Appendix 31: 2021 Natural recovery and active restoration of tundra plant-soil systems Report

Natural recovery and active restoration of tundra plant-soil systems at Agnico Eagle Mines Meliadine site, Nunavut

Prepared for Agnico Eagle Mines Limited

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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) Development of tundra restoration techniques using local native vegetation; iii) Characterization of initial and realized niches of early vascular colonizing vascular plants and actively restored tundra vascular and non-vascular plants on disturbed substrates. In addition to the scientific work, the project included the development of a youth education program and local community engagement in Rankin Inlet and Baker Lake, NU. Although, education materials have been developed (see website), due to logistical issues and COVID-19 education programming and community engagement were not carried out. **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 wastes 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 turfs, 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 education programs 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	Hold community meetings in Rankin Inlet and Baker Lake. Working with Agnico's community relations department identify key groups to host. Our research and restoration of tundra environments will be 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.*"

The University of Saskatchewan has been working in partnership with Agnico Eagle Mines through a NSERC Collaborative Research Development grant to provide the needed information to effectively meet Term and Condition no. 41. To better understand the barriers for revegetation and to identify potential vegetative species for restoration natural colonization of drilling wastes and disturbed substrates by biological soil crusts and native vascular plants was examined from 2018-2021. Primary findings include: i) native plant communities on drilling wastes appear to recover 20-25 years after disturbance; ii) bryophytes are key early colonizing species with generalist ruderal acrocarpous mosses found on boreal and Arctic disturbed substrates; iii) biological soil crusts modify the environment by changing abiotic conditions at the soil surface, which in turn promote development of native vegetation communities; and iv) native legumes appear to play an important role in plant-soil interactions in early successional habitats.

Active restoration trials including ex-situ and in-situ examinations of the efficacy of transplanting upland tundra heath turfs and shredded materials were conducted from 2019-2021. Growth chamber trials indicated that fertilization of adjacent substrates may reduce restoration timelines and highlighted the importance belowground expansion. In-situ restoration efforts at the Meliadine site revealed: i) turfs 40 cm^2 by 10-15 deep support native plant-soil communities that can act as resources for revegetation; ii) shredded materials had poor establishment and may not be appropriate at Meliadine; iii) in general, evergreen shrubs should be avoided due to high transplanting morality; and iv) graminoids should be targeted for transplanting due to extensive belowground expansion.

Soil invertebrate surveys were conducted in 2018 at Meliadine and found soil invertebrate communities, expressed as the density of collembolan, enchytraeids and mites, were relative constant across the upland tundra heath sites, although vegetation and soil characteristics appear to influence species density and should be considered when selecting materials for restoration. An extensive invasive plant species survey was conducted in summer 2019 and no non-native invasive species were observed or identified. In fall of 2019 we launched a website: tundrarestoration.com that provides information on tundra ecology, scientific and traditional Indigenous knowledge of common tundra plants, details and videos of our restoration trials and information on youth education programs. Here we attach technical appendices with project summaries.

Finally, on-going examinations of the plant-soil interactions of early colonizing Arctic legumes has identified nitrogen-fixing legumes as important early colonizers on disturbed substrates at Meliadine. Characterization of nitrogen fixing bacteria associated with these species is currently underway and will be complemented by radioisotope imaging of carbon and nitrogen uptake and translocation.

Together these studies indicate that tundra turfs, biological soil crusts and nitrogen fixing legumes are all candidates for active and passive restoration and both natural recovery and active restoration of local native vegetation can support the development of healthy functioning tundra ecosystems.

Section I: Natural Colonization of Drilling Waste

Natural Recovery of Tundra Vegetation on Drilling Waste

Background

Examining natural colonization of vegetation on disturbed sites is critical to understanding both the barriers to revegetation and to identify potential vegetation species and processes that can support restoration efforts. Extreme environmental conditions and the slow recovery of plant communities and nutrient cycling can impose severe limitations on the recovery of tundra ecosystems following anthropogenic disturbance. Natural recovery can occur following disturbance of tundra systems, but many studies have suggested that recovery of northern ecosystems may take decades, millennia, or may never return to pre-disturbance conditions (Komárková 1983; Harper & Kershaw 1996; Jorgenson et al. 2010). Lower species cover and differences in richness and diversity often follow disturbance on Arctic sites (Harper & Kershaw 1996; Forbes & Jefferies 1999). In the summer of 2018, prior to engaging in active revegetation efforts, a total of 25 drilling waste sites across the primary ecotypes at the Meliadine site were examined for natural vegetative recolonization. The ecotypes included both low-lying and upland hummock-hollow complexes dominated by sedges and heath vegetation, respectively, and upland lichen-heath often with frost boil features. The drilling waste sites ranged in age from one year post-disturbance to 25 years post-disturbance providing insight into the natural recovery and successional trajectories common on this landscape.

Methods

A total of 25 drilling waste sites were examined across a range of ecotypes including low-lying hummock-hollow complexes dominated by sedges, upland hummock-hollow complexes dominated by heath, and upland lichen-heath often with frost boil features (Table 1). Low-lying hummock-hollow complexes dominated by sedges were characterized by high soil moisture, often with standing water surrounding hummock-hollow complexes of approximately 30 cm in height. The primary vegetation found in this ecotype was *Dryas integrifolia*, *Carex aquatilis*, and *Scorpidium scorpioides*. Upland hummock-hollow complexes were similar in size to low-lying complexes; however, due to a high landscape position, had drier soil conditions and were dominated by *Rhododendron lapponicum, Loiseleuria procumbens, Cassiope tetragona,* and species of *Cetraria* lichen. The upland heath ecotype was typically associated with upper slope locations and well-developed frost boil features. The primary vegetation in the upland heath ecotype was *Alectoria ochroleuca*, *Cassiope tetragona, Tomentypnum nitens,* and species of *Cetraria* lichen.

Drilling waste sites were grouped by time since disturbance and included sites created in 1993, 1997-1998, 2008, 2012-2013 and 2017 (n = 5 per age group). Of the sites surveyed, 5 were low-lying sedge, 9 upland heath, 7 transitional between low-lying sedge and upland heath, and 4 lichen-heath ecotypes. Ecotypes were not equally represented across age categories because drilling tended to be localized within the landscape for a given year (Figure 1; Table 1). Older sites (1993, 1997-1998) occurred more frequently in low-lying sedge and sedge-heath transitions, while four of the five 2017 sites were in lichen-heath tundra. Due to the nature of drilling, some sites we sampled were spatially clustered together (i.e., 2008 and 2017 sites).

Table 1. Drilling waste areas surveyed for natural revegetation. Site name, location, year and ecotype of each area surveyed is included. Ecotypes are described as low-lying sedge hummock-hollow (SH), upland heath hummock-hollow (HH) and upland lichen-heath (LH). Some drilling waste areas were in transitional areas between ecotypes (SH/HH).

Site	**Location (UTM)**	**Year**	**Ecotype**
W93-29	15V 0541749 6986813	1993	SH
W93-30	15V 0541809 6967640	1993	SH
W93-32	15V 0541948 6986649	1993	SH
W93-40	15V 0541439 6987661	1993	SH/HH
W93-44	15V 0541647 6986830	1993	SH
M97-155	15V 0540844 6986435	1997	HH
M98-230	15V 0541609 6986957	1998	HH
M98-242	15V 0541675 6986906	1998	SH/HH
M98-243	15V 0541385 6987129	1998	SH/HH
M98-244	15V 0541874 6986759	1998	SH
M08-729	15V 0542342 6986377	2008	HH
M08-734	15V 0542295 6986320	2008	SH/HH
M08-735	15V 0542344 6986314	2008	SH/HH
M08-740	15V 0542435 6986305	2008	HH
M08-741	15V 0542427 6986240	2008	HH
M12-1528	15V 0541565 6986959	2012	HH
M12-1860	15V 0541625 6986905	2012	HH
M12-1863	15V 0541720 6986994	2012	HH
M13-1948	15V 0540492 6986413	2012	SH/HH
GT13-71	15V 0540477 6986460	2013	SH/HH
M17-2394	15V 0540298 6986710	2017	LH
M17-2395	15V 0540375 6986656	2017	LH
M17-2396	15V 0540500 6986571	2017	LH
M17-2398	15V 0540455 6986519	2017	LH
M17-2415	15V 0540371 6986700	2017	HH

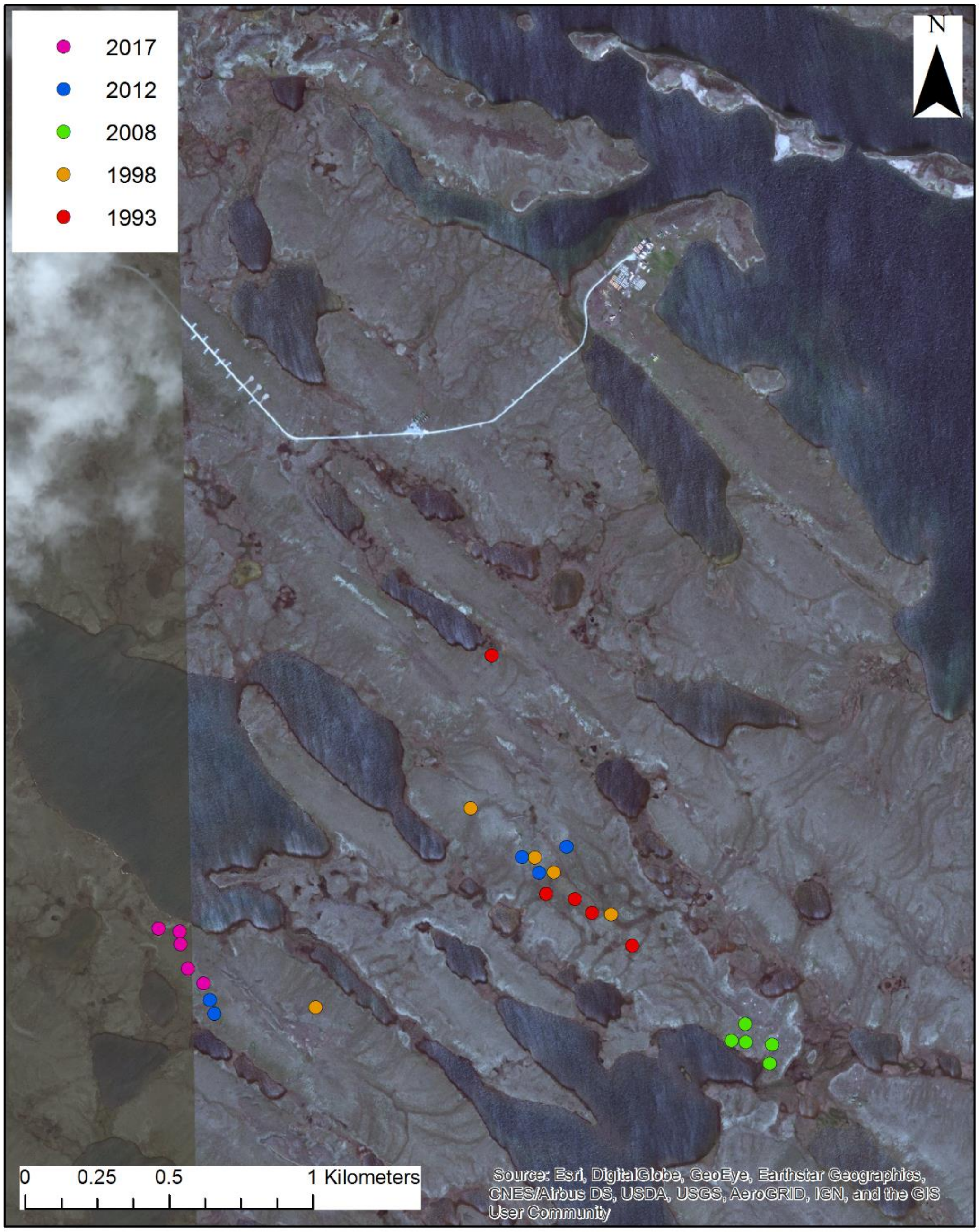

Figure 1. Map of drilling waste areas sampled in 2018. The year of drilling is given for each of the sites surveyed. The Meliadine site is shown for reference and all drilling waste sites were outside of the mine footprint.

The approximate area of each drilling waste site was delineated by determining the extent of drilling waste at each location. The presence of drilling waste was either observed on the surface or below the surface by digging up to about 15 cm in depth. The average size of the drilling waste sites was 21 m x 9 m or 189 m^2. The largest drilling waste area sampled was from 1993 (W93-32) with an area of 1472 m^2. On average the 2008 drilling waste sites surveyed had the smallest area (77 m^2). In general, older drilling waste sites were covered with more vegetation and at times were difficult to locate (Figure 2). However, even at sites created in 1993 small patches of drilling waste were present at the surface (Figure 2). Newer drilling waste sites (i.e., 1-5 years old) generally had large patches of waste visible at the surface (Figure 3).

Figure 2. Small pockets of drilling waste evident at the surface of older mining sites (Left M93-30, Above M93-40).

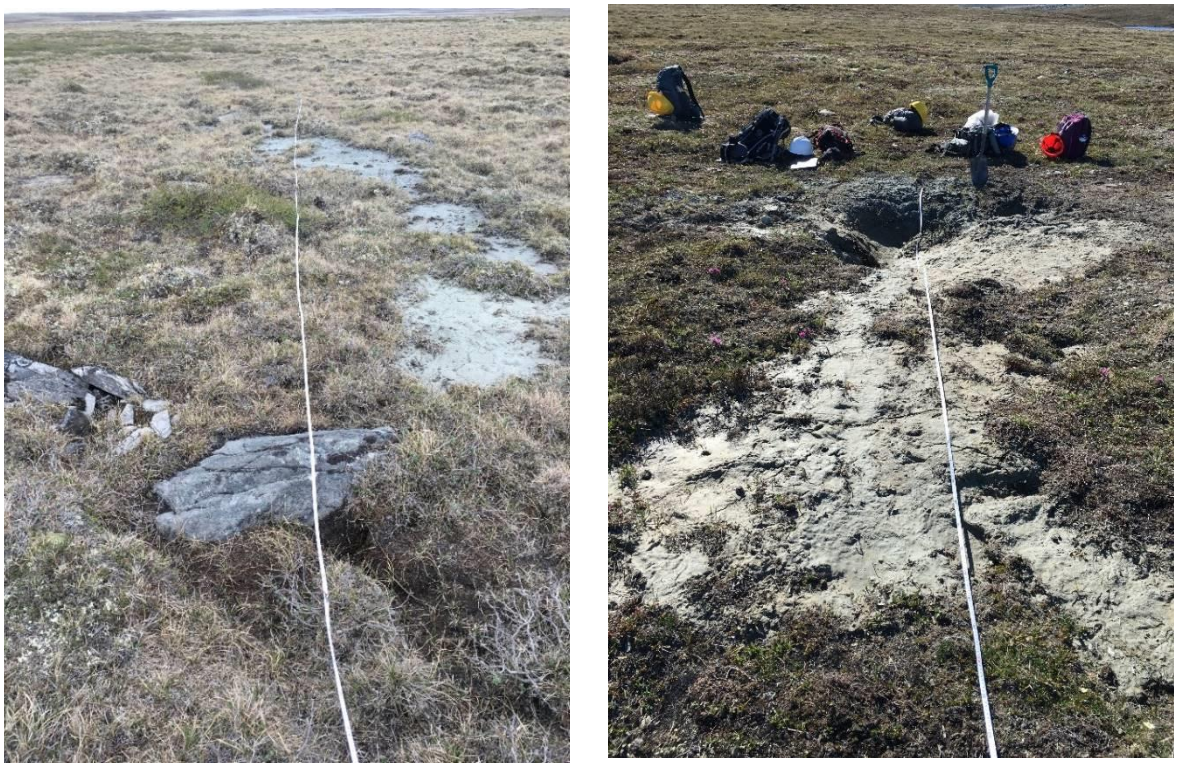

Figure 3. Larger patches of drilling waste evident at the surface on newer drilling sites (left M12-1863; right M17-2398).

Following delineation of each drilling waste site, a transect was placed along the long axis of the waste site and three survey plots were placed at the center of this transect (Figure 4). The three plots were each 1 m^2 and were placed immediately adjacent to one another. In each plot, percent cover was visually estimated for all species present. Following completion of the vegetation survey, a soil/drilling waste sample was taken in the center of each plot and the depth of both drilling waste and organic matter were measured.

At each drilling waste site, a control transect was placed at least 10 m from the edge of the drilling waste (Figure 4). Along the transect three adjacent 1 m^2 plots were placed in undisturbed vegetation of the same type, slope, and aspect as the disturbed area. In control plots, percent cover was visually estimated for all species present and a soil sample was taken in the center of each plot to determine the depth of the organic matter. For all statistical analyses, the average across the three plots on drilling wastes or in control plots was used for each site.

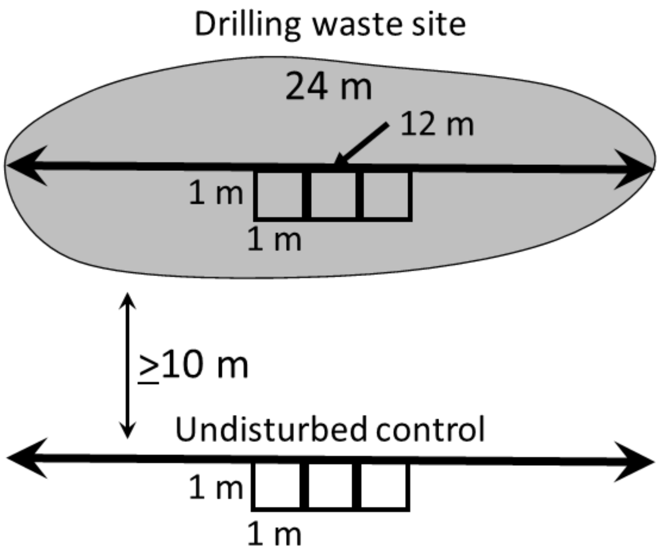

Figure 4. Sampling design at each drilling waste site. Three 1 m^2 plots were placed directly adjacent to one another at the center of the long axis of the delineated drilling waste area (ex. at a drilling waste area 24 m long, the center sampling plot would be placed from 11.5-12.5 m). At each site an undisturbed control transect was placed at least 10 m from the edge of the drilling wastes and 3 control plots (1m^2 each) were placed in undisturbed vegetation.

Results

Substrate Characterization

The average depth of drilling waste and organic matter in disturbed sites was 3.7 ± 3.2 cm and 2.4 ± 3.2 cm, respectively (Figure 5), whereas the average depth of organic matter in control sites was 7.7 ± 2.9 cm. The deepest deposits of drilling waste were > 15 cm and found at M93-40 and M98-230. The deepest organic layer depth was found in a control site at 20 cm. While drilling waste depth was similar across the differently aged sites, higher organic matter accumulation was evident at older sites (Figure 6). Drilling waste depth was similar across all disturbance years and organic matter in undisturbed control plots was also similar across the landscape (Figure 6). The highest amount of organic matter found on drilling wastes was found in sites >25 years old (i.e., 1993), while very low to no organic matter was found on recently disturbed sites (i.e., 2017).

Sites surveyed from 2008 were an anomaly with no organic matter depth detected (Figure 6). It is possible that our sampling design biased placement of survey plots to areas with exposed drilling waste. However, we also found lower percent cover of several species (Table 2) and the highest number of absent species on drilling wastes (Table 3) at 2008 sites. The lack of organic matter accumulation could be due to the physicochemical properties of the drilling wastes inhibiting vegetative establishment or may be associated with the landscape position of 2008 sites. All 2008 sites were in close proximity to one another and were the most south-easterly sites sampled (Figure 1). Differences in landscape conditions (i.e., moisture, aspect, soil properties) in this area may impact natural revegetation. The 2008 sites were classified as heath hummock-hollow or transitional sites between heath hummock-hollow and sedge hummock-hollow. The mid-slope position of these sites would likely contribute to drier soil conditions, which may be limiting vegetative growth and hence organic matter accumulation. To confirm if/why lower organic matter accumulation is occurring in 2008, further survey of 2008 drilling waste sites in other landscape locations and assessment of the physicochemical characteristics of these drilling wastes is needed.

The relatively high organic matter depth measured at 2012 sites is also unexpected (Figure 6). Accumulation of organic matter in tundra ecosystems is generally considered to occur very slowly due to the short growing season and relatively low biomass production each year (Hobbie et al. 2002; Hugelius et al. 2010). It is very unlikely that approximately 4 cm of organic matter would accumulate within 6 years. We noted that on several drilling waste sites, wastes were found in depressions or hollows, with hummocks remaining intact above the level of the wastes. It is highly possible that some slumping is occurring in these hummock-hollow landscapes that could result in the burial of drilling wastes and appearance of organic matter accumulation on the drilling waste surface. These results should be interpreted with caution both due to the possible biasing of measurement due to the sampling design and the low numbers of replication (i.e., n = 5) in each age category.

Figure 5. Drilling waste ~3 cm in depth underlain by an organic soil horizon (left) and organic matter accumulation over drilling waste (right).

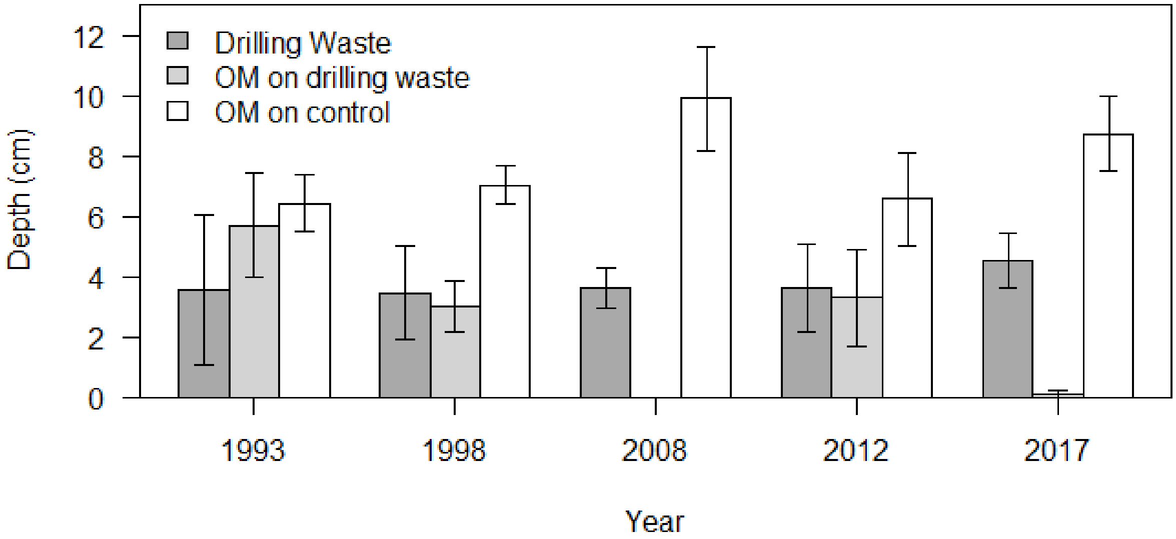

Figure 6. Depth of drilling waste, organic matter found over drilling waste, and depth of organic matter in undisturbed controls plots. Bars are means with standard error (n=5).

Community-level Differences

Recovery of the tundra vegetation at the drilling waste sites was examined through comparison of vegetation in disturbed and undisturbed survey plots at each site, as well as examination of vegetative recovery over time (i.e., across time since disturbance). Community differences between drilling wastes and undisturbed controls over time were explored using non-metric multidimensional scaling ordination (NMDS). NMDS provides an ordination based on a distance or dissimilarity matrix; therefore, objects that are ordinated closer to one another are more similar than those that are farther apart (McCune and Grace, 2002). Overlap between drilling sites from 1993 and 1998 and their respective controls was evident (i.e., overlapping black and red squares and circles) suggesting that 20-25 years following disturbance, plant communities on drilling wastes are similar to undisturbed tundra (Figure 7). More recently disturbed sites from 2008, 2012, and 2017 had distinct communities (i.e., black and red triangles, crosses and x's are separated along the axes) suggesting that >10 years is required for disturbed tundra communities to naturally recover.

To test for the effect of drilling wastes on community composition over time, a PERMANOVA with Euclidean distances was used (Okasanen et al., 2015). PERMANOVA is a non-parametric multivariate analysis of variance that uses distance matrices. Treatment (i.e., drilling waste or control), year (i.e., 1993, 1998, 2008, 2012, 2017) and the interaction of treatment and year were fixed effects in the analysis and species cover was the response. All fixed effects were significantly different indicating that the plant communities are different between drilling wastes and control sites, as well as with time since disturbance ($p < 0.05$). A significant interaction between treatment and year is consistent with the NMDS since we observed that plant communities at older sites (1993, 1998) were similar between drilling waste and control sites, but more recently disturbed communities were not.

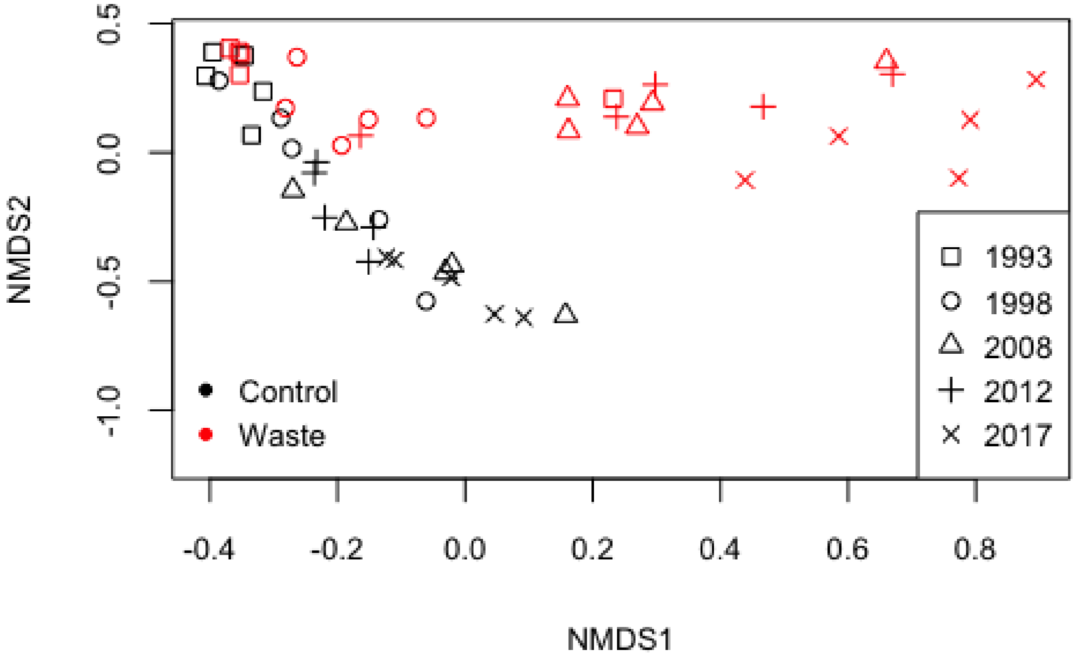

Figure 7. Non-metric multidimensional scaling ordination of species percent cover data on drilling wastes (red) and undisturbed control sites (black) in the disturbance years of 1993 (squares), 1998 (circles), 2008 (triangles), 2012 (crosses) and 2017 (x's). A stress value of ≤ 0.05 indicates a good fit. The stress value for the above ordination was 0.047.

Species Richness

Species richness was compared between the disturbed drilling wastes and undisturbed control plots for each year included in the study. There were significant differences in species richness over time (df = 4, F = 2.96, p = 0.03), between drilling waste and undisturbed controls (df = 1, F = 19.763, p < 0.001), and the interaction between time and drilling waste/control (df = 4, F = 3.24, p = 0.02). Sites disturbed in 2017 had significantly lower species richness compared to their respective controls (p < 0.001); however, there were no significant differences in species richness between disturbed and undisturbed plots for sites in 1993, 1998, 2008 and 2012 (Figure 8). Natural recovery of the tundra ecosystem results in similar species richness within 6 years. This trend suggests that species richness can recover rapidly and similar numbers of species are able to colonize the disturbed area. However, individual species may still be excluded on these recovering drilling wastes due to differences in the life history traits (i.e., functional group, reproduction, and growth rate) of Arctic vegetation.

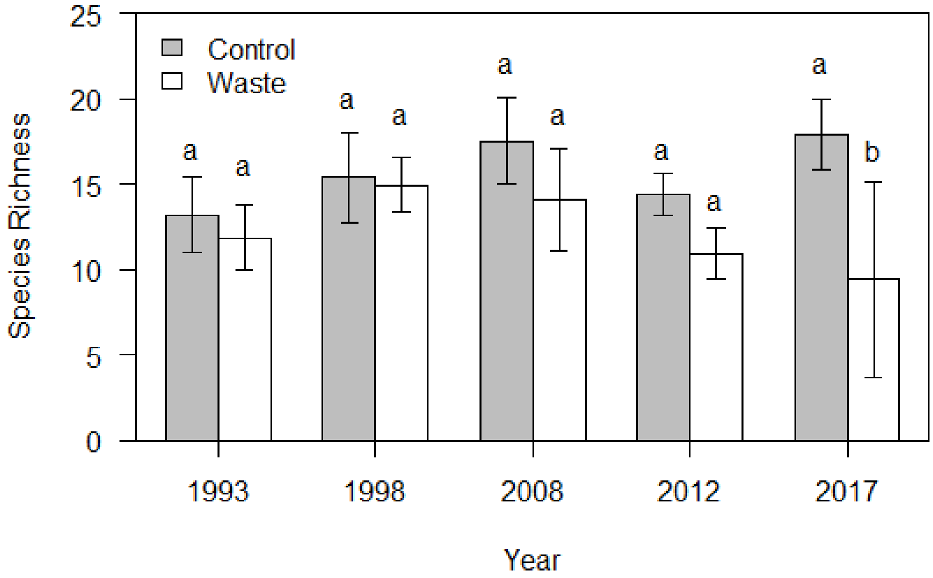

Figure 8. Species richness of drilling wastes and undisturbed control plots in different disturbance years. Both treatment (drilling waste and control) and year (1993, 1998, 2008, 2012, 2017) were significantly different (ANOVA, p <0.05). Different letters represent significant differences (p < 0.05) between control and waste sites within each year from a Tukey HSD post-hoc test. Bars present means with standard deviation (n=5).

Species-level Responses

Delayed dwarf shrub establishment was observed in recently disturbed sites, with the three most abundant ericaceous dwarf shrubs (e.g., *Cassiope tetragona, Dryas integrifolia,* and *Loiseleuria procumbens*) having lower cover 1-2 years following disturbance (Table 2). Although there was variability across years since disturbance, low-lying shrub cover generally increased over time with similar dwarf shrub cover present following 20 years of natural recovery.

Higher cover of *Carex aquatalis* in the early years of recovery are not surprising since sedges can often rapidly establish on disturbed Arctic sites. Previous revegetation studies from Alaskan tundra found that native sedges established on disturbed sites in 5-10 year and had an aboveground biomass equal to undisturbed tundra (Chapin and Chapin, 1980). Sedges are often more dominant in lower-lying landscape positions in tundra ecosystems. Higher sedge cover on drilling wastes were observed only in 2012 and 2017. The 2012 sites were located in sedge (n = 2) or heath (n = 3) dominated hummock-hollow complexes, whereas 2017 sites were in heath dominated hummock-hollow (n = 1) and lichen heath (n = 4) ecotypes. Therefore, sedge establishment of disturbed substrates may be occurring naturally across ecotypes indicating that these species could be useful for active restoration efforts.

One to two years following disturbance, both mosses and lichens had a lower cover on drilling wastes than undisturbed controls. However, bryophytes played an important role in the initial and early colonization of drilling wastes across the landscape. 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. In addition, *Bryum* spp. had higher cover on the drilling wastes compared with the paired undisturbed tundra controls 10-20 years following disturbance. *Bryum* sp. are acrocarpous, or carpet forming, are tolerant of high levels of solar radiation, and are known to establish on disturbed substrates, especially slightly alkaline substrates (McKnight et al., 2013). These early colonizing acrocarpous moss species appear to be the first species in biological soil crusts to establish. Biological soil crusts (BSCs) are communities of mosses, liverworts, lichens, bacteria, cyanobacteria, and fungi commonly found throughout the Arctic, especially on recently disturbed soils (Bowker et al, 2018). Mature BSCs appear to naturally develop approximately 10 years following disturbance with BSCs present on all but one drilling waste site from the disturbance years 1993-2008. 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 (Forbes and Jefferies 1999; Stewart et al. 2011a,b,c). In addition, early bryophyte colonizers provide surface roughness that can trap wind blown seed (Belnap and Harper, 2003). Through wetting and drying cycles, moss mats can also provide a significant input of carbon, nitrogen and phosphorus that in turn promote key ecosystem processes, such as carbon and nitrogen cycling (Stewart 2011a,b).

Other moss species, such as *Scorpidium scorpioides*, have lower establishment on the disturbed substrates. *S. scorpioides* is pleurocarpous, or branched, and tends to be found in moist depressions in the landscape. In addition, saline conditions are known to inhibit or prevent growth of S. *scorpioides* (Vitt et al., 1993). Salinity associated with the drilling waste materials may be inhibiting the establishment of *S. scorpioides* over time. Lichen cover was lower across almost all ages of drilling wastes, which is not surprising as lichens are commonly regarded as slow-growing (Werner, 1990; Seminara et al., 2018). Despite the lower percent cover of lichens on the drilling wastes, there was a clear trend of increasing cover over time. This is exemplified by *Cetraria* sp., which had 20% lower cover on drilling wastes 1-2 years following disturbance but only 1.5% less cover 25 years following disturbance (Table 2).

Figure 9. Early acrocarpous moss establishment on 4-5-year-old drilling waste (left GT13-70) and mature biological soil crust 20-year-old drilling wastes (right M98-230).

Table 2. Difference in the percent cover of species between the drilling waste and paired undisturbed control plots. Values are the average % differences with standard error across all sites within a given year and only species with >5% differences in at least one year are included. Negative values indicate lower cover on the drilling wastes compared with the control and positive values indicate higher cover on the drilling waste compared to the control.

Year	Difference in Species percent cover (average % difference)				
	Dwarf Shrubs			**Sedge**	
	Cassiope tetragona	*Dryas integrifolia*	*Loiseleuria procumbens*	*Carex aquatilis*	
2017	**-9.0 (3.4)**	-4.3 (1.7)	-1.2 (1.2)	**8.6 (0.8)**	
2012	**5.3 (1.8)**	-2.4 (1.6)	0 (0)	1.5 (0.7)	
2008	1.6 (1.0)	2.9 (1.4)	**-9.6 (4.6)**	-0.7 (1.4)	
1998	4.1 (3.7)	**8.4 (3.8)**	0 (0)	-0.3 (3.0)	
1993	-0.6 (0.5)	-1.3 (2.4)	-1.2 (0)	0 (4.5)	
	Mosses		**Lichens**		
	Bryum spp.	*Scorpidium scorpioides*	*Cetraria* sp.	*Alectoria ochroleuca*	Fruticose Lichen
2017	-1.3 (1.2)	-0.3 (0.3)	**-20 (7.7)**	**-6.3 (1.3)**	**-8.7 (4.2)**
2012	4.1 (4.3)	-0.1 (0.1)	**-7.2 (3.1)**	0 (0.6)	-0.4 (0.4)
2008	**8.3 (2.3)**	0 (0)	**-14 (4.7)**	**-7.7 (3.0)**	**-4.1 (0.8)**
1998	**5.3 (2.9)**	-0.3 (0.2)	**-6.4 (3.2)**	-2.2 (1.4)	-1.9 (1.7)
1993	0.9 (1.5)	**-13 (5.4)**	-1.5 (1.1)	0.1 (0.1)	-0.9 (0.4)

Sedges and mosses may be important early colonizers and appear to establish relatively rapidly under natural recolonization. Dwarf shrubs and lichens are likely to require more time for natural recolonization and these plant functional groups may require active restoration efforts if shorter revegetation timelines (i.e., < 20 years) are required.

Presence/absence of plant species had similar trends to those observed for the percent cover differences (Table 3). Investigating presence/absence data is critical to understanding specific species response because it reveals species that could be excluded from disturbed sites. Dwarf shrubs (e.g., *Cassiope tetragona* and *Dryas integrifolia)* and *Salix* spp. were absent from 60-80% of recently disturbed drilling waste sites (i.e., 2012, 2017) (Table 3). Similarly, two moss and several lichen species were absent from 60-100% of drilling waste sites that were disturbed within the last 10 years. As discussed above, moss species may play a critical role in early colonization; however, differences between moss species may be because some species have slow natural recolonization rates. The slow-growing nature of lichens likely accounts for the slow rates of colonization. In addition, some *Carex* spp. seem to be absent from the drilling wastes even after 25 years of natural recovery (Table 3).

Only two species were found to occur on the majority of drilling waste sites in comparison to the paired control plots: *Betula glandulosa and Pedicularis* sp. (Table 3). Both species were found on 60% of the drilling waste at sites disturbed in 1998, and *B. glandulosa* was also found on 20% of the drilling waste sites in 2012. It is possible that both of these species benefit from the exposed substrate and high light conditions on the drilling waste surfaces. A survey of more sites across the landscape is needed to support these initial results, but it is clear that there are species that may be excluded from the drilling wastes and others that may benefit from the disturbance.

Overall tundra disturbed by the deposition of drilling wastes at the Meliadine site appears to recover relatively rapidly (i.e., within 20 years) without any active restoration. The community composition between drilling wastes and the paired undisturbed tundra was similar 20-25 years post disturbance and species richness recovered within 6 years. Due to the different life history characteristics of tundra plants, individual species responses to disturbance were observed. While sedges and mosses may recover more rapidly on these drilling wastes, dwarf shrubs and lichens may require longer to recover. These trends in natural recovery are important for guiding future restoration efforts and techniques. Specifically, targeting sedge and moss species for transplanting and/or seeding of disturbed substrates may be a highly effective strategy for initiating the development of early successional tundra communities.

