

## Appendix 32 : 2020 Tundra Restoration and Natural Recovery Monitoring Report

**Tundra restoration and natural recovery monitoring at Agnico Eagle Mines  
Meliadine site, Nunavut**

**Prepared for Agnico Eagle Mines Limited**

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**Dr. Katherine Stewart and Dr. Alix Conway**

## Contents

<b>List of Tables</b> .....	iii
<b>List of Figures</b> .....	iv
<b>Preface</b> .....	vi
<b>Executive Summary</b> .....	vii
<b>Restoration Trial</b> .....	1
Background .....	1
Methods .....	1
Site Selection .....	1
Site Preparation .....	3
Harvesting Procedure .....	5
Substrate and Vegetation .....	7
Soils and Soil Invertebrates .....	7
Initial Results .....	8
Species cover on plugs .....	8
Soils and Soil Invertebrates .....	9
Future Directions .....	10
<b>Plug Expansion Trial</b> .....	11
Plug Expansion Sampling .....	12
Future Directions .....	13
<b>Key Early Colonizing Vascular Plants</b> .....	15
Background .....	15
Methods .....	16
Field Work .....	16
Initial Results .....	16
Future Directions .....	17
<b>Natural Colonization of Biological Soil Crusts on Drilling Waste Sites</b> .....	19
Background .....	19
2018 Chronosequences .....	19
Methods .....	19
Results .....	20
Microclimate .....	20

Nutrients .....	22
Nitrogen fixation and gas flux .....	24
BSC Community Composition .....	26
2019 Chronosequences.....	28
Methods.....	28
Future Directions .....	29
<b>Invasive Species Survey</b> .....	31
Background .....	31
Methods.....	31
Results.....	31
Future Directions .....	33
<b>References</b> .....	34

## List of Tables

Table 1. Location of the three gravel quarries used as restoration sites (decimal degrees).....	2
Table 2. Particle size distribution of the substrates used at each site. Values reported as percentage of weight. ....	2
Table 3. Description, dimensions (length, width, depth) and diagram of the four treatments applied within the hollow-hummock plot. ....	4
Table 4. Depth of organic and mineral layers across all plugs used at each restoration trial. Values are means with standard deviation (n= 20 plugs per site). ....	5
Table 5. List of species present in ≥75% quadrats (from at least one site, Q1 or Q2) on plug (including both plug and plug+shredded treatment) and shredded material. Q1% and Q2% are the percentage of plots each species were present in at Quarry 1 and Quarry 2, respectively. Bolded species were present in ≥75% quadrats from both sites.....	9
Table 6. Mean Invertebrate abundance (+ standard deviation) in soils collected directly adjacent to plug 10 harvesting locations at each restoration site.....	10
Table 7. Average precipitation and calculated weekly watering regime for the plug expansion trial. Watering regime was calculated by [precipitation/month (in L/m <sup>2</sup> ) multiplied by the size of container, and divided by four weeks].....	12
Table 8. Comparison of gas flux (CO <sub>2</sub> , N <sub>2</sub> O, CH <sub>4</sub> ) measured on BSC samples under dark and light optimal conditions from drilling wastes deposited on site in the years 2012/2013, 2008 and 1997/1998. F values and p values are shown for ANOVA (p<0.05). Bold p values indicate significantly different flux values. .	25
Table 9. Roadside walking survey for non-native invasive species. At each roadside location 50 m on one side of the road was surveyed. Roadside locations were observed when traveling south towards Rankin Inlet (IRS) and when travelling north towards Meliadine (IMN). The latitude and longitude at each location was recorded, as well and the general landscape type. No non-native invasive species were identified. Four unknown (UNK) species were observed that have not yet been identified. The frequency of these species was noted based on the number of individuals observed.....	32
Table 10. A walking and driving survey of areas of high likelihood of non-native invasive species around the main camp and exploration camp. The latitude and longitude at each location was recorded. No non-native invasive species were identified. Three unknown (UNK) species were observed that have not yet been identified. The frequency of these species was noted based on the number of individuals observed. ....	33

# List of Figures

Figure 1. Location of the three restoration trials established in 2019, and associated harvesting/reference systems. ....	2
Figure 2. Four rows of hummock-hollow microtopography at each site (left) and a cross-sectional view of the hummock-hollow complex. ....	3
Figure 3: Stratified random block design at the Emulsion Plant (EMP), Quarry # 1 (Q1) and Quarry #2 (Q2) sites. Treatments (P, S, PS and C) were grouped within blocks (B1-B10) at each site. ....	5
Figure 4. Harvesting and transplanting of plugs and shredded materials. Removing the plugs manually with a flat-head shovel used to cut the dimensions of the plug (A). Harvesting plot after the plug has been removed (B). Harvesting plots will be re-visited for analysis of vegetative encroachment into the disturbed area. Further physical manipulations of the hummock-hollows, to allow the plug surface to sit even with the bottom of the hollow (C). Hand-lifting the plug into position, with fallen material being placed in the bottom of the hollow before placement (D). The 4 cm <sup>2</sup> grid used for shredding vegetative material (E). A Plug + Shredded treatment ready for percent-cover surveying (F). Note the shredded material is only applied to the areas next to the physical plug, not overtop. ....	6
Figure 5. Survey of plugs + shredded layers treatment plot. Shredded layers on either side of the plot were surveyed for percent cover (A and C), as well as, the plug in the center of each plot (B). ....	7
Figure 6. Visual representation of the growth chamber plug expansion trial design (A). Fertilizer was mixed into substrates to ensure even distribution. The mesh screen (12 cm <sup>2</sup> mesh) was placed in front of the freshly cut growing front and allowed for both plug-substrate contact, and root growth out of the plugs. Photograph of plug divided and placed in contact with fertilized or unfertilized substrate (B). ....	11
Figure 7. Sampling of the plug expansion trial. Measuring height, lateral spread and distance from plug of emerging vascular plants on the substrate (A). Biological soil crust cover estimated on the plug and substrate (B). Collecting images of plugs for NDVI (C) Mini-rhizo samplers used to extract root exudates from the organic soils and substrates (D). ....	13
Figure 8. <i>Oxytropis deflexa</i> excavated on a gravel pit. Nodulation is visible on the lateral roots at center left. ....	15
Figure 9. Environmental and plant community factors associated with <i>Oxytropis arctobia</i> , <i>O. deflexa</i> , and <i>O. maydelliana</i> in upland tundra heaths at Meliadine. ....	17
Figure 10. PET-CT imaging of <i>Oxytropis</i> cores collected at Meliadine in summer 2019. ....	18
Figure 11. Data loggers on drilling wastes colonized with BSCs and not colonized with BSCs. ....	19

Figure 12. Microclimate data from co-located BSC covered ground and bare ground areas on an early staged drilling waste site (2013) over the duration of 1 year, expressed in average per Julian day. Photosynthetically active radiation (PAR $\mu\text{mol}/\text{m}^2/\text{s}$ )(A). Soil temperature ( $^{\circ}\text{C}$ ) (B) and soil moisture ( $\text{m}^3/\text{m}^3$ )(C). .....	21
Figure 13. Nutrients in BSCs and soils immediately below BSCs on drilling wastes deposited on the Meliadine site in the years 2012/2013, 2008 and 1997/1998. Bars represent means with standard error. Nutrients in BSCs and soils were compared using a two-way ANOVA including the interaction of year and substrate type (i.e. BSC or underlying soil) followed by Tukey HSD posthoc comparisons across each substrate type. ....	23
Figure 14. Nitrogen fixation by biological soil crusts (BSCs) sampled on drilling wastes created in 2013, 2008 and 1997/1998. Nitrogen fixation was measured via acetylene reduction assays. BSCs on older drilling wastes (1997/1998 and 2008) had significantly higher rates of nitrogen fixation than more recently disturbed sites (2013) (ANOVA, Tukey posthoc, $p < 0.05$ ). ....	24
Figure 15. Gas flux ( $\text{CO}_2$ , $\text{N}_2\text{O}$ and $\text{CH}_4$ ) measured on BSC samples from Meliadine under optimal conditions in the laboratory. Flux values are provided under dark and light conditions at early (2012/2013), mid (2008) and late (1997/1998) drilling wastes. ....	25
Figure 16. Principal component analysis of community composition (OTUs) for bryophytes (Rankin Bryo), lichen (Rankin Lichen), fungi (Rankin Fungi) and COI (invertebrates) (Rankin COI) at early (2012/2013), mid (2008) and late (1997/1998) drilling wastes. ....	27
Figure 17. BSC sampling sites and nutrient monitoring sites at Meliadine in 2019. ....	28
Figure 18. Static vented gas flux chambers on BSCs at drilling wastes from 1997/1998. Gas flux was sampled every 15 minutes over a 1 hour period to determine in-situ $\text{CO}_2$ , $\text{N}_2\text{O}$ and $\text{CH}_4$ . ....	29
Figure 19. Principal components analysis of bryophyte community composition in BSCs on for bryophytes at early (2012/2013), mid (2008) and late (1997/1998) drilling wastes with corresponding environmental vectors. ....	30

## Preface

On June 1, 2018 Agnico Eagle Mines and the University of Saskatchewan were successful in receiving a Natural Sciences and Engineering Research Council (NSERC) Collaborative Research and Development grant. The grant entitled “Tundra Restoration: Niche construction in early successional plant-soil systems” will support on-site and laboratory research from June 2018 to June 2022. The primary objective of this research is to address Term and Condition no. 41 of the Project Certificate for the Meliadine site: *“Prior to the commencement of operations, the Proponent shall develop a progressive re-vegetation program for disturbed areas that are no longer required for operations, such program to incorporate measures for the use of test plots, reseeding and replanting of native plants as necessary.”* Several additional scientific objectives that support this primary objective will also be examined: i) Characterization of initial and realized niches of biological soil crusts and tundra vascular plants across a chronosequence of naturally recolonized drilling waste dumps; ii) Characterization of initial and realized niches of actively restored biological soil crusts on disturbed substrates; iii) Characterization of initial and realized niches of actively restored tundra vascular plants on disturbed substrates. In addition to the scientific work, the project will include the development of a youth education program and local community engagement in Rankin Inlet and Baker Lake, NU.

**Below is a summary timeline for key project activities and deliverables.**

Milestone	Description of activities	Anticipated starting date	Anticipated completion date
Natural recolonization of drilling wastes	Study natural recolonization of drilling waste dumps 2-17 yrs old by biological soil crusts and vascular plants. Specific recommendations of species for restoration will be developed.	2018-06-01	2019-08-31
Active restoration with tundra surface layers	Transplanting of tundra plugs, shredded surface layers and biological soil crusts onto disturbed substrates. Specific recommendations of restoration practice and species for restoration will be developed.	2019-06-01	2021-08-31
Youth education program	Collaboratively develop and deliver an education program for one week in 2019 and 2020 for youth from Rankin Inlet and Baker Lake, NU. The program will focus on arctic ecology, restoration and skills in environmental monitoring and research.	2019-01-31	2021-01-31
Community meetings	We will hold community meetings in Rankin Inlet and Baker Lake. Working with Agnico's community relations department we will identify key groups to host. Our research and restoration of tundra environments will be presented and discussed.	2019-06-01	2021-08-31
Website development	Creation of project website providing information to restoration practitioners and the public on general arctic ecology and restoration practice in the North, as well as key findings from the research.	2019-08-31	2022-06-01
Technical reports for AEM	Detailed technical reports for AEM on the restoration techniques examined. Guidelines and standard operating procedures for on-going monitoring will be included.	2018-11-30	2022-06-01



## Executive Summary

In February 2015, Nunavut Impact Review Board issued a Project Certificate for Agnico Eagle Mines Ltd Meliadine gold project located in low arctic tundra of the Maguse River Upland Ecoregion. As a condition of the Project Certificate AEM is required to develop a reclamation and revegetation program for all project phases as outlined in Term and Condition no. 41: *“Prior to the commencement of operations, the Proponent shall develop a progressive re-vegetation program for disturbed areas that are no longer required for operations, such program to incorporate measures for the use of test plots, reseeding and replanting of native plants as necessary.”*

As part of the on-going NSERC Collaborative Research Development grant between the University of Saskatchewan and Agnico Eagle Mines, we have established three restoration trials in summer 2019 to monitor the success of transplanting intact and shredded tundra material on disturbed areas associated with the Meliadine mine site. Restoration sites at Quarry 1 and 2 are located at ~27 km on the All Weather Access Road (AWAR), and a third site was located at the quarry area before the emulsion plant on site. In late summer 2019 the third site was lost, but the associated harvesting site remains intact. To complement this field trial we have conducted a tundra plug expansion trial in growth chambers at the University of Saskatchewan from January 2020 to June 2020. In 2019, we also initiated a field study examining early colonizing *Oxytropis* species that have been identified as potential local native species for restoration. These trials will assist with our specific objective of characterizing initial and realized niches of actively restored tundra vascular plants on disturbed substrates.

