

**A Guide to Restoring Biological Soil Crusts on Forests and Rangelands in
Western North America**

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Abstract

Biological soil crusts (biocrusts) are a community of organisms that live in the uppermost portions of soil, including cyanobacteria, lichens, and bryophytes. These organisms are valuable to many ecosystems for the ecological functions they provide, including soil aggregation, nutrient cycling, water retention, and habitat creation. Biocrusts are easily degraded by physical forces, and natural recovery may be slow. For these reasons, biocrusts have experienced some decline globally, but researchers have developed numerous methods for restoring them. This manual provides guidelines for how to maximize success of a restoration project. Biocrust material can be collected from the field, ideally in a salvage scenario. It can then be cultivated to increase the amount of material available, and stored if necessary. Steps are explained that can be taken to ensure the collected/cultivated material is ready for distribution, and some ideas for preparation of sites to ensure maximum effect are given. Multiple methods are explained for distributing the biocrust onto a restoration site. Finally, biocrusts should be monitored to evaluate the success of the project. Different monitoring techniques are explained in the manual that have different associated costs and provide differentially useful information.

Chapter 1: Biocrusts and their Restoration Value



1.1 What and where are biocrusts?

Biological soil crusts (commonly referred to as biocrusts) are a living community of photosynthetic and other organisms that exist in and bind the top few millimeters to centimeters of soil substrate (Weber et al 2016). They are estimated currently to cover 12% of the Earth's terrestrial surface, but may decrease by 25-40% over the next 65 years due to anthropogenic climate change and land use practices (Rodriguez-Caballero et al. 2018). The community can be composed of cyanobacteria, lichens, mosses, and liverworts, alone or in any combination, along with heterotrophs. The organisms that dominate the biocrust community vary with climate, soil type and successional stage (Ferrenberg et al. 2015).

Cyanobacteria (blue-green bacteria) are the largest and most diverse group of photosynthetic prokaryotes (Stanier & Stohen-Bazire 1977). They are often the earliest successional component of biocrusts. When wet, cyanobacteria filaments are able to move around in the soil inside polysaccharide sheaths, and in doing so bond the soil together and create an

erosion resistant layer (Budel et al 2016). Cyanobacterial crusts often serve as early successional components as they can recover more quickly after disturbance and provide soil aggregation (Belnap 1993). Later successional cyanobacterial crusts have the ability to fix nitrogen and appear darker in color, altering the albedo of the soil surface (Rutherford et al. 2017).

Lichens are a symbiotic partnership between photosynthetic organisms, such as algae or cyanobacteria, and fungi (Nash 1996). It is important to remember that lichens are made up of multiple species, because the symbiotic partners affect their characteristics (Nash 1996). Lichens in biocrust are highly stress-tolerant, they can survive dessication, extreme temperatures, and high light intensity (Rosentreter et al. 2016). Those with a cyanobacterial photobiont have the unique capability to fix nitrogen, and all lichens provide critical habitat for soil animals (Belnap 1996, Belnap 2002). Lichens require more time to form than other crust types, so are later in succession (Torres-Cruz et al. 2018).

Bryophytes, the non-vascular non-seeding land dwelling microplant group, include mosses, liverworts, and hornworts. While the latter are occasionally found as part of biocrust, mosses are more common. Mosses can capture more than 1000X their dry mass in water, and have special mechanisms to survive desiccation as well, enabling them to exist in arid landscapes (Oliver et al. 1993). Unlike most vascular plants, mosses do not rely on seeds or flowers for sexual reproduction, they instead produce spores (During and Tooren 1987). Dryland mosses, however, rely primarily on asexual reproduction which can be used to the advantage of restoration projects. Because of this, they can be early successional but often persist into late successional biocrust communities. Some mosses have physical structures that enable them to efficiently capture fog for water supply, making them especially common in coastal regions or other areas where fog is frequent but rain may not be (Wu et al. 2014). Some mosses are also capable of capturing atmospheric dust, a valuable ecosystem service (Belnap et al. 2014). They can also serve to retain water, reducing loss to runoff and evaporation. Mosses with epiphytic cyanobacteria augment the nitrogen fixing capability of the community (Zackrisson et al. 2009).

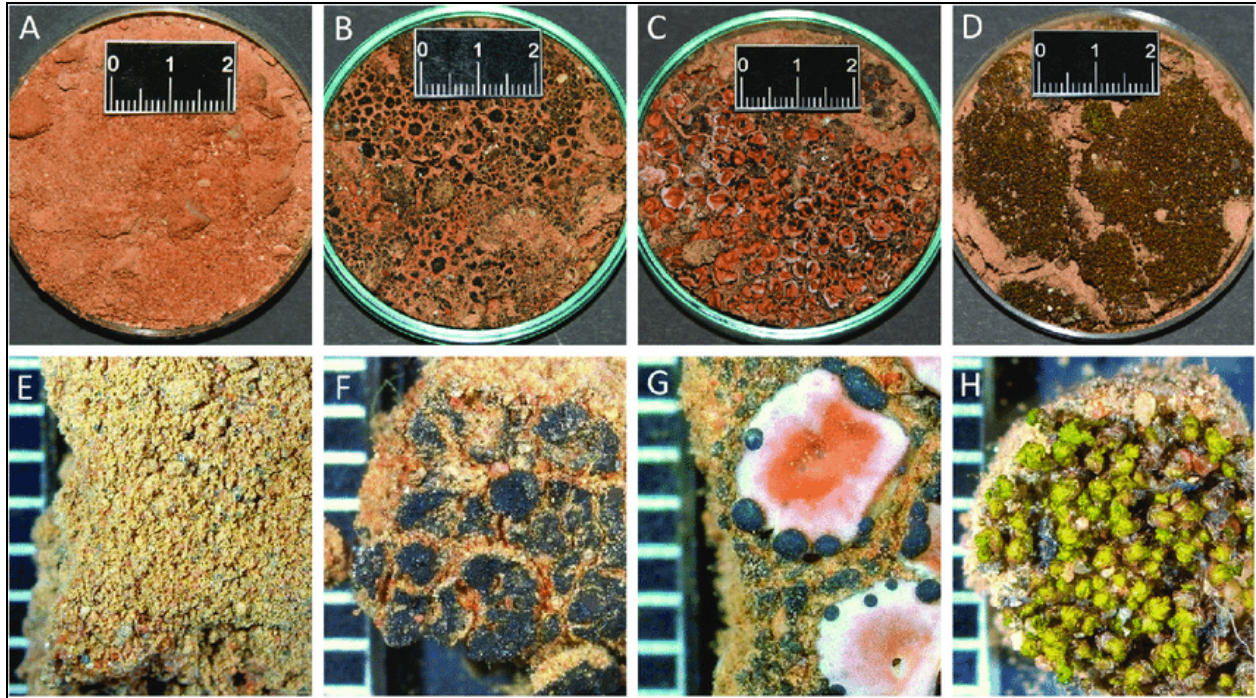


Figure 1. Different types of biocrusts. A-D present the different biocrust types and bare soil in an overview, E-H present close-up views of the respective biocrust types and bare soil, taken with a stereomicroscope (scale = 7 mm). A and E are bare soil; B and F are cyanobacteria-dominated biocrust plus cyanolichens; C and G are chlorolichen-dominated biocrust; D and H are moss-dominated biocrust. Figure and caption from Maier et al. (2018).

As a whole, the biocrust community has multiple traits that determine the types of environments in which they exist. They are photosynthetic, but not tall, meaning they thrive best in areas with high light input at the soil surface, such as the drylands which make up approximately 40% of the world's terrestrial ecosystems (Bowker 2007). In these drylands, which constitute much of the western US, light is not a limited resource because the amount of water is often insufficient to support a dense overstory of vascular plants. Biocrusts, though they have a very shallow depth, can persist in dry soils due to their ability to tolerate complete desiccation. They are capable of dormancy, enabling survival through long periods between precipitation.

1.2 Why should we restore biocrusts?

Biocrusts do not always recover well *without assistance*. When damaged, they can take years to decades or, possibly much longer, to recover, establish and mature (Belnap and Eldridge

2003, Eldridge and Ferris 1999, Belnap 1993). Recovery rate varies widely based on climate, soil, and severity of disturbance, and faster recovery is often possible (Weber et al. 2016). These long time scales combined with lack of understanding have long turned land restoration managers away from focusing on restoration of biocrusts where it has been degraded, and directed attention towards vascular plants instead (Bowker 2007). Because biocrusts provide a myriad of critical ecosystem functions (Table 1), loss or damage of biocrusts diminishes ecosystem health and function. Including biocrusts in a restoration recipe is necessary to restore these functions.

