UNIVERSITY OF WISCONSIN-LA CROSSE

Graduate Studies

EFFECTS OF BIOLOGICAL SOIL CRUSTS ON BACTERIAL DIVERSITY AND SOIL PROPERTIES IN A WISCONSIN SAND PRAIRIE

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for the Degree of Master of Science in Biology

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ABSTRACT

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Sand prairies are a unique variant of the North American tallgrass prairie, found on sandy glacial and riverine deposits in the Upper Mississippi River (UMR) floodplain and elsewhere in the upper Midwest. Biological soil crusts (BSC) -- communities of bacteria, microalgae, fungi, archaea, and mosses, living within the top 3-14 mm of the soil -- provide important ecosystem services in sandy habitats worldwide. However, little is known about BSCs in Midwestern sand prairies. To characterize BSCs on a sand prairie in Wisconsin and quantify their effects on soil properties, soil samples were collected as matched pairs from disturbed, open sand areas (uncrusted) and nearby recovering BSC (crusted). Samples were analyzed for bacterial diversity and physical and chemical properties. Crusted samples contained significantly higher alpha and beta diversity. Proportions of major phyla, Cyanobacteria, Actinobacteria, Acidobacteria, and Bacteriodetes, were similar between crusted and uncrusted soils, but there was greater variation at the class and ordinal levels. Crusted soils had significantly higher organic material, total nitrogen, and total organic carbon, and were more resistant to erosion. These results indicate positive effects of BSCs on sand prairie habitat. These effects could be useful in restoration of sand prairie within the UMR floodplain and other sand prairie areas.

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INTRODUCTION

Biological soil crusts (BSCs), also known as cryptogamic, cryptobiotic, microbiotic, microphytic, microfloral, organogenic, biogenic, and desert algal soil crusts, consist of bacteria, fungi, cyanobacteria, archaea, mosses, and microalgae that live together within the top 3-14 mm of the soil surface (Belnap 1990, 2001, 2006; Belnap and Lange 2003; Garcia-Pichel et al. 2001; Knapen et al. 2007; Knudsen and La Doux 2005; Soule et al. 2009; Zancan et al. 2006). Large, motile filamentous cyanobacteria are the first of these organisms to colonize bare soils, with smaller, less mobile species appearing later (Belnap 2006). The cyanobacteria move to the soil surface in response to wetting of the soil, and move below the surface as the soil dries. This repeated movement causes sticky polysaccharide sheaths from the cyanobacteria to attach to soil particles and form aggregates, thus stabilizing the soils and eventually creating layers (Belnap 2003, 2006; Nunes da Rocha et al. 2015). With available propagules and appropriate climate conditions, these stabilized layers allow for other species to colonize the soils. Next, secondary colonists including archaea, microalgae, and microfungi appear, followed by lichens, mosses, and bryophytes in later successional stages (Belnap 2006; Bu et al. 2013).

Species composition of BSCs often differs on both temporal and spatial scales, with changes in abiotic characteristics such as temperature and water availability as well as amount of disturbance and successional stage. Cyanobacteria have been described in hot deserts on every continent, likely due to the ubiquity of these organisms and their

tolerance of extreme temperatures (Castillo-Monroy and Maestre 2011). Additionally, distribution of mosses is often dictated by clay content and pH of soils, and lichen distribution is affected by air quality and rainfall (Castillo-Monroy and Maestre 2011). In BSCs where levels of disturbance are high, there will likely be an early successional stage present with lower species diversity and a higher number of cyanobacteria and cyanolichens, whereas lower levels of disturbance allow for later successional stages which contain higher species diversity and numbers of lichens and mosses (Neher et al. 2003). Some genera and species can be found on nearly every continent, such as the cyanobacteria *Microcoleus vaginatus* and lichen species *Psora decipiens*, *Collema tenax*, *C. coccophorum*, and *Catapyrenium squamulosum* (Belnap 2003). In addition, some cyanobacterial genera responsible for the initial colonization and formation of BSCs, such as *Microcoleus*, *Nostoc*, and *Scytonema*, are ubiquitous in many different types of BSCs around the world and have been documented in Arizona, USA (Nagy et al. 2005), Mexico (Rivera-Aguilar et al. 2006), Sultanate of Oman (Abed et al. 2010), India (Kumar and Adhikary 2015), Israel (Hagemann et al. 2015), China (Wang et al. 2016; Zhang et al. 2009), the Western Himalayas (Čapková et al. 2016), Germany (Langhans et al. 2009), and Arctic and Antarctic regions (Makhalanyane et al. 2015). Despite the worldwide distribution of these three genera, their presence and abundances may vary among different regions within a continent. This variation of species composition is likely based on levels of disturbance and subsequent effects on successional stage, as well as available propagule densities. *Nostoc* and *Scytonema*, less mobile genera, are thought to be later successional groups compared to *Microcoleus* (Bu et al. 2013). For example, a chlorophyte-dominated BSC in Ohio recovering from previous disturbances contained *M.*

vaginatus but lacked *Nostoc*, while BSCs dominated by cyanobacteria on sandy barrens in Michigan and New Jersey contained both *M. vaginatus* and *Nostoc* (Neher et al. 2003). Ultimately, the abiotic environmental conditions, level and amount of disturbance, and successional stage may drive species diversity of BSCs.

BSCs are common throughout the arid and semi-arid regions of the world, and thanks to the large spatial extent of these habitats, BSCs are estimated to cover 35% of the surface area of the continents (Belnap and Lange 2003). In addition to their worldwide distribution, BSCs have a considerable ecological impact on local ecological communities. BSCs provide many ecosystem services such as increasing soil stability and water holding capacity, dust trapping, increasing the viability of seeds from vascular plants, as well as increasing the relative amounts of nitrogen and carbon in soils (Abed et al. 2013; Belnap 2003, 2006; Gao et al. 2014; Knapen et al. 2007). As bare soils are colonized by cyanobacteria, followed by later successional species, the soil surface becomes rough and is better able to trap nutrient-rich dust and hold water for longer periods of time, thus increasing the fertility of crusts. With the exception of smooth crusts that are dominated by cyanobacteria and found in hyper-arid regions (Belnap 2003), water infiltration is increased and runoff is decreased for areas where BSCs are present (Belnap 2006). The increased roughness of the soil surface also allows for the establishment of vascular plants due to increased stability and fertility of otherwise barren soils (Belnap 2003). Increased biomass of BSC organisms also leads to increased available nitrogen for vascular plants and carbon via nitrogen and carbon fixation by free-living and lichenized cyanobacteria, and some free-living bacteria (Belnap 2003; Hawkes 2003). Additionally, BSCs reduce leaching, thus aiding in the retention of these soil

nutrients (Hawkes 2003). Both nitrogen and carbon are crucial for vascular plant establishment; in regions with low atmospheric deposition and anthropogenic input rates, fixation by soil crust organisms is the only potential source for increased soil fertility (Belnap 2002, 2003; Hawkes 2003). Furthermore, the nitrogen fixing organisms present in BSCs require less moisture compared to symbiotic bacteria associated with vascular plants to fix nitrogen, so BSC organisms are better able to persist in harsh conditions, allowing for greater subsequent vascular plant establishment and growth (Su et al. 2011).

However, BSCs are easily damaged by human activities, and where crusts are destroyed it can take up to 250 years for all of the components of the BSC to reestablish (Belnap 1993). The ecosystem services provided by BSCs are sometimes lost, depending upon the severity of disturbance. Examples include alterations to nitrogen and carbon cycling as well as hydrological regimes, increased vulnerability to drought, and decreases in soil stability, fertility, water holding capacity, and crust cover (Belnap 2003; Bu et al. 2013). As a consequence, disturbance to BSCs will often lead to negative effects on the local vascular plant community (Belnap 2003).

Conversely, due to the ecosystem services they supply, BSCs are considered a powerful tool for the restoration of degraded areas: if crusts can be re-established, many other aspects of ecosystem structure and function will be improved at the same time. To aid in the re-establishment of crusts, inoculation of cyanobacterial species as an attempt to artificially cultivate BSCs has been tested both in laboratory settings and in the field with various results. Some of these studies have shown enhanced and accelerated growth of BSC (Bu et al. 2013), while others report only small, short-term improvements (Bowker 2007). However, given what we know about how early-colonizing species of

cyanobacteria provide the structure and conditions needed for subsequent colonization by other microbial species, it makes sense to identify and use early-successional cyanobacterial species in crust restoration.

While many studies have examined the species diversity of and ecosystem services provided by BSCs in many parts of the world, knowledge about BSCs in the Midwest, and more specifically Wisconsin, is lacking. We are unaware of any studies examining BSCs in WI to-date, so this project was designed to determine the bacterial species composition of soil crusts in Wisconsin sand prairie. Furthermore, it evaluated one aspect of the ecosystem function provided by these crusts, by examining their effects on soil properties. Crust formation and soil properties were tested and measured by performing experiments on collected samples in the field and laboratory. Species diversity was quantified using molecular techniques, identifying species based on 16S bacterial ribosomal gene sequences. Understanding the species diversity of young crust communities allows us to identify the taxa that pave the way for later crust development, provide us with baseline information about crust communities in the Midwest, and facilitate comparisons with similar systems around the world. Furthermore, knowledge of species identifications in young crusts will be useful to identify candidates for future restoration projects. This information will contribute to the overall knowledge of BSCs in the Midwest and be useful in conserving BSCs in Wisconsin.

MATERIALS AND METHODS

Study Site

Samples were collected in April 2015 in sand prairie habitat at the Oak Barrens State Natural Area at Ft. McCoy in Tomah, Wisconsin (coordinates 43.9866˚N, 90.6990˚ W). Sand prairie is a unique variant of the tallgrass prairie, containing a special set of species that can tolerate the droughty conditions caused by sandy soils (Curtis 1959). Distinct areas of biological soil crusts are common in sand prairie sites, interspersed between the relatively sparse and low-statured clumps of vegetation. The State Natural Area at Fort McCoy contains areas with high-quality sand prairie, some of which has been degraded due to repeated military training exercises with large vehicles and firearms. However, some disturbed areas are showing signs of recolonization by BSCs, providing an ideal opportunity to compare the microbial community composition of uncrusted areas to that of areas in which crusts have begun re-forming.

Seven pairs of plots were selected by identifying “blowout” areas with open sand, surrounded by undisturbed and recovering sand prairie, that were at least 30 m apart. Sampling plots were paired to allow for comparison between each uncrusted blowout and the recovering crusted region surrounding it. The crusted areas sampled were immediately adjacent to a blowout, and contained relatively undeveloped crusts compared to areas further from the blowout. The appearance of crust type was recorded at each crusted plot (Table 1). Within each plot, a transect was used to collect four

subsamples at fixed 1 m increments; for crusted plots, cores were positioned at the location closest to the 1 m mark at which 50% of the core would contain BSC. Separate, sterilized 3.18 cm diameter corers were used for each transect. Corers were used to remove the top two centimeters of soil at each sampling location; the four cores from each transect were then combined in a sterile Whirl-Pak bag. Two additional samples were collected from each plot by removing an 8.26 cm diameter core of the top two centimeters of soil using the bottom of a plastic Petri dish (Abed et al. 2013). The top of the Petri dish was then replaced, the dish was labeled and sealed with Parafilm M laboratory film (Bemis Co., Inc., Neenah, WI, USA), and the dish was carefully transported to keep the soil crust sample intact.

Diversity and Structure of Soil Crust Microbial Communities

Culture-independent assessment of microbial diversity was conducted using high throughput DNA sequencing on the Illumina platform. DNA was extracted from each of the combined samples in triplicate using a MO BIO PowerSoil DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA), following manufacturer's instructions. Samples were labeled by site, group (crusted/uncrusted), and replicate, respectively (e.g. 1C1 is from site 1, crusted soil, DNA extract replicate 1, etc.). Fungal amplifications were attempted using primers ITS1F and ITS4, targeting the nuclear ribosomal internal transcribed spacer region (Gardes and Bruns 1993, White et al. 1990). Fungal amplifications failed for most samples, suggesting low fungal DNA quantity; therefore, Illumina sequencing of fungi was not attempted.

The 16S bacterial ribosomal gene was amplified using barcoded Illumina primers targeting the 16S V4 variable region, using the two-step phasing amplicon sequencing

method of Wu et al. (2015). First, a 10-cycle PCR amplification was performed using universal primers 515F and 806R, incorporating primer sequence modifications introduced by Parada et al. (2016) and Apprill et al. (2015). Next, the first step products were used as templates in a second PCR using a set of barcoded Illumina primers containing a 0-7 bp heterogeneity spacer in the forward and reverse primers with primers paired so that the total spacer length equals 7 bp for each amplification. These phased primers increase the overall base heterogeneity in the sequencing run, which increases the precision of cluster discrimination, which in turn increases overall sequence quality and decreases the amount of Φx174 DNA that must be added to the Illumina flow cell in order to increase base heterogeneity. The proportion of sample sequences in the run is therefore increased by reducing the number of Φx174 sequences that are discarded (Fadrosh et al. 2014; Wu et al. 2015). Negative control reactions were conducted to detect potential ambient contamination, and a low-DNA Taq polymerase was used to avoid amplification of bacterial DNA originating from enzyme cloning (AmpliTaq polymerase LD; Life Technologies, Carlsbad, CA, USA). Success of PCR amplifications was assessed using agarose gel electrophoresis. PCR products were purified using Agencourt AMPureXP beads (Beckman Coulter, Inc., Indianapolis, IN, USA) in a volume:volume ratio of 0.7:1 beads: PCR product, and then quantified using the dsDNA HS quantification kit for the Qubit 3.0 fluorometer (Thermo Fisher Scientific).

Equimolar sample pools were constructed and submitted for sequencing, sequence preprocessing, and data analysis to the University of Wisconsin Biotechnology Center DNA Sequencing Facility (Madison, WI, USA). Bacterial/Archaeal amplicons were combined on a single Illumina MiSeq flow cell and sequenced using 2x300 bp read

cycles. Skewer (Hongshan et al. 2014) was used for adapter and quality trimming. Paired end reads were merged using Flash (Magoc and Salzberg 2011), and then quality filtered (Appendix A). Sample demultiplexing, OTU assignment, and diversity comparisons were conducted using QIIME (Caporaso et al. 2010). UClust (Edgar 2010) was used for OTU assignment, with reads clustered against a reference sequence collection using a 97% similarity threshold (Caporaso et al. 2010). Taxonomic assignment of OTU sequences was performed using the RDP classifier. Prior to calculation of phylogenetic diversity and distance metrics, sequences were aligned using PyNAST with singleton OTUs and OTUs that could not be aligned removed, and variable regions removed by filtering alignments.

Microbial diversity and community composition were compared within (i.e., between replicate subsamples) and between samples, as well as between crusted and uncrusted soil types, using measures of alpha diversity, beta diversity, and through comparison of taxonomic compositional profiles. Samples were compared qualitatively using rarefaction curves in order to compare samples/subsamples despite differences in sequencing depth. A rarefaction upper limit of median depth/sample count was used to calculate alpha diversity, and beta diversity was leveled according to the lowest sample depth. Alpha diversity was calculated using four metrics: Chao1, Faith's phylogenetic diversity (PD), observed species, and Shannon diversity. Chao1 (Chao 1984**)**, a richness estimator, was used to estimate the number of species per sample given a finite sampling of the community. This is accomplished by counting how many times each distinct OTU was observed in each sample while also considering the ratio of singletons and doubletons present in each sample. Knowing the proportion of rare species in a sample

gives a better understanding of species diversity compared to only a count of all species present. A phylogenetic analysis was conducted using FastTree (Price et al. 2010) to place OTUs from all samples into a single phylogenetic tree. Phylogenetic diversity (Faith 1992**)** was used to estimate diversity at an evolutionary scale through weighting sample diversity by phylogenetic distance. Phylogenetic diversity is calculated by adding the total tree branch length separating all OTUs in a sample, as well as the total number and abundance of OTUs in that sample. For a given number and abundance distribution of OTUs, a sample with greater branch length (i.e., containing more distantly related OTUs, or representing a greater proportion of the tree of life) is more evolutionarily diverse. Observed species is the count of the number of species in each sample based on OTU counts, and is the simplest metric of diversity used here. Lastly, Shannon diversity is a statistical measure of diversity that takes into account the count of OTUs present in the sample as well as the proportion of each OTU within the sample. Shannon diversity quantifies the probability of being able to predict the species identification of a randomly selected individual from a sample. This probability is based in part on evenness of species, where individual identification from samples with unequal abundances of species are more difficult to predict than for samples with equal abundances. Replicate subsamples were examined as well as different samples and soil types (crusted/uncrusted). Alpha diversity measures at 159,184 sequences/sample for crusted and uncrusted samples were averaged for each site and compared using paired t-tests in SPSS.