*Table 3. Species presence/absence on the drilling wastes compared with paired undisturbed control plots. Negative values represent the total percentage of sites where a species was present in the paired control plots but not on the drilling waste. Positive values represent the total percentage of sites where a species was present on the drilling waste but not the paired control plots. All values are expressed as a percentage of sites where a species was present/absent (i.e., x/5*100). Only species where presence/absence differences were found at > 2 sites (>40%) are included.*

Year	Presence/absence of species on drilling wastes vs. paired controls (% of sites)						
	Shrubs	**Dwarf Shrubs**				**Sedge**	**Forb**
	Betula glandulosa	*Cassiope tetragona*	*Dryas integrifolia*	*Salix spp.*	*Salix reticulata*	*Carex spp.*	*Pedicularis sp.*
2017	0	**-80**	**-80**	20	-20	-20	0
2012	20	**-60**	0	**-60**	**-80**	0	0
2008	0	0	40	0	-20	0	-20
1998	**60**	-40	0	40	-40	-20	**60**
1993	0	-40	20	0	40	**-60**	-40
	Mosses		**Lichens**				
	Tomenthypnum nitens	*Distichium spp.*	*Alectoria ochroleuca*	*Dactylina arctica*	*Thamnolia spp.*	*Xanthoparmelia spp.*	
2017	**-60**	-40	0	**-100**	**-60**	**-60**	
2012	**-60**	-40	-20	-20	-40	-20	
2008	**-60**	**-60**	**-80**	**-60**	**-80**	-20	
1998	0	0	-40	-40	20	-20	
1993	-20	20	0	-0	-20	-20	

Recommendations

Based on the characterization of drilling waste sites and the tundra vegetation present on drilling wastes ranging from 1-25 years, it is evident that natural revegetation is occurring on these wastes. Sources of vegetative materials for recolonization and depth of drilling waste appear to be two of the primary driving factors in determining the extent of recovery. Given these driving factors, we suggest the following practices to promote natural revegetation processes:

1. **Leaving intact tundra vegetation dispersed throughout the area** where drilling wastes are contained is essential. In hummock-hollow tundra, dispersal of drilling wastes into hollows allows for hummocks to stay intact and act as source population for revegetation. Hummocks provide not only aboveground islands for revegetation, but also maintain connectivity with intact belowground soil systems.
2. **Thinly spread drilling wastes appear to undergo revegetation processes more quickly.** In general, a depth of ~3 cm allowed for natural recolonization to proceed. Whereas depth of drilling waste > 15 cm can impede ecosystem recovery with little recovery observed even 20 years after disturbance. Underlying microtopography of drilling waste sites should be considered in managing wastes with an emphasis placed on limiting drilling wastes.
3. Direct colonization of drilling wastes in the first ~5 years is primarily driven by the colonization of acrocarpous mosses. Establishment of these early colonizing moss communities appears to facilitate the development of cohesive and diverse biological soil crusts in later years (~10-20 years). Mature biological soil crusts may act to further stabilize exposed drilling wastes and promote establishment of vascular plant species. **Promoting bryophyte cover in early stages may be beneficial to long-term tundra vegetation establishment.** Surface moisture is essential for establishment of early bryophyte communities and, therefore, practices that promote retention of surface moisture may assist in recovery.

Natural Colonization of Biological Soil Crusts on Mining-Disturbed Sites

Background

Bryophytes play an important role in the initial and early colonization of drilling wastes across the landscape at Meliadine (Previous section; 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. Given the importance of BSCs in early succession, we developed two studies: i) an examination of the establishment and function of BSC macro-phyla (i.e., bryophytes and lichens) and micro-phyla (i.e., bacteria and fungi) on mining disturbed substrates at Meliadine's Arctic site and Detour's boreal site; and ii) an exploration of the niche modifications due to BSC establishment at the Meliadine site.

Study I: BSC establishment and function at Meliadine and Detour

Methods

Site descriptions

The Meliadine mine is a low Arctic site with heath tundra and lichen-heath communities where exploration drilling over the past 30 years has left hundreds of small-scale drilling waste sites. Drilling waste sites range in size and shape (on average ~200 m^2) and include disturbance from drilling and shallow sumps that have been filled with drilling wastes. This drilling waste consists of a mixture of rock and mud along with additives such as surfactants, detergents, and saline solutions used to raise the freezing point, as the majority of drilling occurs in the winter months (Kokelj and GeoNorth Ltd., 2002; Thienpont et al., 2013).

The Detour Lake Mine (50°00'10.9"N 79°41'43.9"W, 280 masl), located in the boreal forest, is an open pit gold mine located in northeastern Ontario, Canada. The property includes multiple legacy borrow pits in addition to the open pit mine and associated infrastructure. These borrow pit were created when the topsoil and vegetation were removed to access the underlying gravel, clay, and sand needed to support the development of mine infrastructure (e.g., roads and platforms). These sites have been left to naturally re-vegetate since their initial disturbance.

The time of mining disturbances at both Detour and Meliadine were well documented, which allowed for a chronosequence of sampling sites in three, 10-year age ranges representing 30 years of recovery (early: 2011-18; mid: 2000-10; late: 1990-99).

Sampling Design

At both mine locations, sampling sites were selected to fit within the three 10-year age classes (early: 2011-18; mid: 2000-10; late: 1990-99), which represents a 30-year post-disturbance chronosequence. At each sampling site (i.e., drilling waste site or borrow pit), three biological soil crust (BSC) samples were collected at least 10 m apart. Due to the small operational scale of BSC communities, this distance between samples allowed us to treat each as an independent sample. BSCs and the underlying substrate were collected in cylindrical sampling cups (7 cm diameter by 6.5 cm depth). Substrate sample depth varied from about 4 cm to 6.5 cm.

Molecular methods

A subset of samples representing each age class were randomly selected for next generation sequencing. In total, 20 BSC samples (10 BSC samples per study site) were sent for molecular analysis at the Centre for Biodiversity Genomics (CBG) at the University of Guelph, Guelph, Ontario, Canada.

A composite of approximately 2 mL of crust was collected from each BSC sample by using a sterile 1mL pipette tip. The collected material was placed in a sterile 5ml tube (Fischer brand AxygenTM Screw Cap Transport Tubes). Samples for the molecular analysis were stored at -80°C at the CBG prior to being processed.

Prior to statistical analysis, each community data set (bryophyte, lichen, bacteria, and fungi) was filtered to remove any sequencing errors and rare species. Filtering focused on removing all operational taxonomic units (OTU) or amplicon sequence variant (ASV) which had a read count lower than 100 in at least one sample. Additionally, zeros were replaced using count-zero multiplicative approach (using CoDaSeq package (Gloor et al., 2016)) and data was normalized using a count-log ratio. This was done following the approach taken by Gloor et al. (2017). Molecular data was treated as compositional.

Gas flux measurements

Gas flux measurements of carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4) were conducted on each whole sample *ex -situ* by use of a Fourier Transform InfaRed Multicomponent Gas Analyzer (FTIR-MGA), which measured rates of flux under dark and light conditions for 10-minute intervals. Rates of nitrogen-fixation were determined through acetylene reduction assays (Stewart et al., 1967; Turner and Gibson, 1980). BSC samples were incubated with 10% (v/v) acetylene for 5 hours under 85 $\mu mol/m^2/s$ light intensity (PAR) in 500 ml mason jars sealed with fitted rubber stoppers. During each incubation, two samples were fitted with fine-wire thermocouples to monitor BSC surface temperature (with an average temperature of 24°C). Ethylene and acetylene concentrations were analyzed through gas chromatography (Scion 456-GC with CompassCDS 3.1 software). Control samples containing (i) BSC without acetylene and (ii) no sample injected with acetylene were collected during each incubation.

Crust and substrate nutrient analysis

Prior to nutrient analysis, each BSC sample was separated into a crust and a substrate layer. A subsample of both the BSC and underlying substrate was extracted through a 1:2.5 soil to water ratio extraction prior to measurements of pH, anions (NO_2^-, NO_3^-, SO_4^{2-}, Cl^-, PO_4^{3-}), and cations (Ca^{2+}, Na^+, Mg^{2+}, K^+, NH_4^+) by ion chromatography (Dionex IC-2000 with Chromeleon 7 software). Both mine locations exhibited a number of samples with nutrients below detection limit (BDLs) values (0.05 ug/g). Nutrients with samples >45% BDLs per location were omitted from further analysis. At Meliadine this included: NO_3^- (crust: 58% BDL, substrate: 100% BDL), NO_2^- (crust: 89% BDL, substrate: 100% BDL), and PO_4^{3-}(crust:47% BDL, substrate:69% BDL). At Detour this included, NO_3^- (crust: 89% BDL, substrate: 85% BDL), NO_2^- (crust:100% BDL, substrate: 100% BDL), PO_4^{3-} (crust:100% BDL, 100% BDL) and NH_4^+ (crust: 81%BDL, substrate: 100% BDL). The remaining material from each layer was air dried for 72 hours and then homogenized in a ball mill (Retsch MM-400, Germany). The crust and substrate layers were then analyzed for total carbon (TC) and total nitrogen (TN) separately through combustion analysis (LECO TruMac CNS Analyzer).

Results

Changes in BSC richness and community composition

Using generalized linear models, we found that richness for all BSC communities was significantly higher at Detour (bryophytes: $p = 0.018$; lichens: $p = 0.007$; bacteria: $p = 0.043$; fungi: $p = 0.001$); however, only bacterial richness was significantly different over time at Detour with higher richness in the mid-age range. At Meliadine, only the richness of bryophytes ($p = 0.033$) and fungi ($p = 0.020$) changed with time, both of which were lower in the mid age range. Results of a PERMANOVA analysis revealed that the composition of each BSC community was significantly different between the two mines and the bryophytes, lichens, bacteria, and fungi of each community changed over the three age ranges (Figure 10).

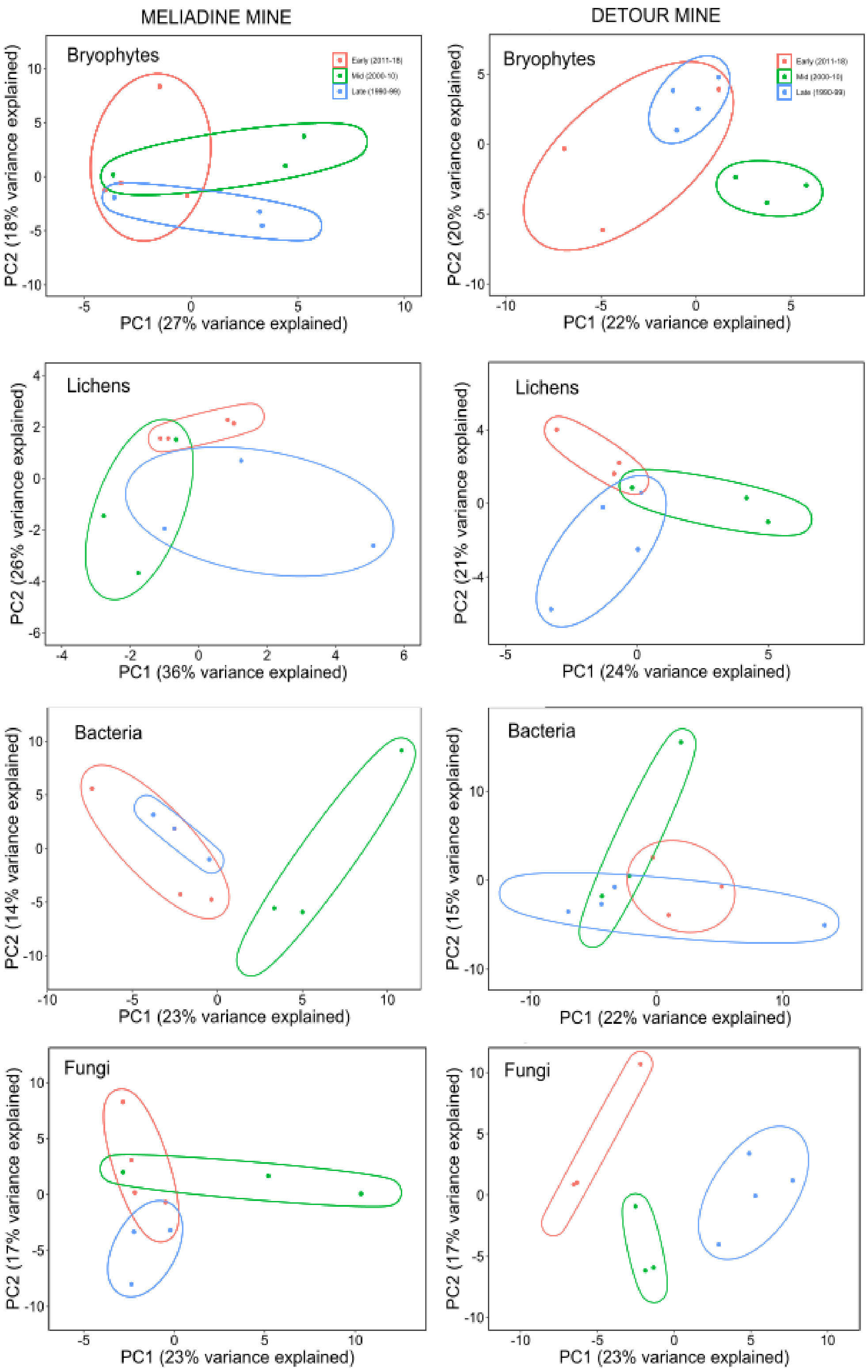

Figure 10. Panel of principal components analysis (PCA) results presented in biplots for each community (bryophytes, lichens, bacteria and fungi) at each study location. Each ellipses represents the community composition trends for each age range [red= early (2011-18), green= mid (2000-10), and blue = late (1990-99)].

Bryophytes

At both mines, the dominant bryophyte species across all three age ranges was the acrocarpous moss *Ceratodon purpureus.* The relative abundance of community assembly of bryophytes at Meliadine illustrates a dominance of the morphologically small acrocarpous mosses in the genera *Ptychostomum,* with the occurrence of morphologically larger pleurocarpous genus *Rhytidum* in the mid age range. The late age range was dominated by physically larger acrocarpous of the genera *Dicranella* and *Distichium*. Although changes in the bryophyte community composition were not significantly different across the age ranges at Meliadine (Figure 10), bryophyte richness was significantly lower in the mid age range. In contrast, community composition of bryophytes was significantly different over time at Detour, with no change in species richness with time. Changes in the Detour bryophyte community composition over time was observed in PCA biplots, with clear separation of the mid age range (Figure 11A). Three bryophyte species corresponded to the top 10 principal component loadings (Figure 11B) in the PCA analysis at Detour. The early and late bryophyte communities were dominated by the mosses *Ceratodon pupureus* and *Dicranella varia*, with an appearance of the moss *Streblotrichum convolutum* in the mid age range. The significant interactions of crust pH (p = 0.0358), light CO_2 flux (p = 0.096), and N_2 fixation (p = 0.005) may be associated with the changing bryophyte community composition over time (Figure 11A).

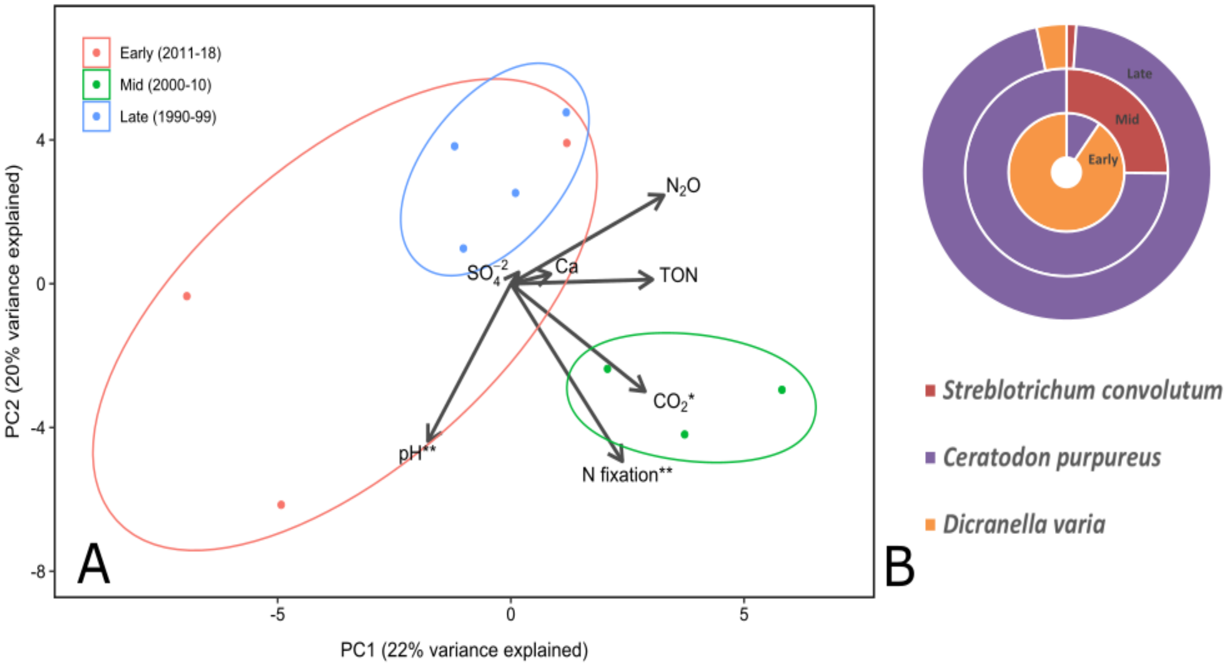

*Figure 11. A: Principal component analysis (PCA) of bryophyte community composition across the three age ranges at Detour study location. Environmental vectors are overlayed on the PCA biplot with variables significant to the analysis highlighted with ** ($p \leq 0.05$) and * ($p \leq 0.01$). B: Bryophyte species composition per age range which correspond to the top 10 principal component loadings.*

Lichens

The composition of lichen communities was significantly different across the age ranges at both mines (Figure 10), with no changes in the species richness over time at either mine. At Meliadine, lichen community composition was driven by fewer genera than at Detour. The lichen species corresponding to the top 10 principal component loadings at Meliadine were the crustose lichens *Thalloidima physaroides* in the early age range and by both *Thalloidima physaroides* and the lichenized fungi *Lecanorales* sp. in the mid age range (Figure 12B). There was an increase in diversity of lichens in the late age range, including the genera *Trapeliopsis* and *Coccocarpia* (Figure 12B). Further, Ca^+ and SO_4^- both increased over time in the crust layer at Meliadine and were the only environmental vectors significant to the lichen community analysis (Ca^+; $p = 0.061$, S SO_4^-; $p = 0.088$) (Figure 12A). The relative abundance of lichens at Detour illustrated that the fruticose lichen genera *Cladonia* was present across all three ages, and there were shifts in dominant lichen genera at each age range. Further, the lichen species corresponding to the top 10 principal component loadings showed the early age range lichen community at Detour was dominated by the fruticose lichens *Cladonia magyarica.* In the mid age range, both *Cladonia magyarica* and *Cladonia conioncraea* were dominant, as well as *Peltigera* spp. The late age community was dominated by the fruticose lichen *Dibaeis baeomyces*. This lichen was abundant in borrow pit and roadside embankments in the boreal forest region around Detour. Moreover, the lichen *Thalloidima physaroides* was also present in the early and mid-age ranges at Detour, similar to Meliadine.

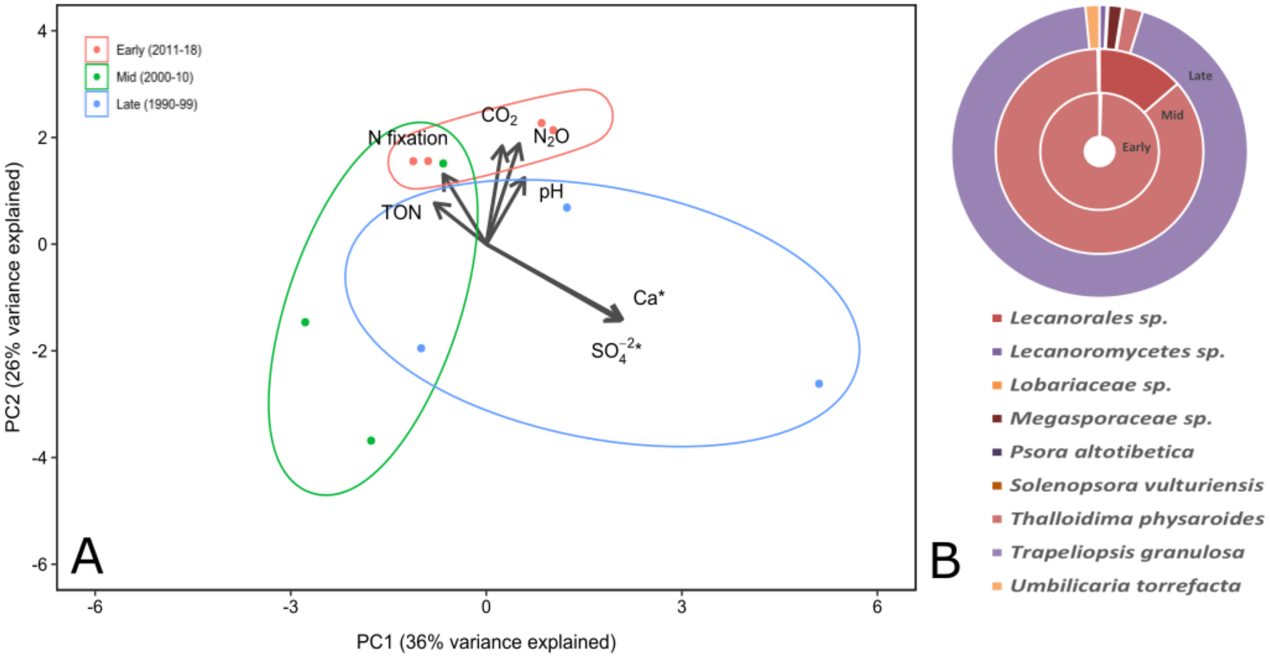

*Figure 12. A: Principal component analysis (PCA) of lichen community composition across the three age ranges (represented by ellipses) at Meliadine study location. Environmental vectors are overlayed on the PCA biplot with variables significant to the analysis highlighted with * ($p \leq 0.1$). B: Lichen species composition per age range corresponding to the top 10 principal component loadings*

Bacteria

At Meliadine, bacteria community composition changed over time while the species richness did not. In contrast, at Detour there were no significant changes in community composition but there were changes in species richness, with significantly higher richness in the mid age range. The differing behaviours in community composition across the ages at both study locations were observed in the PCA biplots (Figure 10). However, there were no significant interactions between the bacteria community composition and any environmental variables. The bacteria species corresponding to the top 10 principal component loading in the early and mid age ranges at Meliadine were dominated by the cyanobacteria species *Kamptonema animale* and *Wilmottia murrayi,* with the proteobacteria species *Asospirilium veronii* and *Brevundimonas variabilis* occurring in the mid age range. Proteobacteria had the highest relative abundance across all age classes and actinobacteria was found only in the late age range at Meliadine. In contrast, at Detour the communities were structured similarly at each age range with a combination of proteobacteria, cyanobacteria, and bacteriodetes. At the early age range acidobacteria *Edaphobacter modeustus* and *Terriglobus saanensis* occurred with the cyanobacteria *Kamptonema animale* and *Wilmottia murrayi* observed in the mid and late age ranges.

Fungi

At both Meliadine and Detour mines, there were significant differences in the community composition of fungi across the age ranges (Figure 10). While there were changes in species richness over time at Meliadine (i.e., lower richness in mid age range), there were no changes in species richness at Detour. The only fungi family to occur at both locations was *Didymellaceae,* which was most abundant in the early and mid age ranges. Community composition was different across the age ranges at both sites indicated by clear separation of the age range ellipses in the PCA biplots (Figure 10); however, there was no significant interaction between the fungi communities and any environmental variables. At Meliadine, the dominant fungal species associated with the top 10 principal component loadings in the early age range was *Thelebolus globosus* with a shift to *Xylariales* sp. in the mid age and *Tertracladium* sp. in the late age range. While at Detour, the fungi *Helotiales* sp. corresponded with the top 10 principal components across all three age ranges, with a dominance of the species *Knufia peltigerae* in the mid and late age ranges.

Interaction between the macro and micro-phyla communities over time

Interactions between the different macro- and micro-phyla communities at each mine were examined with non-metric multi-dimensional scaling (NMDS) ordinations and significant interactions between the bacteria and bryophyte communities were identified at both sites (Figure 13A,B). At Meliadine, the joint plot overlay of the bryophyte order onto the bacterial community NMDS biplot illustrated that two liverwort orders (*Fossombroniales* and *Metzgeriales*) were interacting significantly with the bacterial community composition (p = 0.018 and p = 0.025, respectively) (Figure 13A). While at Detour, four orders of mosses and two liverworts were observed to be influencing the bacterial community composition (Figure 13B). A combination of two mosses (*Porellales* (p = 0.002) and *Hypnales* (p = 0.019)) and two liverworts (*Fossombrionales* (p = 0.001) and *Metzgeriales* (p = 0.001)) were significant to the ordination and most strongly associated and grouped with the overlapping early and late age ranges. Further, the moss *Polytrichales* was observed to be most strongly associated (p = 0.048) with the mid age range bacteria community (Figure 13B).

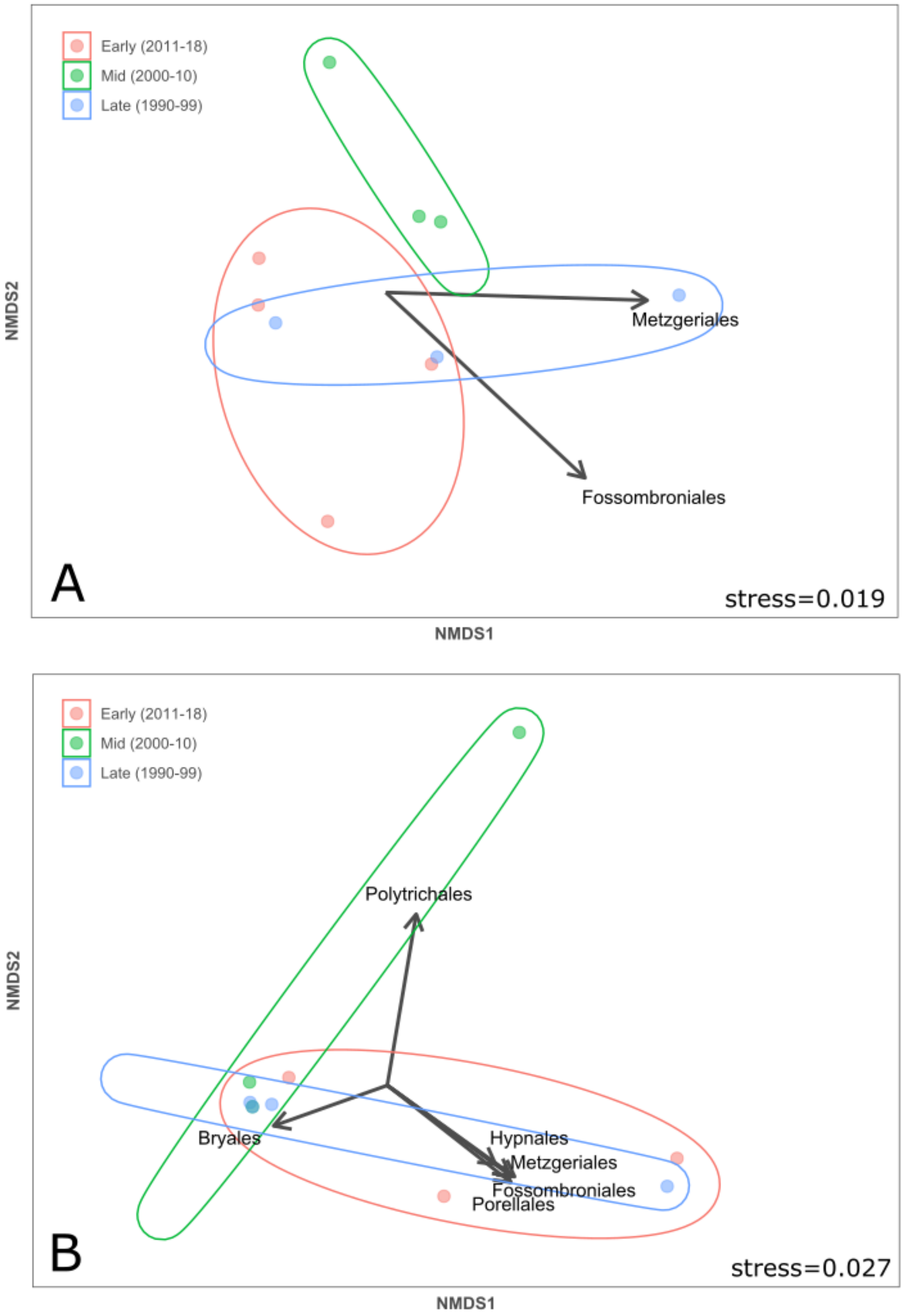

Figure 13. Non-metric dimensional analysis (NMDS) scaling plot of bacteria community composition across the three age ranges at Meliadine Mine (A) and Detour Lake Mine (B). Bryophyte communities at the order level were overlaid in joint plots. Only those orders significant to the ordination were included as vectors (p≤0.05). Vector direction represents the age range where this interaction is most significant.

Changes in BSC function over time

Using linear mixed-effects models (LMEs), we found that gas flux rates of CO_2 (light and dark) and N_2 fixation were significantly higher at Meliadine compared to Detour, while the flux of N_2O (light and dark) as well as CH_4 (light and dark) were not significantly different between the two mines. The flux rates of CO_2 were about 2-4 times higher at Meliadine than Detour. Gas flux and atmospheric nitrogen fixation rates were not significantly different across the age ranges at Meliadine, with the exception of higher light N_2O flux at the mid age range (Figure 14). At Detour, significantly lower rates of CO_2 flux under light conditions were observed in the late age range (i.e., higher net primary productivity) and significantly higher rates of N_2O flux under light conditions were observed in the mid and late ranges. N_2 fixation was also significantly higher in the mid age range at Detour (Figure 14). No clear trends in CH_4 flux over time were observed at either of the study locations.

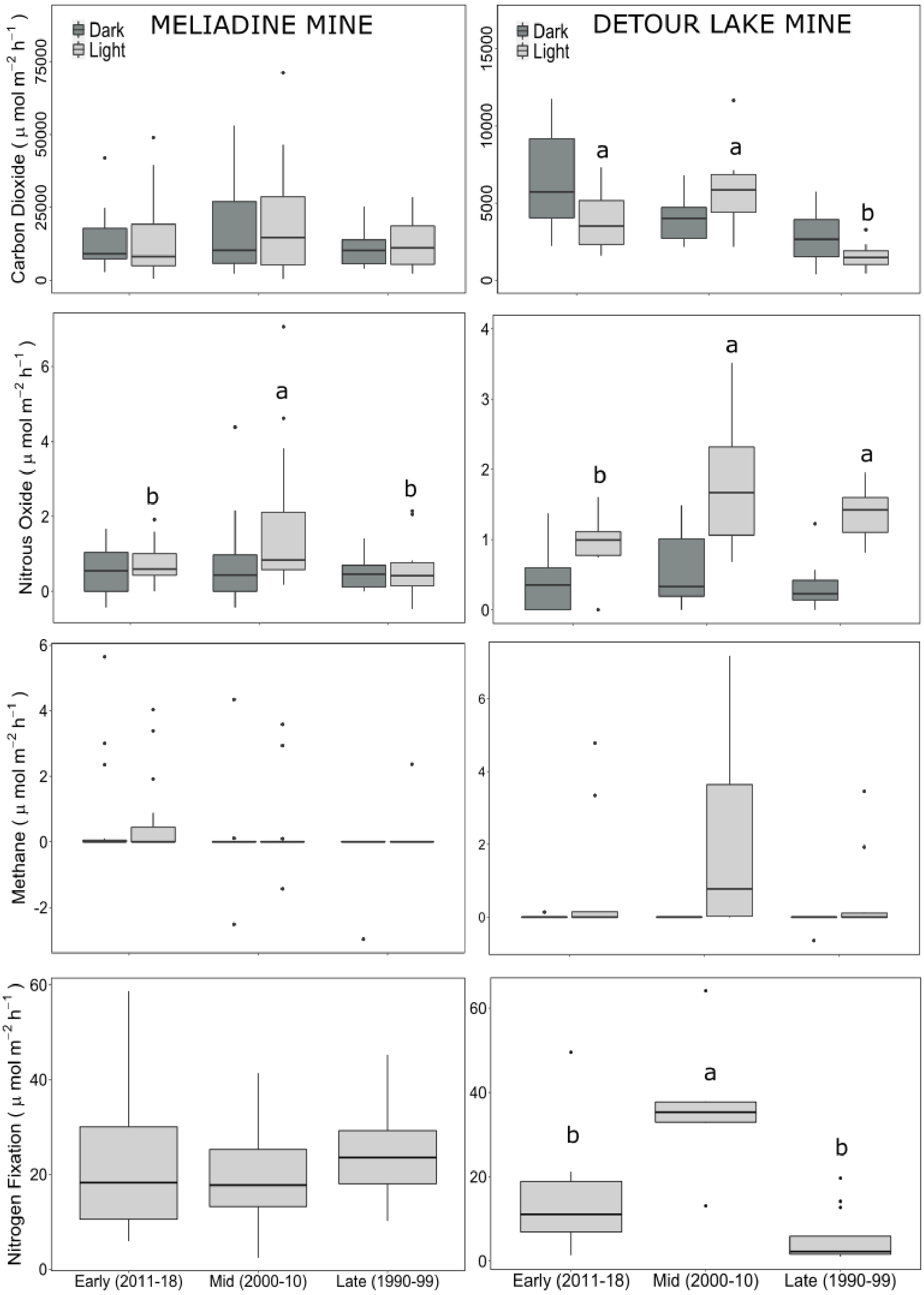
MELIADINE MINE
DETOUR LAKE MINE
Dark
Light
Carbon Dioxide (µ mol m⁻² h⁻¹)
0
25000
50000
75000
0
5000
10000
15000
a
a
b
Nitrous Oxide (µ mol m⁻² h⁻¹)
0
2
4
6
b
a
b
0
1
2
3
4
b
a
a
Methane (µ mol m⁻² h⁻¹)
-2
0
2
4
6
0
2
4
6
Nitrogen Fixation (µ mol m⁻² h⁻¹)
0
20
40
60
0
20
40
60
b
a
b
Early (2011-18)
Mid (2000-10)
Late (1990-99)
Early (2011-18)
Mid (2000-10)
Late (1990-99)

Figure 14. Gas flux of carbon dioxide (CO_2), nitrous oxide (N_2O), methane (CH_4), and nitrogen fixation (N_2 fixation) measured across three age ranges (Early (2011-18), Mid (2000-10) and late (1990-99)) on intact BSC samples collected at Meliadine and Detour Mine locations. Bars represent means with standard error. Significant differences in gas flux rates across the age ranges were determined using linear mixed-effects models. Different letters indicate significant differences in gas flux rates across the three age ranges.

Using liner mixed-effects models, we found that crust and substrate nutrient concentrations were significantly higher at Meliadine, with the exception of the crust concentrations of NH_4^+ and K^+ which did not show significant differences between mines. At both mines, nutrient concentrations were significantly higher in the crust compared to underlying substrate. Nutrients were not significantly different across the age ranges at either site, except for TC at Detour which was significantly higher in the early age range (Figure 15). Despite the lack of significant differences in nutrients across the age ranges, a consistent trend of increasing nutrients over time was observed at both sites (Figure 15). A number of nutrients measured in the crust and substrate layers at both mine sites (PO_4^{3-}, NO_3^-, NO_2^-, NH_4^+,) exhibited low concentrations (i.e., below detection limits).

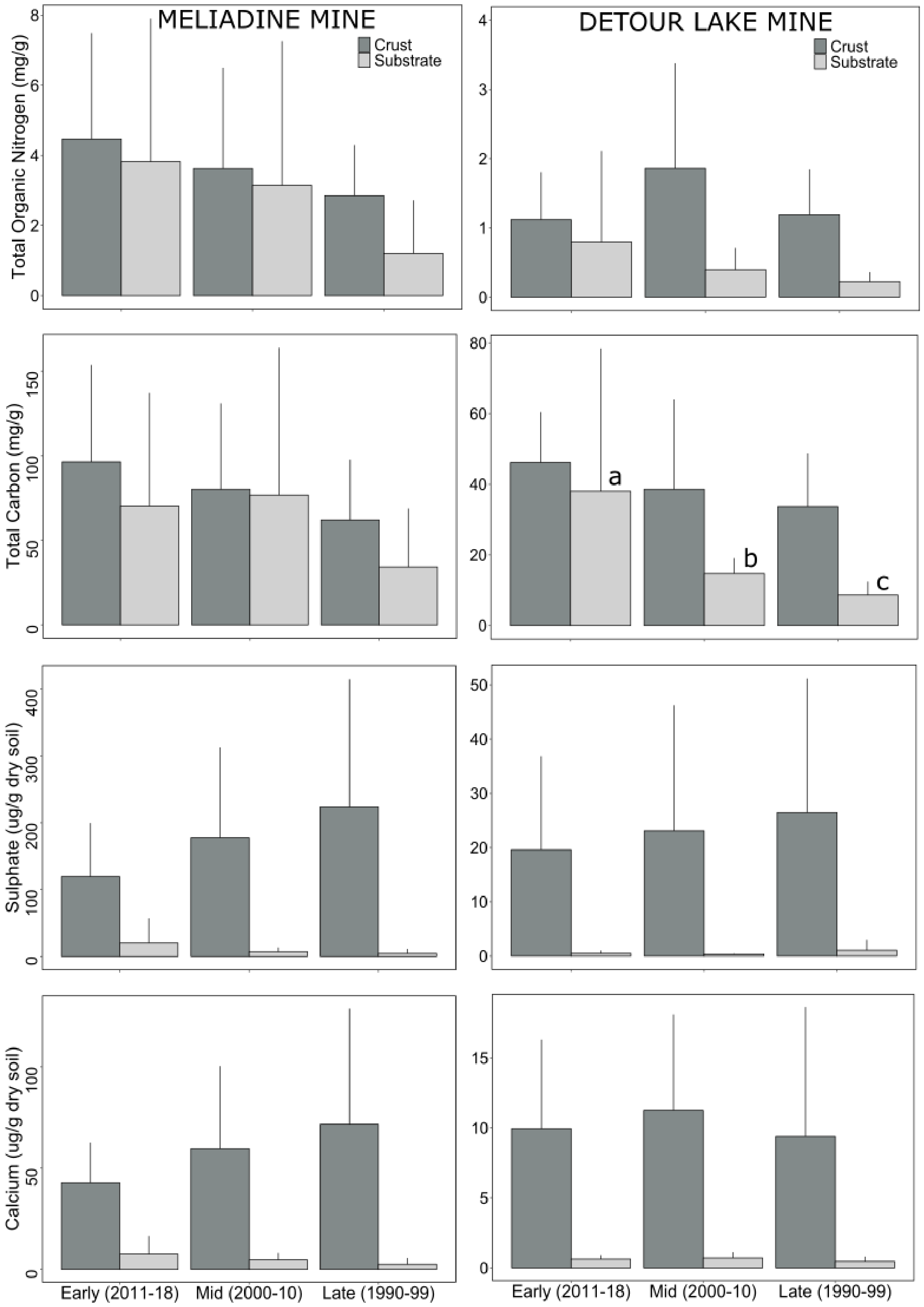
MELIADINE MINE
DETOUR LAKE MINE
Crust
Substrate
Total Organic Nitrogen (mg/g)
0
2
4
6
8
0
1
2
3
4
Total Carbon (mg/g)
0
50
100
150
0
20
40
60
80
a
b
c
Sulphate (ug/g dry soil)
0
100
200
300
400
0
10
20
30
40
50
Calcium (ug/g dry soil)
0
50
100
0
5
10
15
Early (2011-18)
Mid (2000-10)
Late (1990-99)
Early (2011-18)
Mid (2000-10)
Late (1990-99)

Figure 15. Nutrient concentrations measured across the three age ranges (Early (2011-18), Mid (2000-10) and late (1990-99)) in the crust and underlying substrate from BSC samples collected at both Meliadine and Detour Mines. Bars represent means with standard error. The nutrient concentrations were compared using linear mixed-effects model which included the interaction for age range and type (crust or substrate. Nutrient were significantly higher in all crust samples compared to the underlying substrate and annotations indicate significant differences between the age ranges ($p \leq 0.05$).

