Rates of Lateral Expansion of Biological Soil Crusts

by

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ABSTRACT

Biological soil crusts (biocrust) are photosynthetic communities of organisms forming in the top millimeters of unvegetated soil. Because soil crusts contribute several ecosystem services to the areas they inhabit, their loss under anthropogenic pressure has negative ecological consequences. There is a considerable interest in developing technologies for biocrust restoration such as biocrust nurseries to grow viable inoculum and the optimization of techniques for field deployment of this inoculum. For the latter, knowledge of the natural rates of biocrust dispersal is needed. Lateral dispersal can be based on self-propelled motility by component microbes, or on passive transport through propagule entrainment in runoff water or wind currents, all of which remain to be assessed. I focused my research on determining the capacity of biocrust for lateral self-propelled dispersal. Over the course of one year, I set up two greenhouse experiments where sterile soil substrates were inoculated with biocrusts and where the lateral advancement of biocrust and their cyanobacteria was monitored using time-course photography, discrete determination of soil chlorophyll *a* concentration, and microscopic observations. Appropriate uninoculated controls were also set up and monitored. These experiments confirm that cyanobacterial biological soil crusts are capable of laterally expanding when provided with presumably optimal watering regime similar to field conditions and moderate temperatures. The maximum temperatures of Sonoran Desert summer (up to 42 °C), exacerbated in the greenhouse setting (48 °C), caused a loss of biomass and the cessation of lateral dispersal, which resumed as temperature decreased. In 8 independent experiments, biocrust communities advanced laterally at an average rate

of 2 cm per month, which is half the maximal rate possible based on the instantaneous speed of gliding motility of the cyanobacterium *Microcoleus vaginatus*. In a span of three months, populations of *M. vaginatus*, *M. steenstrupii*, and *Scytonema* spp. advanced 1 cm/month on average. The advancing crust front was found to be preferentially composed of hormogonia (differentiated, fast-gliding propagules of cyanobacteria). Having established the potential for laterally self-propelled community dispersal (without wind or runoff contributions) will help inform restoration efforts by proposing minimal inoculum size and optimal distance between inoculum patches.

DEDICATION

To my Mom and Grandma

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INTRODUCTION

Arid lands cover some 45% of Earth's total terrestrial area (1). Exhaustive use of arid lands leaves behind a soil unsuitable for plant life in the case of unsustainable farming (2) and leads to slow recovery time following grazing (3). Human-caused land degradation negatively impacts the resilience of an ecosystem, increasing erosion, contributing to dust storms and reducing biodiversity (4, 5). Large dust storms originating in arid lands have the potential to damage the health of the population beyond its own residents (6, 7). Traditional habitat restoration techniques such as humus addition, revegetation, or trampling management are unsuccessful in arid lands due to slow carbon sequestration rates in arid lands in comparison to wetter and cooler climates (8).

Application of biological soil crust inoculum is a promising tool to restore ecosystem services in degraded soils of drylands. Biological soil crusts (biocrusts; 9) are communities of organisms, including cyanobacteria that live in the top 2 mm niche of soil (10). Cyanobacterial members of biocrust can colonize barren soil (11) and survive a range of high temperatures typical of drylands (12). Biocrust restoration is a relatively new arena of research, with the first attempt of restoration using biological soil crust in the desert field being published in 2009 (13). With the goals of restoring large bare soil areas efficiently, research has been done on mass-producing biocrust in the laboratory and the greenhouse on the basis of replicating the original biocrust cyanobacterial community composition (14-16).

Biocrust cyanobacteria provide a suite of ecosystem services. Cyanobacteria retain nutrients present by decreasing erosion through the binding of soil particles (17), and heterocystous cyanobacteria increase metabolically available nitrogen content via

nitrogen fixation (16). Field and growth chamber experiments on cyanobacterial biocrusts provide evidence that they increase water retention from the top few centimeters to 9 cm at most in depth. Evaporative loss was significantly lower in cyanobacterial crusts in comparison to bare soil (18). Cyanobacteria are one of the first organisms to establish biological soil crust, and are often referred to as biocrust pioneers, owing to their ability to not only disperse through bare soil but to also colonize it (11).

Motility of filaments is a characteristic that allows cyanobacterial pioneers, such as *Microcoleus vaginatus* and *Microcoleus steenstrupii,* to colonize soil more easily, as opposed to non-motile cyanobacteria. Other cyanobacterial biocrust members lacking filament motility (*Scytonema* spp.) can join the community only after the motile pioneers have colonized the soil. Understanding motility of cyanobacterial pioneers can elucidate the self-propelled dispersal rate contributing to dryland restoration.

Although the cyanobacterial molecular mechanism for motility is yet unclear, they do not possess flagella, and reviews suggest it involves polysaccharide excretion coupled with type IV pili (19, 20). Qualitative and quantitative observations clearly point to water presence as the main factor triggering motility and being a requirement for any motility to occur (21-23).

Vertical migration of oscillatorian motile cyanobacteria has been addressed in the literature. A marine cyanobacterium *Microcoleus chthonoplastes* migrated up through the mud at a net rate of 0.08 μm s^{-1} (23) to avoid high light intensity exposure. Oscillatorian cyanobacteria inhabiting desert biocrust have been shown to migrate vertically up to the surface of the soil upon wetting in minutes, exhibiting water taxis (22). The instantaneous gliding speed of a single *Microcoleus chthonoplastes* filament quantified on a glass

surface when provided with light and water was 0.7 μm s^{-1} on average (23). In solid agar, a chosen fast *M. vaginatus* filament traveled 0.4 μm s^{-1} on average (21). In 1979, Campbell came to the logical conclusion that gliding motility and sheath production are some of the factors leading to "spreading of vast communities of the prokaryotic primary producers". However, knowing a single filament's speed does not provide ecologically relevant information on soil colonization by a community of cyanobacteria. To this day there has been no direct measurement of such phenomenon in the natural habitat.

This knowledge gap stands in the way of monitoring biocrust restoration success using nursery-grown inoculum, a community of biocrust forming cyanobacteria that are well suited for field survival. For example, when researchers attempt to restore a disturbed patch of arid land by applying nursery-grown inoculum, the success of the inoculum will not be guaranteed. The recovery could instead be mediated by local biocrust communities, rather than by the man-made inoculum. The knowledge of natural self-propelled dispersal rates will not only aid in distinguishing the source of recovery, but also help design optimal placement of inoculum.

I proposed two questions were proposed to elucidate lateral dispersal of cyanobacterial biocrusts. First, will biological soil crust disperse laterally? I hypothesized that due to lateral motility of certain cyanobacterial biocrust organisms, the crust will disperse laterally at a certain rate that is lower than the maximum rate determined by instantaneous cyanobacterial filament speeds. Secondly, which biocrust organisms will disperse laterally? I hypothesized that motile pioneer cyanobacteria known to live in desert biocrust such as *Microcoleus vaginatus* and *Microcoleus steenstrupii* will disperse laterally, but sessile forms (like *Scytonema* spp.) will lag.

These investigations also aimed to quantify isolated wind-driven aerial dispersal from lateral, self-propelled dispersal. Cyanobacteria can be passively aerially dispersed through dust clouds (24), and possibly, through surface runoff currents. To investigate this self-propelled propagule dispersal rate, I set up greenhouse experiments with control and experimental units. In the experimental units, inoculum was laid down in a small strip along one edge, leaving a much larger area for lateral expansion. The control units had no inoculum and were meant to account for airborne greenhouse dispersal. The units were watered every four days with an automated wicking watering system to simulate local annual precipitation frequency, and sampled monthly from March 2016 to February 2017. Overall growth was monitored by observation and time-lapse photography; discrete samples were also taken regularly at different distances away from inoculum. Chlorophyll *a* analysis was used to quantify cyanobacterial biomass, while individual species were morphologically identified through compound light microscopy.

A known rate of dispersal without wind or runoff contributions should help guide restoration effort in deciding the size of inoculum patches as well as the optimum distance for inoculation away from natural crust. The rate of lateral dispersal can be used to predict natural recovery rates, hence, helping restoration ecologists and land managers to allocate the minimal effort necessary to speed up recovery.

METHODS AND MATERIALS

Two experiments were conducted in the greenhouse. Experiment #1 captured lateral dispersal during the spring season, while experiment #2 captured it for the winter season. Experiment #2 controlled for the location within the greenhouse by rotating the units, while experiment #1 did not. The inoculum cyanobacterial species composition of experiments #1 and #2 differed.

Field Collection

Biological soil crust and soil for greenhouse experiment #1 were collected close to Gold Canyon (AZ) by the foothills of the Superstition Mountains on December 12th, 2015 after it had just rained (700 m elevation, at 33.3923072, -111.3539764). The field location represented Sonoran Desert Upland with a bimodal rainy season that receives most of its precipitation in late summer and winter (Table 1). As confirmed by observation under a dissecting microscope, the crust had few mosses and lichens, mostly being composed of cyanobacteria. The crust was placed into ten 100 cm^2 plates and immediately covered from light to keep it photosynthetically inactive until greenhouse incubation. The biocrust was wet from the field and therefore, it had to be dried uncovered for 3 days under a drying hood to reduce the potential for increased fungi growth during storage. Native gravely and sandy soil (bulk soil) was collected 1 meter away from the crust. For greenhouse experiment #2 biocrust was collected on October 22, 2016. The inoculum was collected no further than 1 km away from the location where it was collected for experiment #1 (no coordinates are available). As confirmed by observation under the dissecting microscope, no moss or lichen growth was observed on

the inoculum, only cyanobacteria. The bulk soil collected on December 12th, 2015 was used as substrate for experiment #1 and #2.

Field Sample Processing

Bulk soil was mixed in a large container and left out to dry in the green house, which had low humidity, for 5 days, then sieved to eliminate pebbles larger than 0.5 mm in diameter and transferred into an autoclavable container. The sieved soil was subjected to 3 consecutive 20-minute solid autoclaving cycles with the soil being mixed with an autoclaved wide metallic spatula between cycles.

To prepare the inoculum, extra loose soil was gently manually shaken off from the biocrust. Then the biocrust was homogenized by gentle crushing with a metal spatula and then mixing. Before application to the experimental units, the biocrust inoculum was stored in dry covered conditions at room temperature for no longer than one month.

