



2022 COSIA LAND EPA

# Mine & In Situ Research Report

PUBLISHED MARCH 2023



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## INTRODUCTION

Canada's Oil Sands Innovation Alliance (COSIA) is the innovation arm of Pathways Alliance, an organization created by Canada's major oil sands producers to advance responsible development of the industry, including addressing the critical challenge of climate change. For more than 10 years, COSIA has brought together innovative thinkers in industry, government, academia and the public to drive innovations that reduce impacts to air, water and land.

**This report is funded by members of COSIA's Land Environmental Priority Area (EPA):**

Canadian Natural Resources Limited (Canadian Natural)  
Cenovus Energy Inc. (Cenovus)  
ConocoPhillips Canada Resources Corp. (ConocoPhillips)  
Imperial Oil Resources Limited (Imperial)  
Suncor Energy Inc. (Suncor)\*  
Syncrude Canada Ltd. (Syncrude)\*\*

For 2022 COSIA published a combined research report, 2022 COSIA Land EPA — Mine and In Situ Research Report. This report summarizes progress for projects related to in situ and mine reclamation of the COSIA Land EPA. The project summaries included in this report do not include all projects completed under the Land EPA.

Please contact the Industry Champion identified for each research project if any additional information is needed.

2022 COSIA Land EPA — Mine and In Situ Research Report. Calgary, AB: COSIA Land EPA.

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\* Teck Resources sold its stake in the Fort Hills project to Suncor Energy in October 2022 and exited the oil sands business and its COSIA membership.

\*\* Suncor Energy assumed operatorship of the Syncrude Joint venture in October of 2021, however Syncrude Research and Development remains a full member of COSIA.

Front cover image of Muskeg Lake, Kearl (Imperial's compensation lake). Image courtesy of Imperial and photographer Jeffrey Short.

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## WETLANDS

## Removing the Wellsite Footprint (iFROG)

**COSIA Project Number:** LJ0216

**In Situ**

**Research Provider:** Circle T Consulting Inc.

**Industry Champion:** ConocoPhillips

**Industry Collaborators:** AOC Leismer Corner Partnership, Canadian Natural, CNOOC Petroleum North America ULC, Harvest Operations Corp., Cenovus, Imperial, Japan Canada Oil Sands Limited, MEG Energy Inc.

**Status:** Year 6 of 7

### PROJECT SUMMARY

The purpose of the industrial Footprint Reduction Options Group (iFROG) is to develop, fund and implement a balanced portfolio of boreal wetlands research projects that:

- Follow the fundamental guiding principles of land stewardship, intelligent research, and collaboration;
- Demonstrate iFROG members are meeting the intent of the applicable wetland research conditions in their respective Environmental Protection and Enhancement Act (EPEA) Approvals (for oil sands in situ projects);
- Contribute to mitigating impacts to wetlands during operations; and
- Increase the knowledge base for, and confidence in, wetland reclamation efforts.

In 2022, two projects were supported: Japan Canada Oil Sands Limited (JACOS) Road Reclamation and Pad TT Road Construction Best Practice. Although a third project, From Dirt to Peat, was not funded in 2022, analyses producing additional results continued in 2022. JACOS oil sands projects were acquired by Greenfire Resources. Therefore, the project has been renamed Greenfire Road Reclamation Study.

#### Greenfire Road Reclamation Study (Year 4 of 5)

The JACOS/Greenfire Road Reclamation Study involves the continuation of reclamation work first initiated in 2010 within a bog peatland. Whereas the initial study restricted treatments to three treatment blocks established on only a subsection of the road, the entire road was reclaimed in the present study and examines two primary reclamation strategies. Partial fill removal is used in both strategies, where only enough fill was removed to establish a revegetation surface with an elevation that is continuous with the adjacent peat. One strategy attempts to establish peatland vegetation on the mineral fill surface, while the second attempts to establish peatland vegetation on varied depths of organic substrate.

The road was divided into two study areas, one for each of the revegetation strategies. Fill on the mineral substrate section was removed until the surface was continuous with the adjacent peatland and the surface was inoculated with fen peatland propagules from a nearby donor site and covered with straw mulch. Fill on the organic substrate



section was excavated to below the elevation of the adjacent peatland to the desired depth of organic substrate, and then filled to the adjacent peatland elevation with stockpiled peat. The peat surface was inoculated with bog peatland propagules from a nearby donor site and covered with straw mulch.

The objectives of the peat substrate study are to evaluate revegetation methods such as substrate depth and fertilization, as well as identify drivers of the emerging plant community (e.g., soil pH, electrical conductivity, water content, and soil and water chemistry).

The objectives of the mineral section are to compare revegetation success on two surface treatments (smooth and lightly scarified), to identify drivers of the emergent vegetation, and assess any physico-chemical effects of the exposed mineral surface on the adjacent peatland.

### **From Dirt to Peat (Concluded)**

This study is a three-year, multi-site, meta-analysis examining ecological recovery in response to a range of reclamation practices over a range of conditions, including both partial and complete removal of fill from roads and pads constructed within wetlands. Seven pads and two linear features are being studied, including the IFROG Canadian Natural pad (2011) and the JACOS/Greenfire road study sites.