Recommendations

There were significant changes over time in both the macro- (bryophytes and lichens) and micro-phyla (bacteria and fungi) of BSC communities in the first 30 years of recovery at both boreal and Arctic mine sites with ubiquitous ruderal species commonly found between sites. The functional traits exhibited by BSCs during early stages of recovery (i.e., 30 years) on disturbed sites are likely driven by compositional differences rather than developmental stage. At both mine sites, the bacterial community appears to be responding to changes in bryophyte communities as they develop over time. Based upon the findings of this study, we recommend the following practices to promote recovery of disturbed Arctic and boreal substrates:

1. Bryophytes likely play an important role in determining early successional BSC community assembly and restoration efforts should **promote bryophyte establishment by targeting generalist species with ubiquitous distribution, such as *Ceratodon purpureus*.**
2. The community assembly of bryophytes changes over time. Small acrocarpous moss species tend to dominate in the earliest stages of vegetative recovery with larger acrocarpous and pleurocarpous mosses establishing later (i.e., 10-30 years post-disturbance). **The growth form of moss species used in restoration efforts should be matched with site-specific post-disturbance legacies.**
3. Although BSCs are generally considered slow growing, BSCs naturally colonized disturbed substrates and initiated functional processes associated with nutrient cycling as early as 10 years since disturbance at both study locations. Changes in functional measurements were associated with colonization by various BSC species. **Therefore, BSC species with high rates of carbon and atmospheric nitrogen fixation, such as cyanolichens and bryophytes with known cyanobacterial associations, should be selected for restoration.**
4. Work remains to be done to better understand the interactions between the macro- and micro-phyla BSC communities in these early stages; however, our study demonstrated that **establishment of generalist ruderal macro-phyla communities may facilitate the establishment of key micro-phyla communities responsible for nutrient cycling processes.**

Study II: Niche modifications due to BSC establishment at Meliadine

Methods

Microclimate Monitoring Sites

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 (M13-2100), two HOBO loggers were placed on exposed drilling waste with no biological soil crusts (BSC) and two loggers were placed on areas colonized by BSCs. At each logger, Photosynthetically Active Radiation (PAR) at the surface, soil temperature and moisture at about 5 cm depth, relative humidity and air temperature at about 10 cm above the ground, and percent wetness at the surface were measured hourly from July 2018 to Oct 2020. Due to COVID-19, we were not able to collect the data from these loggers in summer 2020, but batteries were replaced and data from the loggers was downloaded in summer 2021. Unfortunately, one logger on drilling waste logged for only about 3 months, leaving one pair of loggers with relatively continuous data from Jul 2018- Oct 2020, at which point the remaining loggers failed. Percent cover of BSC was visually estimated at each logger site in 2018 using 5 cm^2 grids in a 25 cm^2 quadrat. Each location was resurveyed in 2021 to examine changes in BSC communities over time and assess microclimatic conditions associated with developing BSC communities.

Chronosequence Monitoring Sites

In summer 2018, biological soil crust (BSC) and underlying substrate samples (5 cm depth) were collected from 5 locations within 9 drilling waste sites (total = 45 samples) for disturbance years between 1997-2013 (see previous section). In summer 2019, 15 additional sites were identified to extend and fill in the chronosequence (Figure 16). At each additional site, three sampling locations were examined for a total of 45 sampling locations. Site observations were recorded (e.g., size of drilling wastes, slope, aspect, proximity to water bodies). Custom made static vented gas sampling chambers were installed to measure *in situ* gas flux under field conditions (Figure 17). 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 location. Samples were returned to the laboratory at the University of Saskatchewan where optimal gas flux measurements and atmospheric nitrogen fixation via acetylene reduction assays were completed. 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.

The BSC samples were inventoried by classic taxonomic methods and are currently undergoing molecular analysis to examine the bacterial (16S), fungal (ITS) and nitrogen fixing community (*nif*H) via Illumina Mi-Seq amplicon sequencing. To better understand the role these early colonizing communities have in driving recovery of ecosystem function, nutrient concentrations of both the BSCs and underlying substrate were determined as: ammonium (NH_4^+), nitrate (NO_3^-), total nitrogen (TN), total carbon (TC), sodium (Na^+), potassium (K^+), magnesium (Mg^+), calcium (Ca^+), chlorine (Cl^-), and sulfate (SO_4^{-2}). 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_2), methane (CH_4), and nitrogen dioxide (N_2O) flux under both dark and light conditions.

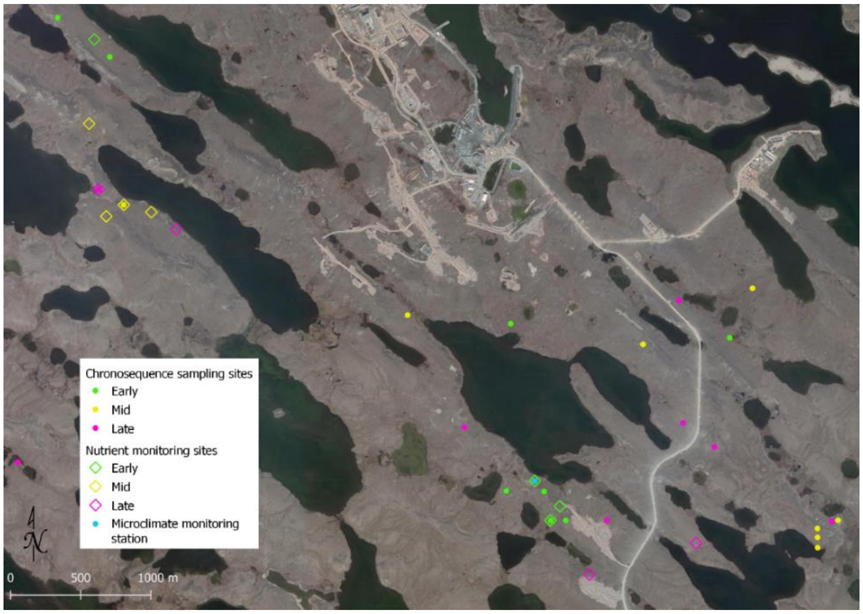

Figure 16. BSC sampling sites and nutrient monitoring sites at Meliadine in 2019.

Figure 17. 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 CO_2, N_2O and CH_4.

Results

Microclimate Monitoring Sites

Maximum daily air and soil temperature, water content, photosynthetically active radiation (PAR), dew point, and relative humidity (RH) showed varying patterns between crusted (i.e., BSCs present) and exposed (i.e., bare) drilling waste surfaces, as well as across years (Figure 18, Figure 19). Water content displayed the most evident differences between crusted and exposed drilling waste, where crusts always had greater water content in all years (Figure 18C). Daily maximum conditions during the growing season (i.e., early-July to early-Aug) had stronger microclimatic trends between exposed and crusted sites (Figure 19). Specifically, exposed drilling waste tended to have higher maximum soil temperatures and lower maximum water content than crusted drilling waste during each year (Figure 19B,C). Though more variable, daily maximum PAR tended to be greater in exposed than crusted drilling waste (Figure 19D). For daily maximum RH, the pattern differed by year: 2018 shows no differences between exposed and crusted drilling waste but in 2019, RH is greater on exposed drilling waste (Figure 19F). Air temperature and dew point show less obvious patterns between crusted and exposed drilling waste (Figure 19A,E). We also explored hourly microclimate conditions during mid-July in 2019 and found similar patterns as described previously: exposed drilling waste tended to have greater soil temperature and RH, and lower water content than crusted soil (Figure 20). Hourly patterns in air temperature, PAR, and dew point were more difficult to discern. These results demonstrate that the microclimate differs between crusted and exposed drilling waste, suggesting that developing BSCs modify their surrounding environment.

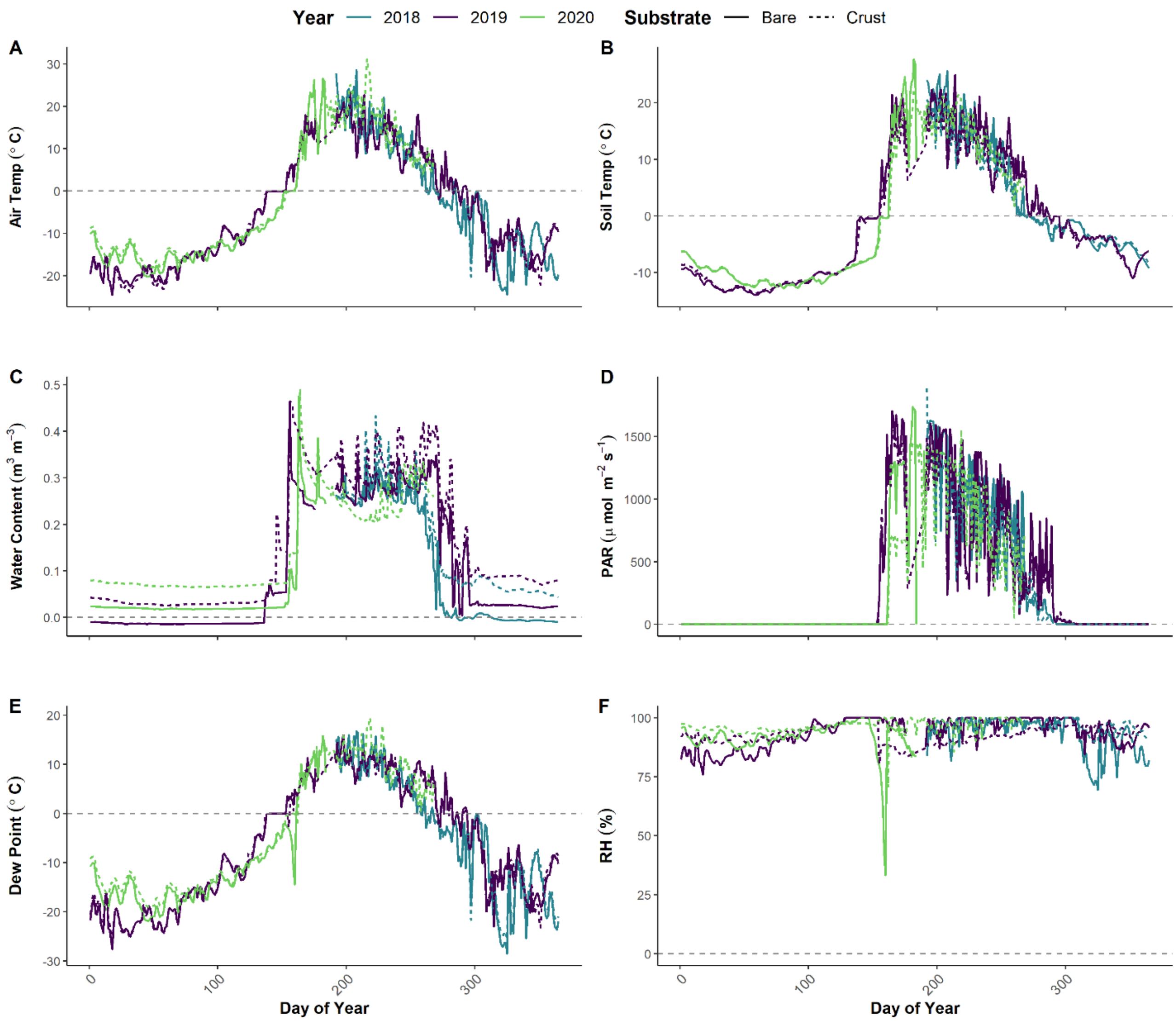

Figure 18. Daily maximum microclimate conditions for both bare (i.e., exposed drilling waste) and crusted (i.e., covered in BSC) substrates over three sampling years at the M13-2100 site.

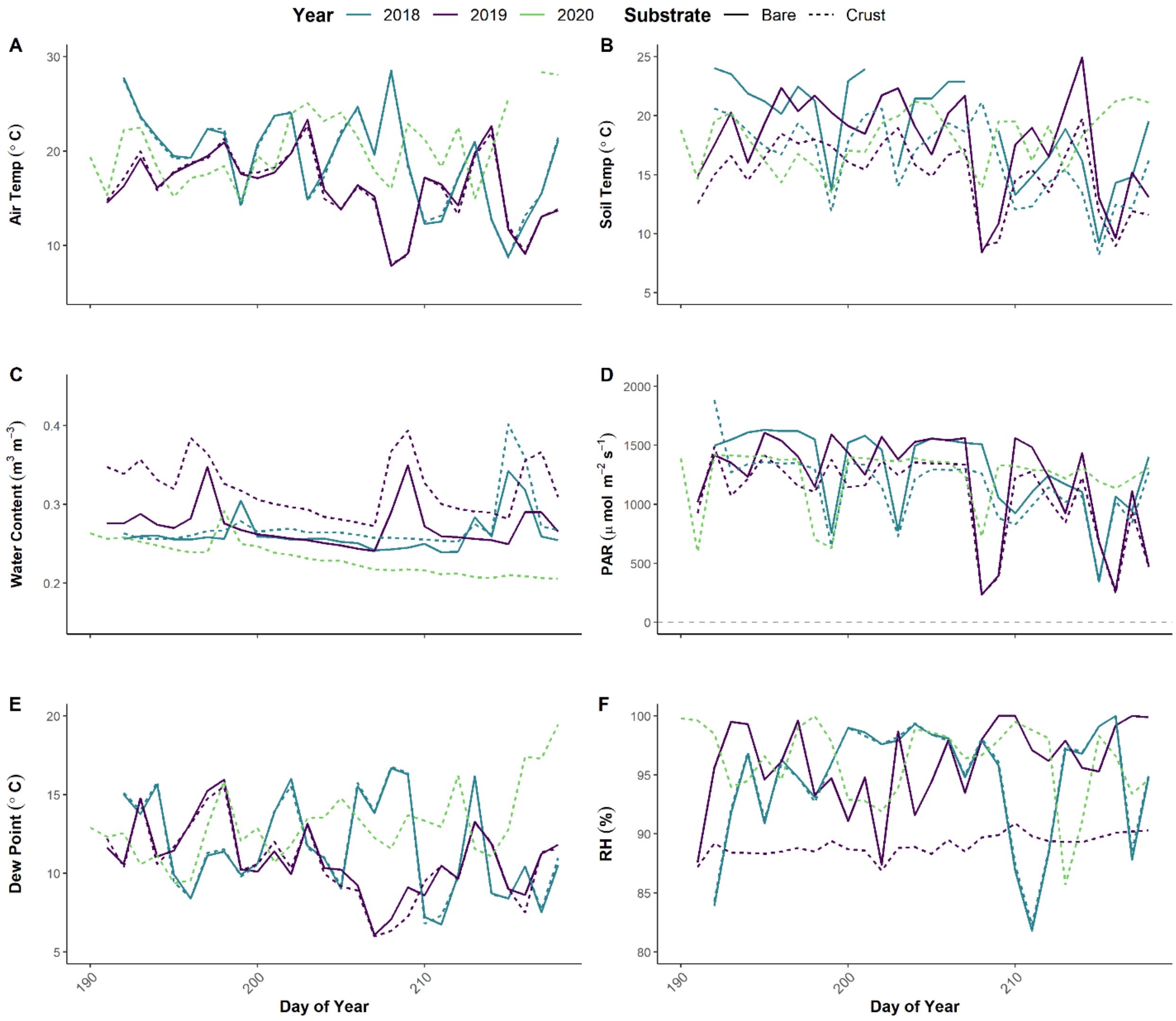

Figure 19. Daily maximum microsite condition for both bare (i.e., exposed drilling waste) and crusted (i.e., covered in BSC) substrates between early-July and early-Aug over three sampling years at the M13-2100 site.

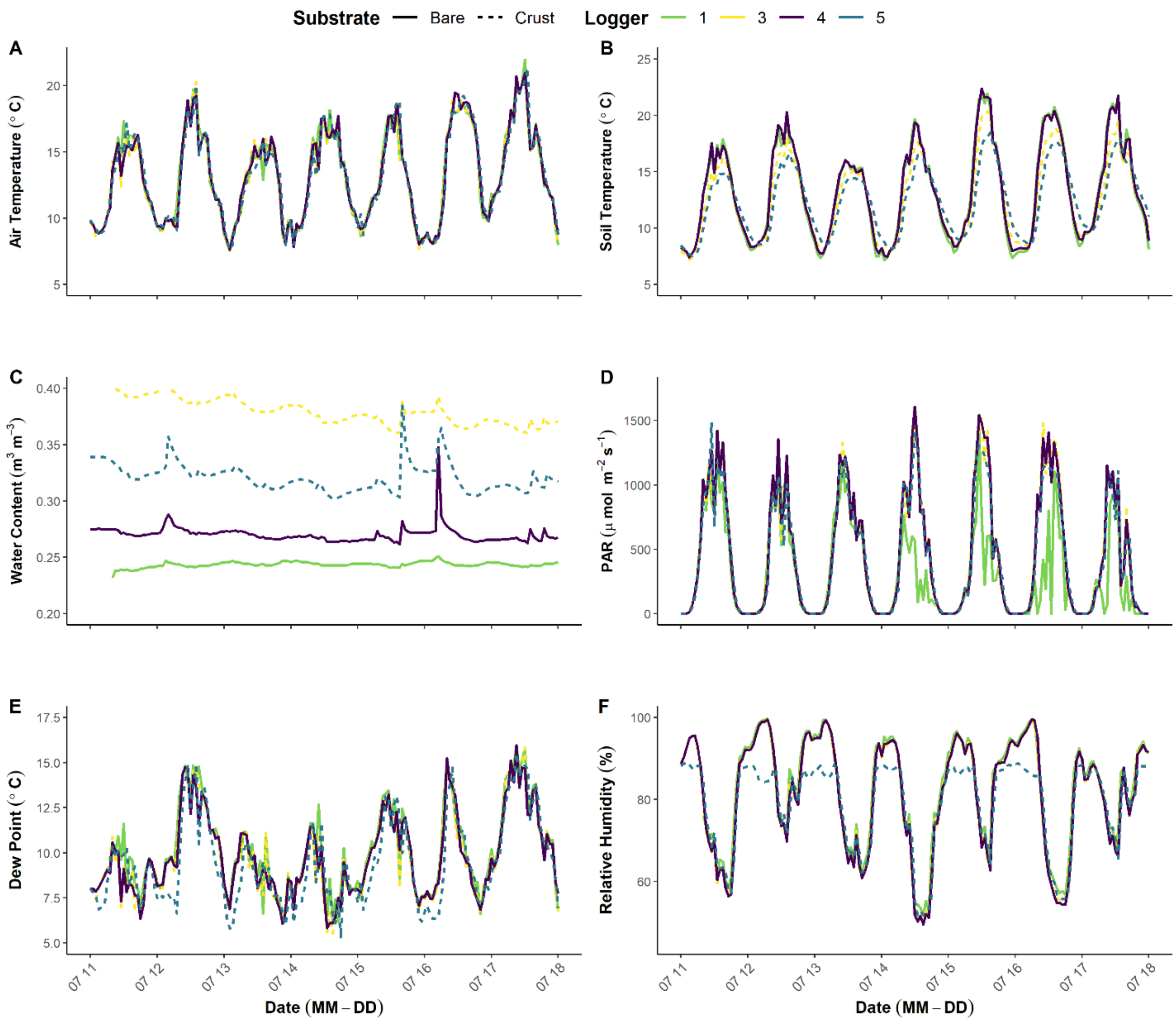

Figure 20. Hourly microclimate conditions of both bare (i.e., exposed drilling waste) and crusted (i.e., covered in BSC) substrates in mid-July at the M13-2100 site.

PERMANOVA indicated that ground cover (as drilling waste, rocks, litter, biological soil crusts, and vascular plant species) of the monitoring sites differed significantly between years (2018 and 2021; $F = 61.59$, $p < 0.01$, $R^2 = 0.11$), substrates (bare vs. crusted drilling waste; $F = 69.22$, $p < 0.01$, $R^2 = 0.12$), and their interaction ($F = 7.39$, $p < 0.01$, $R^2 = 0.01$; Figure 21). However, the beta dispersion test, which assesses if the dispersion of points around the centroids differs among categories, was also significant ($F = 97.90$, $p < 0.01$). As a result, the significant PERMANOVA results could indicate an influence of beta dispersion and/or differences among the centroids of ground cover between substrates and years. That being said, the first two axes of principal coordinates analysis (PCoA) explained about 15% of variation in ground cover and demonstrated clear development of BSCs and vascular species on both bare and crusted drilling waste between 2018 and 2021 (Figure 21). As expected, bare plots in 2018 had greater cover of drilling waste than the other categories. Interestingly, by 2021, bare plots had progressed to resemble the ground cover community of the crusted plots from 2018. For crusted drilling waste, we see further development of the ground cover community with greater cover of moss crust and vascular plant species. Interestingly, variability in ground cover community is much greater in bare 2021 and crusted 2018, than in bare 2018 and crusted 2021. Indicator species analysis supports the results of the PCoA and assessed which ground cover types were associated with which substrate and/or year (Table 4). The presence of plant and woody litter, as well as the ericaceous shrubs *Kalmia polifolia* and *Vaccinium uliginosum* and the forb *Chamerion latifolium* distinguished 2021 from 2018. In addition, crusted drilling waste was associated with the ericaceous shrub *Rhododendron tomenstosum* and the fructiose lichen *Cetraria.* Crusted (both 2018 and 2021) and bare 2021 drilling waste were indicated by moss crust and black lichen crust, suggesting drilling waste was colonized by these groups between 2018 and 2021. Lastly, we see that bare (both 2018 and 2021) and crusted 2018 substrates were typified by presence of drilling waste (Table 4).

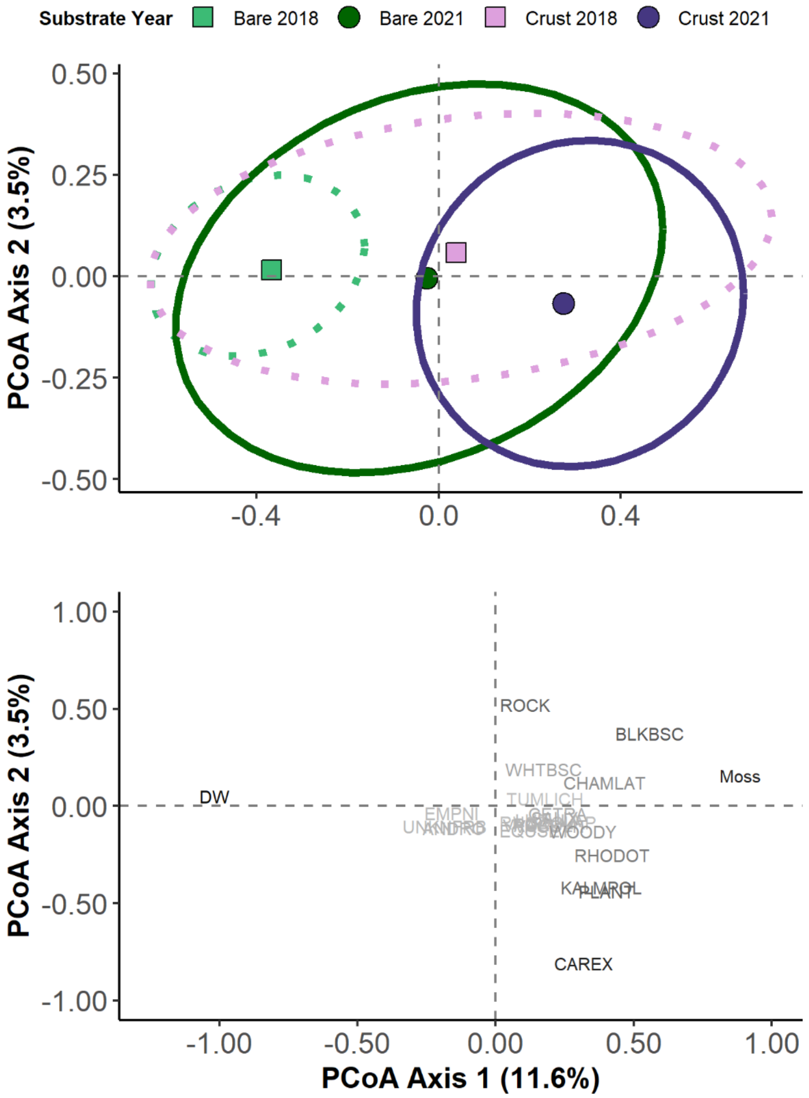

Figure 21. Principal coordinates analysis (PCoA) of ground cover (e.g., rocks, drilling waste, plant litter, BSCs, vascular, and moss species) of both exposed (i.e., bare) and crusted drilling waste at the M13-2100 microclimate monitoring site during two sampling years (2018 and 2021). Presented are the centroids (I.e., average ground cover community per substrate per year) and 95% confidence interval ellipses showing the succession of ground cover over time. Green and purple represent exposed (i.e., bare) and crusted drilling waste, respectively, and lighter squares and darker circles represent 2018 and 2021, respectively.

Table 4. Indicator species analysis results showing the species, substrate, or biological soil crust (BSC) representation of each Year x Substrate group. Corresponds to the PCoA in Figure 21.

Category	**Indicator**
2021 Bare	White Lichen Crust
2021 Crust	Salix
2018 (Bare & Crust)	Unknown Forb
2021 (Bare & Crust)	Plant & Woody Litter, *Kalmia polifolia*, *Chamerion latifolium*, *Vaccinium uliginosum*
Crust (2018 & 2021)	*Rhododendron tomentosum*, Cetraria lichen
Crust (2018 & 2021) & Bare 2021	Moss Crust, Black Lichen Crust
Bare (2018 & 2021) & Crust 2018	Drilling Waste

To explore whether developing BSCs modify the microclimate conditions, we compared the time-series of water content (which had the most evident differences between crusted and bare drilling waste) to changes in percent cover of drilling waste, moss crust, lichen crust, and vascular plants between 2018 and 2021 (Figure 22). There are differences in cover between bare and crusted drilling waste and between the years (Figure 22B), though we were unable to statistically model these changes. In general, drilling waste was greater and moss crust, lichen crust, and vascular plant cover lower in the bare vs crusted soils in each year. Drilling waste decreased for both bare and crusted plots from 2018 to 2021. Vascular plants and lichen crusts increased cover between 2018 and 2021 in both bare and crusted plots, but moss crust only increased in bare plots (Figure 22B).

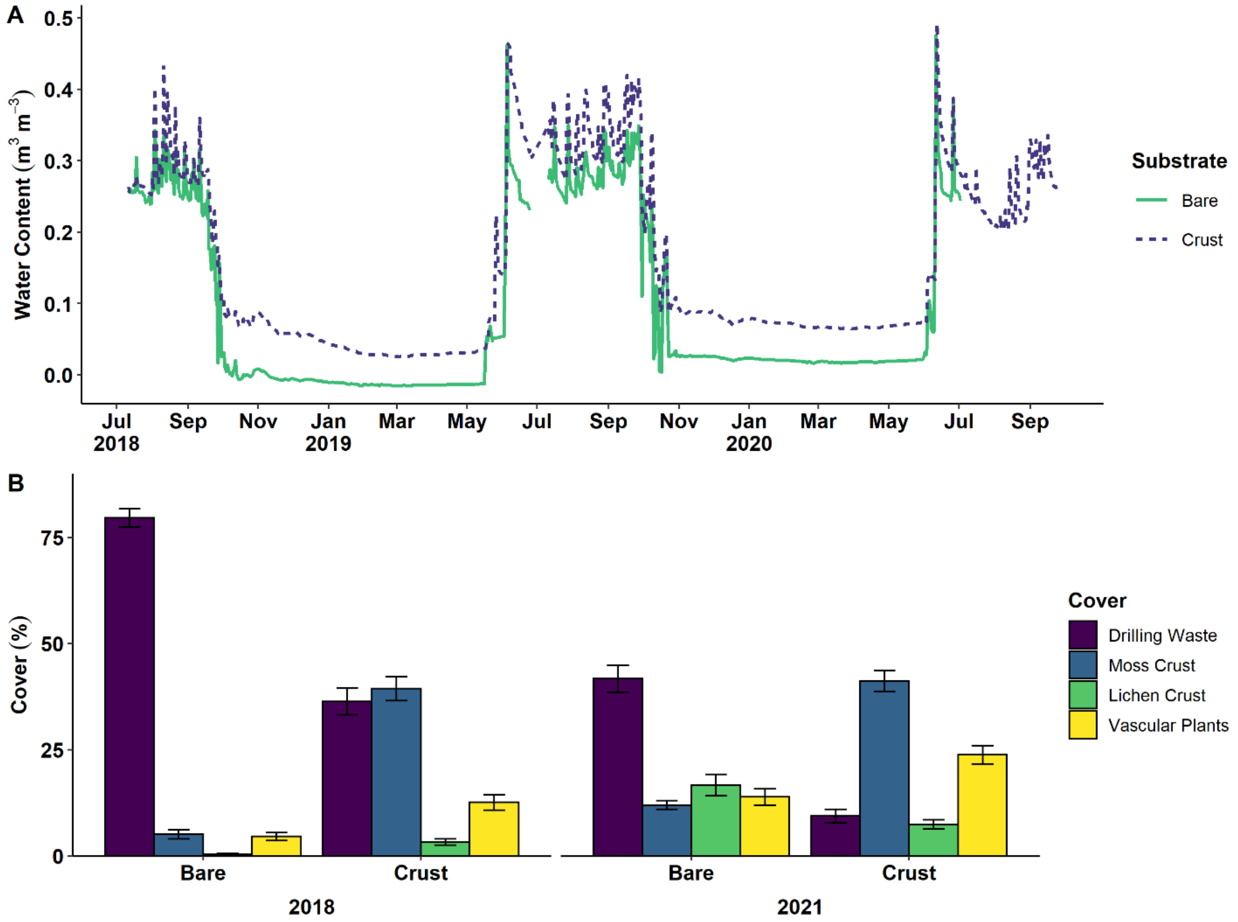

Figure 22. Daily average water content from Jul 2018 to Oct 2020 (A) and average ± standard error percent cover of different ground covers in 2018 and 2021 (B) for bare (i.e., exposed drilling waste) and crusted (i.e., covered in BSCs) substrates at the M13-2100 microclimate monitoring site.

Chronosequence Monitoring

Community Composition

Colonization of drilling waste by byrophytes across the chronosequence of mining disturbance demonstrates no obvious successional trajectory (Figure 23). The first two axes of the principal coordinates analysis explain about 33% of variation in bryophyte community composition, and there is some separation of the older and newer sites along the y-axis, though there is still substantial variation. Specifically, newer sites tended to be associated with greater cover of *Cinclidium latifolium arcticum* and *Barbula convoluta*, and older sites tended to be associated with greater cover of *Cephaloziella*. This is particularly evident when cover of individual bryophytes is examined over the chronosequence (Figure 24). In addition, there are several bryophyte species that are fairly ubiquitous across the chronosequence, including *Ceratodon purpureus, Pohlia nutans,* and *Bryum blindii* (Figure 24). As such, bryophytes are a ubiquitous part of succession on drilling waste, but there is no obvious trajectory of differing species over time, rather a few species that occur throughout the entire chronosequence.

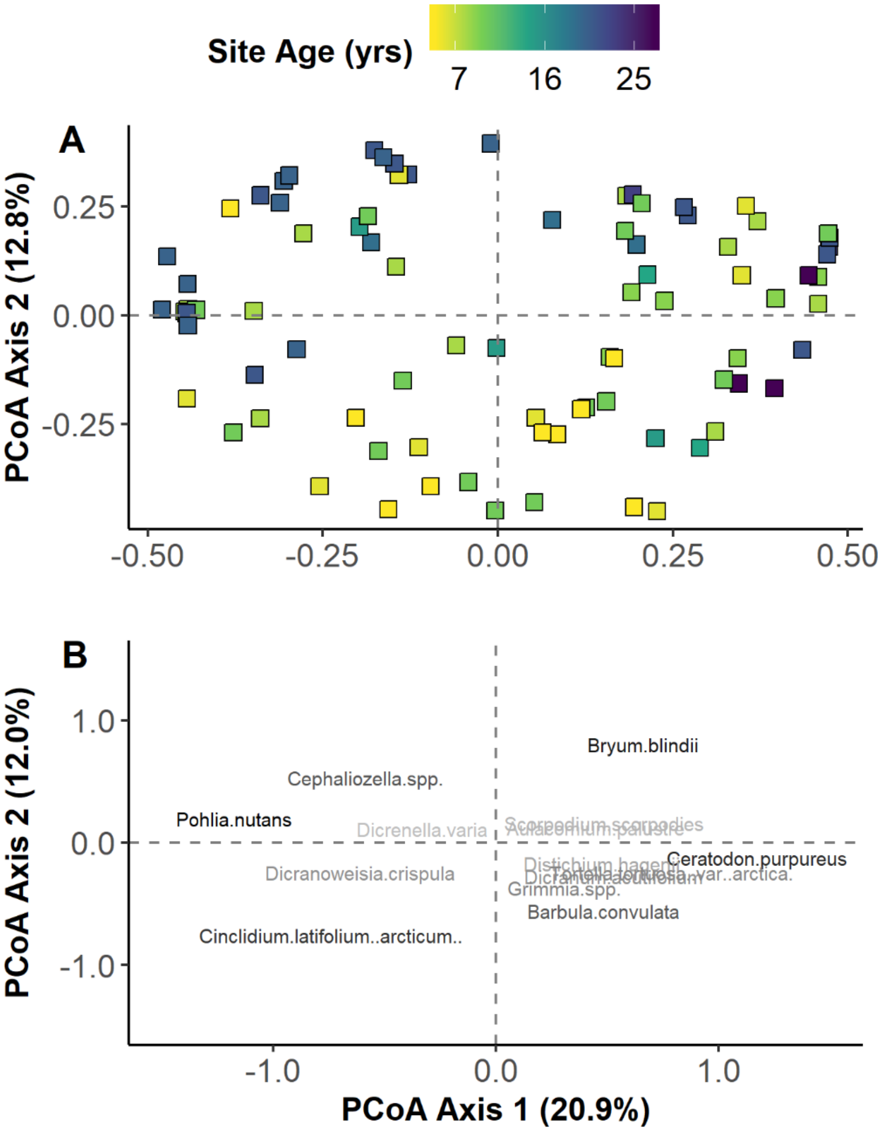

Figure 23. Principal coordinates analysis (PCoA) of bryophyte cover on drilling waste across a chronosequence of mining disturbance. Displayed are the site scores (A) and species scores (B).

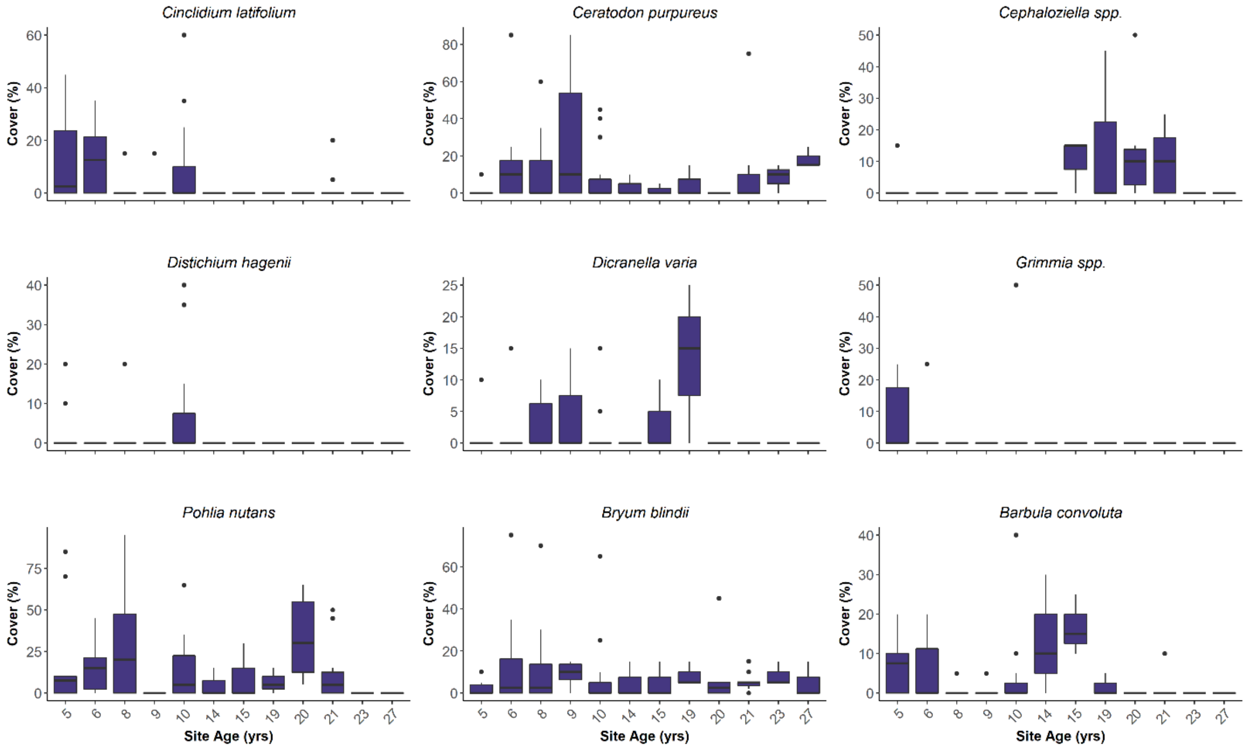

Figure 24. Boxplots showing change in bryophyte cover on drilling waste along a choronosequence of mining disturbance.

In contrast to bryophyte community composition, ground cover (e.g., drilling waste, vascular species, and lichen and moss crusts) over the chronosequence had evident successional trends (Figure 25). The first two axes of the principal coordinates analysis explain about 30% of variation in ground cover, where separation of sites by age occurs along the x-axis. Along the x-axis, there is a gradient of drilling waste and moss crusts on the negative scores to vascular plants and lichen crusts on the positive scores. As such, the colonization of drilling waste after disturbance begins with bryophyte species and moves to more lichen and vascular species as sites get older. Combined with the bryophyte community composition results discussed previously (Figure 23, Figure 24), these data suggest that moss are initial colonizers of drilling waste, but they persist throughout succession and are not entirely replaced by lichen crusts and vascular species, which appear later in the successional trajectory. Indeed, it appears that the lichen crusts and vascular species tend to establish around 20 years post-disturbance.

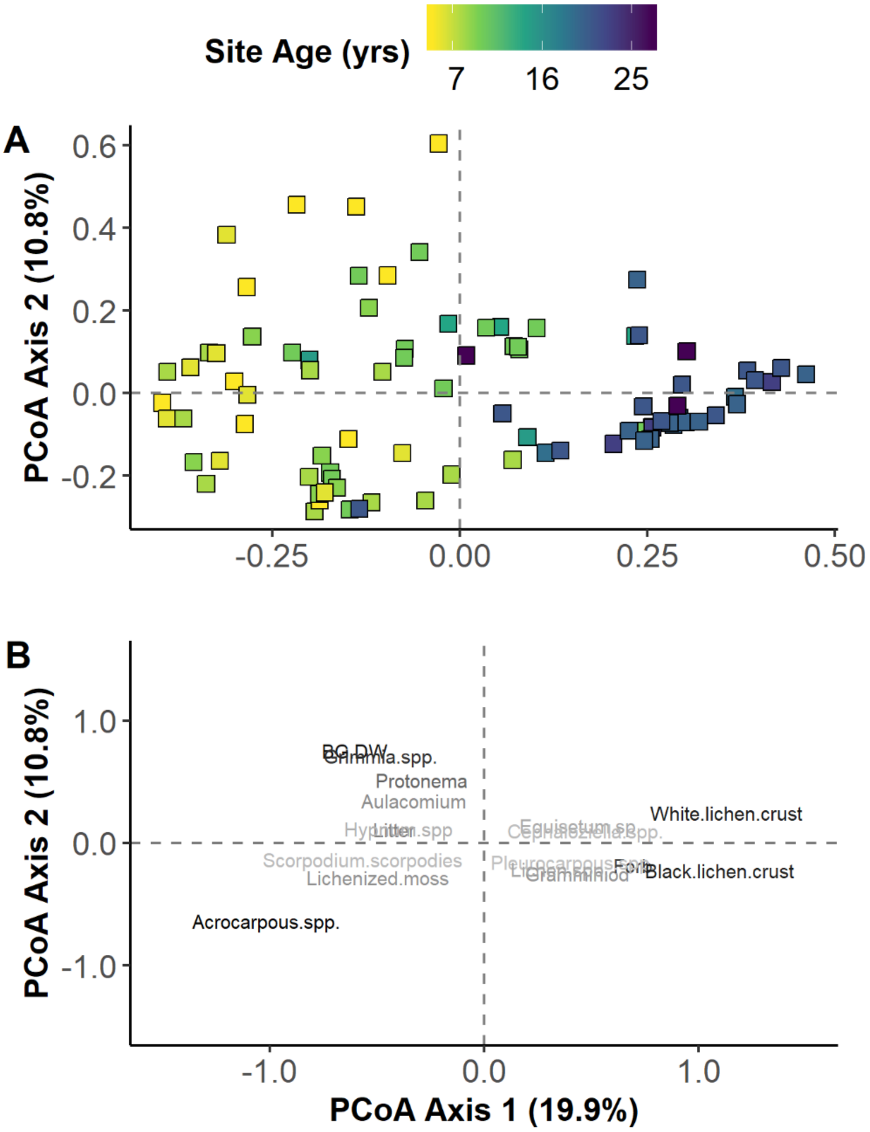

Figure 25. Principal coordinates analysis (PCoA) of ground cover, as drilling waste, vascular species, and lichen and moss crusts, on drilling waste across a chronosequence of mining disturbance. Displayed are the site scores (A) and species scores (B).

