.J., D.B. Knaff, and R. Malkn. 1975. The role of iron-sulfur center and siroheme in spinach nitrite reductase. *Archives of Biochemistry and Biophysics* 169: 102-107.

Bellenger, J.P., F. Arnaud-Neu, Z. Asfari, S.C.B. Myneni, E.I. Stiefel, and A.M.L. Kraepiel. 2007. Complexation of oxoanions and cationic metals by the biscatecholate siderophore azotochelin. *Journal of Biological Inorganic Chemistry* 12: 367-376.

Bellenger, J.P., T. Wichard, A.B. Kustka, and A.M.L. Kraepiel. 2008. Uptake of molybdenum and vanadium by a nitrogen-fixing soil bacterium using siderophores. *Nature Geoscience* 1: 243-246.

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

Belnap, J. 1995. Surface disturbances: Their role in accelerating desertification. *Environmental Monitoring and Assessment* 37, no. 1-3: 39-57.

_____. 2002. Nitrogen fixation in biological soil crusts from southeast Utah, USA. *Biologicla Fertility of Soils* 35: 128-135.

Belnap, J. and D. Eldridge. 2001. Disturbance and recovery of biological soil crusts. In *Biological soil crusts: Structure, function, and management*, ed. J. Belnap and O.L. Lange. 503. Berlin: Springer.

Belnap, J., K.T. Harper, and S.D. Warren. 1994. Surface disturbance of cryptobiotic soil crusts: Nitrogenase activity, chlorophyll content, and chlorophyll degradation. *Arid Soil Research and Rehabilitation* 8: 1-8.

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, no. 3: 348-359.

Berman-Frank, I., J.T. Cullen, Y. Shaked, R.M. Sherrell, and P.G. Falkowski. 2001. Iron availability, cellular iron quotas, and nitrogen fixation in *Trichodesmium*. *Limnology and Oceanography* 46, no. 6: 1249-1260.

Bernroitner, M., M. Zamocky, M. Pairer, P.G. Furtmüller, G.A. Peschek, and C. Obinger. 2008. Heme-copper oxidases and their electron donors in cyanobacterial respiratory electron transport. *Chemistry and Biodiversity* 5, no. 1927-1961.

Bevington, P. and D.K. Robinson. 2002. *Data reduction and error analysis for the physical sciences*. New York: The McGraw Hill Companies, Inc.

Beymer, R.J. and J.M. Klopatek. 1991. Potential contribution of carbon by microphytic crusts in pinyon-juniper woodlands. *Arid Soil Research and Rehabilitation* 5: 187-198.

Bothe, H., O. Schmitz, M.G. Yates, and W.E. Newton. 2010. Nitrogen fixation and hydrogen metabolism in cyanobacteria. *Microbiology and Molecular Biology Reviews* 74, no. 4: 529-551.

Bowker, M.A., J. Belnap, D.W. Davidson, and S.L. Phillips. 2005. Evidence for micronutrient limitation of biological soil crusts: Importance to arid-lands restoration. *Ecological Applications* 15, no. 6: 1941-1951.

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

Bowker, M.A., M.E. Miller, J. Belnap, T.D. Sisk, and N.C. Johnson. 2008c. Prioritizing conservation effort through the use of biological soil crusts as ecosystem function indicators in an arid region. *Conservation Biology* 22, no. 6: 1533-1543.

Brady, N.C. and R.R. Weil. 2002. *The nature and properties of soils*. Upper Saddle River: Prentice Hall.

Burgess, B.K. and D.J. Lowe. 1996. Mechanism of molybdenum nitrogenase. *Chemical Reviews* 96: 2983-3011.

Campbell, J.H., J.S. Clark, and J.C. Zak. 2009. PCR-DGGE comparison of bacterial community structure in fresh and archived soils sampled along a Chihuahuan desert elevational gradient. *Microbial Ecology* 57: 261-266.

Campbell, S.E. 1979. Soil stabilization by a prokaryotic desert crust: Implications for precambrian land biota. *Origins of Life* 9: 335-348.

Dertz, E.A., A. Stintzi, and K.N. Raymond. 2006. Siderophore-mediated iron transport in *Bacillus subtilis* and *Corynebacterium glutamicum*. *Journal of Biological Inorganic Chemistry* 11: 1087-1097.

Essén, S.A., D. Bylund, S.J.M. Holmstrom, M. Moberg, and U.S. Lundstrom. 2006. Quantification of hydroxamate siderophores in soil solutions of podzolic soil profiles in Sweden. *BioMetals* 19: 269-282.

Evans, R.D. and O.L. Lange. 2001. Biological soil crusts and ecosystem nitrogen and carbon dynamics. In *Biological soil crusts: Structure, function, and management*, ed. J. Belnap and O.L. Lange. 263-279. Berlin: Springer-Verlag.

Ferguson-Miller, S., G.T. Babcock, and C. Yocum. 2007. Photosynthesis and respiration. In *Biological inorganic chemistry: Structure and reactivity*, ed. I. Bertini, H.B. Gray, E.I. Stiefel and J.S. Valentine. Sausalito, California: University Science Books.

Garcia-Pichel, F. 2002. Desert environments: Biological soil crusts. In *Encyclopedia of environmental microbiology*, ed. G. Bitton:1019-1023. New York: John Wiley.

Garcia-Pichel, F. and J. Belnap. 1996. Microenvironments and microscale productivity of cyanobacterial desert crusts. *Journal of Phycology* 32: 774-782.

Garcia-Pichel, F., S.L. Johnson, D. Youngkin, and J. Belnap. 2003. Small-scale vertical distribution of bacterial biomass and diversity in biological soil crusts from arid lands in the Colorado Plateau. *Microbial Ecology* 46: 312-321.

Garcia-Pichel, F., A. Lopez-Cortes, and U. Nubel. 2001. Phylogenetic and morphological diversity of cyanobacteria in soil desert crusts from the Colorado Plateau. *Applied and Environmental Microbiology* 67, no. 4: 1902-1910.

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, no. 11: e7801.

Gledhill, M. 2001. Electrospray ionisation-mass spectrometry of hydroxamate siderophores. *Analyst* 126: 1359-1362.

Guerrero, M.G., J.M. Vega, and M. Losada. 1981. The assimilatory nitrate-reducing system and its regulation. *Annual Review in Plant Physiology* 32: 169-204.

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.

Holmström, S.J.M., U.S. Lundstrom, R.D. Finlay, and P.A.W. Van Hees. 2004. Siderophores in forest soil solution. *Biogeochemistry* 71: 247-258.

Howard, J.B. and D.C. Rees. 1996. Structural basis of biological nitrogen fixation. *Chemical Reviews* 96: 2965-2982.

Isichei, A.O. 1990. The role of algae and cyanobacteria in arid lands. A review. *Arid Soil Research and Rehabilitation* 4: 1-17.

Ito, T. and J.B. Neilands. 1958. Products of "Low-iron fermentation" with *Bacillus subtilis*: Isolation, characterization and synthesis of 2,3-dihydroxybenzoylglycine. *Journal of the American Chemical Society* 80, no. 17: 4645-4647.

Jeffries, D.L., J.M. Klopatek, S.O. Link, and J. Bolton. 1992. Acetylene reduction by cryptogamic crusts from a blackbrush community as related to resaturation and dehydration. *Soil Biology and Biochemistry* 24: 1101-1105.

Kalinowski, B.E., L.J. Liermann, S. Givens, and S.L. Brantley. 2000. Rates of bacteria-promoted solubilization of Fe from minerals: A review of problems and approaches. *Chemical Geology* 169: 357-370.

Kim, H.J., D.W. Graham, A.A. DiSpirito, M.A. Alterman, N. Galeva, C.K. Larive, D. Asunskis, and P.M.A. Sherwood. 2004. Methanobactin, a copper-acquisition compound from methane-oxidizing bacteria. *Science* 305, no. 5690: 1612-1615.

Kiss, T. and E. Farkas. 1998. Metal-binding ability of desferrioxamine b. *Journal of Inclusion Phenomena and Molecular Recognition in Chemistry* 32: 385-403.

Kraemer, S.M. 2004. Iron oxide dissolution and solubility in the presence of siderophores. *Aquatic Science* 66: 3-18.

Lancaster, J.R., J.M. Vega, H. Kamin, N.R. Orme-Johnson, W.H. Orme-Johnson, R.J. Krueger, and L.M. Siegel. 1979. Identification of the iron-sulfur center of spinach ferredoxin-nitrite reductase as a tetranuclear center, and preliminary EPR studies of mechanism. *The Journal of Biological Chemistry* 254, no. 4: 1268-1272.

Lankford, C.E. 1973. Bacterial assimilation of iron. *CRC Critical Reviews in Microbiology* 2: 273-331.

Liermann, L.J., R.L. Guynn, A. Anbar, and S.L. Brantley. 2005. Production of a molybdophore during metal-targeted dissolution of silicates by soil bacteria. *Chemical Geology* 220: 285-302.

Liermann, L.J., B.E. Kalinowski, S.L. Brantley, and J.G. Ferrey. 2000. Role of bacterial siderophores in dissolution of hornblende. *Geochimica et Cosmochimica Acta* 64, no. 4: 587-602.

Litos, C., A. Terzis, C. Raptopoulou, A. Rontoyianni, and A. Karaliota. 2006. Polynuclear oxomolybdenum(VI) complexes of dihydroxybenzoic acids: Synthesis, spectroscopic and structure characterization of a tetranuclear catecholato-type coordinated 2,3-dihydroxybenzoate and a novel tridentate salicylato-type coordinated 2,5-dihydroxybenzoate trinuclear complex. *Polyhedron* 25: 1337-1347.

Lockau, W. 1981. Evidence for a dual role of cytochrome c-553 and plastocyanin in photosynthesis and respiration of the cyanobacterium, *anabaena variabilis*. *Archives of Microbiology* 128, no. 3: 336-340.

Martinez, J.S., M.G. Haygood, and A. Butler. 2001. Identification of a natural desferrioxamine siderophore produced by a marine bacterium. *Limnology and Oceanography* 46, no. 2: 420-424.