The study objective is to characterize each site in terms of its functioning, or potential to function, as a healthy peatland based on emerging vegetation communities and peat accumulation potential. Ecological response variables that will be used include vegetation composition, accumulation of organic matter (“peat thickness”), above and below ground biomass productivity, biomass decomposition, peat accumulation potential (calculated) and GHG (greenhouse gas) balance. Explanatory variables include the site-specific treatments, as well as environmental conditions such as local climatic indicators, soil and water chemistry, soil moisture and soil temperature. Reclamation site response variables will be compared with similar variables on reference peatlands within the oil sands areas in which the study sites are located.

### **Pad TT Road Construction Best Practice (Year 5 of 5)**

Devon Canada constructed a road at the Jackfish 2 project (now named Canadian Natural Jackfish 2) intersecting several areas of deep fen peat that are each approximately 180 m long. Timber corduroy was used as road foundation over the soft peat sections in conjunction with several 600 mm steel culverts that were closely spaced within each section. High-density polyethylene (HDPE) pipe bundles or log bundles were installed among the culverts to facilitate additional drainage. Seventeen culverts and seven bundles were installed along the 1.5 km length of road.

Study objectives are to:

- Determine whether the road allows water to pass through effectively as a result of the corduroy and drainage conduit installations;
- Characterize flow rates and patterns in the vicinity and through the road to assess the effectiveness of the type and number of conduits; and
- Assess road performance in the corduroy sections as indicated by progressive road settlement over time and identify any problem areas.



## PROGRESS AND ACHIEVEMENTS

### Greenfire Road Reclamation Study

Drought conditions in 2022 were detrimental to mosses established in previous years. *Scirpus cyperinus* (woolgrass) and *Carex spp.* cover increased in the organic treatment section, while *Trifolium hybridum* (alsike clover) and *Melilotus officinalis* (yellow sweet clover) cover increased in the mineral section. In the organic section, high shade cover by tall *Scirpus* and *Carex spp.* limited bryophyte growth reduced *Sphagnum* moss presence. However, both *Sphagnum* and true mosses are still present in moderate abundance in the deepest organic substrate treatment. True mosses were diminished in the smooth section of the mineral substrate treatment but were surviving under the shade of clover.

A number of temporary water diversion devices were installed along both edges of the mineral section to slow runoff flow along the edges and to redirect water across the road surface to enhance moisture conditions. Increasing moisture should reduce undesirable species and promote bryophyte retention and expansion. Diversion devices will be removed once bryophytes have become sufficiently established to self-regulate varying moisture conditions.

Results from 2022 increased understanding of the range of variables that need to be monitored and managed on road reclamation projects.

### From Dirt to Peat

Treatments where peat donor material was applied, specifically *sphagnum* and true mosses, had greater accumulation of peat than treatments without donor material.

These results identified the importance of active species reintroduction to the success of peatland reclamation.

### Pad TT Road Construction Best Practice

Corduroy and conduits improved road permeability and the road achieved better water balance than typically observed with conventional construction. However, the road was still a barrier to water flow, particularly subsurface water. Water balance across the road was most closely achieved when the general water table was high, such that water could access the conduits and flow through. While depth to water table (DTW) was generally deeper on the downstream than upstream side of the road (Table 1), these differences increased when the general water table was lower. Similarly, hydraulic gradients across the road tended to be steeper during drier conditions than moister conditions.





**Table 1: Depth to water table (cm) at 5 m from upstream and downstream road edges for control, corduroy and conduit transects. The final column is site average water table elevation for each date.**

Date	Control		Corduroy		Culvert		Pipe Bundle		Log Bundle		Average WT Elevation (masl)
	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	
04-Jun-21			-4	6	3	-1	-4	-7	-2	-1	652.58
13-Jul-21	-10	-32	-15	-18	-12	-13	-12	-17	-8	-14	652.48
16-Aug-21	-3	-22	-12	-15	-10	-7	-11	-14	-6	-13	652.50
13-Sep-21	-7	-28	-14	-18	-11	-13	-12	-18	-8	-16	652.47
14-Oct-21	-8	-29	-12	-16	-8	-13	-10	-15	-5	-15	652.49
31-May-22	8	-6	0	-5	-1	1	-2	-4	4	0	652.58
30-Jun-22	10	-8	-2	-7	0	-1	-2	-8	6	-2	652.58
20-Oct-22	-27	-38	-15	-22	-11	-18	-13	-22	-8	-19	652.42
<b>2021 Average</b>	-7.0	-27.8	-11.4	-14.6	-8.8	-9.4	-9.8	-14.2	-5.8	-11.8	
<b>2022 Average</b>	-3.0	-17.3	-5.7	-11.3	-4.0	-6.0	-5.7	-11.3	0.7	-7.0	