Greenhouse Experimental Setup

Experiment #1 consisted of twelve independent incubation units in total and was carried out in a greenhouse facility at Arizona State University, Tempe. The units were positioned from North side to South side of the greenhouse room in two rows of 6 (Fig. 1). Six were experimental units, and 6 were control units. To eliminate the effect of water run-off during wetting, each unit's position was adjusted until it had zero % incline, using a carpenter's level. Each unit consisted of a plastic container nestled inside another container that delivered water through a wicking watering system described in Doherty et al. (14). All containers were 22 cm × 35 cm × 7 cm. The bottom container was connected

to a tube that delivered water from a deionized water reservoir, and had a hole drilled into it to drain excess water away to the sewer drain. The inside container had multiple holes drilled around the bottom perimeter and was provided with a polyester water filter lining, on top of which, sterile soil was placed (Fig. 1). The volume of the sterile soil in all the containers was adjusted to fill up to the 1.5 cm height, so that each container had about 3.9 kg of sterile soil.

Greenhouse experiment #2 was conducted in a 6 unit set-up comprised of 4 experimental and 2 control units. The experimental unit set up was similar to the set up for experiment #1 with minor differences. The units were arranged in one row from West to East side of the green house room, which allowed for a more uniform light exposure. Every 3 weeks the unit locations were rotated to assure that each unit was in a new location after each rotation (this was not done in experiment #1). The rotation made sure that growth would not be dependent on the location on the bench.

Control units. The units contained about 3.9 kg of sterile soil each. The control units did not have inoculum. The purpose of the control units was to monitor contamination of the soil resulting from airborne algae or cyanobacteria that might disperse to the soil.

Experimental units. In addition to the 3.9 kg of sterile soil each, the experimental units had a surface area of 5 cm by 35 cm along one of the long edges inoculated with the homogenized inoculum (Fig. 2). In addition to cyanobacterial bundles, the inoculum contained extraneous materials such as rocks and soil particles lacking cyanobacteria that could not be separated from the cyanobacterial biomass. Knowing that the mass of inoculum did not reflect cyanobacterial biomass but inflated it,

I had to maximize the mass of added inoculum to approach the level of Chl *a* seen in the original biocrust (0.0153 ± .00547 mg/cm^2). To do so, the units were inoculated to 100% of surface area, corresponding to a mass 44 g. While 44 g would theoretically correspond to 251 mg/cm^2 of Chl *a*, the resulting inoculum biomass ranged from 0.00123 mg/cm^2 to 0.0146 mg/cm^2. The inoculum was placed on the experimental units in a dry form that turned into a slurry with the first watering event. One experimental unit in experiment #2 had pieces of inoculum away from inoculum after the first watering event. Therefore, this unit was not included when looking at the rate of dispersal.

Watering regime. All units were watered to match average natural rainfall frequency of field site, where 77 days out of the year received some precipitation (Table 1). Rainfall frequency data from May 1987 to January of 2015 at Apache Junction 5 NE station informed the watering frequency to be every 4 days to soil holding capacity (Western Regional Climate Center). The typical local yearly rainfall pattern can be found in Table 1. Consequently, units received water for 6 minutes at 10 a.m. every 4 days. This way, the units were allowed to completely soak and to dry out thereafter before the next watering. The biocrusts typically remained visibly wet for 4 hours during the Spring, 2 hours during the summer, and up to 6 hours during the fall and winter.

Local Air Temperature. The green house air temperatures were available only for May, June, and July. Therefore, Tempe weather station served as a proxy for temperatures during other months. While the air temperature means in the greenhouse did not significantly differ from the temperature means accessed from the Tempe weather station, the temperature maximums tended to be higher in the greenhouse. Especially during peak summer temperatures, the heating effect in the greenhouse was not

successfully moderated by the greenhouse air circulation system. For a more detailed information on temperature maximums and minimums, refer to Table 2.

Photosynthetically Active Radiation. During three consecutive sunny days in November 2016, 5 light meters were placed along the north to south gradient of the greenhouse facility. This was done to see if certain parts of the room received more light than others. Each light meter was balanced and propped at a height that was not shaded by the unit containers and placed on the bench adjacent to the units. The light meters measured photosynthetically active radiation (PAR) as photosynthetic photon flux density (PPFD) in $\mu mol \cdot m^{-2} \cdot s^{-1}$. The meters took hourly measurements during the daylight hours from 8 a.m. to 5 p.m. Certain positions within the greenhouse were found to have highly differential total PAR exposure. The light meter closest to the south window of the green house received the highest PPFD total based on the average of three days (2615.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$), while two light meters halfway between the south and north position received the lowest total average PPFD (1017.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 1345.2 $\mu mol \cdot m^{-2} \cdot s^{-1}$). To minimize the high light exposure difference effect, experiment #2 plots were set up from east to west (this directional gradient had a more consistent light exposure) and the position of each unit was rotated every three weeks.

Unit Photography

In experiment #1, aerial unit pictures were taken with Nikon Coolpix S7000 monthly in the greenhouse, although time since watering and lighting was not controlled for (Fig. 3). In experiment #2, units were brought down to a dark room 1 day after watering and pictures were taken with a Nikon Coolpix S7000 under a common light

source and conditions. An additional set of pictures for experiment #2 was made in the greenhouse as well. To avoid picture comparison inconsistency, the color match tool was applied in Photoshop.

Lateral dispersal in the experimental units was visually assessed with the aid of Fiji Image J image processor after 3 months of incubation by measuring the width of the lateral dispersal band. Experimental unit pictures from experiment #1 and #2, a total of 9 pictures (one unit was excluded because there was no visible lateral dispersal), were analyzed using color threshold adjustment on min. error setting. Pixels of the picture were set to pass the threshold if the hue (21-233), saturation (80-212), and brightness (11-204) were kept within a certain range of values. Six measurements of width of the lateral dispersal band (Fig. 4, bottom) were taken on each picture with applied threshold to get a range of widths using the calibrated straight line tool in Fiji Image J.

Sampling of Soil for Biomass and Cyanobacterial Identification

Discrete sampling was carried out for microscopy and biomass quantification at different time points and distances. Each unit was sampled at 5 different distances from the inoculum. Any location on the 5 cm-wide inoculum band on experimental units was defined as 0 cm. In the absence of inoculum, this area was defined as 0 cm as well (control units). Four distances away from inoculum were chosen: 3 cm, 6 cm, 9 cm, and 12 cm, along a line orthogonal to the inoculum line.

About a month (28 days) after the first watering event, the time series sampling was started. Samples were taken monthly from March 2016 to August 2016. November

2016 was the final sampling time for experiment #1. Experiment #2 was sampled monthly from December, 2016 to February, 2017.

Microscopy. One experimental unit was chosen to track cyanobacterial presence over time. Using sterile forceps, the top 2 millimeters of soil were sampled around the set distances from inoculum, avoiding areas where prior sampling was done (as denoted by soil core holes), and placed into a microplate. They were all observed in the dissecting microscope. If biomass was visible under the dissecting microscope, it was then further examined under the compound light microscope. For purposes of documentation, pictures of the field view in the compound light microscope were taken with a Nikon Coolpix S7000 camera, held steady in front of the eyepiece. To view the sample under the compound light microscope, 5 or less drops of water were added to the sample with a disposable 1 mL pipette. The sample was then homogenized by pipetting it back and forth, and a wet mount was made from one drop.

Morphological Identification of Cyanobacteria. Cyanobacterial presence or absence was noted. When present, cyanobacteria were identified using morphological characteristics typically used in literature that also identified cyanobacteria from biocrust (4, 25-27). Additionally, AlgaeBase and expert opinion (personal communication, Giraldo, A; Machado, N.) were used to identify *Schizothrix* spp. A list of key morphological characteristics for the identified species can be found in Table 3. The width and length of cells was measured as needed using the eyepiece graticule (ruler visible in one of the eyepieces when viewing a sample) while viewing the organism under the microscope. A Fiji Image J image processor was used to assign the correct scale bar to each magnification.

Biomass determinations. Soil cores were sampled on control and experimental units in an identical manner. Soil was cored at 3 cm intervals along an orthogonal line, starting from the left side of the unit (Fig. 2). Sequential samplings were done from left to right, avoiding areas with cracks in the soil or a hole remaining from a past soil core sampling. All samples were taken with a 1.2 cm in diameter by 1 cm depth core. The soil core samples were stored at 4 ºC wrapped in aluminum foil before chlorophyll *a* extraction. Chlorophyll *a* extraction from soil was used as a proxy for calculating biomass. Chlorophyll has been used as a proxy for photoroph biomass (16, 28). I used an improved method to extract higher level of chlorophyll *a* optimally provided by A. Giraldo Silva (unpublished). Each soil core was ground with mortar and pestle in 90% acetone for 3 minutes until a paste formed. The paste was placed into a 15 mL polypropylene centrifuge tube and 90% acetone was added until the volume reached 10 mL and then vortexed for 1 minute. The centrifuge tube was then stored for 24 hours in the 4 ºC in the dark, after which it was centrifuged at 7, 000 rpm for 8 minutes at 15 ºC. Absorption spectra (from 330 to 1000 nm) of the extracts were run on Shimadzu spectrophotometer and absorbance was recorded at 384 nm, 490 nm, and 663 nm. All absorbance values were corrected for any remaining turbidity by subtracting absorbance value at 750 nm. Absorbance attributable to chlorophyll *a* was corrected for scytonemin interference with the trichromatic equation of Garcia-Pichel and Castenholz (29). Using this value, areal concentrations were back-calculated based on core size and presented in units of mg of Chl *a* per m^2 of crust surface.

Lateral Dispersal Rate Calculation

Chlorophyll *a* concentrations during an appropriate time frame were used to calculate lateral rate of dispersal. Compared to visible greening, chlorophyll *a* is a more accurate determinant of phototrophic biomass. Because chlorophyll *a* was sampled every 3 cm, the precision of the distance measurements was within the 3-cm range. Biomass had to be detected continuously in the experimental unit in order to be considered as laterally dispersed. For example, one experimental unit had to be excluded because it had no biomass detected 3 cm away from the inoculum but did have biomass 6 cm away from the inoculum.