Field and laboratory activities in 2019-2020 also continued to our objective of characterizing initial and realized niches of biological soil crusts (BSCs) and tundra vascular plants across a chronosequence of naturally recolonized drilling waste dumps. In addition to the chronosequence work from 2018, 15 additional sampling sites were surveyed in 2019 and measurements of in-situ gas flux and nutrient availability were added. Analysis of microclimate data, optimal gas flux, nutrients and community composition of BSCs from 2018 samples has provided further insights into the use of BSCs as a target community for passive and active restoration. Peer-reviewed publications and specific recommendations from this work are expected by spring 2021.

An extensive invasive plant species survey was conducted in summer 2019 and no non-native invasive species were observed or identified. These survey results provide a baseline for future monitoring of the Meliadine mine site for invasive plant species. We recommend that in addition to taking preventative measures to reduce the likelihood of introducing invasive species that these surveys be repeated yearly.

In fall of 2019 we launched a website: [tundrarestoration.com](http://tundrarestoration.com) that provides information on tundra ecology, scientific and traditional Indigenous knowledge of common tundra plants, details and videos of our restoration trial and information on our youth education programs.

Due to COVID-19 we were not able to conduct field work on the Meliadine mine site in summer 2020, however, project activities have continued. Additional resources were allocated to the tundra plug expansion trial to gain information regarding patterns of species growth and expansion from the plugs and better understand plant-soil interactions of these expanding communities. *Oxytropis* species in tundra plugs from 2018 have been maintained and together with southern *Oxytropis* will provide the needed materials for methods development and initial growth chamber trials in winter 2021.

# Restoration Trial

## *Background*

Currently many industries operating in northern Canada are faced with the need to restore tundra ecosystems that have been impacted by resource extraction activities. The goal of restoration is often to establish pre-disturbance plant-soil assemblages and promote recovery of key ecosystem functions to ensure long-term ecosystem health. Seeding and fertilization is commonly used in many revegetation efforts. However, the ecological and economic feasibility of this approach in northern tundra environments is limited due to the lack of commercial seed stocks for northern native tundra vegetation and isolated site locations.

Where the goal of restoration is to establish pre-disturbance plant-soil assemblages, the use of locally harvested biological soil crusts and/or tundra organic surface materials may provide a solution to ensure appropriate soil and plant species are represented and sufficient stocks are available. Limited knowledge regarding successional trajectories of arctic vegetation, plant-soil interactions, and traits of specific floral and faunal species reduces the ability of restoration practitioners to utilize proven restoration techniques developed in more southern ecosystems.

In our active restoration trial using transplanted tundra plugs and shredded tundra material we aim to identify restoration techniques (i.e. transplanting intact and shredded materials, and organic and mineral soils, creating microtopography, and providing erosion control) that promote pre-disturbance plant-soil assemblages and recovery of ecosystem functions. We also aim to identify tundra species that colonize rapidly upon transplantation and promote tundra plant-soil community development.

## *Methods*

### Site Selection

Prior to site selection, we visited several disturbed areas associated with the Meliadine mine. These included quarries along the All Weather Access Road (AWAR), old contaminated sites (Hyster Rollover), and quarries of varying sizes within the mine footprint. The three gravel quarries selected for the restoration sites were located to the northwest and southeast of the current operating mine (Figure 1, Table 1). We chose sites that had similar landscape characteristics including slope (i.e. open, flat), aspect, exposure, and surface ground water. Finally, site location was also determined based on the accessibility and quality of required materials, such as substrate used for site preparation (Table 2), and vegetative material harvested for treatments. We chose upland tundra heath as the harvest community, as it is common in the area and has diverse groups of vascular and non-vascular plants, including biological soil crusts.

### AEM Meliadine Restoration Trials

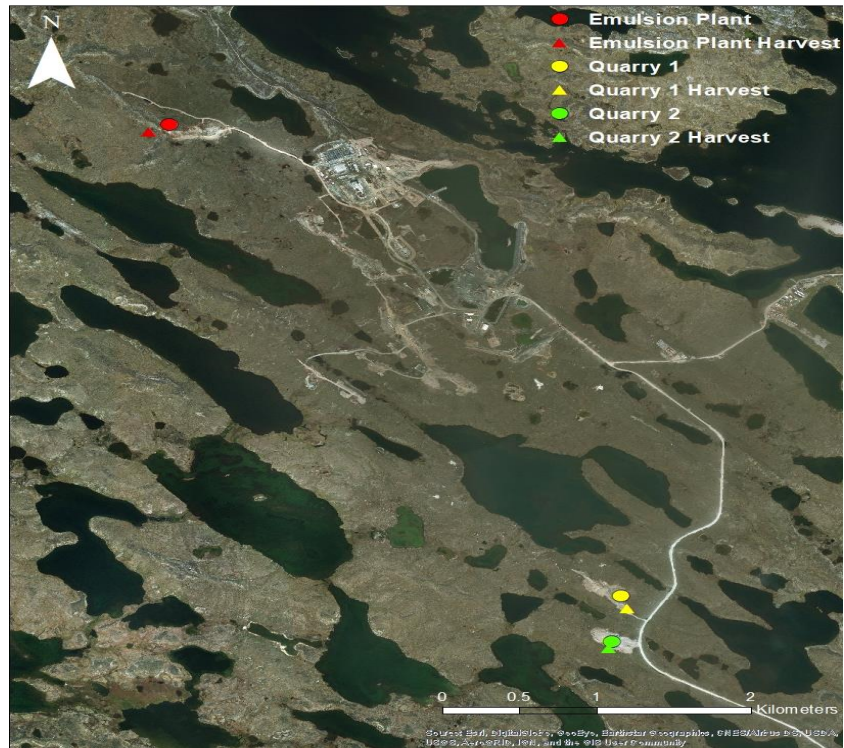


Figure 1. Location of the three restoration trials established in 2019, and associated harvesting/reference systems.

Table 1. Location of the three gravel quarries used as restoration sites (decimal degrees).

Site	Latitude	Longitude
Quarry 1 restoration	63.00436° N	92.19363° W
Quarry 1 harvest	63.00336° N	92.19289° W
Quarry 2 restoration	63.00070° N	92.19492° W
Quarry 2 harvest	63.00019° N	92.19528° W
EMP restoration	62.04261° N	92.25031° W
EMP harvest	63.04206° N	92.25297° W

Table 2. Particle size distribution of the substrates used at each site. Values reported as percentage of weight.

Site	Clay	Silt	Sand	Gravel
EMP	0	6	38	56
Quarry #1	0	9	64	27
Quarry #2	0	8	45	47

## Site Preparation

All three restoration trials (EMP, Q1, and Q2) had four 15 m long rows of material that were used to create a hummock-hollow microtopography. A backhoe-loader was used to excavate quarry substrates and place the materials into each row (15 x 10 m), which were ~50 cm high and spaced approximately 1.5 m apart. Material in the center of each mound was manually removed with shovels as needed, and the sides reshaped to create hummock-hollows characteristic of the surrounding tundra landscape (Figure 2). Hummocks were ~50 cm high, with 0.9 – 1.1 m between the ridges of the hummock. Each row contained 10 treatment plots (0.5 X 1 m) separated by 0.75 m.

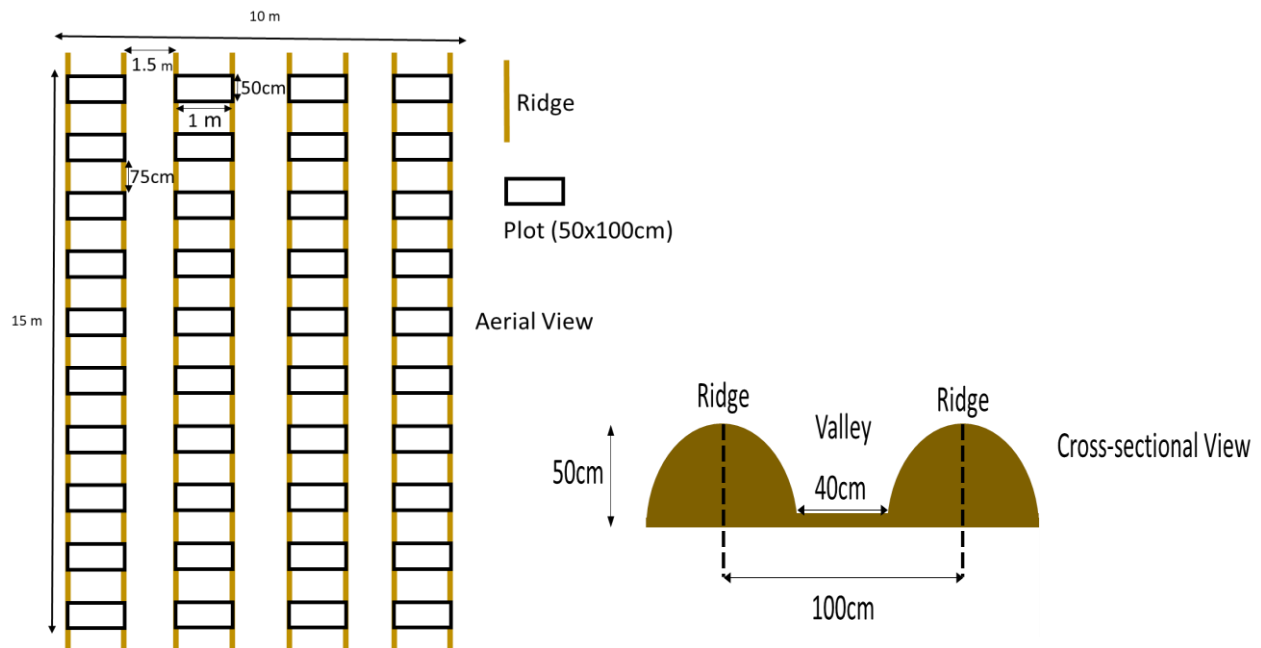






Figure 2. Four rows of hummock-hollow microtopography at each site (left) and a cross-sectional view of the hummock-hollow complex.

Four treatments were applied within the treatment plots: Plugs, Shredded, Plugs and Shredded and Control (Table 3).

Table 3. Description, dimensions (length, width, depth) and diagram of the four treatments applied within the hollow-hummock plot.

Treatment	Description	Dimensions	Diagram
<b>Plugs (P)</b>	Intact vegetative sod placed in bottom (center) of hollow	40 x 40 x ~10-15 cm	
<b>Shredded (S)</b>	Vegetative sods shredded and homogenized, spread over the entire plot	3000 cm <sup>3</sup> shredded material spread across plot at 2 cm depth	
<b>Plugs + Shredded (PS)</b>	Intact vegetative sod placed in bottom (center) of hollow and shredded material spread on hummock sides	40 x 40 x ~10-15 cm and 1800 cm <sup>3</sup> shredded material spread at a 2 cm depth	
<b>Control (C)</b>	Control plot	No material added	

Treatments were grouped based on a stratified random block design. The Emulsion Plant trial and Quarry #1 were blocked across two rows to account for slight differences in the amount and type of substrates deposited, while Quarry #2 was blocked across all four rows to account for differences in substrates (Figure 3).

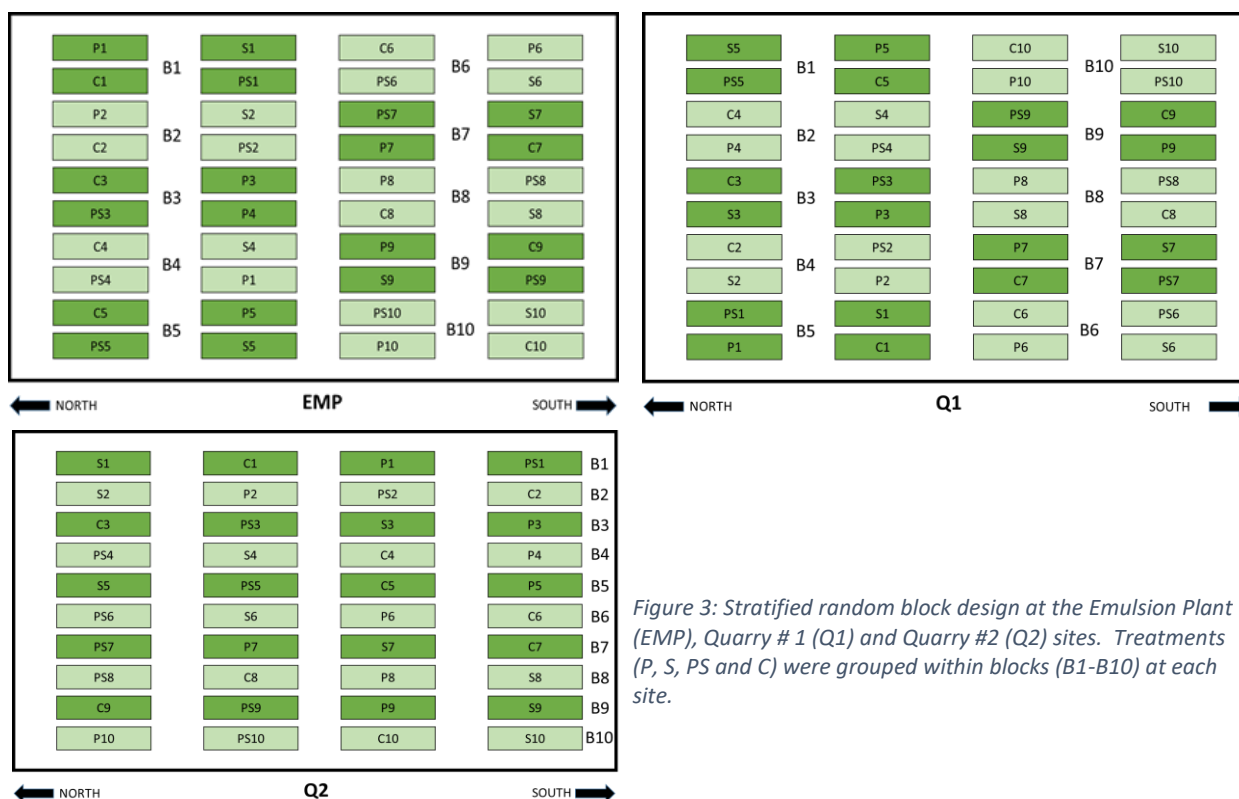


Figure 3: Stratified random block design at the Emulsion Plant (EMP), Quarry #1 (Q1) and Quarry #2 (Q2) sites. Treatments (P, S, PS and C) were grouped within blocks (B1-B10) at each site.