Nutrients

Across the chronosequence, we generally see an increase in anions and cations in BSCs with time (Figure 26, Figure 27). Thus, as crusts develop, there is a concurrent increase in anion and cation concentrations. Interestingly, this increase in crusts does not generally lead to increased anions and cations in the substrate below the crust within the first 30 years of development. For percent nitrogen (N) and carbon (C), as well as pH, we see different patterns than the plant-available nutrients (Figure 28). Specifically, percent N and C, and pH did not change in crusts over time, whereas percent N and C decreased in underlying substrate. pH in the underlying substrate also did not change over time, but crusts tended to have lower pH than the underlying substrate (Figure 28C). These observations are supported by linear mixed-effects and linear regression models, which indicated that there were significant ($p < 0.05$) interactions between site age and substrates in all variables except pH (Figure 28C), which demonstrates that patterns of nutrient concentrations between the crusts and underlying substrate differ. Goodness of fit statistics for each model are generally moderate to strong (R^2M: 0.46-0.71, R^2_{adj}: 0.65), but for percent N and C and phosphate were weaker (R^2M: 0.17 & 0.17, and R^2_{adj}: 0.13), suggesting that our models explained the majority of nutrient variation in most cases.

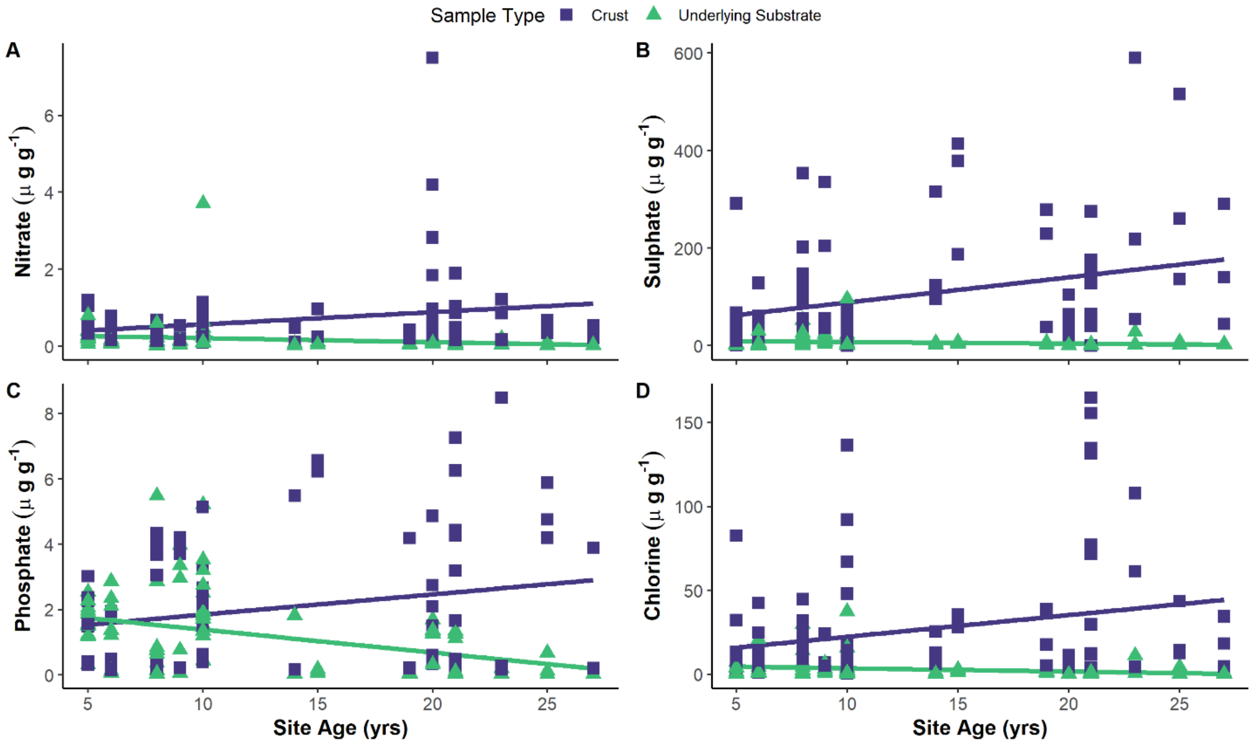

Figure 26. Concentration of anions in the biological soil crusts and underlying substrate across sites disturbed by mining 5-27 years before sampling.

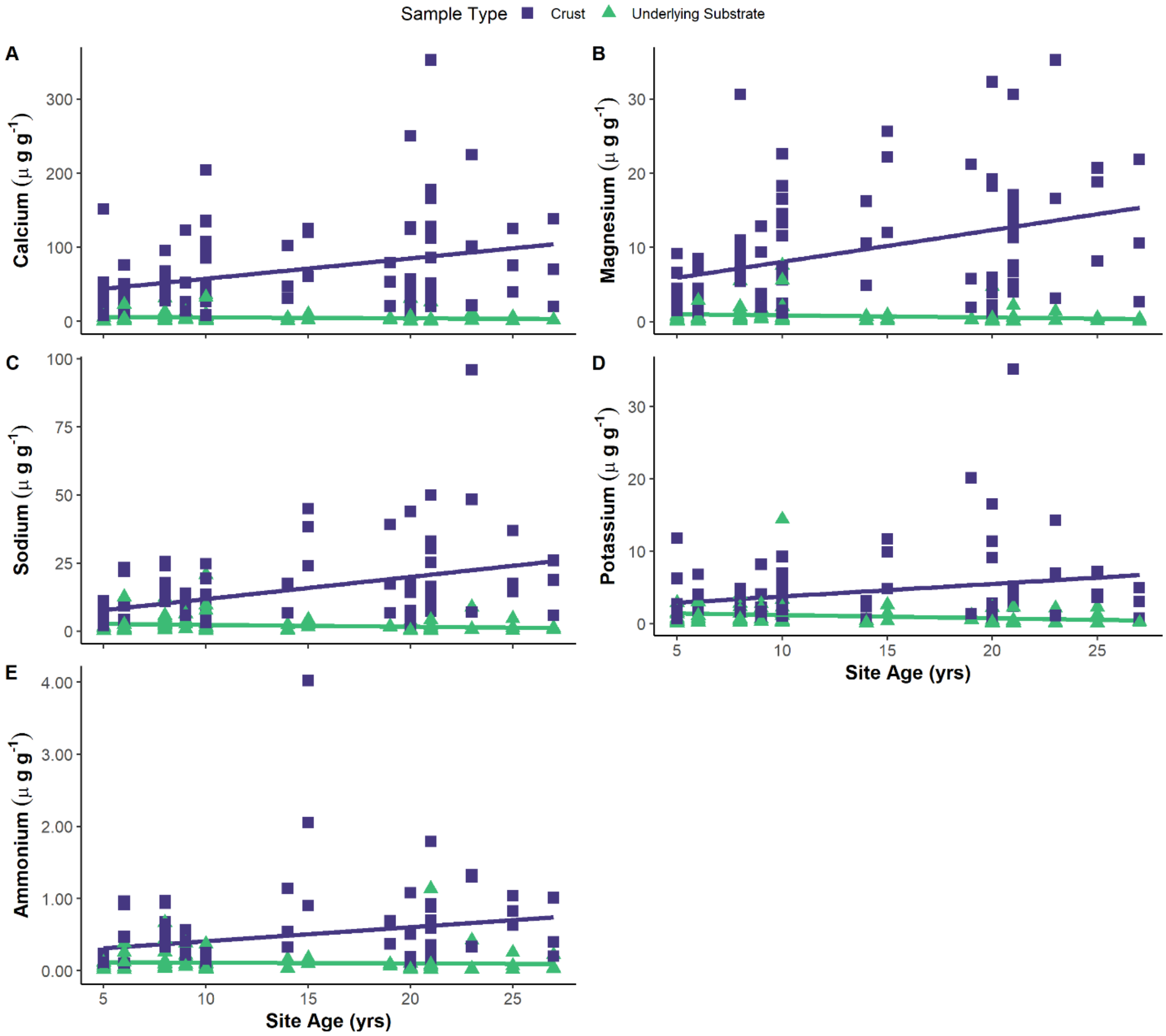

Figure 27. Concentration of cations in the biological soil crusts and underlying substrate across sites disturbed by mining 5-27 years before sampling.

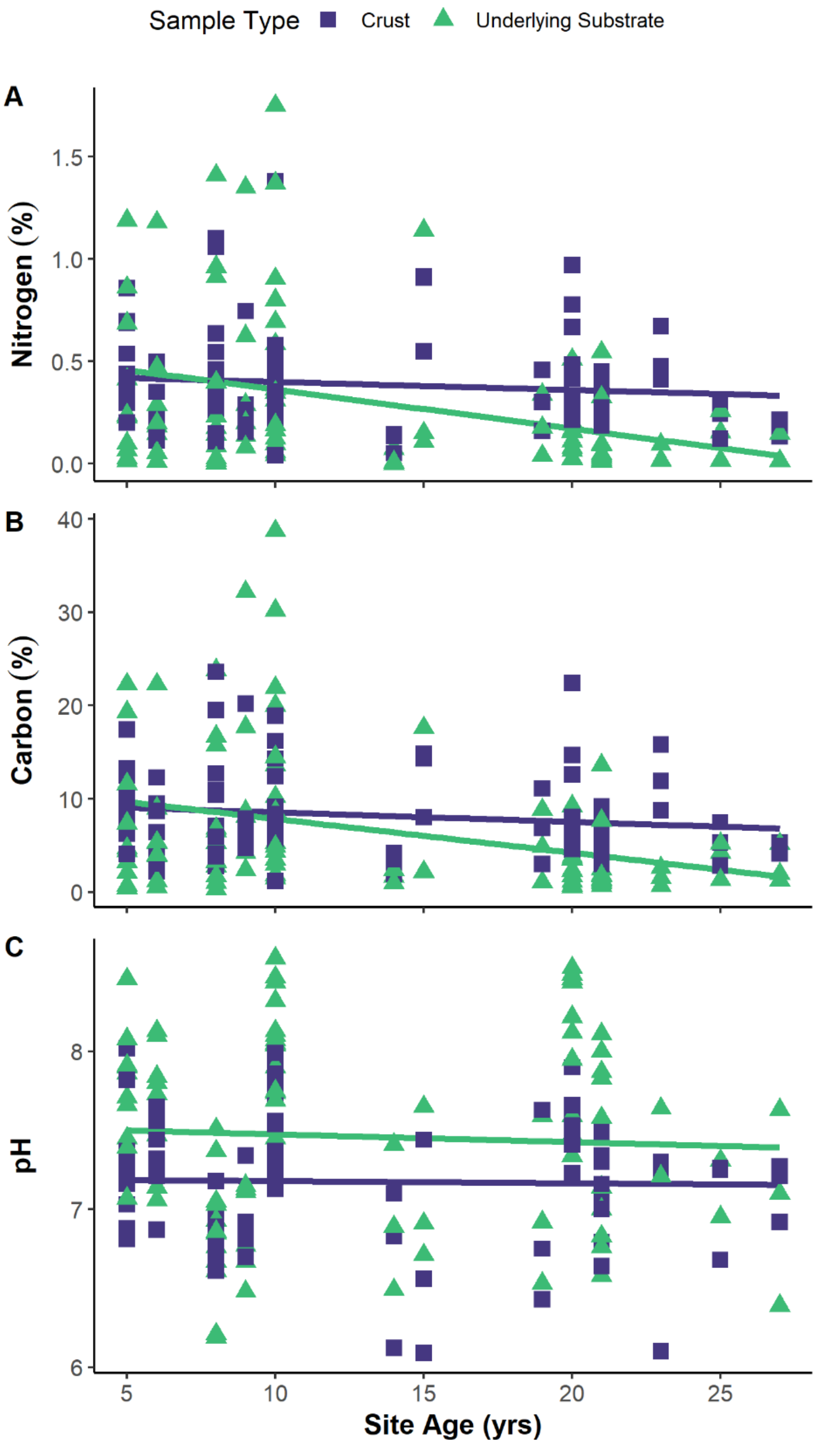

Figure 28. Percent carbon and nitrogen, and pH in biological soil crusts and underlying substrate across sites disturbed by mining 5-27 years before sampling.

Nitrogen fixation and gas flux

Gas flux (CO_2, CH_4, N_2O) of BSCs in light and dark conditions generally did not vary over time under optimal (i.e., laboratory; Figure 29) or field conditions (i.e., *in situ*; Figure 30). In addition, there was little difference between light and dark fluxes. Taken together, these results suggest that gas fluxes are relatively stable during colonization of drilling wastes after disturbance, and that there is relatively little uptake of gasses occurring by these communities. This is supported by linear mixed-effects models of optimal gas flux, which show that age of site was not significant for any model ($p > 0.10$), though light conditions (e.g., fluxes measured in light vs. dark conditions) were significantly different for CO_2 ($p < 0.01$). In contrast, N_2-fixation of crusts increased significantly with site age ($p < 0.01$; $R^2M = 0.21$, $R^2C = 0.33$), suggesting that with BSC development there is an increase in N_2-fixation (Figure 29D). There is a significant albeit weak, positive relationship between N_2-fixation and lichen crust cover (LME: $p = 0.03$, $R^2M = 0.07$, $R^2C = 0.28$; Figure 31), which also tended to increase with site age (Figure 25).

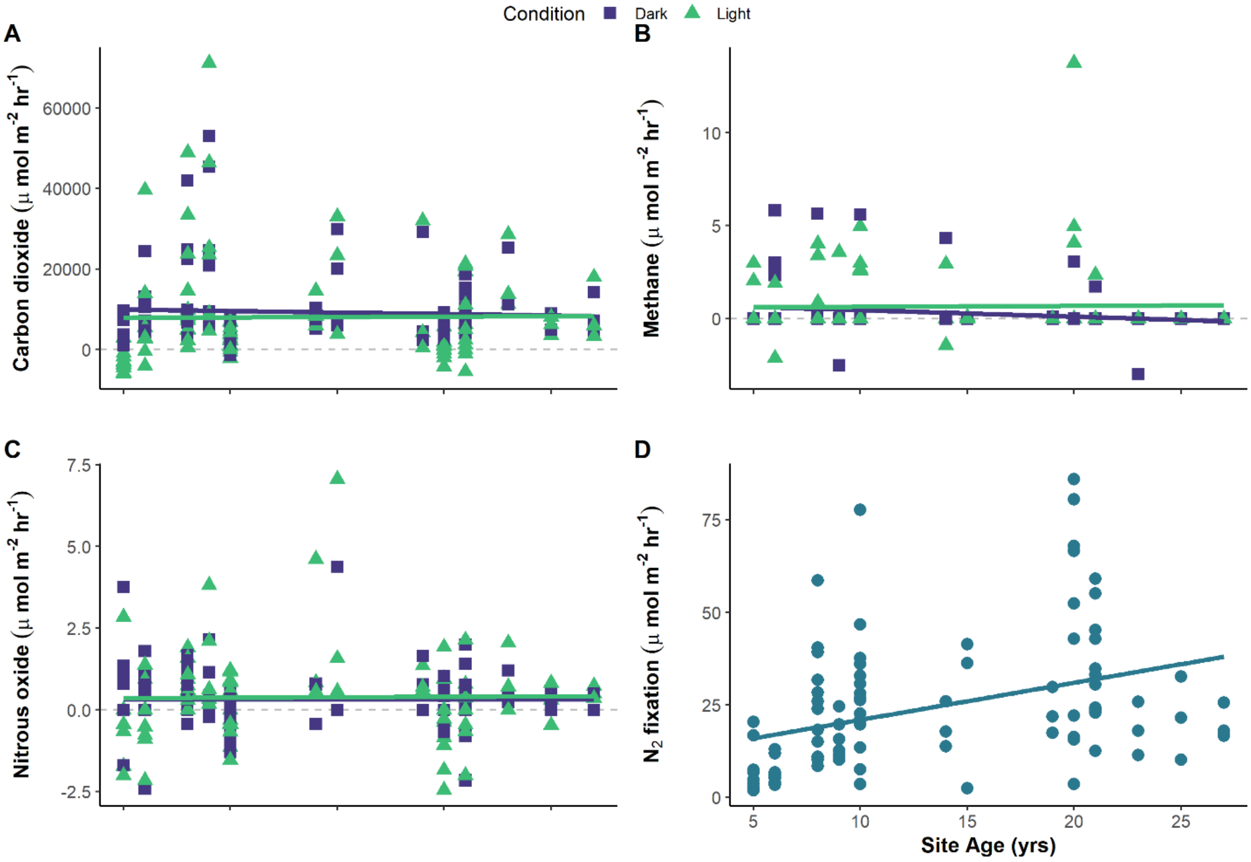

Figure 29. Gas flux and atmospheric nitrogen (N_2) fixation of biological soil crusts (BSCs) sampled on drilling wastes of varying ages measured under laboratory (i.e., optimal) conditions. Fluxes of gases (CO_2, CH_4, and N_2O) were measured under both light and dark conditions.

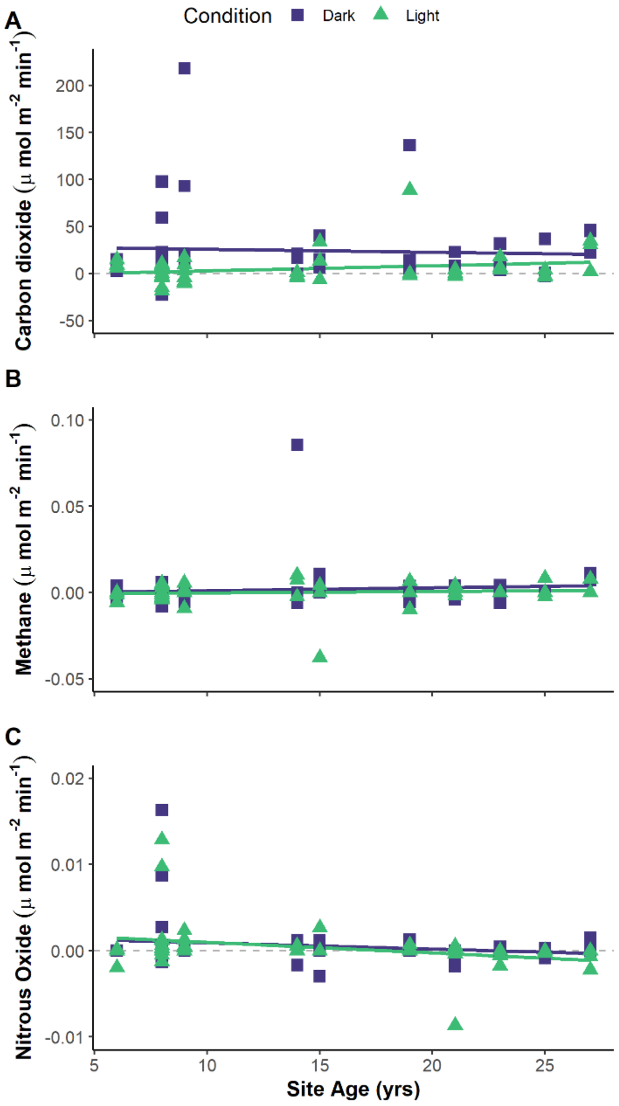

Figure 30. Gas flux of biological soil crusts (BSCs) sampled on drilling wastes of varying ages measured under field (i.e., in situ*) conditions. Fluxes of gases (CO_2, CH_4, and N_2O were measured under both light and dark conditions.*

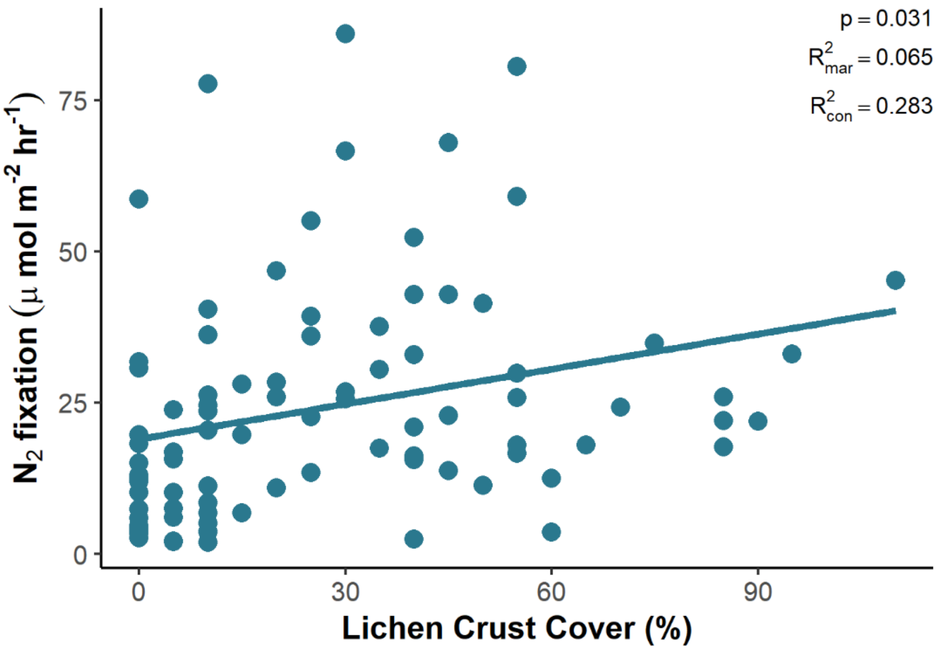

Figure 31. Relationship between in situ *lichen crust cover and nitrogen (N) fixation of biological soil crusts (BSCs) sampled from drilling waste sites of varying ages.*

Section II: Active Restoration of Drilling Waste

Turf Expansion Trial – Growth Chamber Study

Background

Whole-turf or sod transplantation (i.e., harvesting and transplanting intact soil-plant trufs) for restoration has been examined in several Arctic and alpine environments, ranging from alpine grassland and dry alpine heaths (Aradottir & Oskarsdottir 2013; Mehlhoop et al. 2018; Bay & Ebersole 2006), wet alpine (Conlin & Ebersole 2001), to Arctic sedge meadows (Cater et al. 2015; Kidd et al. 2006). The benefits of turf transplants include initiating restoration efforts with established and functional assemblages of native vegetative species (Cater et al. 2015), development of safe sites for seed and spore germination, the inclusion of soil resources (i.e., soil organic matter, plant propagules, soil invertebrates, and microbial communities) (Conlin & Ebersole 2001; Klimeš et al. 2010), maintenance of high species diversity and presence of rare species (Aradottir 2012; Aradottir & Oskarsdottir 2013), and reduced shock to individuals within the transplants (Bay & Ebersole 2006). Furthermore, in remote areas, whole-turf transplants can be carried out with limited equipment. In almost all Arctic environments, plant roots represent the majority of biomass, contribute to soil carbon sequestration and organic matter, and act as long-lasting sources of nutrients for microbial communities (Iversen et al. 2015). Assessing restoration success based only on aboveground growth ignores both the substantial development of belowground structures and considerable contribution to nutrient cycling regimes within Arctic environments (Iversen et al. 2015). Due to the dominance and importance of belowground biomass, and the limited knowledge on belowground expansion, we investigated both above and belowground expansion of turfs harvested from the Meliadine mine site and transplanted adjacent to fertilized or unfertilized bare substrates under growth chamber conditions.

Methods

Turf Harvesting

Plant-soil turfs were harvested from an area of undisturbed upland tundra heath located on Agnico Eagle Mines Meliadine site (63°01'22.9"N 92°11'41.1"W DMS) near Rankin Inlet, Nunavut, Canada, in early July 2019. A spiral search was used to identify eight suitable turfs, with at least 5 meters of separation between each harvested turf. Suitable turfs occurred on the upper part of large hummocks which had relatively level topography and nearly continuous vascular plant cover. Turfs approximately 46 cm long, 28 cm wide, and 10 cm deep were dug out using a flathead shovel and lifted into plastic bins. Turf soils were composed of approximately 8-10 cm of organic soil with two centimeters of mineral soil below. Plant species cover was measured for each turf on-site within three days of harvest using a 0.096 m^2 gridded quadrat (15 grids, 0.0064 m^2 each). Values ranging from 1 to 4 (1 = <25%, 2 = 25-50%, 3 = 50-75%, 4 = >75%) were used to estimate each species' cover in each individual grid square. Turfs were transported to the University of Saskatchewan and placed in growth chambers [18.5hrs light (15^0C)/5.5hrs dark (5^0C) cycle at ~400 µmol, relative humidity 65%].

Experimental Design

Plastic containers (50 cm long x 30 cm wide x 15 cm deep) with drainage holes were lined with a thin layer of cheesecloth. Turfs were scored lengthwise using a flathead shovel and separated by hand to prevent damage to each half. Separated turfs were then placed against one side of a container, with a mesh screen (12 cm^2 openings) placed underneath the turf and across the growing front. In a paired design, one half of each of the eight turfs was placed in contact with a fertilized substrate (10 gm^{-2} N (NH_4NO_3), 5 gm^{-2} P (P_2O_5)), and the other half with unfertilized substrate (Figure 32). Substrates were sourced from a local distributor (Rock Bros, Saskatoon, SK), and fertilized through dry mixing before being placed alongside the fresh growing front. The substrate was added until the surface of the turf and substrate were level (i.e., ~ 10-12 cm depth). The texture of the substrate varied from sand to sand/loam, and the particle size distribution (clay 0%, silt 12-17%, sand 83-88% (Horiba LA950 Particle Size Analyzer, Japan)) was similar to substrates encountered at Rankin Inlet. Each container (turf and substrate) was watered twice per week (643 mL/container/watering) to simulate the natural precipitation regime of Rankin Inlet during the growing season and were kept in growth chambers for a total of 140 days. Turfs were randomly repositioned and photographed weekly within the growth chambers.

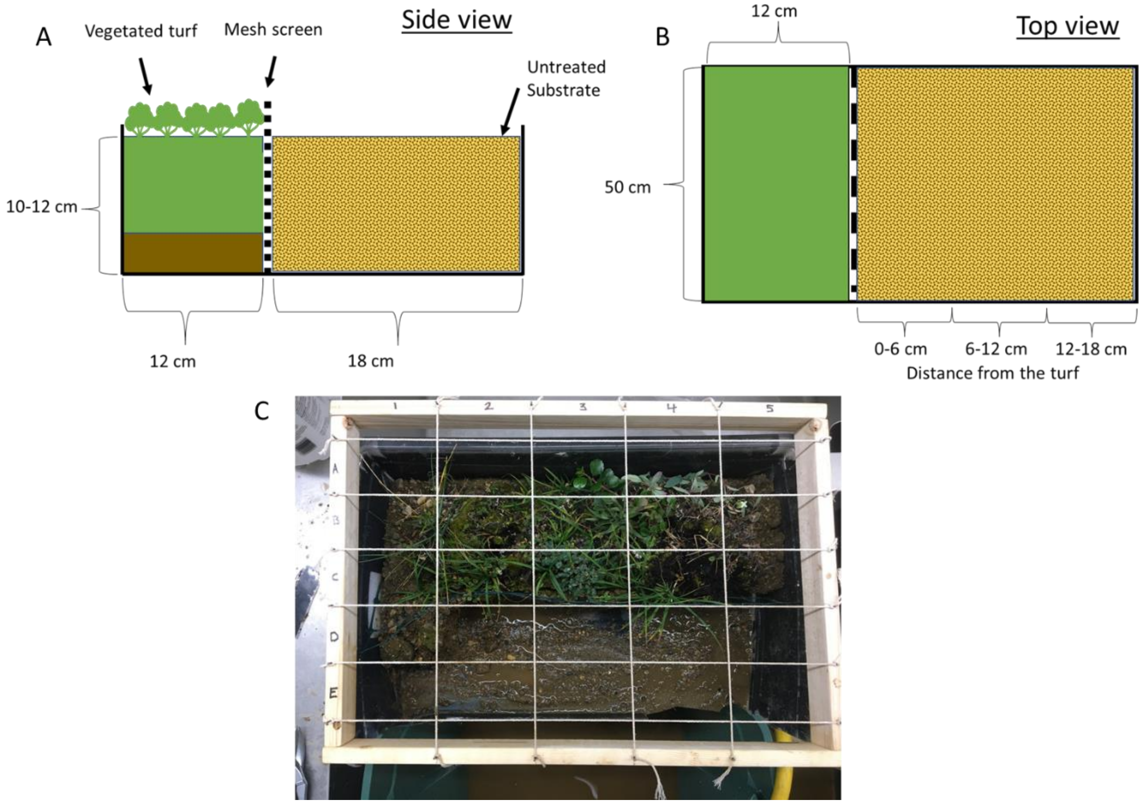

Figure 32. Side view (Panel A), and top view (Panel B) conceptual diagram of experimental treatments. Panel C shows a turf during root harvesting, with gridded quadrat overtop.

After the 140-day incubation period, the turfs were photographed using a multi-spectral camera (Parrot Sequoia, U.S.A) to generate normalized-difference vegetative index (NDVI) values for each container, a measurement used to assess quantity and health of vegetation present within each container. To assess community composition, vegetative growth, and species richness on the turfs and on the substrate, plant species cover across the entire container was measured using a 0.15 m^2 gridded quadrat. All vascular plants, mosses, and lichens were identified to species in each grid. All vascular plants emerging above the substrate (i.e., individual vascular plant stems, hereby referred to as "emergents") were counted in each grid and the height, lateral spread (i.e., the horizontal distance of emergents' growth), and distance of each vascular emergent from the turf (i.e., the mesh screen) were measured. Emergents were harvested at ground level after percent cover measurements, dried for 72 hours at 40^{o}C, and weighed to determine biomass. The side of each container farthest from the turf was cut away, and the substrate in each cell was carefully washed out to harvest the roots in each cell. Collected roots were then dried for 72 hours at 40^{o}C, and subsequently freeze-dried to ensure all moisture was removed. Dried roots were then weighed, before being ground with a ball mill (Retsch MM-400, Germany).

The identity of roots extending into the new substrate was determined using DNA barcoding (Lamb et al. 2016). Root DNA was extracted from ~0.7 g of ground material using a PowerPlant Pro kit (Qiagen, Germany). All operational taxonomic units (OTU's) detected were identified to species level, except for an unidentified *Salix* and *Fabaceae* sp., which were unable to be identified beyond family level.

Results

Aboveground Community Composition and Growth

PERMANOVA indicated that fertilization of the adjacent substrate did not affect the community composition of the turfs after 140 days of growth (df = 31, R^2 = 0.04, p = 0.25), while the composition of the turfs did change over time (df = 31, R^2 = 0.16, p = 0.001) (Figure 33). Significant interactions between treatment and time were not detected. Over time we observed decreases in lichen and plant litter cover and increases in evergreen shrub, moss, and graminoid cover (Table 5). Vegetative community composition on the fertilized substrate was significantly different from the unfertilized substrate (PERMANOVA: df = 15, R^2 = 0.42, p = 0.001) (Figure 34). Mean NDVI of the turfs did not differ between fertilized and unfertilized treatments (Student's t-test: df = 7, t = 0.64, p = 0.5); however, the mean NDVI of the fertilized substrates was significantly higher than unfertilized (Student's t-test: df = 7, t = -3.5, $p < 0.01$) (Figure 35).

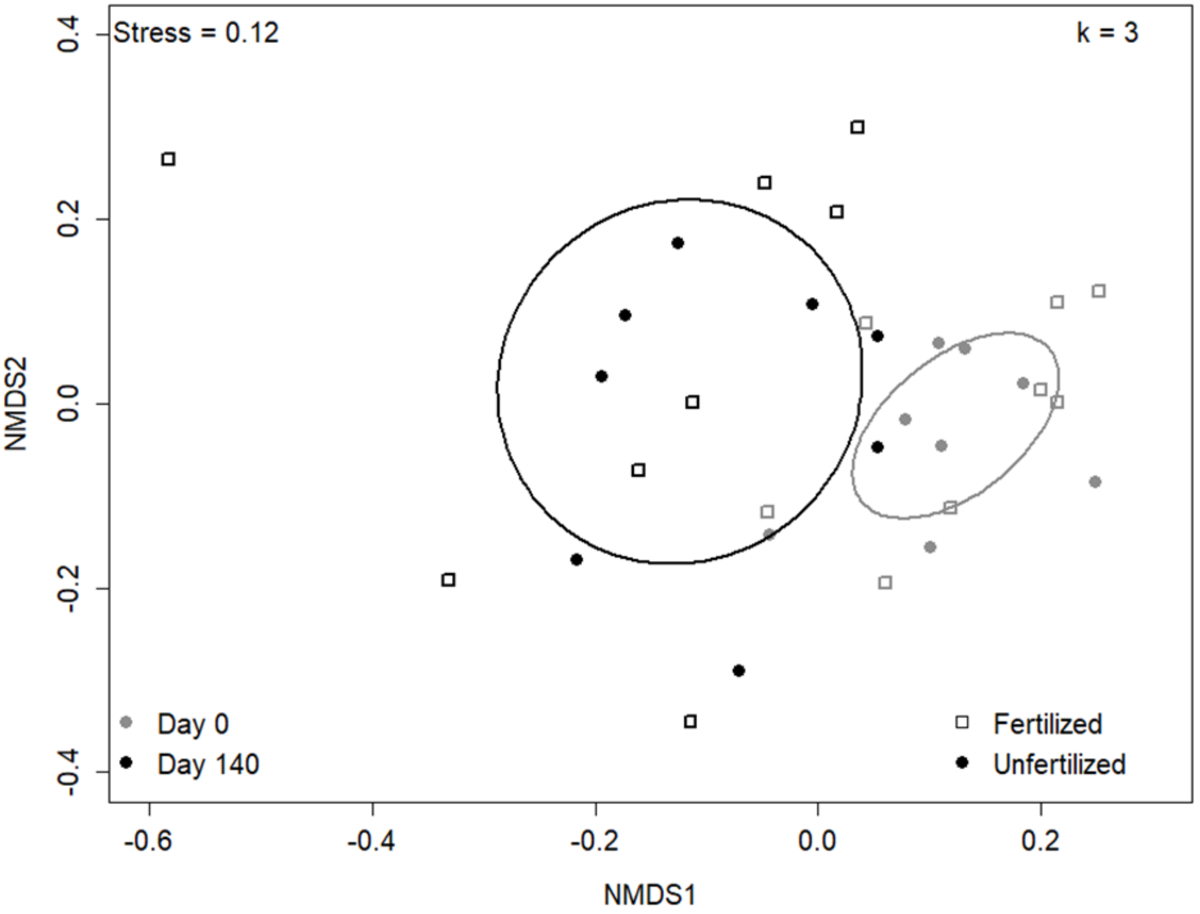

Figure 33. A non-metric multidimensional scaling ordination of turf community composition placed adjacent to fertilized (hollow square) and unfertilized (filled circle) substrates, at the start of the experiment (Day 0- grey) and end of the experiment (Day 140-black).

Table 5. Growth forms, species, litter, and bare ground that contributed to >70% of the overall dissimilarity between Day 0 and Day 140 for turfs and between fertilized and unfertilized substrates on Day 140. The dissimilarity contribution of growth forms and species was identified using SIMPER analysis. Absolute and relative percent differences between Day 0 and Day 140 turfs and between fertilized and unfertilized substrates were calculated. Absolute percent differences are (species cover Day 140 or fertilized – species cover Day 0 or unfertilized)/species cover Day 0 or species cover unfertilized), respectively. Relative percent differences are ((species cover Day 140 or species cover fertilizer/total species cover Day 140 or total species cover fertilizer) - (species cover Day 0 or species cover unfertilized/total species cover Day 0 or total species cover unfertilized)), respectively. Species that were not recorded at Day 0 or within unfertilized treatments but recorded at Day 140 or within fertilized treatments are recorded as 100% increase in absolute cover.