Beta diversity was assessed using two phylogenetic diversity metrics, weighted and unweighted UniFrac distances (Lozupone and Knight 2005; Lozupone et al. 2010).

The UniFrac distance between two samples is a function of the length of phylogenetic branches unique to one of the two samples relative to the total branch length for all OTUs present in one or both of the samples. Unweighted distances use simple OTU presence/absence, whereas the weighted metric accounts for relative OTU abundance, measured as sequence read count. Principal Coordinates ordinations were conducted on the UniFrac distance matrices. Barplots of taxonomic composition were produced and compared across samples at the taxonomic levels of phylum, class, and order.

Soil Properties

To compare the properties of uncrusted and crusted soils, erosion resistance and water holding capacity were tested using the 8.26 cm diameter soil core samples from each plot. Erosion resistance was tested by oven drying a soil core at 100˚C, attaching each piece onto the lid of the Petri dish using water soluble commercial multi-purpose white glue, weighing, and placing the sample outdoors, for two months (Abed et al. 2013). Rainfall data and any disturbances were recorded during this time. After two months, the remaining crust samples were removed, dried and weighed. The proportion of the final weight to the initial recorded weights was calculated for all samples.

Water holding capacity was measured by placing the second 8.26 cm diameter soil core from each plot onto a sieve with mesh size 0.150 mm, and lowering the netting to the surface of distilled water in a container (modified from Abed et al. 2013). Once the free water reached the upper surface of the sample via capillary movement, the samples were removed, allowed to drain completely, weighed, oven dried at 100˚C, and reweighed until a constant weight was reached. The dry weight was used to calculate the field capacity for each sample, expressed as the grams of water per gram of dry soil.

A nutrient analysis was performed for each plot, using soil remaining from the bulked soil cores used for the molecular analysis. Soils were oven dried at 60˚C, ground using a mortar and pestle, and sifted through a sieve with 2 mm mesh size. The prepared samples were sent to the University of Wisconsin – Madison Soil Testing Laboratories for analysis of percent organic material (OM), total nitrogen (TN), and total organic carbon (TOC), as well as the concentration of total available phosphorus (P), nitrate, and ammonium in ppm. Organic material was measured using the weight loss-on-ignition method at 360˚C (Combs and Nathan 1998; Schulte and Hopkins 1996). Samples were digested using sulfuric acid, metal catalyst, and salicylic acid for total nitrogen (Bremmer 1965; Ruzicka 1983). Total organic carbon was measured on a weight percent basis, using a LECO TruSpec CN combustion analyzer according to manufacturer instructions. Phosphorus concentrations were determined using the Bray method (Bray and Kurtz 1945). Nitrate and ammonium were extracted using potassium chloride and analyzed by flow injection (Lachat Instruments 1995; Ruzicka 1983). Nitrogen mole percentages for nitrate and ammonium were used to determine total available nitrogen (AN) for each sample. Soil property data were examined for normality and equality of variances and compared between crusted and uncrusted areas using paired t-tests in SPSS. Arcsine transformations were performed on TOC, erosion resistance, and water holding capacity data to meet the assumption for normality. The Wilcoxon signed-rank test was used to analyze TN data since it did not meet the assumption for normality.

RESULTS

Diversity and Structure of Soil Crust Microbial Communities

Alpha Diversity

The appearance of sampled crusts varied from flat brown cyanobacterial dominated crusts in two sites, to more developed moss/lichen dominated crusts in another (Table 1). All PCR amplifications were successful, with the exception of samples 4C1, 4C2, 6C3, 6U2, and 7U3, decreasing the sample sizes for those sites. Crusted samples contained greater alpha diversity than uncrusted samples for phylogenetic diversity (PD), observed species, and Chao1, but little difference for Shannon diversity (Figure 1). When rarifying to the number of sequences obtained for the least well-represented specimen, it appears that the accumulation curve was not saturated for some of the metrics (Figure 1, Figure 2). Further examination of the variation seen between replicate amplifications from the same soil samples showed the presence of outliers causing high variation among replicates (Figure 2).

Table 1. Descriptions of BSC types for seven paired plots at Fort McCoy State Natural Area in Wisconsin (USA).

Plot number	Description
1	Moss/lichen dominated crust
2	Moss dominated crust
3	Moss dominated crust
4	Flat brown cyanobacterial dominated crust
5	Flat brown cyanobacterial dominated crust
6	Moss dominated crust
7	Moss dominated crust

Significant variation was detected between replicate amplifications from the same plot as well as between different crusted and uncrusted samples (Figure 2, Appendix B). However, when the plot-level means of the four diversity measures for crusted vs. uncrusted areas were compared using paired t-tests, Chao1 ($t_6 = 6.228$, $p < 0.001$; Figure 3a), phylogenetic diversity ($t_6 = 3.5971$, $p = 0.0114$; Figure 3b), and observed species ($t_6 = 5.4747$, $p = 0.0016$; Figure 3c) were all significantly higher in crusted samples than uncrusted ones. Conversely, Shannon diversity showed no significant difference between crusted and uncrusted samples ($t_6 = 0.5015$, $p = 0.6339$; Figure 3d).

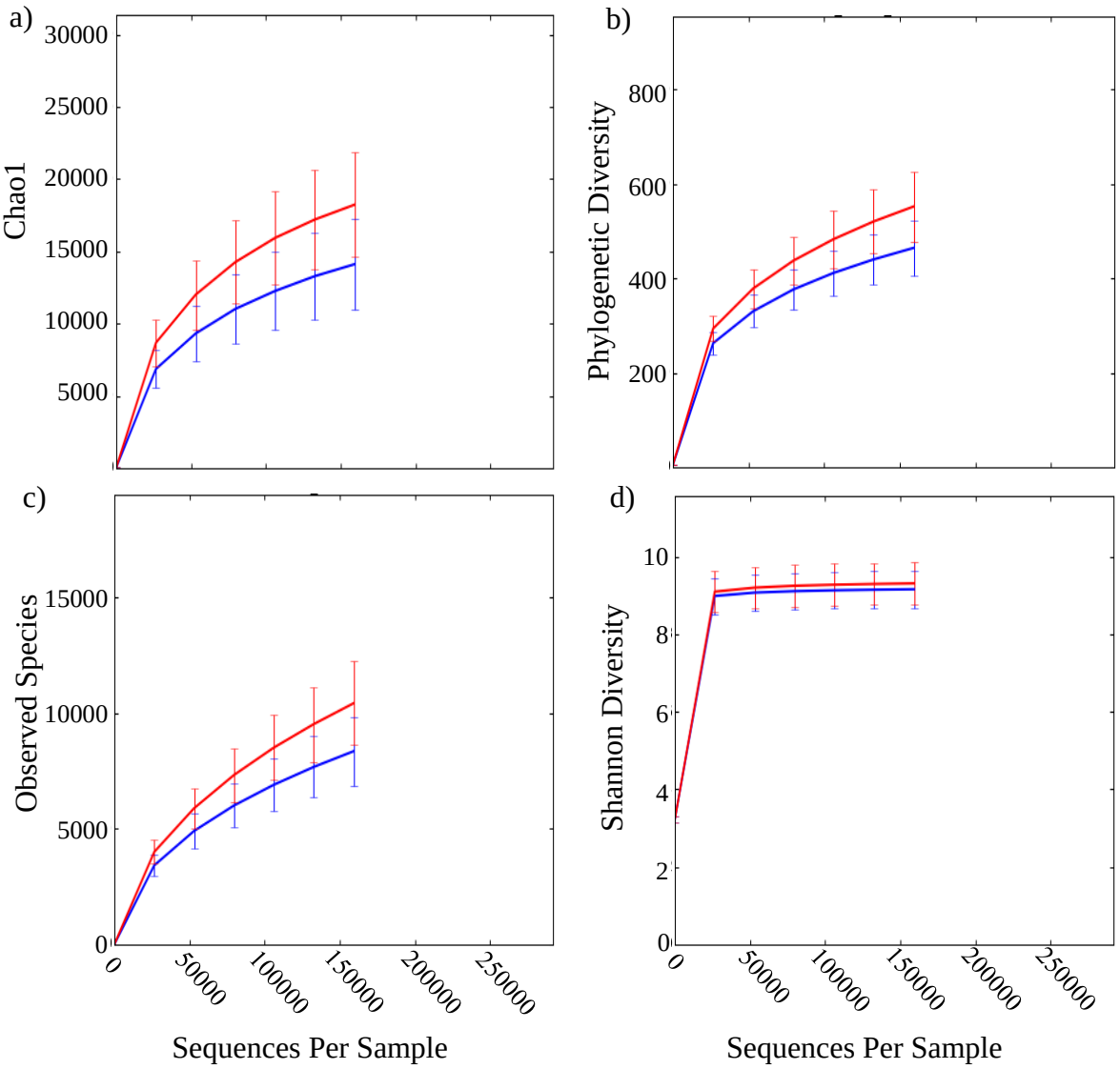


Figure 1. Rarefaction curves (± 1 SEM) comparing alpha diversity between crusted (red) and uncrusted (blue) samples measured by four indices, a) Chao1, b) Faith's phylogenetic diversity (PD), c) observed species, and d) Shannon diversity.

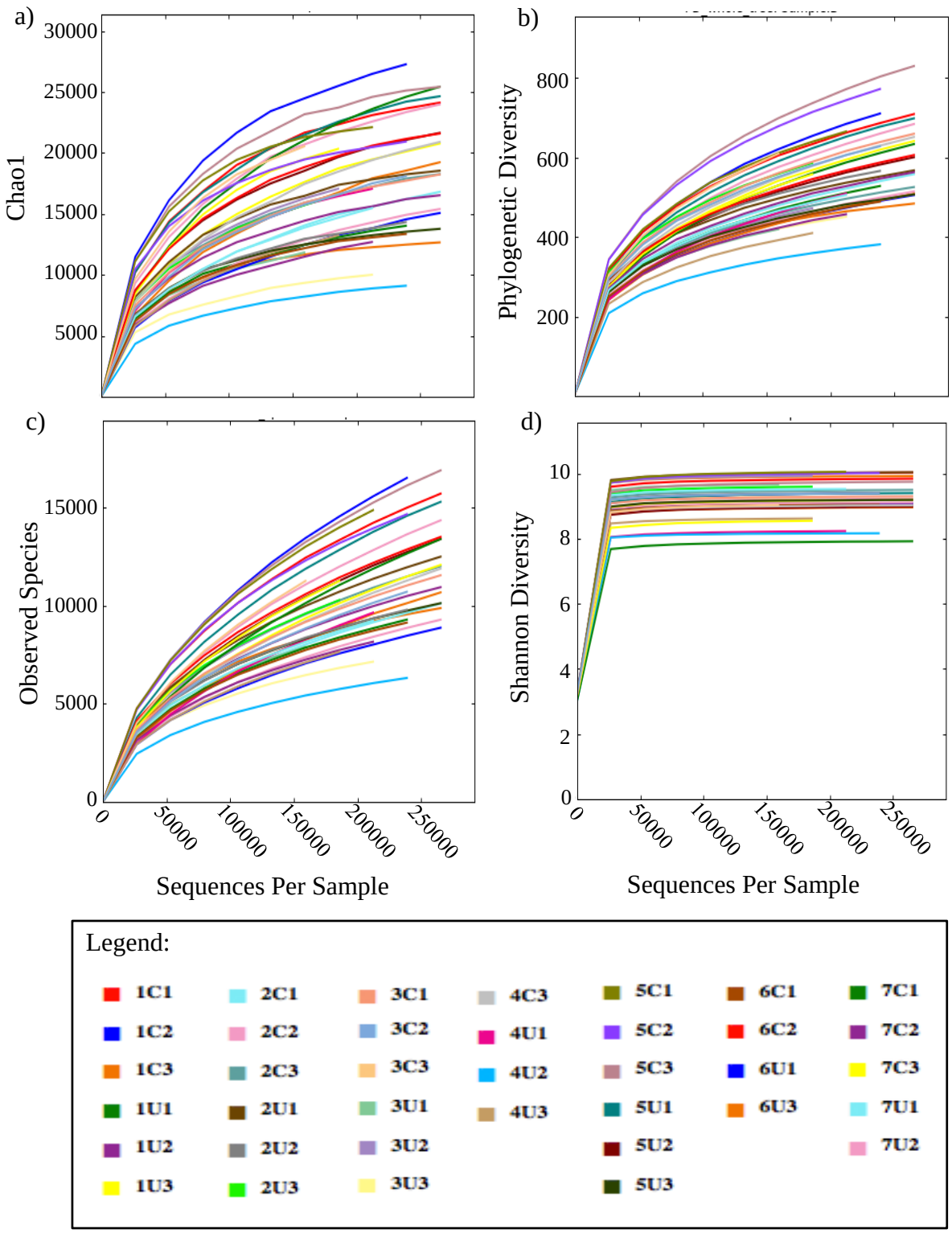


Figure 2. Rarefaction curves for sample level alpha diversity measured by four indices. Samples are labeled by site, group, and replicate, respectively (e.g. 1C1 is from site 1, crusted soil, DNA extract replicate 1, etc.).