Differences were observed among control, corduroy, and conduits in DTW and hydraulic gradients. The largest differences in DTW between sides of the road were observed at the control transect, followed by the log bundle transect. Differences in DTW between sides of the road were relatively similar among the corduroy, culvert, and pipe bundle transects on any given date, but differences were least at the culvert transect on average. Hydraulic gradients across the road were measured within 1.2 m wells slotted over the entire depth, as well as piezometers slotted at 85 cm and 155 cm depths, or roughly the mid-point and bottom of the peat. Hydraulic gradients between sides of the road at the control transect were always very much steeper than the corduroy and conduit transects regardless of depth. Gradient steepness for wells was similar among corduroy, pipe bundle, and log bundle transects, while the culvert transect gradient was least steep for wells (Table 2). Gradient steepness rankings at mid peat depth were corduroy > log bundle > culvert > pipe bundle, whereas rankings for peat bottom were log bundle > pipe bundle > culvert = corduroy (Table 3).

**Table 2: Vertical hydraulic gradients from upstream to downstream side of the road for wells positioned 5 m from the road edge. Value = 1 indicates a gradient parallel with the ground slope, > 1 = steeper than ground slope, < 1 = flatter than ground slope.**

Day of Year	Control	Corduroy	Culvert	HDPE Bundle	Log Bundle	Average WT Elevation (masl)
4-Jun-21		1.09	0.89	1.14	0.95	652.58
13-Jul-21	3.12	1.13	1.05	1.24	1.28	652.48
16-Aug-21	2.83	1.13	0.84	1.14	1.32	652.50
13-Sep-21	3.02	1.17	1.11	1.29	1.37	652.47
14-Oct-21	3.02	1.17	1.27	1.24	1.46	652.49
31-May-22	2.35	1.21	0.89	1.10	1.19	652.58
30-Jun-22	2.73	1.21	1.05	1.29	1.37	652.58
20-Oct-22	2.06	1.30	1.38	1.43	1.51	652.42
<b>2021 Average</b>	3.00	1.14	1.03	1.21	1.28	
<b>2022 Average</b>	2.38	1.24	1.11	1.27	1.35	



**Table 3: Vertical hydraulic gradients from upstream to downstream side of the road for mid-peat depth piezometer positioned 5 m from the road edge. Value = 1 indicates a gradient parallel with the ground slope, > 1 = steeper than ground slope, < 1 = flatter than ground slope.**

Date	Control	Corduroy	Culvert	HDPE Bundle	Log Bundle	Average WT Elevation (masl)
4-Jun-21		2.41	1.00	0.96	1.22	652.58
13-Jul-21		2.96	1.33	0.93	1.39	652.48
16-Aug-21	3.34	2.20	0.89	1.00	1.34	652.50
13-Sep-21	2.81	2.41	1.50	0.96	1.47	652.47
14-Oct-21	3.13	2.85	1.33	1.00	1.26	652.49
31-May-22	3.02	3.17	1.28	1.18	1.22	652.58
30-Jun-22	3.34	2.30	1.00	1.14	1.56	652.58
20-Oct-22	3.45	3.61	1.55	1.14	1.78	652.42
<b>2021 Average</b>	3.09	2.57	1.21	0.97	1.34	
<b>2022 Average</b>	3.27	3.03	1.28	1.16	1.52	

Vertical water gradients measure on each side of the road, rather than across the road differed between sides of the road and varied more among treatments on the upstream side than downstream side. Vertical gradients tended to be upward (meaning greater hydraulic pressure at depth) on the downstream side of the road under most moisture conditions (Table 4), indicating reduced nearer-surface moisture. On the other hand, the gradient was almost always downward (greater hydraulic pressure near surface) on the upstream side at the control transect, indicating increased nearer-surface moisture. The vertical gradient was almost always downward at the pipe bundle transect on the upstream side, but weaker than observed at the control. The gradients did strengthen during lower water table periods. The gradient was predominantly downward at the culvert on the upstream side, but the gradient was very weak. The vertical gradient at the corduroy transect was split between upward and downward on the upstream side but had no distinct pattern in relation to overall water table conditions. The gradient at the log bundle was weak but predominantly upward on the upstream side. Shallower peat at this location may have had more influence on the vertical gradient here. Downward gradients on the upstream side indicate that water tended to build up nearer the surface on the upstream side. Weaker gradients at the culvert indicate somewhat better movement of water across the road to enable a quicker hydraulic equilibrium between deeper and shallower zones of the peat. On the other hand, upward gradients on the downstream side indicate interruption of nearer-surface flow with some flow occurring underneath the road from the upstream to downstream side.



