RESEARCH ARTICLE

# Potential Contribution of Native Herbs and Biological Soil Crusts to Restoration of the Biogeochemical Nitrogen Cycle in Mining Impacted Sites in Northern Canada

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## ABSTRACT

The nitrogen cycle is highly sensitive to pollutants and restoration of this biogeochemical pathway is essential to ensure long-term sustainable ecosystems. Due to their ability to fix nitrogen, some native herb species and Biological Soil Crusts (BSCs) may promote plant community growth and help to establish primary successional processes. In a greenhouse trial, growth and nitrogen fixation rates of yellow mountain aven (*Dryas drummondii*), alpine sweetvetch (*Hedysarum alpinum*), field locoweed (*Oxytropis campestris*) and arctic lupine (*Lupinus arcticus*) were determined in tailings and mining impacted soils with amendments of Rhizobia and biochar. In a growth chamber trial, Pure *Nostoc commune* culture, Dried *Nostoc* spp. and BSC slurries derived from mature soil crusts were applied with and without biochar to tailings. Arctic lupine had the highest biomass and all species except yellow mountain aven showed nitrogen fixation in tailings and mining impacted soils. Nodulation and nitrogen fixation only occurred in herbs given a Rhizobia inoculum, suggesting the use of nitrogen-fixing species in northern reclamation may require microbial amendments. When used in combination with Rhizobia inoculum, biochar may promote nitrogen fixation associated with herb species. BSC slurries from mature soil crusts had significantly higher mean rates of nitrogen fixation (95 μmol N m²/hr) compared with other soil crust species (5–23 μmol N m²/hr). Biochar, Rhizobial inoculants and native nitrogen fixing species can enhance revegetation and nitrogen input in northern mining impacted substrates. However, large scale field experiments are required to identify suitable landscapes and determine how these initial nitrogen fixing species influence subsequent succession.

**Keywords:** biochar, biological soil crusts, native species, nitrogen fixation, mine tailings, soil amendments, revegetation

Mining and processing activities result in large amounts of waste (ex. tailings) that can be toxic and hazardous. Establishment of a plant cover (i.e. phytostabilization) can provide a relatively simple and cost effective way to reduce mobilization and transportation of contaminants via wind and water (Bradshaw 1997, Lan et al. 1998, Ye et al. 2002, Liu et al. 2012). In addition to reducing exposure potential, revegetation of mining impacted sites should allow for the development of diverse self-sustaining plant communities (Shu et al. 2002, Singh et al. 2002, Hao et al. 2004, Sheoran et al. 2010) and aim to restore key biogeochemical pathways that support healthy ecosystem function. The use of indigenous or native species in restoration are preferable to exotic species because they can reestablish natural successional trajectories, are most likely to fit into fully functional ecosystems and are climatically adapted (Li et al. 2003, Chaney et al. 2007, Sheoran et al. 2010). However in northern ecosystems, very few native species are commercially available for use and are often not propagated within the area of origin and thus contain non-native genotypes (Matheus and Omtzigt 2013). In addition, revegetation techniques used with success in more southern locations are often unsuccessful in the North (Snow et al. 2009) for reasons that are not always clear. Using native nitrogen-fixing species for revegetation and restoration of the nitrogen cycle may be a potential strategy to increase restoration success in northern ecosystems impacted by mining activities.

The nitrogen cycle is highly sensitive to pollutants and restoration of this biogeochemical pathway is essential to ensure long-term sustainable ecosystems (Schafer et al. 2007, Bisset et al. 2013). In highly disturbed N-limited ecosystems biological nitrogen fixation is important for

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plant growth and nitrogen-fixing herbs and shrubs are generally believed to increase soil C and N availability and promote a more favorable balance between the production and immobilization of inorganic N (Bradshaw 1993, Myrold and Huss-Danell 2003). Nitrogen fixing legumes are often used in restoration efforts and several northern studies have observed the presence of nitrogen-fixing plants increased soil N content, however these legumes are often not native to the area of restoration (Van Cleve et al. 1993, Wurtz, 1995, Tordoff et al. 2000, Rhoades et al. 2001, Broos et al. 2004, Reichman 2007, Liu et al. 2012). Increases in soil N availability subsequently play a key role in facilitating colonization by later successional species (Chapin et al. 1994, Dobson et al. 1997, Huang et al. 2011), therefore $N_2$-fixing species not only play a key role in plant productivity but also affect plant community development and ecosystem processes at all scales (Zhan and Sun 2011).

Biological Soil Crusts (BSCs) are early successional communities, composed of bacteria, cyanobacteria, algae, mosses, liverworts, fungi, and lichens. BSCs are found to occur naturally throughout northern Canada (Stewart et al. 2011a) and these crusts may act as keystone communities in establishing primary successional processes and returning disturbed ecosystems to a desirable trajectory (Bowker 2007). Unlike commercial stabilization products, initial restoration efforts that incorporate the use of BSCs may offer soil protection, as well as initiate a number of biological processes promoting restoration of ecosystem functions (Bowker 2007, Doudle and Williams 2010). Nitrogen fixing cyanobacteria are common pioneering species during the amelioration and revegetation of degraded ecosystems and have been frequently regarded as biofertilizers and soil conditioners (Rao and Burns 1990, Zimmerman 1993, Acea et al. 2001). Inoculation of soils with cyanobacterial species leads to the formation of organo-mineral aggregates composed of cyanobacterial filaments and extracellular polysaccharides (EPS), where coating, enmeshment, binding and gluing of aggregates and isolated mineral particles significantly improves soil stability (Zimmerman 1993, Neuman et al. 1996, Zulpa de Caire et al. 1997, Acea et al. 2001, Malam Issa et al. 2001, Malam Issa et al. 2007). In addition, EPS increase soil organic matter content and can be an important source of carbon helping to ensure microbial growth and survival in soils by their capacity to buffer nutrient supply to microorganisms closely associated with their surfaces (Zulpa de Caire et al. 1997). BSCs can also change the spatiotemporal pattern of soil moisture and influence re-allocation of moisture by decreasing rainfall infiltration, increasing topsoil water-holding capacity and altering evaporation (Spröte et al. 2010, Li et al. 2010). These changes in hydrologic conditions within the soil are important in controlling floristic and structural changes in vegetation.

