Biological Soil Crusts for Reclamation of Mine Tailings.

by

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Abstract

Biological soil crusts (biocrusts) are soil characterized by desiccation resistant organisms residing in the soil crust. On disturbed landscapes, they can provide key soil building and nutrient cycling processes that provide structure and function to the substrate. To accelerate biocrust establishment on mine tailings at Gibraltar Mines, British Columbia, a series of experiments were implemented to identify amendments and treatments that could assist establishment of biocrust and mosses on tailings sand. Biocrust recruitment was tested using treatments of biocrust inoculation, fertilizers, micro-topology and super-absorbent polymer on 250 cm^2 plots. The results indicated higher chlorophyll content in treatments combining biocrust inoculation, fertilization and micro-topology. Moss recruitment was tested using treatments of wood-flour substrate, fertilizer, shade panels and gel covering on 100 cm^2 inoculated plots. Use of wood flour-based media significantly enhanced moss productivity.

Analytical research was simultaneously conducted to understand the trajectories and characteristics of unassisted generation of biocrust on previously reclaimed mine tailings (6 to 31 years after reclamation), at three different mine sites in Canada: Endako mine (BC), Brenda mines (BC), and Gaspé mines (QC). Biocrusts were surveyed and sampled along transects to understand the environmental conditions allowing biocrust persistence, characterize biocrust morphologies, the effect of biocrust on nutrient dynamics and any possible correlation between biocrust and onsite vegetation. Sampling and analysis consisted of inventorying moss and lichen specimens, PLFA testing on biocrusts, and nutrient testing. On the mine tailings, *Cladonia* sp. was the dominant lichen genus. *Ceratadon purpureus* moss was observed on all sites. Microbial biocrust and moss dominated biocrust had more microbial and arbuscular mycorrhizal biomass than lichen dominated. Microbial biomass correlated positively to biocrust carbon content and negatively against estimated biocrust age. Biocrust samples showed higher Al, P, K, S and Mg concentrations, but lower Fe concentrations, than sub-surface samples. Cumulatively, this project identifies amendments and ecological functions of biocrusts on mine tailings.

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List of Acronyms

SFU	Simon Fraser University
BCIT	British Columbia Institute of Technology
EP	Extra-cellular Polysaccharides
SAP	Super-Absorbent Polymer
PLFA	Phospho-Lipid Fatty Acid
Al	Aluminium
B	Boron
C	Carbon
Ca	Calcium
K	Potassium
Mg	Magnesium
Mn	Manganese
N	Nitrogen
Na	Sodium
P	Phosphorous
S	Sulfur
Zn	Zinc
DMSO	Di-methyl Sulfoxide

1) Introduction

It was identified by project partners with Stantec Consulting Inc. and Taseko | Gibraltar mines, that revegetation activities on mine tailings facilities are challenged by the conditions on the tailings substrate. Biological soil crusts (biocrusts) were known to have the potential to improve conditions by substrate controlling capabilities and desiccation tolerance. However, more knowledge was needed on using biocrusts as a treatment and understanding the potential of biocrusts to lend soil building properties towards mine tailings reclamation. This project was conducted to find answers to such questions, specifically in context to reclamation on mine tailings.

1.1. Ecology of Biocrusts.

According to Belnap et al. (2003) “Biological soil crusts result from an intimate association between soil particles and cyanobacteria, algae, microfungi, lichens, and bryophytes (in different proportions) which live within, or immediately on top of, the uppermost millimeters of soil. Soil particles are aggregated through the presence and activity of these biota, and the resultant living crust covers the surface of the ground as a coherent layer.” Biocrusts are noticeably found across various ecosystems on substrates where stress from desiccation and freezing prevents dominance by vascular plants (Evans & Johansen 1999).

Filamentous cyanobacteria, such as *Microcoleus*, and filamentous algae, such as *Klebsormidium*, are pioneer colonizing communities and essential to substrate stabilization due to their filamentous structure and the secretion of extracellular polysaccharides (EP) that add cohesion to the substrate. *Microcoleus vaginatus* cyanobacteria, are phototrophic early colonizers on bare substrates *and M. vaginatus* is a very common biocrust cyanobacteria in North America (Bowker et al. 2016).The stabilized substrate allows for other cyanobacteria and algae to attach onto, including dark cyanobacteria and diatoms (Budel et al. 2016). The stabilization of the substrate by the microbial communities, provides suitable conditions for lichens and bryophytes to colonize. They successively build more organic and nutrient content, improve water retention and lend protection against erosion (Belnap 2006; Guo et al. 2007; Zhang et al.

2009; Reed et al. 2012; Belnap et al. 2014; Zaady et al. 2014). Layering from gradual deposition has shown biocrusts to creates layers of sequestration (Chen et al. 1980)

The functionality and productivity of biocrusts increases as they approach complete maturity and complexity (Bates et al. 2012; Fischer et al. 2014; Lazaro & Mora 2014; Bowker et al. 2018). Thus, a positive feedback loop is created between biocrust functionality and development (Fischer et al. 2014; Zaady et al. 2014; Lan et al. 2015). The complexity in the microflora of the biocrusts is also reflected in the complexity of micro-fauna such as nematodes, tardigrades, protozoa etc. which are active in the water films formed in the biocrust (Darby & Neher 2016). Hence, biocrust development shows a successional trend of dominance by communities of cyanobacteria/algae → lichen or moss, based on moisture availability and microsite conditions.

The composition and productivity of a biocrust, is bound by the environmental stressors it experiences. Desiccation is a major stressor, dictating the threshold for biocrust maturity. Bowker et al. (2016) summarizes that hydration frequency, intensity and source of hydration dictate the community members of a biocrust. Studies in the Negev desert have shown that cyanobacterial diversity and the ability of biocrusts to host bryophytes, is dependant on the precipitation gradient (Yair et al. 2001; Hagemann 2015). Lan et al. (2015) also modeled that bryophytes are vulnerable to desiccation stress, while lichen are more dependent on substrate type. Grain size has shown to influence the development of cyanobacterial biocrusts and their ability to stabilize the substrate (Rozenstein et al. 2014). Intensity of solar radiation is a stressor, in the form of desiccation and UV damage. Shade-providing features such as aspect and canopy, are important to biocrust functionality at a micro-topological scale that provides partial shade to the moss and lichen specimen in the crust (III et al. 1977, Lobel et al. 2006). Biocrusts are sensitive to physical disturbance but micro-topology from disturbance can assist moss development (Csotonyi & Addicott 2004). Pócs (2009), demonstrates how cyanobacteria specialize and distribute themselves, even in the same biocrust, showing distinct survival strategies based on the type of stressor. Bowker et al. (2005) found biocrust development dependence on the micronutrients; manganese and zinc. Bryophytes and lichen have shown a strong response to modifications to substrate calcium content, pH, alkalinity and hydrological regime (Eldridge & Tozer 1997; Ponzetti & McCune 2001).

Biocrusts are unique due to the properties lent to the soil crust by the biological communities. EP produced by microbial communities aggregate the surrounding soil particles, stabilizes soils to limit erosion and, improve water retention (Mager & Thomas 2011; Adessi et al. 2018; Rossi et al. 2018). The EP also create vesicular porous structures in the substrate that increase albedo and water retention (Rossi 2012). The rhizoids from lichen and mosses, penetrate the soil crust, increase surface area and, break the flow of water and laminar air flow (Belnap & Gillette 1998; Eldridge & Leys 2003; Lazaro & Mora 2014). Biocrusts form horizontal layers as sediment is deposited (Chen et al. 1980). Biocrusts have shown to accumulate carbon, nitrogen and biogenic elements (Beraldi-Campesi et al. 2009; Huang et al. 2011).

1.2. Biocrust Recruitment on Mine Tailings

Exposed mine tailings lack surface stability and are susceptible to erosion by wind and water. Depending on the original mineral composition and processing, varying amounts of metals may be present and this can further make the particulates from the tailings an environmental and health concern when eroded (Hossner & Hons 1992; Csavina et al. 2012). Polster (1997) identified challenges to revegetation on mine tailings, which include steep slopes, adverse substrate chemistry, lack of nutrients, coarse substrate grain sizes, desiccation stress and the need for treatments to improve these conditions and achieve self-sustaining vegetation. Soils in reclaimed sites show multi-decade trajectories towards functional restoration that is hastened by restoration of micronutrients and positively assists revegetation (Benson 2018).

Biocrusts, due to their ecology, can withstand these stressors and occupy tailings substrate. The natural regeneration and potential for assisted development of biocrusts on mine tailings facilities has been investigated in prior research. Studies on mine tailings have shown that naturally occurring biocrusts contribute to carbon sequestration, nitrogen availability, substrate stability, and water retention. (Huang et al. 2001; Fischer et al. 2014; Gypser et al. 2016; Stewart & Siciliano 2015). The link between substrate type and biocrust development is not direct. Gypser et al. (2016), studying mine sites in Chile, noticed no significant impact of the substrate on biocrust development. However, biocrusts have been shown to be sensitive to the composition of their substrate and nutrient availability, which may be modified by addition of substrate amendments.

Experimentally, addition of nitrogen and phosphorous has shown to increase the maturity of biocrusts (Knelman et al. 2014; Zhang et al. 2015).

Biocrust inoculations have been tested to facilitate biocrust establishment and it has been demonstrated that certain amendments aid in productivity of biocrusts. Williams et al. (2017) conducted a lab-based incubator study and successfully demonstrated the ability of tailings substrate to host cyanobacteria foundational to biocrust development. Bu et al. (2018) were able to demonstrate field moss regeneration on loam soil within 2 months. Muñoz-Rojas et al. (2018) observed roughly 35% cyanobacterial cover on mining overburden substrate, within 3 months of using a cyanobacterial inoculation at a concentration of 6 $g.m^{-2}$. Ayuso et al. (2017) demonstrated biocrust establishment using biocrust inoculum at 5%, by area, over a span of 4 months. Hydration and fertilizer additions in laboratory conditions have shown enhanced biocrust recovery as well (Antoninka et al. 2016). Watering, sludge-based inoculation and fertilizer addition have shown increased biocrust productivity (Maestre et al. 2006). These studies shed light on amendments that are useful for aiding biocrust productivity.

Moss and lichen have also been experimentally cultivated for biocrust generation on tailings. Moss cover benefits from cloth covers, watering and micro-sites provided by heterogenous ground cover (Buxton et. al 2005; Lammare 2016). Inventory of lichen species on a mine site in NE Alberta, showed dominance by *Cladonia* and *Peltigra* spp. (Duncan 2011). Lichen regeneration is seen dependant on fragmentation size and, substrate amendments that maximize water retention but allow quicker adhesion of lichen fragment to substrate (Roturier et al. 2007; Duncan 2011; Rapai et al. 2018). Phosphorus has been observed as a nutrient simulator for lichen growth (Vagts and Kinder 1999; McCune and Caldwell 2009). Prolonging hydration and hydrating at night has shown to maximise lichen photosynthesis (Bidussi et al. 2013).

Biocrusts can assist revegetation efforts on mine tailings by supporting germination and survival of seedlings. Small breaks in biocrust mats provide exposed nutrient rich substrate that promotes seedling survival and growth (Beyschlag et al. 2008). Research by Green et al. (2018) has shown support for the fungal loop hypothesis, whereby fungal mycelium connect and facilitate the transfer of nutrients and carbon between vascular plants and biocrusts.

2) Objectives

The aim of this research is to characterize biocrust composition and soil building capacities and, to find amendments that can assist biocrust application as a treatment for reclamation on mine tailings. The analytical component of this project focused on characterizing the natural biocrust recruitment on mine tailings substrate across three reclaimed mine tailings in Canada. The natural biocrust communities were evaluated for their community composition, structure and contribution to ecosystem functioning in the form of carbon content, nitrogen content, pH and inorganic nutrient dynamics between biocrust and sub-surface substrate. The experimental component of this project aimed to identify treatments or amendments that may assist biocrust and moss recruitment on tailings. Cumulatively, this research sought to identify aides in biocrust establishment on mine tailings, while also examining the potential long-term effects of such recruitment on ecosystem functioning of the reclaimed tailings. The specific objectives are as follows:

1. To characterize naturally established biocrust communities at designated mine tailing facilities according to their microbial communities, bryophyte and lichen species composition.

    1.1 To identify naturally established moss and lichen species for use as biocrust inoculums for mine reclamation.

    1.2 To characterize the contribution of biocrusts to soil building through accumulation or leaching of soil nutrients.

2. To promote biocrust community establishment on the mine tailings substrate through addition of biocrust inoculum and amendments.

    2.1 Experiment 1: Test the effects of biocrust inoculation, nutrients and physical amendments on productivity of tailings crust.

    2.2 Experiment 2: Test the effect of *Microcoleus vaginatus* cyanobacteria in aiding biocrust establishment.

    2.3 Experiment 3: Test the effects of substrate and shade on moss establishment on tailings sand.

3. To develop recommendations for potential use and follow-up research on the use of biocrust on mine tailings for reclamation.

3) Methods

3.1. Selection of study sites.

Experiments were conducted on the tailings beach of Gibraltar mines, located near Williams Lake, BC (approx. 52°31'N 122°17'W). The beach had been vegetated through drill-seeding in spring 2019, and a ~250m strip was cleared therein to exposed bare tailings sand, to host the experimental plots. Figure 1 is an image of the strip from an edge with the treatments already applied . Figure 2 is a map of the strip on the tailings facility, as well as the areas from which biocrust samples were collected for inoculation. Gibraltar mines acted as the primary testing site for the experiments. Natural, undisturbed biocrust covers at the site were in forest, forest-edge and grassy-slope features. Biocrust cover was too sparse and insufficient on the grassy slope for use as inoculum in experiments.

To understand the natural composition and substrate effects of biocrusts on mine tailings substrate, biocrusts were sampled at three reclaimed Canadian mine sites; Endako mine, Brenda mines and Gaspé mines. All three locations have been reclaimed and are not currently in operation. Endako mine and Brenda mines are in the Montane Cordillera ecozone while, Gaspé mines is located in the Atlantic Maritime ecozone . Endako mines, operated by Centerra Gold, is a surface molybdenum mine. It is located near Fraser Lake, British Columbia (BC) (approximately 54.02º N/124.05º W) and the region experiences annual precipitation of around 540 mm. Brenda mines, operated by Glencore, is an open-pit copper-molybdenum mine that ceased operations in 1990. It is located near Peachland, BC (approximately 49°52’N/119°58’W) and the region experiences annual precipitation of around 340 mm. Gaspé mines, also operated by Glencore, is a copper mine that ceased mining operations in 1999. It is located adjacent Murdochville, Quebec (approximately 48°57' N/65°30' W) and receives an annual precipitation of around 1190 mm. To calculate the age of the samples taken, for each sample point, the respective mine personnel was contacted for the history of restoration treatments. Replies were received as personal communication in emails. For Brenda mines, a document was provided on the history of restoration treatments. Using these, an age of the biocrust samples was calculated. The samples ranged from 8 to 29 years

at Endako mine, from 18 to 24yrs at Brenda mines and from 15 to 31yrs at Gaspé mines. A complete list of sampling sites and ages is tabulated in Table 4.

3.2. To characterize naturally established biocrust communities at designated mine tailing facilities according to their microbial communities, bryophyte and lichen species composition.

3.2.1. Sampling Design and parameters used.

Endako mine, Brenda mines and Gaspé mines, had varying amounts and levels of biocrust development on their tailings facilities. At each mine site, the tailings facilities were first demarcated and surveyed for three criteria: (a) safe access to the tailings feature, (b) visibly noticeable biocrust cover and, (c) the substrate being tailings sand. Four, three and five sampling areas were identified for Endako, Brenda and Gaspé mines, respectively, meeting these parameters.

Within these sampling areas, transect based sampling was conducted in August 2019. At each of the sampling areas, a transect was outlined and oriented, such that the transect would both: (a) cover the length of the sampling area and, (b) capture the landscape variability within the sampling area, which consisted of changes in slope, aspect and vegetation structure. Sampling points were then established on each of these transects, with the points 400 m apart from each other. The length of the transects varied, with majority of the transects being 400 m (2 points) and the remainder of the sampling areas being less than 400m across. When establishing the end points of a transect, a minimum distance of 10 m was maintained from the edge of the established sampling area, to avoid ‘edge effects’ from adjacent ecological features. At each sampling point, two sub-samples were collected through 4 m x 4 m quadrats set 1 m apart in the direction of travel. Figures 3,4 and 5 are maps of the sampling areas and sampling points therein, for all three sites. Due to heterogeneity of the terrain and the biocrust cover, in some instances it was impractical to set the sampling points at exact 400m intervals. This variation was addressed by installing the quadrats at the

researcher's discretion, at the closest representative spot. Such discretion was employed on sites 3, 14 and 19.

This sampling methodology utilized surveys to identify where biocrust patches were located. The transect approach created uniform distance and buffer between sampling points, while accommodating the varying sizes and topographies of the sampling areas. The sampling distance, quadrat size and number of sub-samples, was decided by the researcher based on the following observations: (a) Biocrust composition (moss and lichen) and landscape features, were visibly similar for at least 400m at all sites, (b) a 4x4m quadrat, often used for vegetation sampling, allowed sample collection while accounting for sparse vegetation, breaks in the biocrust and collection without stripping large strips of the biocrust and, (c) collection of two-subsamples adequately reflected biocrust moss and lichen communities found in and around the sampling points. To test this methodology, a preliminary attempt was conducted on the forest biocrust at Gibraltar mines. Three sampling points were established 400 m apart and within 2 m of the forest edge. Three 4x4 m sub-sampling plots were established. A total of 12 unique moss and lichen specimen were found. After the initial six found at the first sampling point, three unique specimen were found at the second and third sites. The sub-samples for all three sampling points, never yielded any unique specimen after the second sub-sample. Based on this analysis and assumption of forest biocrust being more diverse in their composition compared to dryland biocrust; a sampling distance of 400 m and the use of two sub-samples, was deemed adequate to study biocrusts in dryland scenarios. The summarized data from the analysis is documented in Appendix F.

Samples of mosses and lichen unique at each sampling point, were collected for identification. Biocrust samples, 0 to 0.5 cm deep, and reference samples, 0.5 to 2 cm deep, were collected at each sub-sample, starting from the center of the quadrat and proceeding radially to its edge. Both reference and biocrust samples were from the same subsampling quadrats, but collected at different locations, to minimize cross contamination between samples, especially due to the loose, sandy substrate found underneath the biocrust. They were also collected in small dispersed sections to minimize the scaring of the biocrust patch being sampled. The samples from both sub-samples were combined for every sampling point. The biocrust and respective reference samples collected were 40 in total for the 20 sampling points. This approach to collecting

reference samples, allows accounting for potential variation in chemical composition across the tailings facilities from the mining legacy. Separate tools were used for extracting biocrust and reference samples. Sampling tools were wiped with isopropyl alcohol after every sampling point. This step was missed between sampling point 1 and 2, potentially causing a measure of cross-contamination of nutrients from sampling point 1 to sampling point 2. Each sample was checked for leaf litter and residual plant material, such as roots, to limit samples showing legacy effects from vegetation. This allows a fair comparison between different biocrust samples as there is no metrics being employed to discern between substrate properties acquired from biocrusts against those acquired from vegetation. Samples were bagged in zipper storage bags for off-site processing. Sites where effects from surrounding vegetation could interfere with sample evaluation through the presence of detritus or roots, were not sampled. Two such sites occurred, between sample points 1 and 2 and, sample points 9 and 10. Many sites in Gaspé mines also had visibly dominant lichen cover on the ground but were not sampled due to the substrate underneath being detritus and not tailings substrate.

At each sampling point, notes on biocrust appearance, associated vegetation and aspect (>10^0 slope) were made. In this study, biocrust 'cover type', refers to the dominant community visible, along the successional scale of cyanobacteria/algae → lichen → mosses. These members of are not mutually exclusive of each other, but the progressive appearance corelates to biocrust maturity, as discussed in section 1.2. This was used to identify the year where restoration activities were concluded on the areas sampled. Using the end dates of the restoration treatments for all sites, a baseline start year was established for the biocrusts sampled. When subtracted from the sampling year, it provides an estimate for the age of the biocrust community sampled. Notes were made for each site on the initial restoration treatments and whether the site had received fertilizer treatments in the years thereafter.

3.2.2. Sample processing and analysis

Samples were air-dried for 24 hours and then stored in a freezer within 14 days. Samples were separated for lab testing and appropriately shipped. Phospho-Lipid Fatty Acid (PLFA) analysis by Ward Laboratories Inc., was conducted on all 20 biocrust samples. The BC government's Analytical Laboratory was employed to conduct a Mehlich III extraction on the biocrust and reference samples for the elements Al, B, Ca,

Cu, Fe, K, Mg, Mn, Na, P, S and Zn. Reference sample for sampling site 18 and biocrust sample for sampling site 12 were misplaced for this nutrient testing. A pH test, 1:1 substrate to water, was also conducted on both sets of samples. Additionally, total percent carbon and nitrogen, were also measured through combustion, for the biocrust samples.

Samples of bryophytes and lichen species, were collected in paper envelopes, air-dried and stored in paper envelopes until identification. Visual identification of the bryophytes and lichen was conducted for each site (Schofield 1969; Schofield 1992; Parish et al. 1996; Malcolm 2000).

3.2.3. Statistical Analysis

Results obtained were statistically analyzed through the software R© due to the software being free, open-source and supportive of statistical analysis. The default statistical significance for all tests is $p_{critical} = 0.05$, but p-values close to the critical values are also given consideration and are stated as such in Section 4. For comparing two interval variables, correlations and regressions were used. For comparing a parametric continuous variable across categorical data, t-tests were used. For comparing a non-parametric continuous variable across categorical data, Wilcox tests were used.

3.3. Biocrust community establishment on the mine tailings substrate through addition of amendments.

3.3.1. Experiment 1: Testing effects of biocrust inoculation, nutrients and physical amendments on productivity of tailings crust.

It is hypothesized that amendments of biocrust inoculation, nutrient addition and physical amendments that increase water availability, will increase biocrust establishment on bare mine tailings. To test this hypothesis, a multifactorial experiment was used with the following three levels:

1) Inoculation: Salvaged biocrust inoculum, no inoculum (control).

2) Chemical amendments: Macro-nutrients, macro- and micro-nutrients, no chemical amendments (control).

3) Physical amendments: topology feature, super absorbent polymer (SAP), no physical amendments (control).

Experimental plots were a series 0.5 x 0.5 m square plots, setup linearly across the strip of bare mine tailings, buffered with 0.1m borders and with a gap of 0.5 m between each plot. The design systemically places eight sets of 18 treatment plots, with the placement of the treatments within each replicate set randomized individually. A total of 18 treatments, replicated eight times, yielded 144 plots. Table 1 is the resultant experimental matrix for experiment 1.