McCormack, P., P.J. Worsfold, and M. Gledhill. 2003. Separation and detection of siderophores produced by marine bacterioplankton using high-performance liquid chromatography with electrospray ionization mass spectrometry. *Analytical Chemistry* 75: 2647-2652.

McKnight, D.M. and F.M.M. Morel. 1980. Copper complexation by siderophores from filamentous blue-green algae. *Limnology and Oceanography* 25, no. 1: 62-71.

Mills, M.M., C. Ridame, M. Davey, J. La Roche, and R.J. Geider. 2004. Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. *Nature* 429: 292-294.

Monteiro, B., L. Cunha-Silva, S. Gago, J. Klinowski, F.A.A. Paz, J. Rocha, I.S. Gonçalves, and M. Pillinger. 2010. Crystal and supramolecular structures of dioxomolybdenum(VI) and dioxotungsten(VI) complexes of dihydroxybenzoic acids. *Polyhedron* 29: 719-730.

Murphy, M.J., L.M. Siegel, S.R. Tove, and H. Kamin. 1974. A new prosthetic group participating in six-electron reduction reactions catalyzed by both sulfite and nitrite reductases. *Proceedings of the National Academy of Sciences of the United States of America* 71, no. 3: 612-616.

Neilands, J.B. 1973. Microbial iron transport compounds (siderochromes). In *Inorganic biochemistry*, ed. J.B. Neilands, 1:2-34. New York: Academic Press.

_____. 1981. Microbial iron compounds. *Annual Review of Biochemistry* 50: 715-731.

Peschek, G.A., C. Obinger, and M. Paumann. 2004. The respiratory chain of blue-green algae (cyanobacteria). *Physiologia Plantarum* 120: 358-369.

Peters, W.J. and R.A.J. Warren. 1968. Itoic acid synthesis in *Bacillus subtilis*. *Journal of Bacteriology* 95, no. 2: 360-366.

Powell, P.E., G.R. Cline, C.P.P. Reid, and P.J. Szaniszlo. 1980. Occurrence of hydroxamate siderophore iron chelators in soils. *Nature* 287: 833-834.

Raven, J.A., M.C.W. Evans, and R.E. Korb. 1999. The role of trace metals in photosynthetic electron transport in O2-evolving organisms. *Photosynthesis Research* 60: 111-149.

Ross, A.R.S., M.G. Ikonomou, and K.J. Orians. 2003. Characterization of copper-complexing ligands in seawater using immobilized copper(II)-ion affinity chromatography and electrospray ionization mass spectrometry. *Marine Chemistry* 83: 47-58.

Rossi, F., R. Potrafka, F. Garcia-Pichel, and R. De Philippis. 2012. Role of the exo-polysaccharides in enhancing hydraulic conductivity of biological soil crusts. *Soil Biology and Biochemistry* 46: 33-40.

Rubio, L.M., E. Flores, and A. Herrero. 1999. Molybdopterin guanine dinucleotide cofactor in *Synechococcus* sp. Nitrate reductase: Identification of *mobA* and isolation of a putative *moeB* gene. *FEBS Letters* 462: 358-362.

_____. 2002. Purification, cofactor analysis, and site-directed mutagenesis of *Synechococcus* ferredoxin-nitrate reductase. *Photosynthesis Research* 72: 13-26.

Rueter, J.G., K. Ohki, and Y. Fujita. 1990. The effect of iron nutrition on photosynthesis and nitrogen fixation in cultures of *Trichodesmium* (cyanophyceae). *Journal of Phycology* 26: 30-35.

Rychert, R.C. and J. Skujins. 1974. Nitrogen fixation by blue-green algae-lichen crust in the great basin desert. *Soil Science Society of America Proceedings* 38: 768-771.

Saal, L.B. and O.W. Duckworth. 2010. Synergistic dissolution of manganese oxides as promoted by siderophores and small organic acids. *Soil Science Society of America Journal* 74, no. 6: 2021-2031.

Schwyn, B. and J.B. Neilands. 1987. Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry* 160: 47-56.

Smith, K.S. and J.G. Ferry. 2000. Prokaryotic carbonic anhydrase. *FEMS Microbiology Reviews* 24: 335-366.

Steppe, T.F., J.B. Olson, H.W. Paerl, R.W. Litaker, and J. Belnap. 1996. Consortia N_2 fixation: A strategy for meeting nitrogen requirements of marine and terrestrial cyanobacterial mats. *FEMS Microbiology Ecology* 21: 149-156.

Strauss, S.L., T.A. Day, and F. Garcia-Pichel. 2012. Nitrogen cycling in desert biological soil crusts across biogeographic regions in the southwestern United States. *Biogeochemistry* 108: 171-182.

Szabó, O. and E. Farkas. 2011. Characterization of Mn(II) and Mn(III) binding capability of natural siderophores desferrioxamine b and desferricoprogen as well as model hydroxamic acids. *Inorganic Chimica Acta* 376: 500-508.

Temirov, Y.V., T.Z. Esikova, I.A. Kashparov, T.A. Balashova, L.M. Vinokurov, and Y.B. Alakhov. 2003. A catecholic siderophore produced by the thermoresistant *Bacillus licheniformis* vk21 strain. *Russian Journal of Bioorganic Chemistry* 29, no. 6: 542-549.

Umamaheswari, A., D.R. Madhavi, and K. Venkateswarlu. 1997. Siderophore production in two species of *Nostoc* as influenced by the toxicity of nitrophenolics. *Bulletin of Environmental Contamination and Toxicology* 59: 306-312.

Umena, Y., K. Kawakami, J.-R. Shen, and N. Kamiya. 2011. Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. *Nature* 473: 55-61.

Vitousek, P.M. and R.W. Howarth. 1991. Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry* 13: 87-115.

Wilhelm, S.W. and C.G. Trick. 1995. Physiological profiles of *synechococcus* (cyanophyceae) in iron-limiting continous cultures. *Journal of Phycology* 31: 79-85.

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, no. 2: 973-983.

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 N_2-fixing members of biological soil crusts of the Colorado Plateau, USA. *FEMS Microbiology Ecology* 60: 85-97.

Zerkle, A.L., C.H. House, R.P. Cox, and D.E. Canfield. 2006. Metal limitation of cyanobacterial N_2 fixation and implications for the precambrian nitrogen cycle. *Geobiology* 4: 285-297.

CHAPTER 7

CONCLUSIONS, IMPLICATIONS, AND FUTURE WORK

Prior to my work, very little was known about the geochemical effects of biological soils crusts (BSCs) on the soil solid-phase. Several studies investigated the correlation between crust cover and element distributions (Rogers, 1972; Bowker et al., 2005; Beraldi-Campesi et al., 2009), and a couple looked at the influence of element addition on crust physiology (Bowker et al., 2008b; Hartley & Schlesinger, 2002). But in general, there has been very little work explicitly linking the geochemical environment to the physiology or ecology of biological soil crusts. My work is the first to monitor the real-time effects of crusts on soil element mobility, and to demonstrate the mechanisms by which crusts actively alter soil solution element concentrations. My results provide the first evidence for siderophore production in crusts, and therefore, are also the first report of the ecological roles of siderophore producers and the types of siderophores found in BSCs.

The goal of my initial experiments was to determine whether or not BSCs actively influence soil solution element concentrations, and if changes in soil solution concentrations could provide insight into active biological processes (see Chapters 2 & 3). Using simulated rainfall experiments and N_2 fixation as a measure of metabolism, I was able to assess changes in soil solution concentrations of living crusts under

different metabolic conditions. Several of my experimental studies showed that multiple bioessential elements have lower soil solution concentrations in crusted soils versus killed controls, which reflects overall uptake of elements by crusts that are released into the soil solution when the microbes are killed. The analysis of concentration changes in the soil solution of live crusts revealed that some elements showed evidence of microbial uptake, while others showed evidence of microbial solubilization. In particular, I observed microbial uptake of nickel and zinc (Chapter 3), microbial solubilization of molybdenum (Chapter 2) and copper (Chapter 3), and both uptake and solubilization of potassium and manganese (Chapter 3). My results prove that BSCs actively influence soil solution concentrations of bioessential elements and support the original findings of Beraldi-Campesi et al. (2009) who postulated that microbial solubilization would leave elements susceptible to leaching down the soil profile, which over time, would result in element depletions in the soil solid-phase.

Next, I wanted to know if crusts were limited with respect to molybdenum (Mo), which is required in large quantities for nitrogen (N_2) fixation. I found that Mo addition significantly increased N_2 fixation rates; a clear indication that N_2 fixation in crusts is limited by Mo (see Chapter 4). This is interesting because Mo was solubilized in some crusts (Chapter 2). Together, the evidence for Mo solubilization coupled with Mo limitation of

crust N_2 fixation, exhibit the link between element need for microbial physiology, and microbial element acquisition strategies. Molybdenum is required by crusts in high amounts, and yet the availability of Mo limits N_2 fixation. Therefore, crusts must actively solubilize solid-phase Mo to meet the demands of N_2 fixation.

Given that crusts actively alter soil solution element concentrations, and that they do so to combat metal limitation for critical physiological processes, such as N_2 fixation, I was interested in the strategies crusts use to obtain bioessential elements, like Mo. I hypothesized that crusts produce siderophores to both increase the solubility of elements and to facilitate element uptake. Siderophores are low molecular weight organic molecules that bind iron and other elements with high affinity. Siderophore production is a common strategy used by soil microbes to obtain bioessential elements, however, it had never been investigated in BSCs. I confirmed that multiple organisms produce siderophores in crusts, thus proving for the first time that siderophore production is one mechanism by which crusts obtain bioessential elements (see Chapter 5). I also found that siderophore producers belong not only to dominant microbial groups, such as the cyanobacteria and Alphaproteobacteria,but also to less dominant groups like the Betaproteobacteria and Firmicutes. Cyanobacteria are the primary N_2 fixing organisms in crusts, and they are also the major crust builders, so it is expected that cyanobacteria would

produce siderophores. Butsiderophore production by the less dominant crust members suggests that the heterotrophic bacteria may play a critical role in element cycling within crusts. Half of the siderophore producers I isolated did not closely match any published sequences, indicating that they are likely novel organisms and that the siderophores they produce may be unique as well. The organic chemistry that isolated siderophores coupled with the molecular techniques that identified the siderophore producing microbes revealed that organisms previously thought to be minor players in crust ecology, may play important roles by increasing metal availability. The ecological structure of siderophore producing microbes in the crust system may not have been discovered by biological studies alone.