Table 1. A brief summary of major ways that biocrusts provide ecological function.

Biocrust Effect:	Soil Aggregation	Decrease Albedo	Altered Soil Environment	Dust Trapping	Altered hydrological function
Ecological Function:	Reduced dust emission and water erosion	Increased soil temperature can promote metabolic processes	Shapes development of plant and soil biotic communities	Surface roughness and increased hydroperiod can reduce atmospheric dust	Enhanced water retention and modification of infiltration and runoff pattern

Biocrusts aggregate soil, which can reduce wind and water erosion. Significant impacts on snowpack may be a result from increased atmospheric dust due to biocrust degradation (Painter et al. 2010). They can increase the soil surface temperature, due to darkening which reduces the albedo effect (Gold and Bliss 1995). In cool seasons, these higher temperatures increase rates of metabolic processes such as nitrogen fixation, photosynthesis, and activity of microarthropods (Belnap 2003). Biocrusts can trap atmospheric dust, due to their surface roughness, which enhances fertility (Reynolds et al 2001). Some cyanobacteria components found in a majority of biocrusts can fix nitrogen into the soil, providing a key nutrient to other species (Belnap 2002).

All of the aforementioned traits of biocrust have an effect on ecosystem functioning, but a few particular qualities also affect the success of the vascular plants which dominate ecosystems. The biocrust-plant relationship is complex and is not easily characterized as facilitative or

antagonistic. Facilitative relationships often result from moss crusts, and inhibitive relationships from lichen-dominated crusts (Havrilla et al. 2019). Biocrusts can increase the hydroperiod of the soil surface by trapping water, and their microtopography can create soft, shaded seed beds. The combination of these two factors make biocrusts helpful for the germination of many plant species. However, biocrusts can also serve to seal the soil surface, making it more difficult for some seeds to take root. This effect seems to vary based on seed traits like size, awns, and mucilage (Havrilla et al. 2019). The effect of biocrusts on nitrogen fixation is also important for supply of this essential plant nutrient (Evans and Ehleringer 1993). Established plants in a biocrusted area seem to benefit from increased growth rate and tissue nutrient levels, possibly due to the fixation the biocrust community provides. Biocrusts may even be capable of fending off some invasive species (Slate et al. 2019). Exotic plant seeds, such as those of cheatgrass (*Bromus tectorum*) which are not accustomed to the biocrust are less capable of establishing (Deines et al. 2007). However, Ferrenberg et al. (2018) found biocrust to increase the growth rate of established cheatgrass, highlighting that many biocrust effects on plants are dependent on the life stage of the plants. Native seeds that have coevolved with biocrusts can have self-burial mechanisms which enable them to take advantage of the comfortable habitat the biocrust provides (Havrilla et al. 2019).

While unassisted recovery is slow, research throughout the 21st century has developed a variety of methods for aiding the restoration of biocrusts (Doherty et al. 2015, Blankenship et al. 2019, Zhao et al. 2016). With modern strategies, biocrust restoration is possible. This manual will provide a framework which will allow land managers to evaluate if biocrust restoration is appropriate for their ecosystem, and provide them with the guidance they need to implement restoration projects with biocrusts.

Chapter 2: Planning a Biocrust Restoration Project

2.1 Is biocrust restoration needed and feasible?

Active biocrust restoration may be unneeded in some degraded sites, and it may be infeasible in others. First, it is important to consider the desired outcome of restoration activities. Is the goal to re-establish the presence of well-developed biocrust widely, or the broader objective of restoring ecosystem function? Must the desired outcome be met in full, or is it adequate to establish a trajectory toward the desired outcome? Aiming to restore specific ecosystem functions may require focusing on restoration of biocrust in key areas or use of key species in reintroduction strategies. Once these goals have been conceptualized, estimating whether (and how quickly) an ecosystem may recover to the desired outcome without assistance is the main step in evaluating if active biocrust restoration is warranted.

If the existing biocrust is severely degraded or absent entirely, or has been static in a diminished state for a long period of time, or specific functional traits of biocrust are desired but not currently present, then assisted recovery may be needed. If these conditions are not met, then passive recovery of biocrusts is potentially the better option; perhaps by omitting the disturbance factors that diminish biocrust cover and function, biocrusts can recover on their own. Assisted recovery of biocrusts using active techniques has been shown to be significantly faster than unassisted in multiple scenarios (Chiquoine et al. 2016, Zhao et al. 2016, Belnap 1993), but other studies indicate that it is not certain to succeed (Chandler et al. 2019).

Even when active biocrust rehabilitation is warranted, multiple constraints may limit the ability of land managers to proceed. At this time, feasibility of active biocrust rehabilitation is largely dependent on scale, i.e., the size of the project area. In the USA, the most successful and feasible restoration projects have been targeted towards smaller sites. Larger projects have been implemented and are being advanced but bring challenges with attainment, cultivation, and distribution of material (Zhang et al 2004, Grettarsdottir et al. 2004).

Biocrust material used for restoration activities is commonly referred to as *inoculum* or *propagules*. Obtaining sufficient inoculum for a restoration project is a major part of its feasibility. This is easiest if areas surrounding the restoration site have intact biocrust, and it can be harvested for immediate reuse or be multiplied by cultivation (for collection and cultivation details see Chapter 3). However, if the regions surrounding the site are either completely devoid of inoculum or protected by regulations, sourcing inoculum is a challenge that will require additional consideration (see chapter 3).

2.2 What materials and expertise are needed?

Biocrust restoration projects require some basic resources and equipment to be feasible. To determine if restoration is feasible, it is necessary to take inventory of these and be conscious of how existing equipment can be adapted to aid in biocrust projects. Budgets are often limited, and creative use of existing equipment and facilities can free up money to spend on personnel. A greenhouse is the most useful type of facility for rapidly growing large quantities of biocrust (Doherty et al. 2015), but outdoor growing has also been effective. Irrigation equipment, growing substrates such as burlap, and structures for stacking can also be helpful (see sections 3.2.2 and 3.2.3). Agricultural equipment may aid in the distribution of inoculum in the field, once obtained (Doherty et al. 2019).

Some steps in the biocrust restoration process require training by experienced personnel, or careful learning by reviewing resources in the scientific literature. These primarily are monitoring methods that are used to collect scientific data about the results of a project, so they must be precise and are often tedious. Examples include soil chlorophyll analysis, nutrient analysis, and visual assessment of level of development.

Chapter 3: Where and How to Obtain Biocrust Material

3.1 Collection

Because of their desiccation tolerance (remaining alive in a dry state) and clonal growth abilities, biocrust material can be moved from one site to another fairly easily. Collection is the first step in this process, and is often done by hand with a flat scraping device such as a shovel, dust pan, or flat trowel. Ideally, the device used must be able to precisely scrape the top few millimeters or centimeter of crusted soil and minimize uncrusted soil “scooped” from below. Shallow scraping will ensure a high density of biocrust biomass in the inoculum with minimal dilution. Mechanized equipment may be used for biocrust collection, for example, a front-end loader. This practice can decrease the time invested per amount of inoculum gathered, but is likely to result in decreased quality because a greater depth must be collected, diluting the biocrust gathered with deeper soil. While the actual process of collecting biocrust from the field is fairly simple, multiple considerations must be made to ensure it is done ethically and effectively, described below. These wild harvested materials may be used directly in the field, or to “seed” cultivation systems, or establish cultures. These activities are decreasingly dependent on field collection, for example, once cultures are created there is no need for additional collection of a given strain.

3.1.1 Salvage Rather than Steal

Generally speaking, intact biocrusts performing their ecological function in a landscape should be left alone. Removing healthy biocrust from one area in order to restore another only perpetuates ecological degradation. So how then, can biocrust material be obtained ethically? Salvage harvest is the most effective means (Tucker et al. 2019). Frequently, construction operations or other large developments such as mines or utility scale solar installations threaten intact fields of biocrust. These provide an ideal opportunity for biocrust restoration practitioners to collect viable biocrust material. Salvage harvest has the added benefit of convenience - collectors can take all of the material from a site since it will otherwise be destroyed. If a non-salvage harvest

is deemed necessary, care must be taken to only remove a small percentage of the total biocrust, and to avoid damaging the rest in the process.