Natural recolonization of native plants on mine tailings is often limited since these degraded materials tend to have no aggregate structure, low pH, low organic matter and are deficient in nutrients (N and P), as well as having high toxicity associated with metals and metalloids (Pb, Zn, Cu, Cd, Mn, Ni, and As) (Bradshaw and Johnson 1992, Ye et al. 2002, Petrisor et al. 2004, Huang et al. 2011). Hence, the use of soil amendments may be necessary to allow for successful germination and growth of native species. Several studies have found biochar, a product that results from the oxygen limited pyrolysis of various biological ingredients, can result in significant decreases in the bioavailability of heavy metals associated with mining impacted soils (Namgay et al. 2006, Beesley and Marmiroli 2011, Fellet et al. 2011) and simultaneously improve physical, chemical, and biological soil properties (Laird et al. 2010). Biochar has many benefits for the environment and has been investigated extensively in southern climates, but very few studies have examined its use in northern reclamation and restoration.

The objective of this study is to examine the potential of nitrogen-fixing native herbs, biological soil crusts (BSCs), and biochar soil amendments to improve soil conditions and promote long term revegetative success and nitrogen input in northern mining impacted soils. We conducted two trials: 1) a 3 month greenhouse trial examining four nitrogen-fixing native herb species, and 2) a 101 day growth chamber trial examining nitrogen-fixing BSCs. In the greenhouse trial we compared germination, above and belowground growth, nodulation and nitrogen fixation as response variables of yellow mountain aven (*Dryas drummondii* [Richardson ex Hook.]), alpine sweetvetch (*Hedysarum alpinum* [L.]), field locoweed (*Oxytropis campestris* [L.]) and arctic lupine (*Lupinus arcticus* [S. Watson]) grown in both tailings and mining impacted soils. In the growth chamber trial we compared net photosynthesis, dark respiration and nitrogen fixation as response variables of Pure *Nostoc commune* culture, Dried *Nostoc* spp. and BSC slurries derived from mature soil crusts grown on tailings. We also examined these same response variables for mature BSC slurries grown on mining impacted soils. In the greenhouse trial we examined the impact of Rhizobia inoculums and biochar on the above response variables of four native herb species and in the growth chamber trial we examined the impact of biochar on the above response variables of BSCs. The aim of this study was to identify the optimal initial mix to be used in longer term field studies for mine site restoration.

## Methods

### *Site Description*

Soil amendments and nitrogen-fixing herb species were examined in a greenhouse trial at the Yukon Research Centre, Whitehorse, Yukon. Tailings and mining impacted soils for the greenhouse trial were taken from the Keno Hill Silver District, which is one of the world's highest-grade silver districts located 330 km north of Whitehorse, Yukon,

Canada. It is estimated that approximately 4,050,000 tons of tailings were deposited at a 130-ha site, known as the Valley Tailings, located in the McQuesten River Valley (63°55′26.4N, 135°29′76.1W). The tailings are highly variable with a pH ranging from 5.7 to 8.4 and texture varying from silt loam to sand. The tailings exceed the Canadian Council of Ministers of the Environment (CCME) industrial soil quality guidelines for allowable levels of Antimony (Sb), Arsenic (As), Cadmium (Cd), Copper (Cu), Lead (Pb), Silver (Ag), Titanium (Ti), and Zinc (Zn). Mining impacted soils from Husky SW (63°54′18.9 N, 135°31′45.1W), 1.8 km from Valley Tailings, were also collected on-site. The Husky SW soils are currently being used in an engineered cover design trial as a mining impacted soil. The pH of these soils ranges from 8.0 to 8.4 and they have a loam texture and organic carbon content of 0.47%. The soils exceed the CCME industrial soil quality guideline for allowable limits of As.

The original site consisted of boreal vegetation including small trees (*Picea glauca*, *Picea mariana*, *Populus tremuloides*, and *Populus balsamifera*), as well as, shrubs (*Salix* spp.) and vegtative moss mats (*Sphagnum* spp., *Pluerozium* spp.). Vegetation in the Valley Tailings area was eventually covered by tailings, which range from 0.1 to over 4m in thickness (Keller et al. 2010). Mean January temperature in the Keno area is –26.9°C, while mean July temperature is 15.6°C. However, summer temperatures in the region can exceed 25°C and winter temperatures –50°C. The average total precipitation is 322 mm and discontinuous permafrost is found throughout the area (Clark and Hutchinson 2005).

### *Nitrogen-Fixing Herb Greenhouse Trial*

We used a full factorial design with two soil types (Tailings and Cover), four local native nitrogen-fixing species (yellow mountain aven, alpine sweetvetch, field locoweed, and arctic lupine) and four soil amendments (biochar, Rhizobia, biochar and Rhizobia, and fertilizer only). Each treatment combination had 12 replicates for a total of 384 samples. Each sample comprised of an individual container that was 3.8 cm in diameter, 21cm deep, and had a volume of 164 ml (Ray-Leach Tubes, Stuewe & Sons, Tangent, Oregon).

Seeds from the four local native nitrogen-fixing species were collected within the Keno area throughout August 2012. In addition, for each species the belowground systems of a number of plants were excavated, examined and sampled for Rhizobia or Frankia nodules between August 29–September 3, 2012. Nodules were kept in the refrigerator without light at 4°C until used.

Three different soil amendments and a control treatment were used: biochar (BC); Rhizobia (R); biochar and Rhizobia (BCR); and a control with fertilizer only (C). The biochar (BC) was a phosphorus-rich bone meal biochar (2–14–0) pyrolyzed at a temperature of 450°C for 6 hours. The grain size of the finished product was ≤ 2mm. The biochar (1 kg/m²) was mixed with deinonized (DI) water and 5 ml of biochar slurry was added to each container. Nodules previously collected were masticated in DI water to create Rhizobia slurries or for yellow mountain aven, a Frankia slurry (S. Berch, British Columbia Ministry of Environment Conservation Science Section, pers. comm.). Seeds receiving the R and BCR treatments were soaked for 3 hours within the slurry prior to planting. Slurries contained 1.4 mg/ml of masticated nodules (wet weight). Containers receiving the R treatment were given 2.5 ml of slurry and 2.5 ml of DI water, which was poured onto the surface of each container. All containers received two seeds of one of the four species and fertilizer (19:19:19) at a rate of 110 kg/ha. Control soils were either tailings with fertilizer or mining impacted soils with fertilizer and all treatments were fertilized at the given rate to mimic the current industry standard for restoration being carried out on the Keno site. The trial was initiated on September 11, 2012.

The greenhouse conditions and watering were controlled to reflect typical summer growing conditions in the Keno area. Temperature was 11°C with no light from 22:00–4:00 and 16°C with 175 µmol/m²/s of light from 4:00–22:00. Each replicate was watered every second day with 6 ml of DI.