The maximum distance at which the biomass was detected to laterally disperse was divided by the number of months elapsed to calculate the rate of lateral dispersal. The monitoring of control units helped choose an appropriate time frame for calculating lateral dispersal rate. After visible widespread aerially originated colonies were both visible and detected via chlorophyll *a*, the measurements on experimental units could no longer be trusted.

RESULTS

Aerial dispersal was defined as cyanobacterial growth originating from the air inside the greenhouse. The main source of air movement was the greenhouse ventilation system that either carried cyanobacterial cells from the inoculum inside the greenhouse, or from the air external to the greenhouse.

In contrast, lateral dispersal was when motile cyanobacteria self-propelled in a lateral direction through the soil substrate. Lateral dispersal came from the adjacent community of cyanobacteria, originating from the inoculum that was placed on the experimental units.

Aerial Dispersal in Control Units

Tracking aerial dispersal in control units informed when lateral dispersal in experimental units could no longer be differentiated from aerial dispersal. When control units showed substantial presence of biomass, monitoring of experimental units was stopped.

Experiment #1. No biomass was detected in any control unit until August, although biomass was not quantified in June or July Ffig. 5 A). The first visible signs of growth on the control units were in August, 5 months after the start of the experiment Ffig. 3 I). Yellow-green or dark green colonies then dotted multiple parts of the control units without a distinct spatial pattern (Fig. 3 I). Chlorophyll *a* concentrations of the colonies were similar, independently of the distance they were detected at. Chlorophyll *a* concentrations in control units increased from August to November (Fig. 5 A).

The species present on control plots in August were *Leptolyngbya* spp. in dense clusters, and *Microcoleus vaginatus. Schizothrix* spp. and an unidentified *Microcoleus* species were also detected in November (see fig 6. for microphotographs and Table 3 for their main morphological characteristics).

Aerially sourced colonies were visible on the experimental units in August as well, located away from the front of the laterally advancing biocrust, as seen by the pattern of green cyanobacterial colonies in fig. 3 D. By November, growth reached all the measured distances in experimental units and no longer allowed distinguishing lateral advancement from aerially dispersed sources (Fig. 7 A).

Experiment #2. In the first month, there was biomass detected (Fig. 5 B) in the controls. The first visible signs of dark green growth on one of two control units appeared in January, 2 months after the start of the experiment (fig. 5 B), when I also detected chlorophyll *a* (0.534 ± 0.891 mg/m^2) in 4 out of ten control samples. Three months after the start of the experiment, in 8 out of 10 samples, the controls had 2.61 ± 2.40 mg/m^2 (Fig. 5 B). The main species present then was *Scytonema* spp. Chlorophyll *a* concentration on control units at the end of the experiment (in February 2017), displayed lateral dispersal from the original colonies (Fig. 5 B), indicating that a combination of initial aerial dispersal coupled to a lateral, self-propelled dispersal of this natural inoculum was taking place.

By February 2017, one of the experimental units also displayed growth at all sampling points (fig. 7 B). Additionally, in the rest of the experimental plots, I discovered significant biomass loss caused by an unknown pathogen (Fig. 8). For the above-mentioned reasons, monitoring was halted then.

Inoculum Characterization

In experiment #1, the dominant inoculum forming cyanobacteria were *Microcoleus vaginatus* and *Scytonema* spp., while in experiment #2 they were *Microcoleus steenstrupii* and *Scytonema* spp (Fig. 6). In experiment #1, there was a high variability of chlorophyll *a* concentration among units on inoculum (Fig. 7, A). In March, chlorophyll *a* concentration ranged from 3.46 mg/m^2 to 146 mg/m^2 with the average of 57.6 ± 51.2 mg/m^2 (the standard deviation). Inoculum biomass average fluctuated from March to August, decreasing in May, while increasing in July, and then decreasing again in August. In experiment #2, on average, biomass at inoculum increased from December to January, then decreased in February. Inoculum biomass differences between different sampling times were not significant and resulted from variability.

Lateral Dispersal in Experimental Units

Biocrusts in experimental units in both experiments (Fig. 3 A-E) exhibited a clearly advancing front of biocrust through time, with an edge that was irregularly shaped (Fig. 4, middle and bottom), indicating some level of spatial variability. On average, the biocrust visibly advanced 1.38 ± 1.50 cm in 3 months (Table 4), at which time biocrust biomass was found away from the inoculum on all 10 experimental units (Fig. 7 A). However, only 8 out of the 10 experimental units were considered to have clearly advanced laterally (Table 4). One of six experimental units from experiment #1 presented discontinuous biomass spread, where biomass was detected at 6 cm and 12 cm but not at 3 cm or 9 cm, nor was there any visible lateral advance using the color threshold method. I suspect that this biomass on this experimental unit may have resulted from aerial

contamination, and thus the data was not included in the lateral dispersal calculations. In one experimental unit from experiment #2, inoculum was not placed successfully (see methods); this unit was also not taken into account for calculations. The nature of discrete core sampling with 3 cm intervals resulted in ± 3 cm sensitivity of lateral Chl *a*-based dispersal measurements. Lateral dispersal rate was 2 cm/month on average, as based on chlorophyll *a* concentrations (Table 4).

Experiment #1. I detected advances by Chl *a* in March in one unit. In May, biomass was detected 3 cm and 6 cm away from the inoculum on all units. Biomass reached 23.1 ± 14.7 mg/m^2 at 3 cm and 3.36 ± 2.28 mg/m^2 at 6 cm (Fig. 7 A). Lateral dispersal was detected in 5 out of 6 units. Four units laterally dispersed 2 cm/month, while one dispersed at a faster rate of 3 cm/month.

Experiment #2. Chla *a*–based lateral dispersal was detected just one month after the start of the experiment. By January (2 months), biomass in two units had reached 6 cm in the first 2 months (0.227 mg/m^2 and 2.78 mg/m^2). By February, biomass in two additional units was detectable at 3 cm (1.58 mg/m^2), and 6 cm (0.277 mg/m^2) (Fig. 7 B). In the end, 3 out of 4 units showed detectable advances. Two units dispersed 2 cm/month, while one dispersed 1 cm/month.

Laterally Dispersed Cyanobacteria

Experiment #1. *Microcoleus vaginatus* was present in sheathed bundles containing multiple filaments with 4-6 µm wide trichomes on the inoculum and 3 cm away from the inoculum. The bundles at 3 cm were smaller (contained less filaments) than the ones on the inoculum. *Scytonema* spp. was present as single sheathed filaments

from 8-11 µm in width on the inoculum, and 3 cm away from the inoculum. With increasing distance, *Scytonema* spp. filament density decreased. Cyanobacterial hormogonia of differing widths (3-10 µm) were observed at 3 cm, 6 cm and 9 cm by the 3 months (Fig.6, left). Hormogonia are motile short segments of cells that are released from the vegetative filament as a means of colonizing new substrate (30).

Experiment #2. *M. steenstrupii* was observed as single sheathed filaments not yet forming bundles 3 cm away from the inoculum. *Scytonema* spp. was present as single sheathed filaments from 8-11 µm in width on the inoculum, and 3 cm away from inoculum (Fig. 6, right).

Mortality Events

Experiment #1. From May to July, biomass 3 cm away from inoculum decreased on average, reaching zero in 5 out of 6 units (Fig 7 A). In the early summer, the green color saturation of cyanobacterial growth temporarily receded. *M. vaginatus* filament color changed from green to pale yellow, and in some cases, clear. Meanwhile, *Scytonema* spp. filaments changed the color from light green to orange, possibly signifying higher carotenoid production, not mortality. In August, average biomass at 3 cm recovered to higher than summer biomass concentration level but still lower than May biomass level (17.1 ± 12.6 mg/m^2).

Experiment #2. After two months, bleached cyanobacterial filaments were observed on the inoculum area after the movement of the green band has already occurred (Fig. 8).

Contamination on Experimental Units

Non-cyanobacterial organisms and cyanobacteria detected in abundance but not part of the original inoculum were considered contamination (Fig. 6). Their origin could be from dust particles carried inside via greenhouse ventilation.

Experiment #1. *Leptolyngbya* spp. was present as single thinly sheathed filaments from 1-3 µm in width with oval and beadlike cell shape. *Leptolyngbya* spp. was observed 9 cm away from inoculum 3 months after the start of the experiment (May). *Schizothrix* spp. was present as multiple filaments in sheathed bundles in November. Each trichome was 2.5-3 µm in width. In November, light green moss growth appeared on the inoculum of all experimental units. Visible surface area covered by moss of the inoculum ranged from .03 % to 42 %. Measurement was done using a transparent grid, where each square was 1 cm by 1 cm.

Experiment #2. Two out of 4 units exhibited light green powder like growth at all distances on the unit in February. Light green matched the appearance of unidentified algae under the compound light microscope unconfirmed by other means. In February, *Schizothrix* spp. was the only species detected 6 cm away from the inoculum in February (Fig. 6, right).

Qualitative Assessment of Microtopographical Preferences of Growth

The biocrust tended to laterally disperse with high variability in all units. The replicate that visually dispersed the furthest in 3 months, dispersed 2.32 ± 2.31 cm away from inoculum. Three out of 8 units (that were considered laterally dispersed according to chlorophyll a data) did not have a visual dispersal statistically significant from zero. The

dispersal away from the biocrust edge ranged from no visible dispersal to 6.60 cm in 3 months.

Holes remaining from soil cores had green growth in certain parts that were not too deep for optimal light exposure. From the very beginning, the soil substrate in all units formed characteristic cracks. There was no apparent preference for growing in the cracks but growth did appear along the edges of cracks. Compared to not cracked surfaces, it was not possible to visually assess a pattern of preferential growth. The width of cracks mattered and if it was narrow enough, there was more growth visible on it.