Section	**Growth Form**	**Dissimilarity Contribution**	**Percent Difference Absolute (Relative)**	**Species**	**Percent Difference Absolute (Relative)**
Turf	Lichen	25%	-51% (-14%)	*Thamnolia vermicularis*	-66% (-4%)
				Cetraria nivalis	-44% (-4%)
				Gowardia nigricans	-54% (-3%)
				Alectoria ochroleuca	-40% (-1%)
	Litter	16%	-52% (-10%)	Plant Litter	-51% (-5%)
				Woody Litter	-58% (-6%)
	Moss	13%	+43% (+8%)	*Aulacomnium turgidum*	+42% (+3%)
				Dicranaceae sp.	-27% (-2%)
	Evergreen Shrubs	13%	+15% (+3%)	*Dryas integrifolia*	+47% (+3%)
				Cassiope tetragona	-16% (-1%)
	Graminoids	10%	+95% (+8%)	*Carex sp.*	+100% (+3%)
					+100% (+4%)
				Poa flexuosa	
Substrate	Bare Ground	37%	-55% (-40%)	Bare Soil	-70% (-30%)
				Rocks/Stones	-31% (-11%)
	Moss	27%	+123% (+18%)	*Pohlia nutans*	+160% (+7%)
					+103% (+5%)
				Dicranoweisia crispula	+3% (-1%)
				Aulacomnium turgidum	
	Biological Soil Crusts	18%	+169% (+12%)	Nostoc spp.	+258% (+7%)
					+100% (+4%)
				Cyanobacterial mats	

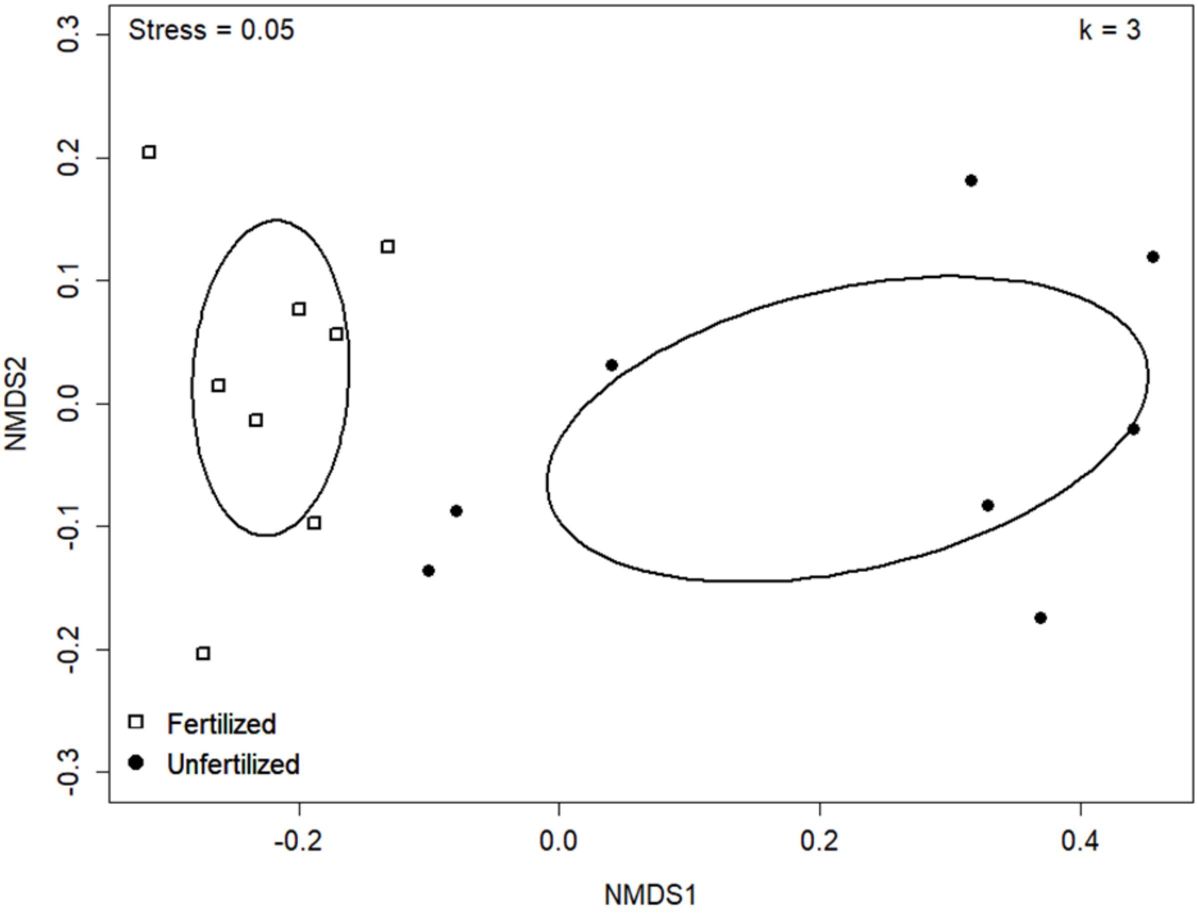

Figure 34. A non-metric multidimensional scaling analysis of substrate community composition of fertilized (squares) and unfertilized (circles) substrates.

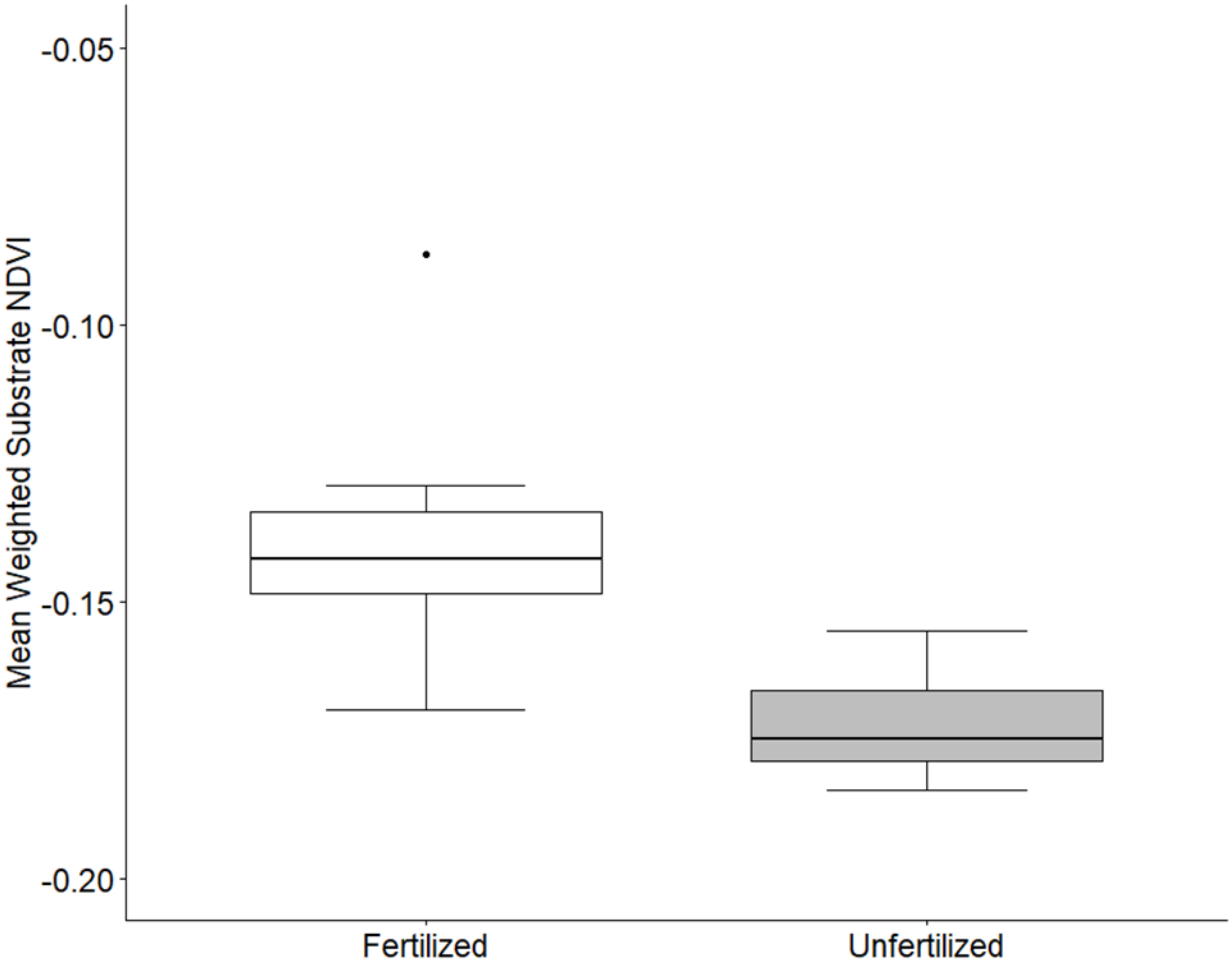

Figure 35. Mean weighted substrate NDVI values between fertilized and unfertilized substrates. Boxes encompass 25-75% percentiles of the data, while whiskers encompass 5-95%. The median is indicated by the black horizontal line, with dots representing points outside of the 95th percentile. Difference letters indicate significantly different NDVI values (Paired t-test, t-stat = -3.5, p < 0.01).

Biological Soil Crusts

BSC cover and species richness on fertilized substrates were significantly higher (Two-way ANOVA: $F_{1,7} = 25$, $p < 0.01$; $F_{1,7} = 15$, $p < 0.01$) with both metrics approximately double that of unfertilized substrates (Table 5). BSC cover and richness were not significantly different with distance from the turf and no fertilizer × distance interaction was detected (Figure 36A). Cover on both substrates was dominated by moss (~63% of total cover), with cyanobacterial mats and lichen species comprising 28% and 8% of total cover, respectively. *Pohlia nutans* (Copper Wire Moss), *Dicranoweisia crispula* (Mountain Pincushion), and *Aulacomnium turgidum* were the most common moss species, while cyanobacterial components included *Nostoc* spp. Most BSC species occurred on both fertilized and unfertilized substrates, except for *Bryum blindii*, which only occurred on fertilized substrates (Figure 36A).

Vascular Plants

We were unable to model vascular species richness, however, we observed similar species richness immediately adjacent to the turfs (0-6 cm) in fertilized and unfertilized substrates (0.33 ± 0.32 and 0.43 ± 0.36, respectively), while at the furthest distance assessed (12-18 cm) fertilized substrates had higher mean vascular richness (0.28 ± 0.41 and 0.03 ± 0.07 respectively). Both fertilized and unfertilized substrates contained similar assemblages of vascular species, namely *Astragalus alpinus*, *Stellaria longipes* (Longstalk Starwort), and *Carex* spp. (Figure 36). *S. longipes* demonstrated the farthest growth from the turf, often emerging against the farthest wall of the container, and was more prevalent in the fertilized compared to the unfertilized substrates (46 vs 3 emergents respectively). Mean aboveground vascular biomass was higher in the fertilized substrates than the unfertilized substrates, with approximately three times more emergents, although no statistical differences between treatments were detected. No effect of fertilizer treatment was detected for any other aboveground measurements (i.e., distance from the turf, mean height, mean lateral spread).

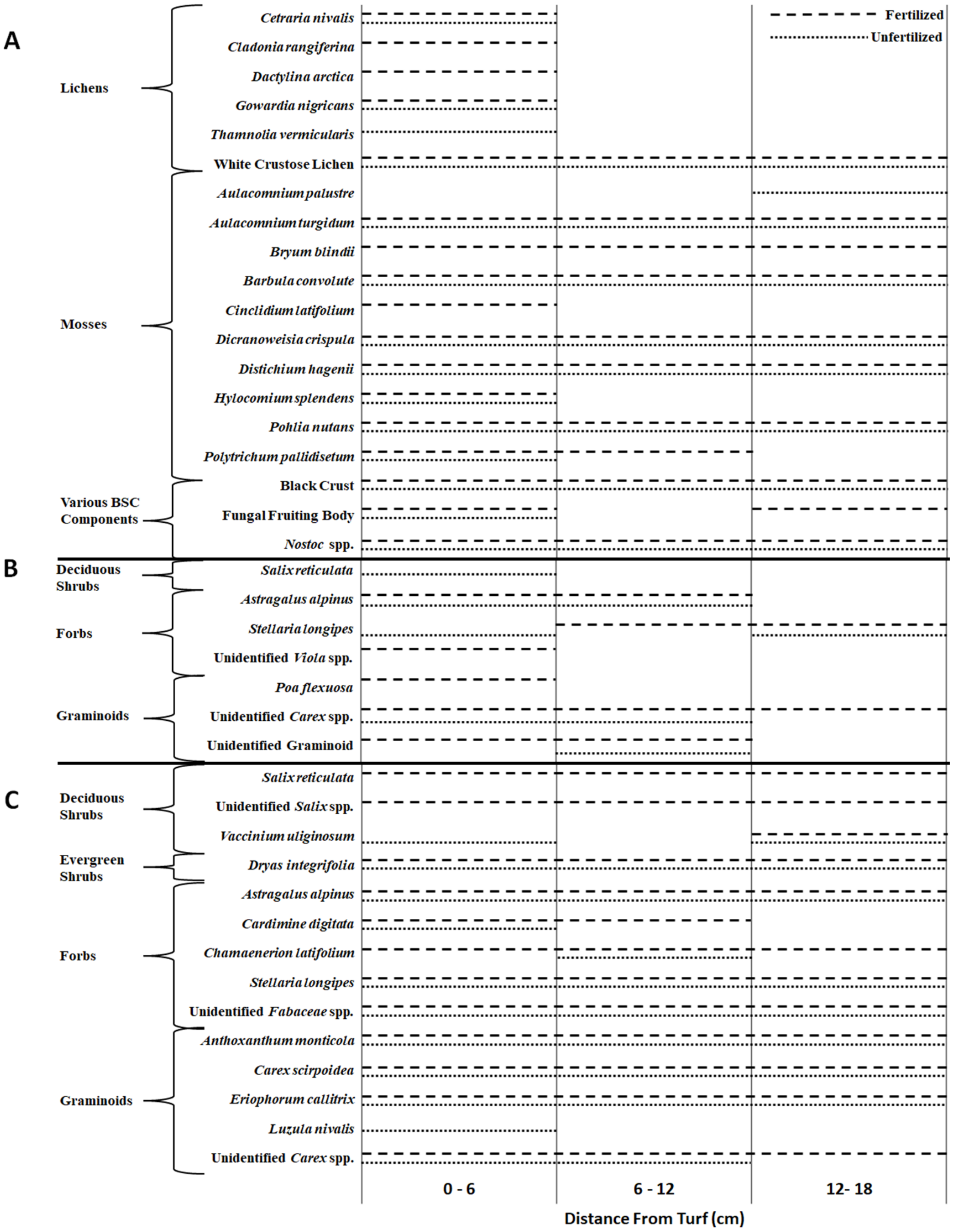

Figure 36. Presence of individual species on/in fertilized/unfertilized substrates at each distance category (0 - 6, 6 - 12, and 12 - 18 cm), separated into biological soil crust (A), aboveground species (B), and belowground species (C). The presence of species within fertilized substrates is represented by dashed lines, and unfertilized substrates are represented by dotted lines.

Belowground Community Composition and Growth

Based on two-way ANOVA, belowground species richness was not significantly different with fertilization, distance, or their interaction. Both fertilized and unfertilized substrates contained the same species, except for *Luzula nivalis* (Arctic Wood Rush), which occurred only within unfertilized substrates. Nine vascular species were detected in greater prevalence within fertilized substrates, most notably *Anthoxanthum monticola* (Alpine Sweetgrass), *S. longipes*, and *Salix reticulata*, which were detected in 56%, 31%, and 18% of fertilized cells, compared to 37%, 13%, and 1% cell within unfertilized substrates respectively. Unfertilized substrates had a greater prevalence of four species, *Dryas integrifolia*, *Astragalus alpinus*, *Eriophorum callitrix* (Arctic Cottongrass), and an unidentified *Fabaceae spp*., being detected in 51%, 20%, 10%, and 14% unfertilized cells, yet only detected in 22%, 8%, 7%, and 11% fertilized cells, respectively. It should be noted that while these species seemingly favored unfertilized substrates, each was found expanding at least 12 cm from the turf in fertilized substrates as well (Figure 36C).

Belowground biomass was significantly higher in the fertilized substrate than the unfertilized substrates and changed with distance from the turf (two-way ANOVA: $F_{1,7} = 6.3$, $p < 0.05$; $F_{2,14} = 17$, $p < 0.001$), and a significant fertilizer × distance interaction was detected (two-way ANOVA: $F_{2,14} = 3.9$, $p < 0.05$). Belowground biomass decreased with distance from the turf, with significantly higher biomass observed at 0 to 6 cm compared with 6 to 12 cm and 12 to 18 cm in both substrates (Figure 37C). Fertilized substrates had more belowground biomass than unfertilized at each distance increment (Student's t-tests: df = 39, t = -3.68, $p < 0.001$; df = 39, t = -5.98, $p < 0.001$; df = 39, t = -4.87, $p <0.001$). The increase in biomass observed within the fertilized substrates at 12 to 18 cm was due to belowground biomass found pressed against the farthest wall of the container, indicating that container size limited belowground expansion in the fertilized treatment. Overall, total belowground biomass was 22-fold (fertilized) and 12-fold (unfertilized) greater than aboveground biomass.

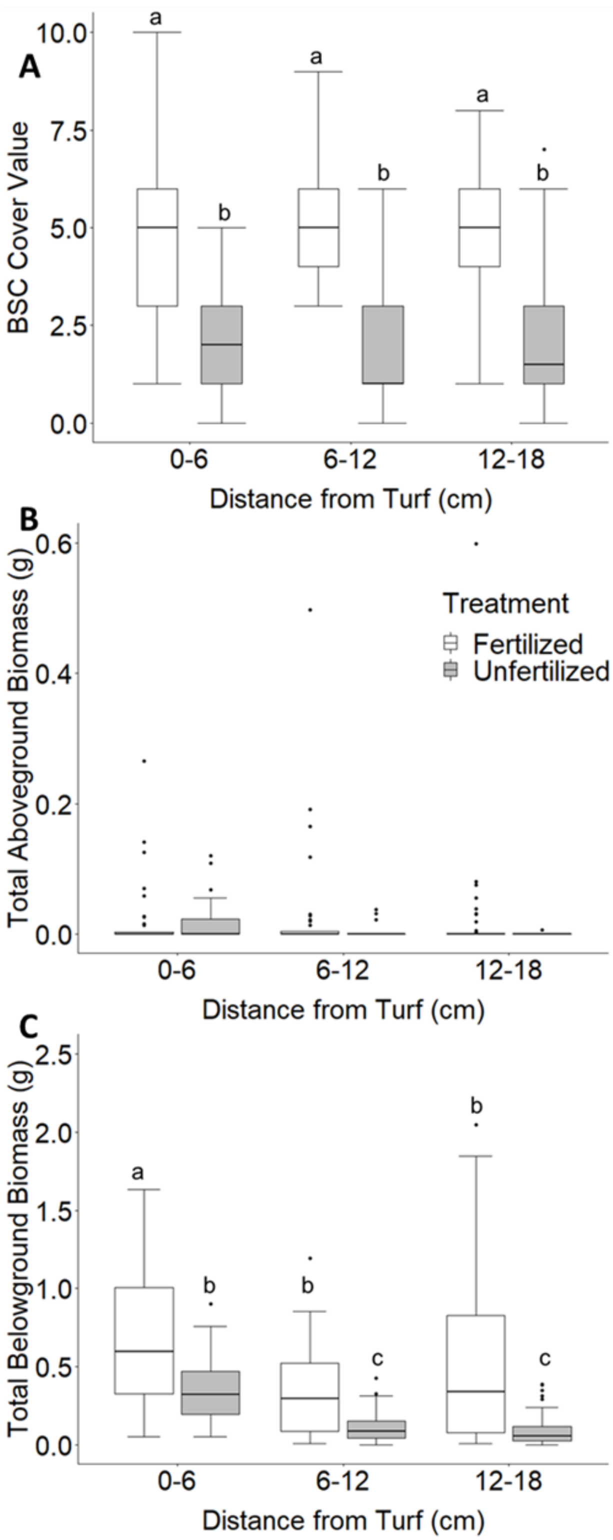

Figure 37. Comparative box plots (untransformed data) of biological soil crust cover (A), aboveground vascular biomass (B), and belowground biomass (C), at each distance category (0-6, 6-12 and 12-18 cm). White boxplots represent fertilized treatments, grey boxplots represent unfertilized treatments. Lower-case letters on boxplots represent significant differences or similarities between groups based on Student's t-tests. The boxplot of biological soil crust cover uses the raw values recorded in each grid.

Recommendations

While fertilization of adjacent substrates did not alter the turf community, greater BSC development and belowground biomass in fertilized substrates, suggests fertilization may decrease timeframes of natural colonization in the turf's immediate surroundings and support the development of later-stage vegetative communities. Turfs in our growth chamber experiment expanded ~150% in total area, with graminoid species predominately responsible for this expansion. Furthermore, belowground expansion far exceeded aboveground. Based on these findings we recommend the following:

1. **Harvesting and transplanting intact, undisturbed tundra turfs aids the rehabilitation of mining-disturbed sites**, as substrates adjacent to turfs were found to be colonized by BSCs and vascular plants after 140 days of incubation in the growth chamber.
2. **Fertilization of substrate adjacent to transplanted tundra turfs: i) allows for maintenance of turf transplant community composition promoting native assemblages of species**; **ii) expedites colonization** of disturbed substrates by BSCs and fast-growing vascular species; and **iii) increases the distance of expansion.**
3. Turfs can act as a **source of BSC propagules** and the forbs ***Stellaria longipes*** and ***Astragalus alpinus*,** the evergreen shrub ***Dryas integrifolia,*** and deciduous shrub ***Salix reticulata*** **are target species for restoration.**
4. **Targeting nitrogen-fixing vascular species** (e.g., *Astragalus alpinus* and the family Fabacaeae) **in turfs promotes expansion into unfertilized substrates** and may facilitate the growth of other species through N addition.
5. Expansion of belowground biomass from turf transplants far exceeds aboveground and is primarily driven by grasses, specifically *Anthoxanthum monticola.* Therefore, **belowground expansion of species, especially grasses should be monitored.**

Turf Expansion Trial– in situ Study

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 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 *in-situ* active restoration trial using transplanted tundra turfs and shredded tundra material, we aim to identify restoration techniques (i.e., transplanting intact and shredded materials, and organic and mineral soils, creating microtopograhpy, and providing erosion control) that promote pre-disturbance plant-soil assemblages and recovery of ecosystem functions. We also aim to identify tundra species that rapidly colonize 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 (i.e. Hyster Rollover), and quarries of varying sizes within the mine footprint. Three gravel quarries were selected, however one site near the emulsion plant (62.04261° N 92.25031° W) was damaged from mine site operations following establishment of the trial in 2019 and is not included in the report. The two remaining restoration sites were located to the northwest and southeast of the current operating mine (Figure 38, Table 6). 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 7), 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.

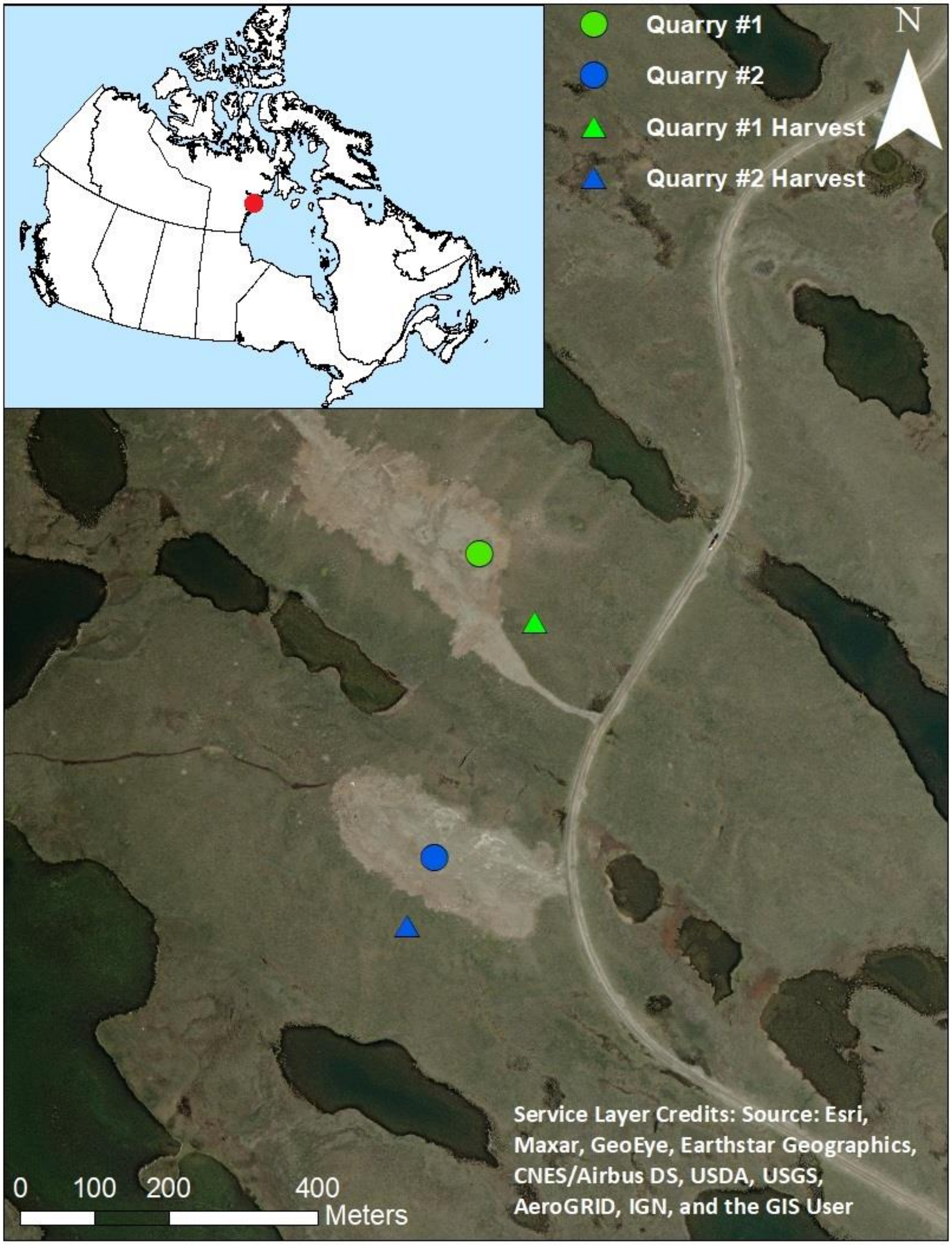

Figure 38*. Location of the two restoration trials established in 2019, and associated harvesting/reference systems. Study sites are located near the coast of Hudson Bay, approximately 25 km northwest of Rankin Inlet, Nunavut, Canada (represented by the red circle on the inset map).*

Table 6. 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

Table 7 Depth values of organic and mineral layers across all turfs used at each restoration trial. Values are means with standard deviation (n= 20 turfs per site).

Restoration Site	Organic Depth (cm)	Mineral Depth cm)	Clay	Silt	Sand	Gravel
Quarry #1	7.0 ± 4.2	3.08 ± 3.52	0%	9%	64%	27%
Quarry #2	9.1 ± 3.2	1.46 ± 2.10	0%	8%	45%	47%

Site Preparation

The restoration trials 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 39). 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.

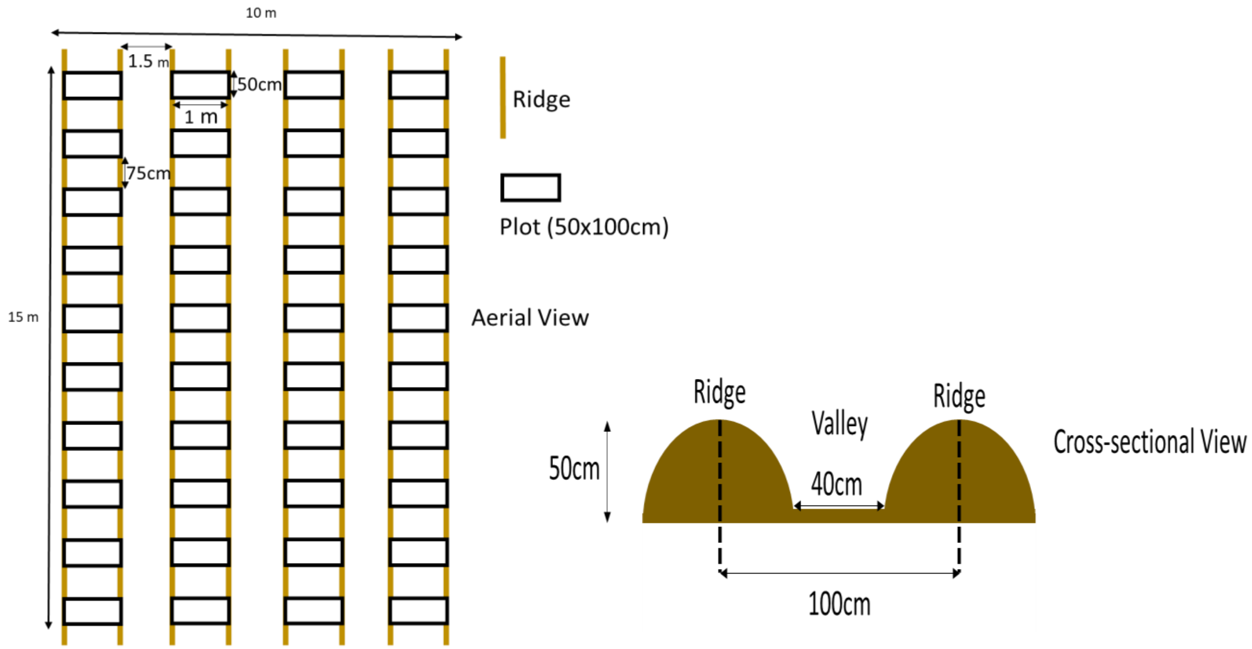

Figure 39. Four rows of hummock-hollow microtopography at each site (left) and a cross-sectional view of the hummock-hollow complex.

Four treatments were applied within the treatment plots: Turfs, Shredded, Turfs and Shredded and Control (Table 8). Treatments were grouped based on a stratified random block design. Quarry #1 was 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 40).

Table 8. Description, dimensions (length, width, depth) and diagram of the four treatments applied within the hollow-hummock plot.

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

S5	B1	P5	C10	B10	S10
PS5		C5	P10		PS10
C4	B2	S4	PS9	B9	C9
P4		PS4	S9		P9
C3	B3	PS3	P8	B8	PS8
S3		P3	S8		C8
C2	B4	PS2	P7	B7	S7
S2		P2	C7		PS7
PS1	B5	S1	C6	B6	PS6
P1		C1	P6		S6

S1	C1	P1	PS1	B1
S2	P2	PS2	C2	B2
C3	PS3	S3	P3	B3
PS4	S4	C4	P4	B4
S5	PS5	C5	P5	B5
PS6	S6	P6	C6	B6
PS7	P7	S7	C7	B7
PS8	C8	P8	S8	B8
C9	PS9	P9	S9	B9
P10	PS10	C10	S10	B10

Figure 40: Stratified random block design at 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 turfs and shredded material was conducted in upland heath communities located near each restoration trial (i.e., 119 m from Q1 and 60 m from Q2). A flat-head shovel was used to cut, lift, and remove the turfs from the harvesting sites (Figure 41). The underlying material was predominantly composed of organic matter; however, an effort was taken to ensure that harvested material also contained underlying mineral layers. Harvesting plots were flagged, spatially referenced, and photographed. Turfs were carefully removed and the depth of organic and mineral soil was measured on each side of the turf. Turfs 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 41B). All measurements were taken at the center of each side.

Turfs (n = 20 per site) were carefully hand-lifted into place, with any fallen material placed in the bottom of the individual hollow (Figure 41C). All turfs were placed with the vegetative surface being level with the surrounding substrate surface. Once placed, the surrounding substrate was pushed against the turfs to ensure good turf-substrate contact (Figure 41D). Three additional turfs were harvested for shredding, with all measurements described above obtained before shredding occurred. Turfs including all soils and vegetative materials were physically separated and sieved through a 4 cm^2 metal mesh screen (Figure 41E). All shredded material was homogenized. Shredded material was measured and dispensed along both internal sides of the hummock surrounding the turfs in the Turf + Shredded treatments (Figure 41F), whereas Shredded treatments had shredded material covering the entire treatment plot. A brief video showing the steps used to create the restoration trial is available at: https://www.tundrarestoration.com/background

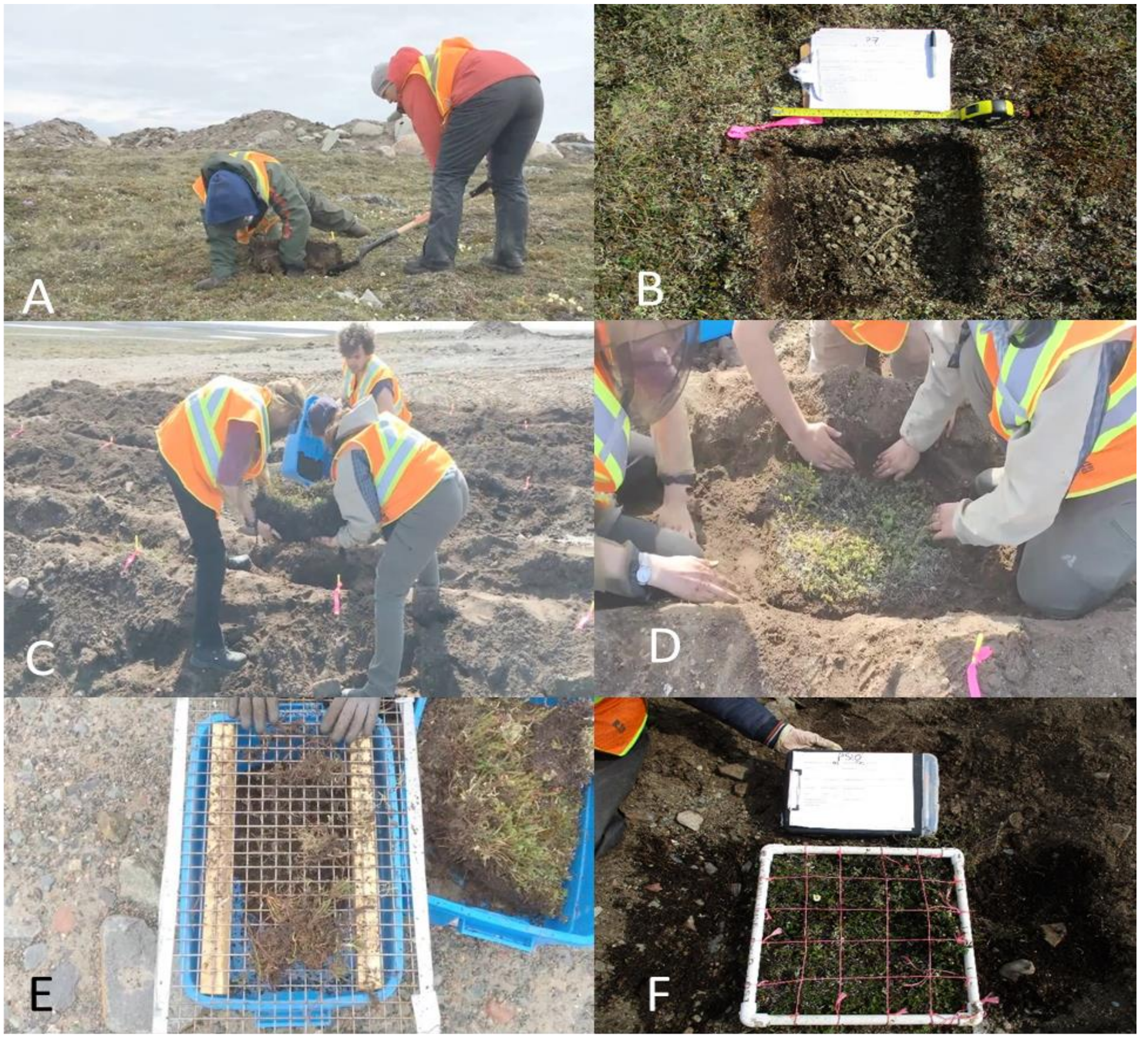

Figure 41. Harvesting and transplanting of turfs and shredded materials. Removing the turfs manually with a flat-head shovel used to cut the dimensions of the turf (A). Harvesting plot after the turf has been removed (B). Harvesting plots will be re-visited for analysis of vegetative encroachment into the disturbed area. Further physical manipulations of the hummock-hollows, to allow the turf surface to sit even with the bottom of the hollow (C). Hand-lifting the turf into position, with fallen material being placed in the bottom of the hollow before placement (D). The 4 cm^2 grid used for shredding vegetative material (E). A Turf + Shredded treatment ready for percent-cover surveying (F). Note the shredded material is only applied to the areas next to the physical turf, not overtop.

Substrate and Vegetation

A 0.16 m^2 gridded quadrat (25 grids, 0.0064 m^2 each) was placed over the top of each Turf, Turf + 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 42). All vascular and non-vascular plants within harvested turfs 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.

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.

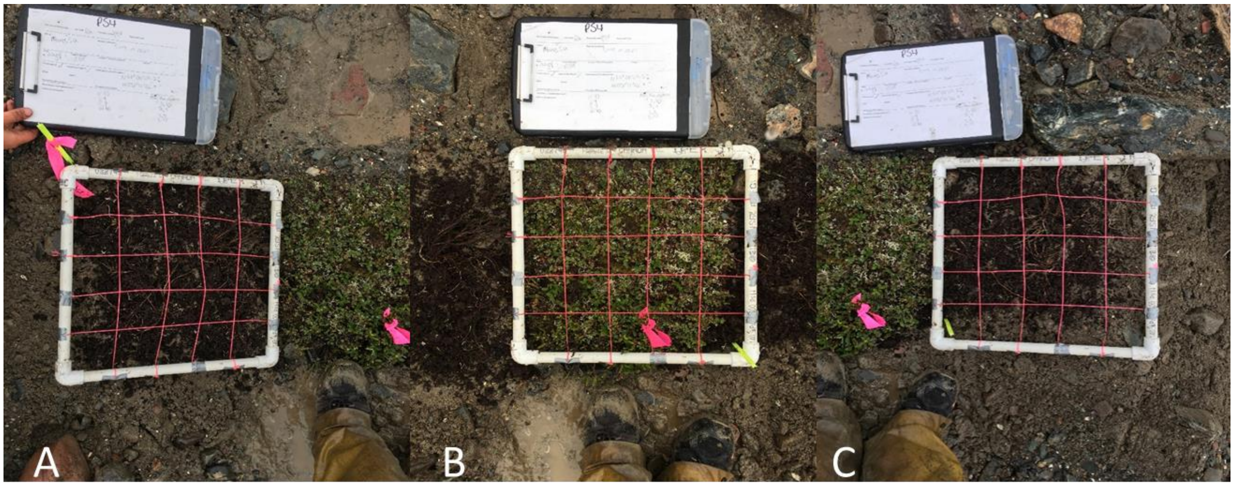

Figure 42. Survey of trufs+ shredded layers treatment plot. Shredded layers on either side of the plot were surveyed for percent cover (A and C), as well as the turf in the center of each plot (B).

Soil samples were collected using shovels immediately beside (i.e., within 1 m) ten randomly chosen turf harvesting locations at each restoration site. Both organic and mineral horizons were sampled, except for turf #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. Further soil samples were collected in 2021, with ten organic and mineral layer samples taken from the same harvest locations as 2019, and organic and mineral composite pencil-core samples from all treatments that included turfs. All samples were transported to the University of Saskatchewan, with a small subsample (~50 ml) stored at -80°C for future molecular work, if necessary. The remaining soil was dried, sieved (4 mm^2), and stored at -20^0C for future analysis. Water extractions were conducted on all soil samples using a 1:4 ratio of soil to Milli-Q water, except for 8 (2019) and 23 (2021) 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^{2+}, Mg^{2+}, K^+, Na^+, NH_4^+) and anions (F^-, NO_3^-, NO_2^-, SO_4^-, Cl^-, PO_4^{3-}). pH was measured using a Mettler Toledo FiveEasy pH meter. Subsamples of each soil will be ground in preparation for total carbon (TC), total organic carbon (TOC), total inorganic carbon (TIC) and total nitrogen (TN). Total nitrogen was analyzed using a LECO TruMac CNS Analyzer, total inorganic carbon was analyzed by an acetic acid pH standard curve (20.2, CSSS, 2008), total carbon was analyzed with a modified high temperature combustion method (21.2, CSSS, 2008), and total organic carbon was calculated by the difference between total carbon and total inorganic carbon. Total carbon and total inorganic carbon samples were analyzed by ALS Environmental (Saskatoon, Saskatchewan, Canada).

Results

Community composition between all treatments

Two years following turf transplantation, PERMANOVA indicated that all four treatments had significant differences in community composition (df = 79, $R^2 = 0.43$, $p = 0.001$) (Figure 43). Post-hoc comparisons revealed that the community compositions of all transplanted treatments were significantly different from the control ($p < 0.001$), with shredded treatments also significantly different from turf and turf + shredded treatments ($p < 0.01$), and turf and turf + shredded treatments statistically similar ($p = 0.99$). Turf and turf + shredded treatments contained the highest species richness, greatest vegetative cover, and greatest flower density. Shredded treatments contained ~1/2 of the species richness, ~1/4 of the vegetative cover, and 1/10 the flowering density of turfs and turf + shredded treatments. All growth forms, except for biological soil crust and lichen cover, were significantly higher in turf and turf + shredded treatments compared to shredded or control treatments.