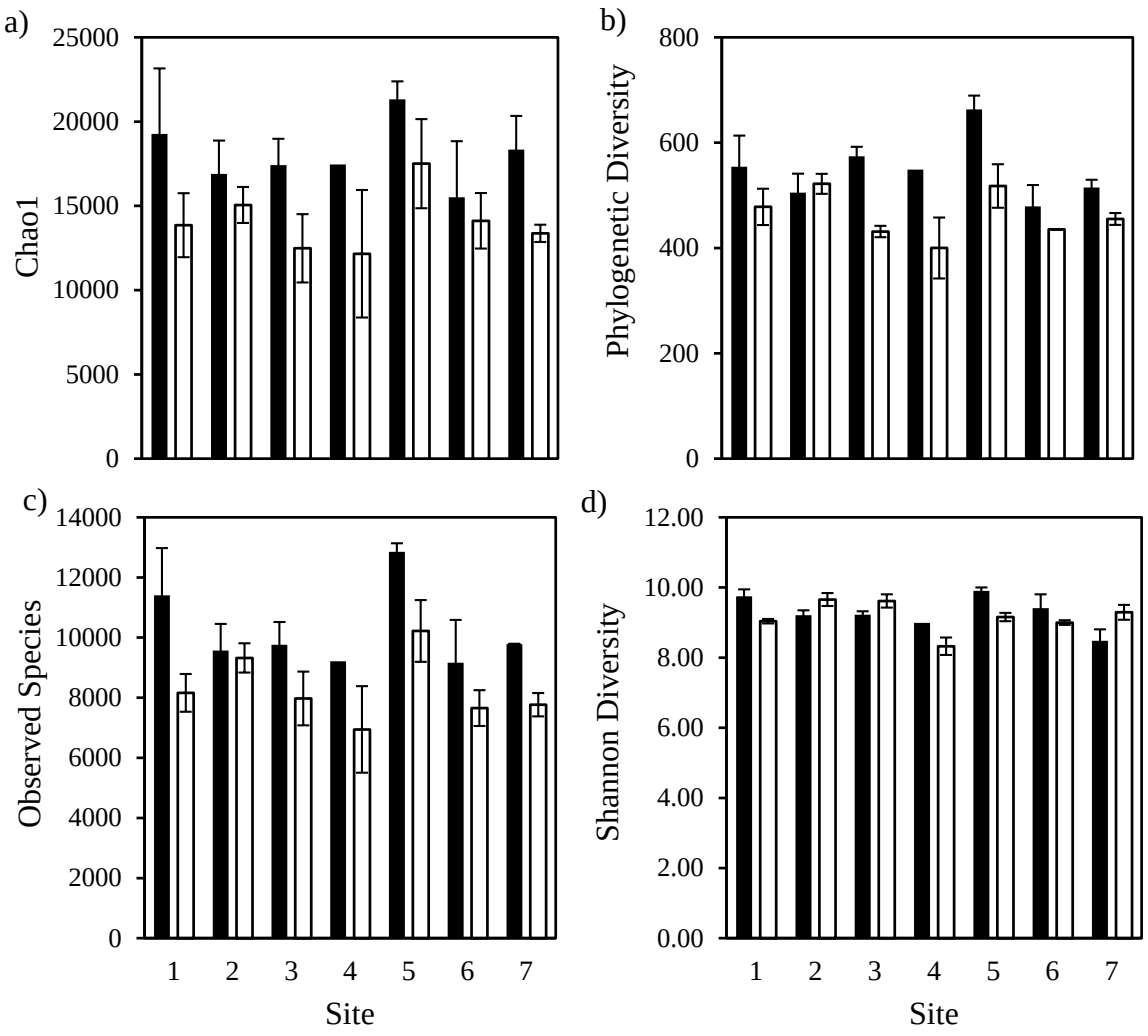
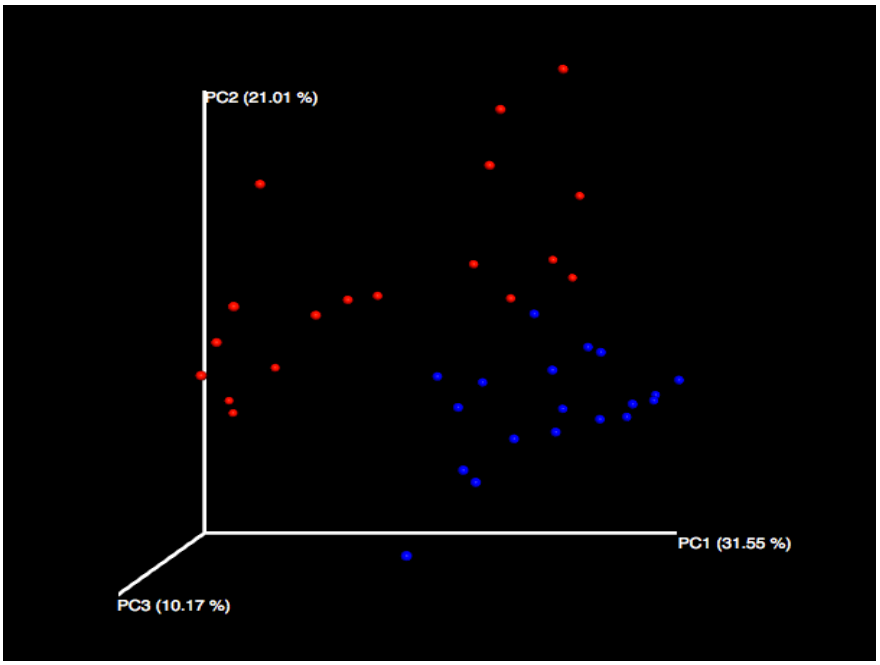


Figure 3. Mean (± 1 SEM) rarefaction estimates comparing alpha diversity between crusted (black) and uncrusted (white) samples measured by four indices. Rarefaction measures at 159184 sequences/sample were used to standardize all four indices. All samples had n=3 except 6C, 6U, and 7U with n=2, and 4C with n=1.

Beta Diversity

Comparisons of similarity in microbial community composition between samples, based on phylogenetic distances, distinguished crusted from uncrusted soils. Taxonomic composition among uncrusted samples was more similar than among crusted samples, as evidenced by tighter clustering of the former in PCoA plots (Figure 4). The first three PCoA axes described a higher proportion of the variation in taxonomic composition between samples using the weighted UniFrac distance compared to the unweighted UniFrac distance, where the weighted measure described 62.73% of the total variance (Figure 4a), and the unweighted measure described 25.22% of the total variance (Figure 4b). Both PCoA plots showed distinct clustering of uncrusted samples compared to crusted samples, but the clustering was more distinct using the weighted UniFrac measure. For the weighted UniFrac, the uncrusted samples contained less diversity than the crusted samples and were more strongly discriminated from the crusted samples by PC1 and PC3 (Figure 4a). The crusted samples were more diverse and were better discriminated by PC2 and PC3 (Figure 4a). The unweighted UniFrac yielded similar results, but described less variation within crusted and uncrusted samples with significant variation along PC1 for both groups, PC2 discriminating uncrusted samples from crusted samples, and PC3 discriminating crusted samples from uncrusted samples, respectively (Figure 4b). The uncrusted samples show higher similarity, as represented by tighter clustering compared to the crusted samples in both PCoA plots; however, tighter clustering was evident in the weighted ordination.

a)



b)

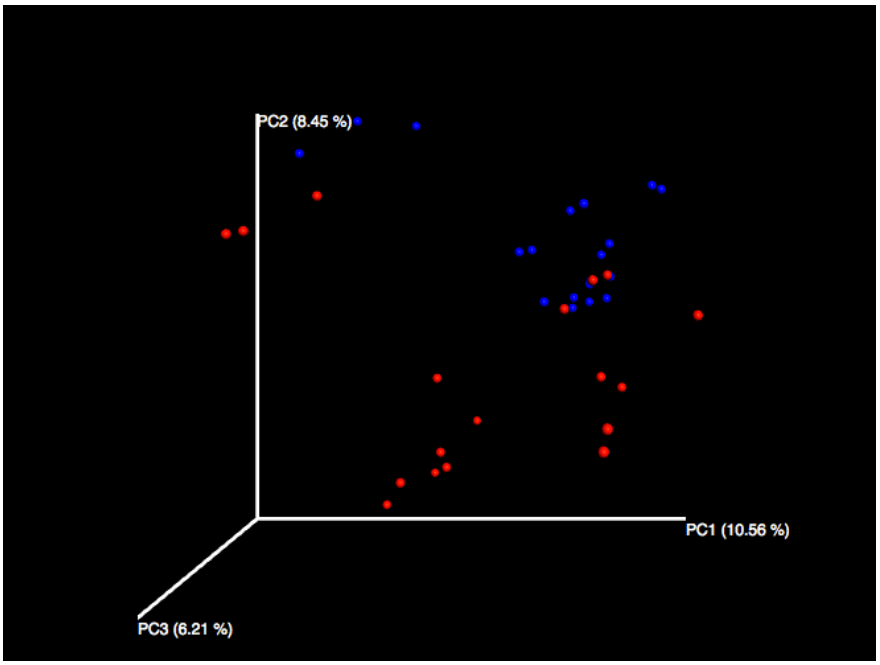


Figure 4. Principal Coordinate Analysis (PCoA) plots of crusted (red) and uncrusted (blue) samples based on both (a) weighted and (b) unweighted UniFrac distance matrices.

Taxonomic Diversity

Sequencing of 16S rRNA allowed for comparison of taxonomic composition of bacteria and eukaryotic chloroplasts for each sample; these comparisons revealed both differences and similarities between crusted and uncrusted soil communities. The most common phyla present across all samples include Cyanobacteria, Proteobacteria, Actinobacteria, Acidobacteria, Bacteriodetes, and Unassigned other with average abundances of 32.6%, 12.9%, 9.7%, 9.3%, 9.2%, and 8.60%, respectively (Figure 5). Actinobacteria present in replicates for plot 1U contained an average ($\pm$ 1 SEM) of 21.3 $\pm$ 3.8%, more than double the average across all samples (Figure 5). Additionally, there were high abundances of Chloroflexi in replicates for plots 1C, 2C, and 6C with averages ($\pm$ 1 SEM) of 9.3 $\pm$ 0.8%, 17.1 $\pm$ 0.7%, and 10.8 $\pm$ 0.3%, respectively.

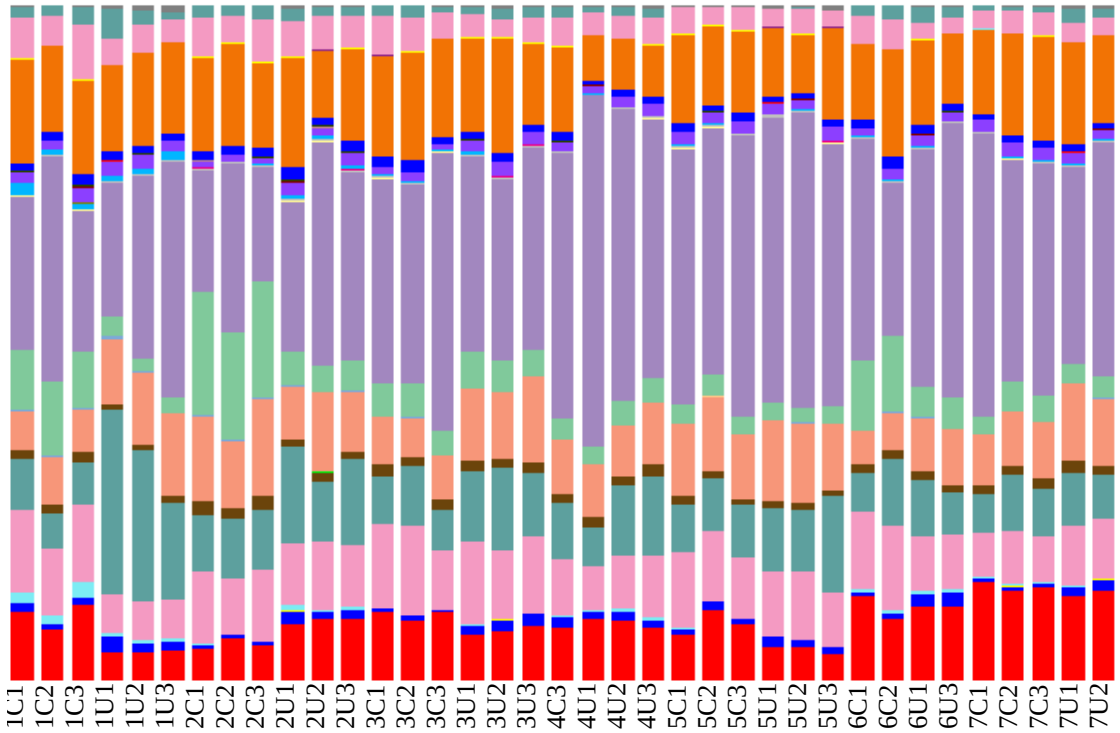


Figure 5. Taxonomic composition of bacterial phyla in crusted and uncrusted samples. As in Fig. 2, samples are labeled by site, group, and replicate, respectively.

Legend:

- Unassigned; **Other**
- Archaea; **Crenarchaeota**
- Bacteria; **AD3**
- Bacteria; **Acidobacteria**
- Bacteria; **Actinobacteria**
- Bacteria; **Armatimonadetes**
- Bacteria; **Bacteriodetes**
- Bacteria; **Chlamydiae**
- Bacteria; **Chlorobi**
- Bacteria; **Chloroflexi**
- Bacteria; **Cyanobacteria**
- Bacteria; **Elusimicrobia**
- Bacteria; **FBP**
- Bacteria; **Firmicutes**
- Bacteria; **Gemmatimonadetes**
- Bacteria; **Nitrospirae**
- Bacteria; **OD1**
- Bacteria; **TM7**
- Bacteria; **Verrucomicrobia**
- Bacteria; **WPS-2**
- Bacteria; **[Thermi]**

The most common classes present across crusted and uncrusted soil samples include chloroplast DNA and Nostocophycideae (phylum Cyanobacteria), Alphaproteobacteria (phylum Proteobacteria), and members of the phylum Actinobacteria (Figure 6). Alphaproteobacteria abundances, with an average of 9.4%, were consistent across all samples. Higher abundances were seen in crusted plots 1, 2, and 6 for the class Ktedonobacteria, (phylum Chloroflexi), with averages of 5.93% and 1.53% for crusted and uncrusted samples, respectively (Figure 6). Overall, abundances of chloroplast OTUs were highest in crusted samples compared to uncrusted samples with an average of 21.07% and 8.18%, respectively. In contrast, an unknown class within the phylum Cyanobacteria exhibited higher average abundances in uncrusted samples (6.82%) than crusted samples (1.78%; Figure 6). Similarly, Nostocophycideae (phylum Cyanobacteria) abundances were highest in uncrusted samples, specifically for plots 2U, 4U, 5U, and 6U (Figure 6) with an average of 9.98% for uncrusted samples, and 3.65% for crusted samples. A similar trend was seen for Synechococcophycideae, another class belonging to the phylum Cyanobacteria, having higher average abundances in uncrusted samples (6.38%) such as 2U, 4U, and 5U, compared to uncrusted samples (1.69%; Figure 6). Lastly, Actinobacteria abundances were highest in 1U with an average ($\pm$ 1 SEM) of 16.9 $\pm$ 3.0% compared to the across sample average of 6.5%, crusted sample average of 4.81%, and uncrusted sample average of 8.04%.

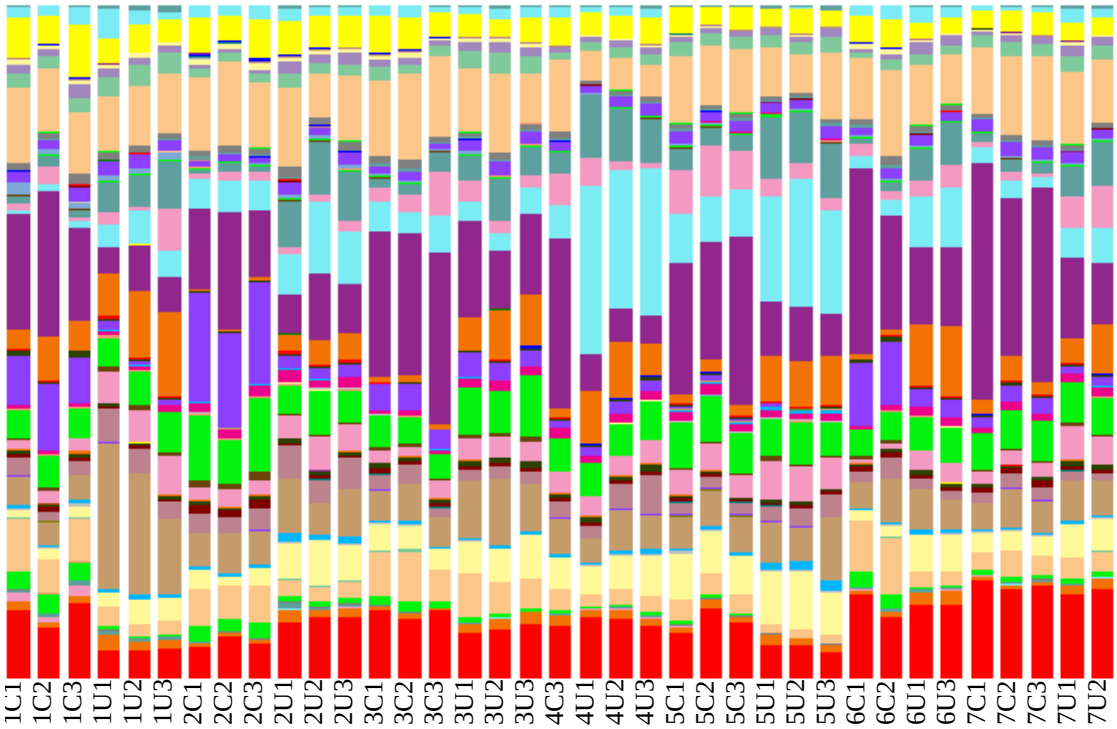


Figure 6. Taxonomic composition of bacterial classes in crusted and uncrusted samples. Samples are labeled by site, group, and replicate, respectively.