DISCUSSION

Lateral Dispersal Rate

Qualitative observation coupled with chlorophyll a concentration time series confirmed that cyanobacterial biological soil crusts are capable of laterally dispersing when provided with a consistent watering cycle. In 8 independent experimental units, biocrusts laterally dispersed 1-3 cm per month (Table 4). As populations grew and space became limited, the constituent cyanobacteria laterally dispersed to colonize new substrate. Motile cyanobacteria dispersed as far as 9 cm distances in 3 months even when having an opportunity to move only during the short pulses of water availability. The watering regime is very critical here for it regulates the metabolic activity (31) and aids in gliding motility (21). Because the chosen water cycle mimics a raining frequency in a bimodal rainy season's year of certain parts of the Sonoran Desert (Table 1), the measured dispersal rate should be relevant to the potential for self-propelled dispersal during the rainy season in the field (i.e, in summer and winter).

When active, Campbell found that a *Microcoleus vaginatus* filament moves at an average rate of 0.4 μm/s (21). This rate consistent with the finding of the current experiment: 2 cm/month on average (Table 4). Considering that in the greenhouse experiment the filaments were active for 28 hours in a month (at optimal temperature), the filaments would be predicted to move as fast as 4 cm/month, if they were moving in one direction along a straight line. Thus, this theoretical rate is only twice as fast as that measured by population advance seen in the greenhouse. Unlike in Campbell's experiment, the filaments in the greenhouse had to disperse through soil, not agar, and

had to endure periods of desiccation. However, Campbell's rate of lateral dispersal of a filament is closer to this experiment's rate than to the lateral dispersal rate of a whole bundle measured by Ana Giraldo Silva, at 0.5 cm/month (personal communication). The above comparisons suggest that *M. vaginatus* most likely rely on laterally dispersing via single filaments and not bundles. It also shows that the lateral population advance is quite effective in its directionality.

In my experiments, lateral dispersal and aerial dispersal eventually combined to make the source of cyanobacterial growth indistinguishable. This merging point was highly variable and appears to depend on temperature and water availability. While aerial dispersal alone affected a large area of bare soil, the biomass on control units never exceeded 8 mg/m^2. Aerial dispersal most likely originated from outside air that cycled through the green house ventilating system. The ventilation system was the only source of air movement because the closed greenhouse room did not allow any wind to enter. The slight air movement then could presumably transport the dust from outside or from the source inoculum contained on units. Although difficult to measure, it is important to consider aerial dispersal resulting from dust storms in the field, for it can substantially increase the rate of recovery when coupled with lateral dispersal.

A population loss pattern reminiscent of viral plaques in culture, consisting of irregular or circular shaped patches of dead cyanobacteria on some experimental units was observed in experiment #2. Follow-up experiments by J. Bethany (unpublished) confirmed that this was due to the presence of pathogenic, infectious agent that attack *M. vaginatus* in culture, as it did in my units (Fig. 8). This is the first report of a biocrust pathogen. The pathogen could be a lysing bacterium, some of which were isolated from a

cyanobacterial bloom (32), or a cyanophage virus, which is common among filamentous cyanobacteria (33). This discovery is important, as it potentially plays a role in the ecology of soil crusts.

Laterally Dispersed Cyanobacteria

As predicted, motile pioneer cyanobacteria known to live in biocrust (4, 9, 26) , such as *Microcoleus vaginatus* and *Microcoleus steenstrupii,* dispersed laterally. *M.vaginatus* and *M. steenstrupii* laterally dispersed by 1 cm/month. The experiments demonstrate that *M. steenstrupii* attained the same dispersal rate as *M. vaginatus*, which contradicts the findings of Boyer (25).

Another species commonly occupying biological soil crust was detected as far as 3 cm away from biocrust within a 3 month period: *Scytonema* spp. While not motile as filaments, *Scytonema* spp. can disperse by developing and releasing motile hormogonia. The discovery of dispersed *Scytonema* spp. filaments in both experiments suggests that its hormogonia were able to disperse and grow into filaments in as little as three months. This leads me to question whether *Scytonema* spp. and *M. vaginatus* or *M. steenstrupii* disperse together as a community. Being motile at as filaments (21) and hormogonia (34), would *M. vaginatus* assist the hormogonia of *Scytonema* spp.in migration? Or would they move as separate entities without cooperation? Regardless of migration strategy, *Scytonema* spp. and *M. vaginatus* were found aggregated together in the soil away from inoculum. Both species stabilize soil via extracellular polysaccharides and heterocystous *Scytonema* spp. fixes nitrogen (35). *Scytonema* spp. can benefit from *M. vaginatus*, because unlike *M. vaginatus*, *Scytonema* spp. cannot colonize bare, unstable soil.

The only species that was could disperse as far as 6 cm in experiment #2 was *Schizothrix* spp. It is difficult to tell whether *Schizothrix* spp. originated from the air, or from the inoculum, although it was not observed microscopically on the inoculum.

Leptolyngbya spp. and *Schizothrix* spp. were the commonly present weedy species that grew abundantly as temperatures lower in August after the July highs (Table 2). They are not known to play an important functional role in biocrust, but they are often recovered in cultures. In addition to the weedy species, species ecologically important to biocrust community successfully dispersed through air to colonize control units. *Microcoleus vaginatus* and *Scytonema* spp. colonized aerially in between 2 (winter) to 5 (spring and summer) months.

The widespread presence of hormogonia at the far edge (Fig. 6, left) of the lateral dispersal front suggests that certain cyanobacteria first must go through cell differentiation before establishing on a new soil area. Segments of a filamentous cyanobacterium that is in a vegetative state differentiate into hormogonia to disperse and start a new colony. Unlike vegetative cells, cells of a hormogonium cannot divide. Once the motile hormogonia have arrived at a suitable substrate, they differentiate back into a vegetative state (30). While the speed of hormogonia has not been measured for any of the species detected in the present study, it has been found to range from 0.7 to 3 μm/s for *Nostoc punctiforme* and *Mastigocladus laminosus* (36, 37), which is higher than that of a filament 0.4 μm/s of a filament (21). This agrees with the fact that some hormogonia were indeed detected by microscopy at distances as far as 9 cm after 3 months of incubation. While the hormogonia were difficult to assign to a particular taxon, wide (8-

10 µm) abundant hormogonia dispersing as far as 9 cm, are most likely the motile hormogonia from *Scytonema* spp.

Lateral and Aerial Dispersal: Seasonal Patterns

Seasonality appeared to play a role in retarding or encouraging aerial dispersal to units. In experiment #1, small green colonies of cyanobacteria observed on the control units, and by August, on experimental units, point to separate and multiple instances of aerial dispersal, but few over time (Fig. 4). However, during winter in the second experiment, large patches of green were seen in certain parts of the control units, as supported by chlorophyll *a* data (Fig. 5 B). Lower average air temperature in November, December, and January than in the spring months, and a higher relative humidity, could have caused a faster aerial dispersal rate. Also, due to lower evaporation rate, the units stayed wet 2 hours longer. A faster aerial dispersal rate means that each colony can grow larger and multiple colonies might join into a larger patch. Eventually, as the bacteria run out of space, they would be expected to start dispersing laterally.

On the other hand, hot summer months with temperatures at times reaching 48°C in the greenhouse, decreased overall biomass both at distances and at the inoculum (Table 2). Yellowing *Microcoleus vaginatus* bundles were most likely suffering from heat intolerance. The watering cycle only worsened the situation for the cyanobacteria. If no water was provided, they would have stayed dormant below the soil. The availability of water caused them to come up to the surface (22), only to be exposed to lethal temperatures and UV radiation, as water quickly evaporated. Similarly, *Microcoleus vaginatus* died at 40 °C in cultures, while other strains including *Scytonema* spp. survived

(12). As soon as outside mean air temperatures in August decreased by 4 °C from July (Table 2), growth visibly reappeared, which was confirmed by chlorophyll *a* measurements. In a 2002 study by Bowker and colleagues, a similar pattern of mortality during the summer and an increase of chlorophyll *a* concentration in the fall was recorded (38).

In 3 months, biomass concentration 3 cm away from inoculum on 3 winter experimental units that had lateral dispersal detected (12.3 mg/m^2) was significantly lower than biomass detected in the spring (23.1 mg/m^2). Colder winter temperatures most likely slowed down the growth rate in experiment #2. Taking lower temperatures into consideration, it is not surprising that one experimental unit from winter had the lowest lateral dispersal rate (1 cm/month). Nevertheless, other conditions could have led to lower growth rates such as lower light exposure and predation by the unknown pathogen.

One interesting observation was that there was high variability in inoculum biomass between experiment #1 units even after homogenization (Fig. 7 A). This may have been caused by the fact that the units received different illumination due to their different locations within the greenhouse. Except for few units where aerial dispersal was present at 12 cm, the dispersal pattern was consistent in 8 replications. Biomass was the highest on inoculum, decreasing towards 6 cm, and reaching 9 cm only in unit 3 after 3 months on incubation. Surprisingly, in the first 2 months, low amounts of biomass were present at distances away from inoculum in some experimental units, even while there was no biomass detected on control units but in 6 out of 24 cores taken at distances away from inoculum. There is a possibility that pieces of inoculum that were not yet firmly attached to the soil floated away during watering. However, the benches on which the

units were attached, and the containers themselves were balanced to have no incline. Indeed, the placing of the inoculum on the bare soil technique could be improved for future similar experimental set ups. Placing a temporary removable barrier for the first two weeks of watering could be one solution.

Recommendations for Biocrust Restoration

Optimal spatial arrangement of inoculum. To maximize surface area available for lateral dispersal, I would recommend to place inoculum patches of irregular shape. When provided with a straight edge, biocrust will disperse in an irregular pattern even on flat surface. Multiple smaller sized patches rather than fewer larger sized patches will lead to faster lateral dispersal with increased edge length. Small aerially dispersed colonies grew and laterally dispersed to combine into larger joined patches very quickly. In 3 months after first noticing aerial dispersal visually, under favorable water and temperature conditions, cyanobacteria have completely dispersed to all the surface area of the unit (Fig. 7, A).

Patches of inoculum should be placed no closer than 2 cm away from each other because this is the minimal rate that cyanobacterial biocrust is capable of dispersing per month on its own without the aid of water run-off, wind, or aerial dispersal. However, such lateral dispersal will happen only if optimal water and temperature conditions are provided.

It is worth to pay attention to the microtopography of the soil surface. To assure lateral dispersal, cyanobacteria need enough surface area. Deep and wide cracks in the

soil could slow down and even block lateral dispersal. On the other hand, more shallow crevices serve as a potential growth area.