**Table 4: Vertical hydraulic gradients on each side of the road at piezometers located 5 m from each road edge. Gradients are between mid and bottom of peat positions. Negative values indicate upward hydraulic pressure (discharge). Positive values indicate downward hydraulic pressure (recharge).**

Hydraulic Gradient (m/m)										
	T1 (Control)		T3 Corduroy		T3 Culvert		T3 HDPE Bundle		T3 Log Bundle	
Date	Upstream	Downstream	Upstream	Downstream	Upstream	Downstream	Upstream	Downstream	Upstream	Downstream
04-Jun-21	n/a	n/a	0.017	n/a	0.051	n/a	n/a	n/a	n/a	n/a
13-Jul-21	n/a	n/a	0.062	-0.158	0.037	0.028	0.020	n/a	n/a	0.017
16-Aug-21	0.015	0.007	-0.059	-0.018	-0.062	-0.030	0.020	0.073	0.029	0.017
13-Sep-21	-0.015	0.033	-0.029	-0.043	0.037	-0.248	0.033	-0.065	-0.123	0.059
14-Oct-21	0.015	0.033	0.047	-0.069	-0.020	-0.219	0.046	-0.092	-0.207	0.003
31-May-22	0.062	-0.007	-0.041	-0.107	-0.006	-0.219	0.033	-0.051	-0.054	-0.011
30-Jun-22	0.046	-0.007	-0.069	-0.145	-0.020	-0.190	0.033	-0.037	-0.040	-0.011
20-Oct-22	0.031	0.007	0.086	-0.069	0.008	-0.030	0.033	0.059	-0.207	-0.011
<b>2021 Average</b>	0.005	0.024	0.008	-0.072	0.009	-0.118	0.030	-0.028	-0.100	0.024
<b>2022 Average</b>	0.046	-0.002	-0.008	-0.107	-0.006	-0.147	0.033	-0.010	-0.100	-0.011

Overall, corduroy alone was less effective in improving road permeability to water flow than it was with the addition of conduits. Conduits performed similarly, but log bundles were less effective than culverts or pipe bundles.

These results provided useful information regarding the selection of conduit types, as well as inferences regarding size and spacing.

## LESSONS LEARNED

### Greenfire Road Reclamation Study

Initial vegetation establishment on the reclaimed surfaces indicates that inoculation with moss fragments from suitable donor sites is an effective tool for revegetating wetland reclamation surfaces.

The comparative vegetation outcomes on the mineral and organic substrate subsections of the road identify some trade-offs that may need to be considered with respect to peatland reclamation planning, specifically with respect to topsoil salvage and management. Revegetating a mineral surface necessitates using fen species because of the neutral to alkaline pH of the substrate. Doing so appears viable with inoculation with appropriate donor species propagules. Furthermore, less excavation and less movement of materials are required when revegetating a mineral substrate left in place versus an organic substrate applied to mineral fill excavated to below the final target elevation. Therefore, it may be preferable to leave more fill in place and revegetate the surface with fen vegetation.

Starting the restoration with fen vegetation results in a long delay in the establishment of bog vegetation (if that is the final reclamation target) because of the time required for vegetation succession from fen to bog. Bog species adapted to acidic soil conditions will not establish until sufficient depth of peat can accumulate to reduce the



influence of alkaline fill on the vegetation community. This ecological pathway to bog vegetation can take a long time depending on fill chemistry, moisture regime, initial vegetation established, climate, and other factors.

Establishment of peat vegetation directly on a mineral substrate may be more sensitive to moisture conditions because the mineral substrate may be less able to mediate moisture conditions. This could leave it more prone to flooding, drying, and with reduced water storage than compared to an organic substrate. Additional moisture management is likely required on revegetated mineral substrates to a greater extent than organic substrates. Alternatively, bog vegetation can immediately be established on a peat substrate of appropriate pH.

There is a concern that a seed bank of undesirable species could accumulate in peat that has been stockpiled for reclamation use and could potentially interfere with bog species development by producing competitive non-target communities. Undesirable species seeds accumulating in peat stockpiles could include local native species from different ecosites, as well as non-local or invasive species introduced over time depending on the location of the stockpile and overall vegetation management on a given site.

Organic substrate depth is also a consideration, since too shallow a substrate may require the same moisture management interventions as a mineral substrate. The additional costs related to starting restorations with bog vegetation on a peat substrate versus mineral substrate (extra excavation and movement of stockpiled materials) may not be worthwhile if bog establishment is delayed by competition from undesirable vegetation, or the organic material is too shallow to be effectively different from a mineral substrate.

### **From Dirt to Peat**

Successful wetland reclamation is a reasonable expectation based on the successful establishment of peatland vegetation and peat accumulation on the range of sites and practices studied. Inoculation with moss fragments from donor sites increases the likelihood of revegetation success and increases similarity between reclaimed sites and adjacent peatlands. Peatland vegetation establishment is equally viable on mineral or peat substrate, but a mix of substrates (both types present, as opposed to blended) increases abundance of peatland species. Inoculation with moss fragments from donor sites hastens peat accumulation.

### **Pad TT Road Construction Best Practice**

All conduits, as well as corduroy contributed to increasing permeability of the road to water flow. However, corduroy alone was less effective than corduroy with the addition of a conduit closer to the ground surface. While conduits were somewhat similar in effectiveness, log bundles seemed less effective than culverts or pipe bundles. Reduced effectiveness of log bundles is likely because flow is limited to the spaces between logs, whereas pipe bundles have the spaces between pipes as well as the open tubular space to accommodate flow. Culverts of course are completely open with very little resistance to flow.