Finally, I began work to characterize the siderophore compounds themselves. I focused my efforts on the isolate that was most closely related to *Nostoc punctiforme*, because it is among the dominant N_2 fixing organisms in the crust, and although siderophore production has been suggested for *Nostoc* spp., it has not been confirmed. Using mass spectrometry techniques, I identified four putative siderophores, and determined that two of these compounds are likely to be true siderophores with molecular weights of 169 and 398 amu, respecitively (see Chapter 6). These molecular weights do not match the molecular weight of

schizokinen, the siderophore supposedly produced by *Nostoc* spp., therefore I conclude these are novel compounds.

Together the results of my work demonstrate that crust microbes directly influence soil element mobility through the production of siderophores. I observed soil solution concentration changes that were consistent with microbial uptake and microbial solubilization. I found that 50% of the siderophore producers I isolated are novel organisms, and evidence suggests that the siderophore compounds they produce are unique as well.

Trace metal evidence for ancient crust communities is likely to be better preserved in the rock record than organic molecules. Beraldi-Campesi et al. (2009) suggested that soils depleted in manganese (Mn), copper (Cu), and zinc (Zn) may represent biosignatures of ancient BSC communities. They proposed that microbial solubilization left these elements vulnerable to leaching losses. My work supports their suggestion, as I observed real-time increases in Mn, Cu, and Zn soil solution concentrations that are evidence of microbial solubilization. Combined with morphological evidence for crusts, element depletions may represent geochemical signatures of biological activity that could be used to effectively study the existence of crusts on ancient Earth, as well as on an older, wetter Mars. That crusts produce siderophores may provide yet another tool for studying ancient crusts, as the presence of organic ligands

leaves a characteristic weathering pattern in minerals (Neaman et al., 2005a, b). More work is required to determine whether certain element depletions are universal among crusts, and to see how element depletions depend on rock-type. However, my results confirm that geochemical biosignatures will be valuable tools for the study of ancient crusts.

Crusts are critical members of arid ecosystems, and increasing crust cover can go a long way to preserving the fertility of arid lands. Bowker et al. (2005) proposed that crust fertilization may enhance crust growth and development, and the results of my work provides insight for crust conservation and management personnel as to which elements may be effective fertilizers. Microbial uptake and solubilization are both evidence that a given metal is required by BSCs. If crusts need a very small proportion of the available soil solution element pool, I would not have been able to see biologically-mediated concentrations changes. Because I observed significant concentration changes for some elements, I infer that a large proportion of the available soil solution element pool of these elements is needed by crusts. If crusts require a large proportion of the available element pool, that element may become limiting, especially since there is little input of fresh material, except that brought in by dust (Reynolds et al., 2001; 2006). Therefore, those elements that showed concentration changes consistent with uptake or solubilization are likely to be or to become limiting to crusts, and are good candidates for crust

fertilization. Before application of a fertilizer is considered for a given location, experiments would need to be conducted to determine which elements show signs of uptake or solubilization at that particular site. Once the potentially limiting elements are identified, experiments would be needed to verify that addition of an element enhances the rate of a particular physiological process relevant to BSC growth. For example, does Mn addition increase rates of photosynthesis? It would also be necessary to optimize the amount of element added, as many elements may serve as fertilizers up to a certain concentration, at which point they become toxic. Finally, other factors such as light and water availability must be considered as contributors to crust growth.

There are several sets of experiments that could provide additional information about siderophore production in BSCs. First, it would be worthwhile to conduct experiments such as those described in Chapter 6, for additional organisms. After the molecular weights of the siderophores are determined and compared to the weights of known compounds, further structural characterization would be needed for the novel compounds. This work would involve nuclear magnetic resonance (NMR) studies and collision experiments using electrospray ionization mass spectrometry (ESI-MS). Once compounds are purified, it would be interesting to determine which metals each compound is capable of binding. Since many of the siderophores produced in BSCs are likely

novel, they may have unique metal-binding abilities that are tailored to the specific metal needs of crust microbes. Lastly, studies of siderophore production in live, intact crusts would shed light on which organisms produce siderophore *in situ*, and provide a more detailed understanding of metal cycling in complex BSC communities.

Overall, my research was the first to show evidence that BSCs actively influence soil element mobility and that the effects of BSCs on elements is directly linked to their physiological requirements. My results are applicable to the understanding of crust biosignatures that could be used to study the existence and evolution of ancient crust communities, as well as to the development of crust conservation and management techniques that could be used to prevent arid land degradation and enhance arid land fertility. My work also provides insight into the types of studies that could enhance our understanding of element cycling within BSCs, which will further our ability to comprehend the past and future roles of crusts in arid ecosystems.

REFERENCES

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, no. 3: 348-359.

Bowker, M.A., J. Belnap, D.W. Davidson, and S.L. Phillips. 2005. Evidence for micronutrient limitation of biological soil crusts: Importance to arid-lands restoration. *Ecological Applications* 15, no. 6: 1941-1951.

Bowker, M.A., G.W. Koch, J. Belnap, and N.C. Johnson. 2008b. Nutrient availability affects pigment production but not growth in lichens of biological soil crusts. *Soil Biology and Biochemistry* 40: 2819-2826.

Hartley, A.E. and W.H. Schlesinger. 2002. Potential environmental controls on nitrogenase activity in biological crusts of the northern chihuahuan desert. *Journal of Arid Environments* 52: 293-304.

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 Science of the United States of America* 98, no. 13: 7123-7127.

Reynolds, R., J. Neff, M. Reheis, and P. Lamothe. 2006. Atmospheric dust in modern soil on aeolian sandstone, colorado plateau (USA): Variation with landscape position and contribution to potential plant nutrients. *Geoderma* 130: 108-123.

Rogers, R.W. 1972. Soil surface lichens in arid and subarid south-eastern australia. *Australian Journal of Botany* 20: 301-316.

COLLECTED REFERENCES

Acea, M.J. and T. Carballas. 1999. Microbial fluctuations after soil heating and organic amendment. *Bioresource Technology* 67: 65-71.

Adriano, D.C. 2001. *Trace elements in terrestrial environments: Biogeochemistry, bioavailability, and risks of metals*. New York: Springer-Verlag.

Aja, S.U., S.A. Wood, and A.E. Williams-Jones. 1995. The aqueous geochemistry of Zr and the solubility of some Zr-bearing minerals. *Applied Geochemistry* 10: 603-620.

Akers, H. 1983. Multiple hydroxamic acid microbial iron chelators (siderophores) in soils. *Soil Science* 135, no. 3: 156-159.

Allen, M.M. and J. Stanier. 1968. Selective isolation of blue-green algae from water and soil. *Journal of General Microbiology* 51: 203-209.

Alloway, B.J. 1995. *Heavy metals in soils.* London, UK: Blackie Academic & Professional.

Anthony, C., M. Ghosh, and C. Blake. 1994. The structure and function of methanol dehydrogenase and related quinoproteins containing pyrrolo-quinoline quinone. *Biochemical Journal* 304: 665-674.

Aparicio, P.J., D.B. Knaff, and R. Malkn. 1975. The role of iron-sulfur center and siroheme in spinach nitrite reductase. *Archives of Biochemistry and Biophysics* 169: 102-107.

Arnalds, O. and S. Archer. 2000. Introduction. In *Rangeland desertification*, ed. O. Arnalds and S. Archer. 1-4. Dordrecht: Kluwer Academic Publishers. 209.

Ashcroft, G.L., D.T. Jensen, and J.L. Brown. 1992. *Utah climate*. Logan, UT: Utah State University.

Barron, A.R., N. Wurzburger, J.P. Bellenger, S.J. Wright, A.M.L. Kraepiel, and L.O. Hedin. 2009. Molybdenum limitation of asymbiotic nitrogen fixation in tropical forest soils. *Nature Geoscience* 2: 42-45.

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, no. 1: 56-67.

Bellenger, J.P., F. Arnaud-Neu, Z. Asfari, S.C.B. Myneni, E.I. Stiefel, and A.M.L. Kraepiel. 2007. Complexation of oxoanions and cationic metals by the biscatecholate siderophore azotochelin. *Journal of Biological Inorganic Chemistry* 12: 367-376.

Bellenger, J.P., T. Wichard, and A.M.L. Kraepiel. 2008. Vanadium requirements and uptake kinetics in the dinitrogen-fixing bacterium *Azotobacter vinelandii*. *Applied and Environmental Microbiology* 74, no. 5: 1478-1484.

Bellenger, J.P., T. Wichard, A.B. Kustka, and A.M.L. Kraepiel. 2008. Uptake of molybdenum and vanadium by a nitrogen-fixing soil bacterium using siderophores. *Nature Geoscience* 1: 243-246.

Bellenger, J.P., T. Wichard, Y. Xu, and A.M.L. Kraepiel. 2011. Essential metals for nitrogen fixation in a free-living N_2-fixing bacterium: Chelation, homeostasis and high use efficiency. *Environmental Microbiology* 13, no. 6: 1395-1411.

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

_____. 1995. Surface disturbances: Their role in accelerating desertification. *Environmental Monitoring and Assessment* 37, no. 1-3: 39-57.

_____. 2001. Factors influencing nitrogen fixation and nitrogen release in biological soil crusts. In *Biological soil crusts: Structure, function, and management*, ed. J. Belnap and O.L. Lange. 241-261. Berlin Heidelberg New York: Springer.

_____. 2002. Nitrogen fixation in biological soil crusts from southeast Utah, USA. *Biological Fertility of Soils* 35: 128-135.

Belnap, J., B. Budel, and O. L. Lange. 2001. Biological soil crusts: Characteristics and distribution. In *Biological soil crusts: Structure, function, and management*, ed. J. Belnap and O. L. Lange. 503. Berlin: Springer.

Belnap, J. and D. Eldridge. 2001. Disturbance and recovery of biological soil crusts. In *Biological soil crusts: Structure, function, and management*, ed. J. Belnap and O.L. Lange. 503. Berlin: Springer.