Optimal timing of inoculum placement. Choosing an optimal time for inoculum placement depends on climatic conditions such as rain and temperature. Inoculum should not be placed into the field during peak summer temperatures. If inoculum is placed into the field during high temperatures for whatever reason, the inoculum can, however survive in a dormant state until temperatures lower. Watering of inoculum should be avoided during temperatures of 40 °C or higher for this can cause mortality. While inoculum can survive without water it will not laterally dispersed unless water is available. To take advantage of the longest wet period of the year, the inoculum would benefit from being placed between late August and September, or as soon as rains start during the monsoon season and as soon as temperatures are cool enough. While during cold temperatures of winter, lateral dispersal rate might be slow, it will speed up during optimal temperatures of spring if provided with water.

CONCLUSION

This study demonstrates the cyanobacterial biocrust's natural ability to expand horizontally. Considering that in the habitat from which the soils and the biocrust were collected (Sonoran Desert upland) it rains about 77 days per year, and extrapolating from my results, one could expect that inoculum would minimally disperse 4 cm per year. This rate however, does not account for possible mortality. I predict the overall dispersal rate of biocrusts to be faster when accounting for aerial transport. This information can guide restoration efforts regarding the distance between inoculated patches that can be left without inoculum, so as to optimally estimate the area that can be restored. I recommend avoiding trampling the few but precious centimeters surrounding more inconspicuous biocrusts for they might have the hard to see cyanobacterial groundworks of an eventually full-fledged biological soil crust.

REFERENCES

1. **Prăvălie, R.** 2016. Drylands extent and environmental issues. A global approach. Earth-Sci. Rev. **161:**259-278. doi: http://dx.doi.org.ezproxy1.lib.asu.edu/10.1016/j.earscirev.2016.08.003.

2. **Qadir, M, Quillérou, E, Nangia, V, Murtaza, G, Singh, M, Thomas, RJ, Drechsel, P, Noble, AD.** 2014. Economics of salt-induced land degradation and restoration. Natural Resources Forum. **38:**282-295. doi: 10.1111/1477-8947.12054.

3. **Guo, Q.** 2004. Slow Recovery in Desert Perennial Vegetation following Prolonged Human Disturbance. Journal of Vegetation Science. **15:**757-762.

4. **Alwathnani, H, Johansen, JR.** 2011. Cyanobacteria in Soils from a Mojave Desert Ecosystem. Monographs of the Western North American Naturalist. **5:**71-89. doi: 10.3398/042.005.0103.

5. **Jiang, L, Yufen, T, Zhijie, Z, Tianhong, L, Jianhua, L.** 2005. Water Resources, Land Exploration and Population Dynamics in Arid Areas: The Case of the Tarim River Basin in Xinjiang of China. Popul. Environ. **26:**471-503. doi: 10.1007/s11111-005-0008-8.

6. **Naimabadi, A, Ghadiri, A, Idani, E, Babaei, AA, Alavi, N, Shirmardi, M, Khodadadi, A, Marzouni, MB, Ankali, KA, Rouhizadeh, A, Goudarzi, G.** 2016. Chemical composition of PM10 and its in vitro toxicological impacts on lung cells during the Middle Eastern Dust (MED) storms in Ahvaz, Iran. Environmental Pollution. **211:**316-324. doi: http://dx.doi.org.ezproxy1.lib.asu.edu/10.1016/j.envpol.2016.01.006.

7. **Pointing, SB, Belnap, J.** 2014. Disturbance to desert soil ecosystems contributes to dust-mediated impacts at regional scales. Biodivers. Conserv. **23:**1659-1667. doi: 10.1007/s10531-014-0690-x.

8. **Lal, R.** 2009. Sequestering carbon in soils of arid ecosystems. Land Degrad. Dev. **20:**441; 441-454; 454.

9. **Belnap, J, Lange, OL.** 2001. Biological soil crusts: structure, function, and management. Springer, New York; Berlin.

10. **Garcia-Pichel, F, Johnson, SL, Youngkin, D, Belnap, J.** 2003. Small-Scale Vertical Distribution of Bacterial Biomass and Diversity in Biological Soil Crusts from Arid Lands in the Colorado Plateau. Microb. Ecol. **46:**312-321. doi: 10.1007/s00248-003-1004-0.

11. **Garcia-Pichel, F, Wojciechowski, MF.** 2009. The evolution of a capacity to build supra-cellular ropes enabled filamentous cyanobacteria to colonize highly erodible substrates. PloS One. **4:**e7801.

12. **Garcia-Pichel, F, Loza, V, Marusenko, Y, Mateo, P, Potrafka, RM.** 2013. Temperature Drives the Continental-Scale Distribution of Key Microbes in Topsoil Communities. Science. **340:**1574.

13. **Wang, W, Liu, Y, Li, D, Hu, C, Rao, B.** 2009. Feasibility of cyanobacterial inoculation for biological soil crusts formation in desert area. Soil Biol. Biochem. **41:**926-929. doi: http://dx.doi.org.ezproxy1.lib.asu.edu/10.1016/j.soilbio.2008.07.001.

14. **Doherty, KD, Antoninka, AJ, Bowker, MA, Velasco Ayuso, S., Johnson, N.C.** 2015. A Novel Approach to Cultivate Biocrusts for Restoration and Experimentation. Ecological Restoration, North America. **33:**13; 13-16; 16.

15. **Maestre, FT, Martín, N, Díez, B, López-Poma, R, Santos, F, Luque, I, Cortina, J.** 2006. Watering, Fertilization, and Slurry Inoculation Promote Recovery of Biological Crust Function in Degraded Soils. Microb. Ecol. **52:**365-377.

16. **Velasco Ayuso, S, Giraldo Silva, A, Nelson, C, Barger, NN, Garcia-Pichel, F.** 2017. Microbial Nursery Production of High-Quality Biological Soil Crust Biomass for Restoration of Degraded Dryland Soils. Appl. Environ. Microbiol. **83:**e02179-16. doi: 10.1128/AEM.02179-16.

17. **Belnap, J, Gardner, JS.** 1993. Soil Microstructure in Soils of the Colorado Plateau: the Role of the Cyanobacterium Microcoleus Vaginatus. The Great Basin Naturalist. **53:**40-47.

18. **George, D, Roundy, B, St. Clair, L, Johansen, J, Schaalje, G, Webb, B.** 2003. The effects of microbiotic soil crusts on soil water loss. Arid Land Research and Management. **17:**113-125.

19. **Burchard, RP.** 1981. Gliding motility of prokaryotes: ultrastructure, physiology, and genetics. Annu. Rev. Microbiol. **35:**497-529. doi: 10.1146/annurev.mi.35.100181.002433.

20. **Wilde, A, Mullineaux, CW.** 2015. Motility in cyanobacteria: polysaccharide tracks and Type IV pilus motors. Mol. Microbiol. **98:**998-1001. doi: 10.1111/mmi.13242.

21. **Campbell, SE.** 1978. Soil Stabilization by a prokaryotic desert crust: implications for precambrian land biota, p. 85. *In* L. Margulis and C. Ponnamperuma (eds.), Proceedings of the Fourth College Park Colloquium on Chemical Evolution. D. Reidel Publishing Company, Dordrecht, Netherlands.

22. **Garcia-Pichel, F, Pringault, O.** 2001. Microbiology: Cyanobacteria track water in desert soils. Nature. **413:**380-381.

23. **Whale, GF, Walsby, AE.** 1984. Motility of the cyanobacterium microcoleus chthonoplastes in mud. British Phycological Journal. **19:**117-123. doi: 10.1080/00071618400650121.

24. **Griffin, DW.** 2007. Atmospheric Movement of Microorganisms in Clouds of Desert Dust and Implications for Human Health. Clin. Microbiol. Rev. **20:**459-477. doi: 10.1128/CMR.00039-06.

25. **Boyer, SL, Johansen, JR, Flechtner, VR, Howard, GL.** 2002. Phylogeny and genetic variance in terrestrial microcoleus (cyanophyceae) species based on sequence analysis of the 16s rrna gene and associated 16s–23s its region1. J. Phycol. **38:**1222-1235. doi: 10.1046/j.1529-8817.2002.01168.x.

26. **Garcia-Pichel, F, López-Cortés, A, Nübel, U.** 2001. Phylogenetic and Morphological Diversity of Cyanobacteria in Soil Desert Crusts from the Colorado Plateau. Appl. Environ. Microbiol. **67:**1902-1910. doi: 10.1128/AEM.67.4.1902-1910.2001.

27. **Rivera-Aguilar, V, Montejano, G, Rodríguez-Zaragoza, S, Durán-Díaz, A.** 2006. Distribution and composition of cyanobacteria, mosses and lichens of the biological soil crusts of the Tehuacán Valley, Puebla, México. J. Arid Environ. **67:**208-225. doi: http://dx.doi.org.ezproxy1.lib.asu.edu/10.1016/j.jaridenv.2006.02.013.

28. **Chiquoine, LP, Abella, SR, Bowker, MA.** 2016. Rapidly restoring biological soil crusts and ecosystem functions in a severely disturbed desert ecosystem. Ecol. Appl. **26:**1260-1272. doi: 10.1002/15-0973.

29. **Garcia-Pichel, F, Castenholz, RW.** 1991. Characterization and Biological Implications of Scytonemin a Cyanobacterial Sheath Pigment. J. Phycol. **27:**395-409. doi: 10.1111/1529-8817.ep10767308.

30. **Maldener, I, Summers, ML, Sukenik, A.** 2014. Cellular Differentiation in Filamentous Cyanobacteria, p. 279. *In* E. Flores and A. Herrero (eds.), . Caister Academic Press, Norfolk, United Kingdom.

31. **Rajeev, L, da Rocha, UN, Klitgord, N, Luning, EG, Fortney, J, Axen, SD, Shih, PM, Bouskill, NJ, Bowen, BP, Kerfeld, CA, Garcia-Pichel, F, Brodie, EL, Northen, TR, Mukhopadhyay, A.** 2013. Dynamic cyanobacterial response to hydration and dehydration in a desert biological soil crust. The ISME Journal. **7:**2178. doi: 10.1038/ismej.2013.83.