Biocrust inoculums were prepared using a mix of biocrust and tailings sand. The biocrust was collected from a non-mined forest edge adjacent to the tailings sand facility at Gibraltar mine (Figure 5). The biocrust was dominated by mosses, with some lichen and liverworts. Salvaged biocrust was cleared of detritus, stones and small vascular plants. A total of 17 L of biocrust was collected experiments 1 and 2 (see section 3.2.3 for experiment 2). Treatments with biocrust inoculation, consisted of 80 mL of this biocrust mixed with 1.25 L of tailings sand on-site in a bucket, yielding a surface expression of 6%inoculation, by area, when applied at a thickness of 0.5 cm.

Nutrient doses were chosen as appropriate to moss development, due to the observed moss dominated biocrust used for the inoculation. Adamo et al. (2007) and

Adamo et al. (2008) used combustion-based measurements of moss elemental composition. Based on values of uncontaminated moss samples from these studies and an observed density of *Ceratadon purpureus* moss across patches on the site, a value of 0.05 $g.cm^{-1}$ was used. A nutrient dose was created for each plot based on this hypothetical moss density, should each plot be entirely covered by the moss. Nitrogen was applied at 1:1 to phosphorus, using ammonium phosphate, since biocrust communities are inherently capable of fixing nitrogen. Table 2 summarizes the measurements and resultant nutrient dose. It was presumed that copper, a micronutrient, was sufficiently present in the tailings sand and was not added. For treatments with only macronutrients, only ammonium phosphate was added. All nutrients were added 30 days after the commencement of the experiment to ensure the biocrust communities had oriented the phototrophic layer prior to nutrient exposure.

'Topology' treatments were made to provide partial shade to the plots in the form of a mound. These treatments were realized through the application of the 1.3 L of tailings sand in a cone of base diameter 15 cm and height of 10 cm. This yielded cone of fixed curved surface areas of 294.524 cm^2. Inoculum added would have a uniform distribution in the mix and have the same expression on the surface; 6%. However, since these were not made using a mold, some variability is expected between the cone geometry. Figure 6 an image of a 'topology' and 'inoculated' treatment.

Potassium polyacrylate was used as the Super-Absorbent Polymer (SAP). Sourced from Autochem Inc., potassium polyacrylate acts as a polymer when hydrated, storing up to 300 times it's weight in water (Gómez 2016). As opposed to 'topology' treatment, the SAP aims to counter desiccation stress not through shading, but by acting as a reserve of additional water. It also contributes to changes in the physical structure of the substrate, when applied on the surface. Preliminary testing on a grab sample of the tailings sand was conducted. 80g of tailings substrate was put in aluminium trays. A surface area of 55 cm^2 was expressed in each tray. The substrate density was calculated as 1.43 $g.cm^{-3}$, with a sandy-loam texture and water carrying capacity of ~0.3 $ml.cm^{-3}$. There were eight treatments made using four doses of SAP applied in two different ways. Potassium polyacrylate (SAP) doses of 0 g, 1 g, 2.5 g and 5 g per 55 cm^2, were applied separately through surface application and mixing within the sand. Three replicates of each of these eight treatments were put in trays and 24 mL of water was added to each. Samples were put in an oven at 60^o C for 12 hours. Weight was

measured at intervals of 0, 6, 9 and 12 hours. Moisture loss was similar for all trays with SAP, but higher than samples without any SAP. For doses >1g, physical crusting was observed. Surface application caused the SAP to crust and then curl the substrate to form a concave cup, when dry. Based on these results, an expression of 2 g per. 50 cm^2, mixed within the substrate, was chosen as the application dose for SAP treatment in experiment 1. This translated to using 10 g of SAP per. treatment plot of 250cm^2.

Wooden skewers were coated with a waterproof coating, marked and placed in the center of each treatment plot, to act as erosion pins. To counter dry periods in the summer, assisted hydration was provided to plots once a day, every week. The experiment was initiated on 5th June 2019 and sampled on 3rd September 2019, yielding a run time of 90 days. At the end of this period, erosion pin measurements were taken from the plots and total erosion/deposition for the period of 90 days, was recorded for each plot. Due to certain plots being damaged by ungulates, randomized sampling was not possible for all the plots. Hence, crust samples, up to 0.5cm deep, were collected radially from the centers of each plot and analyzed for chlorophyll a and b content.

Using the methods outlined by Casear et al. (2018), chlorophyll extraction was performed on the crust samples, using Dimethyl Sulfoxide (DMSO) as the organic solvent. 3 cm^2 of the collected samples were taken. Samples were run through two 90 min dark water bath extraction cycles, using pure DMSO, with the extract poured out after each cycle. Both extracts were pooled together for each sample, and then run through a centrifuge at 5000 rpm for 10 min. Total volume of solution was then measured. Extracts were analyzed in a spectrophotometer to determine chlorophyll a and total chlorophyll content in the samples by taking measurements at 648, 665 and 700 nm wavelengths. Values obtained were put in the following formula from Casear et al. (2018) who personally sourced it from O.L. Lange, to obtain chlorophyll a and chlorophyll a+b values:

Chl.a+b [ug]= ((A665 -A700) x 8.02) + ((A648 – A700) x 20.2) x (Dilution Factor) x (Amount of Solvent)

and,

Chl.a [ug]= ((A665 – A700)x 12.19) x (Dilution Factor) x (Amount of Solvent)

3.3.2. Experiment 2: Testing the effect of *Microcoleus vaginatus* cyanobacteria and in aiding biocrust establishment.

Inoculation with soil cyanobacteria has shown immediate response to biocrust development, in both field and laboratory settings (Zhang et al 2013; Bowker et al. 2016; Velasco Ayumo et al. 2017). *M. vaginatus* is the most abundant cyanobacteria in North American biocrusts and foundational to biocrust establishment (Bowker et al. 2016). Hence, it is hypothesized that addition of filamentous cyanobacteria, *M. vaginatus,* inoculum to the salvaged biocrust inoculum will positively affect its establishment on mine tailings. To test this hypothesis, an experiment was designed with the following treatments:

1) Biocrust (B): Inoculation with salvaged biocrust mixed with tailings sand.

2) Enhanced (E): Inoculation with salvaged biocrust and *Microcoleus vaginatus* cyanobacteria mixed with tailings sand.

3) Control (N): Addition of bare tailings sand without any inoculum.

Experimental plots were a series 0.5 x 0.5 m square plots, setup linearly across a strip of bare mine tailing, buffered with 0.1m borders and with a gap of 0.5 m between each plot. Hence a distance of 1.2 m existed between the centers of each plot. The three treatments were repeated eight times, for a total of 24 plots randomly arranged plots.

M.vaginatus inoculum was prepared from the Microcoleus vaginatus var. cyano-viridis strain obtained from UTEX Culture Collection of Algae. It was cultured in BG-11 medium in both liquid and solid agar plates. Success, in the form of growth of filamentous grees masses (see Figure 7), was achieved in solid agar plates cultured for nine days at 18 ^{o}C and 6850 lux exposure in 18-hour cycles under a fluorescent full-spectrum grow-light. After seven days, light intensity was increased to 7200lux. The resultant *M.vaginatus* culture was mixed in 100 mL dechlorinated water, with 10mL of the resultant solution being added to each designated plot. The remaining 20 mL was retained to calculate dose, since as shown by Zhang et al. (2013); dose is an important factor in biocrust response to this treatment. Inoculation concentration positively affects the colonization rate.

As in experiment 1, wooden skewers were coated with a waterproof coating, marked and placed in the center of each treatment plot, to act as erosion pins. To counter dry periods in the summer, assisted hydration was provided to plots once a day every week. The experiment was initiated on 5th June 2019 and sampled on 3rd September 2019, yielding a run time of 90 days. At the end of this period, erosion pin measurements were taken from the plots. Due to certain plots being damaged by ungulates, randomized sampling was not possible within the plots. Hence, crust samples, up to 0.5 cm deep, were collected from the centers of each plot. Chlorophyll a and b were measured for each plot, just as outlined for experiment 1 (see section 3.2.1).

3.3.3. Experiment 3: Effects of enriched substrate and shade on moss establishment on tailings sand.

The natural biocrust on the site was a moss dominated biocrust canopied by a coniferous forest. It is assumed that the forest provides shade, organic media and higher nutrient sources than the bare tailings sand. It is hypothesized that by replicating features found in the natural habitat, the establishment of salvaged mosses can be hastened. Substrate characteristics, shade and nutrient availability strongly in the forest floor and edge, contrast the bare mine tailings to the forest floor and edge. To test this hypothesis, a multifactorial experiment was conducted using the following levels:

1) Substrate: Enhanced substrate (wood flour and potassium polyacrylate), enhanced substrate with nutrients, tailings sand (control).

2) Shade: Shade panels, psyllium husk cover, no shade (control).

Experimental plots were a series of circular plots of radius 5.64 cm, with a buffer of 10 cm between each plot. The nine resultant treatments were replicated five times, to yield a total of 45 experimental plots. The resultant treatments are tabulated in Table 3.

From the undisturbed forest edge, mats of *Dicranum scoparium* and *Pleuzorium schreberi* were used as inoculum. They were selected due to the harvestable amounts of homogenous mats found at the forest and trail edges on the site. They offer a contrast of ‘mat’ and ‘tuft’ morphology. Based on their preference for shaded habitats, these species are expected to perform worse than mosses like *Ceratadon purpureus* which are more adapted to exposed sites. All plots substrates were mixed with 5cm^2 each of both moss

species, which were trimmed prior to addition. This yielded a 10% inoculation, by surface area, for each plot. ‘Enhanced’ substrate was created using 50 mL of wood flour from Douglas Fir and 2 g of potassium polyacrylate, mixed dry. The enhanced substrate was applied to the appropriate plots as a 0.5 cm layer. For nutrient enrichment, BCD medium as directed by Mitsuyasu (2004), was used at a dose of 50 mL BCD solution per plot. 15 x 15 cm opaque Styrofoam panels were placed in north-south alignment, on the west side of the plots to act as shade panels. 1 g of psyllium husk powder was sprinkled on top of appropriate plots. All plots were sprayed with water or BCD nutrient media, as appropriate. The experiment was run from 31st July to 4th September 2019, for a total of 35 days. Plots were extracted in entirety, as they were only established till 0.5 cm of depth. Using 1 cm^2 of sample from each plot’s center, chlorophyll b measurements were made using the procedure outlined in experiment 1 (see section 3.2.1).

4) Results

4.1. To characterize naturally established biocrust communities at designated mine tailing facilities according to their microbial communities, bryophyte and lichen species composition.

4.1.1. General description of biocrust development on mine tailings

Biocrust development was visually unique amongst the sampling areas, with no clear trend between morphology, composition and landscape. Most sites occurred on a flat aspect i.e. slope<10^{o}. No south-west and north-west facing sites were encountered. Vegetation on the sites was predominantly grasses and encountered at all sampling areas with varying amount of densities. Secondary vegetation in the form of shrubs, saplings and trees was encountered at different sites. Biocrust cover was dominant on bare ground where vegetation was sparse or absent. No correlation or pattern was identified between biocrust cover type, and such vegetation present on the site. Biocrust and vegetation was witnessed in three distinct forms. Most biocrust development occurred as patches between established vegetation, such as on sampling site 2. More extensive mats of biocrust had breaks within them from which grasses emerged, such as sampling site 5. Finally, biocrusts were also seen in riles around mounds of grasses, as seen on sample site 19. All sites sampled, had prominent patches of biocrust, even when surrounding vegetation was dense. Refer to figures in Appendix G for images of samples from Endako and Gaspé.

For site ‘L’, observational evidence of the fine substrate, vegetation and cracked ground, suggested that the biocrust forming on the edge of a tailings pond was frequently inundated, based on which, it is presumed that the biocrust renews itself annually. It is also the only site with distinctly finer sediment. Observations on the visually dominant biocrust cover type were also made for all sites. A note was also made on the landscape features on which biocrust samples were found. Most of the sites were reclaimed using revegetation efforts. These features include overburdens, dam slopes, tree plantation, grassy plain and pool edge slopes Table 4 summarizes these field observations and calculated ages, for each site.

Varying biocrust morphologies were seen, ranging between rugose (roughened surface) and pinnacle (surface with peaks). Figure 8 is that of a moth taking refugia in the shade of the biocrust morphology, at sample plot 5. In this case, the gap in this pinnacle biocrust is acting as refugia for the moth. Morphology was most pinnacled when vegetation was scarce, as was witnessed on plots 4, 5 and 7. Generally, biocrust density and moss composition would be noticeably higher in depressions such as the transition of slope-to-terrace on sand benches.

The age and biocrust cover type for the samples, showed a significant difference ($p=5.63x10^{-4}$) between all categories except 'Moss and Lichen' cover types against 'Moss' and 'Lichen' cover types. The distribution is plotted in Figure 9. An overall transition is seen from 'Dark Crust' microbial crust, to 'Moss' dominant to 'Lichen' dominant biocrust.

4.1.2. Moss and lichen species

Moss and lichen species were collected from the three reclaimed mine sites and identified in lab. An unidentified moss from the *Bryaceae* family and various lichen from the *Cladonia* genus were the most common form of each. Many of these often showed dwarfed gametophyte development. Moss species in the *Polytrichum* spp. and *Ceratadon purpureus* are also seen frequently across all three mine sites, as well as at Taseko | Gibraltar mines. Neither were observed to be dwarfed in any of the samples. *Polytrichum piliferum* was observed in a dense homogenous patch at Brenda mines. A summary of all observed and identified specimen is tabulated in Table 5. The specimen recorded for each sample are tabulated in Appendix A.

4.1.3. Microbial activity observed in biocrusts

Communities measured can be focused into the following groups: Bacteria, fungi, protozoa and undifferentiated. Percent bacteria ranged from 12.03% to 41.65%. Percent fungi ranged from 5.92% to 42.49%. Percent protozoa ranged from 0.17% to 14.33%. Percent 'undifferentiated' biomass ranged from 20.7% to 61.49%. Undifferentiated biomass made a significant amount of every sample, being the largest fraction in most of the samples. The biomasses are listed in Appendix B. When comparing biomass amongst the groups, a negative correlation is seen between percent protozoan and

bacterial biomass, as plotted in Figure 11. Lichen dominated biocrusts show a higher bacteria: protozoa density (triangles in Figure 11).

The total PLFA biomass extracted from each sample, showed a negative correlation with age. The regression ($p=5.96x10^{-4}$, n=18) had an adjusted R^2 value of 0.5024. A slight negative autocorrelation was seen for the linear relationship. Figure 10 plots this relationship, categorising points for the dominant biocrust cover type and mine sites.

Percentage of Actinomycetes in the microbial biomass distribution was significantly different between Gaspé mines and both Brenda mines ($p=1.32x10^{-2}$, n=20) and Endako mine ($p=9.5x10^{-3}$, n=20). This difference was also positively linearly correlated with regional annual precipitation corresponding to each mine ($p=2x10^{-3}$, n=19) and had adjusted R^2 of 0.386. No other significant interaction is seen between measured microbial biomasses and calculated sample age, cover type, surrounding vegetation type, site or amongst microbial groups.

Saprophytic fungi made 79.3% to 100% of the fungal biomass, with a complete 100% for sample 20. Rhizobia biomass was zero for all samples. The remainder of fungal biomass was composed of arbuscular fungi, more than zero in all samples except sample 20. PLFA weights of communities were tested against biocrust cover types, where a significant difference was seen in arbuscular fungi biomass between lichen and dark crusted, lichen and 'lichen and moss' covers and, moss and dark crust covers. The comparison is plotted in Figure 12.

When comparing the calculated age to the ratio of Mono:Poly- saturated fatty acids from the PLFA analysis, a positive correlation is seen ($p=6.89x10^{-4}$, n=15), with an adjusted R^2 of 0.3745, after four samples are removed as outliers.

4.1.4. Carbon and nutrient content

Total carbon content in biocrusts samples ranged from 1.9% to 9.5%, by weight. Samples 16 and 17 were considered as outliers, giving a range of 1.9% to 5.1% in their absence. Influence from surrounding grasses may be contribute to the carbon content in these plots. Total nitrogen content ranged from 0.079% to 0.49%, by weight. However, a significant correlation ($p= 4.11x10^{-15}$, n=20) with $R^2= 0.97$ is seen between the percent C

and N content. A significant ($p=4.79x10^{-4}$, n=18) linear relationship is seen between total microbial biomass and percent carbon in the biocrust samples, as plotted in Figure 13.

Total C/N ratio calculated from this, ranged from 14.76 to 24.05. A significant difference is seen in sites adjacent to active tailings ponds, compared to plantations, overburden and sand benches. A significant difference is also seen for dam slopes against grassy plains, plantation, pool edge and sand benches. The differences are plotted in Figure 14. Since there are only two widely spaced data points for the feature 'pool edge', including the distinct; sample 20, the feature is retained in the dataset for demonstration but the significant relationships to this feature is ignored.

Measurements of the biogenic elements often yielded values below detection limit. Values that were below detection limit, were set as 0.1 mg/kg below the detection limit for that element. For example; if a sample noted manganese as being below detection limit and the detection limit is 20 mg/kg, then the value for that sample was set as 19.9 mg/kg. Compared to leaving the value as empty, it allows using more samples. The measurement from the reference section of a sample (0.5 to 2 cm) were subtracted from the biocrust section of the sample (0 to 0.5 cm). The values obtained are tabulated as new variable in Table 6. Boron values were removed and considered insignificant since all but two samples had a value over the detection limit, with none over the detection limit in the biocrust samples. This method provides a meaningful statistical comparison in differences. The values obtained are conservative for positive differences (addition) but may over-estimate negative differences (loss). During testing, the difference in copper concentrations of sample 16 is treated as an outlier as it heavily skews the data for copper. Original values for nutrient measurements in the biocrust samples, including for boron, are tabulated in Appendix C.

Al, Mg, K, P and S had significantly higher values, while Fe had a significantly lower value in the crust, compared to the reference. The results are plotted in Figure 15. Nutrients also showed correlation in their concentrations in the biocrust samples (0- 0.5 cm depth). Concentrations of Mg, Mn and Zn were correlated to each other. S and P concentrations in the biocrust were also correlated, albeit slightly over the critical p-value. P, however, was also correlated to Zn. These results are tabulated in Table 7. Al concentration was significantly ($p=2.11x10^{-4}$) negatively correlated with pH. When testing correlations between biomasses from PLFA analysis and nutrient levels observed in the

crust, a positive correlation is seen between percent fungal biomass and difference in P ($p=2x10^{-10}$, n=19) with an adjusted R^2 of 0.4403. Linear regression between biocrust age and difference in nutrient concentrations, with age as the independent variable, showed a significant correlation ($p=2.26x10^{-2}$) for calcium, with an adjusted R^2 of 0.2397. Fertilizer upkeep did not show any significant effect on percent C.

pH was also compared for 18 biocrust samples (0 to 0.5 cm) and their reference counterparts (0.5 to 2 cm). It showed a significant difference ($p=9.4x10^{-7}$) between the two. Biocrust samples had a mean pH and standard error of 5.85 ± 0.17, compared to reference samples with a mean pH and standard error of 7.39 ± 0.21. A variable for difference in pH for each sample site (biocrust pH - reference pH), was created, just as was created for the nutrients. This allows comparing pH changes across different substrates since differences are relative. There is a weak (adjusted R^2= 0.2122), but significant ($p=3.12x10^{-2}$) negative regression between difference in pH and age of biocrust.

4.2. Results from biocrust community establishment experiments on the mine tailings substrate through addition of amendments.

4.2.1. Experiment 1: Effects of biocrust inoculation, nutrients and physical amendments on productivity of tailings crust.

Chlorophyll a measurements, used as a proxy for productivity, are tabulated in Appendix D. All treatments, except treatment ‘INT’, have eight replicates, with ‘INT’ having only seven replicates. Treatment ‘NNN’ is the control in this experiment. A slightly significantly ($p=7.02x10^{-2}$) higher chlorophyll a content for treatment ‘IMaT’, which is a combination of inoculation, macronutrients and topology treatments is observed. However, no treatment had a p-value <0.05. Comparing treatment levels for experiment, showed significant differences (p-critical=0.05) for inoculum and nutrient addition. Addition of inoculum had significantly higher chlorophyll a content, than plots that were not inoculated. Addition of macro-nutrients had a significantly higher chlorophyll a content than without. However, there was no significant difference from the addition of micro-nutrients. Similarly, SAP application had no significant effect on the chlorophyll a content in the samples.

Prominent photosynthetic filaments were seen in at least three samples of ‘IMiN’, ‘NMiS and ‘INN’’ treatments. See Figure 16 for images of the observed gametophyte emergence in the ‘IMiN’ and ‘INN’ samples. Field observations also showed prominent green coloration developments after 30 days of nutrient addition to treatments with nutrient addition (see Figure 17 as an example). Erosion trends were starkly different for ‘topology’ treatments, compared to the rest. ‘Topology’ treatments had pronounced erosion, ranging from -0.9cm to -4.7cm. Treatments without ‘topology’ treatment had erosion and deposition values ranging from -1.5cm to 3cm. The data was differentiated based on the ‘topology’ treatment. Amongst ‘topology’ treatments, there is no significant difference in erosion between the treatments. In the subset of data excluding ‘topology’ treatment, a significant reduction in erosion for ‘IMaS’, ‘IMiS’ and ‘NMiS’ treatments is seen, compared to ‘NNN’ treatments. SAP application significantly ($p=2.14x10^{-2}$) reduced erosion, as opposed to.

Amongst the treatments, prominent crusting of the tailings sand was observed. This crusting ranged in strength, with some of the strongest crusts maintaining shape even after chlorophyll extraction procedure. Figure 18 is an image showing the different stages of crusting, with the most prominent on the left, weaker crusting in the middle and no crusting on the right. Samples with gametophyte development, as discussed above, also showed hard crusting, despite the absence of SAP application.

Prominent hardening of the crust was observed for treatments ‘IMaN’, ‘IMiN’, ‘IMiS’, ‘IMiT’, ‘INS’, ‘NMaT’, ‘NMiN’, ‘NMiT’, ‘INN’ and ‘NNS’. It was most frequent for NNS treatments, occurring in 5 of the 8 replicates. In the absence of the SAP, plots with crust formation can be inferred as having establish a biocrust.

4.2.2. Experiment 2: Effect of *Microcoleus vaginatus* cyanobacteria in aiding biocrust establishment.

During the in-lab cultivation of the cyanobacteria, no observable growth was noticed in the liquid BG-11 media. Propagation was successful on solid agar plates, as seen in Figure 7. Chlorophyll a content and erosion, were not significantly different for plots inoculated with biocrust and *M.vaginatus*, compared to plots only inoculated with biocrust. Results are tabulated in Appendix D.