Conduits were most effective at higher water table conditions, where the water table could reach the elevation of the conduit to access the openings and flow through. This limitation impeded subsurface water flow, causing it to build up on the upstream side of the road. Therefore, additional subsurface drainage capacity needs to be integrated into road designs to address subsurface flow. Such designs could include larger diameter culverts, stacked culverts or culvert pyramids, larger pipe and/or log bundles, additional log layers in the corduroy, or rock fill foundations.





All the conduits examined are useful in promoting road permeability, but choice of conduit type and placement should be made based on enhanced assessment of peatland hydrologic conditions prior to road construction. Since most road drainage designs rely primarily on models of surface flow, assessment of subsurface flows should be integrated into drainage designs. Conduit type, size, depth, and spacing could then be selected based on a better estimate of water volumes required to be moved in conjunction with cost and availability of conduit materials.

## PRESENTATIONS AND PUBLICATIONS

No presentation or articles were published in 2022. Several presentations and publications are expected in 2023.

## RESEARCH TEAM AND COLLABORATORS

Institution: Circle T Consulting, Inc.

Principal Investigator: Terry Osko

Name	Institution or Company	Degree or Job Title	Degree Start Date (Students Only)	Degree Completion Date (Students Only)
Line Rochefort	Université Laval	Professor of Peatland Ecology		
Bin Xu	NAIT Centre for Boreal Research	NSERC Industrial Research Chair in Peatland Restoration		
Melanie Bird	NAIT Centre for Boreal Research	Senior Research Technician		
Clayton Gillies	FPInnovations	Senior Researcher		
Christine Isabel	Université Laval	MSc	2019	2021
Pascal Guérin	Université Laval	MSc	2019	2021

Research Collaborators: Dr. Maria Strack, University of Waterloo

# Assessing the Ecological Potential of Wetlands and Surrounding Reclaimed Pads Field Plot

**COSIA Project Number:** LE0078

**In Situ**

**Research Provider:** Vertex Professional Services Ltd.

**Industry Champion:** Suncor

**Industry Collaborators:** Canadian Natural, Cenovus, ConocoPhillips, Imperial, Syncrude

**Status:** Year 1 of 2

## PROJECT SUMMARY

In 2021, Canada's Oil Sands Innovation Alliance (COSIA) retained Vertex Professional Services Ltd. (Vertex) to conduct a pilot of a proposed study of padded conventional oil and gas wellsites with the goal of helping to inform in-place reclamation of in situ pads. The objectives of the proposed study are to:

1. Determine if conventional oil and gas pads reclaimed in place without a padded access road have altered water and vegetation properties of the surrounding peatland
2. Determine key characteristics of pads that can be used to predict if the surrounding peatland may be impacted
3. Determine peatland characteristics that make these wetlands more susceptible to impacts associated with wellpads reclaimed within.

The objectives of the pilot study were to evaluate if the proposed experimental design and methods will address the study objectives.

Three sites were included in the pilot study with each site located in a different peatland type: bog, poor fen, and moderate-rich fen. The pad and surrounding peatland were characterized to address the study objectives. Measurements of the pad included the thickness, underlying peat thickness and physical and chemical properties of the pad. Measurements of water depth, water chemistry, vegetation composition and tree density and growth were taken in the peatland surrounding the pad at various distances and positions relative to the pad and at a reference area.

## PROGRESS AND ACHIEVEMENTS

The pilot study was completed in 2022.



## LESSONS LEARNED

The pilot study (three sites) suggests that while there are differences in the water and vegetation characteristics of the peatland immediately surrounding each of the pads, the differences likely do not extend more than 50 metres from the edge of the pad. The magnitude of the differences is small and likely has not affected the overall function of the peatland.

Changes in water and vegetation characteristics were often more pronounced downstream of the pad than upstream. This was generally consistent for all three site locations (bog, poor fen, and moderate-rich fen)

The methods investigated here have been deemed capable of detecting changes or a lack of change vis-a-vis a statistical power analysis. It is recommended that slight modifications to the plot layout are made for the full study and reference areas are not sampled.

Completing a full study will be advantageous to validate the findings of the pilot study on a broader scale and to address Objectives 2 and 3. The pilot study demonstrated that reclamation of a pad in place is possible with minimal impact to the surrounding peatland, and completion of the study has the potential to allow for the selection of the best ecological and economical reclamation option for in situ pads — be it full removal, partial removal or reclamation in place.

## PRESENTATIONS AND PUBLICATIONS

No presentations or publications in 2022.

## RESEARCH TEAM AND COLLABORATORS

Institution: Vertex Professional Services Ltd.