32. **Chen, H, Fu, L, Luo, L, Lu, J, White, WL, Hu, Z.** 2012. Induction and Resuscitation of the Viable but Nonculturable State in a Cyanobacteria-Lysing Bacterium Isolated from Cyanobacterial Bloom. Microb. Ecol. **63:**64-73. doi: 10.1007/s00248-011-9928-2.

33. **Baker, AC, Goddard, VJ, Davy, J, Schroeder, DC, Adams, DG, Wilson, WH.** 2006. Identification of a Diagnostic Marker To Detect Freshwater Cyanophages of Filamentous Cyanobacteria. Appl. Environ. Microbiol. **72:**5713-5719. doi: 10.1128/AEM.00270-06.

34. **Zhang, X, Feng, J, Wang, G, Xie, S.** 2014. A morphological and phylogenetic study of a filamentous cyanobacterium, Microcoleus vaginatus, associated with the moss Mnium cuspidatum. Symbiosis. **64:**43-51. doi: 10.1007/s13199-014-0301-3.

35. **Yeager, CM, Kornosky, JL, Morgan, RE, Cain, EC, Garcia-Pichel, F, Housman, DC, Belnap, J, Kuske, CR.** 2007. Three distinct clades of cultured heterocystous cyanobacteria constitute the dominant N2-fixing members of biological soil crusts of the Colorado Plateau, USA: N2-fixing cyanobacteria in soil crusts. FEMS Microbiol. Ecol. **60:**85-97. doi: 10.1111/j.1574-6941.2006.00265.x.

36. **Duggan, PS, Gottardello, P, Adams, DG.** 2007. Molecular Analysis of Genes in Nostoc punctiforme Involved in Pilus Biogenesis and Plant Infection. J. Bacteriol. **189:**4547-4551.

37. **Hernández-Muñiz, W, Stevens, SE.** 1987. Characterization of the motile hormogonia of Mastigocladus laminosus. J. Bacteriol. **169:**218-223.

38. **Donkor, VA, Häner, D.** 1995. Protective Strategies of Several Cyanobacteria against Solar Radiation. J. Plant Physiol. **145:**750-755. doi: http://dx.doi.org.ezproxy1.lib.asu.edu/10.1016/S0176-1617(11)81291-5.

39. **Bowker, M, Reed, S, Belnap, J, Phillips, S.** 2002. Temporal variation in community composition, pigmentation, and F-v/F-m of desert cyanobacterial soil crusts. Microb. Ecol. **43:**13-25. doi: 10.1007/s00248-001-1013-9.

APPENDIX A

TABLES

Table 1. Average 29-year record of total monthly precipitation from a weather station adjacent to the field location where biocrust was collected

	Apache Junction, AZ (1987 – 2016)[a]
Month	Total Precip.[b] (mm)
Jan	40
Feb	40
Mar	35
Apr	16
May	7.4
Jun	1.5
Jul	26
Aug	40
Sep	19
Oct	16
Nov	22
Dec	41

a. Source: http://www.wrcc.dri.edu/cgi-bin/cliMAIN.pl?az0288

b. Typical bimodal precipitation pattern that the experimental watering frequency was based on.

Table 2. Temperature mean, max, and min in the greenhouse and at a weather station adjacent to the greenhouse

	Greenhouse (Tempe, AZ)			Tempe weather station adjacent to the greenhouse[a]		
Month	Mean temp. (°C)	Max. temp. (°C)	Min. temp. (°C)	Mean temp. (°C)	Max. temp. (°C)	Min. temp. (°C)
Mar. 2016	-[b]	-	-	21	28	14
Apr. 2016	-	-	-	23	30	16
May 2016	28	43	20	27	33	20
Jun. 2016	34	48	21	35	42	28
Jul. 2016	36	48	25	36	42	30
Aug. 2016	-	-	-	32	39	22
Nov. 2016	-	-	-	18	26	9.2
Dec. 2016	-	-	-	14	22	6.4
Jan. 2017	-	-	-	13	18	7.8
Feb. 2017	-	-	-	19 (2016)[c]	27 (2016)[c]	11(2016)[c]

a. Source: https://gis.ncdc.noaa.gov/maps/ncei/summaries/monthly
b. – represents data that was not available from the greenhouse facilities
c. Because there was no record for February 2017, information from February 2016 was recorded.

Table 3. Morphological characteristics of identified cyanobacteria

Morph. Character.	*Microcoleus vaginatus*[a, b]	*Microcoleus steenstrupii*[b]	*Scytonema* spp.[a, c, e]	*Leptolyngbya* spp.[c]	*Schizothrix* spp.[d, e]
Trichome width (µm)	4-6	3.8 – 4.5	8-11	2-4	2.5 - 5
Sheath	Colorless	Colorless	Yellow-brownish or clear	Thin and clear	Thin
Makes bundles?	yes	yes	no	no	Yes. Narrowed and pointed at the end
Apical cell	Narrowed at the end with calyptra, shorter than the rest. thicker outer wall	Rounded, no calyptra, or elongated if mature	Rounded	Bluntly rounded	Conical, no thick outer wall
Branching	true	true	false	Varies	none
Cell shape	Quadratic, unconstricted	Rectangular, Slightly constricted	rectangular	Oval beadlike cells	Unconstricted or slightly constricted
Necridia?	no	yes	yes	Varies	unknown
Motility	Yes: filaments	Yes	Yes: only hormogonia	Yes	Yes, hormogonia

a. Phylogenetic and morphological diversity of cyanobacteria in soil desert crusts from the Colorado plateau. 2001.

b. Phylogeny and genetic variance in terrestrial Microcoleus (Cyanophyceae) species based on sequence analysis of the 16S rRNA gene and associated 16S-23S ITS Region. 2002.

c. Cyanobacteria in Soils from a Mojave Desert Ecosystem. 2011.
d. AlgaeBase
e. Distribution and composition of cyanobacteria, mosses and lichens of the biological soil crusts of the Tehuacán Valley, Puebla, México. 2006.

Table 4. Lateral dispersal of cyanobacterial crust communities after 3-month incubation during seasons that allowed net growth

Season (Exp. #)	Avg. visible distance dispersed from inoculum based on pictures (cm)[a]	Max. distance from inoculum based on Chl *a* (cm)[b]	Chl *a* at max. distance (mg/m^2)	Dispersal rate based on Chl *a* (cm/month)
Spring (1)	0.417	6	2.23	2
Spring (1)	1.91	6	1.69	2
Spring (1)	2.32	9	5.99	3
Spring (1)	1.83	6	1.90	2
Spring (1)	1.52	6	2.03	2
Winter (2)	1.02	6	16.0	2
Winter (2)	1.23	3	1.58	1
Winter (2)	0.770	6	0.277	2
Mean	1.38	6	3.96	2
St. Dev.	1.50	2	5.13	0.5
Median	0.871	6	1.97	2

a. Average is based on 6 width measurements spanning the whole biocrust boundary from left to right.

b. distance sensitivity is ± 3 cm

APPENDIX B

FIGURES

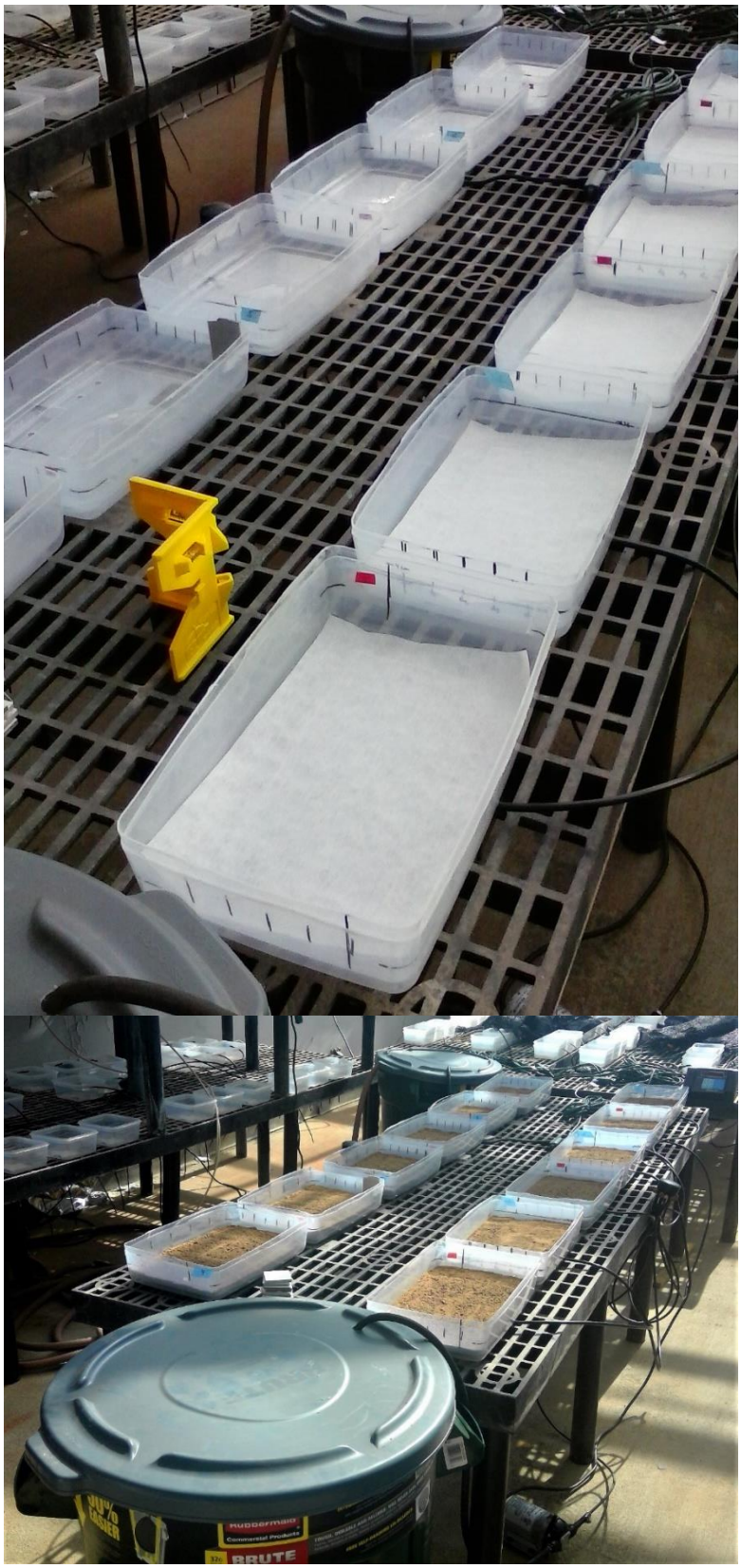


Figure 1.
Experimental setup. **Top:** Units shown before addition of soil with two nestled containers and a water filter (white rectangle inside the container). The yellow instrument is the carpenter's level. Blue labels indicate experimental units, while red labels indicate the controls. **Bottom:** In the foreground is the water reservoir. Plastic containers with soil added and hoses for water distribution comprise the experimental and control units.



