Response of Biological Soil Crusts to Porewater Metal Additions. K. Alexander[1], K. Lui[2], T. Viliboroghi[3], A. Anbar[1,2], F. Garcia-Pichel[4], and H. E. Hartnett[1,2], [1]School of Earth and Space Exploration, Arizona State University, [2]Department of Chemistry and Biochemistry, Arizona State University, [3]School of Sustainability, Arizona State University, [4]School of Life Sciences, Arizona State University

Introduction: The first goal of this study was to determine how nitrogen fixation activity changed as a result of porewater metal additions to biological soil crusts (BSCs). The second goal was to monitor how the porewater metal concentrations change in response to BSC microbial activity. Biological soil crusts are microbial consortia that can contain algae, bacteria, fungi, and mosses [1]. Microbial activity in BSCs provides the primary source of biologically available carbon (C) and nitrogen (N) to the Colorado Plateau ecosystem [2,3,4]. These communities also promote water infiltration and prevent erosion, both of which increase soil fertility [5,6].

The cyanobacteria are important members of the microbial consortium as they are capable of both photosynthesis and nitrogen fixation [1,7]. Biological nitrogen fixation is the conversion of atmospheric nitrogen gas (N_2) to ammonium (NH_4^+). Because N_2 is not biologically available, the production of ammonium by cyanobacteria in BSCs provides the entire community with bioavailable-N.

Organisms capable of biological nitrogen fixation contain genes that code for the enzyme nitrogenase. The reduction of N_2 to NH_4^+ takes place at the active site of nitrogenase, where one of three metals may be used: molybdenum (Mo), vanadium (V), or iron (Fe). Iron is also required in other locations throughout the enzyme. Therefore, the ability to perform N_2 fixation has an absolute requirement for Fe, and an additional requirement for either Mo or V [8].

Fixation of N_2 is an energetically costly process that requires a substantial input of ATP. The Mo-dependent form of nitrogenase has a higher efficiency, using less ATP per molecule of NH_4^+ produced than either the V- or Fe-dependent versions [8]. The majority of N_2 fixing organisms, therefore, utilize the Mo-dependent enzyme. The ability to express the alternative forms of nitrogenase depends first on possession of the genes that code for these enzymes. If an organism possesses the genetic material, it will only produce the V-dependent enzyme in the absence of Mo and the Fe-dependent enzyme when both Mo and V are depleted [9,10,11].

Experimental Design: This work was conducted with crusts collected from the Colorado Plateau near Moab, Utah. These are typical early stage dark crusts; dark crusts are one end-member of crust development dominated by a diverse community of cyanobacteria. All experiments were performed as simulated rainfall events. Water containing either bioavailable-N, Mo, or V was added to individual samples. These elements were added as aqueous solutions of NH_4NO_3, Na_2MoO_4, and Na_3VO_4 respectively.

In both experiments, water alone was added during Week 1 of incubation to establish a baseline for microbial activity. Crusts were allowed to dry, and then water plus bioavailable-N or metal was added during Week 2 to see how microbes responded to additions. During Week 2, water alone was added to some replicates for use as a control.

The first experiment monitored N_2 fixation activity of BSCs using a modified acetylene reduction assay. Crusts were wetted and then incubated in clear plastic jars. Acetylene was added to the jar headspace and ethylene production was measured with gas chromatography.

Metal concentrations in soil porewater samples were measured in the second experiment. Crusts were wetted and porewater was collected [12]. Water samples were filtered through a 0.2 μm Supor^{tm} membrane filter and digested in concentrated nitric acid (to dissolve precipitates) and hydrogen peroxide (to eliminate organics) prior to analysis by inductively coupled plasma mass spectrometry.

Results:

Nitrogen Fixation Assays: Crust organisms showed a change in N_2 fixation activity in response to additions of N, Mo, and V. We confirmed that in the absence of nutrient addition, BSCs fix N_2 under our experimental conditions. With the addition of bioavailable-N, BSC organisms stop N_2 fixation activity (Figure 1). In general, with the addition of Mo, crusts maintain higher N_2 fixation rates than crusts with no added Mo (Figure 2). When V is added, crusts fix N_2 at lower rates when compared to a no-V control (Figure 3).

Metal Concentrations: Metal concentrations showed a biological effect over time. Molybdenum concentrations in crusted soils increased over time in both Weeks 1 and 2. However, the increase and the absolute concentration of Mo in Week 2 were both lower than the changes observed in Week 1. Vanadium concentrations showed similar behavior, and iron concentrations did not show a consistent pattern (data not shown).