Belnap, J. and J.S. Gardner. 1993. Soil microstructure in soils of the colorado plateau: The role of the cyanobacterium *Microcoleus vaginatus*. *Great Basin Naturalist* 53: 40-47.

Belnap, J., K.T. Harper, and S.D. Warren. 1994. Surface disturbance of cryptobiotic soil crusts: Nitrogenase activity, chlorophyll content, and chlorophyll degradation. *Arid Soil Research and Rehabilitation* 8: 1-8.

Belnap, J., J.H. Kaltenecker, R. Rosentreter, J. Williams, S. Leonard, and D. Eldridge. 2001. Biological soil crusts: Ecology and management, ed. United States Department of the Interior Bureau of Land Management, Technical Reference 1730-2.

Beneduzi, A., P.B. Costa, M. Parma, I.S. Melo, M.H. Bodanese-Zanettini, and L.M.P. Passaglia. 2010. *Paenibacillus riograndensis* sp. Nov., a nitrogen-fixing species isolated from the rhizosphere of *triticum aestivum*. *International Journal of Systematic and Evolutionary Microbiology* 60: 128-133.

Beneduzi, A., D. Peres, L.K. Vargas, M.H. Bodanese-Zanettini, and L.M.P. Passaglia. 2008. Evaluation of genetic diversity and plant growth-promoting activities of nitrogen-fixing bacilli isolated from rice fields of south brazil. *Applied Soil Ecology* 39: 311-320.

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, no. 3: 348-359.

Berman-Frank, I., J.T. Cullen, Y. Shaked, R.M. Sherrell, and P.G. Falkowski. 2001. Iron availability, cellular iron quotas, and nitrogen fixation in *Trichodesmium*. *Limnology and Oceanography* 46, no. 6: 1249-1260.

Berman-Frank, I., P. Lundgren, and P.G. Falkowski. 2003. Nitrogen fixation and photosynthetic oxygen evolution in cyanobacteria. *Research in Microbiology* 154: 157-164.

Berman-Frank, I., A. Quigg, Z.V. Finkel, A.J. Irwin, and L. Haramaty. 2007. Nitrogen-fixation strategies and Fe requirements in cyanobacteria. *Limnology and Oceanography* 52, no. 5: 2260-2269.

Bernroitner, M., M. Zamocky, M. Pairer, P.G. Furtmüller, G.A. Peschek, and C. Obinger. 2008. Heme-copper oxidases and their electron donors in cyanobacterial respiratory electron transport. *Chemistry and Biodiversity* 5, no. 1927-1961.

Bertini, I., H.B. Gray, E.I Stiefel, and J.S. Valentine. 2007. *Biological inorganic chemistry: Structure and reactivity*. Edited by I. Bertini, H.B. Gray, E.I. Stiefel and J.S. Valentine. Sausalito, California: University Science Books.

Bevington, P. and D.K. Robinson. 2002. *Data reduction and error analysis for the physical sciences*. New York: The McGraw Hill Companies, Inc.

Beymer, R.J. and J.M. Klopatek. 1991. Potential contribution of carbon by microphytic crusts in pinyon-juniper woodlands. *Arid Soil Research and Rehabilitation* 5: 187-198.

Blindauer, C.A. 2008. Zinc-handling in cyanobacteria: An update. *Chemistry and Biodiversity* 5: 1990-2013.

Blindauer, C.A., M.D. Harrison, A.K. Robinson, J.A. Parkinson, P.W. Bowness, P.J. Sadler, and N.J. Robinsong. 2002. Multiple bacteria encode metallothioneins and SMTA-like zinc fingers. *Molecular Microbiology* 45, no. 5: 1421-1432.

Boison, G., C. Steingen, L.J. Stal, and H. Bothe. 2006. The rice field cyanobacteria *Anabaena azotica* and *Anabaena* sp. Ch1 express vanadium-dependent nitrogenase. *Archives of Microbiology* 186: 367-376.

Booth, I.R. 1985. Regulation of cytoplasmic pH in bacteria. *Microbiological Reviews* 49, no. 4: 359-378.

Booth, I.R., T.A. Krulwich, E. Padan, J.B. Stock, G.M. Cook, V. Skulachev, G.N. Bennett, W. Epstein, J.L. Slonczewski, R.J. Rowbury, A. Martin, J.W. Foster, R.K. Poole, W.N. Konings, G. Schafer, and P. Dimroth. 1999. *The regulation of intracellular ph in bacteria*. ed. D. Chadwick and G. Cardew. Novartis foundation symposium. Chichester: John Wiley & Sons Ltd.

Bothe, H., O. Schmitz, M.G. Yates, and W.E. Newton. 2010. Nitrogen fixation and hydrogen metabolism in cyanobacteria. *Microbiology and Molecular Biology Reviews* 74, no. 4: 529-551.

Bowker, M.A. 2007. Biological soil crust rehabilitation in theory and practice: An underexploited opportunity. *Restoration Ecology* 15, no. 1: 13-23.

Bowker, M.A. and J. Belnap. 2008. A simple classification of soil types as habitats of biological soil crusts on the Colorado Plateau, USA. *Journal of Vegetation Science* 19, no. 6: 831-840.

Bowker, M.A., J. Belnap, V.B. Chaudhary, and N.C. Johnson. 2008a. 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, D.W. Davidson, and S.L. Phillips. 2005. Evidence for micronutrient limitation of biological soil crusts: Importance to arid-lands restoration. *Ecological Applications* 15, no. 6: 1941-1951.

Bowker, M.A., J. Belnap, and M.E. Miller. 2006. Spatial modeling of biological soil crusts to support rangeland assessment and monitoring. *Rangeland Ecology and Management* 59: 519-529.

Bowker, M.A., G.W. Koch, J. Belnap, and N.C. Johnson. 2008b. Nutrient availability affects pigment production but not growth in lichens of biological soil crusts. *Soil Biology and Biochemistry* 40: 2819-2826.

Bowker, M.A., M.E. Miller, J. Belnap, T.D. Sisk, and N.C. Johnson. 2008c. Prioritizing conservation effort through the use of biological soil crusts as ecosystem function indicators in an arid region. *Conservation Biology* 22, no. 6: 1533-1543.

Bowker, M.A., S.C. Reed, J. Belnap, and S.L. Phillips. 2002. Temporal variation in community composition, pigmentation, and Fv/Fm of desert cyanobacterial soil crusts. *Microbial Ecology* 43: 13-25.

Brady, N.C. and R.R. Weil. 2002. *The nature and properties of soils*. Upper Saddle River: Prentice Hall.

Burgess, B.K. and D.J. Lowe. 1996. Mechanism of molybdenum nitrogenase. *Chemical Reviews* 96: 2983-3011.

Butler, A. 1998. Vanadium haloperoxidases. *Current Opinion in Chemical Biology* 2: 279-285.

Butler, C.S. and D.J. Richardson. 2005. The emerging molecular structure of the nitrogen cycle: An introduction to the proceedings of the 10th annual N-cycle meeting. *Biochemical Society Transactions* 33, no. 1: 113-118.

Buttars, S.M., L.L. St. Clair, J.R. Johansen, J.C. Sray, M.C. Payne, B.L. Webb, R.E. Terry, B.K. Pendleton, and S.D. Warren. 1998. Pelletized cyanobacterial soil amendments: Laboratory testing for survival, escapability, and nitrogen fixation. *Arid Soil Research and Rehabilitation* 12, no. 2: 165-178.

Byers, J.E., K. Cuddington, C.G. Jones, T.S. Talley, A. Hastings, J.G. Lambrinos, J.A. Crooks, and W.G. Wilson. 2006. Using ecosystem engineers to restore ecological systems. *Trends in Ecology and Evolution* 21, no. 9: 493-500.

Campbell, J.H., J.S. Clark, and J.C. Zak. 2009. PCR-DGGE comparison of bacterial community structure in fresh and archived soils sampled along a Chihuahuan desert elevational gradient. *Microbial Ecology* 57: 261-266.

Campbell, S.E. 1979. Soil stabilization by a prokaryotic desert crust: Implications for precambrian land biota. *Origins of Life* 9: 335-348.

Capone, D.G. 1993. Determination of nitrogenase activity in aquatic samples using the acetylene reduction procedure. In *Handbook of methods in aquatic microbial ecology*, ed. P.F. Kemp, B.F. Sherr, E.B. Sherr and J.J. Cole: 621-631. New York: CRC Press LLC.

Cejudo, F.J. and A. Paneque. 1986. Short-term nitrate (nitrite) inhibition of nitrogen fixation in *azotobacter chroococcum*. *Journal of Bacteriology* 165, no. 1: 240-243.

Chakraborty, P., P.V.R. Babu, T. Acharyya, and D. Bandyopadhyay. 2010. Stress and toxicity of biologically important transition metals (Co, Ni, Cu and Zn) on phytoplankton in a tropical freshwater system: An investigation with pigment analysis by HPLC. *Chemosphere* 80: 548-553.

Chowdhury, M.R.I. and C.N. Mulligan. 2011. Biosorption of arsenic from contaminated water by anaerobic biomass. *Journal of Hazardous Materials* 190: 486-492.

Clesceri, L.S., A.E. Greenberg, and A.D. Eaton. 1998. *Standard methods for the examination of water and wastewater*. Baltimore, MD, USA: United Book Press, Inc.

Cleveland, C.C., A.R. Townsend, D.S. Schimel, H. Fisher, R.W. Howarth, L.O. Hedin, S.S. Perakis, E.F. Latty, J.C. Von Fischer, A. Elseroad, and M.F. Wasson. 1999. Global patterns of terrestrial biological nitrogen (N_2) fixation in natural ecosystems. *Global Biogeochemical Cycles* 13, no. 2: 623-645.

Cohen, Y. and E. Rosenberg, eds. 1989. *Microbial mats: Physiological ecology of benthic microbial communities*. Washington, DC: American Society for Microbiology.