4.2.3. Experiment 3: Effects of enriched substrate and shade on moss establishment on tailings sand.

The productivity of mosses, quantified by chlorophyll a and b, was compared for levels of shade and substrates. When compared to the control treatment ‘NC’, which had sand as media and no shade treatment, all treatments with organic media had a significantly higher chlorophyll b content. Addition of nutrients did not significantly increase productivity. Furthermore, use of both shade panels and psyllium husk, did not result in significantly different productivity. Figure 19 plots the observed differences as boxplots. The values from the chlorophyll analysis are tabulated in Appendix E.

For the plots, minor evidence of erosion could be seen around the edges of the plots with sand substrate. In contrast, plots with enhanced media, showed aggregate formation and did not show signs of erosion. Treatments with psyllium husk had successfully formed a cover on the plots, but also had sand grains stuck onto the surface.

5) Discussion

5.1. Characterization of biocrust on the mine tailings facilities.

The persistence of biocrust cover on the reclaimed tailings may be indicative of landscape conditions being harsher than the surrounding vegetated patches, allowing biocrusts to form and persist instead of the vegetation. This is notable because the sampled sites underwent deliberate revegetation effort during reclamation. Despite that, each of the sampled sites had significant biocrust cover. The time series of the samples in this study (see Figure 9), shows a transition from microbial → moss → lichen dominated biocrusts. Since this observation is not confirmed through sampling of the same sampling sites across time, an alternative variable may very likely exist; something which is hard to extrapolate with only 18 samples across varying sites. When comparing bryophytes to lichen, it is observed that lichen are more sensitive to prolonged desiccation stress (Lüttge et al. 2011). This could suggest that the biocrusts in this study were becoming increasing less desiccation stressed as time progressed. An experimental investigation by Lan et al. (2015) demonstrated that in biocrusts, moss recruitment depended on microsite moisture retention, whereas lichen recruitment was dependant on silt and cyanobacterial abundance in the biocrust. Further investigation into this will be required to ascertain whether the trend observed in this study is as witnessed, and if it is, what the underlying cause of this trend is at mine tailings.

Lichen community in the biocrust was dominated by *Cladonia* sp. Both morphological variants , club lichen and reindeer mosses, were observed. *Cladonia* lichen have been observed to dominate lichen composition on mine sites and slag dumps (Bačkor & Fahselt 2004; Osyczka & Rola 2013). For moss species, *Bryaceae* sp. were noted as the most common and frequent. However, they were dwarfed and at the time of sampling, lacked sporophytes that could aid in their identification. The arrangement of leaves, response to moisture and leaf morphology, allowed narrowing down the identification to the family, but a complete and confident identification was not possible. In-person comparisons were planned to narrow down the exact ID but were not carried out due to health advisory restricting travel and causing herbarium closure because of the covid-19 pandemic.

When comparing the total biomass obtained through the PLFA analysis, the sampled biocrust show a rich microbial density. Though the samples were restricted to a 0.5 cm depth to specifically sample for biocrusts, the samples showed microbial biomasses comparable to forests and organic soils. The measured microbial biomass was higher at the sampled mine sites than in soils from non-crusted semi-arid regions and, comparable to certain farms and forests studied in Argentina (Yao et al. 2000; Dominchin et al. 2019). A brief literature comparison to compare in this perspective, is documented in Table 8. It compares the microbial capacity of biocrusts measured in this study to organic soils in other ecosystems. As listed in this table, a study by Benson (2018) documented PLFA analysis based microbial biomasses in topsoil at reclaimed sites on Taseko | Gibraltar mines as being lower than those observed in the biocrust samples from the other mine sites in this study. However, the observed matrix of microbial and organic matter is bound to a thin layer on the surface, as opposed to the samples taken at deeper depths in the case of Benson (2018). In an evaluation similar to this study, Li et al. (2016) identified a significant difference in the microbial abundance between moss dominated biocrusts and, crusts dominated by cyanobacteria/algae or lichen. The observation of arbuscular mycorrhizal fungi in the samples supports the observations made in studies by Hawkes (2003) and Green et al. (2008), who demonstrated that arbuscular fungi formed associations between biocrusts and surrounding vegetation. Crenshaw et al. (2008) demonstrated nitrous oxide transfer through arbuscular mycorrhizal associations between grasses and microbial crusts. Hernández-Hernández et al. (2017) studied the make-up of arbuscular mycorrhizal fungi in a neotropical savanna. They suggested that biocrusts host arbuscular mycorrhizal communities which are associated with bare ground and more closely related to those found with annual plant species. Aanderud et al. (2017) noticed a lack of N movement in moss biocrusts, while Zhuang et al. (2015) documented N^{15} movement between plants and biocrusts in the Gurbantunggut Desert.

In the present study, sites dominated with lichen had the lowest abundance of arbuscular fungi. Since biocrusts were sampled up to a shallow depth of 0.5 cm, presence of arbuscular mycorrhizae fungi in these samples is a component of biocrust community at the mine sites. One sample to note, was sample 20, where the presence of arbuscular mycorrhizae was not observed. This is the sample which is concluded to have undergone frequent inundation.

Overall, moss dominated and microbial biocrusts have higher microbial and arbuscular mycorrhizae presence. This makes them a potentially desirable form of biocrust for soil reclamation purposes, especially where deliberate inoculation is conducted. The comparison of biocrust cover types to the calculated sample age (see Figure 9), suggests that biocrust composition on mine tailings succeeds across time, is in the order; microbial biocrust, moss dominated biocrust, lichen dominated biocrust. Cyanobacteria inoculum has shown to establish in an order of weeks from establishment (Sorochkina et al. 2018; Giraldo-Silva 2019). Giraldo-Silva (2019) developed nursing approaches for cultivating filamentous cyanobacteria that can be scaled up. Using an experimental approach similar to this study, Bu et al. (2018) showed successful cultivation of moss biocrust within two months. Lichen components of a biocrust, however, will take multiple growing seasons to establish (Duncan 2014; Roturier et al. 2017; Rapai et al. 2018). These factors weigh in on the choice of morphology and inoculum used for biocrust establishment.

5.2. Carbon and nutrient contribution to substrate.

Li et al. (2016) noted a higher carbon content in crusts that were more mature. Comparison with literature shows that the biocrusts measured at the sampled mine sites had higher carbon content than mineral soils, but much lower carbon content than organic soils. However, the nitrogen content in the sampled biocrusts is on-par with organic soils. This comparison is presented in Table 9.

In this study, the microbial biomass was higher in moss dominated and microbial biocrusts, when compared against lichen dominated biocrusts. Microbial content is highest for sites 1 and 2 (refer Appendix B); which have different cover types but are on the same medium i.e. overburden. Carbon content in this study, did not show any significant trends with the biocrust except, a significant positive correlation with the total microbial biomass (see Figure 13). In comparison, Li et al. (2016) obtained samples from a single 150 x 400 m patch in the Gurbantünggüt desert. This provides them with a more site-specific microbiology and more undisturbed biocrust samples, compared to this study. In the work by Li et al. (2016) a general trend of decreasing bacterial biomasses is

seen with age when excluding fungal biomass. Removal of fungal biomass from the total microbial biomass from the samples in this study did not show this trend.

The sites in this study also showed a total percent carbon higher than those usually observed in the literature (Li et al 2016; Muñoz-Rojas 2018;). The total C/N ratios were consistent with those seen in biocrusts by Huang et al. (2011) at a copper mine tailings, but higher than those recorded by Zhang et al. (2015) in the Gurbantünggüt desert. In this study, total carbon and nitrogen contents were strongly correlated and, the microbial biomass calculated through the PLFA analysis, showed a strong correlation with percent C in the substrate (see section 4.1.4). When comparing the microbial biomass to the calculated age, a succession is seen in the cover type and the data plotted in Figure 10 suggests decreased microbial biomass as the crust becomes older (see section 4.1.3). Cumulatively, it would imply that with age, the biocrusts would lose the productivity associated with the microbial community and in turn, have lower carbon content in them. However, no significant correlation was seen between the carbon content and the calculated sample age or cover type of the biocrusts sampled.

Analysis of biocrust carbon using respiration budget measurements against environmental variables, have shown that biocrusts become carbon sinks only when a threshold of water availability is crossed, while increased desiccation stress also results in a reduced microbial biomass (Housman et al. 2006; Darrouzet-Nardi et al. 2015; Maestre et al. 2015). Housman et al. (2006) demonstrated that more mature biocrusts were more productive and produced more complex organic compounds. This maturity is determined by succession of biocrust cover. In this study, higher microbial biomass was seen in moss dominated biocrusts than lichen dominated biocrusts. Zhang et al. (2015) however, showed that more developed biocrusts, especially lichen dominated biocrusts, had the highest microbial biomass, contradicting this study. The disparity in trends may exist due to higher sample heterogeneity in this study. However, it can also be possible that an independent environmental factor is determining biocrust maturity and microbial biomasses separately. In this study, overburden sites had the highest microbial biomass, while site 13 and 20 were outliers to the correlation between age and total microbial biomass (refer to Figure 10). Site 13 had significantly higher biomass for its calculated age, while site 20 had significantly lower microbial biomass for its calculated age. An underlying sediment-based property may be contributing to the microbial biomasses, with some confounding effect from successional stages of the biocrust itself. It must be

noted that the comparison between microbial biomass and age had a negative autocorrelation. The author does not currently have an explanation or hypothesis for this trend.

Yu et al (2015) studied changes in soil nutrient content after establishment of biocrust around vegetation in the Horqin Sandy Land. They found accumulation of Mg, P and K, similar to this study. However, they also found significantly higher values of Fe and Cu, which is not observed in this study. Their crust samples ranged from 0-1 cm as opposed to the 0-0.5 cm depth in this study. This may prevent them from noticing the effect from leaching. This study notes a correlation between fungal biomass and concentration of P in the crust. Increase of P in biocrusts was also seen by Wu et al. (2013). Bowker et al. (2005) concluded Mn and Zn as limiting nutrients in biocrusts. The correlation with pH observed in this study may suggests that biocrusts with higher pH have higher Al concentrations in them. No reports on Al sequestration by biocrusts were found in the literature. The results in this study are not in complete agreement with Beraldi- Campesi (2009), who suggested a decrease in all nutrients in biocrusts, except C and N. In this study, Ca was not reported as significant for all three sites, but it was significantly higher in the crust for Endako and Gaspé mines. In this study, none of the nutrient concentrations or ratios, correlated to the biomass of any biocrust community member or abiotic parameter. Only Ca concentrations had a significant correlation with age ($p=2.26x10^{-2}$). Further research may be required to ascertain the cause behind these nutrient trends. This study also demonstrated a significant decrease in pH of biocrusts with respect to the subsurface, with a weak but significant, negative correlation between calculated sample age and pH of biocrust. Li et al. (2003) noticed a net increase in biocrust pH with site age, however, in this study the pH was lower in biocrusts, compared to reference samples, and negatively correlated with age.

5.3. Biocrust community establishment experiments on the mine tailings substrate through addition of inoculum and amendments.

5.3.1. Experiment 1: Effects of biocrust inoculation, nutrients and physical amendments on productivity of biocrusts on tailings crust.

In experiment 1, the use of Super Absorbent Polymer (SAP) resulted in a more effective erosion control than all other treatments and levels. It also provided physical hardening of the crust. However, plots without SAP application also showed hardening, with green pigmentation visible when hydrated. This can be inferred as biocrust formation. This biocrust formation was significantly more frequent in plots with nutrient addition to them. Plots without added inoculum and those with the 'topology' treatment, are of particular interest in this group. Due to strong erosion on topology plots and the unaided biocrust formation on uninoculated plots, the seeding of the biocrust in these plots is a natural and non-point phenomena. This suggests that the environment and air around the plots possessed a type of biocrust community member that could deposit and lead to biocrust formation, that hardens and controls the substrate on par with the SAP treatments. Though, it must also be noted that SAP application on landscapes cannot repair itself to physical damage, making biocrusts a more desirable form of substrate control in certain reclamation situations.

The results from this experiment suggest that inoculations and addition of macro-fertilizers (N and P) can increase biocrust productivity. This aligns with prior biocrust recruitment experiments. Velasco Ayuso et al. (2017), used a 5% inoculum by surface area, to successfully establish a biocrust. Sinsabaugh et al. (2015), showed increase in biocrust 'chlorophyll a' productivity by the addition of a nitrate-ammonia mix. Antoninka et al. (2016) showed enhanced biocrust moss succession after addition of nitrogen fertilizer.

The relatively higher production in the individual treatment 'IMiT' (inoculated, micro- and macro- nutrients added, topology feature created for partial shading) suggests that once these treatments are provided, micro-topology may further assist in increasing productivity. Partial shade from micro-topology is seen to act as a refuge for biocrust members in natural landscapes (Csotonyi & Addicott 2004; Wu et al. 2020). It is

possible that the success of the ‘topology’ treatment was hindered to some extent by the increased erosion impeding biocrust establishment. However, the features were created to supply partial shade against desiccation stress and having demonstrated a positive effect, additional or different means may be used to provide partial shade. Maestre et al. (2006) demonstrated how inoculation, watering and fertilization, were all able to enhance biocrust recovery and functioning.

5.3.2. Experiment 2: Effect of *Microcoleus vaginatus* cyanobacteria in aiding biocrust establishment.

Experiment 2 did not show the expected result of enhancing the establishment of biocrust due to the *M.vaginatus* inoculum not successfully propagating in the biocrust, when compared to previously demonstrated experiments in the literature (Antoninka et al 2016; Williams et al. 2017; Muñoz-Rojas et al. 2018). During the cultivation for this experiment, *M.vaginatus* could not be cultured in a liquid media, and instead had to be cultured on solid agar. This corresponds to observations by Giraldo-Silva et al. (2019), who also suggested growth on microbial cellulose and to apply more rigorous hardening procedures to the cultured inoculum before application in the field. This, as demonstrated by Velasco Ayuso et al. (2017) and Muñoz-Rojas et al. (2018), suggests that success from cyanobacterial inoculation will yield more favourable establishment results if native cyanobacteria are cultured to make the inoculum. The successful crusting of samples of Experiment 2 discussed in section 4.2.2. provides insight to this potential. The chlorophyll analysis suggests that the plots with the addition of the cyanobacteria to the biocrust inoculum did not perform better than plots with solely biocrust inoculum. The reference sample for the evaluation of dose of the cyanobacteria inoculum used in this experiment was misplaced in the field, depriving this experiment of the dose of the inoculum used in this experiment. In review of the methods by Giraldo-Silva et al. (2019) and the observed results, it is likely that the culturing methodology used in this study was inadequate. However, in the absence of the reference sample, the adequacy of the dose cannot be verified either. For this reason, the results from the experiment are not discussed further.

5.3.3. Experiment 3: Effects of enriched substrate and shade on moss establishment on tailings sand.

Experiment 3 showed that enriching the substrate with wood flour and SAP resulted in better establishment of moss measured by the amount of chlorophyll a and b, irrespective of nutrient addition (Figure 19). The enhanced media was expected to hold more water and was more favourable to the productivity of the mosses, as opposed to being based only in the tailings sand. Addition of nutrients, including nitrogen, did not show any significant increase in productivity, as was seen by Antoninka et al. (2016). It would be recommended to further replicate this experiment for a longer duration, in the field, to verify that the effects from the addition of the enhanced media persist for complete moss establishment, including the complete development of gametophytes. The use of enhanced media as a treatment may create avenues for supplementing lichen growth. Such growth of lichen was observed at Gaspé mines where, as seen in Figure 21, where extensive patches of lichen were seen growing over a layer of detritus.

Reclaimed parts of the tailings at Gibraltar mine itself had moss cover establishing between hydroseeded grasses. Fire moss (*Ceratadon purpureus*) cover formed between individual grasses. Figure 20 is a photo demonstrating this growth. However, the mosses used as inoculum in Experiment 3, were not comprised of Fire moss or mosses commonly inventoried from sampled biocrusts. Switching the inoculum to these mosses that naturally occur in dryland biocrusts, may allow more pronounced results.

6) Conclusions and Recommendations

6.1. Character of naturally established biocrust communities at designated mine tailing facilities according to their microbial communities, bryophyte and lichen species composition.

Biocrust morphology showed correlation to its microbial composition. The total microbial biomasses and arbuscular mycorrhizal biomass found on the reclaimed tailings sand of the three characterized mine sites, show moss dominated biocrusts as a better alternative to lichen dominated biocrusts. For establishment, filamentous biocrust algae or cyanobacteria and mosses should be used in the inoculum to confer quicker biocrust establishment, as well as provide more ecological functionality to soil crust. As the biocrust samples aged, the introduction and increase in lichen cover was observed, even if it was not the most ecologically functional biocrust cover.

Biocrusts are important for stabilizing substrate and nutrient cycling on desiccation stressed landscapes. This study quantified the soil building capacity through quantifying productivity of the microbial communities and biogenic nutrients contributions. Comparisons of results obtained in this research and values in the literature (see section 5.2) highlight the contribution from microbial activity in the biocrust. While the biocrust accumulates biogenic nutrients, with particular richness of N, the C content is much lower than in organic topsoil. Other functional attributes have also been observed being imparted by biocrusts to soils. Biocrusts overall can reduce drainage but can also increase water retention through moss components (Yair et al 2011; Gypser et al. 2016). Microbial biocrusts have shown to increase soil surface temperatures, but biocrusts also buffer against temperature changes (Couradeau et al. 2016; Guan et al. 2019). Application of biocrusts for soil building should be done with these factors in consideration.

When selecting species for biocrust inoculation at a site, it will be ideal to use those found in the local dryland biocrusts due to their climatic adaption and native occurrence. For mosses, these include *Bryaceae* spp., *Polytrichum* spp and *Ceratadon purpureus* mosses found in the region. *Cladonia* spp. and *Lecanoromycetes* spp. lichen specimen in the region will be appropriate for lichen translating or fragment inoculation.

The moss *Ceratadon Purpureus* was found very consistently across all four sites in this study and may be considered a candidate as a common biocrust moss for Canadian landscapes. Similarly, lichen *Cladonia cariosa* was observed on all sites, except Gibraltar | Taseko mines and may also be a common biocrust component across Canadian landscapes. Inoculation preparation with such species can be beneficial for sites like Taseko | Gibraltar mines which may have minimal or complete lack of dryland biocrusts, resulting in the need for off-site sourcing of inoculum and propagation prior to application.

Revegetation activities can help modify some of the biocrust effects. As discussed in section 4.1, perennial grasses were observed within biocrust patches and are likely to be in symbiosis with the biocrust through the mycorrhizae therein. Furthermore, the insulating and moisture retaining properties of biocrusts are likely to assist these grasses as well. Creating breaks in established biocrust patches, through activities like drill-seeding, will replicate this natural arrangement and provide avenues for increased infiltration amongst biocrust patches. However, biocrusts are also sensitive to physical disturbance and hence, revegetation activities after the establishment of biocrust, will need to consider any potential damage to biocrust development. Plants, as discussed in the fungal loop hypothesis, can increase the carbon content in the soil crust, while the biocrust assists in their survival. The resultant plant litter should also assist in increasing the annual layering of the soil and increased moisture retaining organic content. Overall, vegetating biocrust patches with local perennial grasses has the potential to assist both communities, while improving and speeding up the soil building at the site.

6.2. Promoting biocrust community establishment on the mine tailings substrate through addition of biocrust inoculum and amendments.

Experiment 1 suggests that deliberate inoculation, N and P addition and, micro-topology can individually, and cumulatively, enhance biocrust productivity and establishment. Naeth et al. (2018) also showed micro-topology contributing to vegetation establishment, making it a treatment beneficial to both plants and biocrusts. The SAP, Potassium Polyacrylate, did not contribute to any positive effect in experiment 2, but did not prevent chlorophyll activity in experiment 3. It is suspected that the polymer may be

filling gaps in the sand substrate and preventing filamentous organisms from occupying them. There may be an effective threshold for the use of such polymers that reduces their effectiveness at higher concentrations. Use of such polymers may also act as a barrier to natural biocrust generation. More research would be needed to discern the replicability and cause of this phenomena. Overall, this experiment shed light on the core components in amendments that assist biocrust productivity, but the diminished response of the biocrust in this treatment suggests a lack of appropriate biocrust inoculum. Future use of the treatments in this study should consider a stricter sourcing of inoculum to match the application landscape. This, as noted before, is complementary to the ability to culture adequate inoculum.

From experiment 2, it can be concluded that controlled propagation of *M.vagninatus* cyanobacteria is successful in solid agar instead of liquid medium. However, the results of inoculation with cyanobacteria in the field were likely due to insufficient training of the cyanobacteria. Using biocrusts as a treatment, will inevitably require the creation of specialized inoculum. Biocrusts are ecological communities that undergo succession. The abundance of the pioneering biocrust community members drops as it matures. Further research in the selective isolation, propagation and conditioning of algal or cyanobacterial pioneering communities will allow hastening biocrust establishment and using them as a means of soil stabilization.

For experiment 3, the enhanced media showed strong hospitability to mosses, especially in the filamentous stage of their development. The organic matrix and water retention property can be essential to ameliorate conditions in disturbed or barren soils. However, subsequent experiments should be run for a longer duration to see if the media promotes gametophyte development for mosses, and to what measure the enhanced media can be diluted in its application. Testing the ability to assist seed germination in the field using this media, will also allow exploring the avenue of using grasses and biocrusts in conjunction. Specifically, moss species identified in biocrusts in this report should also be used instead of the ones used in experiment 3. However, presence of harvestable patches of mosses is likely to be a limiting factor and hence, moss culturing may be required before any field application of such experiments.

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8) Tables

Table 1: Treatment matrix for Experiment 1.

INOCULUM	CHEMICAL	PHYSICAL		
		Super Absorbant Polymer (S)	Topology (T)	Blank (N)
Inoculated (I)	Macro- N. (Ma)	IMaS	IMaT	IMaN
	Macro+ Micro- N. (Mi)	IMiS	IMiT	IMiN
	Blank (N)	INS	INT	INN
Not Inoculated (N)	Macro- N. (Ma)	NMaS	NMaT	NMaN
	Macro+ Micro- N. (Mi)	NMiS	NMiT	NMiN
	Blank (N)	NNS	NNT	NNN

Code format: Inoculum- Chemical amendment- Physical amendment.

Table 2: Nutrient doses used in experiment 1.