From December 6–13, 2012 containers were sampled for germination rate, number of observable nodules, above and belowground biomass, and nitrogen fixation. Measurements of $N_2$-fixation were made using acetylene reduction assays (ARA) (Stewart et al. 1967). Plants were harvested from each container (with belowground systems kept intact) and placed in a separate 60 ml amber glass vial with a teflon septa cap. Each amber vial was injected with 10% (v/v) acetylene gas ($C_2H_2$) and incubated in the dark at 20°C for 4 hours. Ethylene concentrations were measured with a portable gas chromatograph (SRI 8610A, Wennick Scientific Corporation, Ottawa, ON, Canada) fitted with a Porapak column (Alltech Canada, Guelph, ON, Canada) and a flame ionization detector (Stewart et al. 2011b,c).

### *Biological Soil Crust Growth Chamber Trial*

Both the mining impacted soils from Husky SW and Valley Tailings used in the greenhouse trial were used in the growth chamber trial. The substrates were collected throughout the summer of 2012 and were autoclaved at 120°C for 1 hour prior to use to provide a sterile medium free of any pre-existing soil microorganisms. Autoclaved soils were lightly packed into petri dishes (8.8 cm diameter, 1.2 cm height, 60.82 cm² surface area) leaving 0.5 cm for addition of slurry treatments to the surface.

Six different treatments were applied as slurries to the Valley Tailings (T): Pure *Nostoc commune* culture (UTEX Culture Collection of Algae, University of Texas) with biochar (NC BC T) and without biochar (NC T), Dried *Nostoc* spp. collected from grasslands near Haines Junction, Yukon with biochar (ND BC T) and without biochar

(ND T), and biological soil crust slurry from mature soil crusts collected at Husky SW with biochar (S BC T) and without biochar (S T). Due to limited growth chamber space and our focus on how the BSC treatments responded on tailings, only two treatments were applied to the Husky SW mining impacted soils (C): Biological soil crust slurry from mature soil crusts collected at Husky SW with biochar (S BC C) and without biochar (S C). Each treatment on each soil type had 10 replicates (i.e. petri dishes) for a total of 80 samples. The *Nostoc commune* (NC) treatment was created by centrifuging *N. commune* held in an aqueous BG-11 growth medium (UTEX Culture Collection of Algae, University of Texas) to provide 0.5 g wet weight of cyanobacteria per replicate. The dried *Nostoc* spp. treatment (ND) was created by passing dried gelatinous globules of macroalgae with cyanobacterial filaments through a 2 mm sieve and adding 0.4 g of dried *Nostoc* spp. to each replicate. Biological soil crusts were collected at the Husky SW site on August 29th, 2012. A visual survey of crusts in the area was conducted and approximately 1 $m^2$ of crust including the dominant lichen (*Stereocaulon* sp.), moss (*Certodon purpureus*), liverworts (not identified), and free-living cyanobacteria (*Nostoc commune*) species were sampled. The top 2–3 cm of the surface were removed with a knife and crusts were placed in plastic containers for transport to the Yukon Research Centre laboratory. Crusts were stored at 5°C under shaded conditions for 12 days until the trial was initiated on September 10, 2012. The biological soil crust slurry was created by homogenizing representative crust fragments, including lichens, through a 2 mm sieve after the underlying soil was removed leaving fragments approximately 1 cm thick. Approximately 6 g wet weight of crust was added per replicate. For treatments receiving bone meal biochar, biochar was added at a rate of 1 kg/$m^2$. All treatments also received a commercial fertilizer (19:19:19) that was pulverised with a mortar and pestle and added at a rate of 110 kg/ha (i.e. 5.9 g per petri dish). For each treatment slurries were created by adding nitrogen fixers/crust, biochar, and fertilizer to 100 ml of DI water and 10 ml of slurry was added to each replicate. Prior to the addition of treatment slurries 10 ml of DI water was added to the substrate surface in each petri dish.

All samples were placed in a growth chamber (Conviron Adaptis A1000, Winnipeg, MB, Canada) on September 10, 2012 which had a diurnal cycle of temperature ranging from 9.9–19.3°C, relative humidity from 47–81% and light from 0–200 $\mu/m^2/s^1$ with darkness from 22:00–4:00 hrs. Growth chamber climatic settings were based on climate data from the last 10 years in the Keno area. DI water (6 ml) was added to each petri dish every second day for the duration of the experiment.

From December 19–22, 2012 petri dishes were randomly selected and net photosynthesis, dark respiration and nitrogen fixation were measured for each replicate. Each petri dish was placed within a 450 ml clear glass incubation chamber and sealed with high vacuum grease. Rates of net photosynthesis and dark respiration were calculated from changes in $CO_2$ concentration within incubation chambers over approximately 30 minutes (LI-840A $CO_2$/$H_2O$ analyzer, Li-Cor, Lincoln, Nberaska, USA). Nitrogen fixation was measured immediately after photosynthesis and respiration using the same ARA method described above, except that samples were incubated for 4 hours under at 20°C and 200 $\mu/m^2/s^1$ of light.

### *Statistical Analyses*

Data from the greenhouse and growth chamber trials were examined to ensure the assumptions of Analysis of Variance (ANOVA) were met and log transformations were performed on some variables. Greenhouse and growth chamber trial data were analyzed using full factorial ANOVAs. For the greenhouse trial five response variables (germination, nodulation, aboveground biomass, belowground biomass, and nitrogen fixation) were examined with substrate, species and treatment (i.e soil amendment) considered independent variables. For the growth chamber trial three response variables were examined (net photosynthesis, dark respiration, and nitrogen fixation). The application of BSCs to mining impacted soils was only conducted for BSCs derived from mature soil crusts; therefore, two sets of analyses were performed for BSCs: 1) all BSC species on tailings only where species and treatment were considered independent variables; and 2) only mature crust slurry on both tailings and mining impacted soils where substrate, species and treatment were considered independent variables. TukeyHSD post hoc tests were carried following all ANOVAs. All analyses were conducted in R (R package version 2.1.50).

## Results

### *Nitrogen-Fixing Herb Greenhouse Trial*

Overall mining impacted soils had significantly lower germination rates (42% vs. 53%) but higher rates of nodulation (3.8 nodules vs. 0.3 nodules) and nitrogen fixation (209 μmol ethylene/$m^2$/hr vs. 23 μmol ethylene/$m^2$/hr) compared with tailings (Table 1). Aboveground and belowground biomass were not significantly different between the two substrate types ($p = 0.47$ and $p = 0.63$ respectively). However, average root length was significantly longer in the tailings (11 cm) compared with mining impacted soils (9 cm) ($p < 0.001$).

Field locoweed had significantly lower germination rates (28%) compared with all other species (yellow mountain aven = 52%, alpine sweetvetch = 51%, arctic lupine = 59%), which did not differ significantly from each other (Table 1). The BC treatment (34%) had lower germination compared with all other soil amendment treatments (BCR = 54%, F = 51%, R = 52%, $p < 0.001$ for all comparisons).