## Harvesting Procedure

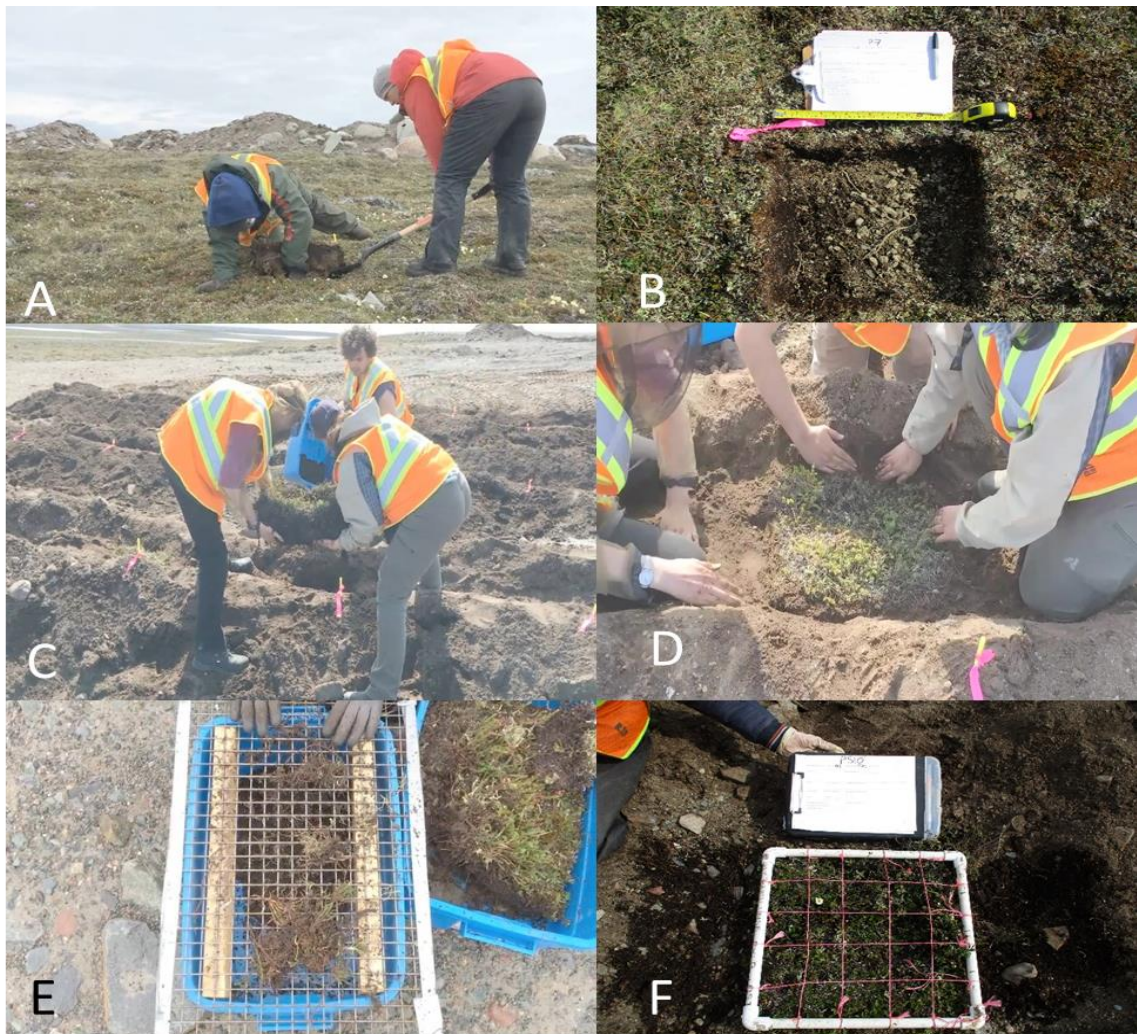
Harvesting of plugs and shredded material was conducted in upland heath communities located near each restoration trial (148 m, 119 m, and 60 m for Emulsion Plant, Quarry #1 and Quarry #2, respectively). A flat-head shovel was used to cut, lift and remove the plugs from the harvesting sites (Figure 4A). The underlying material was predominantly composed of organic matter; however, an effort was taken to ensure that harvested material also contained underlying mineral layers (Table 4). Harvesting plots were flagged, spatially referenced and photographed. Plugs were carefully removed, and the depth of organic and mineral soil was measured on each side of the plug. Plugs were quickly transported to restoration trials in individual bins. At all harvesting plots, the depth of each directional face was recorded for monitoring of future vegetative encroachment from the surrounding tundra (Figure 4B). All measurements were taken at the center of each side.

Table 4. Depth of organic and mineral layers across all plugs used at each restoration trial. Values are means with standard deviation ( $n = 20$  plugs per site).

Restoration Site	Organic Depth (cm)	Mineral Depth (cm)
Emulsion Plant	$9.1 \pm 3.3$	$3.49 \pm 2.62$
Quarry #1	$7.0 \pm 4.2$	$3.08 \pm 3.52$
Quarry #2	$9.1 \pm 3.2$	$1.46 \pm 2.10$



Plugs (n=20 per site) were carefully hand-lifted into place, with any fallen material placed in the bottom of the individual hollow (Figure 4C). All plugs were placed with the vegetative surface being level with the surrounding substrate surface. Once placed, the surrounding substrate was pushed against the plugs to ensure good plug-substrate contact (Figure 4D). Three additional plugs were harvested for shredding, with all measurements described above obtained before shredding occurred. Plugs including all soils and vegetative materials were physically separated and sieved through a 4 cm<sup>2</sup> metal mesh screen (Figure 4E). All shredded material was homogenized. Shredded material was measured and dispensed along both internal sides of the hummock surrounding the plugs in the Plug + Shredded treatments (Figure 4F), whereas Shredded treatments had shredded material covering the entire treatment plot.



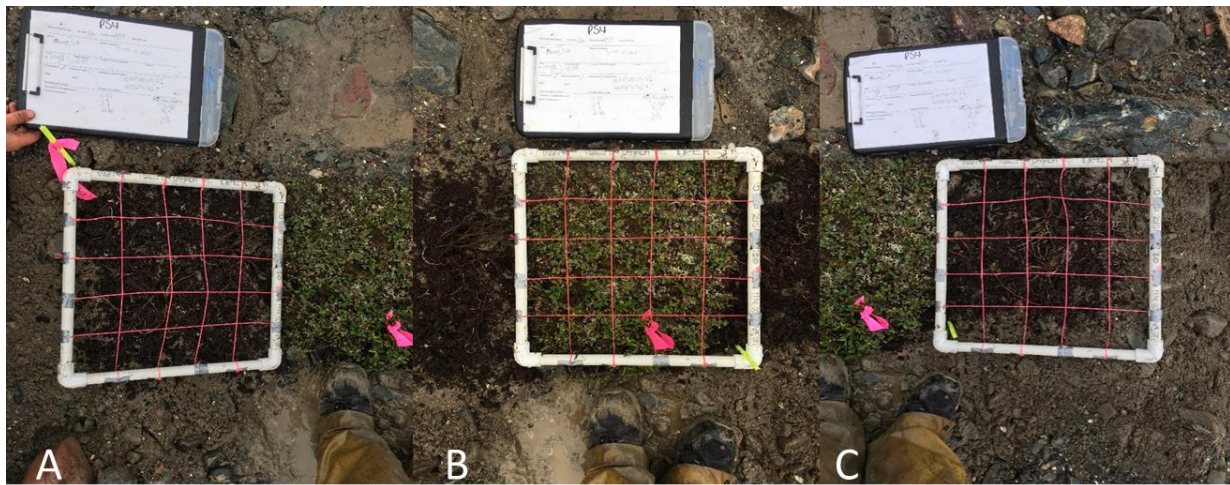
*Figure 4. Harvesting and transplanting of plugs and shredded materials. Removing the plugs manually with a flat-head shovel used to cut the dimensions of the plug (A). Harvesting plot after the plug has been removed (B). Harvesting plots will be re-visited for analysis of vegetative encroachment into the disturbed area. Further physical manipulations of the hummock-hollows, to allow the plug surface to sit even with the bottom of the hollow (C). Hand-lifting the plug into position, with fallen material being placed in the bottom of the hollow before placement (D). The 4 cm<sup>2</sup> grid used for shredding vegetative material (E). A Plug + Shredded treatment ready for percent-cover surveying (F). Note the shredded material is only applied to the areas next to the physical plug, not overtop.*

A brief video showing the steps used to create the restoration trial is available at:

<https://www.tundrarestoration.com/background>

## Substrate and Vegetation

A 0.16 m<sup>2</sup> gridded quadrat (25 grids, 0.0064 m<sup>2</sup> each) was placed over the top of each Plug, Plug + Shredded and Shredded treatment. Values ranging from 1 to 4 (1 = <25%, 2 = 25-50%, 3 = 50-75%, 4 = >75%) were used for estimation of species cover in each individual grid (Figure 5). All vascular and non-vascular plants within harvested plugs were identified to species level whenever possible. Shredded layers were surveyed in the same fashion; however, mosses found within shredded layers were simply identified as bryophytes. For treatments including shredded material, the center plot and the inside of both sides of the hummock were also surveyed.



*Figure 5. Survey of plugs + shredded layers treatment plot. Shredded layers on either side of the plot were surveyed for percent cover (A and C), as well as, the plug in the center of each plot (B).*

After surveying was completed, all treatments were gently covered by a jute-mesh erosion control blanket (Anti-wash GEOJUTE®, Belton Industries, Honea Path, SC). The jute-mesh blanket was chosen due to the ample light penetration (45% - 60%), the ability to protect from erosive forces, water storage capacity (425%) and degradation time of ~1-2 years, depending on climate. Fifteen-centimeter long metal ground staples were driven into the top of each hummock at ~60-70 cm intervals to hold the jute-mesh in place, after which large rocks were positioned over the staples, and substrate material from the hills used to cover the edges of the jute-mesh, with care being taken to prevent movement or burial of shredded layers.

## Soils and Soil Invertebrates

Soil samples were collected using shovels immediately beside (i.e. within 1 m) ten randomly chosen plug harvesting locations at each restoration site. Both organic and mineral horizons were sampled, with the exception of Plug #2 from Quarry #2 where only organic material was sampled. Composite samples of the substrate used to create microtopography at each restoration trial were also taken. All samples were transported to the University of Saskatchewan, with a subsample (~50 ml) stored at -80°C for



future molecular work if necessary. The remaining soil was dried, sieved (4 mm<sup>2</sup>) and stored at -20°C for future analysis. Water extractions were conducted on all soil samples using a 1:4 ratio of soil to Milli-Q water, except for 11 samples that required higher water to soil ratios (i.e. 1:6 or 1:8) due to high amount of organic matter. Extracts were then measured on a Dionex ICS-2000 Ion Chromatograph to determine concentrations of cations (Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, Na<sup>+</sup>, NH<sub>4</sub><sup>+</sup>) and anions (NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, SO<sub>4</sub><sup>-</sup>, Cl<sup>-</sup>, PO<sub>4</sub><sup>3-</sup>). pH was measured using a Mettler Toledo FiveEasy pH meter. Subsamples of each soil were taken for total carbon (TC) and total inorganic carbon (TIC), measured using a LECO C632 Carbon Analyzer, and total nitrogen (TN) using a LECO TruMac CNS Analyzer.

At the same sites chosen for soil sampling, soil invertebrate samples were collected using a slack-hammer to obtain a core (5 cm diameter, 10 cm depth) of organic soil and, if possible, mineral soil. Sampling was done immediately next to the harvesting plot, with the directional side being chosen randomly at each pit. Samples were kept in a chilled, open-air cooler and subsequently transported to the University of Saskatchewan. Invertebrate extractions were performed using a water-flotation technique, with individuals identified to family levels, and the number of individuals recorded. A subsample (~20-25 ml, stored at -20°C) was also taken to be used for DNA analysis.