Salt	Element	Weight of element per plot (250 sq.cm) (g)
Macronutrients Only		
Ammonium Phosphate	Nitrogen	0.234
	Phosphorous	0.517
Macronutrients+ Micronutrients		
Ammonium Phosphate	Nitrogen	0.234
	Phosphorous	0.517
Boric Acid	Boron	0*
Ferric Chloride HexaHydrate	Ferric	0.006
	Chlorine	0.012
Sodium Molybdate Dihydrate	Sodium	0.002
	Molybdenum	0.004
Magnesium Chloride Hexahydrate	Magnesium	0.012
	Chlorine	0.036
Zinc Chloride	Zinc	0.001
	Chlorine	0.001
Manganese Sulphate Monohydrate	Manganese	0.001
	Sulphur	0*

** Values less than 10^{-4} grams.*

Table 3: Treatment matrix for Experiment 3.

SHADE	SUBSTRATE		
	Sand (C)	Organic Media (M)	Organic + Nutrient Media (M+)
No- Shade (N)	NC	NM	NM+
Shade Panels (S)	SC	SM	SM+
Psyllium Husk (P)	PC	PM	PM+
Code format: Substrate- Shade.			

Table 4: Summary of Observational Data and Site Histories.

Mine	Plot ID	Sampling Area	Feature	Visibly Dominant Biocrust Cover Type	Aspect (slope>10 degrees)	Calculated Age (years)	Fertilizer Upkeep
Endako	1	A	Overburden	Moss	N	8	F
	2	A	Overburden	Dark Crust	E	6	F
	3	B	Plantation	Lichen	F	29	F
	4	B	Plantation	Lichen		29	F
	5	C	Sand bench	Moss and Lichen	W	24	F
	6	C	Sand bench	Moss and Lichen	E	24	F
	7	D	Sand bench	Dark Crust	SE	12	F
	8	D	Sand bench	Dark Crust	SE	12	F
Brenda	9	E	Dam slope	Moss		24	T
	10	E	Dam slope	Lichen	NE	23	T
	11	F	plain	Moss		21	T
	12	F	plain	Moss		20	T
	13	G	plain	Moss		19	T
	14	G	plain	Moss		18	T
Gaspé	15	H	plain	Lichen		31	T
	16	H	plain	Lichen		30	T
	17	I	plain	Moss		29	T
	18	J	pool edge	Dark Crust		15	T
	19	K	Dam slope	Lichen	E	26	T
	20	L	pool edge	Moss		1	T

Table 5: Unique moss and lichen specimen observed in biocrusts across all sampling points.

Type	Species	Frequency
Moss	Bryaceae (Family)	13
	Ceratodon purpureus	8
	Polytrichum (Genus)	3
	Polytrichum juniperinum	2
	Polytrichum piliferum	2
	Tortula norvegica	2
	Unknown	3
Lichen	Lecanoromycetes (Family)	12
	Cladonia cariosa	10
	Cladonia (Genus)	9
	Cladonia gracilis	6
	Cladonia cristatella	4
	Lapraria neglecta	3
	Unknown	3
	Cladonia cyanipes	2
	Stereocaulaceae (Family)	2
	Cladonia Stricta	2
	Cladonia fimbriata	2
	Cladonia uncialis	1
	Lapraria neglecta	3

Table 6: Derived nutrient concentration [biocrust layer (0- 0.5 cm depth)-reference layer (0.5-2 cm depth)].

ID	AL (MG/KG)	CA (MG/KG)	CU (MG/KG)	FE (MG/KG)	K (MG/KG)	MG (MG/KG)	MN (MG/KG)	NA (MG/KG)	P (MG/KG)	S (MG/KG)	ZN (MG/KG)
1	80	200	-0.6	-40	110	40	-10	0	40	14	-1
2	30	1230	26.8	30	220	86	11	0	74	33.1	3.7
3	80	700	-4.3	-330	133	70	-59	0	81	18.1	3.3
4	-220	410	0.3	-50	63	90	52	0	-31	0	0.6
5	140	800	-0.2	-70	167	149	20	0	61	23.1	3.8
6	60	1000	-0.3	-130	105	60	30	0	0	96	0.2
7	60	1900	-0.4	-220	336	110	30	0	84	37	2.6
8	30	1100	-1.3	-150	160	70	20	0	43	38	3
9	60	100	-12.7	10	458	78	8	0	210	51.1	12
10	234	-600	-14	250	165	84	11	0	220	16.1	30.3
11	0	-300	-15	-120	85	10	7	0	-7	7.1	-6
13	73	1120	16	-90	278	156	30	0	115	29.1	5
14	114	260	-8	110	92	75	-9	0	67	7.1	0
15	198	-1500	-14	-110	110.1	15	3	5.1	70	17.1	-1
16	80	-1600	-490	-170	68.1	14	-3	1.1	-12	25	-27
17	980	850	33	-130	130.1	37	-7	-36	101	230	14
18	-220	3400	-62	-160	-66.1	-21	2	-5.1	-25	-186	-32
19	347	-1800	5	80	210.1	60	-10	-4.1	50	14	4

Table 7: Inter-nutrient concentration correlations in biocrusts (0-0.5 cm).

Correlations			Sample Size (n)	P- value (critical =0.05)	Correlation Value
Magnesium	-	Manganese	17	$3.074x10^{-5}$	0.8202
	-	Zinc	18	$8.28x10^{-4}$	-0.5867
Manganese	-	Zinc	18	$2.732x10^{-3}$	-0.6478
Phosphorous	-	Potassium	16	$5.265x10^{-2}$	0.4774
	-	Zinc	17	$1.071x10^{-3}$	0.705545

Table 8: Comparison of microbial biomasses of soil surfaces in literature.

Data Source	Location	Community	Depth (cm)	Units of measure	Total PLFA biomass (Mean ± Standard Error)	Microbial Biomass	Method of Extraction
***	Canada (BC)	Biocrust	0-0.5	µg. g^{-1} soil	28.078 ± 2.838		PLFA
Maharjan et al. 2017	Nepal	Forest	0-10	µg C. g^{-1} soil		220	Chloroform fumigation-extraction
		Farm				200	
		Organic				350	
Dominchin et al. 2019	Argentina	Native Forest	0-20	n.mol.% g^{-1} soil		62	PLFA
		Stabilized Agriculture				60	
Yao et al. 2000	China (Longyou province)	Non-cultivated soils	0-20	µg.g^{-1}	16.549 ± 4.288		PLFA
Benson 2018	Taseko | Gibraltar mines, Canada (BC)	Reclaimed top soil (10- 20 yrs)	0-10	ng/g	2243.4± 471.3		PLFA
		Reclaimed top soil (20- 30 yrs)			2355.1± 714.2		
		Reclaimed top soil (30- 40 yrs)			4602.6± 767.3		
		Reference			6332.9± 1383.4		

Table 9: Comparison of topsoil carbon contents across some literature.

Data Source	Location	Ecosystem	Depth	Units	Carbon	Nitrogen
**		Biocrust	0-0.5 cm	mean percentage	1.9- 5.1	0.079-0.49
Laganière 2013	Quebec	Jackpine	0-15 cm	mg/g	200-400	
		Mixedwood	0-15 cm	mg/g	100-200	
Johnson and Wensin 1997	Costa Rica	Forest		mean percentage	5.23	0.43
		Edge		mean percentage	4.09	0.33
		Grass		mean percentage	4.26	0.31
Smith et al. 2000	Quebec	Mineral Soil	0-10 cm	mean percentage	1.56-2.26	0.05-0.08
		Organic Soil	8 cm	mean percentage	39.2	1

9) Figures

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Figure 1: Cleared strip on Gibraltar mines' tailings, with experimental plots setup.

Figure 2: Location of experimental strip on the tailings of Gibraltar mines and sites from where biocrust was salvaged for inoculation purposes.

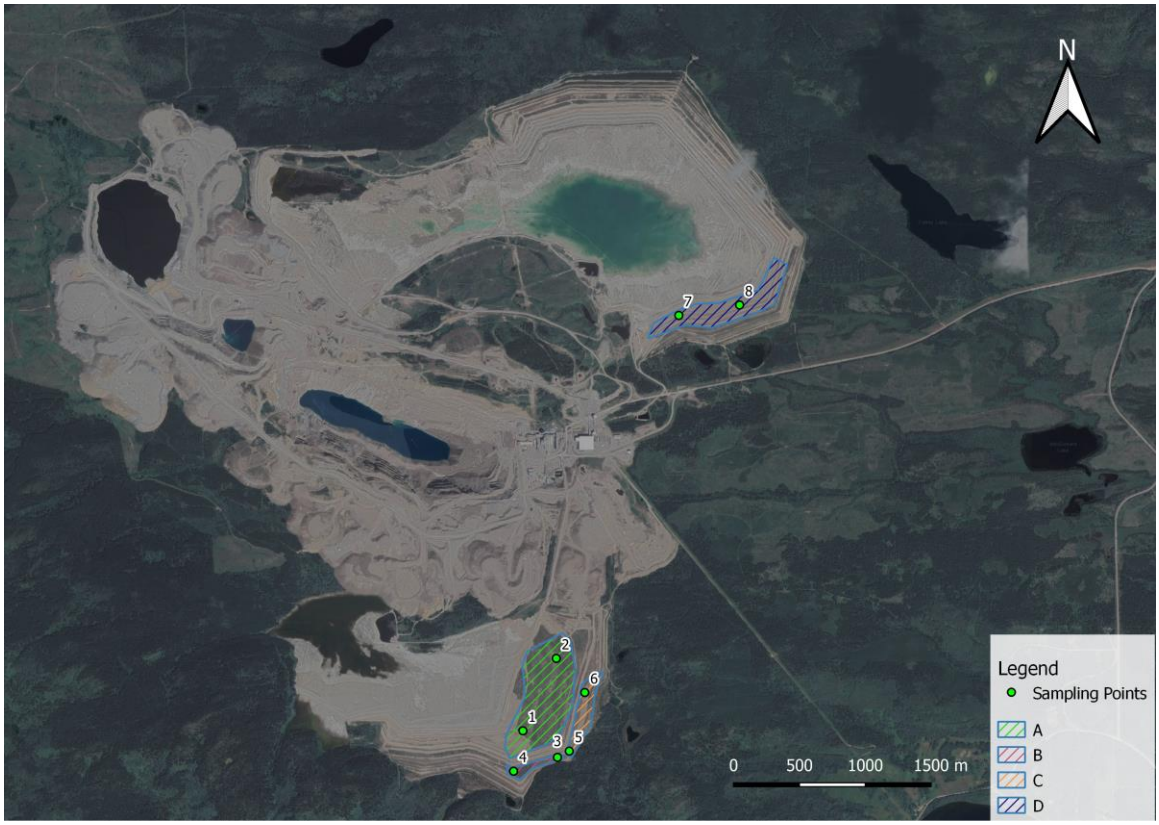

Figure 3: Areas sampled and sampling points at Endako mine

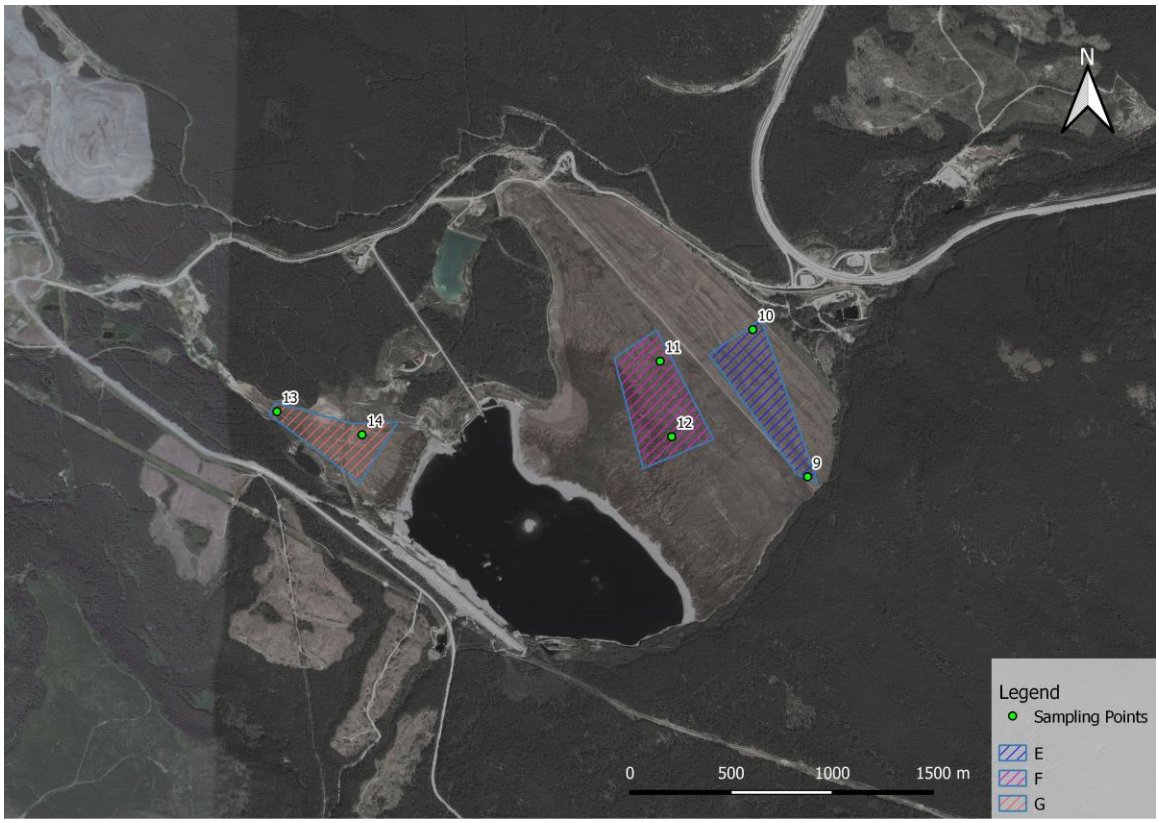

Figure 4: Areas sampled and sampling points at Brenda mines.

Figure 5: Areas sampled and sampling points at Gaspé mines.

Figure 1: Image of a ‘topology’ and ‘inoculated’ treatment right after construction, with an erosion pin emerging from the center of the structure.

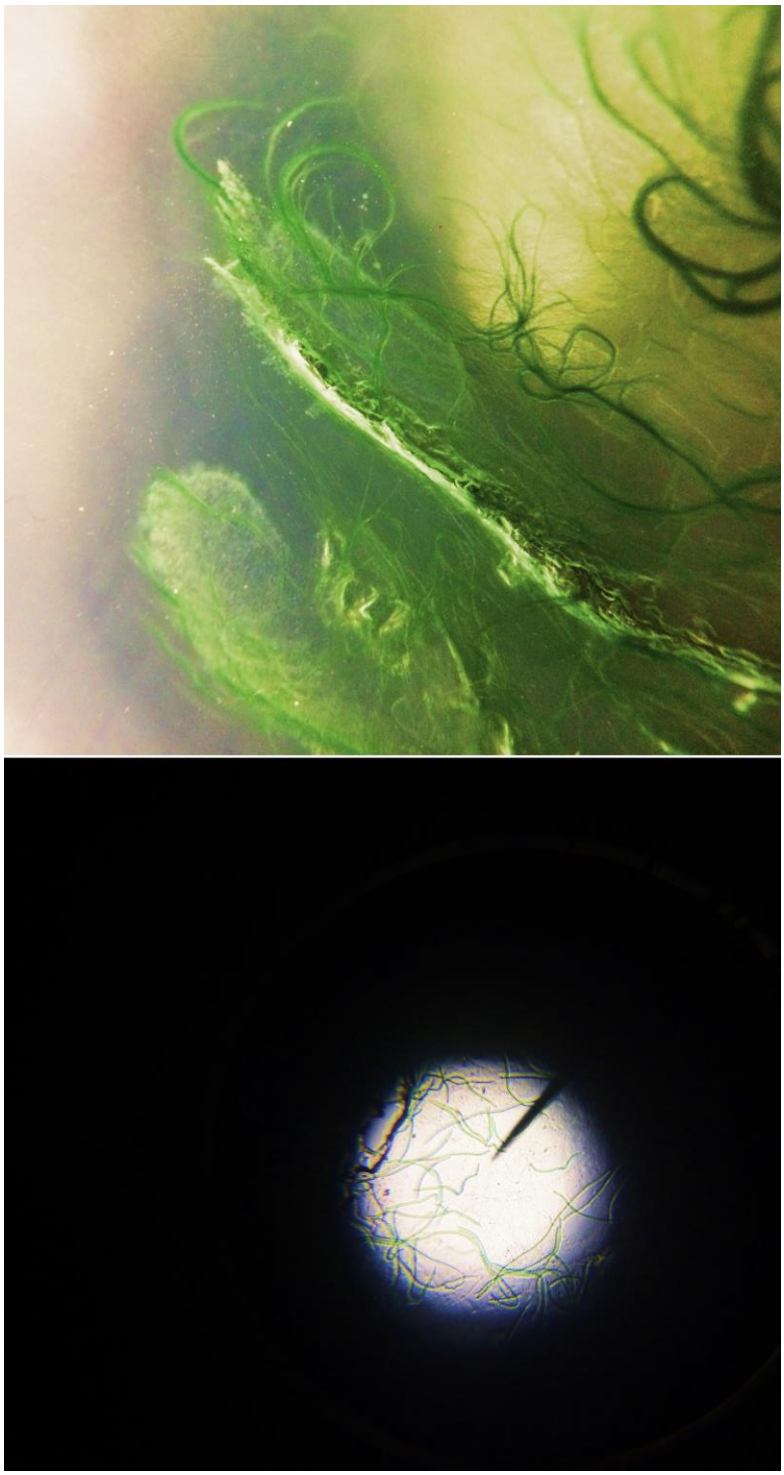

Figure 2: (Left) Microscopic view of filaments of M. vaginatus suspended in a water drop. (Right) Culture of M. vaginatus from plating streak made in solid agar BG-11 medium.

Figure 3: Moth using biocrust morphology as refugia.

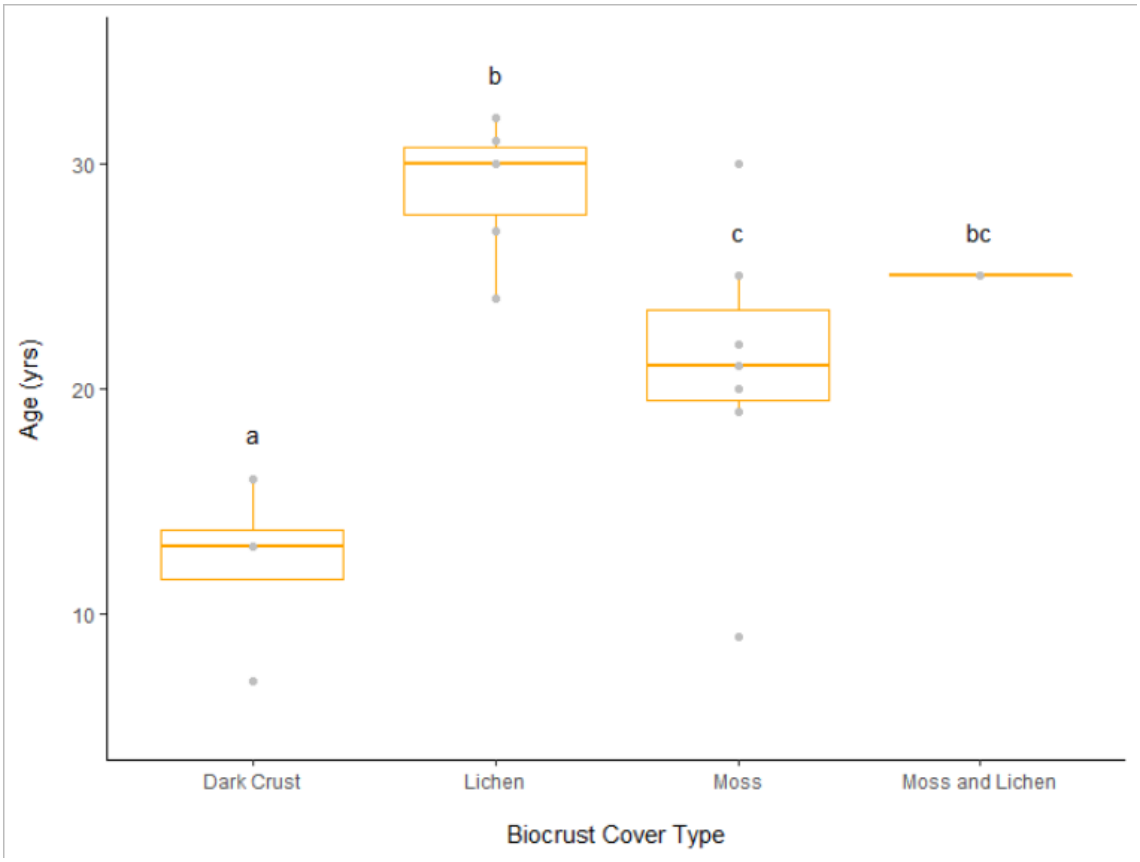

Figure 4: Boxplots on effect of age on biocrust cover types. Significance is tested through t-pairwise t-tests. Difference in letters indicates significant difference. Sample size is 4 for 'Dark Crust', 6 for 'Lichen', 7 for 'Moss' and 2 for 'Moss and Lichen'.