Legend:

- Unassigned; Other; **Other**
- Archaea; Crenarchaeota; **Thaumarchaeota**
- Bacteria; AD3; **ABS-6**
- Bacteria; AD3; **JG37-AG-4**
- Bacteria; Acidobacteria; **Acidobacteria**
- Bacteria; Acidobacteria; **Acidobacteriia**
- Bacteria; Acidobacteria; **Solibacteres**
- Bacteria; Acidobacteria; **Sva0725**
- Bacteria; Acidobacteria; **[Chloracidobacteria]**
- Bacteria; Acidobacteria; **iii1-8**
- Bacteria; Actinobacteria; **Acidimicrobiia**
- Bacteria; Actinobacteria; **Actinobacteria**
- Bacteria; Actinobacteria; **Rubrobacteria**
- Bacteria; Actinobacteria; **Thermoleophilia**
- Bacteria; Armatimonadetes; **Armatimonadia**
- Bacteria; Armatimonadetes; **Chthonomonadetes**
- Bacteria; Armatimonadetes; **[Fimbriimonadia]**
- Bacteria; Bacteriodetes; **Cytophagia**
- Bacteria; Bacteriodetes; **Sphingobacteriia**
- Bacteria; Bacteriodetes; **[Saprospirae]**
- Bacteria; Chlamydiae; **Chlamydiia**
- Bacteria; Chloroflexi; **Anaerolineae**
- Bacteria; Chloroflexi; **C0119**
- Bacteria; Chloroflexi; **Chloroflexi**
- Bacteria; Chloroflexi; **Ktedonobacteria**

Figure 6 legend continued:

- Bacteria; Chloroflexi; **TK10**
- Bacteria; Chloroflexi; **Thermomicrobia**
- Bacteria; Cyanobacteria; **Other**
- Bacteria; Cyanobacteria; **Unknown**
- Bacteria; Cyanobacteria; **Chloroplast**
- Bacteria; Cyanobacteria; **Nostocophycideae**
- Bacteria; Cyanobacteria; **Oscillatoriophycideae**
- Bacteria; Cyanobacteria; **Synechococcophycideae**
- Bacteria; Elusimicrobia; **Elusimicrobia**
- Bacteria; FBP; **Unknown**
- Bacteria; Firmicutes; **Bacilli**
- Bacteria; Gemmatimonadetes; **Gemmatimonadetes**
- Bacteria; Nitrospirae; **Nitrospira**
- Bacteria; Planctomycetes; **Phycisphaerae**
- Bacteria; Planctomycetes; **Planctomycetia**
- Bacteria; Planctomycetes; **vadinHA49**
- Bacteria; Proteobacteria; **Alphaproteobacteria**
- Bacteria; Proteobacteria; **Betaproteobacteria**
- Bacteria; Proteobacteria; **Deltaproteobacteria**
- Bacteria; Proteobacteria; **Gammaproteobacteria**
- Bacteria; TM7; **SC3**
- Bacteria; Verrucomicrobia; **Opitutae**
- Bacteria; Verrucomicrobia; **[Spartobacteria]**
- Bacteria; WPS-2; **Unknown**
- Bacteria; [Thermi]; **Deinococci**

Taxonomic composition also varied considerably at the ordinal level when comparing crusted and uncrusted samples. The Solibacterales, an order belonging to the phylum Acidobacteria, is one group showing such a pattern. On average, crusted samples contained higher abundances of Solibacterales (5.05%) than uncrusted samples (2.64%), with plots 1C, 2C, 3C, 3U, and 6C having the highest abundances (Figure 7). Trends for abundances of cyanobacterial orders varied between crusted and uncrusted samples. There were more Chlorophyta in crusted samples, except plots 2C/2U and 3C/3U, with averages of 11.49% in crusted samples and 6.16% in uncrusted samples, with the highest abundance occurring in plot 7C with an average ($\pm$ 1 SEM) of 26.4 $\pm$ 2.5% (Figure 7), suggesting the presence of eukaryotic organisms. Additionally, there were more Streptophyta present in crusted samples with an average abundance of 9.33% in crusted samples compared to 1.72% in uncrusted samples. However, some cyanobacterial orders were higher in abundance in uncrusted samples. For example, Nostocales comprised 22.0 + 1.3% of sequences in plot 4U and 16.5 + 1.2% in plot 5U with high abundances also present in plots 1U, 2C, 2U, 6U, and 7U, and an average abundance of 6.70% for all samples, 9.72% for uncrusted samples, and 3.52% for crusted samples (Figure 7). Both Synechococcales, with sample 5U1 having the highest amount at 7.2%, and an unknown cyanobacterial order comprising on average 1.78% in crusted samples and 6.82% in uncrusted samples, were also more prevalent in uncrusted samples (Figure 7). Surprisingly, *Microcoleus*, a common cyanobacterium and primary colonizer of BSCs, was absent in all samples except 4U1, 4U2, 5C1, 5C2, and 5C3. Crusted samples, including plots 1C, 2C, 3C, and 6C, contained lower abundances of the order RB41 (phylum Acidobacteria) compared to the respective uncrusted samples, but in some cases

there was no difference (Figure 7). Another difference between communities was the high abundance of Ktedonobacterales (phylum Chloroflexi) for plots 2C and 6C with 12.8 $\pm$ 1.2% and 5.8 $\pm$ 0.1%, respectively, compared to abundances below approximately 0.5% for most other samples (Figure 7). Consistent trends across all samples included Actinomycetales (phylum Actinobacteria) with an average of 6.5% and the highest abundance in plot 1U at 16.93 $\pm$ 3.0%. Additionally, Chthoniobacterales (phylum Verrucomicrobia) and Rhodospirillales (phylum Proteobacteria) were consistent across all samples, with abundances of 3.8% and 4.5%, respectively (Figure 7).

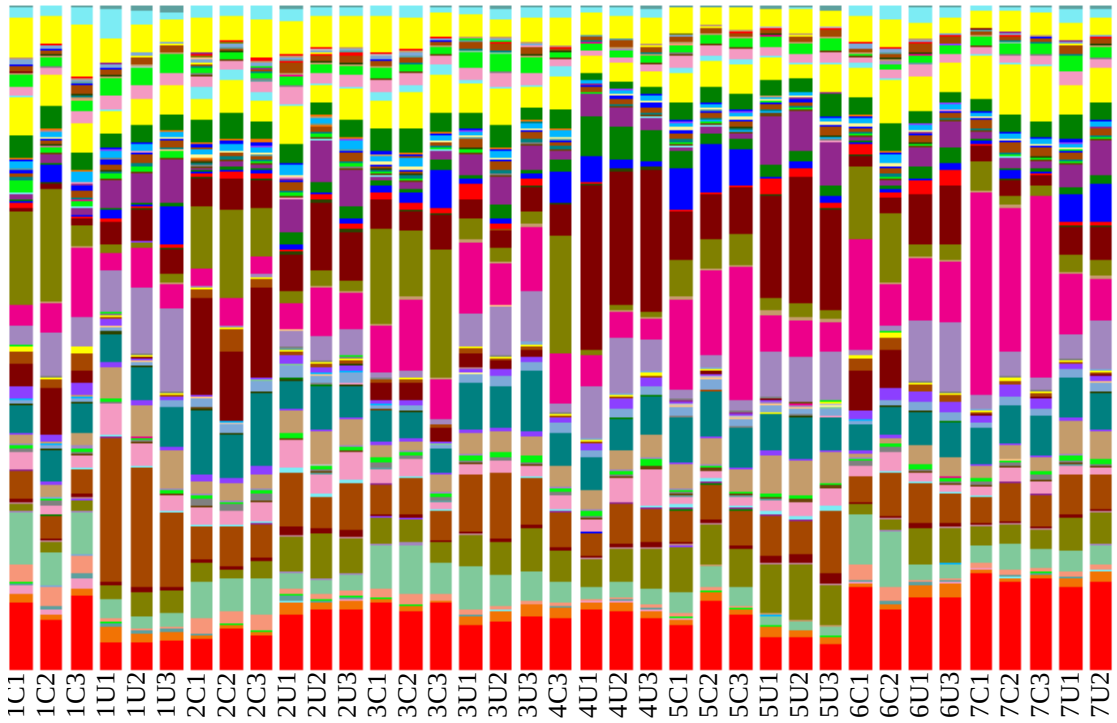


Figure 7. Taxonomic composition of bacterial orders in crusted and uncrusted samples. Samples are labeled by site, group, and replicate, respectively.

Legend:

- Unassigned; Other; Other; **Other**
- Archaea; Crenarchaeota; Thaumarchaeota; **Nitrososphaerales**
- Bacteria; AD3; ABS-6; **Unknown**
- Bacteria; AD3; JG37-AG-4; **Unknown**
- Bacteria; Acidobacteria; Acidobacteria; **iii1-15**
- Bacteria; Acidobacteria; Acidibacteriia; **Acidobacteriales**
- Bacteria; Acidobacteria; Solibacteres; **Solibacterales**
- Bacteria; Acidobacteria; Sva0725; **Sva0725**
- Bacteria; Acidobacteria; [Chloracidobacteria]; **RB41**
- Bacteria; Acidobacteria; iii1-8; **DS-18**
- Bacteria; Actinobacteria; Acidimicrobiia; **Acidimicrobiales**
- Bacteria; Actinobacteria; Actinobacteria; **Actinomycetales**
- Bacteria; Actinobacteria; Rubrobacteria; **Rubrobacterales**
- Bacteria; Actinobacteria; Thermoleophilia; **Gaiellales**
- Bacteria; Actinobacteria; Thermoleophilia; **Solirubrobacterales**
- Bacteria; Armatimonadetes; Armatomonadia; **Armatimonadales**
- Bacteria; Armatimonadetes; Armatimonadia; **FW68**
- Bacteria; Armatimonadetes; Chthonomonadetes; **Chthonomonadales**
- Bacteria; Armatimonadetes; [Fimbriimonadia]; **[Fimbriimonadales]**
- Bacteria; Bacteriodetes; Cytophagia; **Cytophagales**
- Bacteria; Bacteriodetes; Sphingobacteriia; **Sphingobacteriales**
- Bacteria; Bacteriodetes; [Saprospirae]; **[Saprospirales]**
- Bacteria; Chlamydiae; Chlamydiia; **Chlamydiales**
- Bacteria; Chloroflexi; C0119; **Unknown**
- Bacteria; Chloroflexi; Chloroflexi; **AKIW781**

Figure 7 legend continued:

- Bacteria; Chloroflexi; Ktedonobacteria; **B12-WMSP1**
- Bacteria; Chloroflexi; Ktedonobacteria; **Ktedonobacterales**
- Bacteria; Chloroflexi; Ktedonobacteria; **Thermogemmatisporales**
- Bacteria; Chloroflexi; TK10; **B07_WMSP1**
- Bacteria; Chloroflexi; Thermomicrobia; **Ellin6537**
- Bacteria; Cyanobacteria; Other; **Other**
- Bacteria; Cyanobacteria; Unknown; **Unknown**
- Bacteria; Cyanobacteria; Chloroplast; **Chlorophyta**
- Bacteria; Cyanobacteria; Chloroplast; **Stramenopiles**
- Bacteria; Cyanobacteria; Chloroplast; **Streptophyta**
- Bacteria; Cyanobacteria; Nostocophycideae; **Nostocales**
- Bacteria; Cyanobacteria; Nostocophycideae; **Stigonematales**
- Bacteria; Cyanobacteria; Oscillatoriophycideae; **Chroococcales**
- Bacteria; Cyanobacteria; Oscillatoriophycideae; **Oscillatoriales**
- Bacteria; Cyanobacteria; Synechococcophycideae; **Pseudanabaenales**
- Bacteria; Cyanobacteria; Synechococcophycideae; **Synechococcales**
- Bacteria; Elusimicrobia; Elusimicrobia; **FAC88**
- Bacteria; FBP; Unknown; **Unknown**
- Bacteria; Firmicutes; Bacilli; **Bacillales**
- Bacteria; Gemmatimonadetes; Gemmatimonadetes; **Unknown**
- Bacteria; Gemmatimonadetes; Gemmatimonadetes; **Ellin5290**
- Bacteria; Gemmatimonadetes; Gemmatimonadetes; **Gemmatimonadales**
- Bacteria; Gemmatimonadetes; Gemmatimonadetes; **N1423WL**
- Bacteria; Nitrospirae; Nitrospira; **Nitrospirales**
- Bacteria; Planctomycetes; Phycisphaerae; **WD2101**
- Bacteria; Planctomycetes; Planctomycetia; **Gemmatales**
- Bacteria; Planctomycetes; vadinHA49; **DH61**
- Bacteria; Proteobacteria; Alphaproteobacteria; **Caulobacterales**
- Bacteria; Proteobacteria; Alphaproteobacteria; **Ellin329**
- Bacteria; Proteobacteria; Alphaproteobacteria; **Rhizobiales**
- Bacteria; Proteobacteria; Alphaproteobacteria; **Rhodospirillales**
- Bacteria; Proteobacteria; Alphaproteobacteria; **Rickettsiales**
- Bacteria; Proteobacteria; Alphaproteobacteria; **Sphingomonadales**
- Bacteria; Proteobacteria; Betaproteobacteria; **Unknown**
- Bacteria; Proteobacteria; Betaproteobacteria; **A21b**
- Bacteria; Proteobacteria; Betaproteobacteria; **Burkholderiales**
- Bacteria; Proteobacteria; Betaproteobacteria; **SC-I-84**
- Bacteria; Proteobacteria; Deltaproteobacteria; **MIZ46**
- Bacteria; Proteobacteria; Deltaproteobacteria; **Myxococcales**
- Bacteria; Proteobacteria; Deltaproteobacteria; **Spirobacillales**
- Bacteria; Proteobacteria; Gammaproteobacteria; **Legionellales**
- Bacteria; Proteobacteria; Gammaproteobacteria; **Xanthomonadales**
- Bacteria; TM7; SC3; **Unknown**
- Bacteria; Verrucomicrobia; Opitutae; **Opitutales**
- Bacteria; Verrucomicrobia; [Spartobacteria]; **[Chthoniobacterales]**
- Bacteria; WPS-2; Unknown; **Unknown**
- Bacteria; [Thermi]; Deinococci; **Deinococcales**

Soil Properties

Crusted soil samples were significantly more resistant to erosion than uncrusted samples after two months of identical environmental conditions ($t_6 = 7.906$, $p < 0.001$; Figure 8a). On average, uncrusted samples lost nearly six times as much proportional mass as uncrusted samples (Table 2). However, there was no statistically significant difference ($t_6 = 2.181$, $p = 0.072$) in water holding capacity observed between crusted and uncrusted samples (Figure 8b). Additionally, there appeared to be a site-specific interaction occurring for water holding capacity that varied with type of BSC. Crusted samples from plot 1, a moss/lichen BSC, and plot 4, a flat brown cyanobacterial BSC, appeared to hold the same amount of water as their uncrusted pair (Figure 8). Conversely, crusted samples from plots 2, 3, 6, and 7, all moss BSCs, as well as plot 5, a flat brown cyanobacterial BSC, appeared to hold more water than their paired uncrusted plot (Figure 8).