Cole, J.R., B. Chai, R.J. Farris, Q. Wang, A.S. Kulam-Syed-Mohideen, D.M. McGarrell, A.M. Bandela, E. Cardenas, G.M. Garrity, and J.M. Tiedje. 2007. The ribosomal database project (RDP-II): Introducing *myRDP* space and quality controlled public data. *Nucleic Acids Research* 35. Database issue: D169-172. doi: 10.1093/nar/gkl889 [PMID: 17090583].

Cole, J.R., Q. Wang, E. Cardenas, J. Fish, B. Chai, R.J. Farris, A.S. Kulam-Syed-Mohideen, D.M. McGarrell, T. Marsh, G.M. Garrity, and J.M. Tiedje. 2009. The ribosomal database project: Improved alignments and new tools for rRNA analysis. *Nucleic Acids Research* 37. (Database issue): D141-145; doi: 10.1093/nar/gkn879] [PMID: 19004872].

Coleman, J.E. 1998. Zinc enzymes. *Current Opinion in Chemical Biology* 2: 222-234.

Conant, J.B., E.M. Dietz, C.F. Bailey, and S.E. Kamerling. 1931. Studies in the chlorophyll series. V. The structure of chlorophyll a. *Journal of the American Chemical Society* 53, no. 6: 2382-2393.

Coxson, D.S. and K.A. Kershaw. 1983. Rehydration response of nitrogenase activity and carbon fixation in terrestrial *Nostoc communie* in *stipa-bouteloua* grassland. *Canadian Journal of Microbiology* 29: 938-944.

Crowley, D.E., Y.C. Wang, C.P.P. Reid, and P.J. Szaniszlo. 1991. Mechanisms of iron acquisition from siderophores by microorganisms and plants. *Plant and Soil* 130: 179-198.

Csonka, L.N. and A.D. Hanson. 1991. Prokaryotic osmoregulation: Genetics and physiology. *Annual Review in Microbiology* 45: 569-606.

Danin, A. and E. Ganor. 1991. Trapping of airborne dust by mosses in the Negev Desert, Israel. *Earth Surface Processes and Landforms* 16, no. 2: 153-162.

Davidson, D.W., M.A. Bowker, D. George, S.L. Phillips, and J. Belnap. 2002. Treatment effects on performance of N-fixing lichens in disturbed soil crusts of the Colorado Plateau. *Ecological Applications* 12, no. 5: 1391-1405.

De Vos, P., W. Ludwig, K.-H. Schleifer, and W.B. Whitman. 2009. Family iv. Paenibacillaceae. In *The Firmicutes*, ed. P. De Vos, G.M. Garrity, D. Jones, N.R. Krieg, W. Ludwig, F.A. Rainey, K.-H. Schleifer and W.B. Whitman, 3: 269. New York: Springer. 1450.

Dertz, E.A., A. Stintzi, and K.N. Raymond. 2006. Siderophore-mediated iron transport in *Bacillus subtilis* and *Corynebacterium glutamicum*. *Journal of Biological Inorganic Chemistry* 11: 1087-1097.

Dimkpa, C., A. Svato, D. Merten, G. Büchel, and E. Kothe. 2008. Hydroxamate siderophores produced by *Streptomyces acidiscabies* e13 bind nickel and promote growth in cowpea (*Vigna unguiculata* l.) under nickel stress. *Canadian Journal of Microbiology* 54: 163-172.

Doyle, R.J., ed. 1999. *Biofilms*. San Diego: Academic Press.

Drever, J.I. and L.L. Stillings. 1997. The role of organic acids in mineral weathering. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 120: 167-181.

Eady, R.E. 1996. Structure-function relationships of the alternative nitrogenases. *Chemical Reviews* 96: 3013-3030.

Epstein, W. 1986. Osmoregulation by potassium transport in *Escherichia coli*. *FEMS Microbiology Reviews* 39: 73-78.

_____. 2003. The roles and regulation of potassium in bacteria. *Progress in Nucleic Acid Research and Molecular Biology* 75: 293-320.

Essén, S.A., D. Bylund, S.J.M. Holmstrom, M. Moberg, and U.S. Lundstrom. 2006. Quantification of hydroxamate siderophores in soil solutions of podzolic soil profiles in Sweden. *BioMetals* 19: 269-282.

Evans, R.D. and J. Belnap. 1999. Long-term consequences of disturbance on nitrogen dynamics in an arid ecosystem. *Ecology* 80, no. 1: 150-160.

Evans, R.D. and J.R. Ehleringer. 1993. A break in the nitrogen cycle in aridlands? Evidence from delta15N of soils. *Oecologia* 94: 314-317.

Evans, R.D. and O.L. Lange. 2001. Biological soil crusts and ecosystem nitrogen and carbon dynamics. In *Biological soil crusts: Structure, function, and management*, ed. J. Belnap and O.L. Lange. 263-279. Berlin: Springer-Verlag.

Fay, P. and L. De Vasconcelos. 1974. Nitrogen metabolism and ultrastructure in *Anabaena cylindrica*. *Archives of Microbiology* 99: 221-230.

Ferguson, S.J. 1998. Nitrogen cycle enzymology. *Current Opinion in Chemical Biology* 2: 182-193.

Ferguson-Miller, S., G.T. Babcock, and C. Yocum. 2007. Photosynthesis and respiration. In *Biological inorganic chemistry: Structure and reactivity*, ed. I. Bertini, H.B. Gray, E.I. Stiefel and J.S. Valentine. Sausalito, California: University Science Books.

Flores, E. and A. Herrero. 2005. Nitrogen assimilation and nitrogen control in cyanobacteria. *Biochemical Society Transactions* 33, no. 1: 164-167.

Fraústo da Silva, J.J.R. and R.J.P. Williams. 2001. *The biological chemistry of the elements: The inorganic chemistry of life*. New York: Oxford University Press.

Galloway, J.N., F.J. Dentener, D.G. Capone, E.W. Boyer, R.W. Howarth, S.P. Seitzinger, G.P. Asner, C.C. Cleveland, P.A. Green, E.A. Holland, D.M. Karl, A.F. Michaels, J.H. Porter, A.R. Townsend, and C.J. Vorosmarty. 2004. Nitrogen cycles: Past, present, and future. *Biogeochemistry* 70: 153-226.

Garcia-Pichel, F. 2002. Desert environments: Biological soil crusts. In *Encyclopedia of environmental microbiology*, ed. G. Bitton: 1019-1023. New York: John Wiley.

Garcia-Pichel, F. and J. Belnap. 1996. Microenvironments and microscale productivity of cyanobacterial desert crusts. *Journal of Phycology* 32: 774-782.

Garcia-Pichel, F. and R.W. Castenholz. 1991. Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. *Journal of Phycology* 27, no. 3: 395-409.

Garcia-Pichel, F., S.L. Johnson, D. Youngkin, and J. Belnap. 2003. Small-scale vertical distribution of bacterial biomass and diversity in biological soil crusts from arid lands in the Colorado Plateau. *Microbial Ecology* 46: 312-321.

Garcia-Pichel, F., A. Lopez-Cortes, and U. Nubel. 2001. Phylogenetic and morphological diversity of cyanobacteria in soil desert crusts from the Colorado Plateau. *Applied and Environmental Microbiology* 67, no. 4: 1902-1910.

Garcia-Pichel, F., N.D. Sherry, and R.W. Castenholz. 1992. Evidence for an ultraviolet sunscreen role of the extracellular pigment scytonemin in the terrestrial cyanobacterium *Chlorogloeopsis sp*. *Photochemistry and Photobiology* 56, no. 1: 17-23.

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, no. 11: e7801.

Garnham, G.W., G.A. Codd, and G.M. Gadd. 1993. Accumulation of zirconium by microalgae and cyanobacteria. *Applied Microbiology and Biotechnology* 39, no. 4-5: 666-672.

Garrity, G.M., J.A. Bell, and T. Lilburn. 2005. Family vii. Bradyrhizobiaceae *fam. Nov.* In *The proteobacteria*, ed. G.M. Garrity, D.J. Brenner, N.R. Krieg and J.T. Staley, 2: 438-443. New York: Springer.

Gibson, C.E. 1971. Nutrient limitation. *Water Pollution Control Federation* 43, no. 12: 2436-2440.

Gillis, M. and N.A. Logan. 2005. Genus iv. Janthinobacterium. In *The proteobacteria*, ed. G.M. Garrity, D.J. Brenner, N.R. Krieg and J.T. Staley, 2: 636-641. New York: Springer.

Giovannini, G., S. Lucchesi, and M. Giachetti. 1990. Effects of heating on some chemical parameters related to soil fertility and plant growth. *Soil Science* 149, no. 6: 344-350.

Glass, J.B., F. Wolfe-Simon, and A. Anbar. 2009. Coevolution of metal availability and nitrogen assimilation in cyanobacteria and algae. *Geobiology* 7, no. 2: 100-123.

Glass, J.B., F. Wolfe-Simon, J.J. Elser, and A. Anbar. 2010. Molybdenum-nitrogen co-limitation in freshwater and coastal heterocystous cyanobacteria. *Limnology and Oceanography* 55, no. 2: 667-676.

Gledhill, M. 2001. Electrospray ionisation-mass spectrometry of hydroxamate siderophores. *Analyst* 126: 1359-1362.

Goldberg, S. and H.S. Forster. 1998. Factors affecting molybdenum adsorption by soils and minerals. *Soil Science* 163, no. 2: 109-114.

Green, P.N. 2005. Genus i. Methylobacterium. In *The proteobacteria*, ed. G.M. Garrity, D.J. Brenner, N.R. Krieg and J.T. Staley, 2: 567-570. New York: Springer.

Guerrero, M.G., J.M. Vega, and M. Losada. 1981. The assimilatory nitrate-reducing system and its regulation. *Annual Reviews in Plant Physiology* 32: 169-204.

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.

Gustafsson, O., F. Haghseta, C. Chan, J. Macfarlane, and P.M. Gschwend. 1997. Quantification of the dilute sedimentary soot phase: Implications for PAH speciation and bioavailability. *Environmental Science and Technology* 31: 203-209.

Hantke, K. 2001. Bacterial zinc transporters and regulators. *BioMetals* 14: 239-249.