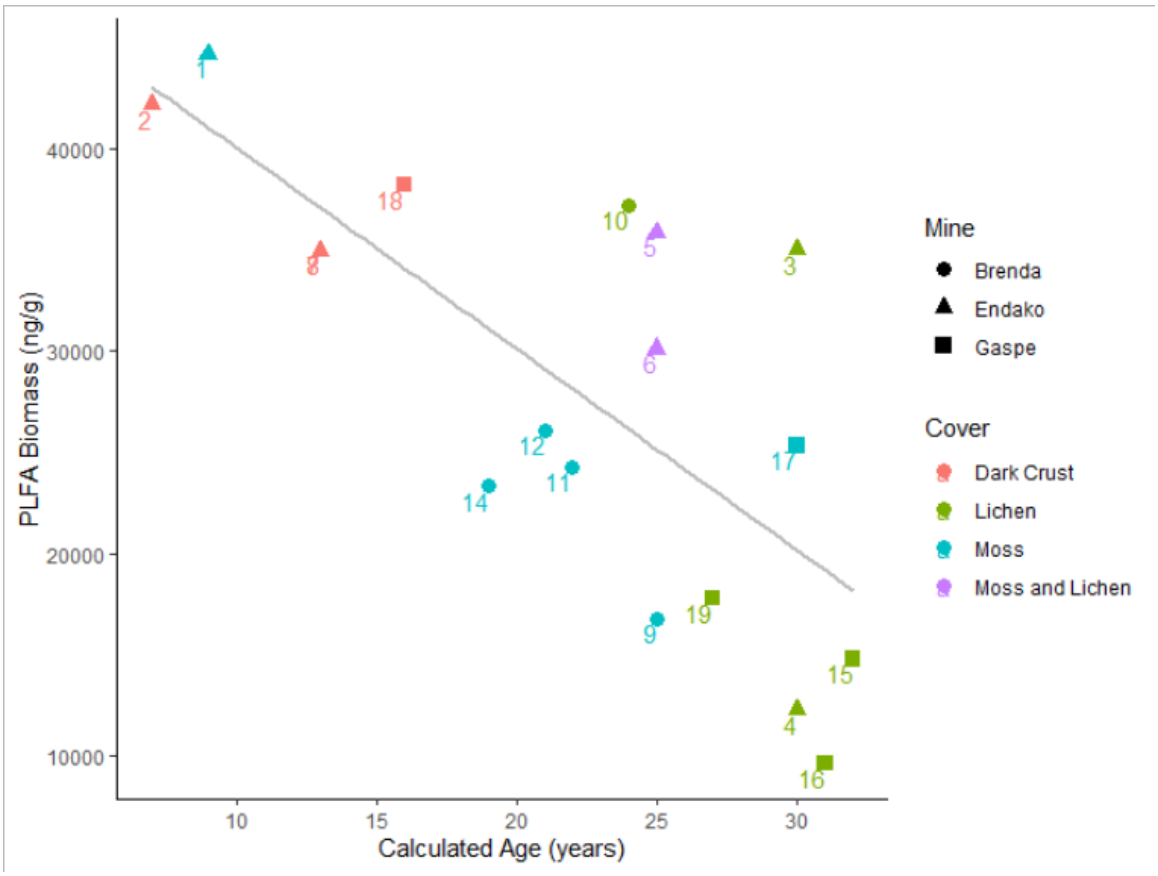

Figure 5: Negative regression between age of samples and total PLFA biomass recorded (p=6x10-4, n=18). Regression is divided by sites and, labeled by sample number and type of cover. Samples 20 and 13 were outliers.

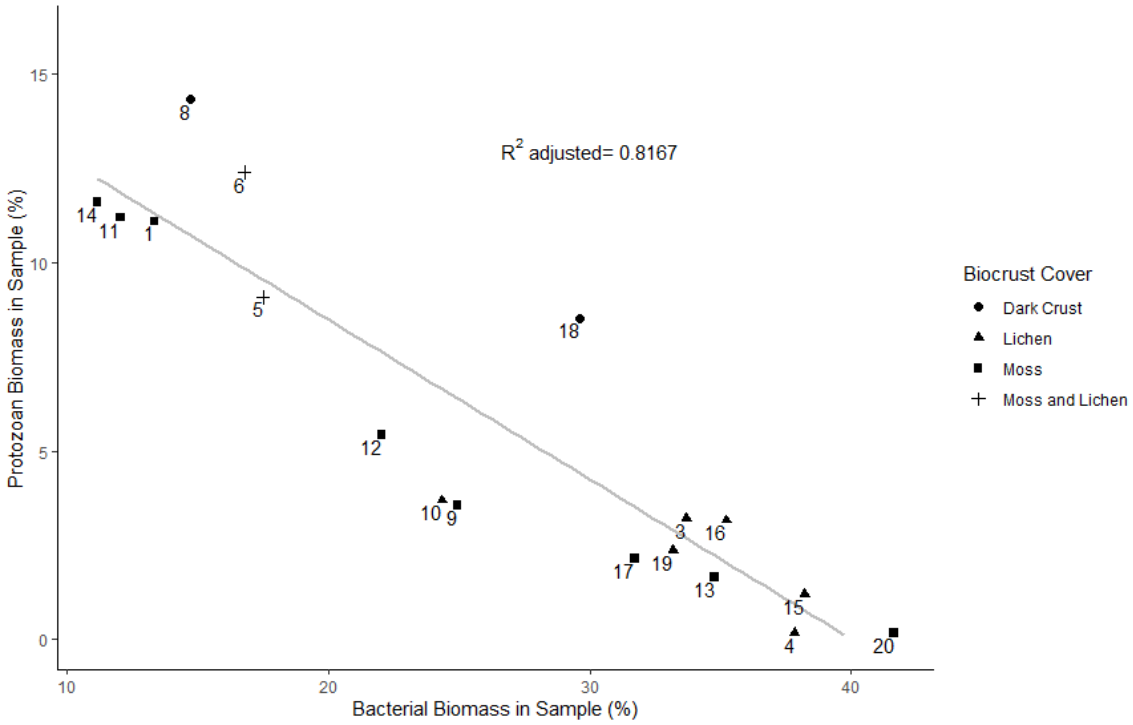

Figure 6: A negative linear correlation, (p=1.65x10-7) is seen between the percent bacterial biomass and protozoan biomass. Points 2 and 7 are excluded as outliers, but correlation remains significant (p=6.7x10-4,n=20), even when included.

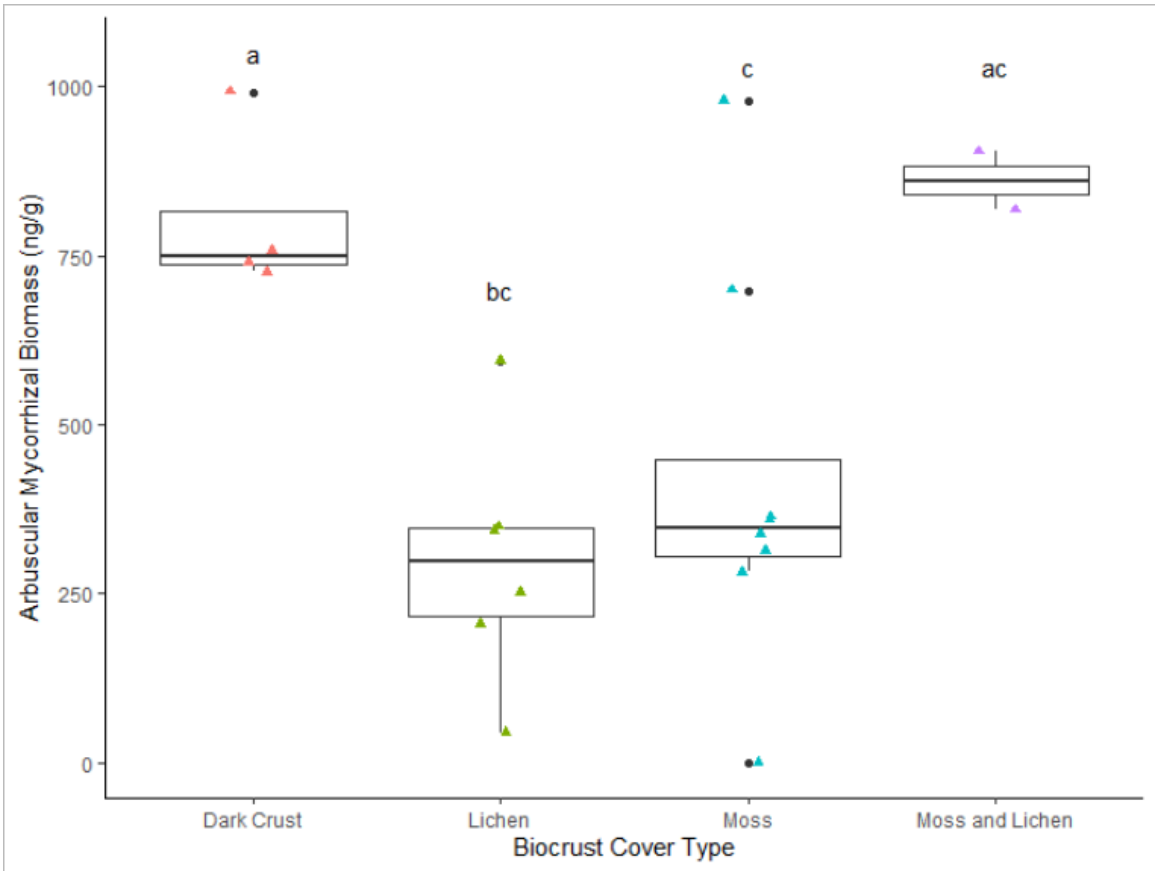

Figure 7: Distribution of arbuscular mycorrhizal biomass across biocrust cover type. Significance denoted by difference in letters.

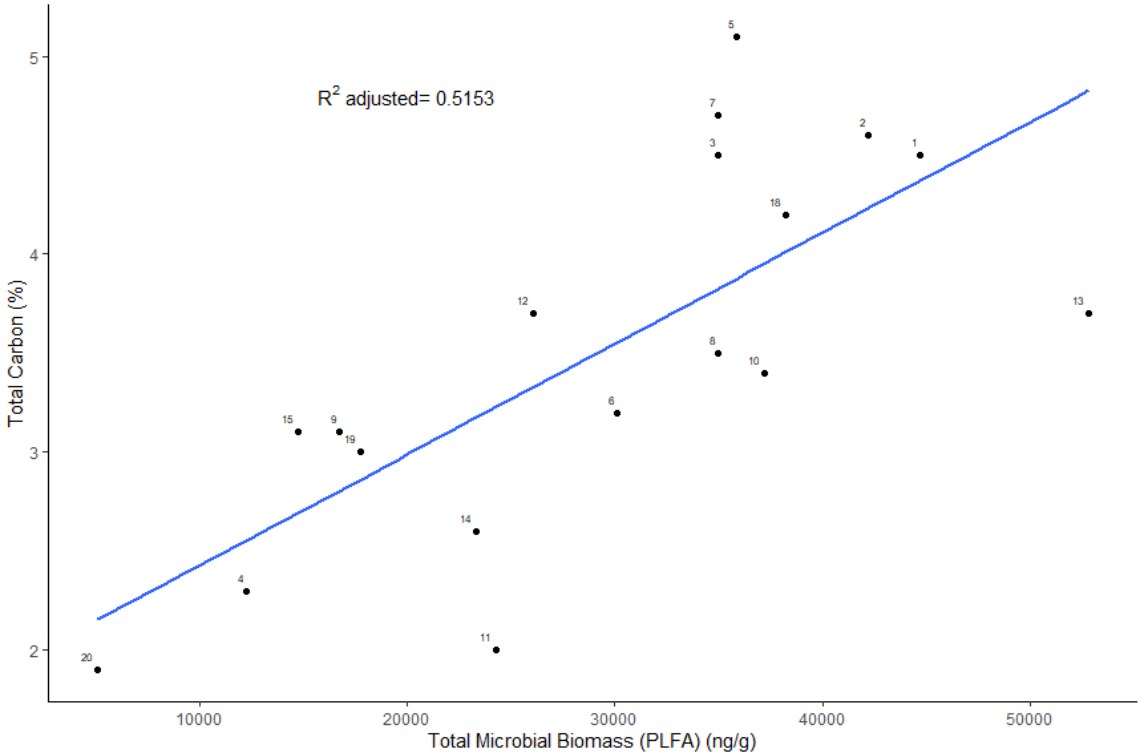

Figure 8: Plot of the linear relationship between total microbial biomass and percent total carbon (0.000479, df=17). Points 16 and 17 were outliers with much higher values of percent C.

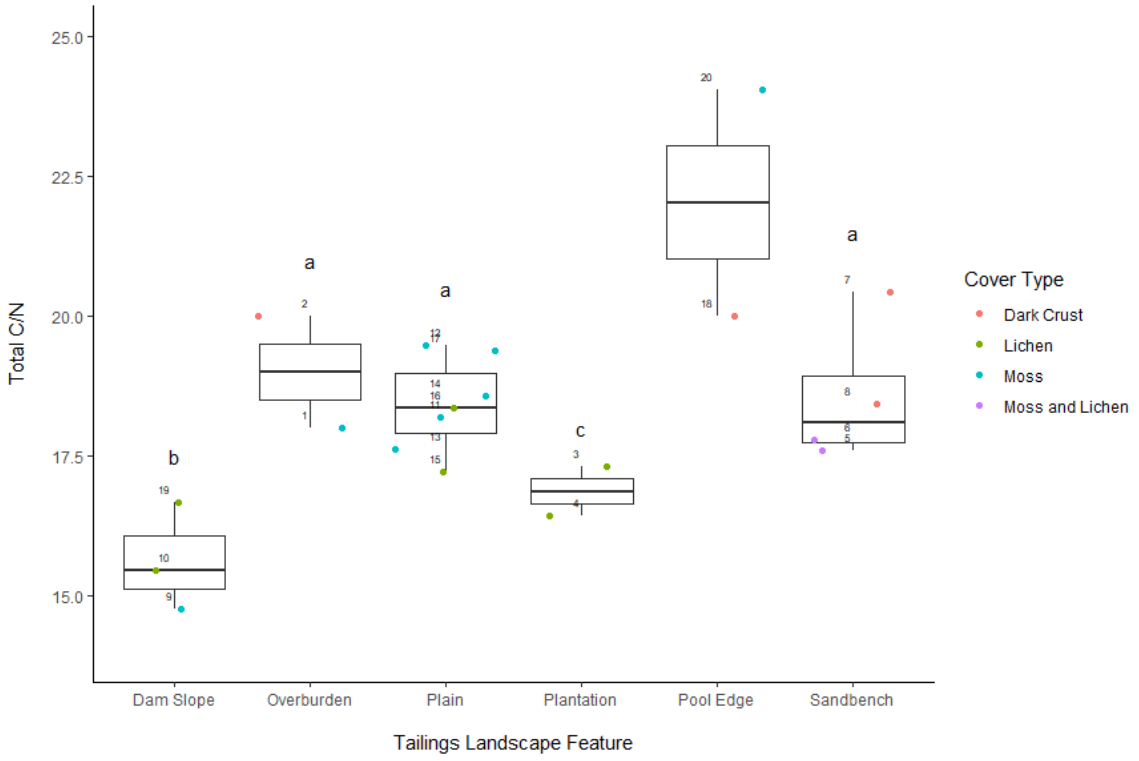

Figure 9: Boxplot demonstrating difference in total C/N in biocrusts between tailings features. Points are colored by biocrust cover type. Difference in letters denotes significant difference. Significance with 'Pool Edge' features is ignored.

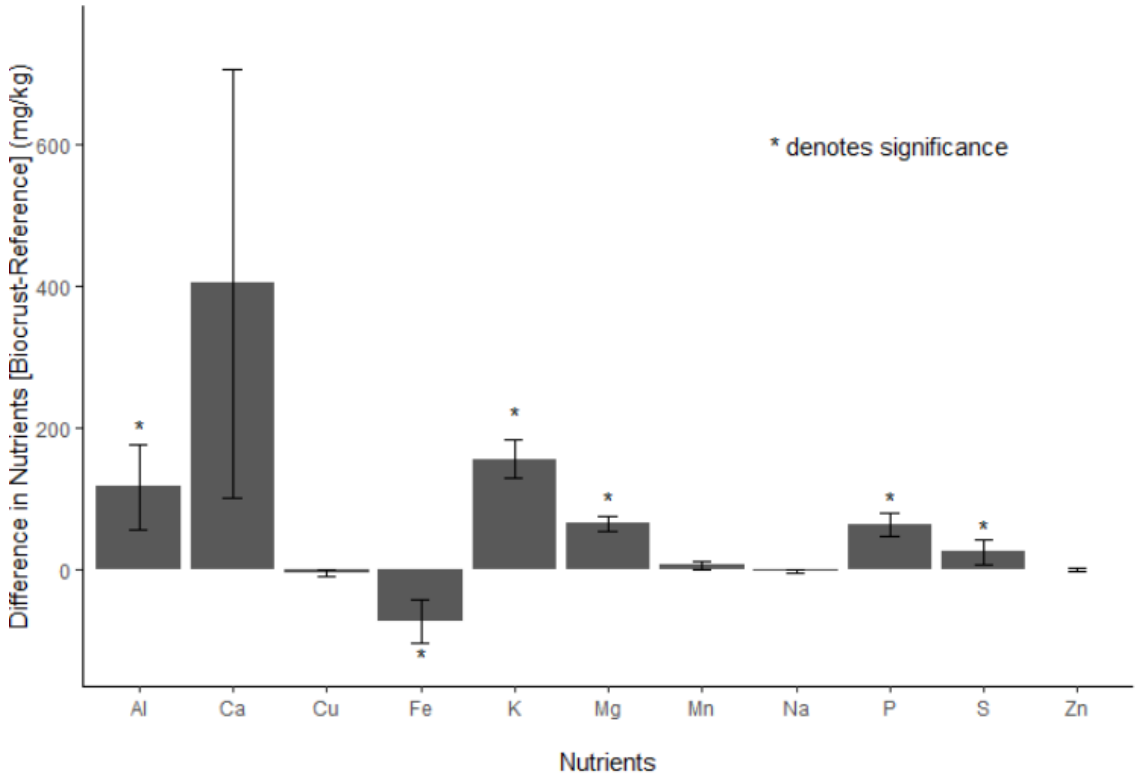

Figure 10: Difference in nutrient concentrations between biocrust and reference samples. Error bars are standard error. '*' denotes significant deviation from mean of zero. Sample size is 18 for all but copper. Sample size for copper is 17.

Figure 11: Gametophyte emergence in 'INN' (left) and 'IMiN' (right) samples in Experiment 1. *Polytrichum juniperinum* moss, dead and new emergent are seen on the right.

Figure 12: Development of green pigmentation on a 'IMiT' treatment plot in experiment 1.

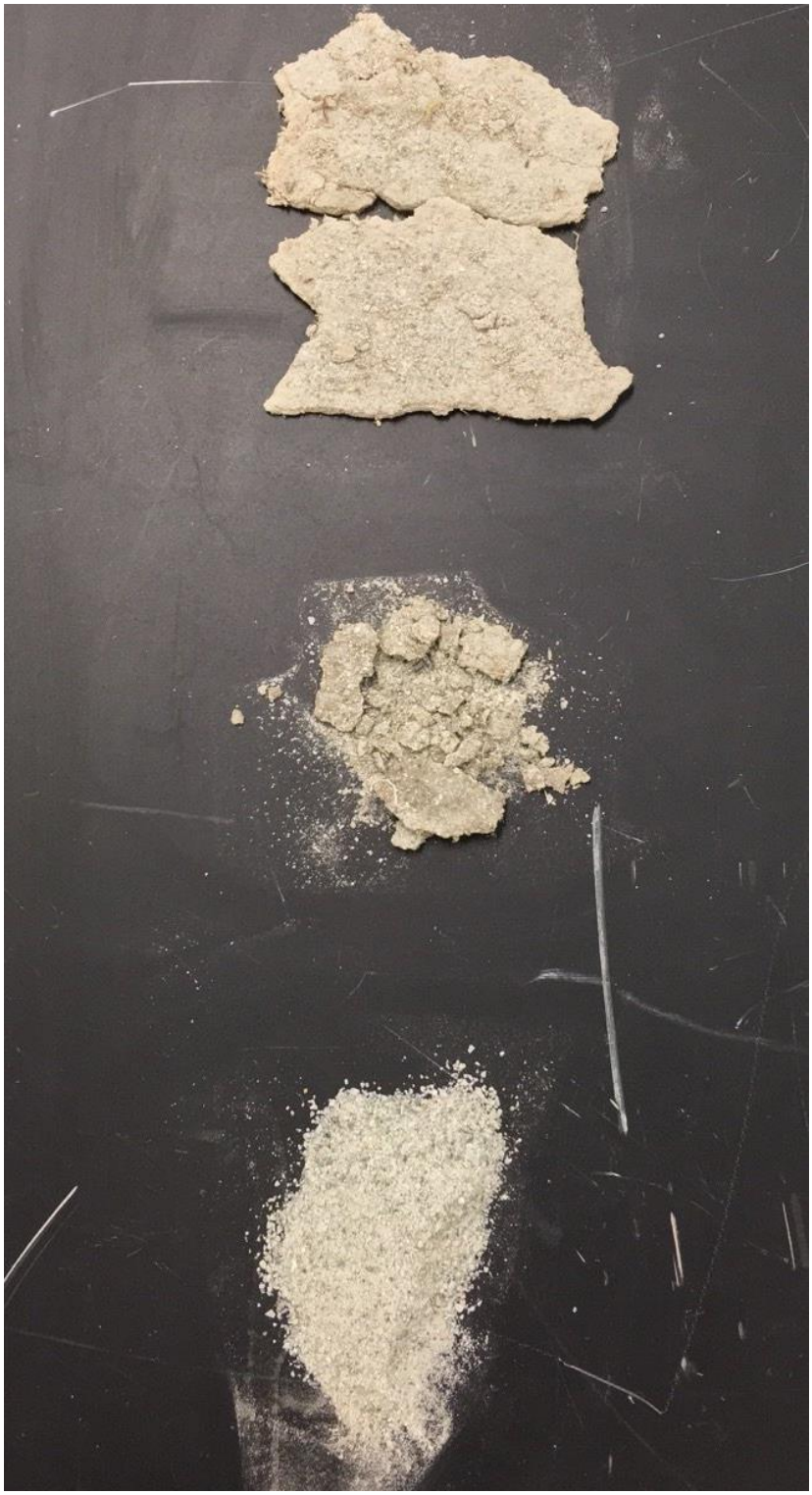

Figure 13:Crust hardening observed in samples, with prominence of hardening increasing from bottom to top.

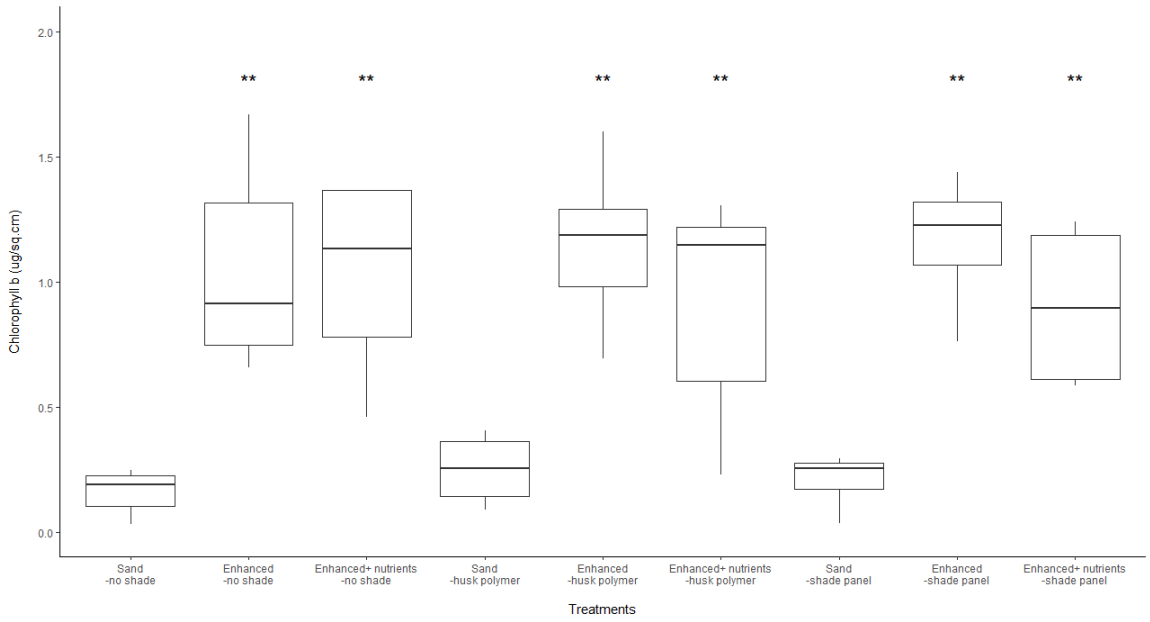

Figure 14: Chlorophyll b distribution, in ug/g, from experiment 3. 'Sand- no shade' is the control and '' denotes significant increase in chlorophyll content.**

Figure 15: Moss cover between grasses on revegetated tailings. Documented on Gibraltar mines.