3.1.2 Selection of an Appropriate Collection Site

Transportation of collected biocrust material can be costly due to the weight of the associated soil, depending on the scale of the project. For this economical reason, if the field-sourced material will be used directly to reinoculate a restoration site, it is best to source restoration material locally. Additionally, locally sourced biocrust has an array of species and genotypes that are able to inhabit the restoration site. If the species composition of historic biocrust at the degraded site is known, it is usually best to source restoration material that mimics that composition. Potential collection sites can be identified by considering the elevation, climate, and topography of the restoration site and searching for similar sites nearby. Alternatively, target species can be established based on the functional goals of the project and surveyed for locally. In some instances when considering the potential effects of climate change, assisted migration of biocrust might be considered (Young et al. 2016). This means purposefully selecting a community of biocrust organisms better adapted to the predicted future climate of the restoration site, rather than the historic one. At this time, this is the subject of research rather than an advisable practice, since the risks and benefits are not fully known.

3.1.3 Storage of Collected Biocrusts

Biocrusts can likely be stored if they are kept dry, although not much is known about long-term storage. Biocrusts that are slightly moist when collected should be allowed to slowly (1-2 weeks) dry in light shade at room temperature or cooler by storing them temporarily on a flat surface like a baking sheet, or permanently in a breathable container like a paper bag (although these post-collection drying methods are difficult at large scales, biocrust could be laid out on a tarp with a shade cloth over it). When biocrusts are wet, they are metabolically active, undergoing both photosynthesis and respiration. But since light is required for photosynthesis, wet biocrusts stored

in the dark can only undergo respiration. This is a recipe for ruining a collected batch of biocrusts. Biocrust material that is fully dry will not be metabolically active and can be stored in dark, airtight or breathable containers and remain alive.

3.2 Cultivation

If collected biocrust material is not sufficient in quantity for a restoration project, it can be cultivated to multiply the available inoculum. While most biocrust species are slow-growing under natural conditions, recent research has revealed several methods for augmenting growth rates, described in detail below.

3.2.1 Preparing Starter Material for Cultivation

Biocrust material collected from the field is probably not in the best state to begin cultivating it. While it will be wet throughout the cultivation period, it should first be dried so that it can be prepared properly. If it has been stored temporarily, it should already be dry (see section 3.1.3). Collected biocrust will have attached soil, and it may be useful to try to mitigate the volume of soil introduced as inoculum by rubbing pieces of biocrust against a sieve to separate that soil. Next, large slabs of biocrusts should be broken up so that it can be evenly distributed as inoculum. This can be done by forcing biocrust through a sieve (usually 2mm). Ideal fragment size is not fully studied, but 2mm fragments have generally been successful. Once the biocrust pieces have been reduced in size, the entire quantity of inoculum should be stirred to homogenize it, ensuring the starter material will be evenly distributed.

3.2.2 Method 1: Greenhouse grown, wicking irrigation

Growing biocrusts in a greenhouse with traditional mist or drip irrigation presents a number of challenges, so using a wicking system can be more economical, repeatable, and reduce disturbance to the biocrust (Doherty et al. 2015). It's repeatability is also desirable in experiments

designed to learn about how best to grow biocrusts. An additional advantage to this system is its scalability - it can be used for a few large growth units or hundreds of small units, allowing for experimentation. At present, wicking irrigation is the most effective way to grow many biocrust species.

To implement a wicking irrigation system, greenhouse temperature should generally be kept below 20°C, as many biocrust organisms grow most efficiently in the cool season. Care should be taken to regulate temperature, as many greenhouses are too hot. If temperature control is difficult, avoid growing in the hot months. Ideally, water should be available from a central hose pipe that branches into smaller drip lines. Most municipal water supplies are too rich in chlorine, which should be filtered out using a charcoal filter (Antoninka et al. 2016). Each drip line runs through a hole drilled in the side of a basin (Fig. 2) This basin should also have a hole drilled in the base to allow for drainage. An additional nesting basin is placed within the lower basin. Multiple small holes must be drilled in the floor of this upper basin, and a wicking liner cloth is placed on its floor. The upper basin is filled part way with a soil substrate, and biocrust inoculum is distributed evenly on top (Fig. 2). Plastic containers ranging from sample cups, to food storage containers to under bed storage bins have been useful as basins in variations of this system. Sand, organic soil amendments, and natural soils have all been employed in systems like these, but have different wicking properties that will affect water-delivery choices.

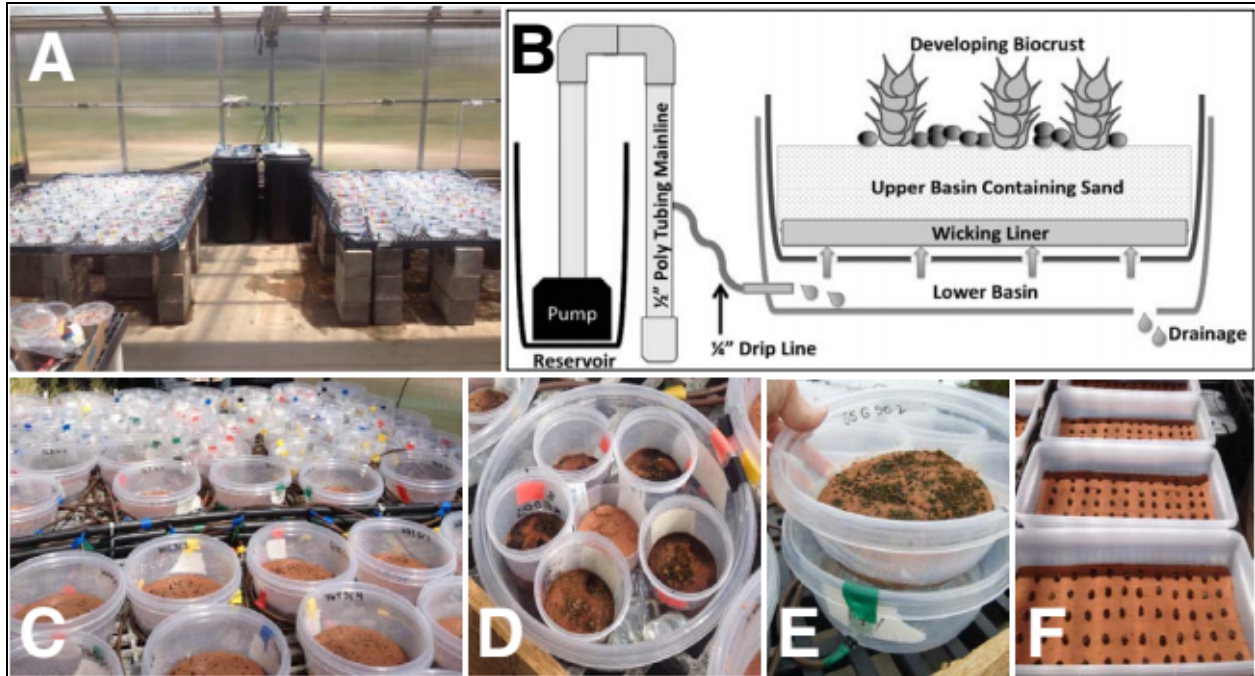


Figure 2. Soil-based cultivation system for biocrust propagation. Reservoirs containing pumps deliver water to two greenhouse benches of experimental units (A). Each unit is composed of a lower and upper basin (B). The lower basin fills and water wicks upward through holes in the upper basin to hydrate soil and biocrust. Bundles of 1/2" poly tubing mainlines deliver water treatments to each table, and 1/4" drip line delivers water to each unit (C). Multiple specimen cups may nest within a unit for randomized factorial experiments (D). Units may be scaled up for bulk cultivation in mid (E) and large sized basins (F). (Doherty et al. 2015).

Water flow to the biocrust units need not be constant, but should occur in brief pulses, each inducing a full hydration. After biocrusts have been hydrated, water from both basins drain, leaving the substrate at field capacity. Length of hydration period is determined by type of substrate, substrate volume, substrate surface area, greenhouse relative humidity, greenhouse temperatures, and watering frequency (Doherty et al. 2015). Longer hydration periods may be realized by rehydrating, before drying can occur; practically, we find that once or twice daily pulses are sufficient to keep a biocrust continuously hydrated on a sand substrate.

A modified version of the wicking irrigation system has been used to grow mosses atop soil over a sheet of fabric such as burlap or weed cloth. This system benefits from placement of 2" thick mineral wool below the fabric, which serves to deliver water at a steady rate, from basins below which do not drain, to the overlying soil over long hydroperiods (Grover et al. 2020). This

approach enables a wicking-based method to work more effectively with organic soils which might otherwise float if irrigated from below. This system for consistent moss growth with less frequent watering events, the possibility of manual rather than automated irrigation, and easy harvest performed by lifting the fabric away, even enabling manual watering instead of timed).