**Table 1. Analyses of Variance comparing germination, nodulation, aboveground and belowground biomass, and nitrogen fixation of native nitrogen fixing herbs by substrate (tailings and mine impacted soil), species (yellow mountain aven, alpine sweetvetch, field locoweed, and arctic lupine), treatment (fertilizer only, biochar and fertilizer, Rhizobia, and fertilizer and biochar, Rhizobia and fertilizer) and all interactions. *Indicates means are significantly different ($p < 0.05$).**

| | Df | Sum Sq | Mean Sq | F | *p* |
|---|---|---|---|---|---|
| ***Germination*** | | | | | |
| Substrate* | 1 | 13184 | 13184 | 11.0 | <0.01 |
| Species* | 3 | 52279 | 17426 | 14.5 | <0.01 |
| Treatment* | 3 | 23646 | 7882 | 6.56 | <0.01 |
| Substrate x Species* | 3 | 12910 | 4304 | 3.58 | 0.01 |
| Substrate x Treatment | 3 | 4594 | 1531 | 1.27 | 0.28 |
| Species x Treatment | 9 | 13261 | 1474 | 1.23 | 0.28 |
| Substrate x Species x Treatment | 9 | 17067 | 1896 | 1.58 | 0.12 |
| ***Nodulation*** | | | | | |
| Substrate* | 1 | 1180 | 1180 | 30.8 | <0.01 |
| Species* | 3 | 1695 | 565 | 14.8 | <0.01 |
| Treatment* | 3 | 910 | 303 | 7.92 | <0.01 |
| Substrate x Species* | 3 | 1259 | 420 | 11.0 | <0.01 |
| Substrate x Treatment* | 3 | 611 | 203 | 5.32 | <0.01 |
| Species x Treatment* | 9 | 3791 | 421 | 11.0 | <0.01 |
| Substrate x Species x Treatment* | 9 | 2565 | 285 | 7.44 | <0.01 |
| ***Aboveground biomass*** | | | | | |
| Substrate | 1 | 0.0005 | 0.0005 | 0.52 | 0.47 |
| Species* | 3 | 0.31 | 0.10 | 108 | <0.01 |
| Treatment* | 3 | 0.02 | 0.01 | 7.99 | <0.01 |
| Substrate x Species | 3 | 0.002 | 0.0006 | 0.63 | 0.59 |
| Substrate x Treatment* | 3 | 0.009 | 0.003 | 3.30 | 0.02 |
| Species x Treatment* | 9 | 0.05 | 0.006 | 6.24 | <0.01 |
| Substrate x Species x Treatment* | 9 | 0.04 | 0.005 | 4.99 | <0.01 |
| ***Belowground biomass*** | | | | | |
| Substrate | 1 | 0.001 | 0.001 | 0.23 | 0.63 |
| Species* | 3 | 1.90 | 0.63 | 120 | <0.01 |
| Treatment* | 3 | 0.08 | 0.03 | 5.04 | <0.01 |
| Substrate x Species* | 3 | 0.06 | 0.02 | 3.50 | 0.02 |
| Substrate x Treatment* | 3 | 0.07 | 0.02 | 4.64 | <0.01 |
| Species x Treatment* | 9 | 0.11 | 0.01 | 2.26 | 0.02 |
| Substrate x Species x Treatment* | 9 | 0.21 | 0.02 | 4.39 | <0.01 |
| ***Nitrogen fixation*** | | | | | |
| Substrate* | 1 | 1.9 | 1.9 | 6.6 | 0.01 |
| Species* | 3 | 10 | 3.5 | 12 | <0.01 |
| Treatment* | 3 | 24 | 7.9 | 28 | <0.01 |
| Substrate x Species* | 3 | 2.8 | 0.9 | 3.2 | 0.02 |
| Substrate x Treatment* | 3 | 6.4 | 2.1 | 7.5 | <0.01 |
| Species x Treatment* | 9 | 12 | 1.3 | 4.6 | <0.01 |
| Substrate x Species x Treatment* | 9 | 7.0 | 0.8 | 2.7 | <0.01 |

Arctic lupine had significantly higher average aboveground biomass and belowground biomass compared with all other species ($p < 0.001$ and $p < 0.05$ for all comparisons respectively, Figure 1A). Alpine sweetvetch had significantly higher average belowground biomass than yellow mountain aven and field locoweed ($p < 0.05$ for both comparisons, Figure 1A). The BC soil amendment treatment had lower average aboveground biomass compared with all other soil amendments ($p < 0.05$ for all comparisons, Figure 1B). The BC soil amendment treatment also had significantly lower average belowground biomass than the BCR and R treatments ($p < 0.05$ for both comparisons, Figure 1B).

Arctic lupine had the highest average rate of nodulation (3.9 nodules) and was significantly higher than both field locoweed (1.3 nodules) and yellow mountain aven (0 nodules) ($p < 0.05$ and $p < 0.01$ respectively, Table 1). Alpine sweetvetch had the second highest average rate of nodulation (3.1 nodules). The R and BCR treatments had higher average rates of nodulation (4.7 and 3.6 nodules