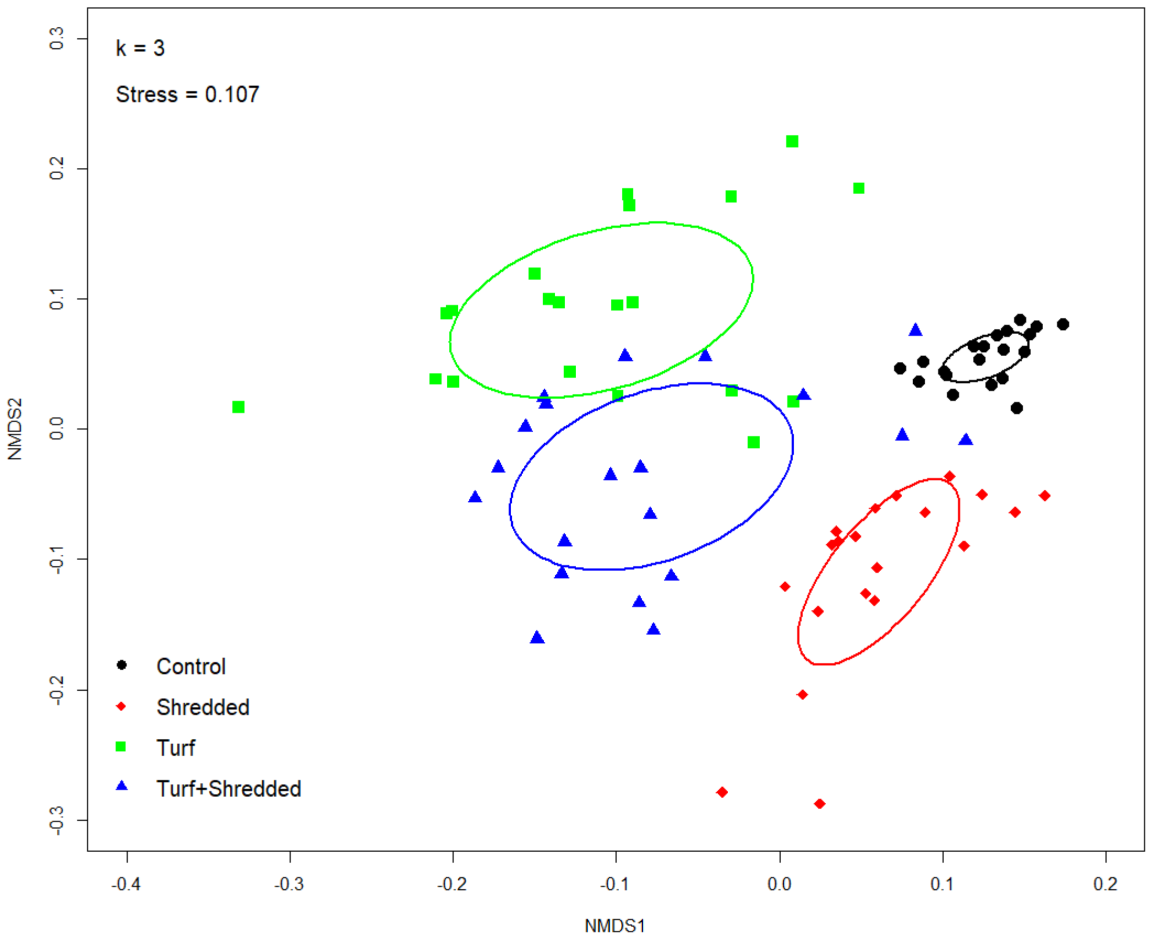

Figure 43. Non-metric dimensional scaling (NMDS) ordination plots of treatment community composition two years after turf transplantation. Treatments are represented by black circles (Control), green squares (Turfs), blue triangles (Turfs + Shredded), and red diamonds (Shredded). Ellipses represent the standard error of the weighted average of scores.

Community composition of shredded materials

Linear mixed-effects models identified that all shredded layers (i.e., shredded treatments, and shredded material from turf + shredded treatments) decreased significantly in species richness ($F_{1, 39}$ = 9.91, $p < 0.01$) two years following transplantation. Cover of living vegetation ($F_{1, 39}$ = 72.68, $p < 0.001$), bryophytes ($F_{1, 39}$ = 57.58, $p < 0.001$), and lichens ($F_{1, 3}$ = 70.92, $p < 0.001$), decreased by at least 50%, while evergreen ($F_{1, 3}$ = 167.69, $p < 0.001$) and deciduous shrub cover decreased by at least 80% ($F_{1, 39}$ = 86.29, $p < 0.001$). Both total litter ($F_{1, 39}$ = 226.96, $p < 0.001$) and bare ground ($F_{1, 39}$ = 167.1, $p < 0.001$) increased in cover over the two years, and the only growth form to increase were forbs ($F_{1, 39}$ = 64.60, $p < 0.001$), which demonstrated a 18-fold increase in cover, likely due to the colonization of volunteer species (i.e., *Descurainia sophioides* (Northern Tansy Mustard), *Stellaria longipes* (Long-stalked Starwort), and several unidentified juvenile individuals).

Community composition of turfs

Using linear mixed-effects models, we determined that turfs (i.e., turf treatments and central plot of turf + shredded treatments) maintained their species richness over two years, although five species that were recorded in 2019 were not recorded in 2021: one deciduous shrub and four forb species. Although overall richness remained unchanged, plant community composition within the turfs changed significantly between 2019 and 2021 (PERMANOVVA: df = 79, $R^2 = 0.16$, $p = 0.001$) (Figure 44). Significant differences were the result of increased cover of abiotic features (i.e., bare ground and rock/stones) ($F_{1, 39} = 27.87$, $p < 0.001$), litter ($F_{1, 39} = 248.6$, $p < 0.001$), and forbs ($F_{1,39} = 14.90$, $p < 0.001$) along with decreases in evergreen shrub ($F_{1,39} = 31.04$, $p < 0.001$), lichen ($F_{1, 3} = 32.96$, $p < 0.001$) moss cover ($F_{1,39} = 7.04$, $p < 0.05$). Turfs between the two sites were similar in composition except that Q1 had higher lichen cover in both 2019 and 2021 ($F_{1.38} = 11.24$, $p < 0.01$), and Q2 had higher cover of deciduous shrubs in 2019 and 2021 ($F_{1,38} = 22.78$, $p < 0.001$). Moss cover was higher at Q2 in 2019 and 2021, but only significantly higher in 2021 ($F_{1,38} = 11.13$, $p < 0.01$).

Evergreen shrubs had the largest decrease in absolute cover (-31%) over the two years compared with other vegetative components (Table 9). Decreases in evergreen shrubs were driven by *Cassiope tetragona* followed by *Rhododendron lapponicum* and *Rhododendron tomatosum*; however, *Empetrum nigrum, Dryas integrefolia* and *Vaccinium vitis-idaea* cover also declined. Lichens had the second largest decrease in absolute cover (-30%) over the two years, which was in part was due to declining cover of *Thamnolia vermicularis, Alectoria ochroleuca, Cetraria* spp.*, Cladonia* sp.*,* and *Dactylina arctica* (Table 9). Overall, mosses declined in absolute cover (-19%); however, while some species decreased (ex. *Hylocomium splendens*, and *Pohlia nutans*), others increased (ex. *Aulacomnium turgidum* and *Bucklandia macrocarpa*) over the two years. Forbs and grasses increased over the two years due to increases in *Astragalus eucosmus, Bistorta vivipara* and *Oxytropis maydelliana, and Carex bigelowii and Carex rupestris*, respectively (Table 9).

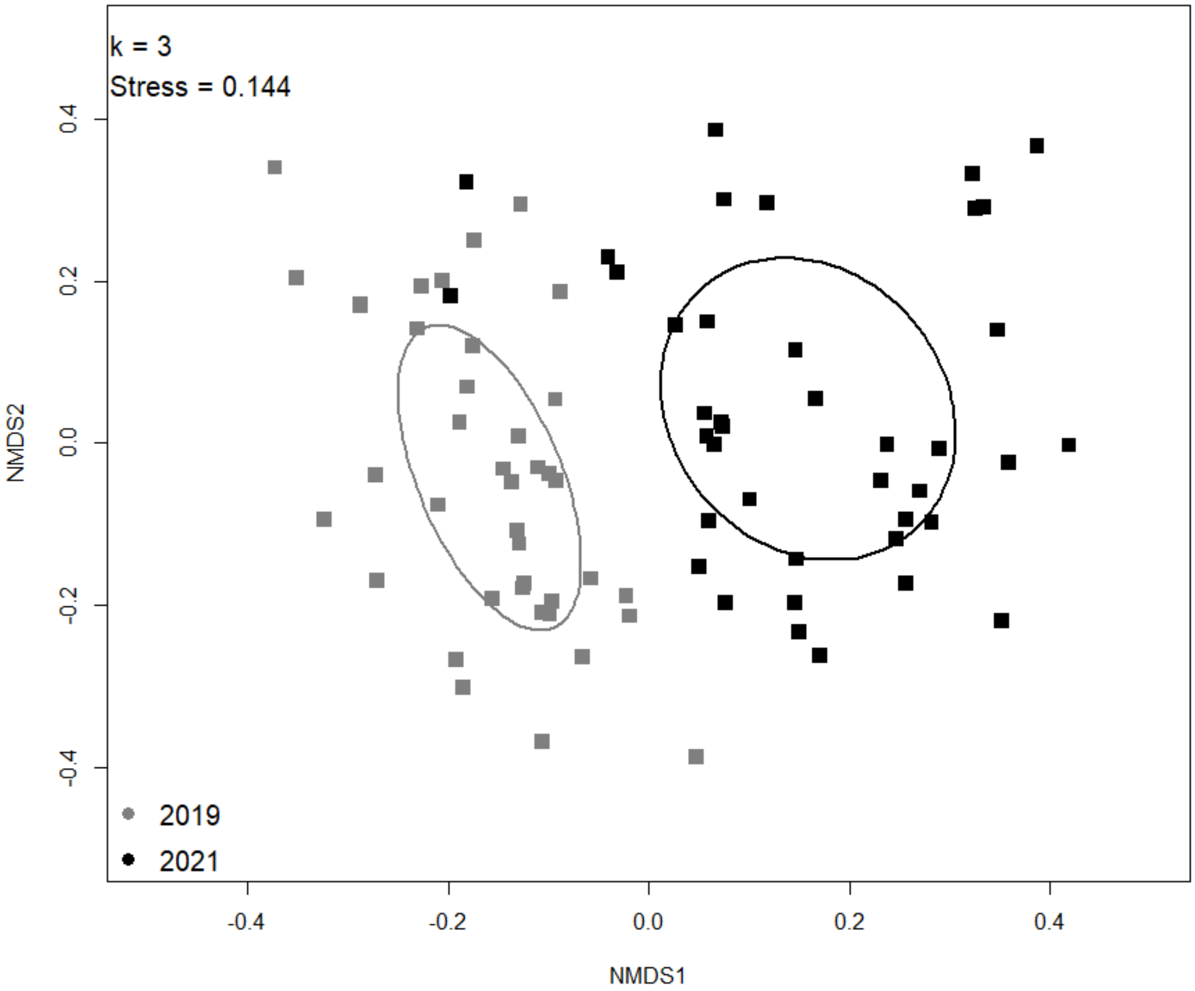

Figure 44. Non-metric Dimensional Scaling (NMDS) ordination plot of community composition of turfs over two years. Squares represent turfs from turf treatments and the central turf from turf + shredded treatments. Years are represented by grey (2019) or black (2021) colors. Ellipses represent the standard error of the weighted average of scores.

Table 9. Percent change of growth forms and species that represented at least 0.5% relative cover across both sites, either in 2019 or 2021, within the center plot of turfs and turf + shredded treatments. Percent difference of absolute cover is presented first, followed by relative cover in parenthesis. Absolute and relative cover are calculated as follows: absolute percent differences are ((species cover in 2021- species cover in 2019)/ species cover in 2019), and relative percent differences are ((species cover in 2021/total species cover in 2021) – (species cover in 2019/total species cover in 2019)). Asterisks denote significant differences between 2019 and 2021.

Growth Form	**Percent Difference Absolute (Relative)**	**Species**	**Percent difference Absolute (Relative)**
Abiotic Features*	+83% (+4%)	Bare Soil	+86% (+3%)
		Rocks/Stones	+110% (+1%)
Litter*	+321% (+13%)	Loose Organic Matter	+58% (+0.2%)
		Plant Litter	+75% (+2%)
		Woody Litter	+10464% (+11%)
Deciduous Shrubs	-1% (-2%)	*Arctous rubra*	-13% (-1%)
		Salix arctica	-16% (-0.4%)
		Salix reticulata	+25% (0.2%)
		Vaccinium uligonosum	-1% (-1%)
Evergreen Shrubs*	-31% (-8%)	*Cassiope tetragona*	-54% (-4 %)
		Dryas integrefolia	-0.4% (-1%)
		Empitrum nigrum	-12% (-0.3%)
		Rhododendron lapponicum	-61% (-2%)
		Rhododendron tomatosum	-43% (-1%)
		Vaccinium vitis-idaea	-8% (-0.3%)
Forbs*	+83% (+2%)	*Astragalus eucosmus*	+58 % (+0.2%)
		Bistorta vivipara	+208% (+1%)
		Cardamine digitata	+455% (+1%)
		Oxytropis arctica	-71% (-1%)
		Oxytropis maydelliana	+263% (+0.5%)
Graminoids*	+63% (+2%)	*Carex bigelowii*	+133% (+1%)
		Carex rupestris	+18% (0.03%)
Lichens*	-30% (-12%)	*Alectoria ochroleuca*	-33% (-3%)
		Cetraria sp.	-38% (-4%)
		Cetraria ericetorum	-34% (-0.2%)
		Cladonia sp.	-75% (-0.5%)
		Dactylina arctica	-26% (-0.4%)
		Gowardia nigricans	-9% (-1%)
		Thamnolia vermicularis	-43% (-3%)
		Unidentified crustose lichen	-16% (-0.2%)
Mosses*	-19% (-2%)	*Aulacomnium turgidum*	+23% (+0.1%)
		Bucklandia macrocarpa	+35% (+0.1%)
		Hylocomium splendens	-66% (-1%)
		Pohlia nutans	-22% (-1%)
		Polytrichum pallidisetum	-91% (-0.5%)
		Rhytidium rugosom	-31% (-0.4%)
		Sanionia uncinate	-10% (-0.1%)

Vegetative Expansion

Turfs and turf + shredded treatments had significant expansion of vascular plants ($F_{3,209}$ = 20.79, p < 0.001) immediately adjacent (i.e., within 0-8 cm) to the turf, whereas little expansion was observed for shredded or control treatments. Similarly, turfs and turf + shredded treatments had significantly higher non-vascular cover than control treatments ($F_{3,209}$ = 10.60, p < 0.001) (Figure 45). The expansion was largely limited to the first eight centimeters away from the turf, with both vascular and non-vascular cover significantly decreasing after this first distance increment (F = 32.26, p < 0.001; F = 8.41, p < 0.001). Vascular and non-vascular species expansion represented ~17% and 6% of total cover within the first 8 cm, respectively, quickly dropping to 4% and 3% after 8 cm. The remaining cover was dominated by abiotic features (bare ground, rock, stones) and litter (Figure 46).

Within eight centimeters from the turfs, the relative contribution of deciduous and evergreen shrubs, forbs and graminoids was nearly equal, with each growth form contributing 27%, 27%, 26%, and 20% of vascular cover. After eight centimeters, forbs and graminoids quickly dominate the expanding vascular cover, demonstrating 46% and 40% relative cover, respectively. Deciduous shrubs represented 11% of the vascular cover after eight centimeters, with evergreen shrubs only representing 3%. A similar pattern was observed with non-vascular cover, with lichens dominating the cover within the first eight centimeters, representing 63% of non-vascular cover, and mosses representing 36%. After eight centimeters, the relative contribution to non-vascular cover switches between the two growth forms, with mosses representing 62%, and lichens 36%.

V. uligonosum (Bog Blueberry), and *E. nigrum* (Crowberry), made the highest contributions (18% and 10%) of the relative vascular cover within 8 cm from the turfs, with all other species representing less than 7% (Figure 46). After 8 cm from the turfs, *S. longipes*, juvenile unidentified forbs, *C. bigelowii* (Bigelow's Sedge), and *P. arctica* (Arctic Bluegrass) contributed the most to the cover of vascular species. Lichen species dominated the relative cover of non-vascular species immediately adjacent to the turfs, with *A. ochroleuca* (Green Witch's Hair Lichen), *C. nivalis* (Crinkled Snow Lichen), *G. nigricans* (Grey Witch's Hair Lichen), *T. vermicularis* (Alpine Whiteworm Lichen) and unidentified crustose lichen growth representing 13%, 20%, 10%, 13%, and 11% of non-vascular cover, respectively (Figure 46). Moss colonization appeared to be in a juvenile stage, with identified mosses representing less than 15% of the non-vascular cover combined, and another 15% represented by unidentifiable mosses. After 8 cm from the turfs, unidentifiable mosses comprised nearly half of all non-vascular cover, although *T. vermicularis* and the crustose lichen growths still contributed over 10% each.

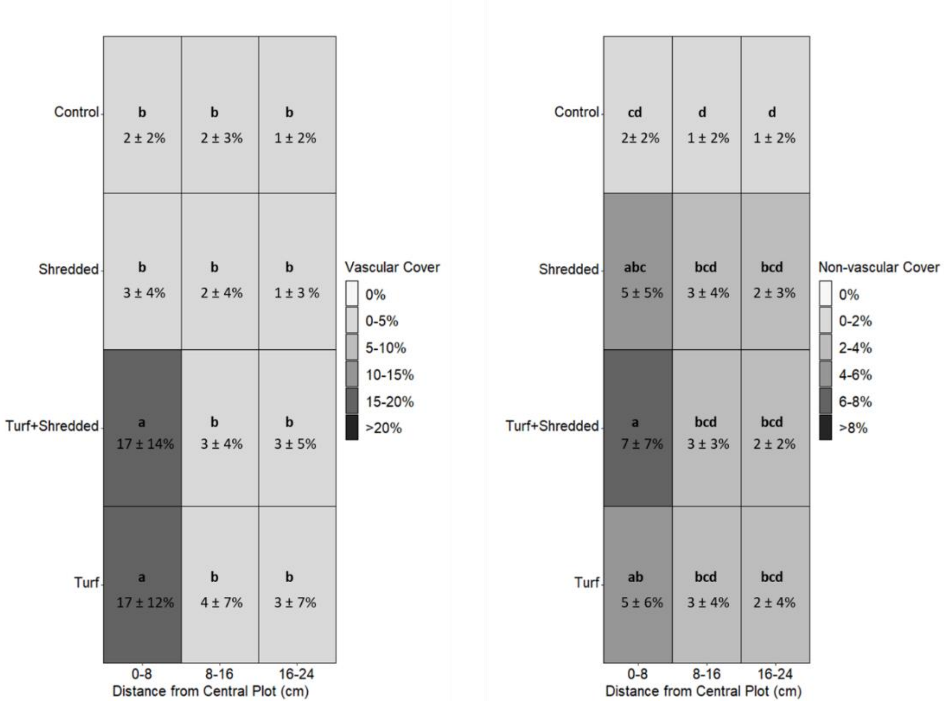

Figure 45. Heatmaps of mean vascular and non-vascular cover expanding from the central plot of each treatment, grouped by distance from the central plot. Significance indicators within boxes represent significant difference between treatments and between distances, with standard deviations presented underneath.

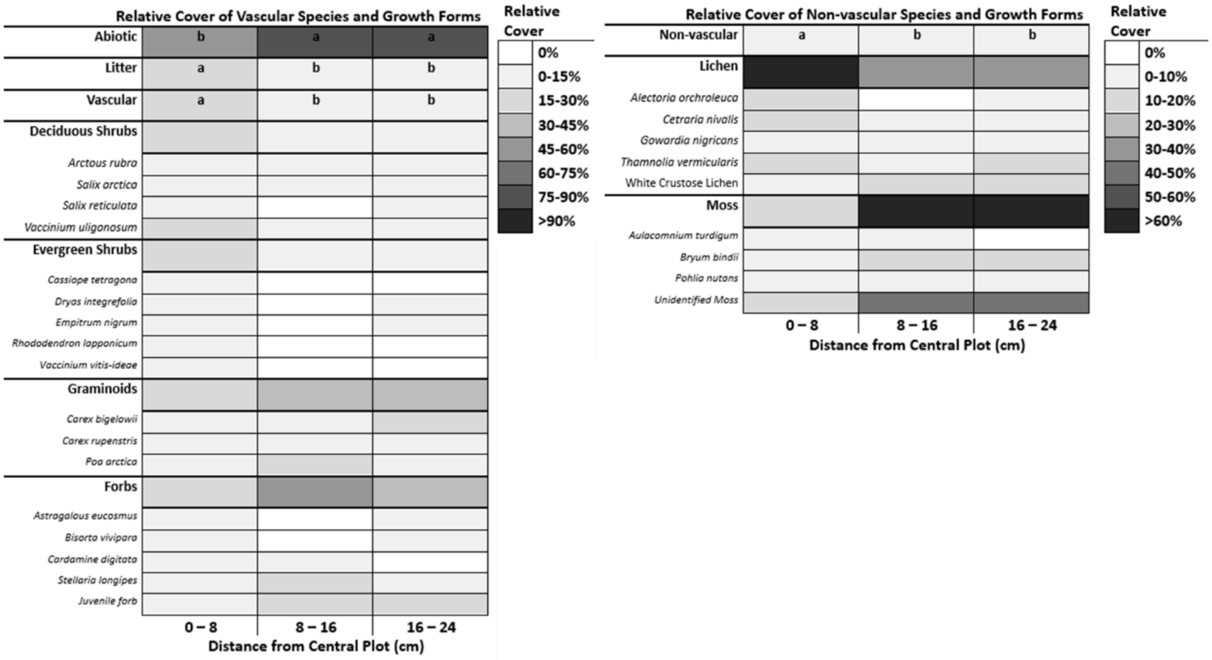

Figure 46. Heatmaps of all vascular and non-vascular species encountered expanding from the turfs, that were observed in at least 10% of the turf and turf + shredded treatment plots at either Quarry #1 or #2. Darker shades represent greater relative contribution to the expanding communities. Abiotic, litter, vascular and non-vascular cover represent cover relative to all species, while deciduous shrubs, evergreen shrubs, graminoids, and forb growth forms represent cover relative to vascular cover, and lichen and moss growth forms represent cover relative to non-vascular cover.

Harvest Site Recovery

After two years, vegetative establishment within harvest plots was minimal. Non-vascular cover from lichens and mosses that had blown into harvest plots accounted for 20% and 8% of the relative cover respectively (Table 10). Vascular plant growth primarily from sidewalls of the harvest plot (i.e., new growth from cut and exposed belowground structures) was composed of evergreen shrubs, deciduous shrubs, forbs, and graminoids at 10%, 8%, 6% and 4% relative cover, respectively. There was evidence of slumping from the sides of some harvest plots, with the average depth of harvest plots decreasing by roughly one centimeter over the two years since harvest (i.e., average of 10.29 cm to 9.13 cm).

Table 10. Mean relative cover and standard deviation of abiotic features, litter, and functional vegetative groups across all harvest plots (n = 46) in 2021. Relative cover was calculated as: cover of functional group/total cover of all functional groups.

Cover	Relative cover and standard deviation
Deciduous Shrub	8% (4.1%)
Evergreen Shrub	10% (4.7%)
Forb	6% (3.9%)
Graminoid	4% (3.3%)
BSC	0.1% (0.9%)
Lichen	20% (7.9%)
Moss	8% (7.3%)

Soil Nutrients

Two years following transplantation, the turfs within turf and turf + shredded center plots demonstrated significant differences in nutrient concentrations from their respective harvesting sites, in both organic and mineral layers. Within organic layers, both sites demonstrated significant increases, approximately 15-fold (Q1) and 3-fold (Q2), in concentrations of Cl^- (ANOVA: $F = 17.80$, $p < 0.001$; $F = 11.21$, $p < 0.001$) and approximately 19-fold (Q1) and 5-fold (Q2) in concentrations of Na^+ (ANOVA: $F = 16.59$, $p < 0.001$; $F = 24.96$, $p < 0.001$). Q1 demonstrated significantly decreased organic nitrogen (ANOVA: $F = 6.57$, $p < 0.01$), whereas Q2 demonstrated 9-fold increases in SO_4^{2+} (ANOVA: $F = 82.46$, $p < 0.001$) and roughly twice the concentration of Ca^{2+} (ANOVA: $F = 10.36$, $p < 0.001$), with significant decreases in NO_3^- (ANOVA: $F = 22.19$, $p < 0.001$) (Table 11).

Differences between transplanted turf and harvest sites within mineral soils were far more frequent than organic layers, with both sites demonstrating significant increases in pH (ANOVA: $F = 48.75$, $p < 0.001$; $F = 114.09$, $p < 0.001$), Cl^- (ANOVA: $F = 74.52$, $p < 0.001$; $F = 20.29$, $p < 0.001$), SO_4^{2-} (ANOVA: $F = 40.32$, $p < 0.001$; $F = 40.32$, $p < 0.001$), Na^+ (ANOVA: $F = 40.77$, $p <0.001$; $F = 73.31$, $p < 0.001$), Ca^{2+} (ANOVA: $F = 22.95$, $p < 0.001$; $F = 9.86$, $p < 0.001$), and inorganic carbon (ANOVA: $F = 23.92$, $p < 0.001$; $F = 23.47$, $p < 0.001$) (Table 11). Mineral soils of turfs at Q1 also increased in NO_3^- (ANOVA: $F = 31.64$, $p < 0.001$), K^+ (ANOVA: $F = 13.62$, $p < 0.001$), Mg^{2+} (ANOVA: $F = 17.93$, $p < 0.001$) and inorganic nitrogen ($F = 21.49$, $p < 0.001$) compared with harvest sites. Substrates used to create the hummock-hollow complexes had higher pH and concentrations of Cl^-, SO_4^{2-}, NO_3^-, Mg^{2+}, Ca^{2+} and Na^+ compared with harvest site mineral soils (Table 11). Mineral soils at both sites demonstrated 2-fold and 10-fold increases in Ca^{2+}, and SO_4^{2+}, respectively. Q1 mineral soils demonstrated 24-fold and 18-fold increases in Cl^- and Na^+, respectively.

Table 11. Means, standard deviations, and significance indicators for pH, and concentrations of chloride, sulfate, nitrate, sodium, ammonium, potassium, magnesium, calcium, total organic nitrogen, total inorganic nitrogen, total organic carbon, and total inorganic carbon within the turf harvesting locations (H) (in 2019 and 2021), turfs within turf and turf + shredded center plots (T), and substrates used at each site, separated by soil layer (Organic, Mineral) and site (Q1 =Quarry 1, Q2 = Quarry 2). TON, TOC, and TIC are presented as g/kg with all other nutrients in mg/kg. Significance indicators are presented as differences between harvesting locations and turfs, within each soil layer, and within each site. Unless stated within parentheses, sample numbers were n=10 for all harvesting sites (except for mineral layer Q2 n=9 in 2019), and n=20 for all turfs in 2021. Total organic and inorganic carbon each contained n=5 samples for each harvest site and turf. Q1 substrate is one composite sample and Q2 substrate is two composite samples. Significant differences were tested with linear models and ANOVA and pairwise differences assessed using least-square means.

Soil Layer	Site	Treatment	pH	Cl^-	SO_4^{2-}	NO_3^-	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	TON	TIN	TOC	TIC
Organic	Q1	H-19	5.8 ± 0.4 ^{ab}	35 ± 22.^{b}	86 ± 93^{a}	2.5 ± 1.4 (9) ^{ab}	23 ± 12 ^{b}	4.2 ± 4.0 ^{a}	27 ± 13 ^{a}	15 ± 12^{a}	77± 73^{b}	11 ± 7.3 ^{a}	6.5 ± 3.9 ^{a}	171 ± 52 ^{a}	1.3 ± 0.67 ^{a}
		H-21	5.3 ± 1.0 ^{b}	52 ± 42 ^{b}	100 ± 114 ^{a}	0.29 ± 0.59 ^{b}	13 ± 11 ^{b}	9.2 ± 9.2 ^{a}	63 ± 43 ^{a}	16 ± 13 ^{a}	108 ± 85 ^{ab}	12 ± 7.2 ^{a}	9.5 ± 9.2 ^{a}	254 ± 112 ^{a}	1.8 ± 1.5 ^{a}
		T-21	6.3 ± 0.5 ^{a}	813 ± 1031 ^{a}	430 ± 478 ^{a}	15 ± 20 ^{a}	439 ± 670 ^{a}	6.7 ± 5.5 (16) ^{a}	52 ± 36 ^{a}	58 ± 91 ^{a}	291 ± 280 ^{a}	4.9 ± 2.6 ^{b}	24 ± 25 ^{a}	123 ± 65 ^{a}	1.2 ± 0.49 ^{a}
	Q2	H-19	6.2 ± 0.3 ^{b}	32 ± 15 ^{b}	53 ± 31 ^{b}	6.1 ± 2.8 (9) ^{a}	24 ± 7.4 ^{b}	4.9 ± 9.4 ^{b}	57 ± 42 ^{a}	24 ± 11 ^{a}	152 ± 65 ^{b}	15 ± 6.3 ^{a}	11 ± 11 ^{b}	250 ± 38 ^{a}	2.6 ± 0.55 ^{a}
		H-21	6.4 ± 0.3 ^{ab}	53 ± 40 ^{b}	45 ± 16 ^{b}	3.1 ± 4.1 ^{b}	35 ± 71 ^{b}	18 ± 11 (8) ^{a}	51 ± 18 ^{a}	12 ± 4.2 ^{b}	119 ± 32 ^{b}	17 ± 5.2 ^{a}	18 ± 11 ^{ab}	334 ± 73 ^{a}	3.0 ± 1.2 ^{a}
		T-21	6.5 ± 0.3 ^{a}	151 ± 215 ^{a}	492 ± 280 ^{a}	0.38 ± 1.2 ^{c}	152 ± 124 ^{a}	20 ± 8.7 ^{a}	69 ± 28 ^{a}	30 ± 15 ^{a}	245 ± 141 ^{a}	13 ± 3.5 ^{a}	21 ± 8.5 ^{a}	279 ± 70 ^{a}	2.8 ± 0.84 ^{a}
Mineral	Q1	H-19	6.5 ± 0.4 ^{b}	4.2 ± 1.6 ^{b}	11 ± 11 ^{b}	0.61 ± 0.30 (7) ^{b}	5.5 ± 1.7 ^{b}	0.27 ± 0.57 ^{b}	0.69 ± 0.94 ^{b}	3.7 ± 2.0 ^{b}	22 ± 12 ^{b}	0.70 ± 0.36 ^{a}	0.70 ± 0.63 ^{b}	11 ± 5.3 ^{a}	0 ± 0 ^{b}
		H-21	5.4 ± 0.6 ^{c}	6.5 ± 6.7 ^{b}	14 ± 13 ^{b}	0.41 ± 1.3 ^{b}	0.30 ± 0.81 ^{c}	1.4 ± 1.2 ^{a}	5.2 ± 7.6 ^{b}	2.5 ± 1.2 ^{b}	21 ± 13 ^{b}	1.6 ± 1.4 ^{a}	1.8 ± 2.2 ^{b}	16 ± 18 ^{a}	0.12 ± 0.27 ^{b}
		T-21	7.2 ± 0.4 ^{a}	156 ± 113 ^{a}	143 ± 93 ^{a}	8.8 ± 6.6 ^{a}	101 ± 83 ^{a}	0.31 ± 0.09 (3) ^{ab}	9.3 ± 4.6 ^{a}	13 ± 8.2 (19) ^{a}	82 ± 34 (19) ^{a}	0.8 ± 0.4 ^{a}	8.9 ± 6.6 ^{a}	14 ± 8.5 ^{a}	1.5 ± 0.61 ^{a}
		Substrate	7.0	10	20	17	10	0	1.6	5.1	41	0.95	17	17	0.90
	Q2	H-19	6.8 ± 0.2 ^{b}	2.9 ± 2.1 ^{c}	10 ± 4.8 ^{b}	1.3 ± 1.1 (5) ^{a}	8.1 ± 1.5 ^{b}	0.02 ± 0.05 ^{c}	0.25 ± 0.74 ^{b}	4.9 ± 1.7 ^{ab}	36 ± 11 ^{b}	0.83 ± 0.43 ^{ab}	0.73 ± 1.1 ^{b}	13 ± 7.4 ^{b}	0.10 ± 0.22 ^{b}
		H-21	6.2 ± 0.4 ^{c}	8.1 ± 6.5 ^{b}	11 ± 7.9 ^{b}	0.99 ± 1.7 ^{a}	1.1 ± 2.2 ^{c}	2.3 ± 2.0 (6) ^{a}	4.6 ± 6.9 ^{ab}	2.7 ± 1.4 ^{b}	30 ± 15 ^{b}	0.17 ± 0.15 ^{a}	2.4 ± 2.9 ^{ab}	44 ± 30 ^{a}	0.48 ± 0.48 ^{b}
		T-21	7.7 ± 0.2 ^{a}	26 ± 27 ^{a}	111 ± 125 ^{a}	3.3 ± 3.5 ^{a}	34 ± 25 ^{a}	0.72 ± 0.49 (17) ^{b}	9.9 ± 9.2 ^{a}	8.1 ± 4.4 ^{a}	89 ± 60 ^{a}	0.51 ± 0.32 ^{b}	3.9 ± 3.5 ^{a}	8.0 ± 3.1 ^{b}	1.8 ± 0.48 ^{a}
		Substrate	7.6 ± 0.4	19 ± 27	111 ± 149	4.8 ± 3.5	26 ± 33	0.80 ± 1.1	0 ± 0	9.0 ± 5.6	79 ± 60	0.23 ± 0.32	2.0 ± 2.8	4.7 ± 1.3	1.8 ± 1.6

Recommendations

Of the three different restoration strategies included in the field trial (turf, turf + shredded, and shredded), turf and turf + shredded treatments contained the highest species richness, greatest vegetative cover, and greatest flower density. Shredded layers did not establish over two years with the cover of living vegetation, bryophytes and lichens all decreasing. Turfs (i.e., turf treatments and central plot of turf + shredded treatments) maintained their species richness over two years although there were significant decreases in evergreen shrubs, lichens, and mosses, and increases in graminoids and forbs. Turfs and turfs + shredded vascular and non-vascular species expanded; however, this was primarily limited to 8 cm from the turf. Harvest sites showed little recovery over the two years with windblown lichens and mosses and in-growth from vascular plants constituting most of the cover. Nutrient concentrations increased in both the organic and mineral layers of turfs; however, increases appear to be driven by quarry substrate nutrient conditions, which were elevated in Cl-, SO42-, NO3-, Mg2+, Ca2+ and Na+ compared with harvest site soils. Based on these findings we recommend the following:

1. Spreading shredded materials was not an effective method likely due to displacement of materials by wind and water, as well as a limited propagule bank. Therefore, the use of intact tundra heath turfs is recommended over the placement of shredded tundra heath.
2. Overall survival of species within turfs was high and reproduction (i.e., flowering) was observed two years after transplanting, therefore tundra heath turfs 40 cm2 by 10-15 cm deep support native plant-soil communities that can act as resources for revegetation of disturbed substrates.
3. Transplanting intact turfs allows for the maintenance of species richness and deciduous shrubs and the growth of graminoids and forbs. However, evergreen shrubs, especially *Cassiope tetragona* and *Rhododendron* spp. had poor post-transplanting survival and should be avoided for future restoration efforts.
4. Graminoids, *Carex bigelowii* and *Carex rupestris* grew within the turfs, *C. bigelowii* and *Poa arctica* had the furthest expansion, indicating that graminoids should be targeted in selecting turf transplant materials.
5. Expansion from turfs by both vascular and non-vascular species was observed, with most expansion occurring within 8 cm of the turf. The forb *Stellaria longipes* along with early moss communities were found to expand the furthest and should be targeted in selecting turf transplant materials.
6. Organic and mineral soils of turfs did not show changes in nutrients to indicate strong shifts in carbon or nitrogen cycling within two years of transplanting. Therefore, key nutrient cycling processes may be maintained to support vegetative growth. However, elevated salts found in disturbed substrates, especially Cl-, SO42-, Ca2+ and Na+, increased in the soils of turfs suggesting that substrate conditions at the site of transplanting must be considered and may require amendment to support turf transplant success.
7. Longer-term monitoring of the vegetation within and expanding from transplanted turfs, as well as the underlying turf soils is needed to determine the long-term (i.e., 10 year) success of this restoration approach.

Section III: Plant-Soil Interactions of Early Colonizing Vascular Species

Identifying Key Early Colonizing Vascular Plants

Background

Initial surveys of the quarries and other disturbed areas at Meliadine in 2019 identified several nitrogen fixing legumes as the most common early vascular species colonizing mining-disturbed substrates. Four common legumes at the Meliadine site are *Oxytropis arctica*, *Oxytropis maydelliana*, *Astragalus alpinus,* and *Hedysarum alpinum*. Excavation of several *A. alpinu*s specimens, the most abundant of the four legumes found on the quarries, revealed nodulation (i.e., root nodules containing nitrogen fixing Rhizobia) (Figure 47), 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 these legumes may be an effective strategy to accelerate plant colonization and soil development. However, little is known about the ecology of Arctic legumes and other key nitrogen-fixing species; therefore, we initiated a series of studies to determine the potential role of these species in restoration.

Plant-soil interactions of native legumes will be examined using the Bio-PET facility at the Saskatchewan Center for Cyclotron Sciences. By dosing samples collected at the Meliadine sites with radioactive [11C]C-CO_2 and [13N]N-N_2 gas, we will be able to capture in real-time the movement of carbon and nitrogen through plant root systems. Characterization of the microbial community at radioactive hotspots detected using Positron Emission Tomography- Computerized Tomography (PET-CT) provides a means to identify bacterial, fungal, and archaeal associated with carbon and nitrogen fixation. Examining plant-soil interactions by identifying carbon and nitrogen fixation and input in early successional environments is being examined through three sub-projects: i) a preliminary 2019 field survey of the plant communities associated with *Astragalus alpinus*, *O. arctica* and *O. maydelliana*; ii) characterization of the native legume nitrogen fixing community at Meliadine in disturbed substrates and intact upland tundra heath; iii) confirmation and characterization of carbon and nitrogen fixation by early vascular nitrogen fixers at Meliadine. Sub-projects ii and iii are on-going with final results expected in late fall 2022.

Figure 47. Astragalus alpinus excavated on a gravel pit. Nodulation is visible on the lateral roots at center left.

Sub-Project I: Preliminary field survey of native legumes

Methods

A field survey to determine the relative abundance of native legumes and the plant communities associated with these legumes was conducted in 2019. Briefly, areas of tundra (at minimum 2m by 5m, but in some cases larger) were searched and the number of native legume individuals of each species recorded. A 50 x 50 cm gridded quadrat was centered over a randomly selected individual, and percent cover was recorded in each 10 cm^2 subquadrat. For each search area, a minimum of one quadrat was surveyed over each species encountered. *Hedysarum alpinum* was not included as a species of interest in this initial survey in 2019.

Results

Astragalus alpinus and *Oxytropis arctica* appear to have contrasting ecologies with *O. arctica* associated with higher vascular plant cover, whereas *A. alpinus* is associated with greater bare rock cover, lower species richness, vascular plant cover and moss cover (Figure 48). Initial colonizing plants such as *O. arctica* and *A. alpinus*, which were naturally found growing on disturbed areas at the Meliadine mine site, are likely the first sources of carbon that can stimulate microbial activity in the soil. The role of these legumes in deposition of carbohydrates is critical to the rhizosphere microbiome and may subsequently drive the establishment and succession of secondary colonizers in the tundra. These results were used to inform the sampling design of sub-project ii, where four legume species were sampled in both disturbed substrates and undisturbed tundra heath. By including several legume species under different habitat conditions, our aim is to identify potential candidates to be used in active restoration of disturbed substrates and better understand nitrogen fixation by these species as revegetation proceeds.