# Boreal Wetland Reclamation Assessment Program (BWRAP): Industrial Research Chair in Oil Sands Wetland Reclamation

**COSIA Project Number:** LE0037

**Mine and In Situ**

**Research Provider:** University of Calgary

**Industry Champion:** Suncor

**Industry Collaborators:** Canadian Natural, Cenovus, ConocoPhillips, Imperial, Syncrude, Teck

**Status:** Year 3 of 5

## PROJECT SUMMARY

The natural landscape of the Athabasca Oil Sands (AOS) region is dominated by wetlands and peatlands. Following the completion of mining activities, reclaiming these landscapes requires ongoing innovation to continue to develop operational best practices for reconstructing forests and wetlands to achieve equivalent land capability. While industry is creating new wetlands in the reclaimed landscape, more work is required to evaluate the success of these efforts and to guide adaptive management. The scientific and technical expertise needed to develop measures of success is being enabled by the Industrial Research Chair (IRC) program — the *Boreal Wetland Reclamation Assessment Program* (BWRAP), led by Dr. Jan Ciborowski.

Dr. Ciborowski's Senior Natural Sciences and Engineering Research Council of Canada (NSERC)/COSIA Industrial Research Chair in Oil Sands Wetland Reclamation was established on April 1, 2020, with support from NSERC, COSIA's oil sands industry partners, and the University of Calgary, to address the issues associated with wetland reclamation following bitumen mining in the AOS region. Ciborowski's research program is developing and testing the *Reclamation Assessment Approach*, a transformational methodology to characterize and assess the ecological condition of young wetlands in AOS reclamation landscapes and to ultimately enable industry to better reclaim land and promote wetland persistence and biodiversity as systems undergo succession.

BWRAP is addressing the following questions:

1. How can industry best predict the early development, biodiversity, and persistence of wetlands in a reclaimed landscape?
2. What environmental or biological indicators best reflect long-term resilience and/or persistence in young wetlands?
3. What reclamation features will promote young wetlands' formation, resilience, and persistence?

COSIA members require an assessment of the effectiveness or 'functionality' of constructed wetlands against reclamation targets. Several attributes are recognized as either modulators or indicators of a wetland's successional state or its environmental or biological condition. This program is measuring a suite of environmental and biological





characteristics of newly formed and maturing wetlands and their surroundings, in order to document the range of natural variation. These ranges will form the basis of comparison against which to assess the ‘success’ of constructed wetlands in the post-mining landscape and by which to determine if adaptive management may be required. The following wetland features are recognized as being important measures of ecological condition:

**Time to Recovery:** Recovery rates of wetlands vary primarily with respect to wetland size. In a meta-analysis of 621 globally-distributed wetland sites, Moreno-Mateus et al. (2012) reported that hydrological features become similar to reference values and vertebrate and macroinvertebrate species recolonize within five to ten years. In contrast, community composition and biogeochemical processes had not fully recovered after 50 years. Further, the rate of recovery was strongly related to wetland area: biological structure in wetlands greater than or equal to 100 hectares (ha) become similar to reference wetlands within five years of reclamation. Perhaps counterintuitively, the meta-analysis found that created wetlands became similar to reference wetlands much more quickly than restored wetlands.

**Water quality Influences:** Water quality constrains the abundance and composition of wetland biota. Most undisturbed wetlands in the AOS have low conductivity, but natural seeps increase salinity and contain halophilic communities. Wetlands forming in saline-sodic overburden storage areas on oil sands leases are also saline enough to influence community composition. Some biota may tolerate higher salinity from natural or runoff sources, possibly due to interactions of the latter with residual bitumen-extraction byproducts.

**Landscape and Microtopography Influences:** Wetland persistence depends on receiving and maintaining an adequate water supply. Evapotranspiration often exceeds precipitation in the AOS, emphasizing the need to trap and store water during precipitation events. Constructed wetlands have been hypothesized to need at least a two- to-one ratio of watershed to wetted area for precipitation to sustain fen habitat in the AOS (Price et al., 2010). Land disturbance (altered forest cover, soil, or drainage pattern) is also a key stressor. For example, roads and culverts alter both hydrology and habitat use by biota. Wetland geometry (e.g., slope, emergent zone width, microtopography) influences the abundance, richness, and distribution of aquatic communities.

**Permanence:** Marsh-like wetlands are a focus of AOS reclamation because they are persistent and relatively easy to construct. However, seeps and naturally-forming minerotrophic wetlands comprise 10% to 17% of the surface area of reclaimed areas (Little- Devito et al., 2019; Hawkes et al., 2020), leading to questions of the determinants of where ‘opportunistic’ wetlands occur and the extent to which they match prescriptions and predictions.

**Biological indicators of wetland condition:** No integrated criteria exist to assess the overall effectiveness of wetland reclamation for the mineable AOS, despite extensive surveys and adoption of biological integrity indices from previous studies (vegetation, aquatic invertebrates, birds, amphibians), and a framework to assess toxicological risks (Arciszewski et al., 2017). Current wetland impact assessment initiatives designed to detect risks to mature off-lease wetlands (difference from wetlands in the Reference Condition Area [RCA]) are not necessarily applicable to young, constructed wetlands or to those formed opportunistically in reclaimed areas.