Figure 2. Top view of a typical experimental unit. Green area denotes growth of biocrusts. Red line is the boundary up to which initial inoculum was placed. To the left, one can see holes left by core sampling along a transect away from the inoculum, sampled at 3 cm intervals (28 days after the first watering event). Successive sampling times occurred in parallel transects.

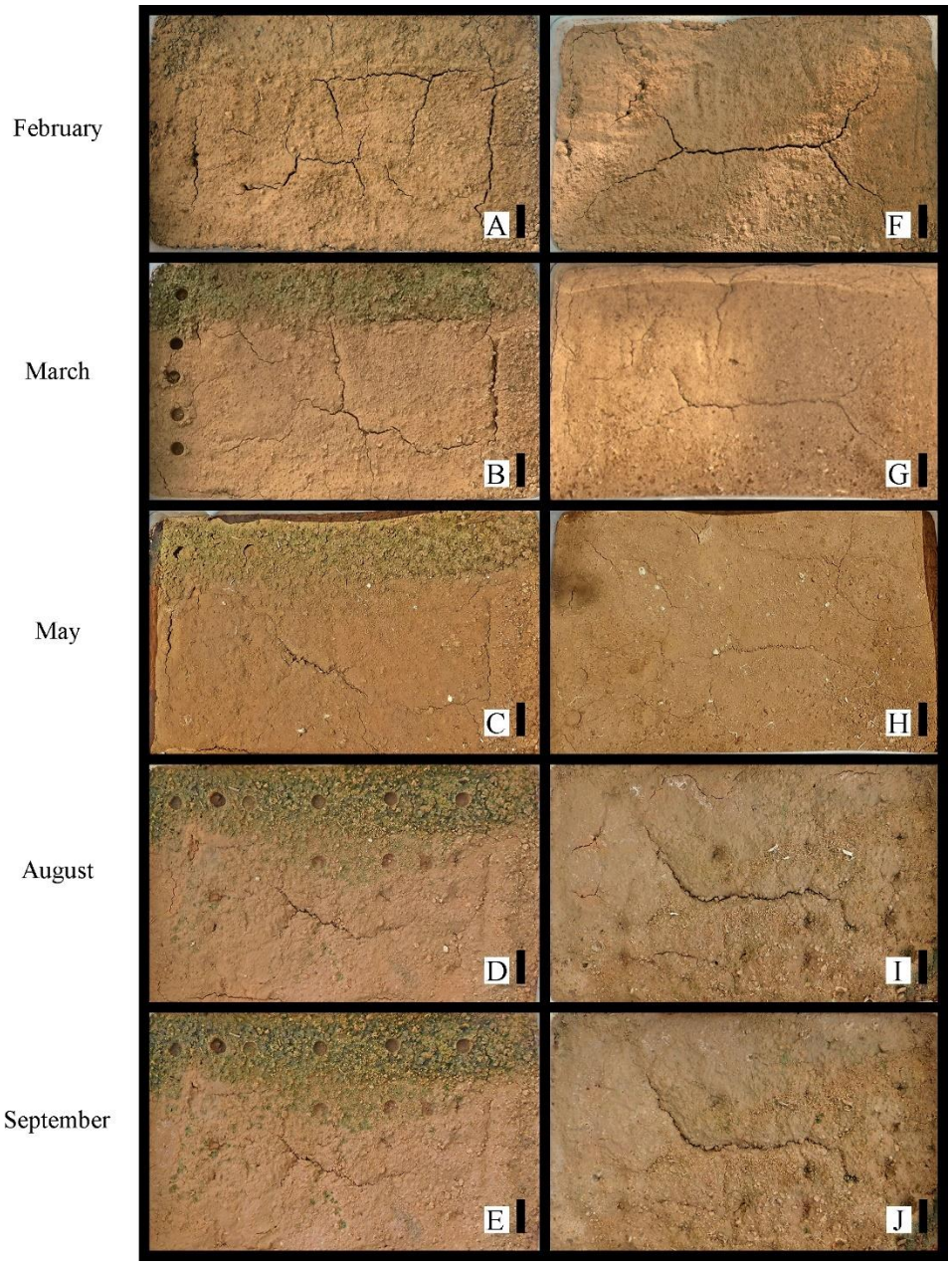


Figure 3. Time course photography of an experimental unit (A – E) and a control (F – J), uninoculated unit during incubation from February to September (2016). Each scale bar represents 3 cm.



Figure 4. Visual analysis of experimental unit with color threshold after 3 months of incubation. Blue line shows the boundary up to which inoculum was initially placed. **Top**: Experimental unit at the start of incubation. **Middle**: Experimental unit after 3 months of incubation. **Bottom**: zoomed in experimental unit after 3 months of incubation with red color marking the threshold assigned using color threshold adjustment in Fiji Image J.

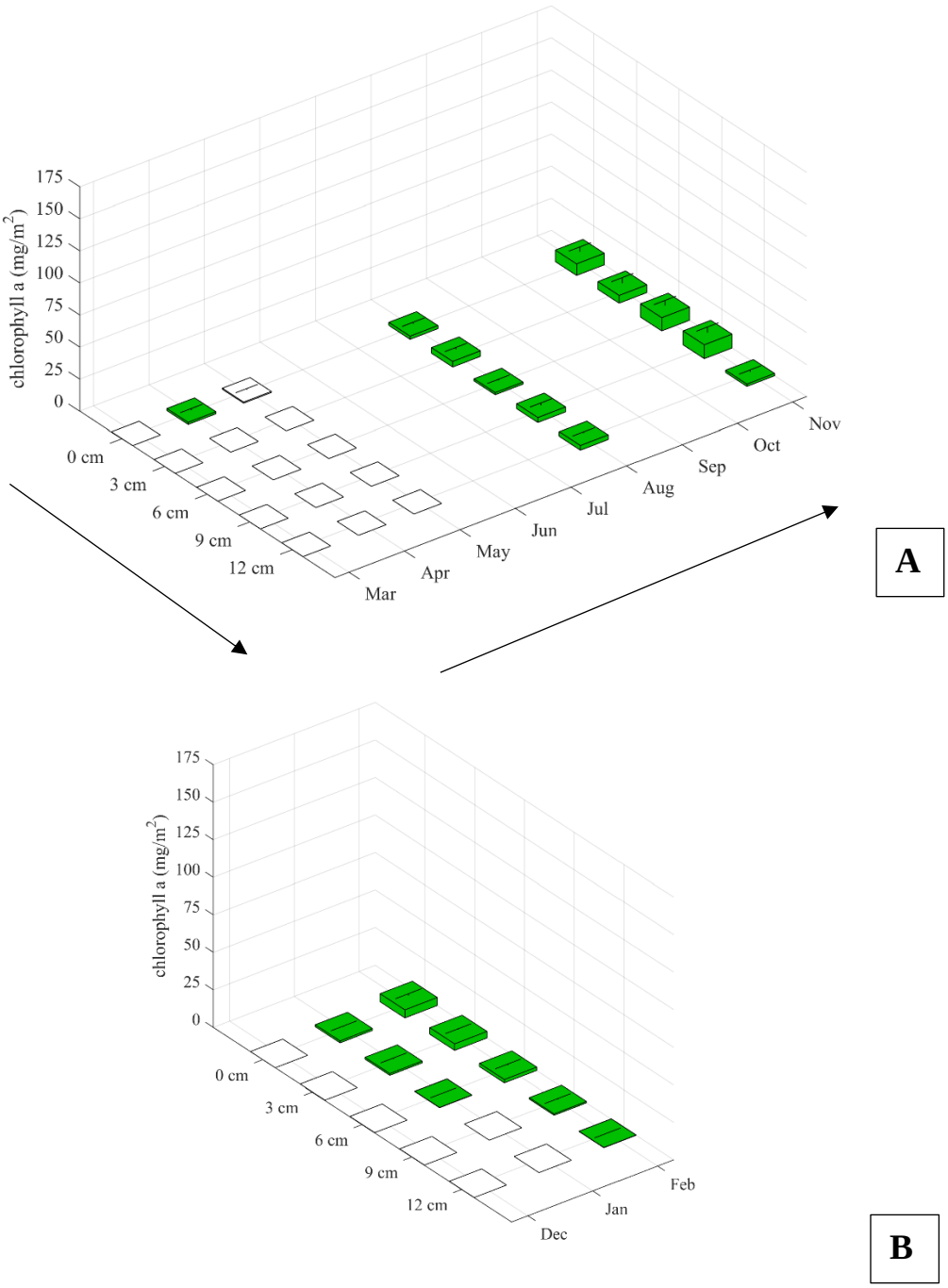


Figure 5. Dynamics of average chlorophyll *a* concentration in control units. Spring, summer, fall (**A**), and winter (**B**). Each bar height is the average of n = 6 (**A**), n = 2 (**B**). Error bars equal one half of standard deviation. Colorless bars indicate averages that did not significantly differ from zero by one standard deviation.