Figure 16: Detritus media underneath lichen cover found in riles between vegetation. Documented at Gaspé site.

Appendix A: Results from Moss and Lichen ID

Table: Species of moss and lichen identified on samples sites.

Mine	Site	Sample ID	Type	ID
Endako	A	1	Moss	Ceratodon purpureus
			Lichen	Lecanoromycetes (Family)
			Lichen	Unknown
		3	Moss	Bryaceae (Family)
			Moss	Ceratodon purpureus
	B	4	Lichen	Cladonia cariosa
			Lichen	Lecanoromycetes (Family)
			Moss	Ceratodon purpureus
			Lichen	Cladonia cariosa
			Lichen	Lecanoromycetes (Family)
			Moss	Polytrichum juniperinum
			Moss	Bryaceae (Family)
			Lichen	Cladonia (Genus)
			Lichen	Cladonia gracilis
		5	Lichen	Lecanoromycetes (Family)
			Lichen	Cladonia cariosa
			Moss	Ceratodon purpureus
			Lichen	Lecanoromycetes (Family)
			Moss	Polytrichum juniperinum
			Moss	Bryaceae (Family)
	C	6	Lichen	Lecanoromycetes (Family)
			Lichen	Cladonia cariosa
			Moss	Bryaceae (Family)
			Lichen	Cladonia (Genus)
	D	9	Moss	Bryaceae (Family)
			Moss	Bryaceae (Family)
			Lichen	Lecanoromycetes (Family)
Brenda	E	10	Moss	Bryaceae (Family)
			Moss	Bryaceae (Family)
		12	Moss	Bryaceae (Family)
			Moss	Polytrichum piliferum
			Moss	Bryaceae (Family)
			Lichen	Cladonia gracilis
			Lichen	Cladonia (Genus)
			Lichen	Lapraria neglecta

			Lichen	Cladonia cariosa
	F	13	Lichen	Cladonia gracilis
			Lichen	Cladonia cariosa
		14	Moss	Tortula norvegica
			Lichen	Lapraria neglecta
			Lichen	Lecanoromycetes (Family)
			Moss	Bryaceae (Family)
			Lichen	Cladonia (Genus)
	G	15	Moss	Ceratodon purpureus
			Moss	Bryaceae (Family)
			Moss	Polytrichum piliferum
			Moss	Bryaceae (Family)
			Lichen	Lecanoromycetes (Family)
			Moss	Unknown
		16	Moss	Unknown
			Lichen	Lapraria neglecta
			Lichen	Cladonia cariosa
Gaspé	H	17	Moss	Ceratodon purpureus
			Moss	Tortula norvegica
			Lichen	Lecanoromycetes (Family)
			Lichen	Cladonia cariosa
			Lichen	Cladonia cristatella
			Lichen	Cladonia (Genus)
			Lichen	Cladonia gracilis
		18	Lichen	Cladonia Stricta
			Lichen	Cladonia cyanipes
			Lichen	Stereocaulaceae (Family)
			Lichen	Stereocaulaceae (Family)
			Lichen	Unknown
			Lichen	Cladonia gracilis
			Lichen	Cladonia cariosa
			Lichen	Cladonia cristatella
			Lichen	Unknown
	I	19	Lichen	Cladonia (Genus)
			Moss	Polytrichum (Genus)
			Lichen	Cladonia gracilis
			Lichen	Cladonia Stricta
			Moss	Polytrichum (Genus)
			Lichen	Cladonia cyanipes
			Lichen	Cladonia fimbriata
	J	20	Moss	Polytrichum (Genus)

			Lichen	Cladonia (Genus)
			Lichen	Cladonia cristatella
			Lichen	Lecanoromycetes (Family)
	K	21	Lichen	Lecanoromycetes (Family)
			Lichen	Cladonia (Genus)
			Moss	Ceratodon purpureus
			Lichen	Cladonia uncialis
	L	22	Lichen	Cladonia cariosa
			Lichen	Cladonia cristatella
			Lichen	Cladonia fimbriata
			Lichen	Cladonia (Genus)
			Moss	Ceratodon purpureus
			Moss	Unknown

Appendix B: Results from PLFA analysis for microbial content of biocrust samples.

Table: Biomasses from PLFA Analysis of Biocrust Samples (0-0.5 cm)

ID	Site	Area	Total Biomass	Total Bacteria Biomass	Total Fungi Biomass	Arbuscular Mycorrhizal Biomass	Protozoa Biomass	Undifferentiated Biomass
1	Endako	A	44701.38	5957.66	8420.85	698.57	4957.51	25365.42
2	Endako	A	42176.52	5639.69	13137.45	740.88	994.84	22404.58
3	Endako	B	35004.41	11797.73	9340.95	594.03	1124.91	12740.8
4	Endako	B	12242.15	4631	1090.76	43.39	21.28	6499.1
5	Endako	C	35893.78	6278.09	11911.55	818.7	3258.18	14445.97
6	Endako	C	30128.17	5054.33	6576.37	903.81	3733.1	14764.38
7	Endako	D	34982.87	21830.76	4782.92	992.2	1127.97	7241.26
8	Endako	D	34976.75	5141.36	7980.29	757.02	5011.45	16843.65
9	Brenda	E	16766.72	4181.43	6516.67	364.65	595.84	5472.76
10	Brenda	E	37240.79	9062.02	15822.15	348.13	1375.36	10981.18
11	Brenda	F	24283.13	2922.27	5891.38	358.15	2717.43	12751.94
12	Brenda	F	26096.62	5748.73	2885.94	338.42	1414.35	16047.48
13	Brenda	G	52815.11	18366.93	12851.42	978.48	874.8	20721.96
14	Brenda	G	23340.66	2601.49	4850.4	283.09	2706.86	13181.9
15	Gaspé	H	14788.2	5653.36	3215.26	253.01	175.96	5743.59
16	Gaspé	H	9610.11	3384.41	2277.38	205.7	303.8	3644.53
17	Gaspé	I	25349.11	8040.01	6661.63	313.8	541.79	10105.67
18	Gaspé	J	38237	11324.68	9438.34	726.4	3261.31	14212.69
19	Gaspé	K	17800.54	5908.63	5373.15	342.12	421.14	6097.6
20	Gaspé	L	5124.93	2134.6	303.51	0	8.52	2678.28

Appendix C: Nurtrient Concentration in biocrust samples.

Table: Nutrient Concentrations in Biocrust Samples (0-0.5 cm) (DL= Detection Limit)

ID	Site	pH	Al	B	Ca	Cu	Fe	K	Mg	Mn	Na	P	S	Zn
1	Endako	6.1	510	< DL	1600	3.7	390	110	180	130	< DL	35	19	6
2	Endako	6.5	460	< DL	870	2.2	290	110	94	99	< DL	46	< DL	< DL
3	Endako	7.77	260	< DL	1100	6.6	830	47	160	130	< DL	49	< DL	3.3
4	Endako	6.6	660	< DL	890	1.4	250	57	120	48	< DL	72	< DL	< DL
5	Endako	8.03	140	< DL	1300	4	560	23	91	110	< DL	24	< DL	< DL
6	Endako	7.96	170	< DL	1100	5.2	640	35	120	160	< DL	< DL	14	2.7
7	Endako	7.91	160	< DL	900	4	660	44	140	180	< DL	26	21	3.1
8	Endako	7.91	130	< DL	1000	5.7	550	50	110	130	< DL	34	< 24	2.2
9	Brenda	7.23	170	< DL	1600	22	420	32	42	50	< DL	220	< DL	18
10	Brenda	7.3	76	< DL	2100	24	320	25	36	56	< DL	140	< DL	9.7
11	Brenda	7.87	140	< DL	1500	31	470	35	56	60	< DL	91	< DL	23
13	Brenda	7.64	77	< DL	980	52	400	22	54	56	< DL	45	< DL	14
14	Brenda	7.45	76	< DL	840	25	280	< 28	35	50	< DL	73	< DL	15
15	Gaspé	7.92	82	< DL	2700	98	610	< DL	12	22	< DL	100	DL	17
16	Gaspé	8.14	230	< DL	3900	850	610	< DL	16	27	DL	44	13	50
17	Gaspé	4.98	320	< DL	450	77	660	DL	23	33	67	99	190	24
18	Gaspé	7.7	220	< DL	2700	150	550	< 86	34	30	15	57	210	45
19	Gaspé	7.75	73	< DL	3500	78	410	DL	19	35	14	150	29	29
20	Gaspé	8.03	380	< DL	4400	930	640	44	34	79	10	43	21	23

Appendix D: Chlorophyll and erosion measurements for Experiments 1 and 2.

Table: Chlorophyll and erosion measurements for Experiments 1 and 2

Experiment	Plot	Treatment	Erosion (cm)	Chlorophyll a ($x10^{-6}$ g. cm^{-1})	Chlorophyll a & b ($x10^{-6}$ g. cm^{-1})	Physical Crusting Observed
1	A1	INN	3	0.3604	0.8756	FALSE
1	A2	NMaS	0	0.7877	1.3486	FALSE
1	A3	E	0	0.9879	1.5908	FALSE
1	A4	NNT	-0.9	0.1615	0.2309	FALSE
1	A5	IMaT	-3.5	3.0634	4.0088	FALSE
1	A6	B	-0.3	0.7471	1.5182	FALSE
1	A7	N	0.6	1.5164	3.1589	FALSE
1	A8	INS		5.5550	8.7753	TRUE
1	A9	NMiT	-4.7	2.6785	4.3984	TRUE
1	A10	NNS	0.3	3.0985	5.6410	TRUE
1	A11	NNN	0.2	6.3600	10.7551	TRUE
1	A12	NMiN	1	5.3891	8.5133	TRUE
1	A13	C				FALSE
1	A14	IMiN	0.3	6.6445	9.7417	TRUE
1	A15	IMaN	0	7.3385	12.3802	TRUE
1	A16	IMaS	0.2	4.8105	6.8542	FALSE
1	A17	IMiS	0.4	1.3919	2.4216	FALSE
1	A18	IMiT	-2.7	7.4322	10.4586	FALSE
1	A19	NMaT	-3.7	7.3174	10.2552	FALSE
1	A20	NMaN	0.1	0.1625	0.2510	FALSE
1	A21	INT				FALSE
1	A22	NMiS	0	0.4504	0.5691	FALSE
1	B1	NMiN	0.4	2.5699	3.5836	FALSE
1	B2	NNN	0.2	0.4991	0.9314	FALSE
1	B3	NMaT	-2.5	11.1167	14.5157	TRUE
1	B4	IMaS	0	0.5465	0.7867	FALSE
1	B5	IMiN	0	0.3912	0.6247	FALSE
1	B6	INT	-3.5	0.2264	0.3552	FALSE
1	B7	IMaT	-4	0.8343	1.0187	FALSE
1	B8	IMaN	0.5	1.0419	1.5628	FALSE
1	B9	B	-3.2	0.2783	0.3868	FALSE
1	B10	NNS	0.3	0.9689	1.5963	FALSE
1	B11	INS	0	0.5078	0.6742	FALSE
1	B12	NMiS	0.2	0.4023	0.6593	FALSE
1	B13	E	0.1	0.7398	1.2325	FALSE

1	B14	NMiT	1	6.3392	9.0248	FALSE
1	B15	IMiS	0	4.2645	6.1142	TRUE
1	B16	N	1.5			FALSE
1	B17	C				FALSE
1	B18	IMiT	-2	0.2735	0.2973	TRUE
1	B19	NMaN		1.5769	2.3473	FALSE
1	B20	NNT	-3	0.1792	0.1645	FALSE
1	B21	NMaS		0.2351	0.3049	FALSE
1	B22	INN	-0.5	1.6005	2.2381	FALSE
1	C1	NMiS	0	0.9076	1.1345	FALSE
1	C2	IMiN	0	6.4892	9.1417	FALSE
1	C3	E	-3	0.2723	0.3010	FALSE
1	C4	IMaT	-2	8.3549	11.1123	FALSE
1	C5	NNN	0	0.4044	0.5543	FALSE
1	C6	INN		0.9064	1.3658	FALSE
1	C7	IMiT	-2.4	9.6590	13.2280	FALSE
1	C8	B	-0.8	0.9609	1.1963	FALSE
1	C9	INS	0.3	1.1818	1.6654	FALSE
1	C10	NMiN		0.9251	1.4680	FALSE
1	C11	IMiS	0.4	1.1551	1.5263	FALSE
1	C12	C				FALSE
1	C13	NMaT	-2.5	8.9734	12.2107	FALSE
1						FALSE
1	C14	NNS	0	0.7730	1.0770	FALSE
1	C15	NMaS	0	0.4644	0.6401	FALSE
1	C16	NNT	-2.4	0.0957	0.0430	FALSE
1	C17	NMiT	-3.5	1.3477	1.8476	FALSE
1	C18	NMaN	0.5	0.7289	0.9544	FALSE
1	C19	IMaN		3.7592	5.8202	FALSE
1	C20	N	0	1.5798	2.4418	FALSE
1	C21	INT	-1	1.6778	2.3820	FALSE
1	C22	IMaS	-1.5	0.9934	1.3367	FALSE
1	D1	NNT	-2.8	0.0741	0.0943	FALSE
1	D2	NNS	0	0.2608	0.3226	FALSE
1	D3	INN	0.3	0.4206	0.6403	FALSE
1	D4	E		0.3842	0.6277	FALSE
1	D5	IMiT	-2.7	11.4668	14.7454	FALSE
1	D6	INS	0.2	3.0045	4.5773	TRUE
1	D7	B	0	6.9409	10.8673	FALSE
1	D8	IMaT	-2	1.5503	2.3211	FALSE
1	D9	NMiN	0.4	4.3096	6.1803	TRUE
1	D10	IMiN	0	10.4684	17.1159	FALSE

1	D11	NNN	0.6	3.5071	6.5949	FALSE
1	D12	IMiS	0	1.0877	1.6476	FALSE
1	D13	NMiT	-2.7	0.5039	0.6465	FALSE
1	D14	NMiS	-0.5	0.2553	0.3237	FALSE
1	D15	IMaN	0.3	0.7155	1.0123	FALSE
1	D16	NMaT	-2.8			FALSE
1	D17	NMaN		0.2417	0.3486	FALSE
1	D18	C				FALSE
1	D19	N	1.3	0.2011	0.2797	FALSE
1	D20	NMaS	0	0.3278	0.4846	FALSE
1	D21	INT	-2.4	0.5160	0.7830	FALSE
1	D22	IMaS	0	0.6143	0.8721	FALSE
1	E1	IMaN		1.4273	1.8856	FALSE
1	E2	C				FALSE
1	E3	NMaN	1.5	0.7600	1.0576	FALSE
1	E4					FALSE
1	E5	IMaS	0	0.3357	0.4245	FALSE
1	E6	IMiN		0.9708	1.5304	FALSE
1	E7	INN	0	0.8581	1.0882	FALSE
1	E8	IMiT	-3	1.6477	2.2918	FALSE
1	E9	IMaT	-1.7	5.6432	7.5512	FALSE
1	E10	NNT	-1	0.1365	0.1837	FALSE
1	E11	B	0.8	0.8290	1.2836	FALSE
1	E12	NMiN	-0.2	0.1989	0.1446	FALSE
1	E13	NNN		0.1764	0.1637	FALSE
1	E14	NMiS		0.3078	0.5086	FALSE
1	E15	NMaS		0.3834	0.4997	FALSE
1	E16	INS	-0.5	0.3850	0.5636	FALSE
1	E17	NMaT	-1.8	1.8091	2.5033	FALSE
1	E18	E				FALSE
1	E19	NMiT	-2.5	2.2357	3.1810	TRUE
1	E20	INT	-3	3.9154	5.8791	FALSE
1	E21	NNS	0	5.0902	7.3707	TRUE
1	E22	IMiS	0	2.1209	3.1724	FALSE
1	F1	NNN	1.5			FALSE
1	F2	E	0			FALSE
1	F3	IMaN	-0.3	0.5793	1.1653	FALSE
1	F4	NMiN	-0.5	0.9175	1.5336	FALSE
1	F5	NMaN	0			FALSE
1	F6	IMaT	-1.2	5.0536	5.9278	FALSE
1	F7	INN	0.3	1.4505	1.8646	FALSE
1	F8	IMaS	0	0.9333	1.5523	FALSE
1	F9	NMaT	-2.4	4.9942	6.7713	FALSE

1	F10	B	0	0.5569	0.9604	FALSE
1	F11	N	-1.1	1.3262	1.7959	FALSE
1	F12	IMiS	0.8	1.6690	2.6031	FALSE
1	F13	NMiT	-3.2			FALSE
1	F14	NMaS		0.8407	1.5435	FALSE
1	F15	NMiS	0	0.7383	0.9788	FALSE
1	F16	IMiN	0.1	1.1440	1.8464	FALSE
1	F17	INS	0	0.4953	0.6969	FALSE
1	F18	INT	-3	0.2809	0.4966	FALSE
1	F19	NNT	-2.3	2.8470	3.9367	FALSE
1	F20	C				FALSE
1	F21	IMiT	-2.1	4.8615	6.8854	FALSE
1	F22	NNS		5.8002	8.5841	TRUE
1	G1	NMiT	-2.4	3.8290	5.7005	FALSE
1	G2	INN	0	2.2865	3.9777	FALSE
1	G3	C				FALSE
1	G4	IMiN		1.2462	2.0846	FALSE
1	G5	NMaS		2.1803	3.5831	FALSE
1	G6	IMaN		1.4927	2.2845	FALSE
1	G7	NNS	0	0.7896	1.3046	TRUE
1	G8	IMiT	-2	3.1926	4.6134	FALSE
1	G9	N	0.3	0.6699	1.1434	FALSE
1	G10	IMaT	3	2.3464	3.5175	FALSE
1	G11	B	0	11.4967	16.3032	TRUE
1	G12	E		8.2411	12.2837	TRUE
1	G13	IMiS		5.7937	8.0726	FALSE
1	G14	INT	2	3.0222	5.0987	FALSE
1	G15	INS	-0.7	1.0155	1.6743	FALSE
1	G16	NNN	0.8	0.3579	0.9065	FALSE
1	G17	NNT	-3	0.4101	0.8072	FALSE
1	G18	NMaN	0	1.9897	3.0944	FALSE
1	G19	IMaS	-0.8	0.7507	1.2814	FALSE
1	G20	NMiS	-1	1.8236	2.6875	FALSE
1	G21	NMiN	0.4	3.6444	4.9742	FALSE
1	G22	NMaT	-3.4	0.6422	0.4225	FALSE
1	H1	IMaS	0.2	1.7661	2.5704	FALSE
1	H2	IMiN	-0.5	0.3239	0.5562	FALSE
1	H3	NMiN	-0.7	0.3696	0.4934	FALSE
1	H4	IMaN	0.7	1.2635	1.7453	FALSE
1	H5	C				FALSE
1	H6	IMaT	-2.5	3.7515	4.9341	FALSE
1	H7	NMaT	-3.6	0.2880	0.4044	FALSE
1	H8	N		0.2327	0.3310	FALSE

1	H9	INS	-0.1	0.1739	0.2393	FALSE
1	H10	NNN	0.1	1.5272	2.8324	FALSE
1	H11	E		1.3902	2.9814	FALSE
1	H12	B	0	1.1352	1.8220	FALSE
1	H13	IMiT	-2.5	1.7371	2.4476	FALSE
1	H14	NMiT	-2.8			FALSE
1	H15	NNT	-2.1			FALSE
1	H16	IMiS	0			FALSE
1	H17	NMaS	0.3			FALSE
1	H18	NMaN	-0.5	0.3098	0.4853	FALSE
1	H19	INN	0			FALSE
1	H20	NNS	0.5			FALSE
1	H21	NMiS	-0.2			FALSE
1	H22	INT	-2.8			FALSE

Appendix E: Chlorophyll measurements for experiment 3.

Table: Chlorophyll measurements for Experiment 3

Plot	Treatment	Chlorophyll a ($x10^{-6}$ g. cm^{-1})	Chlorophyll a & b ($x10^{-6}$ g. cm^{-1})
1	PC	1.00775	1.356132
2	PM	0.675204	1.963871
3	SC	0.334449	0.571707
4	SM+	1.046584	2.215462
5	NC	0.179842	0.264537
6	NM	1.243766	2.157667
7	SM	0.643835	1.812365
8	PM+	0.923368	2.141904
9	PM+	0.158048	0.388281
10	SC	0.271778	0.423438
11	PC	0.375193	0.536591
12	SC	0.418095	0.712763
13			
14	NM	1.350868	3.018092
15	NM+	2.435684	3.800007
16	NM+	1.104448	2.234405
17	PM+	0.489169	1.092404
18	PM	0.373542	1.068223
19	SC	0.088519	0.124082
20	NC	0.302293	0.456542
21	SM+	0.590992	1.832352
22	SM	0.746739	2.184752
23	NC	0.456735	0.702586
24	NM	0.457577	1.205962
25	PC	1.644576	2.049832
26	NM+	0.454508	1.234961
27	PC	0.136879	0.224555
28	PM	1.999099	3.598884
29	NC	0.196104	0.225784
30	SM	0.773763	2.054314
31	PM	0.657263	1.841273
32	SM+	0.464335	1.085418
33	SM	0.879297	1.639974
34	NM	0.402294	1.059802
35	NM+	0.458008	0.915386

36	SM+	0.681938	1.266064
37	NC	0.599789	0.821757
38	SC	0.617191	0.888385
39	NM+	1.124727	2.489273
40	PM	0.510468	1.490789
41	PM+	0.686375	1.992114
42	SM+		
43	PC	0.033371	-0.00476
44	PM+	1.848056	2.993262
45	NM	1.488949	2.803141
46	NC	0.611784	0.838192
47	SC	0.630322	0.907287

Appendix F: Summarized results from preliminary trial of sampling method.