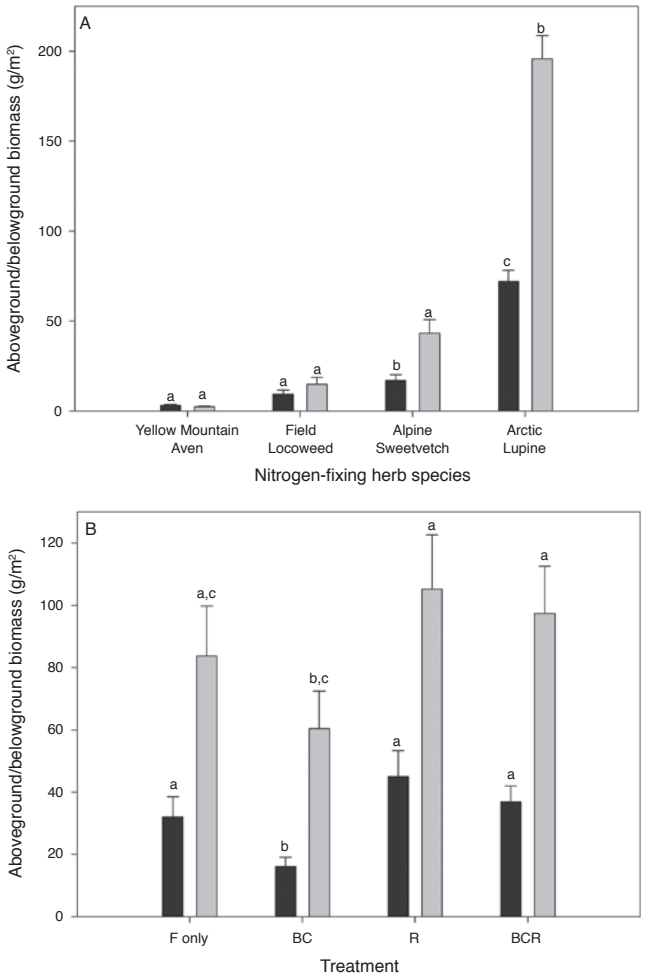


**Figure 1. A. Aboveground (black bars) and belowground (grey bars) biomass of four native nitrogen-fixing herb species (Yellow Mountain Aven, Field Locoweed, Alpine Sweetvetch, and Arctic Lupine) after 12 weeks of growth in a greenhouse trial. B. Aboveground and belowground biomass for each substrate treatment (Fertilizer only (F only), biochar and fertilizer (BC), Rhizobia and fertilizer (R), and biochar, Rhizobia, and fertilizer (BCR)). Bars are means with SE. Different letters indicate significantly different means between species or treatments for comparisons of above or belowground biomass (ANOVA, Tukey post hoc; $p < 0.05$). Different letters do not compare between above and belowground biomass for species or treatments. Overall Arctic Lupine had significantly higher aboveground and belowground biomass than all other species. The BC treatment had lower aboveground biomass compared with all treatments and lower belowground biomass than the BCR and R treatments.**

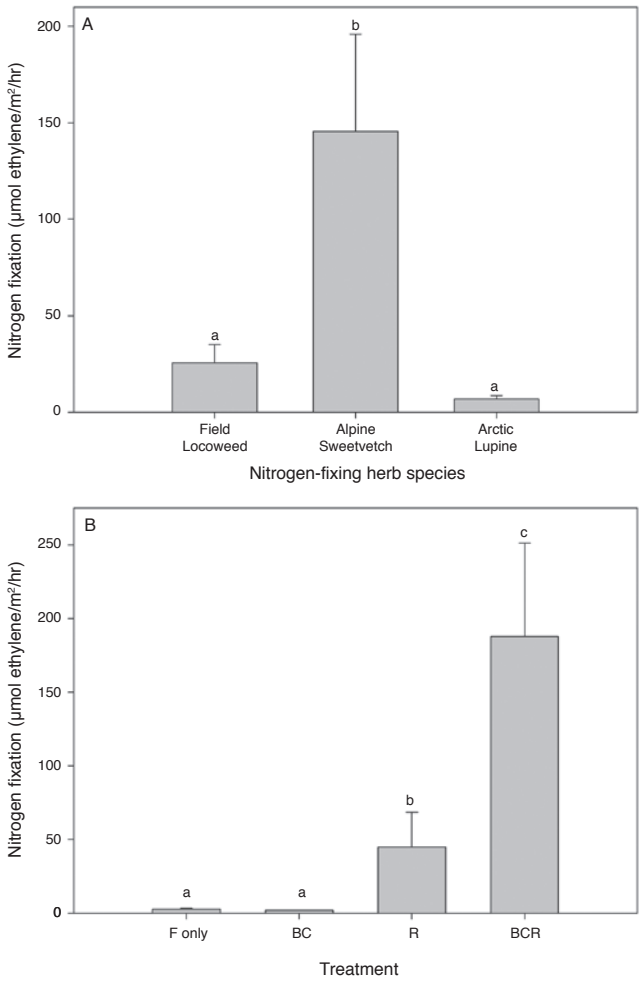


**Figure 2. A. Nitrogen fixation by three herb species after 12 weeks of growth in a greenhouse trial (No fixation was detected for Yellow Mountain Aven so it was not included). B. Nitrogen fixation for each substrate treatment (Fertilizer only (F only), biochar and fertilizer (BC), Rhizobia and fertilizer (R), and biochar, Rhizobia and fertilizer (BCR)). Bars are means with SE. Different letters indicate significantly different means between species or between treatments for nitrogen fixation (ANOVA, Tukey post hoc; $p < 0.05$). Overall Alpine Sweetvetch had significantly higher rates of nitrogen fixation compared to all other species. The BCR treatment had higher rates of nitrogen fixation compared with all other treatments and no significant difference was detected between the F only and BC treatments.**

respectively) than the BC and F treatments (0 and 0.02 nodules respectively, $p < 0.001$ for all comparisons; Table 1).

All species with the exception of yellow mountain aven demonstrated nitrogen fixation after 3 months. Alpine sweetvetch had significantly higher mean rates of nitrogen fixation compared with both field locoweed and arctic lupine ($p < 0.01$, Figure 2A). Overall mining impacted soils had higher rates of nitrogen fixation compared with tailings (209 µmol ethylene/m²/hr vs. 23 µmol ethylene/m²/hr, $p < 0.01$, Table 1). However, only those samples treated with the Rhizobia inoculum in both mining impacted soils and tailings had nitrogen fixation above our detection limit (10 µmol ethylene/m²/hr) (Figure 2B). The highest rates of nitrogen fixation occurred in mining impacted soils

**Table 2. Analyses of Variance comparing net photosynthesis, dark respiration and nitrogen fixation for all biological soil crust treatments by soil crust species (Pure *Nostoc commune* culture, dried *Nostoc* spp., and mature crust slurry), treatment (fertilizer only and biochar and fertilizer), and all interactions. Analyses of Variance comparing net photosynthesis, dark respiration, and nitrogen fixation for mature crust slurry by substrate (tailings and mine impacted soil) treatment (fertilizer only and biochar and fertilizer) and all interactions. *Indicates means are significantly different ($p < 0.05$).**