3.2.3 Method 2: Greenhouse grown, fog chamber irrigation

Biocrust growth in a greenhouse can be an effective method, but irrigation may be challenging and table space limits output (Bowker et al. 2017). The wicking irrigation method described in 3.2.3 is a novel solution, but can be labor and/or equipment intensive. Some biocrust species respond well to growth in a fog chamber. These can be lichens or cyanobacteria, but are most often mosses that are adapted to have fog capturing structures (Doherty et al. 2018).

To implement a fog chamber irrigation system, greenhouse temperature should generally be held below 20°C. Care should be taken to regulate temperature, as many greenhouses are too hot. The first step is the construction of the fog chamber, a visual is provided in Figure 3. Begin by assembling a frame for the fog chamber of PVC pipe. This can vary in size, but will probably be placed on a greenhouse bench. Next, cut the walls of the fog chamber from large sections of fabric with about 30% light transmission. Fabric designed to protect plants from freezing can be used for this. These attach to the PVC pipe frame via clamps. The shade cloth walls serve to contain the fog, but should allow enough light to pass through to permit photosynthesis. Shelves/racks are an essential part of a fog chamber as they allow stacking of biocrust sheets. This can be achieved at a low cost by tying bailing wire across the chamber, upon which light weight shelves can rest.. The final step is to fill the chamber with fog. This is achieved by placing a container of water inside the chamber, and placing a sonication device in that water. The device will require an electricity supply. The water container will need to be filled frequently, so it is best to automate this by utilizing a float valve attached to a continuous water supply.

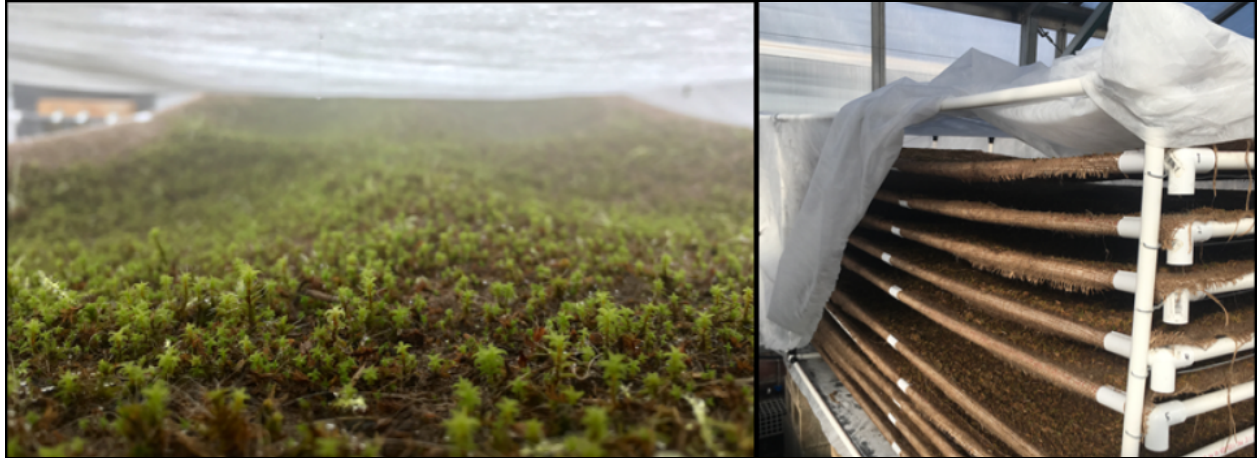


Figure 3. (Left) *Syntrichia ruralis* from MPG Ranch shown on top of burlap cloth in a fog chamber. (Right) Layered moss cloth within a fog chamber (emitter not shown, at rear of chamber). Cover fabric is pulled back to access, but usually seals chamber. Credit K. Doherty.

When the fog chamber is constructed, biocrust can be added. For some moss species, a thin layer of inoculum on burlap alone suffices. Burlap is a useful material for growing biocrusts because it can aid in transportation and outplanting, and will eventually decompose as the biocrust is establishing in the field. If using burlap as a substrate, it is best to suspend it within the fog chamber, for example on PVC frames. Growth requirements of lichens in such a system are untested. It is possible that some species will not be amenable to culture on burlap, and will need a soil substrate. This can be achieved by using shallow plastic containers of any size, filling them with a thin layer of soil, and distributing biocrust inoculum on the surface. These containers can be placed within the fog chamber and can be vertically layered if a proper rack system is used to accommodate the weight and allow free flow of fog. Periodic rotation of shelves may be useful to manage light; alternatively supplemental light sources could be investigated.

3.2.4 Method 3: Outdoor growing

A greenhouse can be helpful in many situations, but is not a requirement for cultivating biocrust. If the proper climate conditions are available with average temperatures below 20°C, outdoor growing systems can be designed. These can still utilize irrigation, but the temperature range will be more variable than inside of a greenhouse. To mitigate the negative effects of

temperature swings and harsh solar input, shading techniques can be used. These allow the biocrust to grow under more stable conditions and reduce drying. An added benefit might be that biocrusts experiencing more variation in temperature and humidity might adapt better to field conditions upon transplant. This hypothesis is still being tested at the time of writing.

Outdoor biocrust growth is best achieved on a flat surface. For a soil surface, first leveling the soil and removing large rocks and existing vegetation, allowing for even watering and removing competition. Weed barrier cloth can be placed on the surface to prevent plant growth and to hold soil in place. Alternatively, paved areas have also been used, and will not require weedcloth. Biocrusts are most effectively grown outdoors when encased between 2 layers of fabric, the bottom being a substrate on which it can grow, or which aids in water distribution, and the top providing shade. The most effective bottom layer is burlap because it can aid in transportation and outplanting, and will eventually decompose as the biocrust is establishing in the field. A thin (few mm) layer of soil can be placed over the burlap to provide nutrients to growing biocrusts, then biocrust inoculum can be distributed across the burlap.

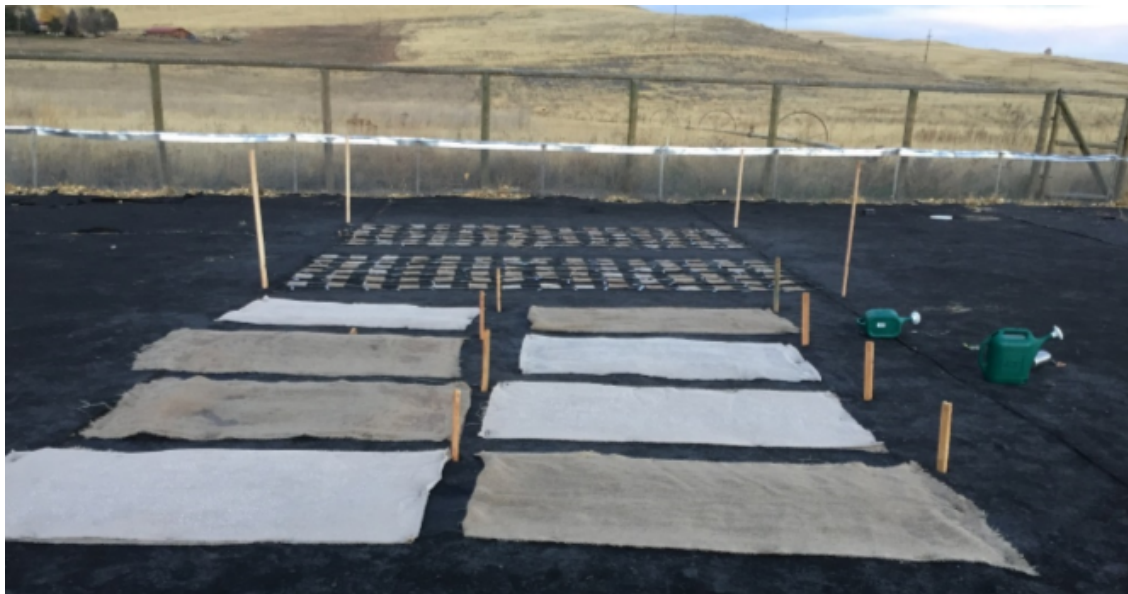


Figure 4. Field cultivation of biocrust mosses using the quesadilla method at MPG Ranch.