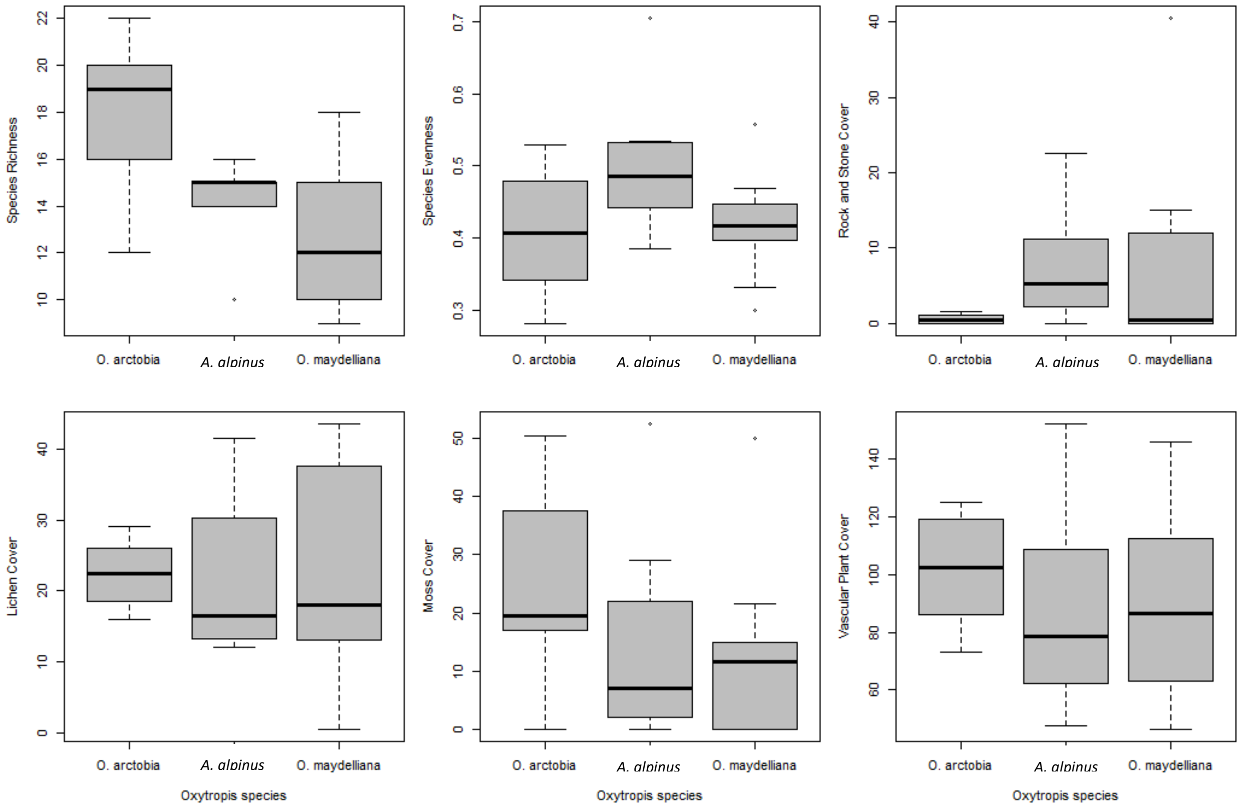

Figure 48. Environmental and plant community factors associated with Oxytropis arctica, Astragalus alpinus, and O. maydelliana in upland tundra heaths at Meliadine.

Sub-Project II: Characterization of the native legume nitrogen fixing community

Methods

In July 2021, we selected and sampled plant specimens and putative nodules from three locations along the 30 km road connecting Rankin Inlet to the Meliadine mine site, the All Weather Access Road (AWAR). Sites on Quarry 1 and Quarry 2 are located at ~27 km on the AWAR and the site on Quarry 3 is located at ~25 km. Because these locations have areas of recent disturbance paired with intact upland heath tundra, they were deemed suitable for the study. Gravel quarries parallel the mine waste, rock, and overburden dumps that will serve as a remnant of mine operations that may require restoration during mine closure.

We collected putative nodules from the field on July 17 and July 18, 2021. Plants were selected across the three quarries, with two collection sites at Quarry 1, two sites at Quarry 2, and one site at Quarry 3 for a total of 5 sites. Collection sites were paired with one site on the quarry and one site approximately 20 m away in upland tundra heath. Legume species were identified in the field and roots carefully dug out to locate putative nodules along the root. Individual putative nodules were pooled in the field from several individuals each of *O. arctica*, *O. maydelliana*, *A. alpinus*, and *H. alpinum*. *H. alpinum* was not always present in the quarries, so was only collected where possible. Following collection, the samples were kept at 4 °C prior to transport back to the University of Saskatchewan and stored at -20 °C upon arrival. Traditionally, studies categorizing rhizobial species first isolate the nodule occupants on agar media, which could result in preferential selection and identification of certain taxa most responsive to culturing (Johnson, 2019). In this project, we propose using a culture independent approach using direct next generation sequencing of 16S rRNA, *nif*H gene, and a conserved bacterial housekeeping gene (i.e., rpoB, atpD, glnII, or recA) to identify all nodule endophytes (Hakim et al., 2018, 2020; Mousavi et al., 2021; Weir et al., 2004).

In July 2021, plant samples of *Oxytropis arctica*, *O. maydelliana*, and *Astragalus alpinus* were collected at five sites: three sites at Quarry 1, one site at Quarry 2, and one site at Quarry 3. At each site, undisturbed tundra and recently disturbed areas were selected and two or more individuals per species were collected from each area. Each individual was carefully dug out of the ground and placed directly into pots approximately 25 cm tall, 11. 4 cm diameter (Figure 49). Samples were packed carefully into coolers with ice packs and stabilized with packing materials to minimize impact during transport to the University of Saskatchewan. At the university, samples were placed in growth chamber set to simulate 18.5 h of daylight with the light intensity set at approximately 400 µmol m^{-2} s^{-1} and a relative humidity at 65%. Plants were minimally watered three times a week for quarry samples and twice a week for tundra samples. The plants collected in July 2021 will be used in completing subproject iii (see below).

Figure 49. Digging up an Oxytropis *plant and placing into pot for transport to University of Saskatchewan.*

Results

To-date, DNA has been extracted from all nodule samples and preliminary steps for 16S and *nif*H sequencing completed. Final results are expected by fall 2022. Characterizing nodule endophytes between the different nitrogen-fixing plant species as well as differences between disturbed and undisturbed areas will inform both species selection for restoration, as well as provide insight into recovery of nitrogen fixation and nitrogen input in disturbed tundra environments. Restoration of this key nutrient cycling process is essential for recovery of functional native plant communities.

Sub-Project III: Confirmation and characterization of carbon and nitrogen fixation

Background

Most non-invasive PET-CT methods have been used for medical applications; however, PET-CT provides an imaging system that allows us to understand intact plant processes under natural conditions (Jahnke et al., 2009; Schmidt et al., 2020). Previous studies conducted using [11C]C-CO_2 with PET imaging have focused on crop plants, which have relatively large leaves compared to Arctic plants. These crop plants were dosed with [11C]C-CO_2 using gas exchange cells that enclosed a single leaf (Jahnke et al., 2009; Kawachi et al., 2011). The short stature of Arctic legumes makes enclosing a single fragile leaf a delicate procedure prone to error and necessitates the design of a specialized acrylic dosing chamber that can enclose an entire plant, allow for photosynthesis, yet exclude substrate. In addition, dosing chambers specialized to allow for dosing of soils (and nodules) while allowing plants to be under light conditions are also needed. To our knowledge, this work is the first carbon and nitrogen radio-isotope work to be completed on nitrogen-fixing Arctic species, which has required methods development and optimization to dose and image plants. 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.

Methods

To detect carbon and nitrogen fixation in plants and movement to belowground, 3 replicates from 3 sites of each species (*O. arctica, O. maydelliana, A. alpinus)* were selected for radio-isotope dosing (n = 9 plants per species). Before dosing, all plants will be moved to the growth chamber in the cyclotron to acclimate. After acclimation, each plant will first be dosed with $^{13}N_2$ then $^{11}CO_2$ the following day with no watering in between. The half-life of these two radioisotopes is short (i.e., ^{11}C half-life 20.4 min, ^{13}N half-life 9.96 min) allowing for sequential dosing.

Cyclotron bombardment of radio-isotope gases ($^{11}CO_2$ and $^{13}N_2$) is ~2GBq (10uA, 2min). Plants will be exposed to the radioactive gas for 30 min, then put into growth chamber for 30-120 min. Afterwards, plants will be placed in the SOFIE GNEXT Positron Emission Tomography –Computed Tomography (PET-CT) scanner using a 10 min acquisition time (Figure 50). Pots will be labelled with copper conductive tape on the outside to orient the pot and locate the nodules within the soil.

After dosing, plants will be extracted from the pot and nodules will be located (Figure 51). Nodules that actively had carbon allocated to them or were actively fixing nitrogen according to PET imaging will be compared to those that were not identified as active in the PET scan. The rhizosphere near these active nodules and near inactive nodules will also be sampled. From both the nodules and rhizosphere soils, we will extract RNA using 16S to compare the species of nodules and rhizosphere soils between the different plant species and between active and inactive nodules.

Figure 50. PET-CT imaging of legume species 2019 proof of concept pilot (left) and PET-CT imaging of Oxytropis maydelliana 2021 finalizing methodology.

Figure 51. Extracted Oxytropis maydelliana *after the PET/CT scan showing rooting structure.*

Preliminary results

Dosing chamber design has now been finalized for both radioisotopes. Initial optimization of the dosing and imaging time series has been conducted for *O. maydelliana*. Approximately 120-150 min after initial exposure is optimal for translocation of ^{11}C belowground (Figure 52). Imaging and sampling of legumes is expected to be completed within the next two months, which will be followed by image processing and molecular sequencing of nodules and soils sampled from radioactive hotspots.

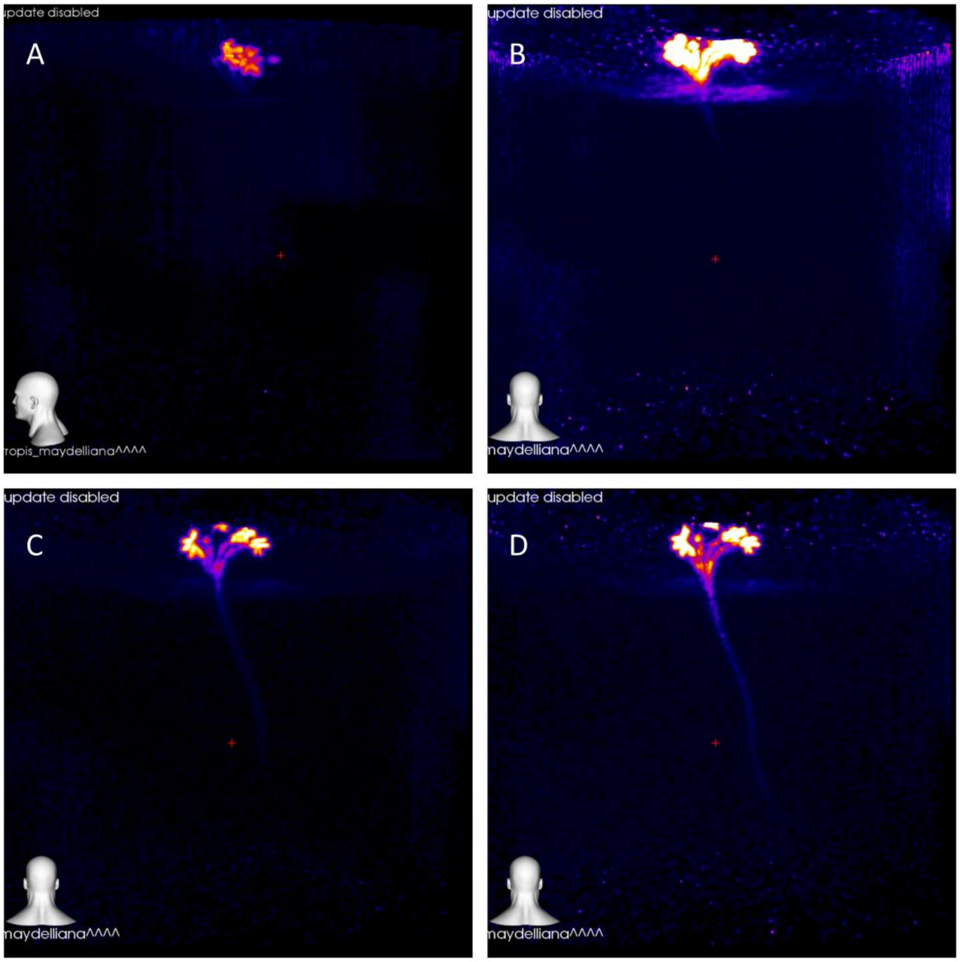

Figure 52. Assimilation and translocation of carbon by Oxytropis maydelliana *60 (A), 90 (B), 120 (C), and 150 (D) minutes post-exposure to the radioisotope* $^{11}CO_2$*.*

Soil Invertebrate Study

Background

Characterization of soil invertebrate communities in the upland tundra heath at the Meliadine site is needed to better understand the key drivers of invertebrate populations in undisturbed tundra. Soil invertebrates play a critical role in nutrient cycling within tundra soils and promoting healthy invertebrate communities is essential to long-term plant-soil establishment and growth. This exploratory study was intended to provide background and baseline data to support further examination of the impact of turf transplantation on soil invertebrate populations within turfs.

Methods

Field sampling

Samples for the invertebrate study were taken from four undistrubed tundra heath sites including the turf harvest sites near the emulsion plant (site A: 62.04261° N 92.25031° W), quarry 1 (site B: 63.00336° N 92.19289° W), quarry 2 (site C: 63.00019° N 92.19528° W) and the ex-situ turf trial (site D: 63.01229° N 92.11411° W). Within each tundra heath site, we randomly selected 10 turf harvesting location for soil and invertebrate sampling. Turf harvest locations were 40 cm^2 plots that represented the local vegetation and had an organic layer depth of at least 10 cm. At each plot, we measured vegetation cover. Specifically, a 0.16 m^2 gridded quadrat (25 grids, 0.0064 m^2 each) (Figure 41) was used for vegetation cover. 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. All vascular and nonvascular plants were identified to species level whenever possible. Soil samples for invertebrate sampling were also collected at each plot by taking 5 cm diameter cores with depths varying between 10 cm and 20 cm containing both organic and mineral soil layers. The plant species were grouped into different plant functional groups common to the tundra ecosystem.

Invertebrate extraction

From the soil cores that were collected at the upland tundra heath sites, mineral soil layers were air-dried for soil properties determination (Figure 53). Invertebrate sampling was done by the water flotation method. To increase the efficiency of invertebrate extraction, ludox(r) am colloidal silica, 30 wt. % suspension in H_2O was used. The invertebrates were counted and viewed under a stereomicroscope and where possible, were identified immediately (Figure 54). Most of the invertebrates could not be identified immediately and were stored in 70% ethanol for subsequent identification with identification keys. The abundance of the invertebrates as mites, collembolans, and enchytraeids collected from the study were determined.

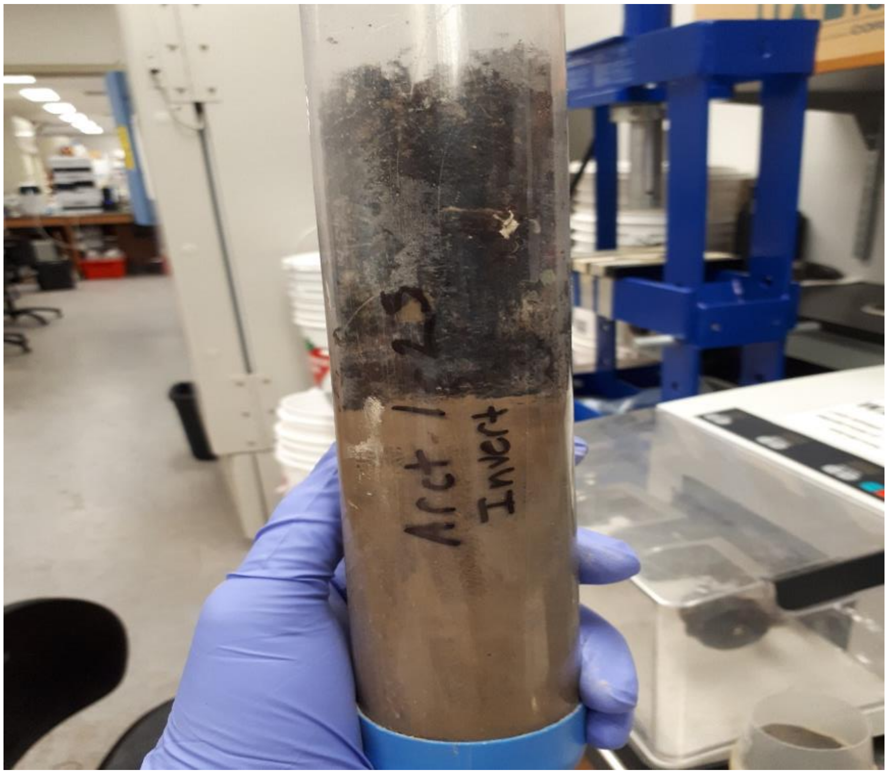

Figure 53. A typical soil core collected from the field from which soil invertebrates were extracted. Sub-samples were taken for soil chemical characterization.

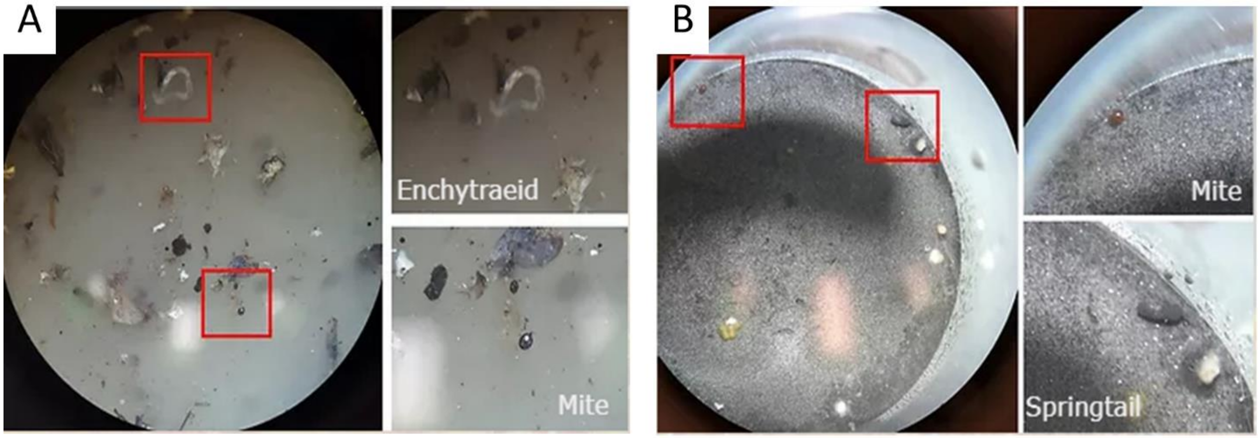

Figure 54. Images of invertebrates found in disturbed and undisturbed soils. Invertebrates (enchytraeid and mite) identified after extraction using the water flotation method (a) and extracted mite and collembolan species kept on plaster of paris medium (b).

Soil chemical analysis

To determine soil pH, 5 g of soil was placed into a 50 ml centrifuge tube and 25 ml of 0.01 M $CaCl_2$ added. The $CaCl_2$ and soil mixture was shaken for 30 minutes and allowed to stand for 2 hours before taking the pH reading on a Mettler Toledo Orion Dual Star pH meter.

Soil cation and anions were determined by placing 2.5 g of soil into a centrifuge tube with 25 mL milli-Q water and shaken for 3 hours at 15 rpm using a rotary shaker. The sample was then centrifuged at 5000 g for 10 minutes and subsequently filtered with a 0.45 µm filter. Afterwards, samples were split into half and analyzed for anions (Dionex IonPac AS18 column) and cations (Dionex IonPac CS12A column) by ion chromatography with a DionexICS-2000 using the Chromeleon7 software at the Department of Soil Science, University of Saskatchewan, Canada.

Results

Differences in vegetation cover, substrate factors, and soil nutrients among sites

The vegetation species surveyed in the study area varied according to plant functional groups (Table 12). Lichen had the highest vegetation cover (44.3 ± 4.8%) in site A, whereas graminoids had the lowest (3.3 ± 0.9%). The highest and lowest cover for site B was lichen (46.5 ± 2.7%) and forbs (2.0 ± 0.6%), respectively. In site C, lichens had the highest vegetation cover (34.5 ± 2.9%), and forbs were the lowest (4.4 ± 1.4%). For site D, lichen had the highest percent vegetation cover (34.3 ± 3.3%), and the forbs had the lowest (9.0 ± 2.3%). Lichen had the highest percent vegetation cover (39.9 ± 3.2%) when all sites are considered whereas forbs (4.8 ± 1.5%) had the lowest cover.

Table 12. Percentage vegetation cover of plant functional groups (e.g., evergreen shrubs, deciduous shrubs, forbs, graminoid, lichen and moss) with their representative plant species names found in four sites in Rankin Inlet.

Plant Functional Group	Percentage vegetation cover				Species	Abbreviation
	Site A	Site B	Site C	Site D		
Evergreen shrubs	16 ± 1.9	21.2 ± 2.4	15.8 ± 1.7	14.4 ± 1.8	*Cassiope tetragona*	CASSTET
					Rhododendron lapponicum	RHODLAP
					Rhododendron tomentosum	RHODTOM
					Arctostaphylos alpina	ARCALP
					Vaccinium myrtilloides	VACCMYR
Deciduous shrubs	18.5 ± 2.5	20.8 ± 2.3	29.1 ± 2.0	17.4 ± 2.9	*Salix* spp.	SALISP
Forbs	3.9 ± 1.8	2 ± 0.6	4.4 ± 1.4	9 ± 2.3	*Astragulus alpinus*	ASTRALP
Graminoids	3.3 ± 0.9	2.1 ± 0.9	5.4 ± 1.4	9.1 ± 1.4	*Carex* sp.	CARETF
Lichens	44.3 ± 4.8	46.5 ± 2.7	34.5 ± 2.9	34.3 ± 3.3	*Cetraria nivalis*	CERTSP
					Cladonia sp.	CUPLICH
					Alectoria nigricans	BLKHAR
Moss	14 ± 1.8	7.4 ± 1.8	10.9 ± 1.8	15.8 ± 2.3	Mat of Moss	MOSSMAT

Vegetation cover, substrate factors, and soil nutrients differed significantly among the four study sites (PERMANOVA: $p < 0.05$). Non-metric multidimensional scaling (NMDS) revealed that vegetation cover of the sites A, B, C were fairly similar but differed from site D (Figure 55A). NMDS also demonstrated that substrates were fairly distinct among sites (Figure 55B) and that soil nutrients of site D were different from that of sites A, B, and C, which were fairly similar in comparison (Figure 55C). Percent cover of the evergreen *Rhododendron lapponicum* explained the most variation in vegetation cover among sites (55%), followed by the lichen *Alectoria nigricans* (53%). Deciduous shrubs explained the least amount of variation in vegetation cover (3.4%) (Figure 56). Bare soil (61%) and plant litter (60%) explained more variation among sites than other substrate factors, whereas woody litter explained the least amount of variation (20%). For soil nutrients, magnesium, sodium, and chloride ions explained >70% variation among sites and nitrates explained the least (33%) (Figure 56).

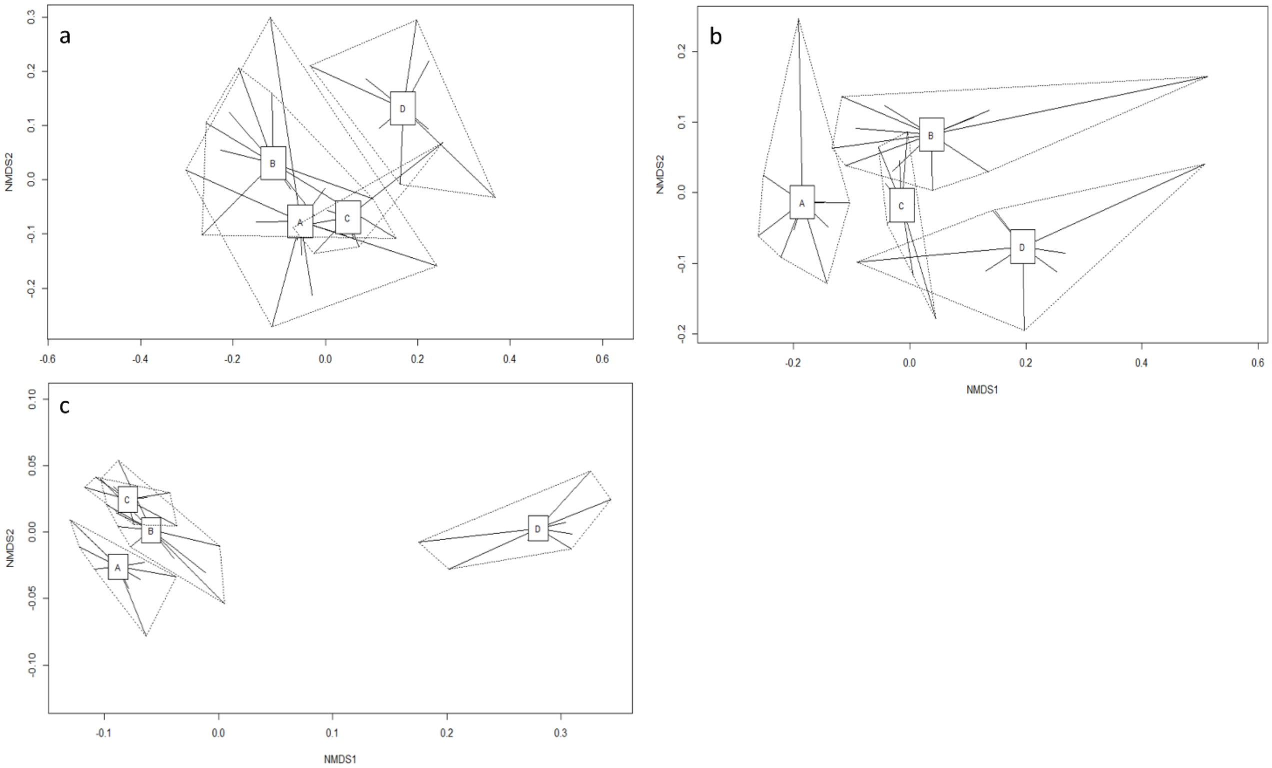

Figure 55. Non-metric multidimensional scaling (NMDS) ordination of sites (A,B,C,D) based on: vegetation cover (a), substrates (b), and soil nutrients (c) in Rankin Inlet revealing similarities or differences between sites. The permutational multivariate analysis of variance (PERMANOVA) indicated significant differences among sites (p < 0.01).

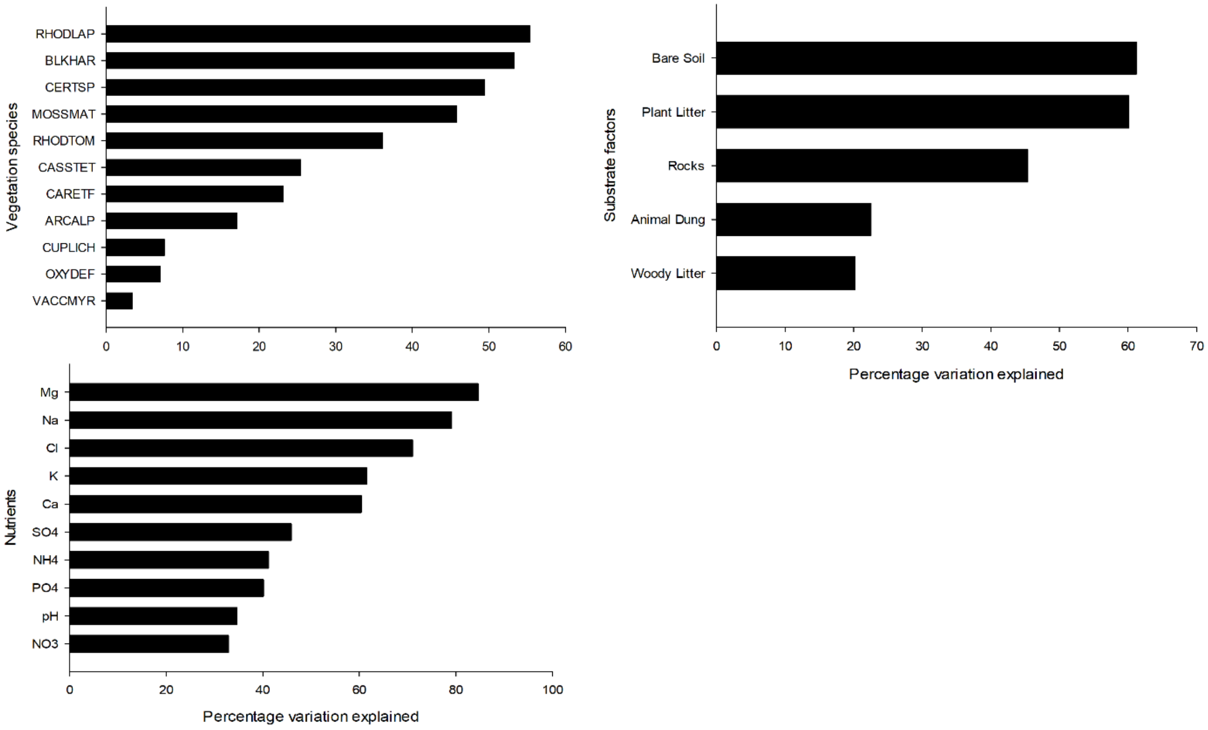

Figure 56. Proportion of the variation explained by variables that make up the vegetation cover (top left panel), substrate factors (top right panel) and soil nutrients (bottom panel). See Figure 55 for separation of study sites based on these categories.

Invertebrate population density

Despite evident differences in vegetation cover, substrates, and nutrients (Figure 55), invertebrate density varied minimally across the four sites. Indeed, collembolan, enchytraeid, and total invertebrate density did not differ among sites (Figure 57B,C,D). However, mite density was significantly lower at site C than in the three other sites, which were generally very similar (Figure 57A).

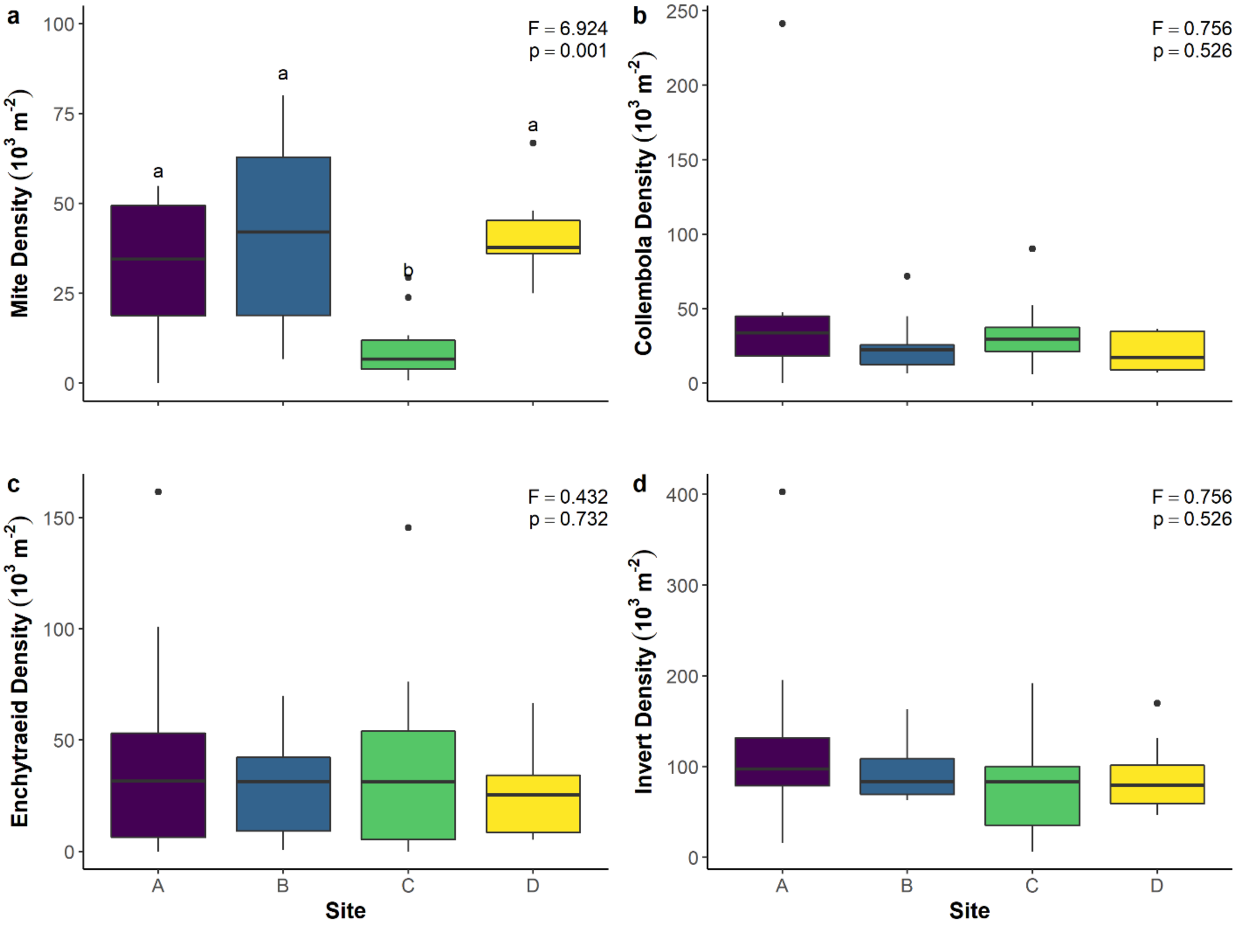

Figure 57. Boxplots showing the population density of mites (a), collembolans (b), enchytraeids (c), and the sum of the soil invertebrates (d) in four sites within the Rankin Inlet. Presented are the F *and* p *values from one-way ANOVAs . Significant pairwise differences denoted by differing lowercase letters.*

Influence of vegetation cover and edaphic factors on invertebrate density

A partial redundancy analysis with site as a conditional factor was conducted (Figure 58). Due to an extreme outlier in collembolan density, one sample was removed from analysis. We used stepwise variable selection of the vegetation cover, substrates, and nutrients to determine which variables should be included in the final model. Of the 26 original variables, five remained in the model after variable selection: cover of the three vegetation species BLKHAR (*Alectoria nigricans*), VACCMYR (*Vaccinium myrtilloides*) and CASSTET (*Cassiope tetragona*), soil pH, and cover of rocks. The final model was significant (ANOVA: $F = 3.44$, $p = 0.002$) and combined, the five variables explained about 25% of variation in invertebrate community density ($R^2_{adj} = 0.25$, $F = 3.44$, $p = 0.001$). Of these variables, *A. nigricans* (ANOVA: $F = 5.03$, $p = 0.009$) and rock cover (ANOVA: $F = 6.25$, $p = 0.002$), as well as pH (ANOVA: $F = 3.09$, $p = 0.045$) were significantly influencing invertebrate community density. Specifically, greater *A. nigricans* cover and lower pH led to greater mite community density and increasing rock cover was associated with increased enchytraeid and collembolan density. Though not significant, *C. tetragona* cover tended to have a negative relationship with mite density and increasing *V. myrtilloides* cover was associated with increasing collembolans and enchytraeids. Not surprisingly based on Figure 57, the four sites showed little separation in terms of density of the invertebrate community: there was a lot of overlap among sites and variability within each site.

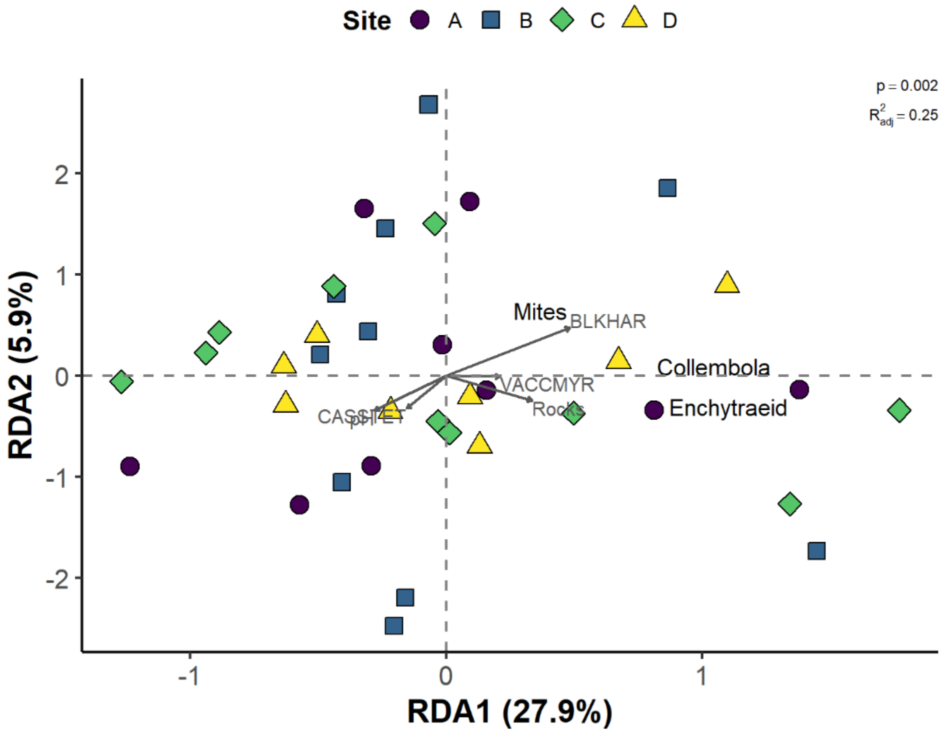

Figure 58. Partial redundancy analysis (RDA) demonstrating the influence of vegetation (BLKHAR, VACCMYR, CASSTET), rocks, and pH on invertebrate community density. Presented are the goodness of fit (R^2_{adj}) and p value for the RDA model. Site was used as a conditional factor in the model. BLKHAR = Alectoria nigricans*, VACCMYR =* Vaccinium myrtilloides*, CASSTET =* Cassiope tetragona.