| Comparison of All Biological Soil Crusts | Df | Sum Sq | Mean Sq | F | *p* |
|---|---|---|---|---|---|
| ***Net photosynthesis*** | | | | | |
| Species | 2 | 0.00002 | 0.00001 | 0.12 | 0.89 |
| Treatment | 1 | 0.0003 | 0.0003 | 2.81 | 0.10 |
| Species x Treatment | 2 | 0.0003 | 0.00007 | 0.80 | 0.45 |
| ***Dark respiration*** | | | | | |
| Species | 2 | 0.03 | 0.02 | 1.89 | 0.16 |
| Treatment | 1 | 0.001 | 0.001 | 0.14 | 0.71 |
| Species x Treatment | 2 | 0.02 | 0.01 | 1.40 | 0.26 |
| ***Nitrogen fixation*** | | | | | |
| Species* | 2 | 18.0 | 9.0 | 109.7 | <0.01 |
| Treatment* | 1 | 0.38 | 0.38 | 4.68 | 0.03 |
| Species x Treatment* | 2 | 2.51 | 1.25 | 15.3 | <0.01 |
| **Comparison of Mature Crust Slurry Only** | **Df** | **Sum Sq** | **Mean Sq** | **F** | ***p*** |
| ***Net photosynthesis*** | | | | | |
| Substrate | 1 | 0.00005 | 0.00005 | 1.12 | 0.30 |
| Treatment | 1 | 0.0000001 | 0.000000009 | 0.0002 | 1.0 |
| Substrate x Treatment | 1 | 0.0001 | 0.0001 | 3.23 | 0.08 |
| ***Dark respiration*** | | | | | |
| Substrate | 1 | 0.01 | 0.01 | 1.61 | 0.21 |
| Treatment* | 1 | 0.03 | 0.03 | 4.75 | 0.04 |
| Substrate x Treatment | 1 | 0.0003 | 0.0003 | 0.05 | 0.83 |
| ***Nitrogen fixation*** | | | | | |
| Substrate | 1 | 0.02 | 0.02 | 0.27 | 0.60 |
| Treatment | 1 | 0.01 | 0.01 | 0.25 | 0.62 |
| Substrate x Treatment | 1 | 0.05 | 0.05 | 0.83 | 0.40 |

with the BCR treatment and average nitrogen fixation was significantly higher for the BCR treatment than the R treatment regardless of substrate types ($p < 0.01$, Figure 2B). The highest rate of nitrogen fixation detected was for alpine sweetvetch in mining impacted soils with the BCR treatment, which was significantly higher than for arctic lupine under the same conditions (815 µmol ethylene/$m^2$/hr vs. 12 µmol ethylene/$m^2$/hr, $p = 0.02$, Table 1).

## *Biological Soil Crust Growth Chamber Trial*

Establishment and growth of biological soil crusts derived from mature crusts on-site (S) were highly successful on both tailings and mining impacted soils within the growth chamber. Lichens, mosses, *Nostoc* spp. and the occasional recruitment of naturally occurring grasses were observable after 10 weeks of incubation (Figure 3).

There were no significant differences in net photosynthesis or dark respiration between the different cyanobacterial soil amendments ($p = 0.89$ and $p = 0.16$ respectively, Table 2). However, biochar may influence both photosynthesis and respiration, although these differences are likely strongly influenced by the substrate type and cyanobacterial amendment. When only treatments in tailings were considered, treatments with biochar tended to have higher rates of net photosynthesis (–1.25 ppm $CO_2$/min vs. –0.33 ppm $CO_2$/min, $p = 0.09$). While slurries from mature soil crusts (S) with biochar had lower rates of dark respiration on both tailings and mining impacted soils (1.21 ppm $CO_2$/min vs. 2.76 ppm $CO_2$/min, $p = 0.04$, Table 2).

All nitrogen fixing slurries created from biological soil crusts harvested from on-site at Keno (S) had significantly higher rates of nitrogen fixation compared with both the pure *Nostoc commune* culture (NC) and dried *Nostoc* sp. slurries (ND), with the exception of the ND and S fertilizer only treatment on tailings, which were not significantly different at $p = 0.05$ (Figure 4). Inclusion of biochar in the ND treatment resulted in significantly lower nitrogen fixation rates (Figure 4). There were no significant differences in nitrogen fixation between the substrate types (mining impacted soils vs. tailings) for the mature soil crusts slurry ($p = 0.60$, Table 2). In addition, there were no significant difference in treatment (fertilizer only vs. biochar) for the mature soil crust slurry ($p = 0.62$, Table 2).

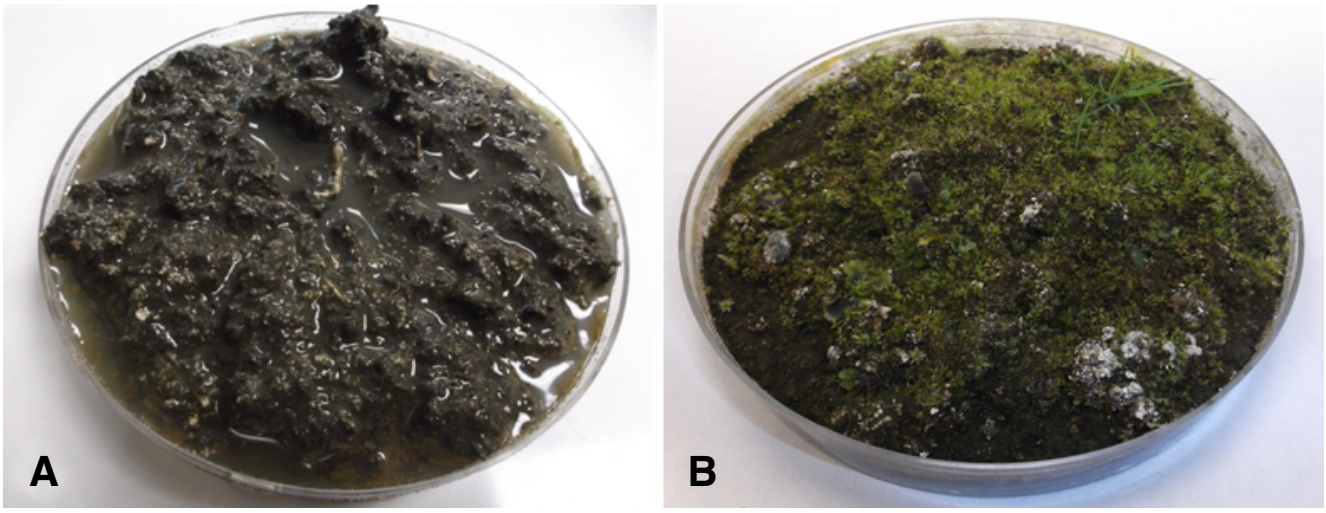


**Figure 3. Establishment and growth of biological soil crust from a slurry derived from mature crust found at Keno Hills on mining impacted soils immediately after application (A) and following 10 weeks of incubation in the growth chamber (B).**

## Discussion

Revegetation of mining impacted sites, particularly in northern environments, has several challenges due to high levels of acidity, low nutrient content, especially N, low water-holding capacity, low organic matter, and short growing seasons (Ye et al. 2002, Chan et al. 2003, Petrisor et al. 2004, Matheus and Omtzigt 2013). Reestablishment of natural successional trajectories on reclaimed areas is important and the processes of nutrient accumulation are critical towards the development of soils that support a properly functioning ecosystem. The use of local native nitrogen-fixing herbs and BSCs in restoration is currently an underexploited opportunity and exploration of techniques that leads to large-scale implementation are needed (Maestre et al. 2006, Bowker 2007). In our greenhouse and growth chamber trials we identified northern nitrogen-fixing species and soil amendments that show potential for restoration of the biogeochemical nitrogen cycle.