Once the biocrust floor is ready, shade should be established over it. Shade cloth varies in material and percent light filtration. Use a light colored cloth that allows 50-75% light to pass through. The shade cloth can be applied to the biocrust sheets in two ways: laid directly on top (“quesadilla” method) or suspended from wire hoops (hoop house method). A quesadilla approach may result in biocrust organisms becoming intertwined with the shade cloth fibers, resulting in difficulty removing the cloth. To mitigate this, wire “hoops” can be used to support the shade cloth above the growing bed of crust (Fig. 5).



Figure 5. Outdoor biocrust growing beneath shade cloth in quesadilla style (left) and raised hoop-house style (right).



Figure 6 (left). Large-scale harvest of biocrust quesadillas at a multi-acre biocrust farm in Utah. Figure 7 (right). Cloth quesadillas ready for distribution after transport in Indian Creek, Utah.

Chapter 4: Readyng Biocrust Material for Field Application

4.1 Hardening

Cultivated biocrust grows most quickly under stable temperatures with reduced UV input and a routine water supply, but will not receive those conditions in the field once applied. To avoid shocking the organisms by transporting them directly to a more stressful natural environment, a “hardening” phase may be beneficial (Giraldo-Silva et al. 2020), but has not been universally supported (Bowker et al. 2019).

At MPG Ranch, a study is underway that tests chemical additives to moss inoculum produced using various methods for their ability to harden the inoculum and ready it for the field. These compounds being tested are sucrose (which may serve as a placeholder for water, an antifreeze, and an energy source) and abscisic acid (which may activate drought response genes, protect membranes during drying, and soften stresses). These may induce a hardening effect and improve field success later on. Preliminary results after one year have shown that abscisic acid may promote establishment in moss cultivated outdoors.

4.2 Sieving Considerations

Cultivated or field collected biocrust may be clumpy or otherwise heterogeneous. It is sometimes effective to decrease the size of biocrust/soil aggregates so that they can be evenly and homogeneously distributed on restoration sites. This is best done by pouring and forcing inoculum through a 2-4mm sieve which seems to be a size that does not damage organisms but allows for even distribution. Larger sieves are easier to use, but for truly large quantities of biocrust inoculum sieving may be too time consuming.

4.3 Cloth Roll Transportation

As described in Chapter 3, multiple circumstances may benefit from growing biocrust atop some fabric (weed barrier, burlap, or both) and beneath shade cloth. This can also aid in easy transportation of biocrusts to restoration sites. These materials can effectively be rolled to create a “biocrust sod”. Leaving the base cloth, soil, biocrust, and shade cloth in place, roll around a sturdy PVC pipe in the center to serve as the core and carry handles. A reasonable size for this is cloth sections about 1x3m, resulting in rolls weighing 20-30kg. Examples of this in figures 6 and 7.

Chapter 5: Habitat Preparation and Amelioration

Biocrusts distributed on a restoration site may struggle to establish, so some habitat preparations, or ameliorations may promote establishment. Without these ameliorations, biocrust inoculum may be subject to removal by wind, flooding, or animals, or may succumb to stress. These tactics may allow the biocrust organisms to establish more quickly, which should result in earlier achievement of restoration goals.

5.1 Surface roughening

Micro-aspect can be important in determining biocrust community characteristics and function (Bowker et al. 2002). At a small scale, a mix of biocrust microhabitat types can be created using hand tools, such as a cement trowel, to make 2cm wide troughs diagonally across plots every 5 cm. This is easily achieved in a fine textured soil, but troughs will not hold up in coarse soil without additional support (Fig. 9). Possibly, similar texturing can be achieved at larger scales using hand tools like rakes or with heavy machinery, though these have not been extensively tested. One medium-scale test is currently underway in which a modified sod-roller was used to imprint biocrust-inoculated surfaces in the Mojave Desert. A test at MPG Ranch using furrows produced by a drill-seeder did not promote establishment, possibly because the furrows were too deep (Figure 8). However, an imprinter did enhance establishment (Doherty et al. 2019; please see Chapter 6 for additional details).



Figure 8. A tractor pulled a seed drill (left) and imprinter (right).



Figure 9. Surface roughening and polymer application (left, credit A. Antoninka) and soil imprinting (right).

5.2 Shading

Shading after application of biocrusts can help reduce desiccation induced by UV intensity, partially removing the water limitation that often inhibits growth (Antoninka et al. 2019). Local materials such as logging slash could plausibly be used to provide this shade, though this approach has not yet been shown to be effective (Young et al. 2018). Vascular plants are also plausible shade elements. Alternatively, shade cloth can be used and has been tested, but is likely restricted to smaller scale applications (Antoninka et al. 2020, Fick et al. 2020). See section 3.2.4 for shade details about shade cloth use.

5.3 Water addition

Addition of water at the time of inoculation can aid in the establishment of biocrust propagules by encouraging them to bind with and grow into the soil. This can be achieved by using a misting sprayer, water truck or by timing inoculation with a precipitation event. Regardless of the method, care must be taken to ensure that the amount of water is sufficient for biocrust growth but is not excessive to the point that runoff and erosion occurs. Continuous irrigation in the

field is a more intensive option that may be beneficial, but results have not been strongly in favor of it over one time watering (Bowker et al. 2019).

5.4 Soil stability additives

Biocrust inoculum applied to loosely aggregated soil surfaces may aid stabilization. However, projects of this nature can benefit from some additional material addition to provide initial stabilization. This facilitates biocrust establishment and can prevent loss of inoculum to erosion. Materials that have been used include physical erosion barriers (straw, jute net) and tackifiers (organic polymers, psyllium).

5.4.1 Straw & jute net

Straw borders can slow overland flow of water and soil, and are frequently used in erosion control after construction operations. At a smaller scale, they can also provide a more stable area for biocrust recovery. Straw checkerboards have been used to stabilize sand dunes in Mongolia and China and were found to be one of the best habitat modifications methods per effort put in (since other methods can require a high level of effort) for biocrust recovery (Zhang et al. 2004).

At MPG Ranch, Slate et al. (2019) found that jute cloth increased moss and lichen cover by five to eight times compared to bare ground. The jute net is a fabric commonly used in erosion control, and its properties may aid biocrust establishment with or without inoculation if it is set up in a way that blocks sediment flow across restoration sites (Bowker et al. 2019). The gridlike pattern of jute can slow overland flow of sediment, and its texture can offer a substrate for biocrust binding. It was also found to increase moss cover under almost all conditions by Condon and Pyke (2016).

5.4.2 Organic polymers and psyllium

Organic polymers or polyacrylamides are commonly used as a method to reduce dust on roads, as well as to stabilize road banks or other soils subject to erosion. They can also be applied over biocrust inoculum to facilitate its adherence and establishment (Fig. 10). Polyacrylamides can be selected based on the following criteria: 1) documented use and effectiveness at soil stabilization in the peer reviewed literature and 2) UV and biodegradability. Because of those criteria the following polymers have been used, although other companies offer comparable products: 1) Dirt Glue (aqueous acrylate polymer emulsion), 2) SoilTac (vinyl copolymer emulsion) and 3) TerraLoc (polyvinyl alcohol). Dirt glue and SoilTac were not found to impede biocrust development when used at a rate of 1 part polymer : 8parts water (Antoninka et al. 2020).



Figure 10. Addition of polyacrylamide to experimental plot. Photo credit: A. Faist.

Psyllium husk is often sold in health food markets as a dietary supplement, but its properties can also aid in the stabilization of soil and establishment of biocrusts and is commercially produced for this purpose in a product called M-Binder. Psyllium can be added to a restoration area immediately before or after biocrust inoculum and adhered into place with water. It helps to nearly instantaneously form large aggregates of soil that better resist erosion, and that may speed biocrust colonization. Some studies have shown that psyllium may be the most effective tackifier, compared to polyacrylamides or guar (Blankenship et al. 2019, Fick et al. 2019). However, Chandler et al. (2019) did not find the additive to be effective. In some instances, psyllium has induced a black fungal crust that is yet to be fully understood.

Chapter 6: Biocrust Material Distribution

6.1 Calculating the amount of material to add

The amount of biocrust inoculum to add depends on multiple factors, including how much inoculum is available, and what a project budget allows for. The objective is to add the minimum amount necessary to generate the expected outcome because it will be more cost effective, and that way more land can be treated. It is hoped that the biocrust materials then grow to attain their potential cover.