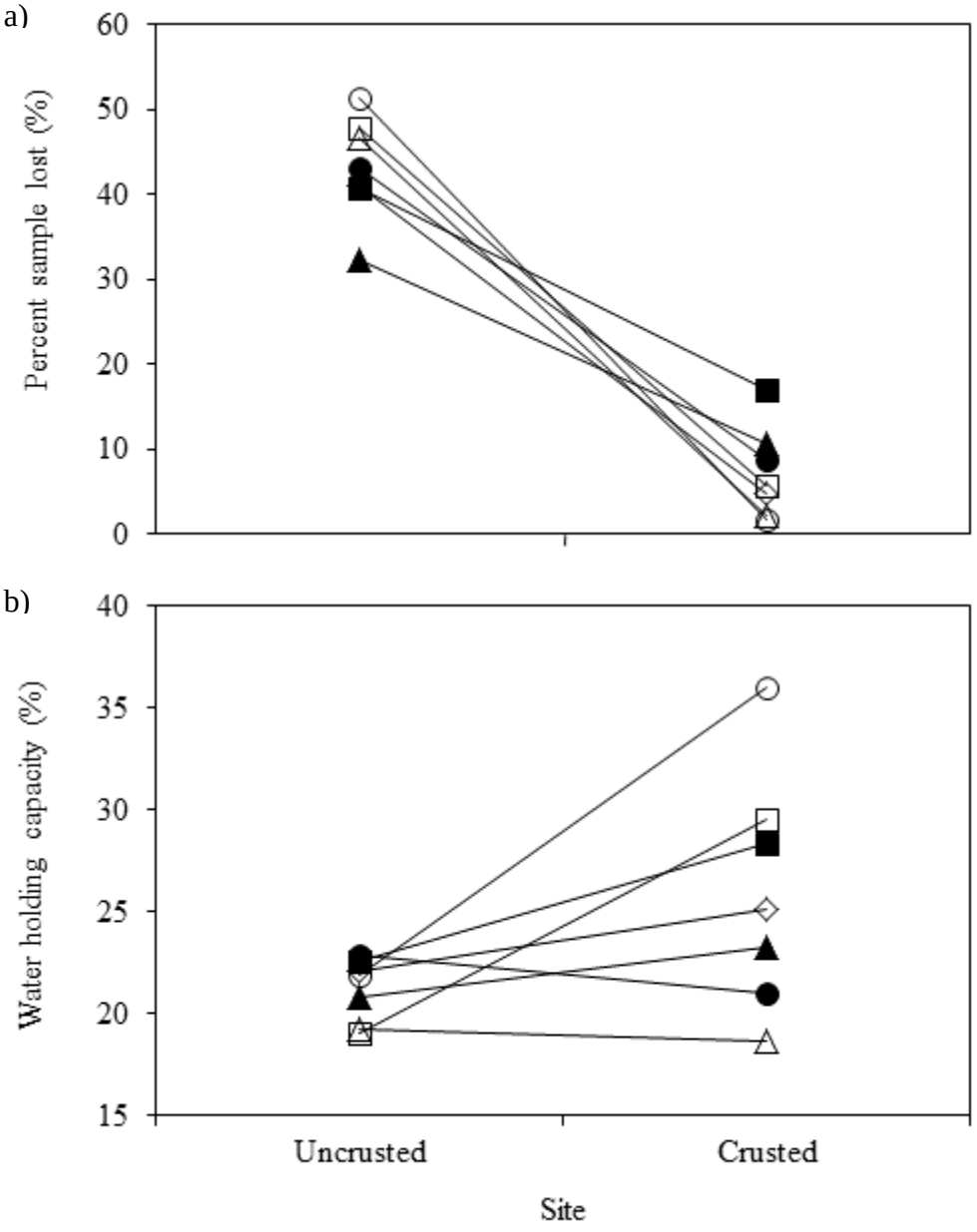


Figure 8. Erosion resistance measured as (a) percent sample lost and water holding capacity measured as (b) percent water held relative to sample weight for seven paired plots at Fort McCoy State Natural Area Oak Barrens in Wisconsin, collected April 2015. Sites were selected by identifying areas where biological soil crust (BSC) had been removed by a disturbance (uncrusted) and pairing it with the earliest adjacent recovering crusted areas (crusted). Cyanobacterial dominated BSCs were found in plots 4 (closed circle) and 5 (open diamond), moss dominated BSCs were found in plots 2 (closed triangle), 3 (open square), 6 (open circle), and 7 (closed square), and moss/lichen dominated BSC was found in plot 1 (open triangle).

Table 2. Mean (± 1 SEM) soil chemical and physical characteristics for seven paired plots at Fort McCoy State Natural Area in Wisconsin (USA), collected April 2015. Sites were selected by identifying areas where biological soil crust (BSC) had been removed by a disturbance (uncrusted) and pairing it with the earliest adjacent recovering crusted areas (crusted).

Plot type	Organic material (%)	Total organic carbon (%)	Total nitrogen (%)	Available nitrogen (ppm)	Available phosphorus (ppm) [a]	Erosion resistance (%) [b]	Water holding capacity (%) [c]
Uncrusted	0.3 ± 0.02	0.10 ± 0.02	0.01 ± 0.01	2.1 ± 0.2	24 ± 4	43.30 ± 2.34	21.23 ± 0.63
Crusted	0.8 ± 0.08	0.40 ± 0.08	0.04 ± 0.00	2.2 ± 0.2	20 ± 4	7.29 ± 2.03	25.99 ± 2.39

[a] Bray method
[b] Measured as percent sample lost
[c] Measured as percent water held relative to sample weight

Crusted samples differed from uncrusted samples with significantly higher percentages of OM ($t_6 = 7.066$, $p < 0.001$; Figure 9a) and TOC ($t_6 = 3.842$, $p = 0.008$; Figure 9b). Organic material concentrations were over two times greater in crusted samples than in uncrusted samples, and crusted samples contained three times more TOC (Table 2). Moreover, there was a significant difference for mean TN ($Z = 2.384$, $p = 0.004$; Figure 9c) where crusted samples contained four times more TN than uncrusted samples (Table 2), and three of the uncrusted soil values were below the detection limit of 0.01 ppm. Both crusted and uncrusted samples had similar concentrations of AN ($t_6 = 0.554$, $p = 0.600$; Figure 9e). Differences in available P concentrations between crusted and uncrusted samples were non-significant ($t_6 = 1.011$, $p = 0.351$; Figure 9d; Table 2).

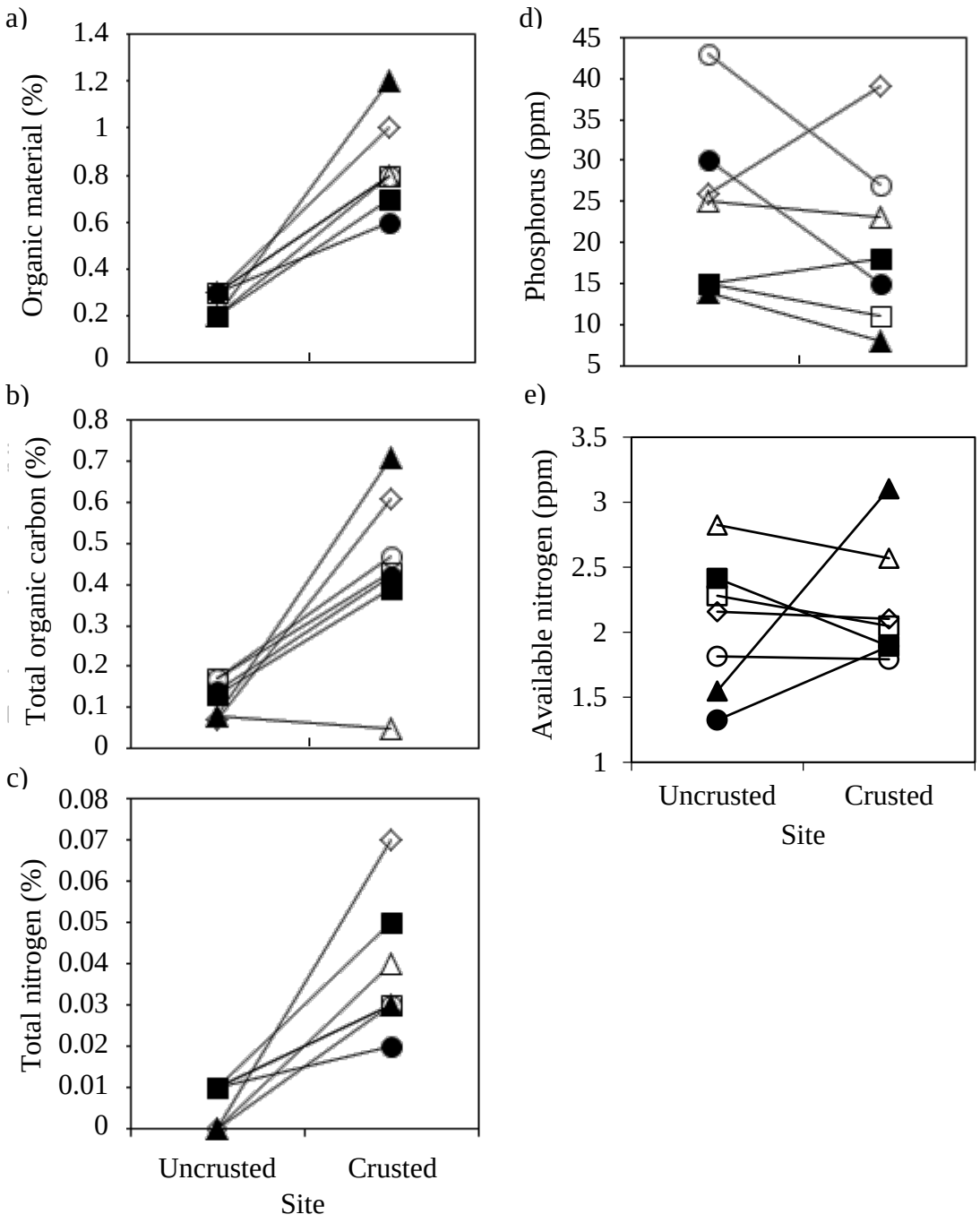


Figure 9. Organic material (a), total organic carbon (b), and total nitrogen (c) percentages and phosphorus (d) and available nitrogen (e) concentrations for seven paired sites at Fort McCoy State Natural Area Oak Barrens in Wisconsin, collected April 2015. Sites were selected by identifying areas where biological soil crust (BSC) had been removed by a disturbance (uncrusted) and pairing it with the earliest adjacent recovering crusted areas (crusted). Cyanobacterial dominated BSCs were found in plots 4 (closed circle) and 5 (open diamond), moss dominated BSCs were found in plots 2 (closed triangle), 3 (open square), 6 (open circle), and 7 (closed square), and moss/lichen dominated BSC was found in plot 1 (open triangle).

DISCUSSION

This study aimed to quantify differences between uncrusted and crusted soils at sites where disturbances resulted in areas where BSC had been removed and crusts were in the earliest stage of re-establishment. Overall, our results indicate that crusted soils differ from their respective uncrusted soils in microbial diversity and several structural and functional characteristics.

Diversity of Soil Crust Microbial Communities

Alpha Diversity

As expected, crusted samples contained significantly greater bacterial and archaeal diversity than uncrusted samples, as measured by Faith's phylogenetic diversity, Chao1, and observed species richness (Figure 3). Many studies have demonstrated a similar increase in microbial species richness over successional time, as species colonize disturbed areas; however, the time frame in which this process happens varies depending on abiotic factors, such as potential evapotranspiration (Belnap 2003). Additionally, site stability, elevation coupled with precipitation, as well as the frequency and severity of disturbances can affect the recovery sequence of disturbed BSCs (Belnap 2003). For example, estimated years for recovery of cyanobacteria biomass in sites where BSC was completely removed varied from 50-100 years in the Mojave Desert to 14-34 years in the Colorado Plateau (Belnap 2003).

Beta Diversity

Similarly, when compared between plots and across samples, crusted samples were more diverse and less similar to each other, while uncrusted samples were less diverse and more similar to each other. This trend was seen with both the weighted and unweighted UniFrac phylogenetic distance metrics (Figure 4). Differing disturbance and recolonization histories for each of the seven pairs of plots may explain why the crusted areas were more different from one another than uncrusted areas. Steven et al. (2015) also found that bacterial community composition changed after various disturbances to a long-term field experiment. Over time, repeated trampling led to decreases in both bacterial, and more specifically, cyanobacterial abundances. Alternatively, other disturbances such as soil warming had little effect on bacterial community composition, and a combination of soil wetting and warming resulted in a greatly altered bacterial community and decreased cyanobacterial abundances (Steven et al. 2015). Results from both this study and Steven et al. (2015) suggest that different disturbance histories lead to different kinds of communities as BSCs recover.

The weighted UniFrac analysis, which indicated a stronger difference among sample communities, emphasizes abundant OTUs, whereas the unweighted analysis gives equal weight to all OTUs, which can cause the rare OTUs to be overemphasized. Furthermore, the weighted analysis provides a quantitative measure of diversity among plots which can likely be associated with ecosystem function. In contrast, the unweighted analysis provides a qualitative measure and is useful in identifying the presence of factors that may be limiting the presence of taxa across all plots (Lozupone and Knight 2008), such as soil characteristics, climate, and disturbance (Belnap 2003).

Finally, it is important to note that our replicate amplifications revealed a high level of variation within individual plots; a single amplification would not provide an accurate depiction of taxonomic structure or beta diversity. To capture maximum heterogeneity of samples, using replicate amplifications, in addition to increasing sequencing depth for each amplification, would provide a more accurate analysis of within-sample diversity, as well as between-sample diversity and taxonomic structure of BSCs.

Taxonomic Diversity

Taxonomic composition among replicate samples and among plots varied considerably. The major bacterial phyla present across all samples, including Cyanobacteria, Proteobacteria, Actinobacteria, Acidobacteria, and Bacteriodetes, were similar to the major bacterial phyla found in the Sonoran Desert in Arizona and the Colorado Platueau (Nagy et al. 2005). Examination of the composition of classes and orders of bacteria present in each sample show greater variation between crusted and uncrusted samples than was observed at the phylum level. This suggests that BSCs in uncrusted soils are either recovering from previous disturbances, or being invaded by nearby species, and that the time and level of these disturbances may have varied across samples, which is reflected in site specific trends. The presence of chloroplast OTUs suggests the presence of green algae or mosses, which was supported by the visible presence of mosses in samples collected at plots 1, 2, 3, 6, and 7. The large proportion of Cyanobacteria present in both crusted and uncrusted samples suggests that, as expected, the BSCs we sampled in crusted soils are in an early stage of succession. Higher abundances of members from Nostocales, including *Nostoc*, in uncrusted samples

suggests that this cyanobacterium may be partially responsible for the initial soil formation rather than *Microcoleus*, as the latter was absent in nearly all samples. *Nostoc*, a common BSC organism, can be found in all types of habitats and spends a majority of its life cycle on the soil surface (Belnap et al. 2001). An unknown class of cyanobacteria with higher abundances in uncrusted plots may also be responsible for the earliest colonization of BSCs at this study site. Proteobacteria, members of the second largest phylum present in both crusted and uncrusted samples, contain species which are both phototrophic and diazotrophic. Carbon- and nitrogen-fixing members of both Cyanobacteria and Proteobacteria play a large role in N and C fixation (Chen et al. 2003, Nagy et al. 2005), which would be important in the recovery of both disturbed BSCs and early successional BSCs. In addition, the relatively large abundance of Actinobacteria is also likely to be important for the recovery of BSCs here as members of this phylum play roles in OM decomposition, which would aid in nutrient cycling. Future work examining past, annual aerial photographs of the study site would be useful to determine the age of disturbance and its possible effect on taxonomic composition and diversity. Our failed attempts to amplify fungal DNA suggest that fungi are not yet major components of either the crusted or uncrusted soils at this site, or that the methods used in this study were unable to successfully amplify fungal DNA present in the samples. This trend is not consistent with the literature, where fungi often are found in fewer numbers but greater biomass than bacteria (Belnap 2001). Further work examining the presence of fungi in BSCs associated with sand prairie should be pursued.