Of the species examined here, arctic lupine may be an important species to consider in restoration efforts aimed at promoting biomass accumulation and N input, since arctic lupine had the highest above and belowground biomass and rates of nodulation. We also found no differences in biomass between the tailings and mining impacted soils, indicating that some species may be useful on a range of mining impacted substrates. Myrold and Huss-Danell (2003) found that plots with Lupine had about twice as much soil N in the top 0.15 m of mineral soil in comparison with control plots without nitrogen fixers (i.e. 187 g N /m² vs. 97 g N /m²) and annual input from Lupine plots were 3.9 g N m²/yr 20 years after establishment in a degraded forest site in northern Sweden. The estimated rate of nitrogen fixation for arctic lupine observed in our study was ~ 0.5 g N m²/yr (i.e. 6.73 µmol ethylene m²/hr with a 3:1 conversion ratio assuming 8 hours of fixation over a 3 month growing season), which is substantially lower, however our plants were in mining impacted soils and tailings and measurements were taken only three months after seeding. Toxic tailings often have very low fixation rates (i.e. < 1 g N m²/yr) (Liu et al. 2012) although there are reports of rates up to 150 kg N ha/yr (Skeffington and Bradshaw 1980, Liu et al. 2012).

Nodulation and nitrogen fixation only occurred in native herb samples that were given a Rhizobia inoculum,

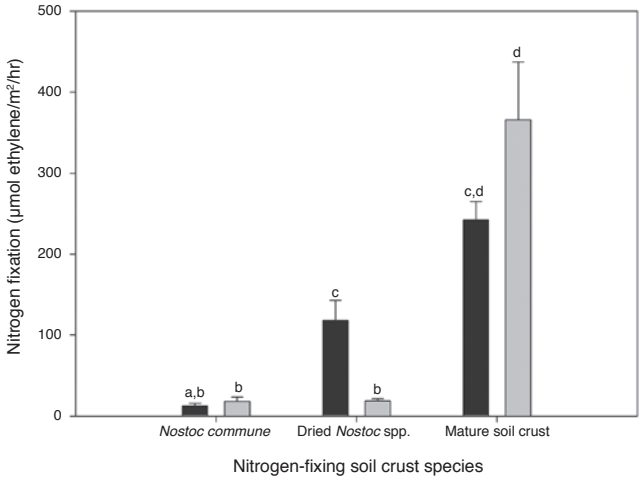


**Figure 4. Nitrogen fixation rates of biological soil crust slurry species after 101 days in a growth chamber experiment on tailings. All soil crust species were added as slurries with fertilizer only (F; black bars) or biochar with fertilizer (BC; grey bars). Bars are means with SE. Different letters indicate significantly different means (ANOVA, Tukey post hoc; $p < 0.05$, except for mature soil crust and dried *Nostoc* spp. with fertilizer only where $p = 0.05$).**

indicating that nodulation in these soils is unlikely to occur naturally within a few months and the use of nitrogen-fixing species in northern reclamation may require microbial amendments. We found higher nodulation and nitrogen fixation rates for both the R and BCR treatments, but the BCR treatment had significantly higher nitrogen fixation rates. Robertson et al. (2012) did not find any differences in the rate of nodulation of lodgepole pine (*Pinus contorta var. latifolia*) or sitka alder (*Alnus viridis ssp. sinuata*) in subboreal forest soils amended with biochar. However, nitrogen fixation was observed to continue for longer in biochar-treated systems vs. non-biochar treated systems. Through modification of the soil habitat (i.e. potential increase in energy availability, water and protective microsites), biochar has been shown to increase soil microbial biomass (Liang et al. 2010, O'Neill et al. 2009, Lehmann et al. 2011). Therefore, by combining a Rhizobia inoculum with biochar, growth and function may be promoted resulting in higher rates of nitrogen fixation and hence N input on mine impacted sites.

The biochar only treatment (BC) had lower rates of germination and aboveground and belowground biomass. Biochar is known to reduce bulk density and increase water holding capacity and nutrient availability due to increased cation exchange capacity, which can promote plant growth (Lehmann et al. 2011). However, under less harsh environmental conditions (i.e. species watered every second day) and in soils with higher water-holding capacity, biochar may play a less important role in promoting germination and growth. In another study conducted in-situ on the Valley Tailings we found that the combined treatment of dolomite lime (54.6% $CaCO_3$, 41.5% $MgCO_3$) and phosphorus-rich bone meal biochar resulted in higher germination and aboveground biomass of native grasses (Stewart et al. 2013). Under field conditions where moisture is limiting, therefore, biochar may play a more important role in germination and growth. In addition, bone meal biochar may reduce the availability of toxic heavy metals and/or provide a long-term slow-release source of phosphorus. It should be noted that this liming effect has been shown to increase As mobility and restrict revegetation (Beesely et al. 2011). Therefore, care should be taken to determine how various types of biochar may interact with the elements and conditions at a given site and influence plant uptake of contaminants and nutrients.

The establishment, growth and nitrogen fixation of BSCs applied as slurries derived from mature crusts on-site (S) was highly successful with establishment of lichens, mosses and *Nostoc* spp. globules within three months. Natural recovery rates of BSCs may be very slow. Under favorable conditions it can take 5–7 years for algal and bacterial components to re-establish and estimates range from a few years to 100 years for mature crust development (St. Clair et al. 1986, Belnap 1993). However, inoculation has shown to significantly hasten recovery (Belnap 1993) and may be a means to accelerate the restoration process on mine tailings (Spröte et al. 2010, Liu et al. 2012). For example, Xiao et al., (2008) demonstrate that it is feasible to inoculate and cultivate artificial BSCs using a method of crushing and broadcast sowing natural BSC collected on-site. In their study, artificial crust coverage reached 30–60% and the main BSCs components were the same as the collected crusts. Our study indicates that under laboratory conditions, tailings and mining impacted soils inoculated with BSCs leads to well established soil crust communities in a relatively short period of time.

The effectiveness of cyanobacterial inoculum as a biofertilizer for accelerating soil recolonization following disturbance is dependent upon both the class of inoculums (i.e. types of cyanobacteria present) and the type of soil (Acea et al. 2001). While nitrogen fixation rates for mature soil crust slurries were relatively high, nitrogen fixation from dried *Nostoc* sp. (ND) and pure *Nostoc commune* culture (NC) were very limited. Nitrogen fixation rates for NC were below our detection limits and it is likely that these cyanobacteria were unable to survive and/or fix nitrogen on the tailings and mining impacted soils. The rapid transition from an aqueous (i.e. growth medium) to solid (i.e. tailings and soils) environment may account for the low success rate with this type of amendment. In the environment these cyanobacteria are exposed to natural drying and wetting cycles and *Nostoc* cells produces EPS in response to stress conditions. However, laboratory cultures that have not been exposed to these desiccation cycles generally have only small amounts of EPS and therefore, may be highly sensitive to desiccation (Tamaru et al. 2005).