## *Initial Results*

### Species cover on plugs

A total of 67 plant species were identified on the tundra plugs. Voucher specimens were collected for the majority of the unknown species, which were identified in the winter of 2020. At Quarry 1, species richness for all plugs (plug and plug + shredded treatment) was 18.9 (± 0.95) (mean ± standard error) and 8.5 (± 0.37) for shredded material. Similarly, at Quarry 2, species richness for plugs was 21 (± 0.53) and 8.6 (± 0.30) for shredded material.

Considering only species that were found in ≥ 75% quadrats on plugs in either Quarry 1 or Quarry 2, there were six shrub species, five lichen species, two bryophytes and one sedge (Table 5). Of these, seven species (four lichen, three shrubs) were present in ≥ 75% quadrats at both sites. On the shredded treatment, there were a total of six species found in ≥ 75% quadrats in shredded material; three lichens, two shrubs and the general category of bryophytes. In addition, the broad category of roots were present in the majority of shredded material (96 and 100% for Quarry 1 and 2, respectively). On the plugs, the three vascular species with the highest average cover were *Cassiope tetragona*, *Dryas integrifolia* and *Vaccinium myrtilloides* and the top three lichen species were *Alectoria nigricans*, *Alectoria ochroleuca* and *Cetraria nivalis*. On the shredded material, vascular species identification was difficult, however the two shrub species found most frequently (*Dryas integrifolia* and *Vaccinium myrtilloides*) also had the highest cover. Lichen species with the highest cover on shredded material were *Alectoria ochroleuca*, *Cetraria nivalis* and *Thamnolia vermicularis*. Bryophytes and roots were also a major component of shredded material.

Table 5. List of species present in ≥75% quadrats (from at least one site, Q1 or Q2) on plug (including both plug and plug+shredded treatment) and shredded material. Q1% and Q2% are the percentage of plots each species were present in at Quarry 1 and Quarry 2, respectively. Bolded species were present in ≥75% quadrats from both sites.

Material	Species	Common name	Category	Q1 %	Q2 %
Plug	<i>Dicranum moss sp.</i>	Fork mosses	Bryophyte	40	85
	Moss mat	Bryophyte	Bryophyte	55	85
	<b><i>Alectoria nigricans</i></b>	Witch's hair lichen (black)	Lichen	100	85
	<b><i>Alectoria ochroleuca</i></b>	Witch's hair lichen (yellow)	Lichen	100	100
	<b><i>Cetraria nivalis</i></b>	Fruticose lichen	Lichen	100	100
	<i>Dactylina arctica</i>	Finger lichen	Lichen	75	70
	<b><i>Thamnolia vermicularis</i></b>	Whiteworm lichen	Lichen	100	95
	<i>Carex sp.</i>	Sedge	Sedge	55	80
	<b><i>Arctostaphylos rubra</i></b>	Bearberry	Shrub	75	75
	<b><i>Cassiope tetragona</i></b>	Heather	Shrub	80	90
	<i>Dryas integrifolia</i>	Mountain avens	Shrub	70	95
	<i>Rhododendron lapponicum</i>	Lapland rosebay	Shrub	50	95
	<i>Salix reticulata</i>	Net-veined willow	Shrub	25	85
	<b><i>Vaccinium myrtilloides</i></b>	Blueberry	Shrub	95	100
Shredded	<b>Various Bryophytes</b>	Bryophyte	Bryophyte	90	94
	<b><i>Alectoria ochroleuca</i></b>	Witch's hair lichen (yellow)	Lichen	98	98
	<b><i>Cetraria nivalis</i></b>	Fruticose lichen	Lichen	98	100
	<b><i>Thamnolia vermicularis</i></b>	Whiteworm lichen	Lichen	88	98
	<b>Roots</b>		Roots	96	100
	<i>Dryas integrifolia</i>	Mountain avens	Shrub	20	90
	<i>Vaccinium myrtilloides</i>	Blueberry	Shrub	70	82

## Soils and Soil Invertebrates

Soils sampled directly adjacent to plug harvesting locations have been analyzed in the University of Saskatchewan laboratory for pH, cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{NH}_4^+$ ), anions ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{SO}_4^-$ ,  $\text{Cl}^-$ ,  $\text{PO}_4^{3-}$ ), total nitrogen, total carbon and total organic carbon. All laboratory soil analyses from the 2019 restoration trial have been completed and data is currently being compiled for presentation and statistical analysis.

Soil invertebrate have been extracted for soils and counted by type (i.e. oribatid mites, predatory mites, collembolans and enchytraeids) (Table 6). There was high variability in the invertebrates extracted from these soils indicated by the high standard deviation at each site. Some invertebrates have been identified to species including *Opiella nova*, *Orthonychiurus folsomi*, *Folsomia fimetaria* and *F. candida*. A summary publication of invertebrate communities found in this work is in preparation.

Table 6. Mean Invertebrate abundance (+ standard deviation) in soils collected directly adjacent to plug 10 harvesting locations at each restoration site.

Restoration site	Invertebrate abundance			
	Orbatid mites	Predatory mites	Collembolans	Enchytraeids
EMP	36 $\pm$ 25	0.4 $\pm$ 1.3	39 $\pm$ 30	33 $\pm$ 33
Q1	37 $\pm$ 29	0.09 $\pm$ 0.3	19 $\pm$ 12	25 $\pm$ 21
Q2	10 $\pm$ 10	0.09 $\pm$ 0.3	34 $\pm$ 23	42 $\pm$ 57

### Future Directions

Due to COVID-19 travel and research restrictions, monitoring of the restoration trial was not conducted in summer 2020. We propose to monitor the two remaining restoration trials (Q1 and Q2) in summer 2021. Plant species cover will be surveyed as described above. In addition, recovery of harvesting locations at all sites will be examined by assessing recolonization of pits where plugs were extracted from. Soil and invertebrate samples will be taken from undisturbed upland health adjacent to the restoration trials to account of interannual variation and a soil/invertebrate cores will be taken from the center of each plug within the restoration trials. Comparison of individual species within transplanted plugs will provide information on the survival at an individual species level. We expect that the survival of graminoid and forbs within the plugs will likely be higher than survivorship of shrubs. Expansion of bryophytes and establishment of graminoids is expected for the shredded treatment. Assessment of changes in community composition and richness will help determine the efficacy of this method in providing stable and expanding resources islands that can promote revegetation of disturbed arctic tundra. The substrate up to  $\sim$  50 cm will be excavated around a subset of plugs to examine root expansion from the plugs. Root samples will be taken to determine biomass and root species (see methods below). Assessment of soil nutrients and invertebrate two years following transplanting will provide insight into the ability of these transplanted plugs to maintain healthy soil conditions and nutrient cycling.

## Plug Expansion Trial

To better understand plug expansion and the plant-soil interactions of the belowground arctic plant communities, a growth chamber experiment was conducted. Eight plant-soil plugs, measuring approx. 46 cm long and 28 cm wide and containing at least one of either *Oxytropis deflexa* or *Oxytropis arctobia* were harvested from across the Meliadine site (See Key Early Colonizing Plants- Methods below). Plant species cover data were collected also using the previously described procedure. Plugs were then transported to the University of Saskatchewan and placed in Phytotron growth chambers [18.5hrs light (15°C)/5.5hrs dark (5°C) cycle at ~400  $\mu\text{mol}$ , relative humidity 65%]. Plant species cover data was re-measured prior to initiation of the growth chamber trial to account for any change since harvest. Plugs were separated lengthwise using the same wide-head shovels used for original extraction, to create two plugs approx. 46 cm long and 14 cm wide, each containing one freshly cut growing front, were used in a paired experimental design (Figure 6). Separated plugs were placed on one side of a 50 cm wide x 40 cm long plastic container and a mesh (12 cm<sup>2</sup> mesh size) screen placed across the growing front. Sand/gravel of the same texture size as Q1 and Q2 was sourced from Saskatoon distributors and placed into the other half of the container. A fertilizer treatment [10 gm<sup>-2</sup> N (NH<sub>4</sub>NO<sub>3</sub>), 5 gm<sup>-2</sup> P (P<sub>2</sub>O<sub>5</sub>)] was examined in the paired design, with one half of each plug placed in contact with fertilized substrate and the other half with unfertilized substrate. (Figure 6).

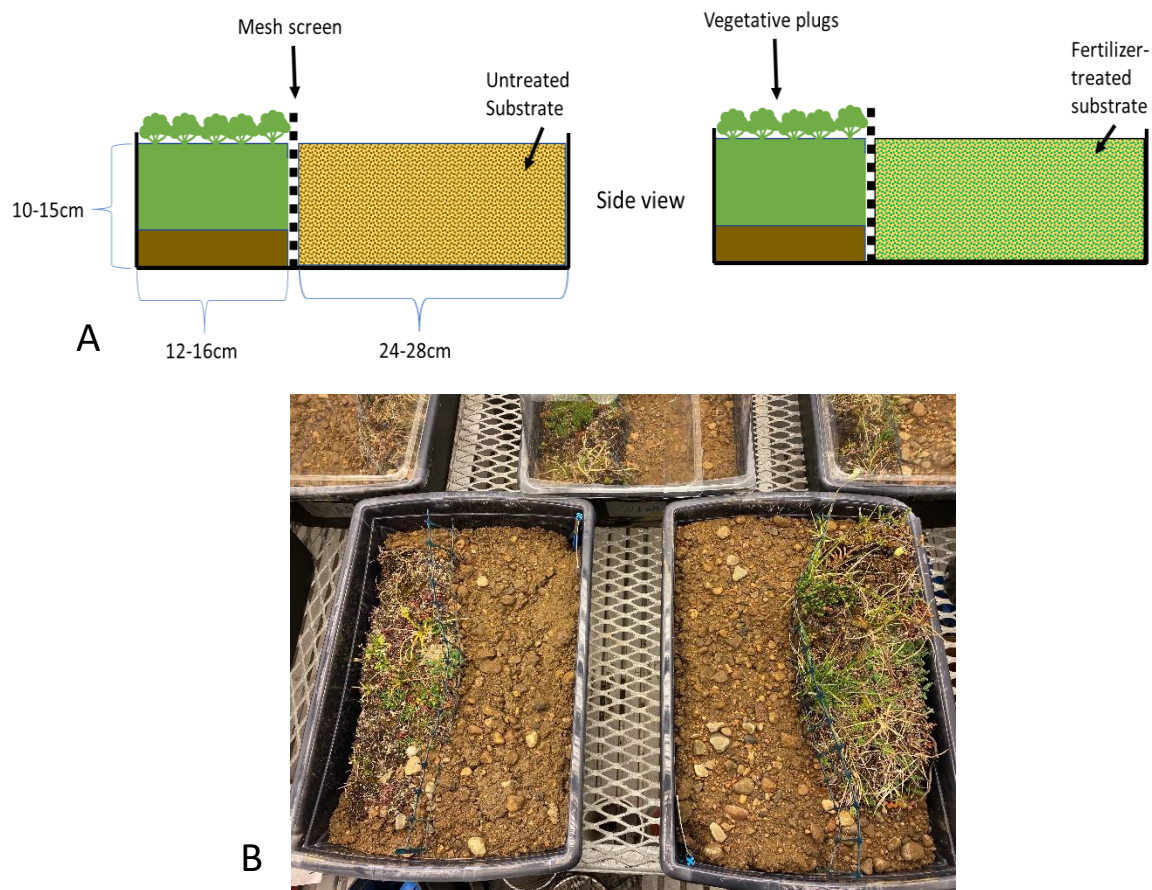


Figure 6. Visual representation of the growth chamber plug expansion trial design (A). Fertilizer was mixed into substrates to ensure even distribution. The mesh screen (12 cm<sup>2</sup> mesh) was placed in front of the freshly cut growing front and allowed for both plug-substrate contact, and root growth out of the plugs. Photograph of plug divided and placed in contact with fertilized or unfertilized substrate (B).

Plugs and substrates were regularly watered at weekly intervals (Table 7) to simulate the natural precipitation regime of Rankin Inlet. The watering regime was based on an average value of June and July precipitation (8.575L/m<sup>2</sup>), although watering was adjusted to account for growth chamber conditions as needed.