Soil Properties

We found strong evidence that BSCs in Wisconsin sand prairies alter soil properties in positive ways. For example, erosion resistance was significantly higher in samples from crusted plots (Table 2). The presence and increased diversity of organisms in crusted soils therefore appears to aid in stabilizing the soil surface compared to uncrusted soils. Many crust species secrete polysaccharides that bind soil particles together and increase soil stability (Belnap 2003). The amount of polysaccharide increases with increasing crust biomass (Belnap 2003), and is also likely to increase with the successional stage of the BSC. For example, Abed et al. (2013) found that after performing soil erosion experiments, uncrusted soils lost 28.16 $\pm$ 6.0% of their mass compared to cyanobacterial crusts that lost 6.17 $\pm$ 1.2% and lichen crusts that lost 3.12 $\pm$ 2.1% in the Sultanate of Oman. Similar results were found in this study, where uncrusted soil samples lost 43.30 $\pm$ 2.34% of their mass compared to crusted soil samples, which lost 7.29 $\pm$ 2.03% (Table 2). The areas used in this study, containing the earliest-recovering BSC with minimal crust formation at each plot, ranged from early-successional cyanobacterial BSCs to more developed crusts including lichens and mosses. Future studies should further examine and compare different successional stages to disturbed areas without crust to separate different effects of biodiversity on erosion resistance.

While there was no significant difference between uncrusted and crusted samples for water holding capacity; five samples from crusted plots appeared to hold more water than their paired uncrusted sample. Of the remaining two pairs where samples from uncrusted plots appeared to hold a similar amount of water, the crust types present were

moss/lichen (open triangle) and flat, brown cyanobacteria (closed circle; Figure 8b). Surveys of the literature have also shown variation in the effects of BSC on hydrological processes. For example, Abed et al. (2013) found that crusted soils held more water at field capacity compared to uncrusted soils. In contrast, Castillo-Monroy and Maestre (2011) found that while some BSCs increased water infiltration and decreased runoff, others decreased water infiltration, or had no effect on these factors. Additionally, Belnap (2006) further separated effects of BSCs on water infiltration and runoff based on BSC type and found a decrease in infiltration and increase in runoff for BSCs in hyperarid regions, conflicting results in arid regions, and an increase in infiltration and a decrease in runoff in semiarid regions. Differences in soil particle size distribution may also mediate the effect of soil crust development on soil hydrology. In this study, there may have been site specific interactions occurring, indicating that abiotic factors such as soil particle size may be affecting both water holding capacity and erosion resistance based on the overlap of slopes for crusted and uncrusted soils across plots. Future studies should address the role that species diversity plays in addition to abiotic factors in hydrological processes in Wisconsin BSCs.

BSCs play a significant role in C and N fixation in arid and semi-arid ecosystems. Belnap and Lange (2003) found that cyanobacteria present in BSCs secreted approximately 50% of the C fixed during photosynthesis into the surrounding soil, leading to a 300% increase of organic carbon. The same trend was seen in this study, with crusted samples containing 400% OM and more than 250% TOC compared to uncrusted samples (Table 2). All crusted samples also contained more TN than uncrusted samples, but no significant difference was seen for AN. Furthermore, measures for TN for three

uncrusted plots were below the detection limit of 0.01ppm, suggesting that BSC organisms play a significant role in N fixation. These results likely demonstrate that N is being fixed by some BSC organisms in the crusted soils compared to uncrusted soils and also being held within living organisms, resulting in no difference in the amount available as ammonium or nitrate in the soil for uncrusted soils.

BSC organisms are the main biological contributors of N in arid and semi-arid regions (Castillo-Monroy and Maestre 2011) for two reasons. First, some BSC organisms fix N, which increases the fertility of these soils. Belnap (2002) found that approximately 80% of fixed N is released to the surrounding soil in as little as minutes to hours, where other BSC organisms can utilize it. Secondly, polysaccharides secreted by cyanobacteria not only aid in stabilizing soils, but their negative charge aids in retention of important positively charged nutrients (Belnap 2003). The presence of Cyanobacteria and Proteobacteria found in the crusted and uncrusted soils in this study – some of which can fix N and C – in addition to the deposition of polysaccharides in the soil surface by Cyanobacteria, further support these trends seen in arid and semi-arid regions.

The positive effect of BSC organisms on N cycling, such as increased total nitrogen in soils, was seen in this study and has been well documented in the literature. Hawkes (2003) examined the effects of BSCs and arbuscular mycorrhizal fungi (AMF) on N cycling in Florida, and found that N fixed by cyanobacteria was retained within the BSC rather than leaching out of the system, ultimately resulting in additional N uptake by heterotrophic bacteria, AMF, and vascular plants. While trends between successional stage and species diversity on soil properties have been identified in BSCs in the United

States, this information is lacking in the Midwest. More work needs to be done to determine the roles of BSC organisms in N cycling in Wisconsin sand prairie.

In addition to C and N fixation, BSCs play an important role in nutrient capture. In arid and semi-arid ecosystems where concentrations of OM are low, the presence of BSC is often associated with an increase in OM and other important nutrients such as nitrogen, phosphorus, and potassium (Belnap 2003) via dust trapping with increased roughness of the soil surface by BSCs. While there was no significant difference between phosphorus concentrations in crusted and uncrusted samples at 24 ppm and 20 ppm, respectively, another study performed in Wisconsin in a candidate meadow for sand prairie restoration found ambient phosphorus concentrations at 22 ppm (Paluch et al. 2012). These similar values suggest that the phosphorus concentrations found at our study site are consistent with other sand prairies in western Wisconsin. Future studies focusing on successional stage and resulting crust morphology on dust trapping would provide better insight into the roles BSCs play in nutrient capture in Wisconsin sand prairie.

Here we have demonstrated that even very early-successional BSCs play a positive role in soil stability and fertility for sand prairie habitat. This study has also identified taxa that likely promote future crust development and could be used for both conservation and restoration of sand prairie habitat in Wisconsin. Finally, understanding the biodiversity and effects of BSCs on soil properties provided a baseline for comparisons of BSCs in the Midwest to those in similar systems around the world. Future studies should further examine the roles that BSCs play on sand prairie habitat in Wisconsin for use in management, conservation, and restoration of this unique habitat.

REFERENCES

Abed, R.M.M., S.A. Kharusi, A. Schramm, and M.D. Robinson. 2010. Bacterial diversity, pigments and nitrogen fixation of biological desert crusts from the Sultanate of Oman. FEMS Microbiology Ecology 72: 418-428.

Abed, R.M.M., A.M. Al-Sadi, M. Al-Shehi, S. Al-Hinai, and M.D. Robinson. 2013. Diversity of free-living and lichenized fungal communities in biological soil crusts of the Sultanate of Oman and their role in improving soil properties. Soil Biology & Biochemistry 57: 695-705.

Apprill, A., S.P. McNally, R. Parsons, and L. Weber. 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquatic Microbial Ecology 75: 129-137.

Belnap, J. 1990. Microphytic crust: 'Topsoil' of the desert. Permaculture Drylands Journal 10: 4-14.

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

Belnap J. 2001. Microbes and microfauna associated with biological soil crusts. Pages 167-174 in J. Belnap and O.L. Lange, editors. Biological Soil Crusts: Structure, Function, and Management. Springer-Verlag, Berlin.

Belnap, J. 2002. Nitrogen fixation in biological soil crusts from southeast Utah, USA. Biology and Fertility of Soils 35: 128-135.

Belnap, J. 2003. The world at your feet: desert biological soil crusts. Frontiers in Ecology and the Environment 1: 181-189.

Belnap, J. 2006. The potential roles of biological soil crusts in dryland hydrological cycles. Hydrological Processes 20: 3159-3178.

Belnap, J., B. Büdel, and O.L. Lange. 2001. Biological soil crusts: characteristics and distribution. Pages 15-16 in J. Belnap and O.L. Lange, editors. Biological Soil Crusts: Structure, Function, and Management. Springer-Verlag, Berlin.

Belnap, J. and O.L. Lange. 2003. Structure and functioning of biological soil crusts: A synthesis. Page 471 in J. Belnap and O.L. Lange, editors. Biological Soil Crusts: Structure, Function, and Management. Springer-Verlag, Berlin.

Bowker, M.A. 2007. Biological soil crust rehabilitation in theory and practice: an underexploited opportunity. Restoration Ecology 15: 13-23.

Bray, R.H. and L.T. Kurtz. 1945. Determination of total, organic, and available forms of phosphorus in soil. Soil Science 59: 39-45.

Bremmer, J.M. 1965. Total nitrogen. Pages 1149-1178 in C.A. Black et al., editors. Methods of Soil Analysis, part 2. American Society of Agronomy, Inc., Madison, Wisconsin.

Bu, C., S. Wu, Y. Xie, and X. Zhang. 2013. The study of biological soil crusts: hotspots and prospects. Clean Soil Air Water 41: 899-906.

Čapková, K., T. Hauer, K. Řeháková, and J. Doležal. 2016. Some like it high! Phylogenetic diversity of high-elevation cyanobacterial community from biological soil crusts of western Himalaya. Microbial Ecology 71: 113-123.

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

Castillo-Monroy, A.P. and F.T. Maestre. 2011. The biological soil crust: recent advances in the knowledge of its ecological structure and function. Chilean Journal of Natural History 84: 1-21.

Chao, A. 1984. Non-parametric estimation of the number of classes in a population. Scandinavian Journal of Statistics 11: 265-270.

Chen, W.-M., L. Moulin, C. Bontemps, P. Vandamme, G. Béna, and C. Boivin-Masson. 2003. Legume symbiotic nitrogen fixation by β-Proteobacteria widespread in nature. Journal of Bacteriology 185: 7266-7272.

Combs, S.M. and M.V. Nathan. 1998. Soil organic matter. Pages 57-58 in J.R. Brown, editor. Recommended Chemical Soil Test Procedure for the North Central Region. NCR Publication No. 221 (revised). Missouri Agricultural Experiment Station SB 1001. Columbia, MO.

Curtis, J.T. 1959. The Vegetation of Wisconsin: An Ordination of Plant Communities. The University of Wisconsin Press, Madison, Wisconsin, USA.

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

Fadrosh, D.W., B. Ma, P. Gajer, N. Sengamalay, S. Ott, R.M. Brotman, and J. Ravel. 2014. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. Microbiome 2: 6.

Faith, D.P. 1992. Conservation evaluation and phylogenetic diversity. Biological Conservation 61: 1-10.

Gao, G.-L., G.-D. Ding, B. Wu, Y.-Q. Zhang, S.-G. Qin, Y.-Y. Zhao, Y.-F. Bao, Y.-D. Liu, L. Wan, and J.-F. Deng. 2014. Fractal scaling of particle size distribution and relationships with topsoil properties affected by biological soil crusts. PLoS ONE 9: e88559.

Garcia-Pichel, F., A López-Cortéz, and U. Nübel. 2001. Phylogenetic and morphological diversity of cyanobacteria in soil desert crusts from the Colorado Plateau. Applied and Environmental Microbiology 67: 1902-1910.

Gardes, M. and T.D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113-118.

Hagemann, M., M. Henneberg, V.J.M.N.L. Felde, S.L. Drahorad, S.M. Berkowicz, P. Felix-Henningsen, and A. Kaplan. 2015. Cyanobacterial diversity in biological soil crusts along a precipitation gradient, northwest Negev Desert, Israel. Microbial Ecology 70: 219-230.

Hawkes, C.V. 2003. Nitrogen cycling mediated by biological soil crusts and arbuscular mycorrhizal fungi. Ecology 84: 1553-1562.

Hongshan, J., L. Rong, D. Shou-Wei, and Z. Shuifang. 2014. Skewer: a fast and accurate adapter trimmer for next-generation sequencing paired-end reads. BMC Bioinformatics 15: 182.

Knapen, A., J. Poesen, P. Galindo-Morales, S. De Baets, and A. Pals. 2007. Effects of microbiotic crusts under cropland in temperate environments on soil erodibility during concentrated flow. Earth Surface Processes and Landforms 32: 1884-1901.

Knudsen, K. and T. La Doux. 2005. Lichen flora of the southwestern Mojave Desert: Key's Ranch, Joshua Tree National Park, San Bernardino County, California, USA. Evansia 22: 103-109.

Kumar, D. and S.P. Adhikary. 2015. Diversity, molecular phylogeny, and metabolic activity of cyanobacteria in biological soil crusts from Santiniketan (India). Journal of Applied Phycology 27: 339-349.

Lachat Instruments. 1995. Total Kjeldahl Nitrogen in Soil/Plant. QuickChem Method 12-107-04-1-B.

Langhans, T.M., C. Storm, and A. Schwabe. 2009. Community assembly of biological soil crusts of different successional stages in a temperate sand ecosystem, as assessed by direct determination and enrichment techniques. Microbial Ecology 58: 394-407.

Lozupone C. and R. Knight. 2005. UniFrac: a new phylogenetic method for comparing microbial communities. Applied and Environmental Microbiology 71: 8228–8235.

Lozupone, C.A. and R. Knight. 2008. Species divergence and the measurement of microbial diversity. FEMS Microbial Reviews 32: 557-578.

Lozupone, C., M.E. Lladser, D. Knights, J. Stombaugh, and R. Knight. 2010. UniFrac: an effective distance metric for microbial community comparison. ISME Journal 5(2): 169-172.

Magoc, T., and S. Salzberg. 2011. FLASH: Fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27: 2957-2963.

Makhalanyane, T.P., A. Valverde, D. Velázquez, E. Gunnigle, M.W. Van Goethem, A. Quesada, and D.A. Cowan. 2015. Ecology and biogeochemistry of cyanobacteria in soils, permafrost, aquatic and cryptic polar habitats. Biodiversity and Conservation 24: 819-840.

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

Neher, D.A., T.L. Walters, E. Tramer, T.R. Weicht, R.M. Veluci, and K. Saiya-Cork. 2003. Biological soil crust and vascular plant communities in a sand savanna of northwestern Ohio. Journal of the Torrey Botanical Society 130: 244-252.

Nunes da Rocha, U., H. Cadillo-Quiroz, U. Karaoz, L. Rajeev, N. Klitgord, S. Dunn, V. Truong, M. Buenrostro, B.P. Bowen, F. Garcia-Pichel, A. Mukhopadhyay, T.R. Northen, and E.L. Brodie. 2015. Isolation of a significant fraction of non-phototroph diversity from a desert Biological Soil Crust. Frontiers in Microbiology 6:277.

Paluch, E.C., M.A. Thomsen, and T.J. Volk. 2012. Effects of resident soil fungi and land use history outweigh those of commercial mycorrhizal inocula: testing a restoration strategy in unsterilized soil. Restoration Ecology 21: 380-389.

Parada, A.E., D.M. Needham, and J.A. Fuhrman. 2016. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environmental Microbiology 18: 1403-1414.

Price, M.N., P.S. Dehal, and A.P. Arkin. 2010. FastTree 2 – approximately maximum-likelihood trees for large alignments. PLoS ONE 5: e9490.

Rivera-Aguilar, V., G. Montejano, S. Rodríguez-Zaragoza, and A. Durán-Díaz. 2006. Distribution and composition of cyanobacteria, mosses and lichens of the biological soil crusts of the Tehuacán Valley, Puebla, México. Journal of Arid Environments 67: 208-225.