Table: **Unique specimen progressively across three forest biocrust sites 400 m apart.**

Site	Unique Specimen
1	6
2	3
3	3

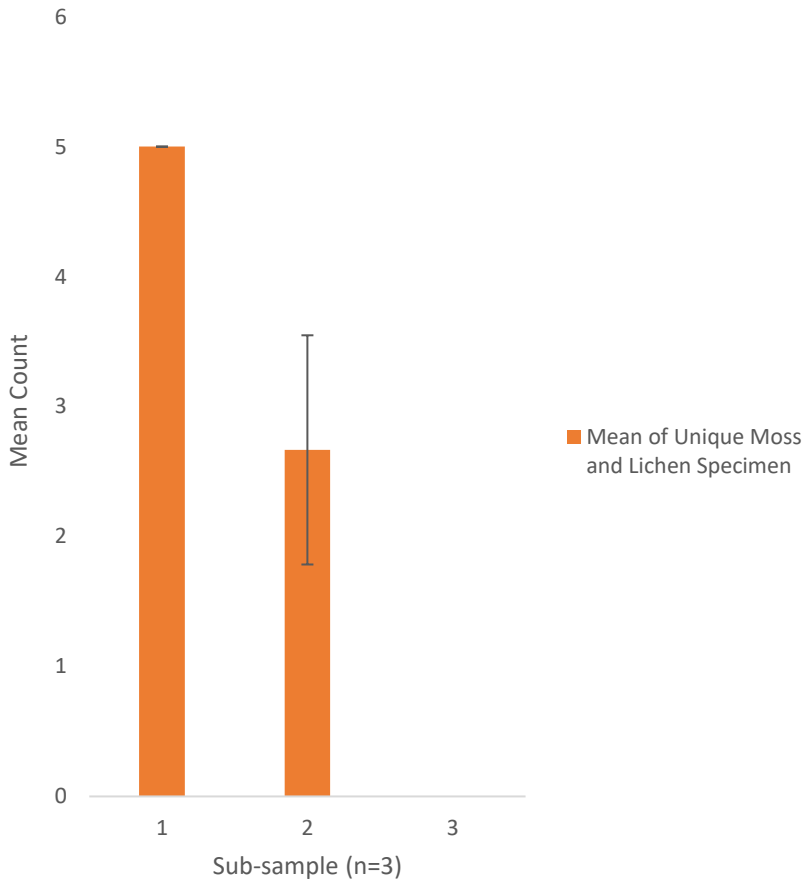

Figure: Comparison of mean unique samples found per. Sub-sample across three sample points.

Appendix G: Photos of sites in Endako and Gaspé.

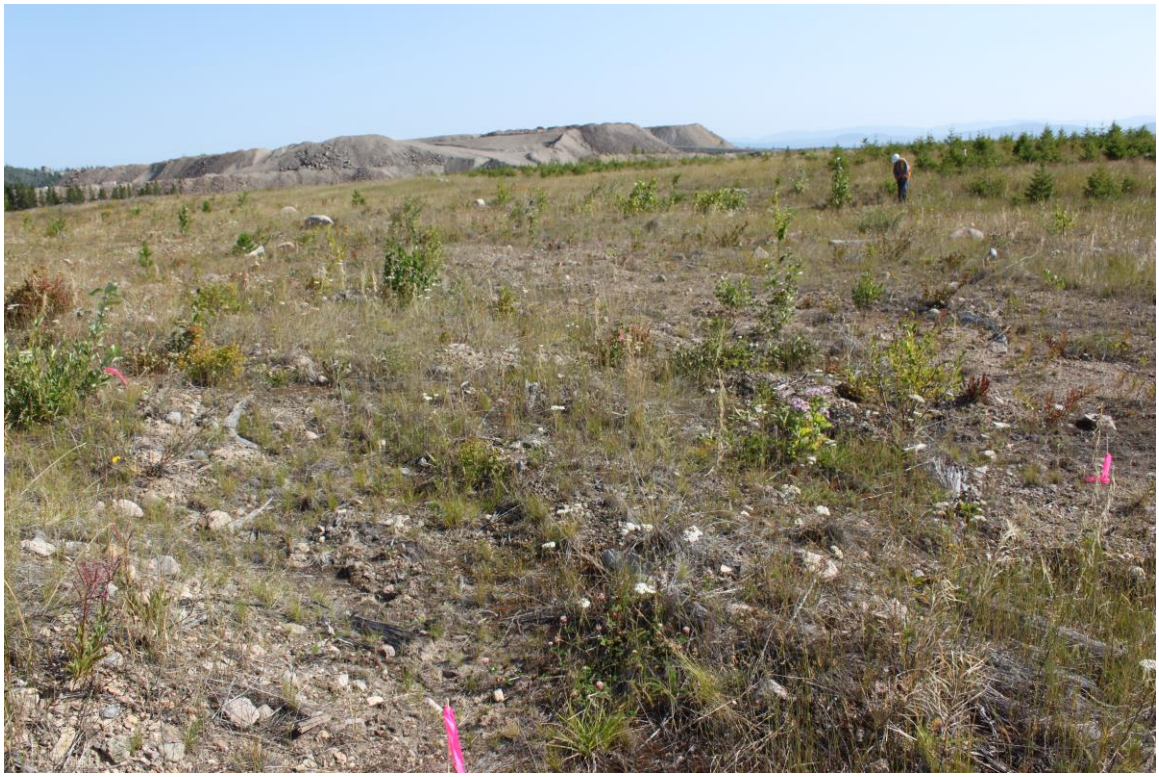

Figure: Site 1.

Figure: Site 3.