*Table 7. Average precipitation and calculated weekly watering regime for the plug expansion trial. Watering regime was calculated by [precipitation/month (in L/m<sup>2</sup>) multiplied by the size of container, and divided by four weeks]*

Month	Average precipitation at Rankin Inlet (liters/m <sup>2</sup> )	Weekly watering amount (liters)
June	26.6	1.31
July	42.0	2.10
August	57.4	2.87
September	42.9	2.15

### *Plug Expansion Sampling*

Plugs were incubated from January 30, 2019 to June 17<sup>th</sup>, 2020 (~ 4 ½ months). Our incubation time was extended to due COVID-19, which prevented us from harvesting our plugs at an earlier date (i.e. 3-month incubation). Prior to harvest species, height, lateral spread and distance from the plug were recorded for all plants emerging aboveground on the substrate portion of the plug (Figure 7A). Biological soil crust (i.e. mosses, lichens) cover was estimated on the substrate and samples have been taken for identification in winter 2021 (Figure 7B). Images were taken for each plug, including the visible red and near-infrared bands in order to create a Normalized Difference Vegetation Index (NDVI) (Figure 7C). NDVI is an indicator of photosynthetically active biomass and can be used to indicate vegetation health and productivity. Each plug was sampled for root exudates using mini-rhizo samplers. Four mini-rhizo samplers were installed, two in the organic materials of the intact plug and two in the adjacent sand/gravel substrate (Figure 7D). Plugs were watered to field capacity and left for 24 hours prior to extracting exudate samples. Exudate samples were frozen (-20°C) and will be analyzed in winter 2021. Soil samples were also taken at each mini-rhizo sampler location and frozen (-80°C) for potential future molecular analysis of soil microbes. The influence of expanding roots on the soil surrounding soils can be examined through determining root exudates and corresponding soil microbial communities (i.e. molecular analysis of soil bacteria and fungi).





Figure 7. Sampling of the plug expansion trial. Measuring height, lateral spread and distance from plug of emerging vascular plants on the substrate (A). Biological soil crust cover estimated on the plug and substrate (B). Collecting images of plugs for NDVI (C) Mini-rhizo samplers used to extract root exudates from the organic soils and substrates (D).

Roots exiting the mesh screen were mapped and sampled using a grid approximately 8 cm x 8 cm extending the depth of the substrate (~10-15 cm). Substrate was carefully washed away to extract the roots in each grid section. Roots were then dried and weighed to determine biomass of roots in each grid section. DNA is currently being extraction from the root samples using a PowerPlant Pro kit (Qiagen, Germany). Extracted DNA will be amplified using the trnL c-1 forward primer and trnL h-1 reverse primer and sequenced using an Illumina MiSeq platform. Sequencing bioinformatics will follow the pipeline developed by Lamb et al. (2016) for the determination of tundra species responsible for root expansion.

### Future Directions

Once data collection has been completed on the plug expansion trial, plug expansion will be examined via emergent vascular species, biological soil crusts, root biomass and identification of roots to species with distance from the intact plug. Results will provide insight into candidate tundra species for restoration that can rapidly expand from locally harvested materials. In addition, changes in species

cover of the intact plugs, emergent vascular species, biological soil crusts, root biomass and NDVI will be compared between plugs adjacent to fertilized versus unfertilized substrate. The overall impact of fertilizing adjacent substrate on plug growth and expansion will be assessed, which may provide recommendations for fertilizer use in future on-site restoration. Specific species responses to fertilization of adjacent substrates will also be assessed to determine if certain species or groups of species increase or decrease with the addition of fertilizer. Changes in species composition of arctic plant communities with fertilization needs to be considered when the restoration goal is to establish pre-disturbance composition. Results from root expansion sampling of the restoration field trial in summer 2021 will be compared with results from the growth chamber plug expansion trial.



## Key Early Colonizing Vascular Plants

### *Background*

Initial surveys of the quarries and other disturbed areas identified *Oxytropis deflexa* as the most common species colonizing. *Oxytropis* is a genus of native legumes common in tundra environments; several species are common in the tundra around Meliadine. These species include *Oxytropis deflexa*, *O. arctobia*, and a third tentatively identified as *Oxytropis maydelliana*. Excavation of a number of *O. deflexa* specimens on the gravel pits revealed nodulation (Figure 8), suggesting active nitrogen fixation by these plants. This combination of apparently rapid early colonization and nitrogen fixation suggests that encouraging colonization of disturbed rocky areas by *Oxytropis* may be an effective strategy to accelerate plant colonization and soil development.



Figure 8. *Oxytropis deflexa* excavated on a gravel pit. Nodulation is visible on the lateral roots at center left.

Little is known about the ecology of Arctic *Oxytropis*, therefore we initiated a series of studies to determine the potential role of these species in restoration. In these studies we are focusing on *Oxytropis deflexa* and *O. arctobia*, as these species are both very common at Meliadine and appear to have a contrasting ecology. *O. deflexa* appears to be the primary colonizer of bare rock substrates, while *O. arctobia* is a later successional species.



Our *Oxytropis* studies began in summer 2019 and consisted of 1) a field survey of the plant communities associated with *Oxytropis deflexa* and *O. arctobia*, and 2) controlled environment investigations of *Oxytropis* ecology at the University of Saskatchewan. Initial results are reported here; continuation of this work was planned for 2020 fieldwork at Meliadine but was not possible due to COVID -19. Alternative research undertaken in summer 2020 is discussed below in future directions.

## *Methods*

### Field Work

A field survey to determine the relative abundance of *Oxytropis* species and the plant communities associated with each *Oxytropis* species was begun in 2019. Briefly, areas of tundra (at minimum 2m by 5m, but in some cases larger) were searched and the number of *Oxytropis* individuals of each species recorded. A 50cm by 50cm gridded quadrat was centered over a randomly selected *Oxytropis* individual, and percent cover was recorded in each 10cm<sup>2</sup> subquadrat. For each search area, a minimum of one quadrat was surveyed over each *Oxytropis* species encountered.

We harvested tundra plugs from intact upland tundra heath, with brown soil, near the old gatehouse along the AWAR. This location is generally representative of the upland heath tundra plant community at Meliadine. We collected 40 x 40 cm, with a depth of ~10-15 cm, intact tundra plugs with either *Oxytropis arctobia* or *Oxytropis deflexa* in the centre of the plug. Four replicates of each species was collected, for a total of eight tundra plugs. Once at the site, we began a random search spiral and collected the first *O. arctobia* or *O. deflexa* encountered. We continued the spiral search, with the next plug located at least 5 m distance from the previous plug and alternating species until four replicates of each were collected. Plugs were dug out by shovel, lifted into plastic bins, and kept watered until transported to the University of Saskatchewan. Species cover, using a 40 cm<sup>2</sup> gridded quadrat, was completed at the mine site within three days of collection.

### *Initial Results*

*Oxytropis deflexa* and *Oxytropis arctobia* appear to have contrasting ecologies with *O. arctobia* associated with higher vascular plant cover, whereas *Oxytropis deflexa* is associated with greater bare rock cover, lower species richness, vascular plant cover and moss cover (Figure 9).

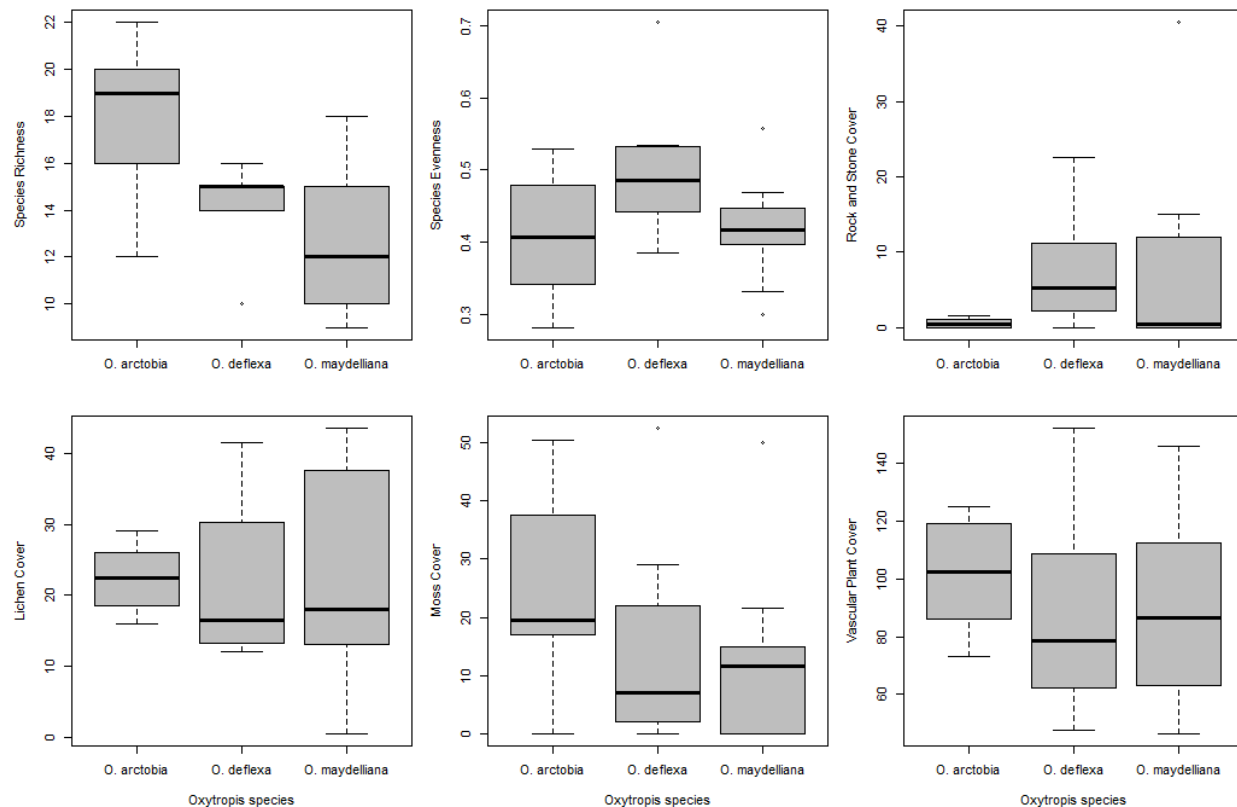


Figure 9. Environmental and plant community factors associated with *Oxytropis arctobia*, *O. deflexa*, and *O. maydelliana* in upland tundra heaths at Meliadine.

## Future Directions

Due to COVID-19 summer 2020 field work was not completed on this project. As an alternative, *Oxytropis spp.* were collected locally near Saskatoon, SK to support future work in better understanding *Oxytropis* ecology, and for use in methods development in winter 2021. Plugs with *Oxytropis* collected at Meliadine in summer 2019 were reallocated for use in the plug expansion trial (see above). Care was taken not to damage *Oxytropis* in plugs and live *Oxytropis* species from Meliadine are being maintained within the remaining plug materials in growth chambers at the University of Saskatchewan.

Using locally collected and Arctic *Oxytropis* we will be developing methods to examine rooting architecture and  $^{11}\text{CO}_2$  and  $^{13}\text{N}_2$  radio-isotope movement in *Oxytropis*-soil systems using Positron Emission Tomography –Computed Tomography (PET-CT). Initial images have been taken following dosing of *Oxytropis* with positron-emitting isotopes to detect carbon fixation ( $^{11}\text{CO}_2$ ) and nitrogen fixation ( $^{13}\text{N}_2$ ) in plants, and movement to belowground and microbial communities in soils (Figure 10). Through co-registration of radioactive hot spots with biological samples and subsequent molecular sequencing of soil bacterial and fungal communities important plant-soil interactions that promote recovery of key soil processes in early successional systems can be identified. Restoration practices and selection of species for restoration will be informed by an improved understanding of these arctic plant-soil interactions and nutrient cycling processes.



*Figure 10. PET-CT imaging of Oxytropis cores collected at Meliadine in summer 2019.*

# Natural Colonization of Biological Soil Crusts on Drilling Waste Sites

## *Background*

Bryophytes play an important role in the initial and early colonization of drilling wastes across the landscape at Meliadine (AEM Technical Report 2018). Moss species, particularly *Bryum* spp. were found on 88% of the drilling waste sites and were only absent at three sites all of which were disturbed in 2017. Biological soil crusts (BSCs) are communities of mosses, liverworts, lichens, bacteria, cyanobacteria and fungi that are commonly found throughout the arctic, especially on recently disturbed soils. These early colonizers and the subsequent development of mature biological soil crusts likely play an important role in increasing water hold capacity and maintaining soil moisture at the surface, which is critical for soil nutrient cycling processes and germination of vascular plants from seed. Here we provide results from 2018 laboratory work and 2019 field work. For details of the 2018 field work see AEM Technical Report 2018. In 2019 we have expanded our chronosequence work to better understand what mechanisms could be driving BSC colonization.

## *2018 Chronosequences*

### *Methods*

In summer 2018, two microclimate monitoring sites were established on drilling wastes from 2013. One monitoring site was lost due to data logger failure and flooding. On the remaining site, two HOBO loggers were placed on exposed drilling waste with no BSCs and two loggers were placed on areas colonized by BSCs. At each logger Photosynthetically Active Radiation (PAR) at the surface, soil temperature at ~5 cm depth, soil moisture at ~5 cm depth, relative humidity at ~10 cm above the ground and percent wetness at the surface is being measured. Due to COVID-19 we were not able to collect the data from these loggers in summer 2020, but batteries were replaced in the loggers allowing for an additional year of data to be collected. In summer 2021 we plan to download both the 2019-2020 and 2020-2021 data. Percent cover of BSC by type was visually estimated at each logger site in 2018 and 2019 using 5cm<sup>2</sup> grids in a 25cm<sup>2</sup> quadrat. This will allow us to examine the microclimatic conditions associated with these developing BSC communities.



Figure 11. Data loggers on drilling wastes colonized with BSCs and not colonized with BSCs.