In one study, no extra benefit was found from adding more than 20% cover (Antoninka et al 2019). An experiment is underway that added an entire gradient of 0-40% crust cover, in 2% increments. Cover is only one basis of measuring how much crust to add, one could also base decisions on the amount of chlorophyll added, for example. No matter what this is based on, it is convenient and practical to add inoculum by volume or mass. So, it should first be determined what needs to be induced on the ground in terms of cover or chlorophyll, then a conversion factor is used to determine how much volume or mass to add per unit area. For example, if inoculating a one square meter area to a desired rate of 20% biocrust cover 1cm deep: $(100\text{cm} \times 100\text{cm}) \times (1\text{cm depth}) \times (0.2) = 2,000\text{cm}^3$ of inoculum = 2 liters.

6.2 Distribution strategies

Biocrust inoculum needs to be delivered to and distributed across a restoration site to induce a recovery response. Multiple methods of achieving this have been tried and can be categorized as: dry broadcast, hydroseeding/slurry, and machine-dispersed. Aircraft dispersal is plausible, but has never been tested.

The simplest and most commonly used method for distributing biocrust material is dry hand broadcast. This is easily done by hand-carrying the inoculum in a container, obtaining smaller

volumes at a time (e.g. handfuls, small cups, etc.), and sprinkling it onto the restoration surface. Most experiments to date have sought even distribution, rather than heterogeneous distribution (e.g. bands or islands), though it is unknown if there is an advantage to one or the other. This is an easy method to control and costs little in materials, but its slow speed and potential labor costs may hinder scaling up. Machine-assisted broadcasting has been suggested as a means to scale this process to larger areas, but has never been directly tested (Doherty et al. 2019).

Hydroseeding is a hybrid technique aimed at dispersing seed and controlling erosion commonly used after construction activity. It applies seed, water, and a tackifier (see 5.4.2) and often inert organic materials like straw to a soil surface through a pressurized hose. Since biocrust propagules are characteristically similar to some seeds, this technique may be an efficient way to distribute inoculum, especially on erosion-prone sites. One study reported that mosses were promoted the most by hydroseeding, compared to manual sowing (Lorite et al. 2019).

Because biocrust propagules are similar to some seeds, interest is increasing in borrowing machine distribution technology from the agricultural industry for large scale restoration projects. A rangeland seed drill was shown to be completely ineffective for distribution of inoculum at MPG Ranch, Montana, likely because biocrusts were buried in furrows (Doherty et al. 2019). In contrast, imprinting at the same locations followed by inoculum broadcast had substantial positive effect on biocrust establishment rates, probably due to the microhabitat it created (Doherty et al. 2019). Thus, there may be opportunities to combine imprinting and machine-assisted broadcasting for applications at medium to large scales.

As described in sections 3.2.3 and 4.3, moss cloth can aid in the distribution of biocrust. The cloth addition can help to provide initial stabilization of soil and can suppress invasive plants that may exist on the restoration site. Doherty (2019) found that cultivated moss cloth placed face-down had the highest rate of establishment. Cloth rolls can be a convenient way to transport biocrust inoculum from cultivation site to restoration site.

Chapter 7: Monitoring to Evaluate Restoration Effects

When a restoration project of any kind is implemented, it is important to monitor the results of the efforts for two reasons: 1) adaptive management of the project and 2) applying lessons learned to future restoration efforts. Success of a project can be considered in a variety of ways, which are ideally developed before a project is implemented. Some may choose to focus on whether a specific metric is met (i.e. 50% biocrust cover), and others may attempt to evaluate if ecosystem functions are restored (i.e. if the substrate is more resistant to erosion). This chapter will provide details on multiple monitoring methods that have been used, inclusive of how they are applied, the speed and efficiency of conducting them, and what aspects they can reveal about the success of the project. This chapter provides only a summary, as this has recently been the subject of an entire review (Mallen-Cooper et al. 2020).

7.1 Biocrust cover

Ocular estimates of biocrust cover are perhaps the simplest method for evaluating the success of a restoration project. In essence, this is done by looking at the soil surface with a trained eye and making an observation about the percent of the area that is covered by biocrust organisms rather than bare soil or other objects such as plants or plant litter. In order to estimate cover effectively, the observer must be able to visually distinguish biocrust organisms. Some efforts may simply evaluate total biocrust cover, while other operations may seek to categorize cover by type (i.e. moss, lichen, cyanobacteria) and others may even desire species level cover estimates.

Visual aids can assist in getting accurate biocrust cover estimates (Fig. 11). These are usually physical frames that at minimum identify the boundary of a sampling point (0.5x0.5m, edges of a pot, etc.), and may also provide gridlines. Gridlines aid in observation of large area because they allow the observer a visual reference by which to “count” biocrust cover. For example, a 0.5x0.5m frame may be divided into 100 squares, allowing easy visualization of what percentage of the soil surface is covered by biocrust. Because the viewer must often position themselves closer to the soil surface to estimate biocrust cover, we recommend smaller quadrats

(0.25 x 0.25m - 0.5 x 0.5m) that those typically used of vascular plants. Multiple quadrats should be placed in an unbiased arrangement, read and averaged to estimate the cover of larger areas.

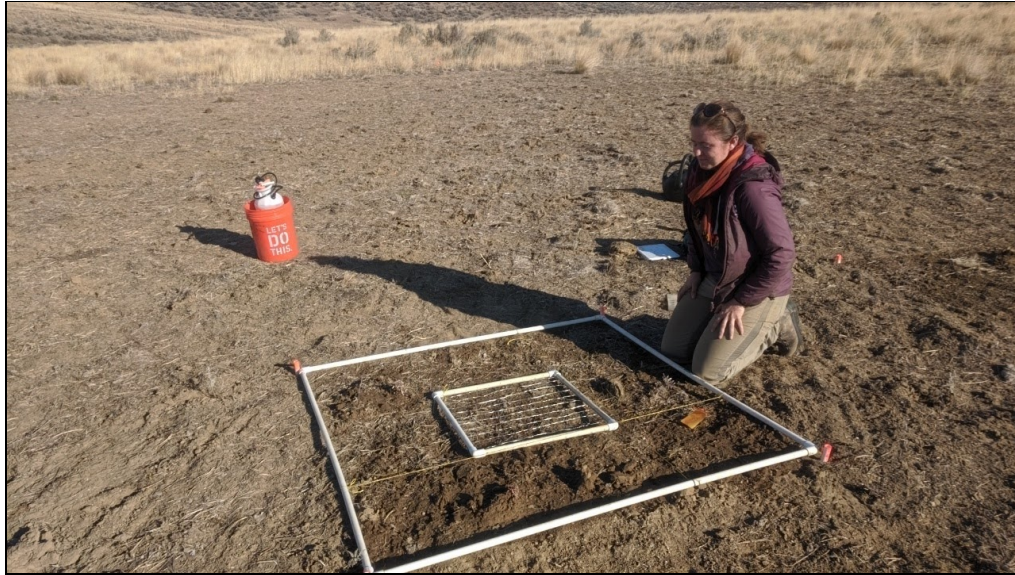


Figure 11. Observation of biocrust cover using a grid frame. Credit: H. Grover of L. Bailey

Biocrust ocular cover estimation requires a knowledge of the visual appearance of organisms, which is fairly easy to train. It requires minimal tools, and can be done reasonably quickly. However, other methods of quantifying biocrust presence may be faster (i.e. photo analysis) and reporting biocrust cover alone does not yield specific information about ecosystem services.

Alternatives to ocular cover that achieve a similar goal in point or line intercept evaluation. Point intercept measurement is done by dropping a pin at predetermined points in a gridded frame (e.g. grid intersections) or along a transect tape (a variation termed line-point intercept) and recording what type of surface the tip of the pin strikes. These can be tallied for quantitative analysis and extrapolation to the whole area. Line intercept methods use a transect tape along which an observer records the point at which a biocrust (or other) interception begins and ends. Total length intercepted can be divided by total length sample to arrive at cover.

7.2 Chlorophyll

Chlorophyll is a pigment used by photosynthetic organisms to obtain energy from solar radiation. Chlorophyll is present in biocrust organisms, often in consistent ratios, scaling with biomass. As such, it can serve as a proxy for the amount of photosynthetic material in the soil. It can be extracted using laboratory techniques and quantified to help provide an accurate metric of biocrust abundance (Green et al. 1998). Procedures for chlorophyll analysis are detailed below - be warned that the process requires extensive lab equipment including a mechanized shaker, centrifuge, and spectrophotometer (Castle et al. 2010).