Ruzicka, J. 1983. Flow injection analysis – from test tube to integrated microconduits. Analytical Chemistry 55: 1040A-1053A.

Schulte, E.E. and B.G. Hopkins. 1996. Estimation of soil organic matter by weight loss-on-ignition. Pages 21-31 in F.R. Magdoff, M.A. Tabatabai, and E.A. Hanlon Jr., editors. Soil Organic Matter: Analysis and Interpretation. Soil Science Society of America, Madison, Wisconsin.

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

Steven, B., C.R. Kuske, L.V. Gallegos-Graves, S.C. Reed, and J. Belnap. 2015. Climate change and physical disturbance manipulations result in distinct biological soil crust communities. Applied and Environmental Microbiology 81: 7448-7459.

Su, Y.-G., X. Zhao, A.-X. Li, X.-R. Li, and G. Huang. 2011. Nitrogen fixation in biological soil crusts from the Tengger Desert, northern China. European Journal of Soil Biology 47: 182-187.

Wang, J., J.-T. Bao, X.-R. Li, and Y.-B. Liu. 2016. Molecular ecology of *nifH* genes and transcripts along a chronosequence in revegetated areas of the Tengger Desert. Microbial Ecology 71: 150-163.

White, T.J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White, editors. PCR Protocols. Academic Press, London.

Wu, L., C. Wen, Y. Qin, H. Yin, Q. Tu, J.D. Van Nostrand, T. Yuan, M. Yuan, Y. Deng, and J. Zhou. 2015. Phasing amplicon sequencing on Illumina Miseq for robust environmental microbial community analysis. BMC Microbiology 15: 125.

Zancan, S., R. Trevisan, and M.G. Paoletti. 2006. Soil algae composition under different agro ecosystems in North-Eastern Italy. Agriculture, Ecosystems, and Environment 112: 1-12.

Zhang, B., Y. Zhang, J. Zhao, N. Wu, R. Chen, and J. Zhang. 2009. Microalgal species variation at different successional stages in biological soil crusts of the Gurbantunggut Desert, northwestern China. Biology and Fertility of Soils 45: 539-547.

APPENDIX A

DATA ANALYSIS SUMMARY DATA GENERATED BY UNIVERSITY OF WISCONSIN BIOTECHNOLOGY CENTER BIOINFORMATICS RESOURCE CENTER

Appendix A. Data analysis summary data generated by University of Wisconsin Biotechnology Center Bioinformatics Resource Center. Sample IDs consist of site number + soil type + replicate; e.g., sample ID 1C1 denotes site 1, crusted soil plot, DNA extract replicate 1. Group designation “groupC” denotes crusted soils; “group U” denotes uncrusted soils.

Name	Group	Raw Sequence Reads	Merged Paired-end Reads	Primer Trimmed Amplicons	Quality Filtered Amplicons	Median Length (base pairs)
1C1	groupC	301662	298818	370	296996	299.00
1C2	groupC	289985	287766	449	286021	299.00
1C3	groupC	332913	330384	644	328083	299.00
2C1	groupC	319345	317151	425	315116	299.00
2C2	groupC	308402	305737	335	304028	299.00
2C3	groupC	293299	290966	382	289299	299.00
3C1	groupC	296611	293930	393	291931	299.00
3C2	groupC	295828	293609	463	291546	299.00
3C3	groupC	195449	193868	228	192754	299.00
4C3	groupC	343456	340887	531	338505	299.00
5C1	groupC	289581	286233	328	284601	299.00
5C2	groupC	317433	314994	390	313229	299.00
5C3	groupC	323745	321036	483	318999	299.00
6C1	groupC	303160	301122	492	299496	299.00
6C2	groupC	317481	313503	290	311763	299.00
7C1	groupC	330885	328470	536	326425	299.00
7C2	groupC	344367	341858	575	339804	299.00
7C3	groupC	257530	254468	307	253118	299.00

1U1	groupU	258356	256134	248	254592	299.00
1U2	groupU	250253	248167	374	246628	299.00
1U3	groupU	311030	308740	432	306654	299.00
2U1	groupU	299111	295059	369	293276	299.00
2U2	groupU	281705	279319	329	277597	299.00
2U3	groupU	214802	212810	312	211247	299.00
3U1	groupU	177266	175976	332	174839	299.00
3U2	groupU	230302	227400	258	226039	299.00
3U3	groupU	255870	253598	315	251944	299.00
4U1	groupU	261669	259553	433	257758	299.00
4U2	groupU	272447	270346	492	268575	299.00
4U3	groupU	228295	224694	240	223530	299.00
5U1	groupU	358028	354179	388	352201	299.00
5U2	groupU	318462	316027	461	314022	299.00
5U3	groupU	330739	328451	572	326448	299.00
6U1	groupU	312563	310283	424	308232	299.00
6U3	groupU	307812	305358	460	303311	299.00
7U1	groupU	251407	249043	351	247259	299.00
7U2	groupU	326484	324236	427	322011	299.00

APPENDIX B

RAREFACTION MEASURES COMPARING ALPHA DIVERSITY BETWEEN CRUSTED AND UNCRUSTED SAMPLES MEASURED BY FOUR DIVERSITY INDICES

Appendix B. Rarefaction measures comparing alpha diversity between crusted and uncrusted samples measured by four diversity indices. Sample IDs consist of site number + soil type + replicate; e.g., sample ID 1C1 denotes site 1, crusted soil plot, DNA extract replicate 1. Indices were calculated at ten steps (numbers of sequences sampled), denoted by the ten values in the Seqs/Sample column corresponding to each Sample ID.

Sample ID	Seqs/Sample	Phylogenetic Diversity	Chao1	Observed Species	Shannon Diversity
1C1	10.0	5.344	44.400	9.600	3.242
1C1	26539.0	316.879	10199.383	4768.300	9.742
1C1	53068.0	412.807	14384.504	7020.200	9.865
1C1	79597.0	477.021	16853.432	8710.100	9.916
1C1	106126.0	527.592	19017.306	10151.000	9.954
1C1	132655.0	568.857	20366.937	11352.100	9.973
1C1	159184.0	605.267	21663.613	12446.800	9.993
1C1	185713.0	633.654	22348.054	13345.900	10.002
1C1	212242.0	662.666	23101.917	14223.000	10.013
1C1	238771.0	686.740	23645.763	14996.000	10.021
1C1	265300.0	709.358	24130.151	15714.900	10.029
1C2	10.0	5.072	37.600	9.100	3.119
1C2	26539.0	315.304	11468.288	4731.500	9.088
1C2	53068.0	412.607	16120.254	7233.600	9.227
1C2	79597.0	482.027	19370.480	9165.000	9.282
1C2	106126.0	537.685	21659.192	10783.500	9.316
1C2	132655.0	584.012	23423.703	12212.300	9.347
1C2	159184.0	620.941	24479.168	13451.300	9.364
1C2	185713.0	654.341	25502.759	14550.500	9.377
1C2	212242.0	684.693	26489.160	15575.400	9.388
1C2	238771.0	710.734	27273.916	16515.100	9.398
1C2	265300.0	nan	nan	nan	nan
1C3	10.0	5.960	51.800	9.900	3.302
1C3	26539.0	272.279	6841.620	3881.800	9.720
1C3	53068.0	331.365	8701.105	5345.900	9.807
1C3	79597.0	370.769	9750.210	6353.700	9.846
1C3	106126.0	399.660	10663.658	7160.700	9.866
1C3	132655.0	420.769	11241.844	7801.100	9.888
1C3	159184.0	437.063	11666.245	8323.300	9.894
1C3	185713.0	451.715	12038.869	8815.000	9.902
1C3	212242.0	464.698	12268.140	9209.100	9.911

1C3	238771.0	474.456	12482.495	9561.900	9.913
1C3	265300.0	484.247	12667.833	9878.400	9.918
1U1	10.0	5.356	45.400	9.700	3.262
1U1	26539.0	261.439	6411.609	3324.900	8.848
1U1	53068.0	330.777	8576.810	4736.700	8.923
1U1	79597.0	378.469	10095.306	5772.000	8.957
1U1	106126.0	414.936	10902.071	6568.800	8.979
1U1	132655.0	442.740	11627.829	7241.300	8.988
1U1	159184.0	469.802	12448.814	7867.500	9.003
1U1	185713.0	491.754	13099.060	8388.700	9.009
1U1	212242.0	511.003	13612.969	8852.700	9.014
1U1	238771.0	528.381	14031.055	9292.500	9.019
1U1	265300.0	nan	nan	nan	nan
1U2	10.0	5.053	33.950	9.000	3.114
1U2	26539.0	246.003	5937.105	3139.900	8.798
1U2	53068.0	306.869	7677.030	4413.600	8.872
1U2	79597.0	348.494	9098.340	5350.100	8.905
1U2	106126.0	378.781	10012.157	6099.400	8.929
1U2	132655.0	403.582	10753.621	6706.800	8.935
1U2	159184.0	422.974	11494.612	7253.400	8.951
1U2	185713.0	442.282	12191.047	7739.500	8.958
1U2	212242.0	457.362	12699.966	8165.200	8.961
1U2	238771.0	nan	nan	nan	nan
1U2	265300.0	nan	nan	nan	nan
1U3	10.0	5.081	39.600	9.400	3.194
1U3	26539.0	277.226	7786.559	3521.900	8.964
1U3	53068.0	362.800	10959.200	5227.000	9.068
1U3	79597.0	421.037	13293.669	6484.500	9.101
1U3	106126.0	466.289	14985.090	7549.600	9.124
1U3	132655.0	505.147	16417.104	8495.800	9.144
1U3	159184.0	541.478	17617.288	9365.700	9.160
1U3	185713.0	568.461	18737.175	10127.600	9.169
1U3	212242.0	594.871	19471.591	10824.400	9.176
1U3	238771.0	619.709	20126.046	11484.800	9.184
1U3	265300.0	641.440	20779.464	12081.800	9.188
2C1	10.0	5.208	34.700	9.100	3.127
2C1	26539.0	267.046	6388.943	3317.200	8.815
2C1	53068.0	337.290	8827.016	4732.600	8.901

2C1	79597.0	384.956	10486.340	5768.600	8.936
2C1	106126.0	423.188	11906.791	6639.800	8.957
2C1	132655.0	452.989	12968.464	7364.600	8.975
2C1	159184.0	479.592	14057.505	8022.100	8.980
2C1	185713.0	502.690	14968.422	8621.500	8.993
2C1	212242.0	521.899	15597.592	9151.000	8.998
2C1	238771.0	540.553	16219.063	9638.700	9.003
2C1	265300.0	558.250	16816.683	10115.800	9.008
2C2	10.0	5.218	39.600	9.400	3.194
2C2	26539.0	295.959	9315.850	4051.200	8.973
2C2	53068.0	385.136	13109.983	6035.400	9.073
2C2	79597.0	448.351	15815.688	7617.500	9.129
2C2	106126.0	498.250	17888.017	8913.500	9.155
2C2	132655.0	541.009	19703.190	10090.900	9.176
2C2	159184.0	576.032	20699.638	11082.300	9.190
2C2	185713.0	606.028	21716.680	11991.800	9.200
2C2	212242.0	634.362	22581.203	12831.100	9.207
2C2	238771.0	660.352	23347.861	13638.600	9.220
2C2	265300.0	684.323	23984.233	14352.700	9.227
2C3	10.0	5.573	42.200	9.600	3.242
2C3	26539.0	267.218	7896.401	3866.300	9.266
2C3	53068.0	333.303	10897.771	5580.700	9.363
2C3	79597.0	375.512	12438.363	6848.700	9.409
2C3	106126.0	409.811	13892.125	7914.300	9.432
2C3	132655.0	435.852	15028.725	8799.600	9.450
2C3	159184.0	459.461	15920.915	9603.200	9.462
2C3	185713.0	478.986	16597.679	10268.600	9.471
2C3	212242.0	496.378	17233.825	10917.300	9.481
2C3	238771.0	512.030	17900.706	11487.800	9.486
2C3	265300.0	525.759	18261.628	12010.400	9.493
2U1	10.0	6.009	48.600	9.800	3.282
2U1	26539.0	291.898	8192.172	4094.400	9.796
2U1	53068.0	361.870	11062.274	5859.900	9.898
2U1	79597.0	409.093	13277.693	7196.600	9.944
2U1	106126.0	442.755	14604.772	8252.000	9.972
2U1	132655.0	473.571	15773.829	9188.300	9.989
2U1	159184.0	497.067	16481.140	9993.000	10.004
2U1	185713.0	518.286	17383.971	10713.700	10.011

2U1	212242.0	536.706	17831.844	11369.200	10.022
2U1	238771.0	552.296	18249.845	11957.600	10.029
2U1	265300.0	567.671	18545.212	12501.000	10.034
2U2	10.0	5.510	46.000	9.600	3.234
2U2	26539.0	290.587	6712.433	3597.000	9.201
2U2	53068.0	367.167	8883.544	5116.800	9.293
2U2	79597.0	414.915	10323.550	6170.100	9.333
2U2	106126.0	453.632	11354.811	7041.800	9.355
2U2	132655.0	484.101	12161.570	7748.200	9.374
2U2	159184.0	509.218	12956.520	8376.600	9.385
2U2	185713.0	529.972	13374.653	8872.000	9.391
2U2	212242.0	549.917	13836.541	9360.600	9.401
2U2	238771.0	566.309	14332.703	9793.900	9.406
2U2	265300.0	nan	nan	nan	nan
2U3	10.0	5.040	45.400	9.700	3.262
2U3	26539.0	312.321	7867.381	3999.000	9.378
2U3	53068.0	395.525	10521.347	5716.700	9.482
2U3	79597.0	450.705	12266.798	6962.100	9.522
2U3	106126.0	494.091	13842.077	7982.100	9.549
2U3	132655.0	529.872	14819.396	8841.300	9.567
2U3	159184.0	559.187	15719.796	9596.700	9.581
2U3	185713.0	586.259	16645.974	10288.300	9.591
2U3	212242.0	nan	nan	nan	nan
2U3	238771.0	nan	nan	nan	nan
2U3	265300.0	nan	nan	nan	nan
3C1	10.0	5.384	39.000	9.500	3.222
3C1	26539.0	299.837	7504.801	3680.200	9.084
3C1	53068.0	386.148	10224.350	5308.800	9.174
3C1	79597.0	444.042	12319.732	6546.500	9.213
3C1	106126.0	487.317	13678.354	7509.300	9.237
3C1	132655.0	528.153	14853.811	8397.600	9.257
3C1	159184.0	560.865	15925.029	9171.400	9.267
3C1	185713.0	589.292	16634.285	9834.600	9.275
3C1	212242.0	616.489	17233.369	10466.700	9.285
3C1	238771.0	639.137	17727.533	11030.400	9.291
3C1	265300.0	659.700	18239.082	11550.900	9.296
3C2	10.0	5.440	48.600	9.800	3.282
3C2	26539.0	296.560	7217.213	3552.900	9.195