BSC and underlying substrate samples (~5cm depth) were collected from three drilling waste sites for each of the following disturbance years: 2012/2013, 2008 and 1997/1998. All BSC samples were kept in the Phytotron facility (chamber conditions of: 20 hr light/4 hr dark, 15 C light/5 C dark, ~400 umol, 65% relative humidity) prior to measurements.

To better understand the role these early colonizing communities have in driving recovery of ecosystem function, BSCs and underlying soil nutrients were determined. Ammonium (NH<sub>4</sub>), nitrate (NO<sub>3</sub>), total nitrogen (TN), total carbon (TC), sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), chlorine (Cl), and sulfate (SO<sub>4</sub><sup>-2</sup>) of both BSC and substrates immediately underlying BSCs were measured. Gas flux measurements and acetylene reduction assays (a proxy for atmospheric nitrogen fixation) of all sampled BSCs were measured under optimal conditions in the laboratory. A Fourier Transform InfaRed-Multicomponent Gas Analyzer was used to determine carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrogen dioxide (N<sub>2</sub>O) flux under both dark and light conditions.

To characterize BSC composition a combination of approaches is being used; traditional taxonomic identification for the macro communities (bryophytes and lichens) in addition to new molecular techniques for the sequencing of lichens (ITS), bryophytes (ITS3-ITS4), bacteria (16S), fungi (ITS) and invertebrates (COI). DNA was extracted from a subset of three samples per age range (2012/2013, 2008 and 1997/1998) and sent to the Canadian Centre for DNA Barcoding for sequencing and bioinformatics. Each of the community data set was filtered to remove sequencing errors and remove rare species. We chose to filter out all OTUs which did not have a read count greater than 100 in at least one sample. We followed the analysis flow from Gloor et al., 2017, which involves replacing zeros using a count-zero multiplicative approach and normalizing data based on count-log ratio (CLR). Principle component analysis was run on the CLR data to explore differences in each BSC community components across the disturbance age ranges.

## *Results*

### Microclimate

Microclimate monitoring of the 2013 drilling waste site provided insight into the abiotic conditions under which BSCs are developing, as well as, how BSCs are modifying their immediate environment. PAR increased sharply at Julian day 150 (~May 30th, 2019) indicating snowmelt had reached a stage where probes at ground level were exposed and decreased to 0 umol/m<sup>2</sup>/s at Julian day 275 (~Oct 2<sup>nd</sup>, 2019) indicating snow accumulation had completely covered the probes (Figure 12). Arctic cyanobacteria associated with BSCs can continue to fix nitrogen and carbon at light levels as low as 50 umol/m<sup>2</sup>/s and can reach light saturation at light levels as low as 100 umol/m<sup>2</sup>/s. Therefore, given sufficient moisture BSCs may be fixing atmospheric N and C over approximately 4 months. Temperature and moisture followed a similar overall pattern with the highest values observed between June and October. At the height of growing season PAR values reached between 600-700 umol/m<sup>2</sup>/s, and temperature and moisture within the top 5 cm soil reached 15-20°C and 0.3-0.5 m<sup>3</sup>/m<sup>3</sup> respectively (Figure 12). The highest moisture conditions occurred early in the growing season between Julian day 160-180 (~June 9-29<sup>th</sup>), which is likely due to snowmelt. Moisture is the main limiting factor for BSC function (i.e. N and C fixation) and is also a driving factor in successful artificial propagation (Stewart and Siciliano, 2015). This suggests that although BSC activity occurs at least periodically between June-

October, early summer (i.e. June) is likely a critical time for BSCs development and activity. Promoting BSC activity and growth via fertilization and/or transplanting should occur early within the arctic summer to maximize inputs modulated through BSCs and BSC expansion.

Comparison of abiotic conditions in areas covered with BSCs versus bare ground shows slightly elevated PAR inputs in areas with bare ground and almost no difference in surface soil temperature (Figure 12). Since the drilling waste site studied was relatively young (i.e. ~5 years old) it is not surprising that BSC cover was not well enough developed to provide any insulation of surface soils. At older sites with well-developed BSC cover, we expect that the BSCs are insulating soils below, which may assist with surface stability and promoting root growth of vascular plants. Accumulation of organic materials at the surface of the soil can help to reduce temperature fluctuations in soils. BSC cover did lead to higher moisture retention throughout the year (Figure 12). Maintenance of higher surface moisture is essential for providing niches into which later successional arctic plant species can establish.

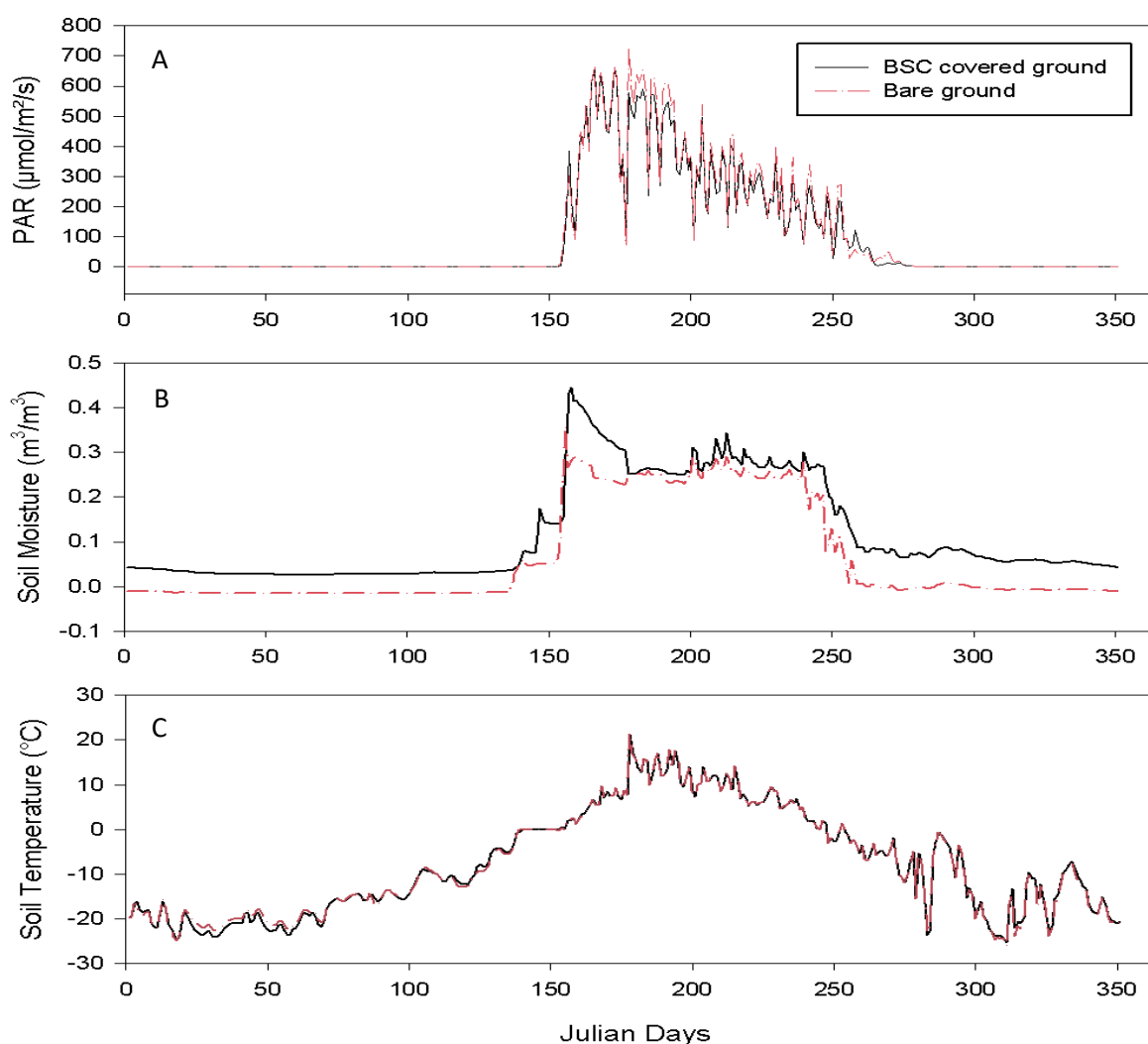


Figure 12. Microclimate data from co-located BSC covered ground and bare ground areas on an early staged drilling waste site (2013) over the duration of 1 year, expressed in average per Julian day. Photosynthetically active radiation (PAR  $\mu\text{mol}/\text{m}^2/\text{s}$ )(A). Soil temperature ( $^{\circ}\text{C}$ ) (B) and soil moisture ( $\text{m}^3/\text{m}^3$ )(C).

## Nutrients

Nutrient analysis of BSCs collected in 2018 found cation and anion values were always higher in BSCs than underlying soils (Figure 13). Significant interactions between year and substrate (i.e. BSC or underlying soil) occurred for Na, K, Cl, and  $\text{SO}_4^{2-}$  (ANOVA with Tukey HSD post hoc test,  $p < 0.05$ ). All nutrients increased in the BSCs over time, demonstrating the role of BSCs in accumulating nutrients at these disturbed sites with time. However, only Na and Mg showed statistically significant higher values with time since disturbance. In soils, different patterns across years were observed for different nutrients. Both Na and  $\text{SO}_4^{2-}$  had were significantly higher in 2008 drilling wastes, which are likely related to operational practices. Cl decreased with time and was significantly lower in underlying soils in samples from 1997/1998, again likely related to materials used during the drilling process.

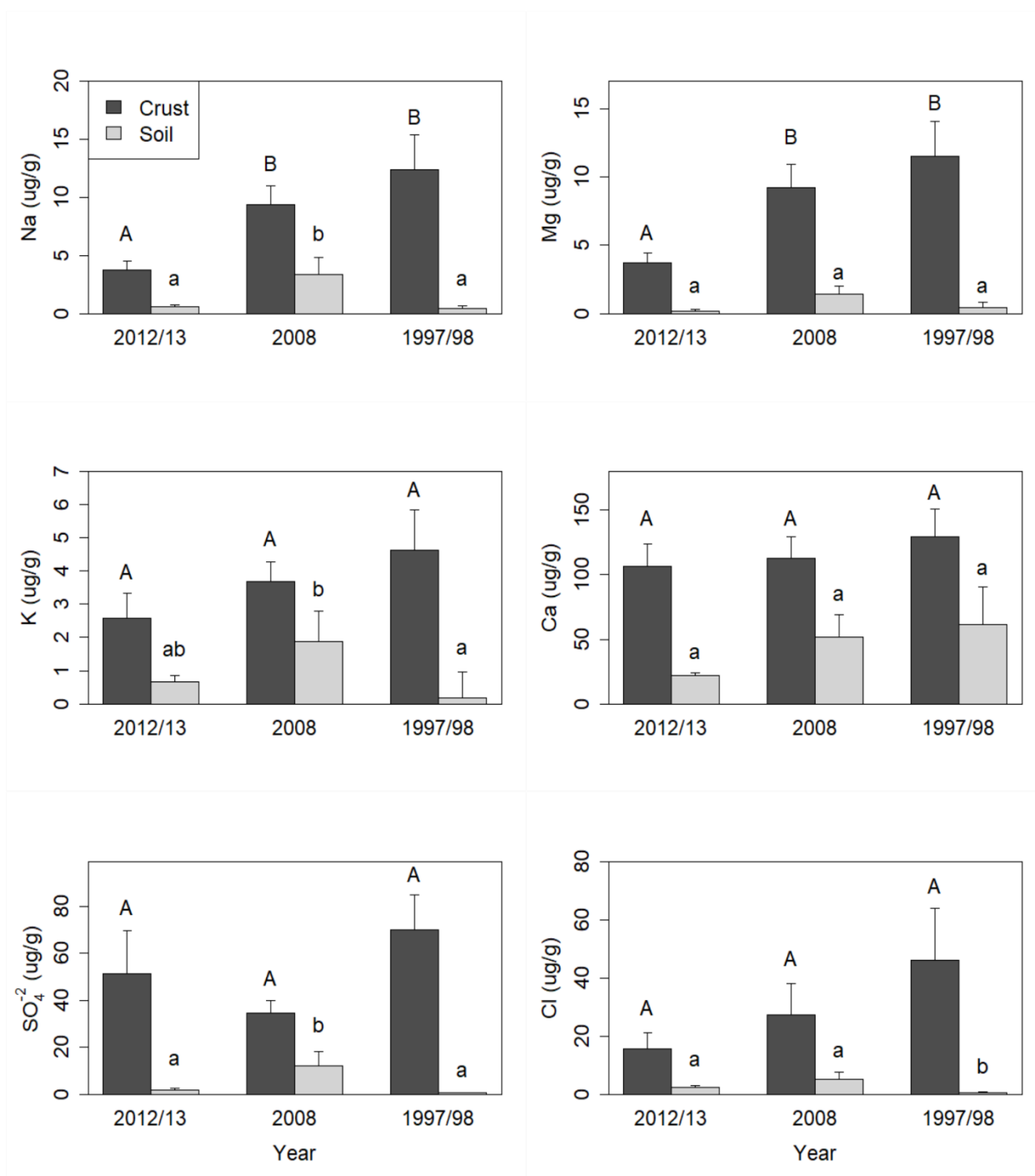


Figure 13. Nutrients in BSCs and soils immediately below BSCs on drilling wastes deposited on the Meliadine site in the years 2012/2013, 2008 and 1997/1998. Bars represent means with standard error. Nutrients in BSCs and soils were compared using a two-way ANOVA including the interaction of year and substrate type (i.e. BSC or underlying soil) followed by Tukey HSD posthoc comparisons across each substrate type.