_____. 2005. Bacterial zinc uptake and regulators. *Current Opinion in Microbiology* 8: 196-202.

Hartley, A.E. and W.H. Schlesinger. 2002. Potential environmental controls on nitrogenase activity in biological crusts of the northern Chihuahuan desert. *Journal of Arid Environments* 52: 293-304.

Hille, R. 1996. The mononuclear molybdenum enzymes. *Chemical Reviews* 96: 2757-2816.

Holmes, A.J., A. Costello, M.E. Lidstrom, and J.C. Murrell. 1995. Evidence that particular methane monooxygenase and ammonia monooxygenase may be evolutionarily related. *FEMS Microbiology Letters* 132: 203-208.

Holmström, S.J.M., U.S. Lundstrom, R.D. Finlay, and P.A.W. Van Hees. 2004. Siderophores in forest soil solution. *Biogeochemistry* 71: 247-258.

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.

Howard, J.B. and D.C. Rees. 1996. Structural basis of biological nitrogen fixation. *Chemical Reviews* 96: 2965-2982.

Idris, R., R. Trifonova, M. Puschenreiter, W.W. Wenzel, and A. Sessitsch. 2004. Bacterial communities associated with flowering plants of the ni hyperaccumulator *thlaspi goesingense*. *Applied and Environmental Microbiology* 70, no. 5: 2667-2677.

Isichei, A.O. 1990. The role of algae and cyanobacteria in arid lands. A review. *Arid Soil Research and Rehabilitation* 4: 1-17.

Ito, T. and J.B. Neilands. 1958. Products of "Low-iron fermentation" With *Bacillus subtilis*: Isolation, characterization and synthesis of 2,3-dihydroxybenzoylglycine. *Journal of the American Chemical Society* 80, no. 17: 4645-4647.

Jacobitz, S. and P.E. Bishop. 1992. Regulation of nitrogenase-2 in *Azotobacter vinelandii* by ammonium, molybdenum, and vanadium. *Journal of Bacteriology* 174, no. 12: 3884-3888.

Jacobson, M.R., R. Premakumar, and P.E. Bishop. 1986. Transcriptional regulation of nitrogen fixation by molybdenum in *Azotobacter vinelandii*. *Journal of Bacteriology* 167, no. 2: 480-486.

Jeffries, D.L., J.M. Klopatek, S.O. Link, and J. Bolton. 1992. Acetylene reduction by cryptogamic crusts from a blackbrush community as related to resaturation and dehydration. *Soil Biology and Biochemistry* 24: 1101-1105.

Joerger, R.D. and P.E. Bishop. 1988. Bacterial alternative nitrogen fixation systems. *Critical Reviews in Microbiology* 16: 1-14.

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, no. 1: 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, no. 3: 680-689.

Jones, K. 1977. The effects of moisture on acetylene reduction by mats of blue-green algae in sub-tropical grassland. *Annals of Botany* 41: 801-806.

Joshi, F.R., G. Archana, and A.J. Desai. 2006. Siderophore cross-utilization amongst rhizospheric bacteria and the role of their differential affinities for Fe3+ on growth stimulation under iron-limited conditions. *Current Microbiology* 53: 141-147.

Jurkevitch, E., Y. Hadar, and Y. Chen. 1992. Differential siderophore utilization and iron uptake by soil and rhizosphere bacteria. *Applied and Environmental Microbiology* 58, no. 1: 119-124.

Kalinowski, B.E., L.J. Liermann, S. Givens, and S.L. Brantley. 2000. Rates of bacteria-promoted solubilization of Fe from minerals: A review of problems and approaches. *Chemical Geology* 169: 357-370.

Kang, B.T. and A. Sajjapongse. 1980. Effect of heating on properties of some soils from southern Nigeria and growth of rice. *Plant and Soil* 55: 85-95.

Kennedy, C. and A. Toukdarian. 1987. Genetics of azotobacters: Applications to nitrogen fixation and related aspects of metabolism. *Annual Review in Microbiology* 41: 227-258.

Kentemich, T., G. Danneberg, B. Hundeshagen, and H. Bothe. 1988. Evidence for the occurrence of the alternative, vanadium-containing nitrogenase in the cyanobacterium *Anabaena variabilis FEMS Microbiology Letters* 51: 19-24.

Khan, A., R. Geetha, A. Akolkar, A. Pandya, G. Archana, and A.J. Desai. 2006. Differential cross-utilization of heterologous siderophores by nodule bacteria of *Cajanus cajan* and its possible role in growth under iron-limited conditions. *Applied Soil Ecology* 34: 19-26.

Kim, H.J., D.W. Graham, A.A. DiSpirito, M.A. Alterman, N. Galeva, C.K. Larive, D. Asunskis, and P.M.A. Sherwood. 2004. Methanobactin, a copper-acquisition compound from methane-oxidizing bacteria. *Science* 305, no. 5690: 1612-1615.

Kiss, T. and E. Farkas. 1998. Metal-binding ability of desferrioxamine b. *Journal of Inclusion Phenomena and Molecular Recognition in Chemistry* 32: 385-403.

Kraemer, S.M. 2004. Iron oxide dissolution and solubility in the presence of siderophores. *Aquatic Science* 66: 3-18.

Kumar, J.I.N. and C. Oommen. 2012. Removal of heavy metals by biosorption using freshwater alga *Spirogyra hyalina*. *Journal of Environmental Biology* 33: 27-31.

Kuske, C.R., L.O. Ticknor, M.E. Miller, J.M. Dunbar, J.A. Davis, S.M. Barns, and J. Belnap. 2002. Comparison of soil bacterial communities in rhizospheres of three plant species and the interspaces in an arid grassland. *Applied and Environmental Microbiology* 68, no. 4: 1854-1863.

Lacava, P.T., M.E. Silva-Stenico, W.L. Araújo, A.V.C. Simionato, E. Carrilho, S.M. Tsai, and J.L. Azevedo. 2008. Detection of siderophores in endophytic bacteria *Methylobacterium* spp. Associated with *Xylella fastidiosa* subsp. *Pauca Pesquisa Agropecuária Brasileira* 43, no. 4: 521-528.

Lancaster, J.R., J.M. Vega, H. Kamin, N.R. Orme-Johnson, W.H. Orme-Johnson, R.J. Krueger, and L.M. Siegel. 1979. Identification of the iron-sulfur center of spinach ferredoxin-nitrite reductase as a tetranuclear center, and preliminary EPR studies of mechanism. *The Journal of Biological Chemistry* 254, no. 4: 1268-1272.

Lane, D.J. 1991. 16s/23s rRNA sequencing. In *Nucleic acid techniques in bacterial systematics*, ed. E. Stackebrandt and M. Goodfellow:115-175. New York, NY: John Wiley and Sons.

Lankford, C.E. 1973. Bacterial assimilation of iron. *CRC Critical Reviews in Microbiology* 2: 273-331.

Liermann, L.J., R.L. Guynn, A. Anbar, and S.L. Brantley. 2005. Production of a molybdophore during metal-targeted dissolution of silicates by soil bacteria. *Chemical Geology* 220: 285-302.

Liermann, L.J., B.E. Kalinowski, S.L. Brantley, and J.G. Ferrey. 2000. Role of bacterial siderophores in dissolution of hornblende. *Geochimica et Cosmochimica Acta* 64, no. 4: 587-602.

Litos, C., A. Terzis, C. Raptopoulou, A. Rontoyianni, and A. Karaliota. 2006. Polynuclear oxomolybdenum(VI) complexes of dihydroxybenzoic acids: Synthesis, spectroscopic and structure characterization of a tetranuclear catecholato-type coordinated 2,3-dihydroxybenzoate and a novel tridentate salicylato-type coordinated 2,5-dihydroxybenzoate trinuclear complex. *Polyhedron* 25: 1337-1347.

Littlechild, J. 1999. Haloperoxidases and their role in biotransformation reactions. *Current Opinion in Chemical Biology* 3: 28-34.

Lockau, W. 1981. Evidence for a dual role of cytochrome c-553 and plastocyanin in photosynthesis and respiration of the cyanobacterium, *Anabaena variabilis*. *Archives of Microbiology* 128, no. 3: 336-340.

Logan, N.A. and P. De Vos. 2009. Genus i. Bacillus. In *The firmicutes*, ed. P. De Vos, G.M. Garrity, D. Jones, N.R. Krieg, W. Ludwig, F.A. Rainey, K.-H. Schleifer and W.B. Whitman, 3:21 New York: Springer.

Luque, F. and R.N. Pau. 1991. Transcriptional regulation by metals of structural genes for *Azotobacter vinelandii* nitrogenases. *Molecular and General Genetics* 227: 481-487.

Madigan, T.M., J.M. Martinko, and J. Parker. 2003. *Biology of microorganisms*. Edited by T.D. Brock. Upper Saddle River, NJ: Prentice Hall.

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.

Marques, A., C. Pires, H. Moreira, A. Rangel, and P. Castro. 2010. Assessment of the plant growth promotion abilities of six bacterial isolates using *Zea mays* as an indicator plant. *Soil Biology and Biochemistry* 42: 1229-1235.

Martinez, J.S., M.G. Haygood, and A. Butler. 2001. Identification of a natural desferrioxamine siderophore produced by a marine bacterium. *Limnology and Oceanography* 46, no. 2: 420-424.

McCormack, P., P.J. Worsfold, and M. Gledhill. 2003. Separation and detection of siderophores produced by marine bacterioplankton using high-performance liquid chromatography with electrospray ionization mass spectrometry. *Analytical Chemistry* 75: 2647-2652.

McDonald, I.R. and J.C. Murrell. 1997. The methanol dehydrogenase structural gene *mxaf* and its use as a functional gene probe for methanotrophs and methylotrophs. *Applied and Environmental Microbiology* 63, no. 8: 3218-3224.

McKnight, D.M. and F.M.M. Morel. 1980. Copper complexation by siderophores from filamentous blue-green algae. *Limnology and Oceanography* 25, no. 1: 62-71.

McMaster, J., C.D. Garner, and E.I. Stiefel. 2007. Molybdenum enzymes. In *Biological inorganic chemistry structure and reactivity*, ed. I. Bertini, H.B. Gray, E.I. Stiefel and J.S. Valentine: 518-544. Sausalito, California: University Science Books.