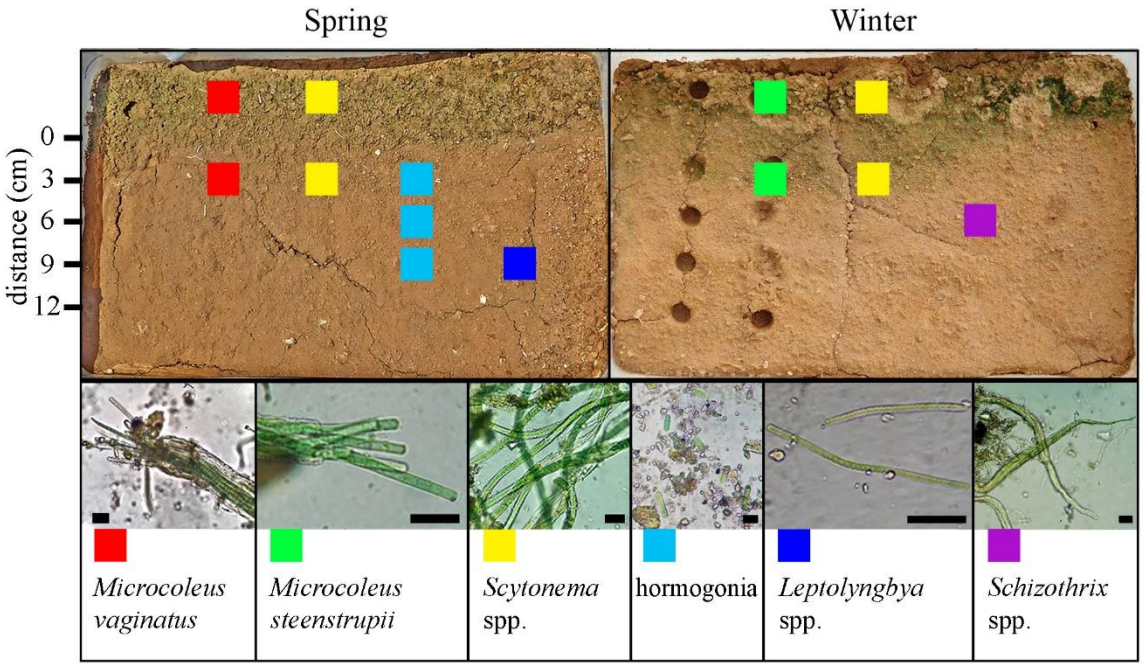


Figure 6. Cyanobacteria detected on experimental units after 3 months of incubation. Each colored square underneath a microscopy picture represents that cyanobacteria on the experimental unit. Squares are located on the experimental unit according to the distance at which the cyanobacteria were detected. Each bar on the microscopy picture is equivalent to 20 µm. Red: *Microcoleus vaginatus* (400 magnification. Green: *Microcoleus steenstrupii* (1000 magnification). Yellow: *Scytonema* spp. (400 magnification). Light blue: hormogonia (400 magnification). Dark blue: *Leptolyngbya* spp. (1000 magnification). Purple: *Schizothrix* spp. (400 magnification).

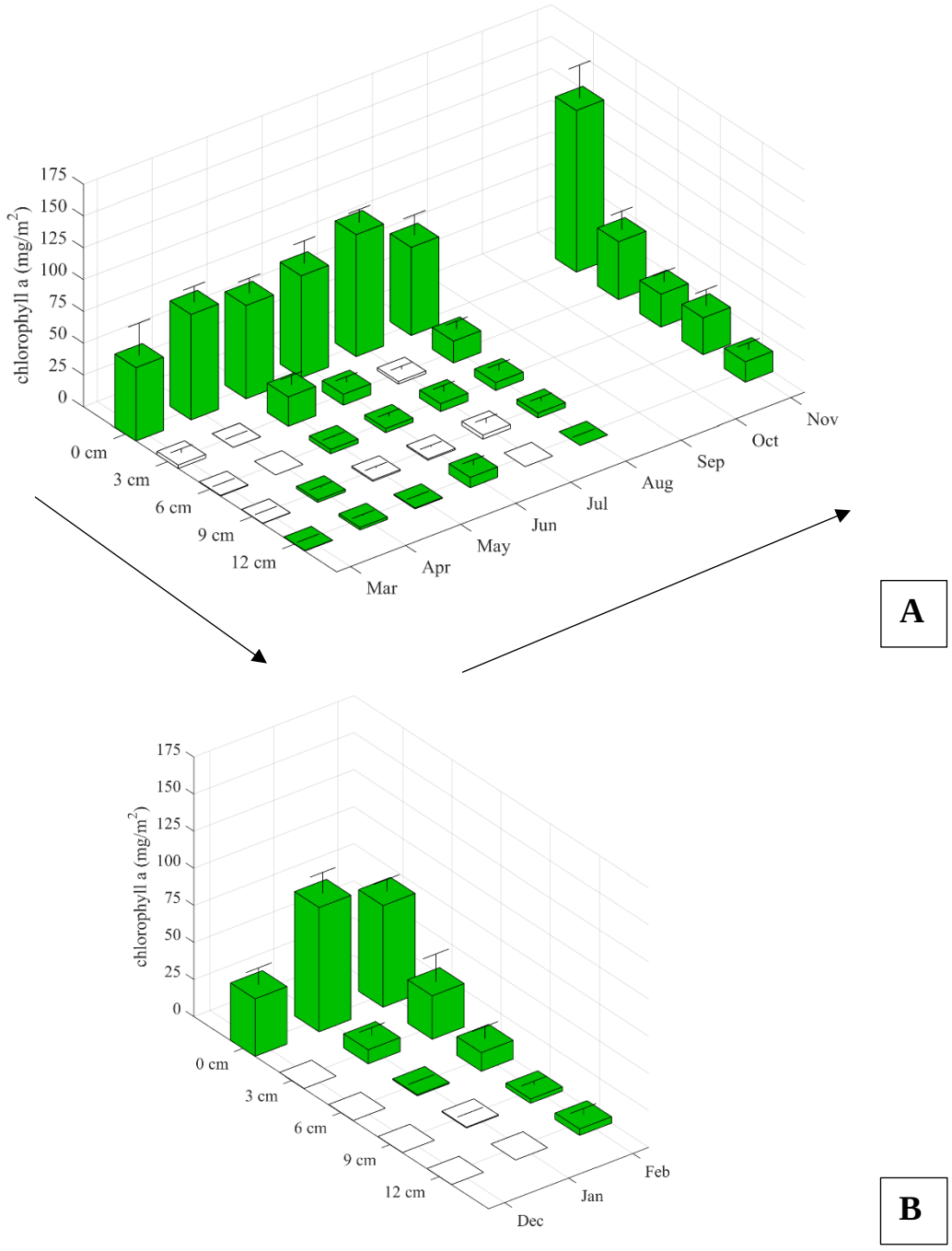


Figure 7. Dynamics of average chlorophyll *a* concentration in experimental units. Spring, summer, and fall (**A**); winter (**B**). Each bar height is the average of n = 6 (**A**), n = 4 (**B**). Error bars equal one half of standard deviation. Colorless bars indicate averages that did not significantly differ from zero by one standard deviation.

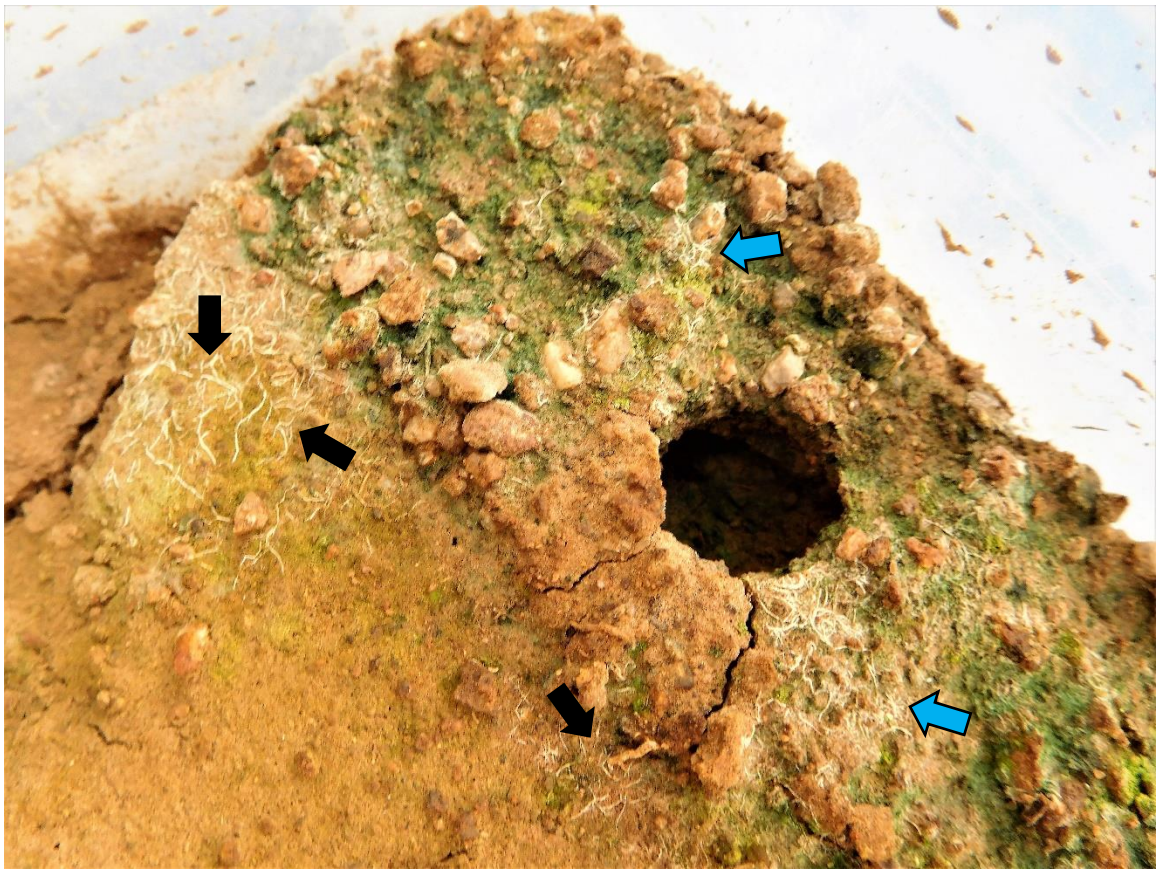


Figure 8. Close-up of mortality events detected in experimental units during Winter. Arrows indicate bleached cyanobacterial filaments (white) both on inoculum (blue arrows) and dispersed away from inoculum (black arrows)