Concentrations of Mo and V behaved comparably in crusted control samples to which water minus

bioavailable-N was added in Week 2. In these samples, iron concentrations increased through Weeks 1 and 2 (data not shown).

Discussion:

Nitrogen Fixation Assays: When bioavailble-N is added to crusted samples, they stop N_2 fixation activity. This indicates that under natural conditions, the community is limited with respect to bioavailable-N. Maintenance of higher N_2 fixation rates with addition of Mo implies that crust organisms use the Mo-dependent version of nitrogenase and that they are limited with respect to Mo. Decreased rates of N_2 fixation under V addition suggests that if any crust organisms utilize the V-dependent form of nitrogenase, that their V requirements are met by the V available in the soil. It is possible that rates decrease as a result of V-toxicity, or that V competes with the uptake and utilization of available Mo, thus exacerbating Mo-limitation.

Metal Concentrations: Changes in Mo and V concentrations indicate that microbial activity influences the availability of these metals in the soil porewater. Increases in concentration suggest mobilization by crust organisms, and decreases in concentration indicate uptake by crust organisms. Similarities between conditions of water plus bioavailable-N and water minus bioavailable-N imply that metal requirements are similar regardless of the nitrogen source. Analyses are underway to measure changes in soil porewater metal concentrations under Mo and V addition.

Summary: Acetylene reduction experiments showed that N_2 fixation activity by BSC organisms responds to porewater metal additions. Analysis of porewater geochemistry indicated that biological activity affects the concentration of metals

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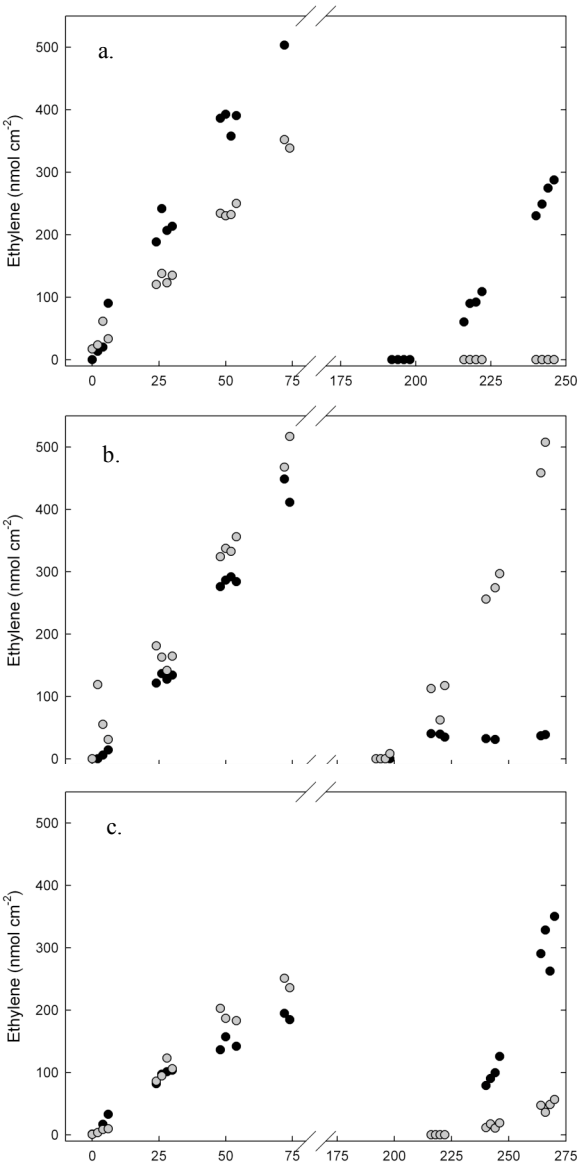

Figure 1. Ethylene production in crusted samples. Week 1: 0-75 h, Week 2: 200-275 h. Water alone was added to all samples in Week 1. a. Black: water –N added in Week 2. Without the addition of bioavailable-N, N_2 fixation continues. Grey: water +N added in Week 2. Addition of bioavailable-N causes N_2 fixation to stop.
b. Black: water –Mo added in Week 2. Without added Mo, N_2 fixation rates drop. Grey: water +Mo added in Week 2. Addition of Mo maintains high N_2 fixation rates.
c. Black: water –V added in Week 2. Without added V, high N_2 fixation rates continue. Grey: water +V added in Week 2. When V is added, N_2 fixation rates drop.