To conduct chlorophyll - based monitoring, soil core samples must be obtained first. The soil may be dry or moist at the time of coring, if it is moist it must be stored frozen or dried at room temperature in the dark before storing. Cores can be taken with a 1cm diameter tube approximately 5mm in depth, yielding a minimum of 3g of soil. Alternatively, multiple smaller cores can be obtained and pooled in a single composite sample or at least 3g. Samples should be taken from predetermined points within a biocrust plot. The samples can be stored isolated from each other in plastic bags, and must be kept in a dark, cold environment. A cooler with ice can be used for temporary transportation to a freezer, which is required for long-term storage.

Once in a lab with proper equipment, soil chlorophyll samples must be processed in isolated containers and never mixed, but can be done in batches. All processing should be done in minimal light to help prevent degradation of chlorophyll. The procedures are as follows:

Start water bath (500ml beaker of water on a hot plate in fume hood)(65c)

1. Grind soil sample in mortar and pestle until homogenous, and place 3g soil into labeled 15mL screw-cap vial. Weigh and record the soil amount for all 10 samples in the chla template file.
2. Pipette 6mL of Ethanol solution to each vial and shake gently by hand.
3. Boil samples in a water bath for 5 minutes, begin timing once samples actually begin to boil.

Make sure to loosen caps to allow heat to escape but not enough to allow evaporation. (If the water is too hot, samples may boil over.)

4. Remove vials from the water bath and allow to cool for 10 minutes. Make sure samples are in the dark.

5. Tighten caps and place all vials horizontally in the shaker and let them shake for 20 minutes.

6. After shaking, centrifuge samples at 4000rpm for 10 minutes.

7. Carefully pour supernatant into separate labeled vial. This sample is ready for analysis on the spectrophotometer. Place extracts covered in the fridge until part 2 is complete.

*If samples or extracts need to be held overnight, they must be kept in the fridge at 4°C.

8. Repeat steps 2-7 for second extraction on the pellet.

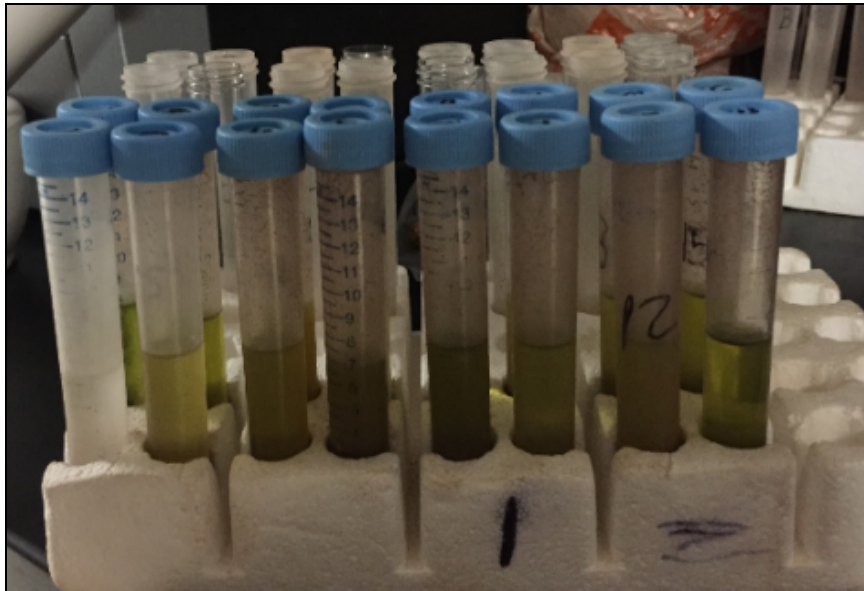


Figure 12. Chlorophyll supernatant ready for spectrophotometry.

After the chlorophyll has been extracted into ethanol (Fig. 12), it is ready for pipetting into a spectrophotometric plate. The spectrophotometry procedure is complicated and should be learned in consultation with an experienced instructor or by reading published scientific literature. Chlorophyll extraction can provide accurate quantitative results if done correctly, but is a very labor intensive form of monitoring.

7.3 Soil aggregate stability

Biocrusts can affect soil aggregate stability, which in turn affects multiple hydrological and biological characteristics of an ecosystem. They do this by binding soil particles together with fibrous structures (Belnap & Budel 2016). Quantitatively evaluating aggregate stability on soil surfaces with different levels of crusting can convey how effectively biocrusts are aggregating the soil.

A common technique to assess aggregate stability in the field is the soil stability kit (Herrick et al. 2001). This kit can be purchased or crafted from affordable, widely available materials (Figure 13). Use of the soil stability kit involves collecting multiple soil aggregates collected in an unbiased way from within a sample unit such as a field plot. The individual aggregates are placed on wire mesh sieves and then dipped in water during a timed procedure, during which their stability is assessed and rated by the observer. Testing must be conducted on dry soils. Detailed instructions are available online or included with purchased kits.



Figure 13. Soil aggregates on slake kit strainers made from PVC pipe and wire mesh (left) and a complete slake kit with 18 soil aggregates ready for evaluation (right). Photo credit NRCS.

7.4 Soil tensile strength and compressional strength

The aggregation of soil provided by biocrusts can increase resistance to erosion by water, but biocrusts may be damaged by compressional forces (crushing) or shear stresses. The ability of a crusted surface to resist these forces can be tested using some tools (Garcia-Pichel et al. 2016). One is a fruit pressure tester aka “pocket penetrometer” designed to quantitatively evaluate the softness and ripeness of fruits. It can also determine the resistance to compression of a soil surface. To do a

penetrometer test, one must choose from different tip sizes in accordance with the type and thickness of the biocrust, and soil characteristics. Smaller tips may break the surface preemptively, larger tips distribute pressure more. The penetrometer is pressed into the (ideally dry) soil to a depth of .25 inches, or until surface tension is broken (depending on which is most informative in a given study region). The penetrometer gives a reading of the force (in kg) required to do so. Record the number of kg required to push into the soil. Higher numbers indicate more resistant surfaces. After testing, pull up a small part of the soil surface adjacent to the hole and observe if it is crusted or not (does it hold together, are organisms present?).

Horizontal shear (tensile) strength can also be tested using a Torvane (Li et al. 2010). See figure 14 for instructions from the Torvane manufacturer for testing soil surfaces. Ideally this test is performed on moist soils, which can be quickly achieved with a spray bottle. As with the penetrometer test, one must choose from different vane sizes in accordance with the type and thickness of the biocrust, and soil characteristics. Smaller vanes may break the surface preemptively, larger tips distribute pressure more.

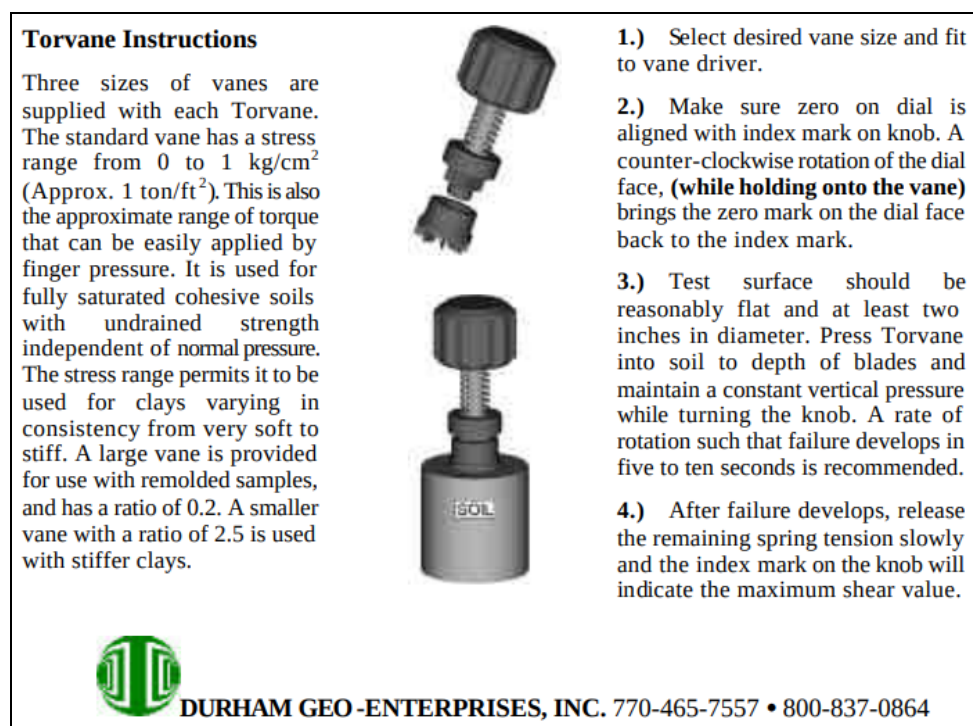


Figure 14. Torvane device to assess sheer strength. Photo credit Durham Geo-Enterprises.