Higher rates of nitrogen fixation associated with BSC treatments derived from mature crusts on-site (S) may also be related to moisture dynamics. Nitrogen fixation changes with crust succession due to changes in species composition, increases in biomass during BSC development and increasing polysaccharide material, which slows water loss and lengthens active time (Belnap 1996, Zhao et al. 2010). *Nostoc* spp. which can fix substantial amounts of N tend to colonize BSCs only after substantial cover of moss is developed and may account for higher rates of fixation observed in older crusts (Zhao et al. 2010). Nitrogenase activity from BSCs ranges from 1 μmol N $m^2$/hr–340 μmol N $m^2$/hr (Belnap 1996, 2002, Zhao et al. 2010) with rates of 11 μmol N $m^2$/hr measured in an arctic tundra environment (Stewart et al. 2011b). The average N fixation rate for our slurry treatments (S) was 95 μmol N $m^2$/hr (i.e. 286μmol ethylene $m^2$/hr with a 3:1 conversion ratio). Zhao et al. 2010 estimated that N input via later successional, moss-dominated crust in a rehabilitated grassland ecosystem was 4 kg N ha/yr, which is similar to BSCs inputs estimated in an undisturbed arctic tundra environment (3.4–7.1 kg N ha/yr) (Stewart et al. 2011b). Our laboratory experiment does not allow us to scale-up to ecosystem inputs, highlighting the need for larger scale in-situ trials of BSC inoculation on mining impacted substrates.

Assessment of BSC establishment can be difficult, since visual measurements fail to quantify the amount of cyanobacteria/green algae present (Belnap 1993) and measurement of processes such as photosynthesis and respiration do not directly reflect cyanobacterial abundance or diversity. Quantification of Bacterio-chllorophyll *a* or spectophotometrically determined chollorphyll *a* provide a more comprehensive means to determine the establishment of crusts. In some studies, chlorophyll *a* content was significantly related to both net $CO_2$ exchange and nitrogen fixation (Maestre et al. 2006). Examination of *nif* H gene (encodes the iron protein subunit of the nitrogenase enzyme) diversity and abundance, as well as, molecular characterization of BSC communities are needed to better determine crust development and composition (Maestre et al. 2006, Zhan and Sun 2011, Stewart et al. 2011c). Diversity of *nif* H genes in tailings has been correlated with the amount of organic C, water content and available Zn (Zhan and Sun, 2011).

In addition to nitrogen fixation, BSCs either alone or in combination with native herbs play a highly important role in providing substrate stability. Although, physical and chemical techniques are available to reduce wind and water erosion, phytostabilization can provide a relatively simple and cost effective way to reduce mobility of mining impacted substrates (Bradshaw 1997, Lan et al. 1998, Ye et al. 2002, Liu et al. 2012). BSCs enhance aggregation of soil particles and protect the soil surface thereby reducing wind and water erosion (Bowker et al. 2007), while plant canopies reduce aeolian dispersion and roots help to prevent water erosion (Yang et al. 2014). Further studies that incorporate measurements of runoff, infiltration (Xiao and Zhao 2008, Xiao and Zhao 2010), extracellular polymer secretions and aggregate stability (Malam Issa et al. 2001, Malam Issa et al. 2007) on disturbed northern sites inoculated with artificial BSCs are needed.

The role of BSCs in seed germination and vascular plant growth also requires consideration. We had relatively high germination rates for all of the herb species examined here with the exception of field locoweed, which is likely due to limitations in our seed preparation procedure that did not optimize seed coat scarification. However, we did not examine the combined use and potential interactions of BSCs and native nitrogen-fixing herbs together. Establishment of native pioneer species can not only improve soil characteristics by enhancing organic content and supplying needed nutrients, especially nitrogen (N), but may also reduce long-term soil toxicity, so that more sensitive plants can establish leading to a healthier more diverse ecosystem (Chan et al. 2003). Several studies have found that the microtopography and higher moisture retention associated with BSCs provide favourable microsites for vascular plant germination (DeFlaco et al. 2001, Su et al. 2009), while other studies have observed BSCs to have no impact or a negative impact on germination rates (Hawkes 2004, Li et al. 2004). Characterization and quantification of artificial BSC development both over time, as well as, their role in facilitating native herb species establishment on mining impacted substrates warrants further investigation.

## Conclusions

Currently there are very few native species available for restoration in northern Canada. In our greenhouse and growth chamber trials we identified three nitrogen-fixing herb species (alpine sweetvetch, field locoweed and arctic lupine) and one biological soil crust treatment (mature soil crust slurry) that appear to be able to establish and contribute to N and C cycling processes in northern mining impacted substrates. While our study indicates that native nitrogen-fixing herbs and biological soil crusts demonstrate a strong potential for use in restoration efforts in the North, we only examined the use of native nitrogen-fixing herbs and BSCs only under greenhouse and growth chamber conditions. There are several challenges that must be addressed to be able to employ these approaches on mine sites, including determination of germination, growth and nitrogen fixation rates of herbs under harsher on-site conditions, and the culturing of sufficient volumes of BSCs and subsequent propagation of crusts that fix nitrogen on-site.

## Implications for Practice

- Our results suggest local native nitrogen fixing herbs and BSCs have the ability to establish, grow, and fix nitrogen in northern mining impacted soils.
- Where a combination of biomass accumulation and nitrogen fixation are needed arctic lupine shows a strong potential for use in restoration efforts.
- Where nitrogen fixation is the main aim of restoration on cover soils, alpine sweetvetch is a desirable species, however alpine sweetvetch roots are a food source of grizzly bears and caution should be used in seeding alpine sweetvetch where disturbance of engineered soil covers is a concern.
- Inoculation of nitrogen fixing herbs appear to require Rhizobia inoculums in ensure short term nodulation and nitrogen fixation.
- Bone meal biochar appears to promote nitrogen fixation when a Rhizobia inoculum is also present, however due to potential negative impacts on growth use of biochar needs to be considered in reference to soil conditions and may be appropriate where a soil conditioner is needed to improve soil texture.
- BSCs derived from mature soil crusts appear to establish rapidly and fix relatively high amounts of nitrogen on tailings and should be consider in restoration of early successional species.

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