Mills, M.M., C. Ridame, M. Davey, J. La Roche, and R.J. Geider. 2004. Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. *Nature* 429: 292-294.

“Moab, Utah.” 38º38'41.72"N 109º37'39.39"W. Elevation 4605 feet. Google Earth. Image date: September 14, 2011. Access date: March 31, 2012.

Monteiro, B., L. Cunha-Silva, S. Gago, J. Klinowski, F.A.A. Paz, J. Rocha, I.S. Gonçalves, and M. Pillinger. 2010. Crystal and supramolecular structures of dioxomolybdenum(VI) and dioxotungsten(VI) complexes of dihydroxybenzoic acids. *Polyhedron* 29: 719-730.

Murphy, M.J., L.M. Siegel, S.R. Tove, and H. Kamin. 1974. A new prosthetic group participating in six-electron reduction reactions catalyzed by both sulfite and nitrite reductases. *Proceedings of the National Academy of Sciences of the United States of America* 71, no. 3: 612-616.

Nakatsua, C.H., V. Torsvik, and L. Øvreås. 2000. Soil community analysis using DGGE of 16s rDNA polymerase chain reaction products. *Soil Science Society of America Journal* 64: 1382-1388.

Nalewajko, C., K. Lee, and T.R. Jack. 1995. Effects of vanadium on freshwater phytoplankton photosynthesis. *Water, Air, and Soil Pollution* 81: 93-105.

Neaman, A., J. Chorover, and S.L. Brantley. 2005. Implications of the evolution of organic acid moieties for basalt weathering over geological time. *American Journal of Science* 305: 147-185.

_____. 2005. Element mobility patterns record organic ligands in soils on early earth. *Geology* 33, no. 2: 117-120.

Neilands, J.B. 1973. Microbial iron transport compounds (siderochromes). In *Inorganic Biochemistry*, ed. J.B. Neilands, 1:2-34. New York: Academic Press.

_____. 1981. Microbial iron compounds. *Annual Review of Biochemistry* 50: 715-731.

Nies, D.H. 2003. Efflux-mediated heavy metal resistance in prokaryotes. *FEMS Microbiology Reviews* 27: 313-339.

Palaniappan, P., P.S. Chauhan, V.S. Saravanan, R. Anandham, and T. Sa. 2010. Isolation and characterization of plant growth promoting endophytic bacterial isolates from root nodule of *Lespedeza* sp. *Biology and Fertility of Soils* 46: 807-816.

Parker, D.L., G. Sposito, and B.M. Tebo. 2004. Manganese(III) binding to a pyoverdine siderophore produced by a manganese(II)-oxidizing bacterium. *Geochimica et Cosmochimica Acta* 68, no. 23: 4809-4820.

Parlak, M. 2011. Effect of heating on some physical, chemical and mineralogical aspects of forest soil. *Journal of the Bartin Faculty of Forestry* 13, no. 19: 143-152.

Pau, R.N., L.A. Mitchenall, and R.L. Robson. 1989. Genetic evidence for an *Azotobacter vinelandii* nitrogenase lacking molybdenum and vanadium. *Journal of Bacteriology* 171, no. 1: 124-129.

Paulsson, M., B. Nyström, and H. Blanck. 2000. Long-tern toxicity of zinc to bacteria and algae in perophyton communities from the river Göta Älv, based on a microcosm study. *Aquatic Toxicology* 47: 243-257.

Pérez-Miranda, S., N. Cabirol, R. George-Téllez, L.S. Zamudio-Rivera, and F.J. Fernández. 2007. O-CAS, a fast and universal method for siderophore detection. *Journal of Microbiological Methods* 70: 127-131.

Peschek, G.A., C. Obinger, and M. Paumann. 2004. The respiratory chain of blue-green algae (cyanobacteria). *Physiologia Plantarum* 120: 358-369.

Peters, W.J. and R.A.J. Warren. 1968. Itoic acid synthesis in *bacillus subtilis*. *Journal of Bacteriology* 95, no. 2: 360-366.

Philippot, L. 2002. Denitrifying genes in bacterial and archaeal genomes. *Biochimica et Biophysica Acta* 1577: 355-376.

Pimentel, D. 2000. Soil erosion and the threat to food security and the environment. *Ecosystem Health* 6, no. 4: 221-226.

Piña, R.G. and C. Cervantes. 1996. Microbial interactions with aluminum. *BioMetals* 9: 311-316.

Powell, P.E., G.R. Cline, C.P.P. Reid, and P.J. Szaniszlo. 1980. Occurrence of hydroxamate siderophore iron chelators in soils. *Nature* 287: 833-834.

Priest, F.G. 2009. Genus i. Paenibacilus. In *The Firmicutes*, ed. P. De Vos, G.M. Garrity, D. Jones, N.R. Krieg, W. Ludwig, F.A. Rainey, K.-H. Schleifer and W.B. Whitman, 3: 269-279. New York: Springer.

Puigdefábregas, J. 1998. Ecological impacts of global change on drylands and their implications for desertification. *Land Degradation and Development* 9: 393-406.

Quintana, J.R., V. Cala, A.M. Moreno, and J.G. Parra. 2007. Effect of heating on mineral components of the soil organic horizon from a spanish juniper (*Juniperus thurifera* l.) woodland. *Journal of Arid Environments* 71: 45-56.

Rashedul, I.M., M. Madhaiyan, H.P.D. Boruah, W. Yim, G. Lee, V.S. Saravanan, Q. Fu, H. Hu, and T. Sa. 2009. Characterization of plant growth-promoting traits of free-living diazotrophic bacteria and their inoculation effects on growth and nitrogen uptake of crop plants. *Journal of Microbiology and Biotechnology* 19, no. 10: 1213-1222.

Raven, J.A., M.C.W. Evans, and R.E. Korb. 1999. The role of trace metals in photosynthetic electron transport in O_2-evolving organisms. *Photosynthesis Research* 60: 111-149.

Raza, W. and Q. Shen. 2010. Growth, fe3+ reductas activity, and siderophore production by *paenibacillus polymyxa* sqr-21 under differential iron conditions. *Current Microbiology* 61: 390-395.

Reddy, K.J. and S.P. Gloss. 1993. Geochemical speciation as related to the mobility of F, Mo and Se in soil leachates. *Applied Geochemistry* Supplmentary Issue 2: 159-163.

Redfield, E., S.M. Barns, J. Belnap, L.L. Daane, and C.R. Kuske. 2002. Comparative diversity and composition of cyanobacteria in three predominant soil crusts of the Colorado Plateau. *FEMS Microbiology Ecology* 40: 55-63.

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 Science of the United States of America* 98, no. 13: 7123-7127.

Reynolds, R., J. Neff, M. Reheis, and P. Lamothe. 2006. Atmospheric dust in modern soil on aeolian sandstone, Colorado Plateau (USA): Variation with landscape position and contribution to potential plant nutrients. *Geoderma* 130: 108-123.

Rogers, R.W. 1972. Soil surface lichens in arid and subarid south-eastern Australia. *Australian Journal of Botany* 20: 301-316.

Rosenberg, D.R. and P.A. Maurice. 2003. Siderophore adsorption to and dissolution of kaolinite at ph 3 to 7 and 22°C. *Geochimica et Cosmochimica Acta* 67, no. 2: 223-229.

Ross, A.R.S., M.G. Ikonomou, and K.J. Orians. 2003. Characterization of copper-complexing ligands in seawater using immobilized copper(ii)-ion affinity chromatography and electrospray ionization mass spectrometry. *Marine Chemistry* 83: 47-58.

Rossi, F., R. Potrafka, F. Garcia-Pichel, and R. De Philippis. 2012. Role of the exo-polysaccharides in enhancing hydraulic conductivity of biological soil crusts. *Soil Biology and Biochemistry* 46: 33-40.

Roy, N. and P.K. Chakrabartty. 2000. Effect of aluminum on the production of siderophore by *Rhizobium* sp. (*Cicer arietinum*). *Current Microbiology* 41: 5-10.

Rubio, L.M., E. Flores, and A. Herrero. 1999. Molybdopterin guanine dinucleotide cofactor in *Synechococcus* sp. Nitrate reductase: Identification of *mobA* and isolation of a putative *moeB* gene. *FEBS Letters* 462: 358-362.

_____. 2002. Purification, cofactor analysis, and site-directed mutagenesis of *Synechococcus* ferredoxin-nitrate reductase. *Photosynthesis Research* 72: 13-26.

Rueter, J.G., K. Ohki, and Y. Fujita. 1990. The effect of iron nutrition on photosynthesis and nitrogen fixation in cultures of *Trichodesmium* (cyanophyceae). *Journal of Phycology* 26: 30-35.

Rychert, R.C. and J. Skujins. 1974. Nitrogen fixation by blue-green algae-lichen crust in the great basin desert. *Soil Science Society of America Proceedings* 38: 768-771.

Saal, L.B. and O.W. Duckworth. 2010. Synergistic dissolution of manganese oxides as promoted by siderophores and small organic acids. *Soil Science Society of America Journal* 74, no. 6: 2021-2031.

Saito, M.A., T.J. Goepgert, and J.T. Ritt. 2008. Some thoughts on the concept of colimitation: Three definitions and the importance of bioavailability. *Limnology and Oceanography* 53: 276-290.

Sañudo-Wilhelmy, S.A., A.B. Kustka, C.J. Gobler, D.A. Hutchins, M. Yang, K. Lwiza, J.A. Burns, D.G. Capone, J.A. Raven, and E.J. Carpenter. 2001. Phosphorus limitation of nitrogen fixation by *Trichodesmium* in the central atlantic ocean. *Nature* 411: 66-69.

Sanz, A.P., C. Moreno-Vivián, J.M. Maldonado, A. González-Fontes. 1995. Effect of a constant supply of different nitrogen sources on protein and carbohydrate content and enzyme activities of *Anabaena variabilis* cells. *Physiologia Plantarum* 95: 39-44.

Schlesinger, W.H. 1996. *Biogeochemistry, an analysis of global change*. San Diego: Academic Press.