Figure: Site 4

Figure: Site 5

Figure: Site 6

Figure: Site 7

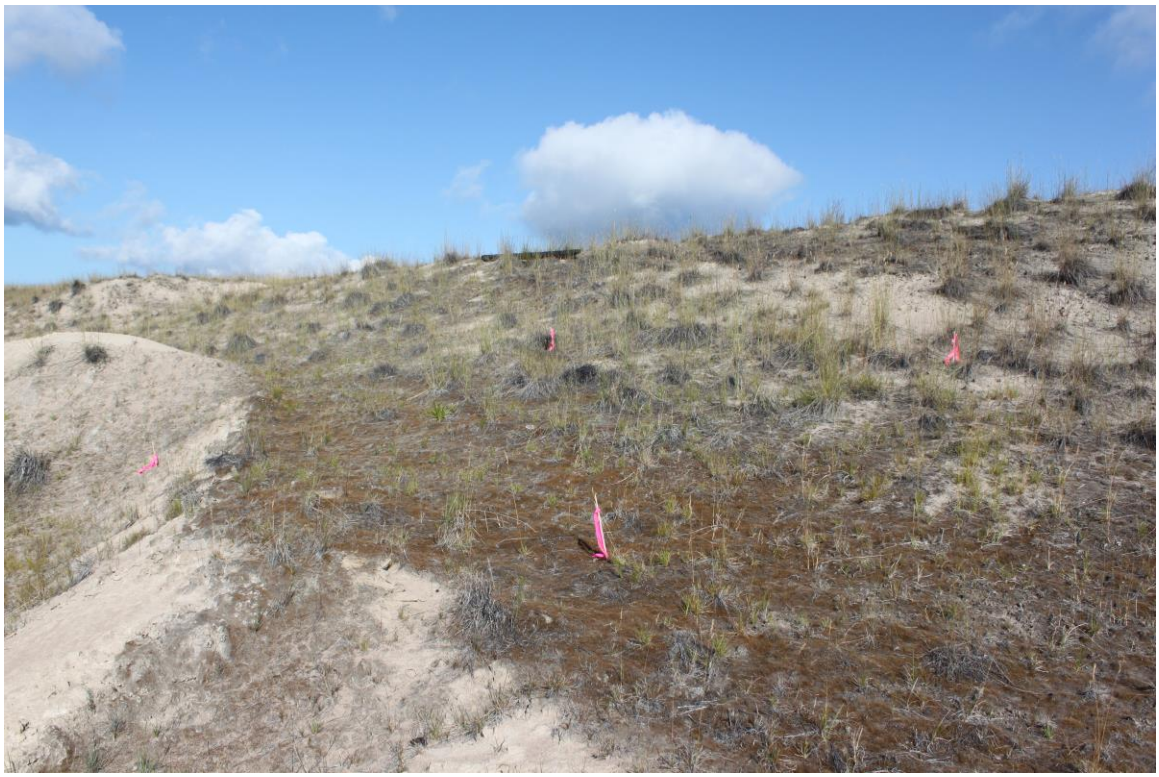

Figure: Site 8

Figure: Site 15

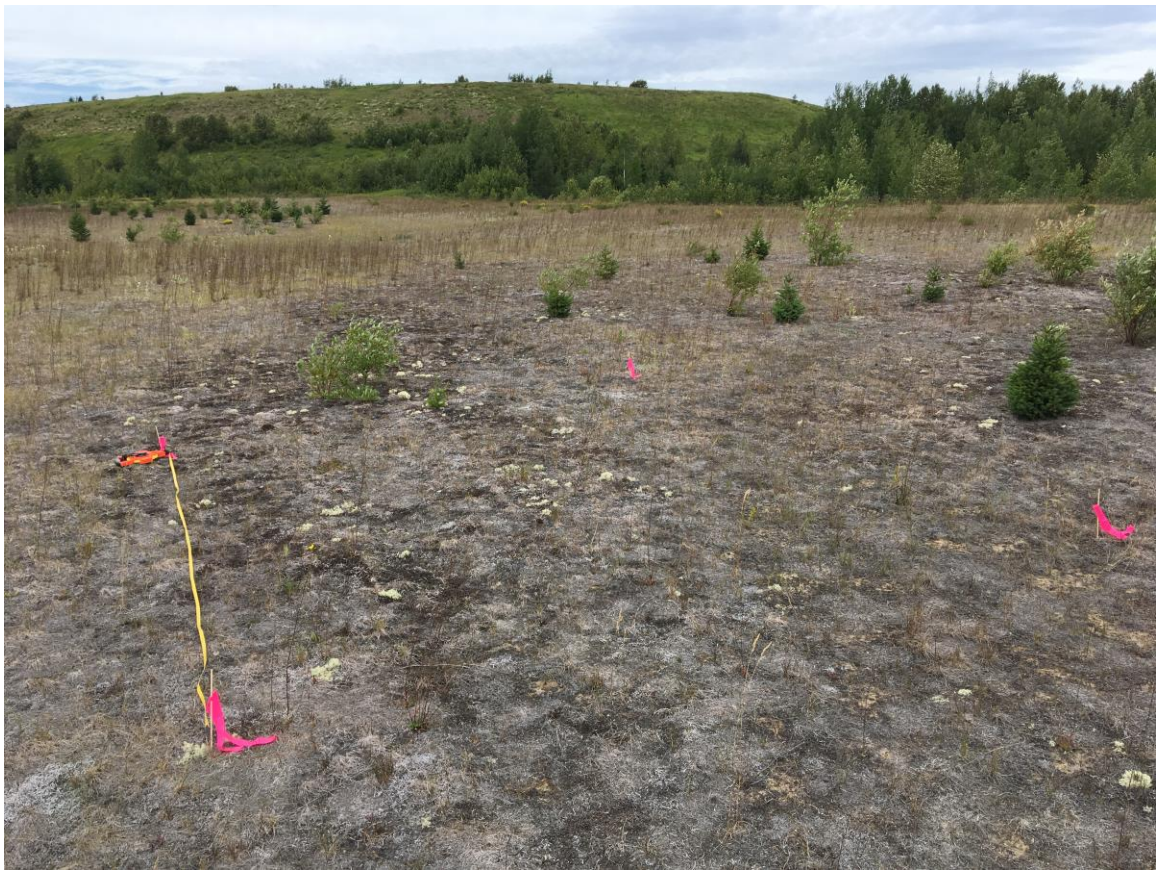

Figure: Site 16

Figure: Site 17

Figure: Site 19

Figure: Site 20

Appendix H: R script used.

```
#Formal script for Applied Research Project (ARP) on Biocrust Restoration on Mine Tailings facilities.

#Splitting display pane:

par(mfrow=c(2,2))

#This script lends from a rough draft where initial exploration of the data was done. This report replaces and
    out-performs that former script.

#Sections of the script are written to correspond investigation and narration in the ARP report.

# Comments tagged with '***' are marking code that yielded a significant result.

#Run code in entire blocks (delineated by headings), since variables in the 't' series are reused outside each
    block of code. 't' series variables are temporary holders.

#Datasets can be found here:
    https://drive.google.com/drive/folders/16Mx1EBO4fxVnVEImUKHYylcxu3a9Fnze?usp=sharing

#libraries and packages

install.packages("ggplot2")

install.packages("lmtest")

install.packages("lmerTest")

install.packages("sciplot")

install.packages("scmamp")

install.packages("PerformanceAnalytics")

install.packages("ggfortify")

install.packages("stringi")


library(ggplot2)

library(lmtest)

library(lmerTest)

library(lattice)

library(sciplot)

library(scmamp)

library(ggfortify)

library(cluster)

library(stringi)

library("PerformanceAnalytics")


#Loading parent datasets:

#Analytical data on sampled mine sites

ana<-read.csv("Analytical_Data.csv",header=T)

summary(ana)

#Experiment 1 and 2: Incolumum and amendment tests on tailings of Gibaltar

exp12<-read.csv("exp1 and 2.csv", header=T)
```

```
exp12<-exp12[exp12$Chloro.a..ug.cm..2.!="#N/A",]
summary(exp12)
#Experiment 3: Moss vegetative propagation on tailings of Gibraltar
exp3<-read.csv("exp3.csv",header=T)
summary(exp3)

#ANALYTICAL
#(1) Inter-community effects.
#Distinctions in biocrusts
y<-ana[,c(16,19,21,27,33,37,41)]
t1<-prcomp(y)
summary(t1)
autoplot(t1,data=ana,loadings.label=T,label=ana$ID,center=T,colour='Feature',frame=F)

#(2) Effects of Age:
#Effects of Age- On PLFA biomass.
plot(ana$Age..yrs.,ana$Total.Biomass)
plot(lm(ana$Age..yrs.~ana$Total.Biomass))# plot 20 and 13 are outliers
t1<-ana[-c(20,13),]
plot(lm(t1$Age..yrs.~t1$Total.Biomass)) #Negative auto-correlation.
summary(lm(t1$Age..yrs.~t1$Total.Biomass)) # ***p=0.000596, n=17, R2=0.5317, Adj. R2= 50.24
cor.test(t1$Age..yrs.,t1$Total.Biomass) #corr=0.7292
plot(t1$Age..yrs.~t1$Total.Biomass)
#Effects of Age- Fungus:Bacteria
plot(lm(ana$Age..yrs.~ana$Fungi.Bacteria)) #plot 20 and 2 are outliers.
t1<-ana[-c(20,2),]
plot(lm(t1$Age..yrs.~t1$Fungi.Bacteria))
summary(lm(t1$Age..yrs.~t1$Fungi.Bacteria)) #p=.368
#Effects of Age- on protozoa:bacteria
plot(lm(ana$Age..yrs.~ana$Predator.Prey)) #non-linear q-q plot
summary(lm(ana$Age..yrs.~ana$Predator.Prey)) #p=0.323
plot((ana$Age..yrs.~ana$Predator.Prey))
#Effects of Age- on Saturated-Unsaturated Fats
plot(lm(ana$Age..yrs.~ana$Sat.Unsat))
t1<-ana[-4,]
t2<-t1[-6,]
plot(t2$Age..yrs.~t2$Sat.Unsat)
plot(lm(t2$Age..yrs.~t2$Sat.Unsat))
summary(lm(t2$Age..yrs.~t2$Sat.Unsat))#p=0.54869
#Effects of Age- on percent C
```

```
cor.test(ana$percent.C,ana$Age..yrs.)
plot(lm(ana$Age..yrs.~ana$percent.C))
summary(lm(ana$Age..yrs.~ana$percent.C))
t1<-ana[-c(20,17,16),]
plot(lm(t1$Age..yrs.~t1$percent.C))
summary(lm(t1$Age..yrs.~t1$percent.C)) #p=0.07 The strongest linear effect.
ggplot(t1,aes(x=t1$Age..yrs.,y=t1$percent.C))+
  geom_point()+
  geom_text(label=t1$ID)+
  geom_smooth(stat="smooth",formula=y~x)
cor.test(ana$percent.N,ana$Age..yrs.)
#Effect of Age- on pH
cor.test(ana$upper.pH..1.1.Soil.Water.,ana$Total.Biomass)#p=0.5142
#Effect of Age- on Nutrients
plot(lm(ana$Age..yrs.~ana$Al_diff))
summary(lm(ana$Age..yrs.~ana$Al_diff))#p=0.197
plot(lm(ana$Age..yrs.~ana$Ca_diff))
summary(lm(ana$Age..yrs.~ana$Ca_diff))#***p=0.0226, r2-adj.=0.2397
plot(lm(ana$Age..yrs.~ana$Cu_diff)) #sample 16 and 2 are outliers.
t1<-ana[-c(16,2),]
summary(lm(t1$Age..yrs.~t1$Cu_diff))#p=0.2
plot(lm(ana$Age..yrs.~ana$Fe_diff))
summary(lm(ana$Age..yrs.~ana$Fe_diff))#p=0.65
plot(lm(ana$Age..yrs.~ana$Mg_diff))
summary(lm(ana$Age..yrs.~ana$Mg_diff))#=0.608
plot(lm(ana$Age..yrs.~ana$Mn_diff))#4 is an outlier
t1<-ana[-4,]
plot(lm(t1$Age..yrs.~t1$Mn_diff))
summary(lm(t1$Age..yrs.~t1$Mn_diff))#p=0.195
plot(lm(ana$Age..yrs.~ ana$Na_diff)) #sample 17 is an outlier
t1<-ana[-17,]
plot(lm(t1$Age..yrs.~t1$Na_diff))
summary(lm(t1$Age..yrs.~t1$Na_diff))#p=0.295
plot(lm(ana$Age..yrs.~ana$P_diff))
summary(lm(ana$Age..yrs.~ana$P_diff))#p=0.959
plot(lm(ana$Age..yrs.~ ana$S_diff))#sample 17 is an outlier
summary(lm(ana$Age..yrs.~ana$S_diff))#p=0.287
plot(lm(ana$Age..yrs.~ana$Zn_diff)) #16 is an outlier
t1<-ana[-16,]
summary(lm(ana$Age..yrs.~ana$Zn_diff))#p=0.816
```

```
#Effect of Age- on Cover type
shapiro.test(ana$Age..yrs.)#p=0.2095
summary(aov(ana$Age..yrs.~ana$Dominant.Cover.Component)) #*** p=0.00569
pairwise.t.test(ana$Age..yrs.,ana$Dominant.Cover.Component,p.adjust='bon') #*** All except 'moss+licen' vs lichen
kruskal.test(ana$Age..yrs.~ana$Dominant.Cover.Component) #*** p=0.007423
#Effect of Age on Mono:Poly Fats
plot(lm(ana$Mono.Poly~ana$Age..yrs.)) #20,4,5 and 6 are outliers
t1<-ana[-c(20,4,6),]
t2<-t1[-5,]
plot(lm(t2$Mono.Poly~t2$Age..yrs.))
summary(lm(t2$Mono.Poly~t2$Age..yrs.))#*** p=0.006, r2=0.4276, r2-adj=0.3868
cor.test(t2$Mono.Poly,t2$Age..yrs.) #cor=0.6539
plot(t2$Age..yrs.,t2$Mono.Poly)
#effects of age on gram+:gram-
plot(lm(ana$Gram.pos.Gram.neg~ana$Age..yrs.))
summary(lm(ana$Gram.pos.Gram.neg~ana$Age..yrs.))#p=0.5269
#effects of age on gram-
plot(lm(ana$Gram.neg.biomass~ana$Age..yrs.))
t1<-ana[-7,]
plot(lm(t1$Gram.neg.biomass~t1$Age..yrs.))
summary(lm(t1$Gram.neg.biomass~t1$Age..yrs.))#p=0.588

#(3) Inter-Community Effects and correlations with nutrients
#Correlation of protozoa and bacteria
plot(lm(ana$Bacteria.percent~ana$Protozoan.percent)) #7 and 2 maybe outliers
t1<-ana[-c(7,2),]
summary(lm(t1$Bacteria.percent~t1$Protozoan.percent)) #***p=1.65x10^-7, r2-adj=0.8167
#Correlation between nutrients and Community
#percentage to nutrient diff
t1<-ana[,c(1,3,10,17,19,21,25,29,31,34,35,37,38,39,59,60:71,73,72)]
chart.Correlation(t1, histogram=F, pch=19)
#fungi vs and P
plot(lm(ana$P_diff~ana$Total.Fungi.percent))#positive autocorrelation
plot(lm(ana$P_diff~ana$Total.Fungi.Biomass)) #10 is an outlier
t1<-ana[-10,]
plot(lm(t1$P_diff~t1$Total.Fungi.Biomass))
summary(lm(t1$P_diff~t1$Total.Fungi.percent))
summary(lm(ana$P_diff~ana$Total.Fungi.percent)) #***p=0.0016, adjusted R2=0.4403
summary(lm(ana$P_diff~ana$Total.Fungi.percent)) #***p=0.0016, adjusted R2=0.4403
```

```
summary(lm(ana$P_diff~ana$Total.Fungi.Biomass)) #***p=0.0327,adjusted r2=0.2081
plot(ana$P_diff,ana$Total.Fungi.percent)
  #with biomass
plot(lm(ana$upper.P~ana$Total.Fungi.Biomass))
t1<-ana[-10,]
plot(lm(t1$upper.P~t1$Total.Fungi.Biomass))
summary(lm(t1$upper.P~t1$Total.Fungi.Biomass))#p=0.886
#Effects on percent C by fungi
summary(lm(ana$Total.Fungi.Biomass~ana$percent.C)) #p=0.7249
summary(lm(ana$Arbuscular.Mycorrhizal.Biomass~ana$percent.C)) #p=0.7177
#effect of cover type on Mono:Poly Fats
shapiro.test(ana$Mono.Poly)#p<0.05
kruskal.test(ana$Mono.Poly~ana$Dominant.Cover.Component)#p=0.1953
#Effects of C by PLFA total
plot(lm(ana$Total.Biomass~ana$percent.C))
t1<-ana[-c(17,16),]
plot(lm(t1$Total.Biomass~t1$percent.C))
summary(lm(t1$Total.Biomass~t1$percent.C))#***p=0.000479, adj r2=0.5153
summary(lm(t1$Undifferentiated.Biomass~t1$percent.C))#p=0.0228, adj.r2=0.2393

#(4)Nutrient-Nutrient trends.
#Preliminary evaluation from correlation matrix produced in section (2) of code.
#Aluminium with sulfur
plot(lm(ana$upper.Al~ana$upper.S))#17 is an outlier
t1<-ana[-17,]
cor.test(t1$upper.Al,t1$upper.S)#p=0.6933
#aluminium with percent C
plot(lm(ana$upper.Al~ana$percent.C)) #17 is an outlier
cor.test(t1$upper.Al,t1$percent.C) #p=0.5653
#copper with carbon
plot(lm(ana$upper.Cu~ana$percent.C))#17 and 16 are outlier
t1<-ana[-c(17,16),]
cor.test(t1$upper.Cu,t1$percent.C) #p=0.7333
#potassium and phosphorous
plot(lm(ana$upper.K~ana$upper.P))
t1<-ana[-c(10,9),]
cor.test(t1$upper.K,t1$upper.P)#*p=0.05265
#magnesium and manganese
plot(lm(ana$upper.Mg~ana$upper.Mn)) #6 is an outlier
t1<-ana[-6,]
```

```
cor.test(t1$upper.Mg,t1$upper.Mn) #***p=3.074x10^-5
#mn and mg vs. zinc
plot(lm(ana$upper.Mg~ana$upper.Zn))
plot(lm(ana$upper.Mn~ana$upper.Zn))
#10 is an outlier
t1<-ana[-10,]
cor.test(ana$upper.Mg,ana$upper.Zn)#***p=0.000828
cor.test(ana$upper.Mn,ana$upper.Zn)#***p=0.002732
#sulfur and percent C
plot(lm(ana$upper.S~ana$percent.C))
t1<-ana[-c(17,16),]
cor.test(t1$upper.S,t1$percent.C)#p=0.5626
#P and Zinc
plot(lm(ana$upper.P~ana$upper.Zn)) #9 is an outlier
t1<-ana[-9,]
cor.test(t1$upper.P,t1$upper.Zn)#***p=0.001071
plot(t1$upper.P,t1$upper.Zn)
#C/N ratio
t1<-ana$percent.C/ana$percent.N
max(t1)
min(t1)
shapiro.test(t1)#p=0.1937
summary(aov(t1~ana$Dominant.Cover.Component))#p=0.132
summary(aov(t1~ana$Feature))#***0.00176
TukeyHSD(aov(t1~ana$Feature))#***pool-dam,pool-plain,pool-plantation
pairwise.t.test(t1,ana$Feature,p.adjust='none')#*** overburden vs dam slope, plain, pool edge, sand bench.
        pool edge vs [all]
plot(t1~ana$Feature)
summary(aov(t1~ana$Surrounding.Veg..Cover))#p=0.266
  #with microbes
plot(lm(t1~ana$Total.Bacteria.biomass+ana$Total.Fungi.Biomass+ana$Undifferentiated.Biomass))
t2<-ana[-c(20,7),]
summary(lm((t2$percent.C/t2$percent.N)~t2$Total.Bacteria.biomass+t2$Total.Fungi.Biomass+t2$Undiffere
        ntiated.Biomass))
summary(lm((t2$percent.C/t2$percent.N)~t2$Undifferentiated.Biomass)) #p=0.0468
plot((t2$percent.C/t2$percent.N),t2$Undifferentiated.Biomass) #Too cluttered.
#percent N by feature
t1<-aov(ana$percent.N~ana$Feature)
summary(t1)#p=0.86
```

```
#(5)Effect of Dominant Biocrust Cover Type and surrounding vegetation
summary(lm(ana$percent.C~ana$percent.N))#*** p=4.11x10^-15, r2 adj.=0.97
#effect on gram+ and -
shapiro.test(ana$Gram.pos.Gram.neg)#p>0.05
shapiro.test(ana$Gram.neg.percent)#p<0.05
shapiro.test(ana$Gram.neg.biomass)#p<0.05
kruskal.test(ana$Gram.neg.percent~ana$Dominant.Cover.Component)#p=0.2829
kruskal.test(ana$Gram.neg.percent~ana$Surrounding.Veg..Cover)#p=0.3772
kruskal.test(ana$Gram.neg.biomass~ana$Dominant.Cover.Component)#p=0.3699
kruskal.test(ana$Gram.neg.percent~ana$Surrounding.Veg..Cover)#p=0.3772
summary(aov(ana$Gram.pos.Gram.neg~ana$Dominant.Cover.Component))#p=0.152
summary(aov(ana$Gram.pos.Gram.neg~ana$Surrounding.Veg..Cover))#p=0.538
#effects on fungi
shapiro.test(ana$Total.Fungi.Biomass)#p=0.7675
summary(aov(ana$Total.Fungi.Biomass~ana$Dominant.Cover.Component))#p=0.615
summary(aov(ana$Total.Fungi.Biomass~ana$Surrounding.Veg..Cover))#p=0.231
shapiro.test(ana$Rhizobia.Biomass)#p=0.713
shapiro.test(ana$Arbuscular.Mycorrhizal.Biomass)#p=0.153
summary(aov(ana$Total.Fungi.Biomass~ana$Dominant.Cover.Component))#p=0.615
t1<-aov(ana$Arbuscular.Mycorrhizal.Biomass~ana$Dominant.Cover.Component)
summary(t1)#***p=0.00612
TukeyHSD(t1) #Lichen vs DC, Moss-lichen vs lichen, moss vs DC
summary(aov(ana$Arbuscular.Mycorrhizal.Biomass~ana$Surrounding.Veg..Cover))#***p=0.016
TukeyHSD(aov(ana$Arbuscular.Mycorrhizal.Biomass~ana$Surrounding.Veg..Cover))#grasses vs sparse.
pairwise.t.test(ana$Arbuscular.Mycorrhizal.Biomass,ana$Surrounding.Veg..Cover)
#Arbs on C
cor.test(ana$percent.C,ana$Arbuscular.Mycorrhizal.Biomass)#p=0.7177
#Effects on total
shapiro.test(ana$Total.Biomass)#p>0.05
summary(aov(ana$Total.Biomass~ana$Dominant.Cover.Component))#p=0.228
summary(aov(ana$percent.C~ana$Dominant.Cover.Component))#p=0.988
cor.test(ana$percent.N,ana$Arbuscular.Mycorrhizal.Biomass)#p=0.7756

#(6)Effect of site or annual precipitation.
#Effect of site- on percent C
shapiro.test(ana$percent.C)#p<0.05
kruskal.test(ana$percent.C~ana$Site)#p=0.262
cor.test(ana$Annual_precip_mm,ana$upper.S)
#Effect of site- on percent N
```

```
shapiro.test(ana$percent.N)#p<0.05
kruskal.test(ana$percent.N~as.factor(ana$Annual_precip_mm))#p=0.4382
#Effect of annual precip on- nutrients (conservative included)
#sample 20 is already excluded.
cor.test(ana$Annual_precip_mm,ana$Al_diff) #p=0.14
cor.test(ana$Annual_precip_mm,ana$Ca_diff)#p=.4443
cor.test(ana$Annual_precip_mm,ana$Cu_diff)#p=.107
cor.test(ana$Annual_precip_mm,ana$P_diff) #p=0.1549
cor.test(ana$Annual_precip_mm,ana$S_diff) #p=0.8871
cor.test(ana$Annual_precip_mm,ana$Fe_diff) #=.3334
cor.test(ana$Annual_precip_mm,ana$Mg_diff) #***p=0.00967, cor=-0.5918
cor.test(ana$Annual_precip_mm,ana$Zn_diff)#*** p=0.04898, corr=-0.47
#Insignificant for Na and B as well.
#effect of precip on bacteria
summary(aov(ana$Actinomycetes.percent~ana$Site)) #***p=0.00604
pairwise.t.test(ana$Actinomycetes.percent,ana$Site)
plot(lm(ana$Actinomycetes.percent~ana$Annual_precip_mm)) #***p=0.00206, adjusted r2=0.386
#Independant Variable: Total Biomass (Biocrust PLFA weight as proxy for development)
#Effect of biomass- on percent C
cor.test(ana$Total.Biomass,ana$percent.C)#p=.775
#Effect of biomass- on percent N
cor.test(ana$Total.Biomass,ana$percent.N)#p=.7906
#Correlation between PLFA and percent C
cor.test(ana$Total.Biomass,ana$percent.C)#p=0.775

#Effect of biocrusts on nutrients
wilcox.test(ana$Al_diff,mu=0) #p=0.02019 ***
wilcox.test(ana$Ca_diff,mu=0) #p=0.1964
t1<-ana[-16,]
wilcox.test(t1$Cu_diff,mu=0) #p=0.3317
wilcox.test(ana$P_diff,mu=0) #***p=0.001782
wilcox.test(ana$S_diff,mu=0) #***p=0.004496
wilcox.test(ana$Fe_diff,mu=0) #***p=0.02782
wilcox.test(ana$Mg_diff,mu=0) #***p=0.0004181
wilcox.test(ana$Mn_diff,mu=0) #p=0.1165
wilcox.test(ana$Zn_diff,mu=0) #p=0.2366
wilcox.test(ana$Na_diff,mu=0) #p=0.4982
wilcox.test(ana$K_diff,mu=0) #***p=2.289x10^-5

#Effect on pH
```

```
t1<-ana[-20,]
shapiro.test(t1$upper.pH..1.1.Soil.Water.) #p=0.4333
shapiro.test(t1$pH..1.1.Soil.Water.) #p=001006
t.test(t1$upper.pH..1.1.Soil.Water.,t1$pH..1.1.Soil.Water.,var.equal = F) #***p=9.412x10^-7
mean(t1$upper.pH..1.1.Soil.Water.)
se(t1$upper.pH..1.1.Soil.Water.)
mean(t1$pH..1.1.Soil.Water.,na.rm=T)
se(t1$pH..1.1.Soil.Water.)
#Effect of age on pH
t1<-ana$upper.pH..1.1.Soil.Water.-ana$pH..1.1.Soil.Water. #crust-ref
summary(lm(t1~ana$Age..yrs.)) #***p=0.0312, adj-r2=0.2122
plot(lm(t1~ana$Age..yrs.))
plot(t1,ana$Age..yrs.)



#EXPERIMENTAL
#subsetting exp 1:
exp1<-exp12[exp12$Chem!="#N/A",]
exp1<-exp1[exp1$Chloro.a..ug.cm..2.!="#N/A",]
exp1$Chloro.a..ug.cm..2.<-as.numeric(exp1$Chloro.a..ug.cm..2.)
shapiro.test(as.numeric(exp1$Chloro.a..ug.cm..2.)) #p<0.05
#Effect of Treatment on Chlorophyll a levels
pairwise.wilcox.test(exp1$Chloro.a..ug.cm..2.,exp1$Treatment,p.adjust="none")#*p=0.0721 for NNN vs
    IMaT
t1=(aov(exp1$Chloro.a..ug.cm..2.~exp1$Inoc*exp1$Chem*exp1$Phys))
summary(t1)#*** p=0.0046 for Inoc, p=0.00769 for Chem vs Phys
TukeyHSD(t1) #oof
#Effect of levels on chloro a
kruskal.test(exp1$Chloro.a..ug.cm..2.~exp1$Inoc)#***p=0.009218
TukeyHSD(aov(exp1$Chloro.a..ug.cm..2.~exp1$Inoc)) #Inoc vs. non-Inoc - p=0.0076287
kruskal.test(exp1$Chloro.a..ug.cm..2.~exp1$Chem)#***p=0.03392
TukeyHSD(aov(exp1$Chloro.a..ug.cm..2.~exp1$Chem))#*** no chem vs micro+macro - p=0.0244193
kruskal.test(exp1$Chloro.a..ug.cm..2.~exp1$Phys)
#Effect of Treatments on Erosion
#new dataset just for erosion. Cleaned to remove NULL values for erosion
ert<-read.csv("erosion.csv",header=T)
#Removing topology from dataset
er<-ert[ert$Phys!="topo",]
t1<-ert[ert$Phys=="topo",]
shapiro.test(er$Erosion) #p<0.05
```

```
bartlett.test(er$Erosion,er$Treatment) #p<0.05
pairwise.t.test(er$Erosion,er$Treatment,p.adjust="none") # *** vs NNN: p<0.05 for IMaS, INS,NMiS
pairwise.t.test(t1$Erosion,t1$Treatment,p.adjust="none") # vs NNT: No sig
kruskal.test(er$Erosion~er$Inoc) #p=0.0528
kruskal.test(er$Erosion~er$Chem) #p=0.5603
kruskal.test(er$Erosion~er$Phys) #***p=0.03783
TukeyHSD(aov(er$Erosion~er$Phys)) #*** p=0.021383 for SAP-no_phys
#Physical Crusting
cr<-read.csv("crusting.csv",header=T)
barplot(cr$Frequency,names.arg=cr$Treatment) #Need to learn frequency stats
#Experiment 2
exp2<-exp12[exp12$Experiment==2,]
exp2$Chloro.a..ug.cm..2.<-as.numeric(exp2$Chloro.a..ug.cm..2.)
shapiro.test(exp2$Chloro.a..ug.cm..2.) #p=0.2235
pairwise.t.test(exp2$Chloro.a..ug.cm..2.,exp2$Treatment,p.adjust="none") #no sig.
shapiro.test(exp2$Erosion) #p<0.05
kruskal.test(exp2$Erosion~exp2$Treatment) #p=0.184
#Experiment 3
exp3<-na.omit(exp3)
plot(exp3$Chl.a,exp3$chl.a.b) #damn straight

#Chloro a
shapiro.test(exp3$chl.a)#p=0.07779. Presming non-parametric, due to result of hist(exp3$chl.a)
pairwise.t.test(exp3$Chl.a,exp3$treatment,p.adjust="none") #*** p<0.05 for NC vs NM+.
#Cholo.b
shapiro.test(exp3$chl.b) #p<0.05
pairwise.wilcox.test(exp3$chl.b,exp3$treatment,p.adjust="none") #***p<0.05 for NC vs NM, NM+, PM, PM+,
        SM, SM+.
TukeyHSD(aov(exp3$chl.b~exp3$treatment))

#Data from Yao et al. 2000
#total PLFA nmol.g-1
t1<-c(2.37,5.35,4.53,10.11,24.88,22.66,29.86,42,21,2.73)
mean(t1)
sd(t1)/sqrt(length(t1))

#DELIVERABLES (Figures for thesis report)
#Figure:plot of correlation between %percent protozoa and bacteria
t1<-ana[-c(2,7),]
```

```
#summary(lm(t1$Bacteria.percent~t1$Protozoan.percent)) #plotting this relationship. Points 7 and 2 are
        outliers.
ggplot(t1,aes(x=t1$Bacteria.percent,y=t1$Protozoan.percent,shape=t1$Dominant.Cover.Component))+

        geom_smooth(method="lm",se=F,inherit.aes=F,aes(x=t1$Bacteria.percent,y=t1$Protozoan.percent
        ),color='gray')+
  geom_point(size=2)+
  theme_classic()+
  geom_text(label=t1$ID,hjust=1,vjust=1.2)+
  labs(title=" ",x="Bacteral Biomass in Sample (%)",y="Protozoan Biomass in Sample (%)",shape="Biocrust
        Cover")+
  ylim(0,16)+
  annotate("text",label=expression(paste("R"^"2"," adjusted= 0.8167")),x=30,y=13)


#Figure: Plotting difference in nutrient concentrations for each sample.
t1<-ana2[-16,]#For outlier in Cu
avg<-
        c(mean(ana$Al_diff,na.rm=T),mean(ana$Ca_diff,na.rm=T),mean(t1$Cu_diff,na.rm=T),mean(ana$F
        e_diff,na.rm=T),mean(ana$K_diff,na.rm=T),mean(ana$Mg_diff,na.rm=T),

        mean(ana$Mn_diff,na.rm=T),mean(ana$Na_diff,na.rm=T),mean(ana$P_diff,na.rm=T),mean(ana$S
        _diff,na.rm=T),mean(ana$Zn_diff,na.rm=T))
se<-
        c(se(ana$Al_diff),se(ana$Ca_diff),se(t1$Cu_diff),se(ana$Fe_diff),se(ana$K_diff),se(ana$Mg_diff),s
        e(ana$Mn_diff),se(ana$Na_diff),
    se(ana$P_diff),se(ana$S_diff),se(ana$Zn_diff))
l<-c("Al","Ca","Cu","Fe","K","Mg","Mn","Na","P","S","Zn")
t2<-data.frame(avg,se,l)
ggplot(t2,aes(x=l,y=avg))+
  geom_bar(stat="Identity")+
  theme_classic()+
  labs(x="\nNutrients",y="Difference in Nutrients [Biocrust-Reference] (mg/kg)",title="\n")+
  ylim(-120,750)+
  geom_errorbar(aes(ymin=avg-se,ymax=avg+se),width=0.2)+
  annotate("text",x=9,y=600,label="* denotes significance",size=4)+
  annotate("text",x=1,y=200,label="*")+
  annotate("text",x=6,y=100,label="*")+
  annotate("text",x=9,y=100,label="*")+
  annotate("text",x=10,y=60,label="*")+
  annotate("text",x=5,y=220,label="*")+
  annotate("text",x=4,y=-120,label="*")


#Figure: Plotting change in cover type with Age
```

```
ggplot(ana,aes(x=ana$Dominant.Cover.Component,y=ana$Age..yrs.))+
  geom_boxplot(color='orange')+
  geom_point(colour='grey')+
  labs(x="\nBiocrust Cover Type",y="Age (yrs)\n")+
  theme_classic()+
  ylim(5,35)+
  annotate("text",x=1,y=18,label="a",size=4)+
  annotate("text",x=2,y=34,label="b",size=4)+
  annotate("text",x=3,y=27,label="c",size=4)+
  annotate("text",x=4,y=27,label="bc",size=4)

#Figure: Plotting Effect of Age on Mono:Poly
t1<-ana[-c(4,7),]
ggplot(t1,aes(x=t1$Age..yrs.,y=t1$Mono.Poly))+
  geom_point()+
  theme_classic()

#Figure: Experiment 2
ggplot(exp3,aes(x=exp3$treatment,y=exp3$chl.b,fill=exp3$treatment))+
  geom_boxplot()+
  theme_classic()+
  labs(x="\nTreatments",y="Chlorophyll b (ug/sq.cm)\n")+
  scale_x_discrete(label=c("Sand\n-no shade","Organics\n-no shade","Organic+ nutrients\n-no shade",
                           "Sand\n-husk polymer","Organics\n-husk polymer","Organic+ nutrients\n-husk polymer",
                           "Sand\n-shade panel","Organics\n-shade panel","Organic+ nutrients\n-shade panel"))+
  scale_fill_manual(values=c("White","White","White","White","White","White","white","white","white"))+
  theme(legend.position = "none")+
  annotate("text",x=2,y=1.8,label="**",size=6)+annotate("text",x=3,y=1.8,label="**",size=6)+
  annotate("text",x=5,y=1.8,label="**",size=6)+annotate("text",x=6,y=1.8,label="**",size=6)+
  annotate("text",x=8,y=1.8,label="**",size=6)+annotate("text",x=9,y=1.8,label="**",size=6)+
  ylim(0,2)

#Figure: scatter plot of PLFA vs Age
t1<-ana[-c(20,13),]
Cover<-t1$Dominant.Cover.Component
ggplot(t1,aes(x=t1$Age..yrs.,y=t1$Total.Biomass,shape=t1$Site,color=Cover))+
  geom_point(size=3)+
  geom_text(label=t1$ID,hjust=1,vjust=1.2)+
  geom_smooth(inherit.aes =
          F,aes(x=t1$Age..yrs.,y=t1$Total.Biomass),method="lm",se=FALSE,color="grey")+
```

```
  theme_classic()+
  labs(x="Calculated Age (years)",y="PLFA Biomass (ng/g)",shape="Mine")+
 # scale_colour_discrete(t1$Site)+
  theme(plot.title=element_text(hjust=0.5))
 # scale_y_continuous(labels = function(x){paste0(x/1000)}) #The original units were ng/g. Here, they are
            divided by 1000 and labeled as ug/g.

#Figure: PCA and microbial biomass
Age<-ana[,11]
Total<-ana[,16]
Bacteria<-ana[,19]
Actino.<-ana[,21]
Fungi<-ana[,27]
Protozoa<-ana[,33]
Undiff<-ana[,37]
Sat_Unsat<-ana[,40]

y<-cbind(Age,Total,Bacteria,Actino.,Fungi,Protozoa,Undiff,Sat_Unsat)
t1<-prcomp(y)
summary(t1)
autoplot(t1,data=ana,loadings.label=T,center=T,colour='Feature',frame=F)+
  theme_classic()+
  geom_text(label=ana$ID,hjust=0.5,vjust=-1)+
  labs(color="Tailings Feature")

#Figure
ggplot(ana,aes(x=ana$Feature,y=ana$percent.C/ana$percent.N))+
  geom_boxplot()+
  geom_jitter(aes(color=ana$Dominant.Cover.Component))+
  theme_classic()+
  labs(x="\nTailings Landscape Feature",y="Total C/N\n",color="Cover Type")+
  geom_text(label=ana$ID,hjust=1.5,vjust=-1,size=2)+
  ylim(14,25)+
  annotate("text",x=1,y=17.5,label="b")+
  annotate("text",x=2,y=21,label="a")+
  annotate("text",x=3,y=20.5,label="a")+
  annotate("text",x=4,y=18,label="c")+
  annotate("text",x=6,y=21.5,label="a")

#Figure; arbuscular fungi across biocrust cover types
```

```
ggplot(ana,aes(x=ana$Dominant.Cover.Component,y=ana$Arbuscular.Mycorrhizal.Biomass))+
  geom_boxplot()+
  theme_classic()+
  geom_jitter(aes(color=ana$Dominant.Cover.Component),width=0.1,shape='triangle',size=1.7)+
  theme(legend.position='none')+
  labs(x="Biocrust Cover Type",y="Arbuscular Mycorrhizal Biomass (ng/g)")+
  ylim(0,1050)+
  annotate("text",x=1,y=1050,label="a")+
  annotate("text",x=2,y=700,label="bc")+
  annotate("text",x=3,y=1030,label="c")+
  annotate("text",x=4,y=1030,label="ac")

#Figure: PLFA biomass vs Percent Carbon
t1<-ana[-c(17,16),]
ggplot(t1,aes(x=t1$Total.Biomass,y=t1$percent.C))+
  geom_point()+
  theme_classic()+
  labs(x="Total PLFA Biomass (ng/g)",y="Percent Carbon")+
  geom_smooth(method="lm",se=F,inherit.aes=F,aes(x=t1$Total.Biomass,y=t1$percent.C))+
  geom_text(label=t1$ID,hjust=1.5,vjust=-1,size=2)+
  annotate("text",x=20000,y=4.8,label=expression(paste("R"^"2"," adjusted= 0.5153")))
```