Recommendations

Overall, we found soil invertebrate communities, expressed as the density of collembolan, enchytraeid and mites, were relatively constant across the upland tundra heath sites at Meliadine. However, this exploratory study did find that changes in vegetation cover, substrate factors including rock cover, and soil pH may impact soil invertebrate communities. Based on the findings of this study, we provide the following recommendations:

1. Mites appear to be negatively influenced by cover of *Cassiope tetragona* and lower soil pH, but positively influenced by the lichen *Alectoria nigricans*. Therefore, **selection of turf materials for restoration efforts should avoid *C. tetragona*, not only due to a potential negative impact on soil mites, but also due to poor transplant survival.**
2. Enchytraeid and collembolan density was positively influenced by increasing rock cover and the cover of *Vaccinium mytrilloides*. **Further research is needed to better determine the key vegetative and soil drivers of tundra invertebrate communities.**
3. Although we did not assess soil invertebrate communities in transplanted turfs, we provide reference data for these invertebrates at harvest locations in undisturbed tundra heath. **Assessment of soil invertebrate communities within transplanted turfs is recommended to determine the impact of transplanting on invertebrate communities.**

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

We examined 25 drilling wastes sites ranging in age from 1-25 years across the landscape at Meliadine, NU. At each of these sites, we surveyed all species present in six 1 m^2 plots (3 on drilling wastes, 3 in undisturbed tundra). Within these 150 survey plots, no invasive species were detected. In addition to these plots, we 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 (30k m/hr on the road into site, and posted speed limits within the mine footprint) along all roadways in the study area. During road surveys, we searched for invasive and non-native plants. All roads were driven twice, to ensure both roadsides were surveyed. Based on a 2010 report from the Canadian Endangered Species Conservation Council, there are 14 non-native plant species that have been recorded in Nunavut (Table 13). While looking for species previously recorded as invasive in Nunavut, we took samples of any plants we could not identify in the field to confirm their identification.

On the main access road, we stopped every 2 km for an informal roadside walking survey. At each informal roadside survey, we recorded latitude and longitude, walked 50 meters along both sides of the road and recorded if any non-native invasive species were encountered (Table 14). Within the mine footprint, areas of high likelihood of invasive species colonization, such as intersections, were surveyed through formal roadside surveys (Table 15). At each formal roadside survey location, we recorded the latitude and longitude, describe the local habitat with reference to native species nearby, and walked 50 m 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.

Table 13. Non-native invasive plant species identified by the Canadian Endangered Species Conservation Council that have been found in Nunavut.

Scientific name	**Common name**
Carum carvi	Wild Caraway
Taraxacum officinale	Common Dandelion
Sonchus arvensis	Field Sow Thistle
Leucanthemum vulgare	Oxeye Daisy
Thlaspi arvense	Field Pennycress
Capsella bursa-pastoris	Shepherd’s Purse
Barbarea vulgaris	Yellow Rocket
Amaranthus retroflexus	Green Amaranth
Hordeum vulgare	Common Barley
Puccinellia distans	Spreading Alkali Grass
Vicia cracca	Tufted Vetch
Papaver somniferum	Opium Poppy
Plantago major	Common Plantain
Polygonum aviculare	Prostrate Knotweed

Table 14. 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 as the general landscape type. No non-native invasive species were identified. Four unknown (UNK) species were observed that were not 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	

Table 15. 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
Main camp				
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
Exploration camp				
II1	63°01'22.6"	092°11'38.0"		Drove ll1 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

Results

No invasive or non-native species were identified in any of 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. Four unknown species were not identified because voucher specimens lacked flowers or other necessary identifying features. The location and frequency of these unknown species have been recorded (Table 14).

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 15). 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 (Table 15).

Northern Tansy Mustard (*Descurainia sophoides*), a weedy species was observed at several locations on-site, including larger patches near exploration camp and extensive patches near the active restoration trial especially at Quarry #1 (Figure 59). *D. sophoides* is a biennial herb that commonly colonizes gravel bars and disturbed soils and is not considered an invasive species in Nunavut. Relative to other tundra vegetation the above height, lateral spread, and biomass of *D. sophoides* is extensive and this species may be useful for short term cover, providing organic soil inputs on disturbed substrates. Although, we observed this species growing directly adjacent to the undisturbed upland tundra heath we did not observe any encroachment into the tundra. Care should be taken to not confuse *D. sophoides* with Flixweed (*Descurainia sophia*), which is considered an introduced species and has been observed at the Meadowbank Mine, along the AWAR and at Bake Lake tank farm (Golder 2021). Due to visual similarity of the two species, on-going monitoring of *Descurainia* spp. on-site is recommended.

Figure 59. Northern Tansy Mustard (Descurainia sophoides) growing in a large patch on Q1 (A) and directly adjacent to undisturbed upland tundra heath (B) as well as within the active restoration trial at Q1 (C) .

Recommendations

We did not observe any non-native invasive species in our 2019 survey. Based upon our survey we recommend the following.

1. **Non-native plant surveys should be conducted at least once during the growing season each year.** Locations with unidentified species for this survey should be examined to confirm none of these species are invasive.
2. **Special attention should be given to *Descurainia* spp.** both due to the potential to use Northern Tansy Mustard (*Descurainia sophoides*) as a fast-growing cover species and to ensure the non-native Flixweed (*Descurainia sophia*) does not become present.
3. **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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Technical Appendices

Natural Recovery of Tundra Vegetation on Drilling Waste at Meliadine

Introduction

Examining natural colonization of vegetation on disturbed sites is critical to understanding both the barriers to revegetation and to identify potential vegetative species and processes that can support restoration efforts. In the summer of 2018, prior to engaging in active revegetation efforts waste sites across the primary ecotypes at the Meliadine site were examined for natural vegetative recolonization. The drilling waste sites ranged in age from one year post-disturbance to 25 years post-disturbance providing insight into the natural recovery and successional trajectories common on this landscape.

Methods & Analysis

At 25 drilling waste sites transects were placed across the site and three, 1 m^2 survey plots were placed at the center. Control transects were placed at least 10 m from the edge of the drilling waste in undisturbed tundra. In each plot, percent cover was estimated for all plant species present and averaged by transect (Figure 1).

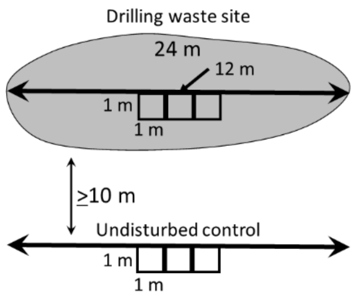

Figure 1. Example transect at a drilling waste site and adjacent undisturbed tundra.

Revegetation of drilling waste was examined using non-metric multidimensional scaling ordination (NMDS), an ordination technique where points that are closer together have more similar plant community than those that are farther apart. Permutational Analysis of Variance (PERMANOVA) tested if statistically significant differences in plant community occurred between treatments over time. To assess changes among individual species over time, percent difference between drilling waste and undisturbed plots were calculated.

Key Results

Community-level Response

Plant community appears to recover 20-25 years after disturbance. Specifically, plant communities between drilling sites and controls from 1993 and 1998 were similar (i.e., overlapping black and red squares and circles; Figure 2) whereas more recently disturbed sites from 2008, 2012, and 2017 had distinct communities (i.e., black and red triangles, crosses and x's are separated along the axes; Figure 2). PERMANOVA confirmed this with significantly different plant communities between drilling waste and control sites over time since disturbance ($p < 0.05$).

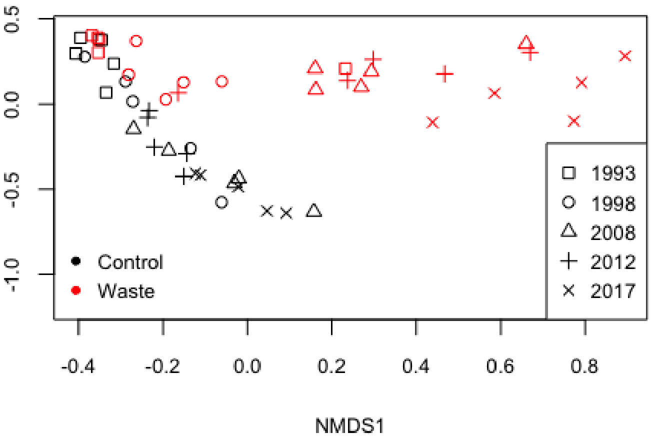

Figure 2. NMDS of species cover data on drilling wastes and undisturbed control sites in disturbance years of 1993, 1998, 2008, 2012, and 2017. Stress value of the plot was 0.047, indicating a good fit.

Species-level Response

Dwarf shrubs had 1-9% lower cover on drilling waste than undisturbed tundra 1-2 years after disturbance. In general dwarf shrubs increased on drilling wastes over time and by 25 years, was similar to undisturbed tundra (about 1% difference; e.g., *Dryas integrifolia*).

Sedge cover was 1.5 to 8% greater on drilling wastes in 2012 and 2017 and was fairly similar between disturbed and control sites at older sites (about 1% difference; e.g., *Carex aquatilis*).

One to two years following disturbance, mosses and lichens had 0.3-20% lower cover on the drilling waste than undisturbed plots. Some moss species (e.g., *Bryum* spp.) recover quickly and establish at higher percentages on drilling wastes (4-8% greater cover). Other moss species (e.g., *Scorpidium scorpioides*) have consistently lower establishment on disturbed substrates (0.1-13%).

Lichen cover was lower on drilling wastes than control plots across almost all time points (0.4-20%). However, there was a clear trend of increasing lichen cover on drilling waste over time (e.g., *Cetraria* sp.).

Conclusions & Recommendations

Based on the characterization of drilling waste sites and the tundra vegetation present on drilling wastes ranging from 1-25 years, it is evident that natural revegetation is occurring on these wastes. Sources of vegetative materials for recolonization and depth of drilling waste appear to be two of the primary driving factors in determining the extent of recovery. Given these driving factors, we suggest the following practices to promote natural revegetation processes.

1. **Leave areas of intact tundra vegetation dispersed throughout the area** where drilling wastes are contained.
2. **Thinly spread drilling wastes appear to undergo revegetation processes more quickly.** In general, a depth of ~3 cm allowed for natural recolonization to proceed. Whereas depth of drilling waste > 15 cm can impede ecosystem recovery with little recovery observed even 20 years after disturbance. Underlying microtopography of drilling waste sites should be considered in managing wastes with an emphasis placed on limiting drilling wastes.
3. Direct colonization of drilling wastes in the first ~5 years is primarily driven by the colonization of acrocarpous mosses. Establishment of these early colonizing moss communities appears to facilitate the development of cohesive and diverse biological soil crusts in later years (~10-20 years). Mature biological soil crusts may act to further stabilize exposed drilling wastes and promote establishment of vascular plant species. **Promoting bryophyte cover in early stages may be beneficial to long-term tundra vegetation establishment.**

Natural colonization of biological soil crusts at Detour and Meliadine mine sites

Introduction

Biological soil crusts (BSCs) are communities of mosses, liverworts, lichens, bacteria, cyanobacteria, and fungi that are commonly found on recently disturbed soils. Given the importance of BSCs in early succession we examined of the establishment and function of BSC macro-phyla (i.e. bryophytes and lichens) and micro-phyla (i.e. bacteria and fungi) on mining disturbed substrates at Meliadine's Arctic site and Detour's boreal site.

Methods & Analysis

Two mines were assessed for natural colonization of biological soil crusts (BSC) on disturbed areas: 1. Meliadine, a low Arctic mine near Rankin Inlet, Nunavut; 2. Detour Lake, a boreal mine in northeastern Ontario. At both mines, sampling sites were selected in three age classes: early (2011-18), mid (2000-10), and late (1990-99). At each site (i.e., drilling wastes at Meliadine or borrow pits at Detour), three BSC samples, including 4-6.5 cm of underlying substrate, were collected at least 10 m apart.

Samples were assessed for changes in both community composition (i.e., moss, lichen, fungi, and bacteria) and function (i.e., gas flux and nutrient concentrations) over time.

Key Results

BSC Community Composition

At Meliadine, bryophyte communities across the age ranges did not differ significantly, though the dominant moss was the generalist species *Ceratodon purpueus* at both mines. Bryophytes at Detour did differ across the age ranges, and corresponded to changes in crust pH, CO_2 flux, and nitrogen-fixation (Figure 1A).

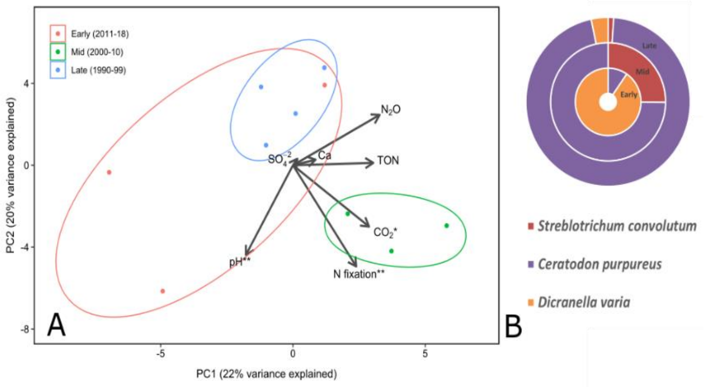

Figure 1. Principal component analysis (PCA) of bryophyte community composition across three age ranges at Detour mine (A). Environmental vectors are overlayed on the PCA biplot with variables significant to the analysis highlighted with ** (p≤0.05) and * (p≤ 0.01). In addition, important bryophyte species per age range (B).

Lichen communities differed across the age ranges at both mines, with shifts in dominant lichens over time (e.g., Figure 2B). The generalist lichen *Dibaeis baeomyces* was common in early age ranges at both mines. At Meliadine, lichen community in the late age range was associated with increased calcium and sulphate in BSCs (Figure 2A).

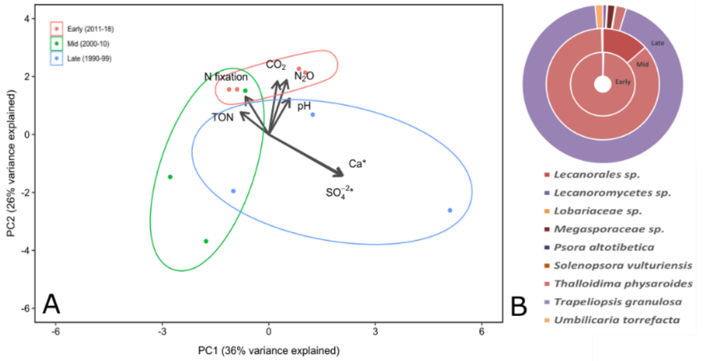

Figure 2. PCA of lichen community composition across three age ranges at Meliadine mine (A). Environmental vectors are overlayed on the PCA biplot with variables significant to the analysis highlighted with * (p≤ 0.10). In addition, important lichen species per age range (B).

Fungal community differed across age ranges at both mines. However, bacterial community only differed with age at Meliadine. Neither fungal or bacterial communities at either mine were associated with gas fluxes or nutrients concentrations.

Interactions between bryophyte and bacterial communities were observed at each mine suggesting that bryophytes facilitate bacterial community development.

BSC Function

Gas fluxes tended to be greater at Meliadine than Detour, but few trends across age ranges at each mine were evident. However, N_2O was greater during the mid age range under light conditions at each site and CO_2 tended to decrease with increasing age at Detour.

Nutrient concentrations of BSC and underlying substrates were generally higher at Meliadine than Detour, and crusts tended to have greater nutrients than underlying substrates at both mines. Though not significant ($p > 0.05$), a consistent trend of increasing nutrients in crusts over time was observed at both mines.

Conclusions & Recommendations

There were significant changes over time in both the macro (bryophytes and lichens) and micro (bacteria and fungi)-phyla of BSC communities in the first 30 years of recovery at both boreal and Arctic mine sites with ubiquitous ruderal species commonly found between sites. The functional traits exhibited by BSCs during early stages of recovery (i.e. 30 years) on disturbed sites are likely driven by compositional differences rather than developmental stage. At both mine sites, the bacterial community appears to be responding to changes in bryophyte communities as they develop over time. Based upon the findings of this study we recommend the following practices to promote recovery of disturbed Arctic and boreal substrates.

1. Bryophytes likely play an important role in determining early successional BSC community assembly and restoration efforts should **promote bryophyte establishment by targeting generalist species with ubiquitous distribution, such as *Ceratodon purpureus*.**
2. The community assembly of bryophytes changes over time. Small acrocarpous moss species tend to dominate in the earliest stages of vegetative recovery with larger acrocarpous and pleurocarpous mosses establishing later (i.e. 10-30 years post-disturbance). **The growth form of moss species used in restoration efforts should be matched with site-specific post-disturbance legacies.**
3. Although BSCs are generally considered slow growing, at both study locations BSCs naturally colonized disturbed substrates and initiated functional processes associated with nutrient cycling as early as 10 years since disturbance. Changes in functional measurements were associated with colonization by various BSC species. **Therefore, BSC species with high rates of carbon and nitrogen fixation, such as cyanolichens and bryophytes with known cyanobacterial associations should be selected for restoration.**
4. Work remains to be done to better under the interactions between the macro and micro-phyla BSC communities in these early stages, however our study demonstrated that **establishment of generalist ruderal macro-phyla communities may facilitate the establishment of key micro-phyla communities responsible for nutrient cycling processes.**

Biological soil crusts as ecosystem engineers at Meliadine

Introduction

Biological soil crusts (BSCs; soil surface communities of bryophytes, lichens, bacteria, and fungi) are initial colonizers in disturbed terrestrial systems that can promote colonization of vascular species and soil development by modifying their surroundings (e.g., soil stabilization, moisture retention). In Canada's low Arctic, resource exploration and extraction are major disturbances and understanding BSC development post-disturbance is crucial to inform restoration efforts and promote ecosystem resilience and function.

Methods

We monitored microclimatic conditions (e.g., photosynthetically active radiation (PAR), soil temperature and moisture, relative humidity, and air temperature) and BSC and vascular plant cover on crusted and bare drilling wastes over 3 years. In addition, we examined changes in BSC and vascular plant cover along a chronosequence of mining disturbance (from 1992 to 2013).

Key Results

Drilling wastes with BSCs (i.e., crusted) had higher surface soil water content and lower surface temperature and PAR compared with bare drilling wastes (Figure 1). Vegetation cover differed between bare and crusted drilling wastes and between the years. In general, drilling waste was greater and moss crust, lichen crust, and vascular plant cover lower in the bare vs. crusted soils in each year. Vascular plants and lichen crusts increased cover between 2018 and 2021 in both bare and crusted plots which led to a decrease in exposed drilling wastes (Figure 1).

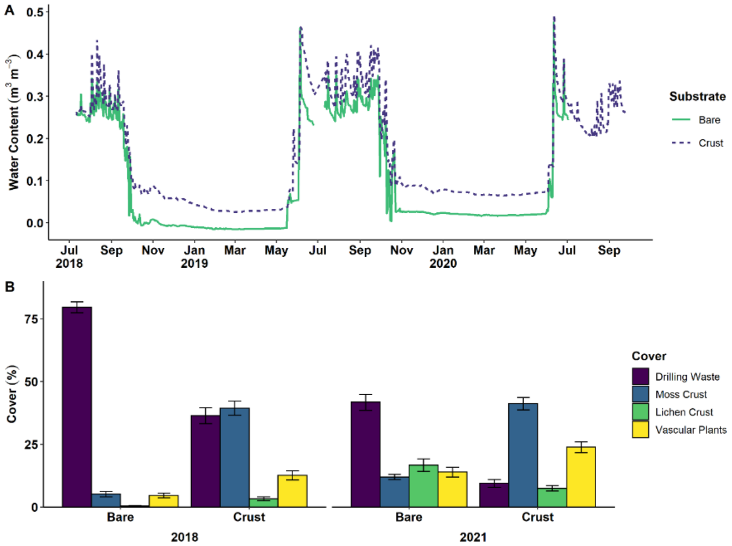

Figure 1. Daily average water content from Jul 2018 to Oct 2020 (A) and average ± standard error percent cover of different ground covers in 2018 and 2021 (B) for bare (i.e., exposed drilling waste) and crusted (i.e., covered in BSCs) substrates at the M13-2100 microclimate monitoring site.

Across the chronosequence, drilling waste and moss crusts were more abundant on newer sites whereas vascular plants and lichen crusts had greater cover on older sites (Figure 2). As such, the colonization of drilling waste after disturbance begins with bryophyte species and moves to more lichen and vascular species around 20-years post-disturbance, though moss does not entirely disappear.

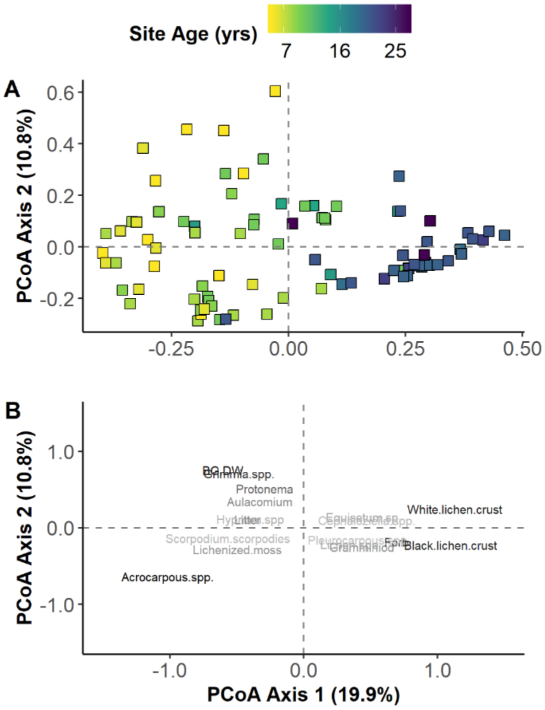

Figure 2. Principal coordinates analysis (PCoA) of ground cover, as drilling waste, vascular species, and lichen and moss crusts, on drilling waste across a chronosequence of mining disturbance. Displayed are the site scores (A) and species scores (B).

Conclusions & Recommendations

Expansion of our chronosequence work at Meliadine in 2019, revealed similar trends to those observed in the 2018 chronosequnce work at Meliadine and Detour. Bryophyte and BSC establishment was evident at early stages post-disturbance. Colonization of drilling waste after disturbance begins with bryophyte species at the Meliadine site and moves to more lichen and vascular species as sites get older. Lichen crusts and vascular species tend to develop around 20 years post-disturbance. Increasing water content and reduced temperatures at the soil surface were also observed with increasing BSC establishment over time. Based on these findings we recommend the following.

1. BSC establishment modifies the surrounding environment by increasing water content and reducing temperature at the soil surface. Therefore, **BSCs cover should be promoted on disturbed substrates to improve the abiotic conditions and promote the establishment of tundra plant species.**
2. Bryophytes are a ubiquitous part of vegetative succession on drilling waste, but there is no clear change in bryophytes species over time with these earlier colonizers persisting throughout succession. A few species occur throughout the entire 30-year chronosequence, including ***Ceratodon purpureus, Pohlia nutans,*** **and *Bryum blindii,* which should be targeted in active restoration.**

Vegetative growth and belowground expansion from transplanted low-Arctic whole tundra turfs

Introduction

Whole-turf transplantation (i.e., harvesting and transplanting intact soil-plant plugs) initiates restoration with established and functional assemblages of native plant species. Turfs provide sites for seed and spore germination, soil resources and high species diversity, while reducing shock to individuals within the transplants. Above and belowground expansion of turfs harvested from Meliadine and transplanted adjacent to fertilized or unfertilized bare substrates was examined to determine the efficacy of this approach.

Methods

Plant-soil turfs including biological soil crusts (BSCs), vascular plants, and soils were harvested from undisturbed upland tundra heath at Meliadine. Turfs were transported to University and Saskatchewan, placed in growth chambers, and exposed to fertilized and unfertilized substrates. The goals were to determine the effect of fertilization on plant community composition, above and belowground biomass, and expansion from the turf into adjacent substrates.

Key Results

Aboveground Response to Fertilization

BSC cover was significantly higher on fertilized than unfertilized substrates ($F_{1,7} = 25$, $p < 0.01$) (Figure 1). Cover on both substrates was dominated by moss (~63%).

Both fertilized and unfertilized substrates had similar assemblages of vascular species: *Astragalus alpinus*, *Stellaria longipes*, and *Carex* spp. *S. longipes* demonstrated the farthest growth and was more prevalent in the fertilized substrates. Mean aboveground vascular biomass was generally higher in fertilized substrates.

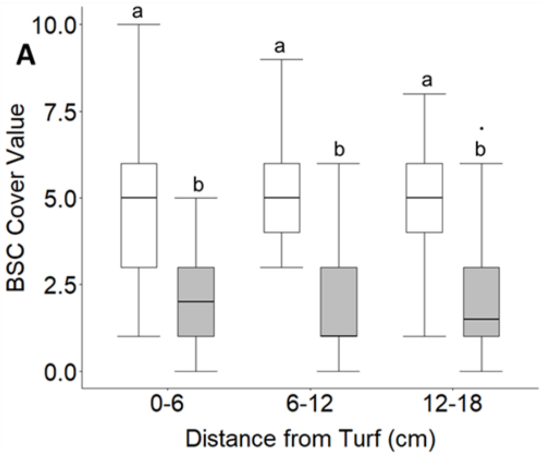

Figure 1. Comparative box plots of BSC cover at each distance category (0-6, 6-12 and 12-18 cm). White and grey boxes represent fertilized and unfertilized substrates, respectively. Letters on boxplots represent significant differences or similarities between groups.

Belowground Response to Fertilization

Nine vascular species were detected in greater prevalence within fertilized substrates, notably *Anthoxanthum monticola*, *S. longipes*, and *Salix reticulata*. Unfertilized substrates had a greater prevalence of *Dryas integrifolia*, *Eriophorum callitrix*, and N-fixing species like *Astragalus alpinus* and an unidentified *Fabaceae spp*.

Belowground biomass was significantly higher in fertilized substrates and changed with distance from the turf ($F_{1,7} = 6.3$, $p < 0.05$; $F_{2,14} = 17$, $p < 0.001$) (Figure 2). Belowground biomass decreased with distance from the turf. Fertilized substrates had more belowground biomass than unfertilized at each distance increment (df = 39, t = -3.68, $p < 0.001$; df = 39, t = -5.98, $p < 0.001$; df = 39, t = -4.87, $p < 0.001$). Overall, total belowground biomass was 22-fold (fertilized) and 12-fold (unfertilized) greater than aboveground biomass.

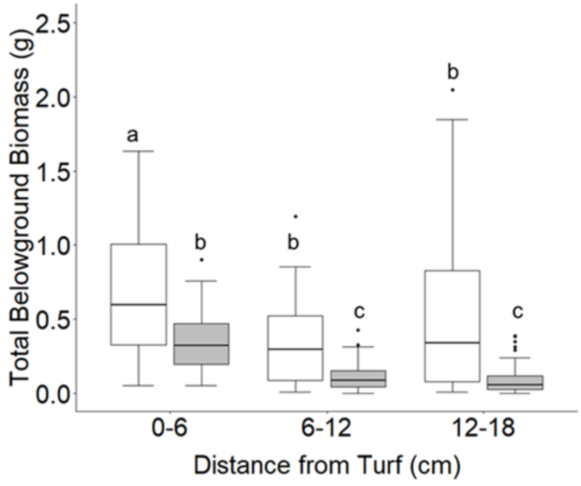

Figure 2. Comparative box plots of belowground biomass at each distance category (0-6, 6-12 and 12-18 cm). White and grey boxes represent fertilized and unfertilized substrates, respectively. Letters on boxplots represent significant differences or similarities between groups.

Conclusions & Recommendations

While fertilization of adjacent substrates did not alter the turf community, greater BSC development and belowground biomass in fertilized substrates, suggests fertilization may decrease timeframes of natural colonization in the turf's immediate surroundings and support the development of later-stage vegetative communities. Turfs in our growth chamber experiment expanded ~150% in total area, with graminoid species predominately responsible for this expansion. Furthermore, belowground expansion far exceeded aboveground. Based on these findings we recommend the following.

1. **Harvesting and transplanting intact, undisturbed tundra trufas aids the rehabilitation of mining-disturbed sites**, as substrates adjacent to turfs were found to be colonized by BSCs and vascular plants after 140 days of incubation in the growth chamber.
2. **Fertilization of substrate adjacent to transplanted tundra turfss: i) allows for maintenance of turf transplant community composition promoting native assemblages of species**; **ii) expedites colonization** of disturbed substrates by BSCs and fast-growing vascular species; and **iii) increases the distance of expansion.**
3. Turfs can act as a **source of BSC propagules** and the forbs ***Stellaria longipes*** and ***Astragalus alpinus*,** the evergreen shrub ***Dryas integrifolia*,** and deciduous shrub ***Salix reticulata* are target species for restoration.**
4. **Targeting nitrogen-fixing vascular species** (e.g., *Astragalus alpinus* and the family Fabacaeae) **in turfs promotes expansion into unfertilized substrates** and may facilitate the growth of other species through N addition.
5. Expansion of belowground biomass from turf transplants far exceeds aboveground and is primarily driven by grasses, specifically *Anthoxanthum monticola.* Therefore, **belowground expansion of species, especially grasses should be monitored.**

Active restoration of native plant-soil systems using tundra turfs at Meliadine

Introduction

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 native tundra vegetation and isolated site locations. Our aim was to examine if *in-situ* active restoration using transplanted tundra turfs and shredded tundra material could promote pre-disturbance plant-soil assemblages and identify tundra species that rapidly colonize upon transplantation and promote tundra plant-soil community development.

Methods

Hummock-hollow microtopography was created on two disturbed quarry sites and four treatments were applied: Turfs, Shredded and Turfs + Shredded and Control. Turfs (40 cm^2 by 10-15 cm deep) were harvested from undisturbed upland tundra heath and shredded material was composed of three shredded turfs. Percent cover of all species was assessed at the time of transplanting in 2019 and two years later in 2021. Soil samples taken at harvest sites (2019, 2021) and from transplanted turfs (2021). Recovery of harvest sites was monitored in 2021.

Key Results

Two years following turf transplantation, PERMANOVA indicated that all four treatments had significant differences in community composition (df = 79, $R^2 = 0.43$, $p = 0.001$) (Figure 1). Turf and turf + shredded treatments contained the highest species richness, greatest vegetative cover, and greatest flower density.

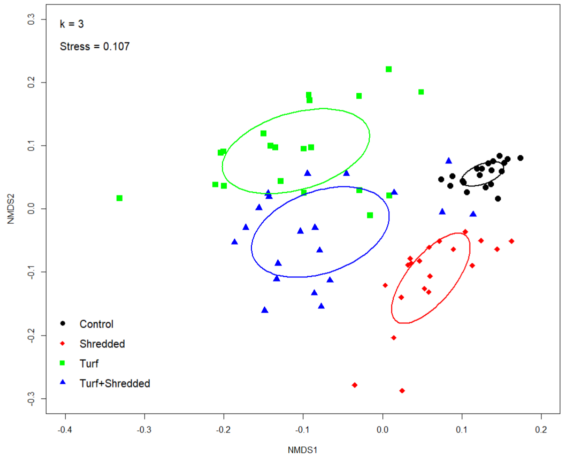

Figure 1. Non-metric dimensional scaling (NMDS) ordination plots of treatment community composition two years after turf transplantation. Treatments are represented by black circles (Control), green squares (Turfs), blue triangles (Turfs + Shredded), and red diamonds (Shredded). Ellipses represent the standard error of the weighted average of scores.

Shredded layers had poor establishment with decreased in species richness, bryophytes, lichens, evergreen and deciduous shrubs after two years. While turfs maintained species richness and bryophytes and evergreen shrub cover decreased to a less degree.

Turfs and turf + shredded treatments had significant expansion of vascular plants ($F_{3,209} = 20.79$, $p < 0.001$) immediately adjacent (i.e., within 0-8 cm) to the turf, whereas little expansion was observed for shredded or control treatments.

After two years vegetative establishment within harvest plots was minimal with windblown lichen (20%) and moss (8%) and in-growth of evergreen shrubs (10%) with the highest relative cover.

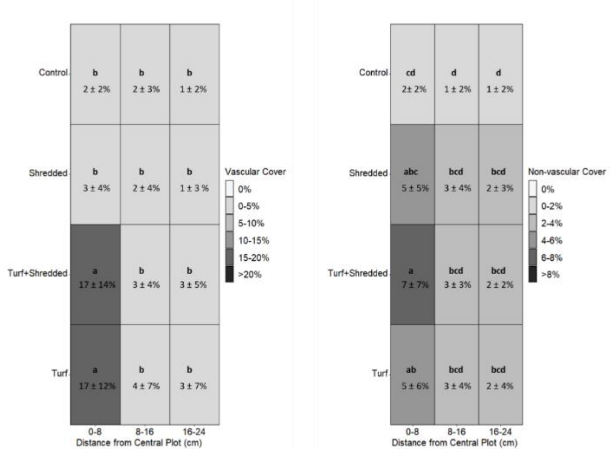

Figure 2. Heatmaps of mean vascular and non-vascular cover expanding from the central plot of each treatment, grouped by distance from the central plot. Significance indicators within boxes represent significant difference between treatments and between distances, with standard deviations presented underneath.

Nutrient concentrations increased in both the organic and mineral layers of turfs, however, increases appear to be driven by quarry substrate nutrient conditions, which were elevated in Cl^-, SO_4^{2-}, NO_3^-, Mg^{2+}, Ca^{2+} and Na^+ compared with harvest site soils.

Conclusions & Recommendations

Of the three different restoration strategies included (turf, turf + shredded and shredded), turf and turf + shredded treatments contained the highest species richness, greatest vegetative cover, and greatest flower density. Shredded layers did not establish over two years with the cover of living vegetation, bryophytes and lichens all decreasing. Based on these findings we recommend the following.

1. Spreading shredded materials was not an effective method likely due to displacement of materials by wind and water, as well as a limited propagule bank. Therefore, **the use of intact tundra heath turfs is recommended over the placement of shredded tundra heath.**
2. Overall survival of species within turfs was high and reproduction (i.e., flowering) was observed two years after transplanting, therefore **tundra heath turfs 40 cm^2 by 10-15 cm deep support native plant-soil communities that can act as resources for revegetation of disturbed substrates.**
3. Transplanting intact turfs allows for the maintenance of species richness and deciduous shrubs and the growth of graminoids and forbs. However, **evergreen shrubs, especially *Cassiope tetragona* and *Rhododendron spp.* had poor post-transplanting survival and should be avoided if future restoration efforts.**
4. Graminoids, *Carex bigelowii and Carex rupestris* grew within the turfs, C. bigelowii and *Poa arctica* had the furthest expansion, indicating that **gramminoids should be targeted in selecting turf transplant materials.**
5. Expansion from turfs by both vascular and non-vascular species was observed, with most expansion occurring within 8 cm of the turf. **The forb *Stellaria longipes* along with early moss communities were found to expand the furthest and should be targeted in selecting turf transplant materials.**

Drivers of soil invertebrate communities at Meliadine

Introduction

Characterization of soil invertebrate communities in the upland tundra heath at the Meliadine site is needed to better understand the key drivers of invertebrate populations in undisturbed tundra. Soil invertebrates play a critical role in nutrient cycling within tundra soils and promoting healthy invertebrate communities is essential to long-term plant-soil establishment and growth. This exploratory study was intended to provide background and baseline data to support further examination of the impact of turf transplantation on soil invertebrate populations within turfs.

Methods

At four undisturbed upland heath tundra sites, we randomly selected 8-10, 1 m^2 plots that represented the local vegetation. At each plot, we measured vegetation and substrate (i.e., rocks, bare ground, etc.) cover and took 5 cm diameter and 10-20 cm deep soil core to determine invertebrate community density and soil nutrients.

Key Results

Soil invertebrate density varied minimally across the four sites: collembolan, enchytraeid, and total invertebrate density did not differ among sites. Mite density was significantly lower at site C than in the three sites, which were generally very similar (Figure 1).

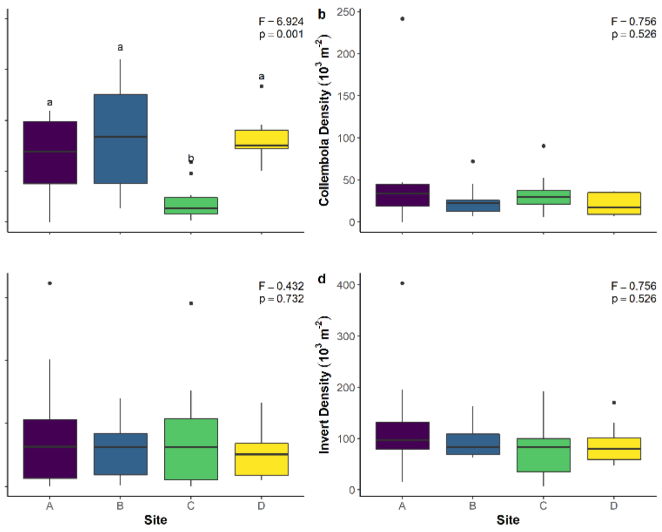

Figure 1. Density of mites (a), collembolans (b), enchytraeids (C), and total invertebrate community (d) at four upland heath tundra sites at Meliadine.

Redundancy analysis revealed five important drivers of invertebrate community: cover of three vegetation species (the lichen *Alectoria nigricans* and the shrubs *Vaccinium myrtilloides* and *Cassiope tetragona*), soil pH, and cover of rocks. The final model was significant (F = 3.44, p = 0.002) and combined, the five variables explained about 25% of variation in invertebrate community density (R^2_{adj} = 0.25, F = 3.44, p = 0.001). Greater *A. nigricans* cover and lower pH led to greater mite density and increasing rock cover was associated with increased enchytraeid and collembolan density. Though not significant, *C. tetragona* cover had a negative relationship with mite density and increasing *V. myrtilloides* cover was associated with increasing collembolans and enchytraeids.

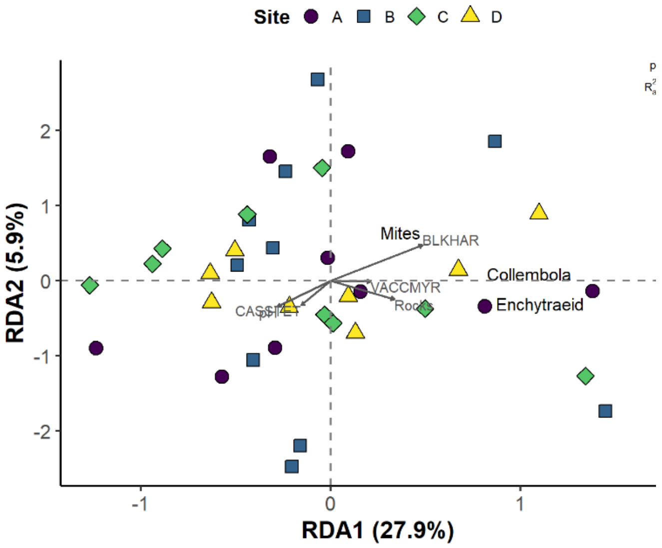

Figure 2. Redundancy analysis demonstrating the important drivers (grey arrows) of invertebrate community at each site (coloured points) and the association between the different invertebrates (Mites, Collembolans, and Enchytraeids) and the drivers. BLKHAR = Alectoria nigricans*, VACCMYR =* Vaccinium myrtilloides*, CASSTET =* Cassiope tetragona.

Conclusions & Recommendations

Overall, we found soil invertebrate communities, expressed as the density of collembolan, enchytraeid and mites, were relative constant across the upland tundra heath sites at Meliadine. However, this exploratory study did find that changes in vegetation cover, substrate factors including rock cover and soil pH may impact soil invertebrate communities. Based on the findings of this study we provide the following recommendations.

1. Mites appear to be negatively influenced by cover of *Cassiope tetragona* and lower soil pH, and positively influenced by the lichen *Alectoria nigricans*. Therefore, **selection of turf materials for restoration efforts should avoid *C. tetragona*, not only due to a potential negative impact on soil mites, but also due to poor transplant survival.**
2. Enchytreid and collembolan density was positively influenced by increasing rock cover and the cover of *Vaccinium mytrilloides*. **Further research is needed to better determine the key vegetative and soil drivers of tundra invertebrate communities.**
3. Although we did not assess soil invertebrate communities in transplanted turfs, we provide reference data for these invertebrates at harvest locations in undisturbed tundra heath. **Assessment of soil invertebrate communities within transplanted turfs is recommended to determine the impact of transplanting on invertebrate communities.**