Most values for  $\text{NH}_4$  and  $\text{NO}_3$  were below detection limits (95% and 65% for  $\text{NH}_4$  and  $\text{NO}_3$ , respectively). The few detectable measurements of  $\text{NH}_4$  were in BSCs. Similarly, crusts had more detectable  $\text{NO}_3$ , and followed similar patterns to other nutrients measured with higher values in older BSCs (mean = 0.31, 0.59, 1.68  $\mu\text{g/g}$  for 1997/98, 2008, 2012/13). Drilling waste sites are strongly



nitrogen limited, therefore, organic N inputs from BSCs may be essential for plant community development.

### Nitrogen fixation and gas flux

Nitrogen fixation rates were determined for BSCs collected on drilling wastes of varying ages (2013, 2008, 1997/1998) in 2018 using acetylene reduction assays (Figure 14). When incubated under optimal conditions, higher rates of nitrogen fixation were found for BSCs collected on older drilling wastes, indicating that nitrogen fixing species, such as cyanobacteria, are likely increasing in BSC communities over time. Due to the nitrogen limited nature of the drilling wastes these early colonizing nitrogen fixers likely play a key role increasing available nitrogen in the wastes, which will promote establishment of vascular plants. As we observed in the 2018 technical report, natural colonization by BSCs appears to be relatively rapid at the Meliadine site, however, actively promoting conditions that foster BSC establishment and BSC nitrogen fixation (i.e. retention of moisture in surface soils) could be a way to simply promote natural recovery of tundra vegetation communities.

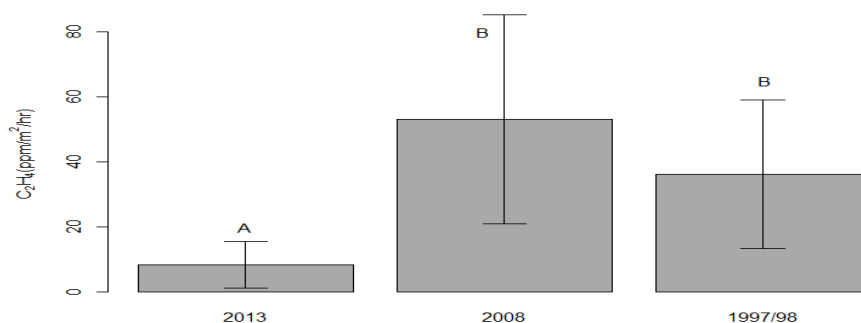


Figure 14. Nitrogen fixation by biological soil crusts (BSCs) sampled on drilling wastes created in 2013, 2008 and 1997/1998. Nitrogen fixation was measured via acetylene reduction assays. BSCs on older drilling wastes (1997/1998 and 2008) had significantly higher rates of nitrogen fixation than more recently disturbed sites (2013) (ANOVA, Tukey posthoc,  $p < 0.05$ ).

Gas flux measurements of BSCs under optimal conditions revealed the majority of flux was not significantly different across the age ranges (Figure 15). Light N<sub>2</sub>O was the only gas to show significant differences between the drilling wastes of different ages and these differences were between the late and mid age ranges (1997-98 and 2008) (Table 8). Overall there was a trend towards higher rates of gas flux at mid age ranges (2008), which may correspond with the most productive BSC communities. Limited differences in gas flux under optimal conditions may be related to the low nutrient status of the underlying drilling wastes. Longer in-situ incubations may provide insight into gas flux in these BSC communities and the associated ecosystem processes (i.e. photosynthesis, respiration, nitrogen fixation, nitrification, denitrification and methanogenesis).

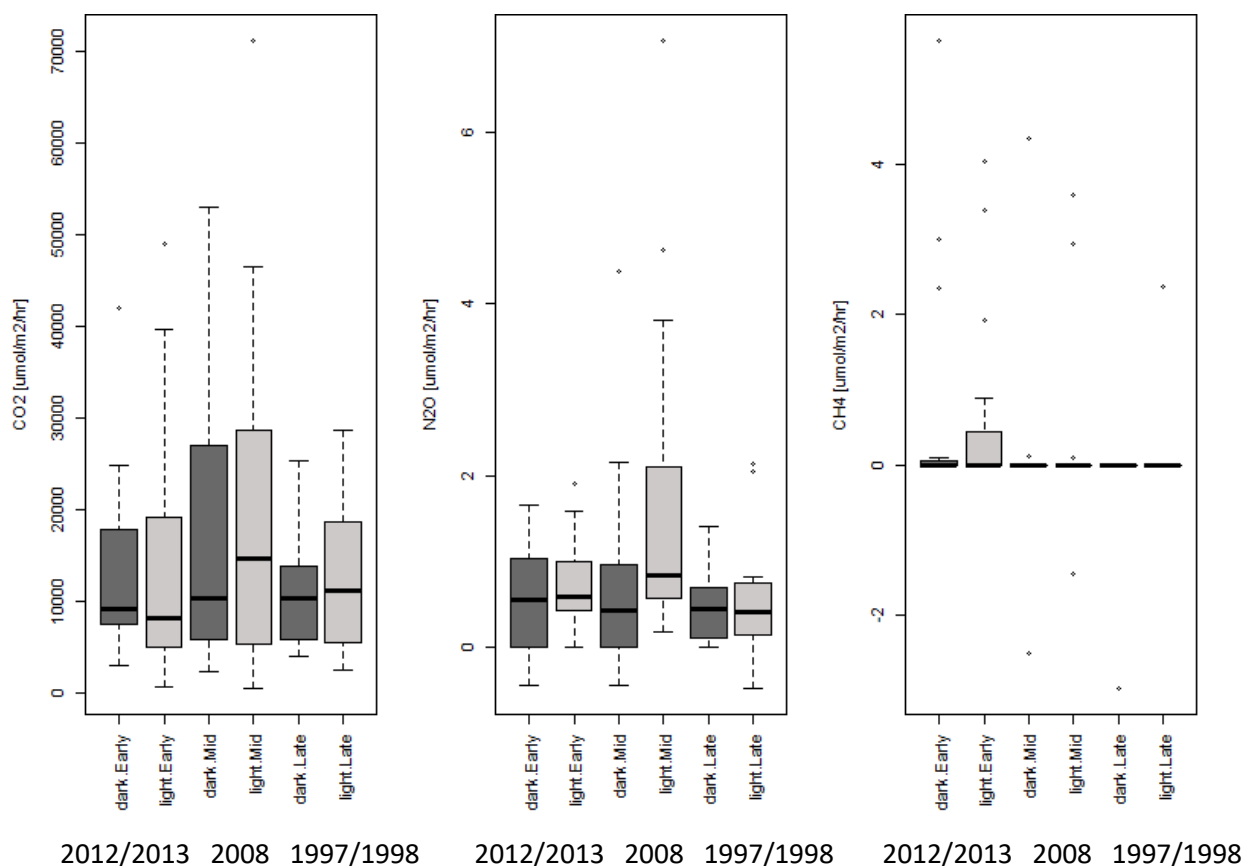


Figure 15. Gas flux (CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub>) measured on BSC samples from Meliadine under optimal conditions in the laboratory. Flux values are provided under dark and light conditions at early (2012/2013), mid (2008) and late (1997/1998) drilling wastes.

Table 8. Comparison of gas flux (CO<sub>2</sub>, N<sub>2</sub>O, CH<sub>4</sub>) measured on BSC samples under dark and light optimal conditions from drilling wastes deposited on site in the years 2012/2013, 2008 and 1997/1998. F values and p values are shown for ANOVA (p<0.05). Bold p values indicate significantly different flux values.

Gas Flux	Age range		
	df	F	p
Dark CO <sub>2</sub>	2	0.4243	0.6636
Light CO <sub>2</sub>	2	0.2954	0.7495
Dark N <sub>2</sub> O	2	0.0223	0.978
Light N <sub>2</sub> O	2	4.818	<b>0.0291</b>
Dark CH <sub>4</sub>	2	1.443	0.275
Light CH <sub>4</sub>	2	0.8049	0.4703
Net Photosynthesis (light-darkCO <sub>2</sub> )	2	0.2409	0.7897

## BSC Community Composition

Comparison of community composition for each BSC component was done using molecular sequencing (i.e. Operational Taxonomic Units (OTUs) were identified to family, genus or species level). We explored the OTU data for differences in each BSC component to determine if/how bryophytes, lichens, fungi and invertebrate composition were changing across time. Bacterial community data is currently being processed and will be included in future analyses. Principal component analysis (PCA) revealed distinct clustering of species on early (2012/2013), mid (2008) and late (1997/1998) drilling wastes for bryophyte, lichen and fungal species indicating shifts in BSC community composition over time. Significant differences in community composition were identified for bryophytes, lichens and fungi (PERMANOVA,  $p < 0.1$  bryophytes,  $p < 0.05$  lichen and fungi). Currently, we are examining factor loadings of individual OTUs/species on the PCA axes to identify key species' differences between the age ranges. This analysis will provide insight into species that are foundational immediately following disturbance and provide a better understanding of the successional pathways of these BSC communities. In addition, we are working to link these changes in community composition with environmental variables (see future directions below). The results from this study will allow us to recommend key BSC species that would be ideal for restoration, as well as, suggest soil treatments that would promote establishment of BSCs and BSC development.

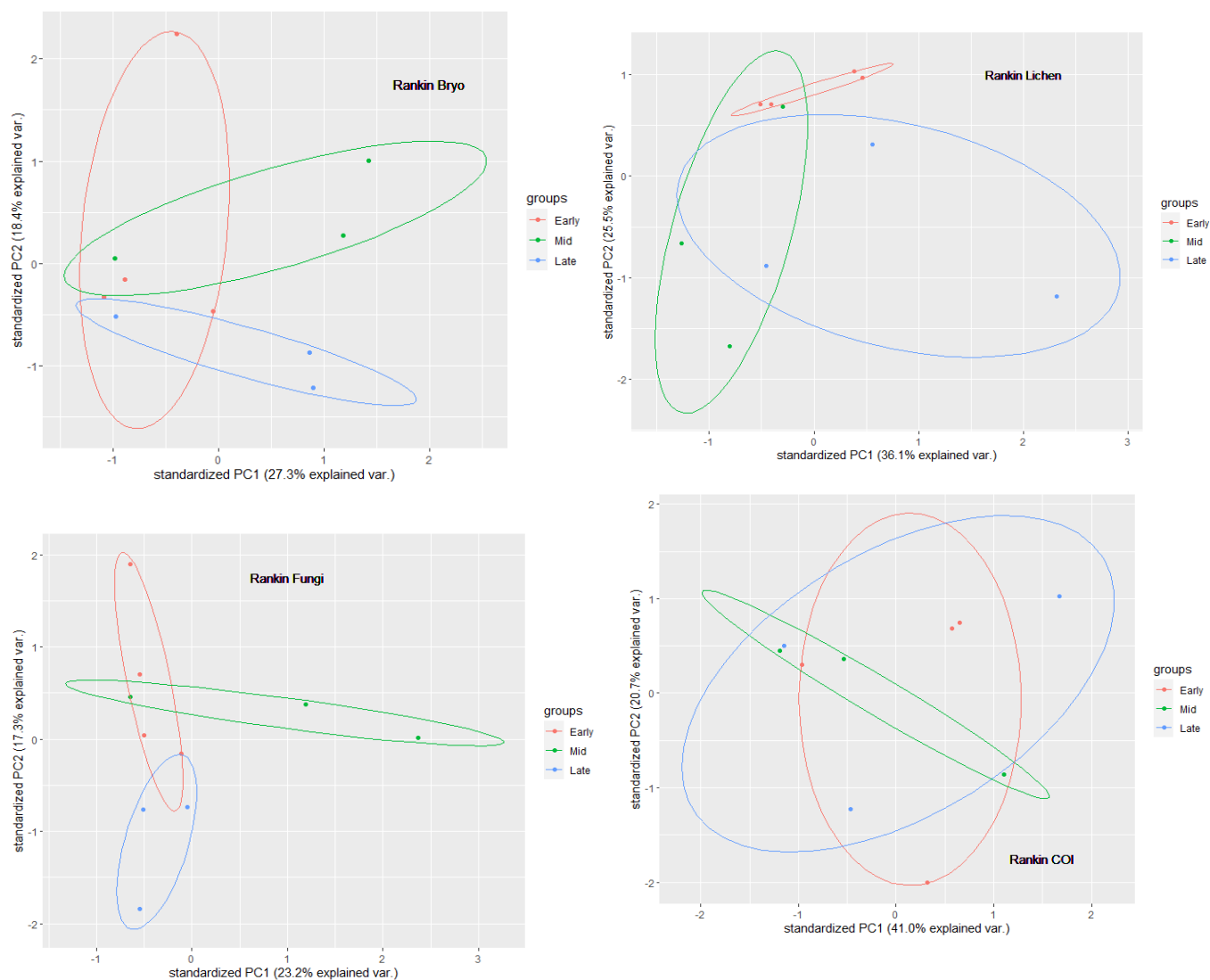


Figure 16. Principal component analysis of community composition (OTUs) for bryophytes (Rankin Bryo), lichen (Rankin Lichen), fungi (Rankin Fungi) and COI (invertebrates) (Rankin COI) at early (2012/2013), mid (2008) and late (1997/1998) drilling wastes.