7.5 Photo monitoring

Photographs taken at time intervals, or simply beginning and end of the monitoring period can be very useful for analysis of restoration success. Photographic analysis can be done in two ways: 1) using photos solely for visual comparison of biocrust appearance, and 2) doing quantitative analysis on photos with computer programs. The former is as simple as reviewing photos and looking for general trends in biocrust appearance. The latter is explained further below.

A normalized difference vegetation index (NDVI) has grown in popularity for easy assessment of vegetation cover throughout many fields of ecology. An R package called CrustCover has been developed for analyzing images. The premise of the technique is that if a true color photo is paired with an infrared filtered photo, the amount of surface area that appears green can be calculated (Fig. 15). This procedure has been adapted to work for biocrust monitoring, with some success (Fischer 2012). Biocrust organisms are not always vibrantly green, so computer analysis must be sensitive. Photos must be taken while biocrust is wet, because dessicated biocrust is often not green or less green. Other biocrust photo methods include threshold-based darkness indices (Bowker et al. 2008), and an index based on red and blue bands (Karnieli 1997).

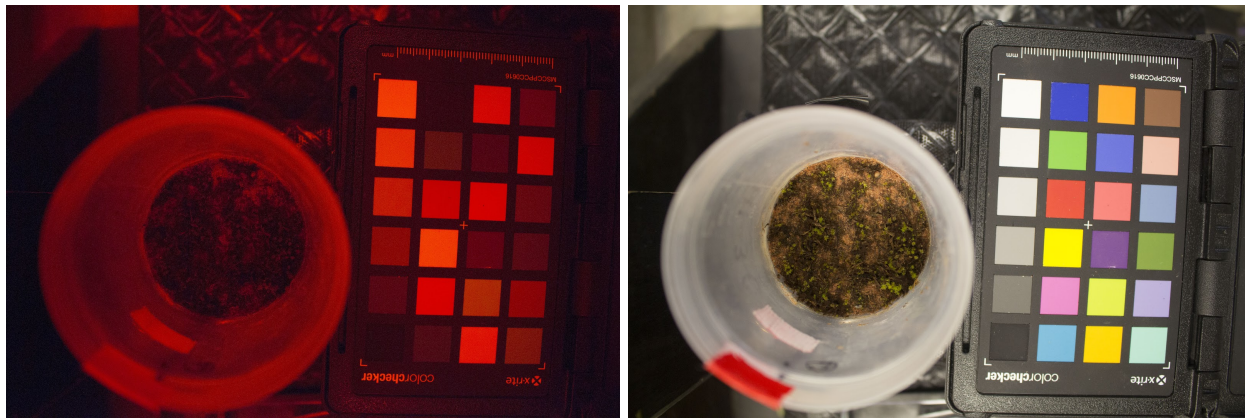


Figure 15. Infrared image (left) and true color image (right) of a small plot of biocrust moss. Color checker board must be present for reference. Photo credit MC. Rengifo-Faiffer.

Photographic analysis of biocrusts can be very effective in evaluating the success of a restoration project. Automated analysis is primarily focused on comparing the amount of surface cover in a more controlled and faster way than visual cover estimates in the field. Photos can also

convey the level of development of a biocrust community, especially if species can be identified in the photo.

7.6 Level of development

Belnap et al. (2008) developed a means of visually assessing and classifying biocrust development by ordinal levels. This is an easy to train, inexpensive method for monitoring results of a restoration project.

Level of Development can be used across large areas and is reasonable even if practitioners have limited familiarity with species of biocrust. Users must familiarize themselves with the appearance of different successional states of biocrusts and possibly prepare a photo guide for reference (figure 16).

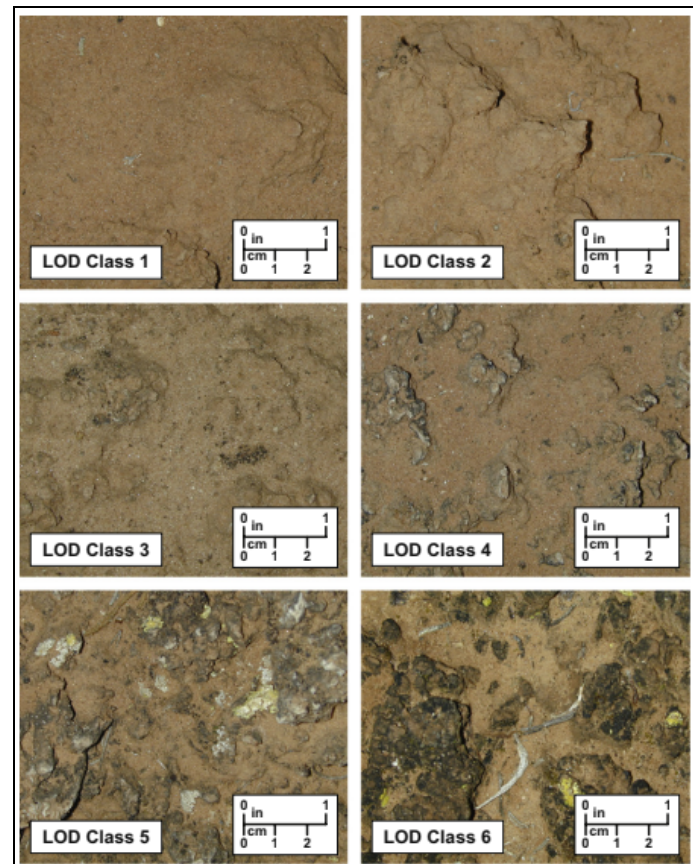


Figure 16. Level of Development classes from Belnap et al. (2008)

7.7 Soil nutrients

A potential benefit of restoring biocrust is improvement of soil nutrient levels via its nutrient cycling abilities (Zhang et al. 2016). If this is a goal of a project, nutrient levels can be monitored across the gradient of restoration. Measurement of soil nitrogen and organic matter in particular can indicate soil fertility, and biocrust development can affect this (Xiao and Veste 2017,

Ferrenberg et al. 2018). Soil samples must be collected to a consistent depth, using a coring device. To obtain total concentrations, samples need to be processed in an elemental analyzer capable of carbon and nitrogen determination. Perhaps more useful are measurements of organic carbon, and plant-available nitrogen fractions. Multiple methods (available, total, and exchangeable nutrients, measured based on various types of extractions) exist making such measurements attainable for many labs, and processing can be outsourced to most service labs if necessary (Robertson et al. 1999). Depending on available equipment, the cost of these measurements can vary.

Chapter 8: Ethics

Ethics are an important part of all forms of land management and ecosystem science, not only in forestry but also in the field of rangeland ecology which this paper focuses on. This concept has long been considered crucial to the well being of mankind and was originally made famous in Aldo Leopold's "The Land Ethic". Part of ethical land management is restoration of degraded ecosystems, a concept which appears in many professional codes of ethics under different wording. This manual focuses on restoring species diversity and ecosystem function on degraded land, and has components that describe how to do so in an ethical way.

Many would argue that to remove a critical part of an ecosystem is ethically wrong, and research has increasingly shown that biocrust degradation is an example of this. The logical next step is to restore the biocrust if possible, and we now know better than ever before how to do so in an efficient, effective, and ethical way. For example, some issues arise with harvest of biocrust starter material. The source location and species composition must be carefully considered. Intact biocrust populations will undergo destruction if they are harvested for use as restoration material, an unethical tradeoff. To mitigate this, we aim to harvest biocrust as salvage, meaning that it is already slated for an unfortunate fate of destruction, perhaps at a construction site. When restoring biocrust, we must consider if the species we inoculate with should be native to the site, or if the site may be better served by using species that are better adapted to future climate (i.e. assisted migration).

Throughout my education at NAU and my experience working on this project, I have learned a great deal about the professional ethics that a land manager and ecosystem scientist should have. I have made it a personal goal of mine to dedicate the rest of my life to stewarding the land that I live and recreate on. This will occasionally be through more indirect approaches, but one of the most direct ways to ethically care for land is to restore it when degraded, and I will seek to incorporate this in my career going forward.

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