3C2	53068.0	380.737	9858.047	5121.100	9.272
3C2	79597.0	440.760	12091.301	6330.900	9.319
3C2	106126.0	481.619	13529.150	7258.600	9.338
3C2	132655.0	520.101	14737.596	8118.100	9.358
3C2	159184.0	551.500	15764.917	8846.500	9.368
3C2	185713.0	580.415	16582.567	9522.800	9.379
3C2	212242.0	606.447	17507.998	10147.600	9.386
3C2	238771.0	628.556	18096.735	10709.200	9.393
3C2	265300.0	nan	nan	nan	nan
3C3	10.0	4.584	38.000	9.400	3.202
3C3	26539.0	305.214	9671.178	4025.500	8.800
3C3	53068.0	402.979	13557.146	6076.700	8.907
3C3	79597.0	472.245	16316.478	7695.400	8.966
3C3	106126.0	524.526	18242.093	9040.100	8.994
3C3	132655.0	570.232	19443.571	10198.000	9.010
3C3	159184.0	609.848	20550.898	11266.900	9.031
3C3	185713.0	nan	nan	nan	nan
3C3	212242.0	nan	nan	nan	nan
3C3	238771.0	nan	nan	nan	nan
3C3	265300.0	nan	nan	nan	nan
3U1	10.0	5.588	55.000	10.000	3.322
3U1	26539.0	254.580	6481.325	3559.700	9.486
3U1	53068.0	311.641	8356.760	4964.900	9.583
3U1	79597.0	349.415	9533.385	5916.200	9.609
3U1	106126.0	377.646	10526.209	6705.300	9.635
3U1	132655.0	400.665	11163.054	7352.600	9.652
3U1	159184.0	419.705	11838.811	7899.600	9.660
3U1	185713.0	nan	nan	nan	nan
3U1	212242.0	nan	nan	nan	nan
3U1	238771.0	nan	nan	nan	nan
3U1	265300.0	nan	nan	nan	nan
3U2	10.0	5.567	55.000	10.000	3.322
3U2	26539.0	263.606	7834.829	3909.200	9.723
3U2	53068.0	327.732	10684.870	5585.000	9.819
3U2	79597.0	371.254	12742.246	6867.300	9.866
3U2	106126.0	403.964	14127.098	7890.400	9.891
3U2	132655.0	430.711	15319.389	8787.700	9.906
3U2	159184.0	452.866	16267.643	9561.900	9.919

3U2	185713.0	472.097	17068.955	10255.100	9.927
3U2	212242.0	nan	nan	nan	nan
3U2	238771.0	nan	nan	nan	nan
3U2	265300.0	nan	nan	nan	nan
3U3	10.0	5.350	55.000	10.000	3.322
3U3	26539.0	254.613	5292.219	3037.800	9.142
3U3	53068.0	312.050	6747.484	4161.400	9.203
3U3	79597.0	350.881	7551.329	4918.200	9.228
3U3	106126.0	378.254	8247.599	5527.800	9.246
3U3	132655.0	402.483	8927.305	6039.800	9.260
3U3	159184.0	421.249	9342.676	6462.800	9.267
3U3	185713.0	437.007	9718.541	6830.800	9.272
3U3	212242.0	450.868	10013.560	7146.200	9.277
3U3	238771.0	nan	nan	nan	nan
3U3	265300.0	nan	nan	nan	nan
4C3	10.0	5.501	47.600	9.700	3.262
4C3	26539.0	285.685	7824.350	3492.100	8.811
4C3	53068.0	369.283	10725.709	5131.500	8.901
4C3	79597.0	429.262	12892.360	6384.000	8.941
4C3	106126.0	475.573	14723.050	7467.700	8.965
4C3	132655.0	513.975	16009.355	8361.000	8.978
4C3	159184.0	548.982	17458.817	9212.900	8.991
4C3	185713.0	578.678	18473.612	9945.300	8.998
4C3	212242.0	606.099	19414.555	10656.700	9.013
4C3	238771.0	629.946	20221.223	11281.200	9.016
4C3	265300.0	651.700	20907.121	11891.200	9.021
4U1	10.0	4.612	32.200	9.100	3.134
4U1	26539.0	241.999	7070.290	2964.200	8.046
4U1	53068.0	312.861	10180.997	4468.700	8.127
4U1	79597.0	363.278	12151.014	5636.900	8.162
4U1	106126.0	402.304	13592.278	6648.500	8.186
4U1	132655.0	433.106	14753.361	7497.700	8.200
4U1	159184.0	461.468	15766.644	8291.800	8.214
4U1	185713.0	487.435	16461.338	8999.600	8.220
4U1	212242.0	509.504	17066.329	9647.900	8.230
4U1	238771.0	nan	nan	nan	nan
4U1	265300.0	nan	nan	nan	nan
4U2	10.0	4.494	27.300	8.700	3.027

4U2	26539.0	208.552	4343.730	2450.200	8.032
4U2	53068.0	258.429	5835.954	3406.500	8.090
4U2	79597.0	289.016	6652.053	4075.900	8.121
4U2	106126.0	311.243	7273.730	4594.200	8.132
4U2	132655.0	330.367	7835.234	5035.900	8.146
4U2	159184.0	346.419	8219.781	5426.500	8.151
4U2	185713.0	359.459	8603.336	5752.200	8.156
4U2	212242.0	371.292	8891.804	6052.500	8.159
4U2	238771.0	381.599	9111.075	6318.000	8.163
4U2	265300.0	nan	nan	nan	nan
4U3	10.0	5.013	44.300	9.500	3.214
4U3	26539.0	232.025	5817.742	2941.000	8.463
4U3	53068.0	286.539	7919.386	4177.700	8.535
4U3	79597.0	323.350	9493.700	5122.200	8.568
4U3	106126.0	352.085	10754.843	5892.600	8.589
4U3	132655.0	374.319	11830.891	6548.000	8.599
4U3	159184.0	392.280	12477.878	7113.500	8.609
4U3	185713.0	409.927	13251.241	7646.900	8.616
4U3	212242.0	nan	nan	nan	nan
4U3	238771.0	nan	nan	nan	nan
4U3	265300.0	nan	nan	nan	nan
5C1	10.0	5.223	45.400	9.700	3.262
5C1	26539.0	321.970	11118.541	4759.200	9.758
5C1	53068.0	419.334	15207.004	7209.400	9.890
5C1	79597.0	483.281	17742.502	9034.700	9.952
5C1	106126.0	537.675	19432.316	10603.800	9.986
5C1	132655.0	576.098	20518.304	11865.200	10.006
5C1	159184.0	611.402	21309.737	13011.300	10.027
5C1	185713.0	639.781	21750.612	13989.300	10.039
5C1	212242.0	665.621	22113.828	14872.700	10.050
5C1	238771.0	nan	nan	nan	nan
5C1	265300.0	nan	nan	nan	nan
5C2	10.0	5.435	51.800	9.900	3.302
5C2	26539.0	343.037	10481.527	4693.100	9.715
5C2	53068.0	455.771	14006.477	7030.900	9.851
5C2	79597.0	531.187	16090.114	8761.200	9.914
5C2	106126.0	591.272	17533.406	10145.500	9.945
5C2	132655.0	638.487	18608.173	11300.800	9.965

5C2	159184.0	678.718	19468.394	12305.400	9.978
5C2	185713.0	714.419	20034.510	13189.500	9.993
5C2	212242.0	744.361	20481.049	13945.800	10.004
5C2	238771.0	772.465	20934.412	14663.200	10.011
5C2	265300.0	nan	nan	nan	nan
5C3	10.0	4.934	48.600	9.800	3.282
5C3	26539.0	344.351	11103.968	4760.900	9.423
5C3	53068.0	459.036	15610.336	7237.900	9.556
5C3	79597.0	539.926	18279.551	9131.200	9.624
5C3	106126.0	603.674	20350.064	10714.300	9.656
5C3	132655.0	655.003	21773.018	12054.000	9.683
5C3	159184.0	699.006	23173.802	13247.700	9.703
5C3	185713.0	736.721	23720.083	14274.700	9.714
5C3	212242.0	772.045	24599.790	15254.100	9.727
5C3	238771.0	803.633	25121.318	16126.200	9.736
5C3	265300.0	830.150	25427.707	16898.600	9.743
5U1	10.0	5.419	45.400	9.700	3.262
5U1	26539.0	304.452	10219.468	4296.700	9.118
5U1	53068.0	397.500	14287.728	6490.200	9.243
5U1	79597.0	461.831	16774.781	8155.400	9.283
5U1	106126.0	512.764	18632.986	9547.600	9.317
5U1	132655.0	555.400	20442.389	10813.100	9.347
5U1	159184.0	590.844	21482.121	11882.700	9.361
5U1	185713.0	623.304	22572.680	12845.300	9.373
5U1	212242.0	652.484	23435.533	13764.800	9.382
5U1	238771.0	677.709	24209.778	14580.000	9.390
5U1	265300.0	698.601	24653.153	15294.600	9.396
5U2	10.0	5.300	40.200	9.400	3.202
5U2	26539.0	278.542	8728.856	3886.200	8.730
5U2	53068.0	353.620	12212.874	5765.000	8.832
5U2	79597.0	410.799	14472.000	7236.100	8.886
5U2	106126.0	451.008	16175.003	8447.200	8.913
5U2	132655.0	486.127	17498.672	9506.800	8.933
5U2	159184.0	514.945	18538.563	10440.200	8.946
5U2	185713.0	539.391	19665.541	11271.300	8.957
5U2	212242.0	562.478	20531.067	12051.300	8.965
5U2	238771.0	582.222	21078.012	12738.100	8.975
5U2	265300.0	600.852	21652.284	13402.500	8.980

5U3	10.0	5.180	44.400	9.600	3.242
5U3	26539.0	262.195	6750.410	3626.400	8.979
5U3	53068.0	327.025	8920.676	5137.100	9.084
5U3	79597.0	368.969	10435.466	6195.500	9.119
5U3	106126.0	401.091	11212.118	7029.000	9.141
5U3	132655.0	424.954	11972.973	7718.900	9.156
5U3	159184.0	447.455	12496.680	8337.900	9.167
5U3	185713.0	464.817	12897.703	8870.100	9.172
5U3	212242.0	482.225	13252.898	9346.900	9.178
5U3	238771.0	494.027	13542.070	9746.100	9.185
5U3	265300.0	507.226	13780.113	10129.400	9.189
6C1	10.0	5.633	45.400	9.700	3.262
6C1	26539.0	250.881	6207.671	3286.700	8.852
6C1	53068.0	313.085	8437.117	4668.500	8.939
6C1	79597.0	357.654	9841.179	5678.400	8.974
6C1	106126.0	391.307	10814.674	6486.700	8.999
6C1	132655.0	417.229	11578.616	7126.500	9.005
6C1	159184.0	438.286	12184.943	7743.000	9.020
6C1	185713.0	458.847	12764.618	8275.400	9.026
6C1	212242.0	474.870	13070.109	8726.900	9.033
6C1	238771.0	489.049	13359.604	9137.800	9.038
6C1	265300.0	nan	nan	nan	nan
6C2	10.0	5.021	45.400	9.700	3.262
6C2	26539.0	287.830	8746.524	4147.200	9.592
6C2	53068.0	363.536	12132.149	6023.600	9.697
6C2	79597.0	417.447	14605.759	7466.300	9.746
6C2	106126.0	458.170	16301.169	8659.900	9.773
6C2	132655.0	491.576	17803.172	9666.400	9.787
6C2	159184.0	519.437	18836.965	10589.900	9.803
6C2	185713.0	545.798	19763.083	11438.600	9.816
6C2	212242.0	567.358	20600.270	12182.500	9.825
6C2	238771.0	587.506	21160.824	12868.300	9.831
6C2	265300.0	606.261	21599.468	13508.200	9.839
6U1	10.0	5.394	42.800	9.500	3.214
6U1	26539.0	245.998	5693.010	2934.600	8.921
6U1	53068.0	310.029	7870.139	4174.800	8.992
6U1	79597.0	352.659	9379.844	5063.100	9.022
6U1	106126.0	384.532	10466.168	5793.500	9.038

6U1	132655.0	411.512	11500.713	6462.400	9.056
6U1	159184.0	436.094	12462.837	7060.900	9.065
6U1	185713.0	456.364	13286.534	7568.900	9.073
6U1	212242.0	473.594	13823.472	8017.700	9.074
6U1	238771.0	490.946	14558.085	8469.700	9.080
6U1	265300.0	505.671	15076.606	8878.500	9.086
6U3	10.0	5.794	42.200	9.600	3.242
6U3	26539.0	242.276	6701.325	3157.400	8.759
6U3	53068.0	305.341	9515.815	4619.800	8.847
6U3	79597.0	350.201	11862.254	5783.300	8.887
6U3	106126.0	384.235	13386.856	6706.000	8.905
6U3	132655.0	410.520	14758.459	7520.100	8.923
6U3	159184.0	434.403	15759.942	8253.600	8.933
6U3	185713.0	455.795	16756.802	8925.600	8.940
6U3	212242.0	477.056	17922.008	9592.300	8.953
6U3	238771.0	492.672	18549.484	10129.100	8.955
6U3	265300.0	508.905	19239.967	10679.000	8.960
7C1	10.0	5.927	33.000	8.800	3.034
7C1	26539.0	268.785	8510.923	3555.600	7.679
7C1	53068.0	351.601	12353.915	5379.000	7.774
7C1	79597.0	410.889	15442.839	6834.100	7.823
7C1	106126.0	456.584	17539.903	8054.700	7.849
7C1	132655.0	497.226	19547.948	9154.900	7.872
7C1	159184.0	529.691	21060.977	10136.300	7.885
7C1	185713.0	560.237	22450.327	11054.400	7.897
7C1	212242.0	587.709	23609.571	11882.900	7.907
7C1	238771.0	611.502	24604.900	12658.100	7.910
7C1	265300.0	633.889	25432.137	13400.600	7.918
7C2	10.0	4.799	45.400	9.700	3.262
7C2	26539.0	271.096	7277.361	3630.500	8.837
7C2	53068.0	344.903	9741.476	5224.000	8.933
7C2	79597.0	394.201	11408.509	6384.700	8.958
7C2	106126.0	428.003	12682.300	7304.600	8.984
7C2	132655.0	458.921	13582.327	8096.900	9.003
7C2	159184.0	485.385	14440.201	8806.900	9.018
7C2	185713.0	510.155	15158.706	9418.100	9.023
7C2	212242.0	527.999	15614.087	9976.600	9.029
7C2	238771.0	547.034	16216.558	10493.300	9.035

7C2	265300.0	563.200	16532.200	10944.600	9.040
7C3	10.0	5.394	29.400	9.200	3.162
7C3	26539.0	275.513	8430.427	3809.300	8.329
7C3	53068.0	356.934	12236.661	5667.100	8.415
7C3	79597.0	415.031	14948.794	7156.700	8.473
7C3	106126.0	461.181	16972.852	8373.900	8.499
7C3	132655.0	498.787	18503.590	9478.500	8.523
7C3	159184.0	529.704	19500.929	10401.500	8.533
7C3	185713.0	557.828	20343.369	11264.300	8.545
7C3	212242.0	nan	nan	nan	nan
7C3	238771.0	nan	nan	nan	nan
7C3	265300.0	nan	nan	nan	nan
7U1	10.0	6.076	51.800	9.900	3.302
7U1	26539.0	267.128	6582.635	3461.700	9.333
7U1	53068.0	335.588	8813.990	4909.700	9.423
7U1	79597.0	378.103	10464.989	5944.600	9.458
7U1	106126.0	414.731	11914.367	6811.900	9.482
7U1	132655.0	442.596	12886.621	7526.200	9.496
7U1	159184.0	466.331	13881.011	8156.600	9.506
7U1	185713.0	486.946	14697.762	8731.400	9.514
7U1	212242.0	505.799	15478.646	9237.600	9.520
7U1	238771.0	nan	nan	nan	nan
7U1	265300.0	nan	nan	nan	nan
7U2	10.0	5.617	47.600	9.700	3.262
7U2	26539.0	253.723	6006.616	3080.800	8.933
7U2	53068.0	316.528	8039.841	4344.200	8.997
7U2	79597.0	360.810	9535.059	5309.000	9.039
7U2	106126.0	394.027	10955.683	6110.600	9.056
7U2	132655.0	421.068	11987.891	6785.800	9.069
7U2	159184.0	443.892	12856.768	7377.700	9.081
7U2	185713.0	464.762	13666.224	7926.500	9.088
7U2	212242.0	483.317	14299.576	8416.200	9.096
7U2	238771.0	499.386	14921.120	8868.900	9.100
7U2	265300.0	514.602	15404.260	9289.100	9.105