### **Overall Objective: Formulating a Reclamation Assessment Approach for oil sand reclaimed wetlands**

Since reference locations in the RCA focus on ‘climax’, a stable state, or a mosaic of successional states, recovering or newly reclaimed areas require a different frame of reference. The BWRAP is compiling data from suites of wetlands at early time points since their formation or creation. These data, essential as a frame of reference for assessing developing landscapes, are being collected and summarized to document the range of natural variation in indicator



variables for opportunistically-forming and reclaimed wetlands. Such information will inform guidelines that will determine whether adaptive management may be needed to achieve closure outcomes (maximize the likelihood of a wetland becoming functional and exhibiting the desirable ecological properties of natural systems).

Over the course of the three-phase, five-year BWRAP program, up to 120 candidate reclaimed wetlands (minerotrophic fens, swamps, and marsh-like areas) approximately three, eight, 20- and 40-years post-formation, and 'mature' (age-indeterminate), will be assessed. Some of these age-states are similar to those used for assessing upland forest stands in Alberta and broadly correspond to times since various pilot reclamation projects were undertaken by COSIA partners.

**Phase 1 — Recruiting and Database Creation:** The first phase entails compiling and harmonizing existing data — a 20-year record of research conducted on natural and reclaimed wetlands in and around the Fort McMurray oil sands leases. As well, remote sensing imagery of reclaimed lease areas and reference areas collected by the partner companies will be analyzed and used to create an inventory of the number, size, age, and permanence of the constructed and opportunistic reclaimed wetlands. A representative set of wetlands varying in age, size, permanence, disturbance history, and water quality will then be selected for field studies over the next three years (Phase 2).

**Phase 2 — Field Investigations:** Each year, teams of fieldworkers will assess a suite of approximately 40 wetlands (minerotrophic fens and swamps and marsh-like areas) using in situ instrumentation, field sampling, and drone surveys to assess wetland morphometry, water chemistry and balance, and riparian disturbance. The biological condition of each wetland will be characterized by surveying the communities of aquatic invertebrates, aquatic vegetation, and birds.

**Phase 3 — Data Compilation, Analysis and Synthesis:** During the third phase, the environmental data will be compiled to align the wetlands of different ages with respect to three gradients of environmental stress — permanence, water quality, and topographic heterogeneity (disturbance). Differences in the composition of biota among wetlands across each stress gradient will be used to identify benchmarks of biological characteristics (bioindicators) of each wetland age class, distinguishing 'acceptable', 'intermediate', and 'unacceptable' classes of wetland health. Successful wetlands will have environmental conditions and associated biota characteristics of 'acceptable' conditions for their successional stage of development. These features (and the landscape features that promote or sustain them) can be used to guide future reclamation protocols and ultimately provide objective criteria by which to anticipate the longer-term persistence of reclaimed wetlands.

## PROGRESS AND ACHIEVEMENTS

### Phase 1 - Recruiting and Database Creation

In 2022, working in collaboration with the Boreal Ecosystem Recovery and Assessment (BERA) NSERC Alliance-funded project, project researchers developed and beta-tested a georeferenced, relational database that is accessible by both on-campus and off-campus collaborators.

The database is housed on the University of Calgary virtual machine servers with an architecture designed to be compatible with information from the Alberta Biodiversity Monitoring Institute, with whom project researchers will



ultimately share data. Syncrude and Suncor have provided detailed imagery and mapping information from which various mapping products can be derived by team members.

The imagery of reclaimed lease areas and reference areas was used to identify suitable wetlands to sample during the 2022 field season. The success in locating 40 suitable study sites was greatly aided by recommendations from Syncrude and Suncor field staff and other local experts.

## Phase 2 - Field Investigations

In 2022, three new graduate students, four undergraduate students and several summer research assistants joined BWRAP and completed the field training necessary to begin their research in mine lease areas. In early May, lab members revisited the 40 wetlands first instrumented in 2021 to re-deploy data loggers. They subsequently located and sampled the open water, emergent vegetation, and wet meadow zones of an additional 40 young wetlands. Data loggers were moved from their spring position to the new set of wetlands in late June to record daily variations in water level, conductivity, dissolved oxygen, temperature, and photoperiod and were left in place until late September. Loggers were removed to avoid overwinter freezing.

The combined suite of 80 wetlands surveyed in 2021 and 2022 encompassed a broad range of ages (2 years to > 40 years), hydroperiods (ranging from ephemeral to permanent), salinities (specific conductance ranging from < 2,000 uS/cm to > 7,000 uS/cm) and disturbance types. Equal numbers of wetlands situated within and outside of mine-lease areas were sampled. However, the distribution of sample points along each stress gradient differed between the suite of wetlands sampled within versus those assessed outside of reclaimed areas. In particular, the most saline wetlands were more prevalent in reclaimed areas than in reference areas. Similarly, a greater proportion of wetlands in reclaimed areas had short hydroperiods than were found in reference areas.