Schloss, P.D., S.L. Westcott, T. Ryabin, J.R. Hall, M. hartmann, E.B. Hollister, R.A. Lesniewski, B.B. Oakley, D.H. Parks, C.J. Robsincon, J.W. Sahl, B. Stres, G.G. Thallinger, D.J. Van Horn, and C.F. Weber. 2009. Introducing mothurs: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology* 75, no. 23: 7537-7541.

Schulten, J.A. 1985. Soil aggregation by cryptogams of a sand prairie. *American Journal of Botany* 72, no. 11: 1657-1661.

Schumacher, B.A. 2002. Methods for the determination of total organic carbon (TOC) in soils and sediments: U.S. Environmental Protection Agency, Washington, DC. 1-23.

Schwyn, B. and J.B. Neilands. 1987. Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemistry* 160: 47-56.

Sheridan, D. 1981. *Desertification of the United States*. Council on environmental quality. Washington, D.C.: U.S. Government Printing Office.

Silva-Stenico, M.E., F.T.H. Pacheco, J.L.M. Rodrigues, E. Carrilho, and S.M. Tsai. 2005. Growth and siderophore production of *Xylella fastidiosa* under iron-limited conditions. *Microbiological Research* 160: 429-436.

Silvester, W.B. 1989. Molybdenum limitation of asymbiotic nitrogen fixation in forests of pacific northwest america. *Soil Biology and Biochemistry* 21, no. 2: 283-289.

Simionato, A.V.C., C. Simo, A. Cifuentes, P.T. Lacava, W.L. Araujo, J.L. Azevedo, and E. Carrilho. 2006. Capillary electrophoresis-mass spectrometry of citrus endophytic bacteria siderophores. *Electrophoresis* 27: 2567-2574.

Smith, K.S. and J.G. Ferry. 2000. Prokaryotic carbonic anhydrase. *FEMS Microbiology Reviews* 24: 335-366.

soilcrust.org. Southwest Biological Science Center. USGS Canyonlands Research Station. Access date: April 18th, 2012.

Solomonson, L.P., M.J. Barber, and W.D. Howard. 1984. Electron paramagnetic resonance studies of the molybdenum center of assimilatory NADH:Nitrate reductase from *Chlorella vulgaris*. *The Journal of Biological Chemistry* 259, no. 2: 849-853.

Sroga, G.E. 1997. Regulation of nitrogen fixation by different nitrogen sources in the filamentous non-heterocystous cyanobacterium *Microcoleus* sp. *FEMS Microbiology Letters* 153: 11-15.

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. *Reclamation and Revegetation Research* 4: 261-269.

Steppe, T.F., J.B. Olson, H.W. Paerl, R.W. Litaker, and J. Belnap. 1996. Consortia N_2 fixation: A strategy for meeting nitrogen requirements of marine and terrestrial cyanobacterial mats. *FEMS Microbiology Ecology* 21: 149-156.

Strauss, S.L., T.A. Day, and F. Garcia-Pichel. 2012. Nitrogen cycling in desert biological soil crusts across biogeographic regions in the southwestern United States. *Biogeochemistry* 108: 171-182.

Suelter, C.H. 1970. Enzymes activated by monovalent cations. *Science* 168, no. 3933: 789-795.

Sutherland, L., J. Cairney, M.J. Elmore, I.R. Booth, and C.F. Higgins. 1986. Osmotic regulation of transcription: Induction of the *proU* betaine transport gene is dependent on accumulation of intracellular potassium. *Journal of Bacteriology* 168, no. 2: 805-814.

Szabó, O. and E. Farkas. 2011. Characterization of Mn(II) and Mn(III) binding capability of natural siderophores desferrioxamine b and desferricoprogen as well as model hydroxamic acids. *Inorganic Chimica Acta* 376: 500-508.

Tamagnini, P., R. Axelsson, P. Lindberg, F. Oxelfelt, R. Wünschiers, and P. Lindblad. 2002. Hydrogenases and hydrogen metabolism of cyanobacteria. *Microbiology and Molecular Biology Reviews* 66, no. 1: 1-20.

Tamura, K., J. Dudley, M. Nei, and S. Kumar. 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24: 1596-1599.

Temirov, Y.V., T.Z. Esikova, I.A. Kashparov, T.A. Balashova, L.M. Vinokurov, and Y.B. Alakhov. 2003. A catecholic siderophore produced by the thermoresistant *Bacillus licheniformis* vk21 strain. *Russian Journal of Bioorganic Chemistry* 29, no. 6: 542-549.

ter Steeg, P.F., P.J. Hanson, and H.W. Paerl. 1986. Growth-limiting quantities and accumulation of molybdenum in *Anabaena oscillarioides* (cyanobacteria). *Hydrobiologia* 140: 143-147.

Terry, R.E. and S.J. Burns. 1987. Nitrogen fixation in cryptogamic soil crusts as affected by disturbance. 369-372.

Thiel, T. 1993. Characterization of genes for an alternative nitrogenase in the cyanobacterium *Anabaena variabilis*. *Journal of Bacteriology* 175, no. 19: 6276-6286.

Tovar-Sanchez, A., S.A. Sanudo-Wilhelmy, A.B. Kustka, S. Agusti, J. Dachs, D.A. Hutchins, D.G. Capone, and C.M. Duarte. 2006. Effects of dust deposition and river discharges on trace metal composition of *Trichodesmium* spp. In the tropical and subtropical North Atlantic Ocean. *Limnology and Oceanography* 51, no. 4: 1755-1761.

Tuit, C., J. Waterbury, and G. Ravizza. 2004. Diel variation of molybdenum and iron in marine diazotrophic cyanobacteria. *Limnology and Oceanography* 49, no. 4: 978-990.

Umamaheswari, A., D.R. Madhavi, and K. Venkateswarlu. 1997. Siderophore production in two species of *Nostoc* as influenced by the toxicity of nitrophenolics. *Bulletin of Environmental Contamination and Toxicology* 59: 306-312.

Umena, Y., K. Kawakami, J.-R. Shen, and N. Kamiya. 2011. Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. *Nature* 473: 55-61.

Vallee, B.L. and D.S. Auld. 1990. Zinc coordination, function, and structure of zinc enzymes and other proteins. *Biochemistry* 29, no. 24: 5647-5659.

van Hees, P.A.W., U.S. Lundström, and R. Giesler. 2000. Low molecular weight organic acids and their Al-complexes in soil solution-composition, distribution and seasonal variation in three podzolized soils. *Geoderma* 94: 173-200.

Vitousek, P.M., K. Cassman, C.C. Cleveland, T.E. Crews, C.B. Field, N.B. Grimm, R.W. Howarth, R. Marino, L. Martinelli, E.B. Rastetter, and J.I. Sprent. 2002. Towards an ecological understanding of biological nitrogen fixation. *Biogeochemistry* 57/58: 1-45.

Vitousek, P.M. and R.W. Howarth. 1991. Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry* 13: 87-115.

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, no. 16: 5261-5267.

Wedepohl, K.H. 1995. The composition of the continental crust. *Geochimica et Cosmochimica Acta* 59, no. 7: 1217-1232.

West, N.E. and J. Skujins. 1977. The nitrogen cycle in North American cold-winter semi-desert ecosystems. *Oecologia Plantarum* 12, no. 1: 45-53.

White, A.E., Y.H. Spitz, D.M. Karl, and R.M. Letelier. 2006. Flexible elemental stoichiometry in *Trichodesmium* spp. And its ecological implications. *Limnology and Oceanography* 51, no. 4: 1777-1790.

White, S., G. Boyd, F.S. Mathews, Z. Xia, W. Dai, Y. Zhang, and V.L. Davidson. 1993. The active site structure of the calcium-containing quinoprotein methanol dehydrogenase. *Biochemistry* 32: 12955-12958.

Wichard, T., B. Mishra, S.C.B. Myneni, J.P. Bellenger, and A.M.L. Kraepiel. 2009. Storage and bioavailability of molybdenum in soils increased by organic matter complexation. *Nature Geoscience* 2: 625-629.

Wiebe, K. 2003. *Linking land quality, agircultural productivity, and food security.* Washington, DC: USDA Economic Research Service, Agricultural Economic Report No. 823.

Wilhelm, S.W. and C.G. Trick. 1995. Physiological profiles of *synechococcus* (cyanophyceae) in iron-limiting continous cultures. *Journal of Phycology* 31: 79-85.

Wolfe-Simon, F., J.S. Blum, T.R. Kulp, G.W. Gordon, S.E. Hoeft, J. Pett-Ridge, J.F. Stolz, S.M. Webb, P.K. Weber, P.C.W. Davies, A.D. Anbar, and R.S. Oremland. 2011. A bacterium that can grow by using arsenic instead of phosphorus. *Science* 332: 1163-1166.

Xiao, B., Q. Wang, Y. Zhao, and M. Shao. 2011. Artificial culture of biological soil crusts and its effects on overland flow and infiltration under simulated rainfall. *Applied Soil Ecology* 48: 11-17.

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, no. 2: 973-983.

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 N_2-fixing members of biological soil crusts of the Colorado Plateau, USA. *FEMS Microbiology Ecology* 60: 85-97.

Young, J.P.W. 1992. Phylogenetic classiication of nitogen-fixing organisms. In *Biological nitrogen fixation*, ed. G. Stacey, R.H. Burris and H.J. Evans. New York: Chapman and Hall, Inc.

Zahn, J.A., D.M. Arciero, A.B. Hooper, and A.A. DiSpirito. 1996. Evidence for an iron center in the ammonia monooxygenase from *Nitrosomas europaea*. *FEBS Letters* 397: 35-38.

Zehr, J.P., B.D. Jenkins, S.M. Short, and G.F. Steward. 2003. Nitrogenase gene diversity and microbial community structure: A cross-system comparison. *Environmental Microbiology* 5, no. 7: 539-554.

Zerkle, A.L., C.H. House, R.P. Cox, and D.E. Canfield. 2006. Metal limitation of cyanobacterial N_2 fixation and implications for the precambrian nitrogen cycle. *Geobiology* 4: 285-297.

Zhao, Y., S.-M. Bian, H.-N. Zhou, and J.-F. Huang. 2006. Diversity of nitrogenase systems in diazotrophs. *Journal of Integrative Plant Biology* 48, no. 7: 745-755.