## 2019 Chronosequences

### Methods

In summer 2019, 15 additional sites were identified for further sampling (Figure 17). Three age ranges corresponding to those surveyed in 2018 (2012/2013, 2008, 1997/1998) were selected and five sites with 3 sampling locations per site were examined for a total of 45 sampling locations. At each site three areas with BSC cover and no vascular plant growth were identified. Site observations were recorded (ex. size of drilling wastes, slope, aspect, proximity to water bodies). Custom made static vented gas sampling chambers were installed to measure gas flux under field conditions (Figure 18). Five gas samples were collected over a 1 hour period (15 minute intervals) under both light and dark conditions. Light and temperature were measured every 15 minutes throughout the gas sampling period. Following gas flux measurements, BSC samples were collected from each sampling location. Samples were returned to the laboratory at the University of Saskatchewan where optimal gas flux measurements and nitrogen fixation via acetylene reduction assays were completed as described above in 2018. The BSC samples are currently being inventoried by classic taxonomic methods and a subset may be used for molecular analysis. Nutrients, pH, total carbon and total nitrogen have also been determined for each BSC and underlying soil sample as described above. Plant root simulator (PRS) probes used to measure available nutrients over a given time period were installed at 18 sites for 16 days during the 2019 field season. Sites chosen were a combination of paired BSC and bareground areas at eight early, five mid and five late age range sites. Probes were inserted into the crust between the underlying substrate and crust layer (Figure 17).



Figure 17. BSC sampling sites and nutrient monitoring sites at Meliadine in 2019.



*Figure 18. Static vented gas flux chambers on BSCs at drilling wastes from 1997/1998. Gas flux was sampled every 15 minutes over a 1 hour period to determine in-situ  $\text{CO}_2$ ,  $\text{N}_2\text{O}$  and  $\text{CH}_4$ .*

### *Future Directions*

Data from both the 2018 and 2019 Chronosequence work will be processed and analyzed in winter and spring 2021. Field data collection for this aspect of our project is almost complete with only microclimate data and associated bryophyte cover data to be collected in summer 2021. In early winter 2021 we expect to submit our first manuscript for peer-review. Our manuscript will examine changes in BSC communities over time on drilling wastes and link these changes with environmental conditions and BSC processes (ex. photosynthesis and nitrogen fixation). Initial results indicate that increasing rates of nitrogen fixation are accompanied by changes in community composition of bryophytes and nutrients appear to accumulate in BSCs over time (Figure 19). Publication of this manuscript will support methodologies used in subsequent work and will also provide a foundation for creating a series of applied restoration recommendations based on these findings. Specifically, we will aim to identify key BSC species that would be ideal candidates for active or passive restoration and create recommendations for soil and surface treatments to promote BSC establishment and growth.

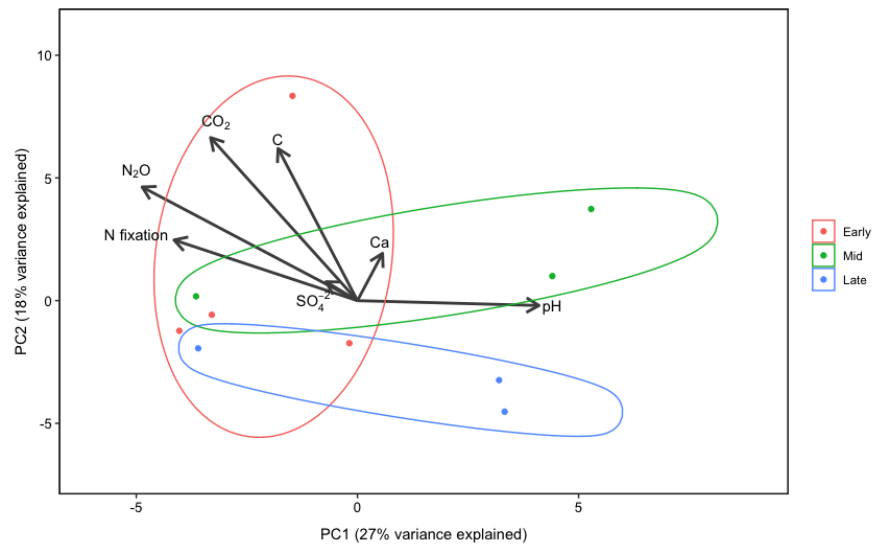


Figure 19. Principal components analysis of bryophyte community composition in BSCs on for bryophytes at early (2012/2013), mid (2008) and late (1997/1998) drilling wastes with corresponding environmental vectors.



# Invasive Species Survey

## *Background*

We conducted an invasive plant survey in summer 2019 upon request from AEM to support meeting Condition 37. We will provide and implement “*protocols for reducing Project-related effects to plant populations and communities, primarily through the mitigation and management of invasive species, and includes both environmental and follow-up monitoring*” as required by the Project Certificate (No.006, Condition 37) for the Meliadine site. The survey was intended to provide early detection of any non-native invasive species present on the Meliadine site and provide the distribution and abundance of any invasive species found. A targeted monitoring approach, which focuses on high probability sites of initial invasive species establishment was used.

## *Methods*

Invasive plant monitoring followed the survey protocol outlined by Oldham 2006 for exotic plants along Northwest Territories highways. Our survey study area included the footprint of the Meliadine mine, as well as, the main access road from Rankin Inlet to the Meliadine site. Road surveys consisted of driving at a low speed (30km/hr on the road into site, and posted speed limits within the mine footprint) along all roadways in the study area. During road surveys, invasive and non-native plants were searched for. All roads were driven twice, to ensure both roadsides were surveyed.

On the main access road, we stopped every 2km for an informal roadside walking survey. At each informal roadside survey, we will recorded latitude and longitude, walked 50 meters along both sides of the road and recorded if any non-native invasive species were encountered (Table 9). Within the mine footprint, areas of high likelihood of invasive species colonization, such as intersections, were surveyed through formal roadside surveys (Table 10). At each formal roadside survey location, we recorded the latitude and longitude, describe the local habitat with reference to native species nearby, and walked 50m in all directions looking for non-native invasive species. Additional locations with a high likelihood of invasive species colonization within the mine footprint, such as the exploration camp and main camp, were also surveyed by the formal approach with a focus on the perimeter of the disturbed area.

## *Results*

No invasive or non-native species were identified in any our 2019 surveys. The roadside driving survey was conducted in 2 km segments in each direction from Meliadine to Rankin Inlet and from Rankin Inlet to Meliadine. Driving at slow speeds (30 km) four individuals observed roadside plant species. Any unknown species identified in the roadside driving species were identified in fall 2019 through voucher specimens. None of the unknown species identified were invasive plants. Every 2 km roadside walking surveys were completed and non-native invasive species were identified (Table 9). Four unknown species have not yet been identified because voucher specimens lacked flowers or other necessary identifying features. The location and frequency of these unknown species have been recorded and can be visited to collect voucher specimens for identification in summer 2021.



Table 9. Roadside walking survey for non-native invasive species. At each roadside location 50 m on one side of the road was surveyed. Roadside locations were observed when traveling south towards Rankin Inlet (IRS) and when travelling north towards Meliadine (IMN). The latitude and longitude at each location was recorded, as well and the general landscape type. No non-native invasive species were identified. Four unknown (UNK) species were observed that have not yet been identified. The frequency of these species was noted based on the number of individuals observed.

Roadside location	Latitude (N)	Longitude (W)	Landscape	UNK Species (frequency)
II1	63°01'22.6"	092°11'38.0"	Upland heath & wetland	UNK grass_2 (5-15)
IRS0	63°01'18.0"	092°11'23.2"	Upland heath & wetland	
IRS1	63°00'28.8"	092°11'41.1"	Upland heath & wetland	UNK grass_2 (<5) UNK grass_5 (<5)
IRS2	62°59'39.7"	092°10'29.5"	Upland heath & wetland	
IRS3	62°59'01.2"	092°08'30.8"	Upland heath& wetland	
IRS4	62°58'04.4"	092°07'33.3"	Upland heath & wetland	UNK grass_5 (<5)
IRS5	62°57'15.3"	092°06'13.8"	Upland heath- large rocks	
IRS6	62°56'27.2"	092°04'59.6"	Upland heath- disturbed	
IRS7	62°55'47.4"	092°03'25.9"	Upland heath-very rocky	UNK forb_10 (<5)
IRS8	62°54'40.9"	092°03'31.0"	Upland heath-very rocky	UNK grass_5 (<5)
IRS9	62°53'43.8"	092°04'11.5"	Wetland	
IRS10	62°52'46.9"	092°05'23.8"	Upland heath	
IRS11	62°52'20.1"	092°07'11.8"	Rocky slope- upland heath	UNK forb_12 (<5)
IRS12	62°51'29.1"	092°08'35.6"	Upland heath	
IRS13	62°50'12.2"	092°08'30.5"	Upland heath	
IRS14	62°49'43.2"	092°07'02.8"	Upland heath	
IMN30	63°01'18.0"	092°11'23.2"	Upland heath	
IMN28	63°00'28.8"	092°11'41.1"	Upland heath	
IMN26	62°59'39.7"	092°10'29.5"	Upland heath	
IMN24	62°59'01.2"	092°08'30.8"	Upland heath	
IMN22	62°58'04.4"	092°07'33.3"	Upland heath	
IMN20	62°57'15.3"	092°06'13.8"	Upland heath	
IMN18	62°56'27.2"	092°04'59.6"	Upland heath	UNK forb_10 (5-15)
IMN16	62°55'47.4"	092°03'25.9"	Upland heath	
IMN14	62°54'40.9"	092°03'31.0"	Upland heath-very rocky	
IMN12	62°53'43.8"	092°04'11.5"	Upland heath	
IMN10	62°52'46.9"	092°05'23.8"	Upland heath & wetland	
IMN8	62°52'20.1"	092°07'11.8"	Upland heath- very rocky	
IMN6	62°51'29.1"	092°08'35.6"	Upland heath	
IMN4	62°50'12.2"	092°08'30.5"	Upland heath-rocky	
IMN2	62°49'43.2"	092°07'02.8"	Upland heath-rocky	

In addition to the roadside surveys, we also completed a survey of areas within the mine footprint that were likely to have invasive species (i.e. disturbed areas and areas of high traffic) (Table 10). No non-native invasive species were observed within the areas surveyed on-site. Three unknown species remain unidentified due to voucher specimens lacking key identifying features. The location and frequency of these unknown species have been recorded and can be visited to collect voucher specimens for identification in summer 2021.

Table 10. A walking and driving survey of areas of high likelihood of non-native invasive species around the main camp and exploration camp. The latitude and longitude at each location was recorded. No non-native invasive species were identified. Three unknown (UNK) species were observed that have not yet been identified. The frequency of these species was noted based on the number of individuals observed.

Location	Latitude (N)	Longitude (W)	UNK Species (frequency)	Notes
<b>Main camp</b>				
IMC1	63°02'23.1"	092°13'30.9"		Walked IMC1 to IMC2 checked under buildings, walked to gravel-tundra transition
IMC2	63°02'26.7"	092°13'45.5"		Walked IMC2 to IMC1 walking west, around dorms
<b>Exploration camp</b>				
II1	63°01'22.6"	092°11'38.0"		Drove II1 to IEXP1 -first intersection inside camp to beginning of Exp
IEXP1	63°01'35.7"	092°10'26.9"		Drove IEXP1 to II1 from Exp to first intersect
IEXP2	63°01'43.1"	092°10'15.3	UNK grass_16 (5-15)	Walked IEXP2 to IEXP3 edge around main building and between dorm wings
IEXP3	63°01'46.9"	092°10'05.0"	UNK forb_10 (>15)	Walked IEXP3 to IEXP4 edges around V and N wing
IEXP4	63°01'44.3	092°10'10.5"	UNK grass_16 (<5) UNK forb_10 (5-15)	Walked IEXP4 to IEXP2

### *Future Directions*

We recommend that surveys in these areas be conducted once during the growing season to monitor for non-native invasive species. In summer 2021 we will return to any location with unidentified species to provide confirmation that none of these species are invasive. Best practices should be followed to prevent the introduction and spread of invasive species on the Meliadine site. Preventative measures include: Inspecting and cleaning vehicles or equipment entering the site, maintaining healthy native roadside vegetation and continually surveying high likelihood areas for invasive species establishment. In addition, the use of local vegetation and soils for restoration can prevent the spread of invasive species introduced through commercial seed mixes and soil amendments.

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