The 2022 field data are being uploaded to the geodatabase and analyzed. However, preliminary summaries broadly corroborate the 2021 interpretations of archival data. Plant community richness and biomass of dominant species appear to increase with age, whereas community composition shows no clear pattern of change. Soil salinity has a complex influence on biomass. In the youngest wetlands (< 5 years), biomass was uniformly low across the salinity gradient. Yet, a salinity threshold was clearly apparent in older wetlands. Biomass at maturity increased as a function of age in low-conductivity wetlands but remained low in wetlands that had saline soils. Similar patterns are evident in the relative frequency of occurrence of three families of snails in the wetlands surveyed in 2022. They were absent in the youngest wetlands surveyed, and older saline wetlands (conductivity > 2,200 uS/cm) but were abundant in older, less saline wetlands.

Analyses of surface and groundwater radon concentration and isotopes of oxygen and deuterium clearly show that wetlands vary greatly in the proportion of water derived from the surface (precipitation, overland, and stream flow) versus groundwater sources. This information will contribute to an assessment of each wetland's resilience to interannual and seasonal variation in precipitation.

Surveys of vegetation provided estimates of community composition, biomass, and relative cover of the three zones. In all, over 160 species have been identified across the 80 wetlands. The dominant species (those with consistently greatest cover) were Blue Joint Grass (*Calamagrostis canadensis*), three sedge species (*Carex aquatilis*, *C. atherodes*,



and *C. utriculata*), Softstem Bulrush (*Schoenoplectus tabernaemontani*), and Cattail (*Typha latifolia*). Over 200 benthic macroinvertebrate samples were collected and preserved over the course of the summer. Processing and enumeration are in progress. Avian surveys were not conducted in 2022.

### **Phase 3 - Data Compilation, Analysis and Synthesis: State-and-Transition modelling to synthesize and interpret wetland development & persistence:**

Tasks/objectives are scheduled to be addressed beginning in 2023. Their implementation and the success of the objectives will entail and depend on:

- populating the database with archival and recently collected field data, including data anticipated for the third year of fieldwork;
- identifying ‘reference’, ‘degraded’ and ‘at risk’ biological community states relative to each gradient of environmental stress for four classes of wetland age;
- forming and convening workshops of a Created Wetland Research & Information Group Community of Practice to corroborate putative biological classes and their relationship to environmental gradients; and
- parameterizing state-and-transition models of wetland development for each biological attribute.

## **LESSONS LEARNED**

This program is still in the early stages, with only the first field season and part of the second season completed. Therefore, few conclusive statements can be made. One learning is that site selection is difficult because although recently collected imagery has excellent resolution, ground-truthing is still required to confirm the presence and status of each site as young wetlands are inherently variable. Current fieldwork has also found that many aquatic invertebrate taxa can occupy local natural wetlands with much greater salinity ranges than have been documented in preliminary studies of lease area landscapes.

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## PRESENTATIONS AND PUBLICATIONS

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## RESEARCH TEAM AND COLLABORATORS

Institution: University of Calgary

Principal Investigator: Dr. Jan J. H. Ciborowski

Name	Institution or Company	Degree or Job Title	Degree Start Date (Students Only)	Degree Completion Date (Students Only)
Camille Sinanan	University of Calgary	BWRAP Admin Manager		
Mir Mustafizur Rahman	University of Calgary	Post-Doctoral Fellow/ Research Scientist		
Jeremy Hartsock*	Southern Illinois University	Post-Doctoral Fellow		
Qing Ye (Richard) Zeng	University of Calgary	Post-Doctoral Research Associate –Programmer/ Developer		
Gillian Donald	University of Calgary	Research Scientist - State-and-Transition modeling; Community of Practice Co-Convener		
Zach Wang	University of Calgary	Research Assistant (Drones)		
Ian Perry	University of Calgary	Research Assistant (Drone pilot)		
Kwok Kei (Maverick) Fong	University of Calgary	Technician - Programmer/Developer		
Erik Biederstadt	University of Calgary	MSc	2021	2023
Amanda Luzardo	University of Calgary	BSc	2017	2022
Abisola Allison	Mount Royal University	BSc	2018	2022



Nika Tovchyrhechko	University of Calgary	Hon. BSc	2018	2023
Courtney Smith	University of Calgary	BSc (thesis)	2017	2022
Megan Mercia	University of Calgary	Hon. BSc	2016	2021
Elizabeth Gillis	University of Calgary	MSc	2021	2023
Ashlee Mombourquette	University of Calgary	MSc	2020	2022
Brenten Vercruysse	University of Calgary	MSc	2019	2022
Michael Wendlandt	University of Calgary	MSc	2020	2022
Steven Blair	University of Calgary	BSc	2015	2020
Elizabeth Gillis	University of Calgary	Hon. BSc (thesis)	2016	2021
Liam Mebesius	University of Calgary	Hon. BSc (thesis)	2016	2021
Emily Moore	University of Calgary	Hon. BSc (thesis)	2016	2021
Manveet Waraich	Mount Royal University	BSc	2018	2022
Matthew Ellis	University of Calgary	BSc	2018	2022
Genevieve Rodrigues	University of Calgary	BSc (thesis)	2019	2023
Maeve Corcoran	University of Calgary	BSc (thesis)	2019	2023
Kyle Filyk	Mount Royal University	BSc	2020	2024
Sean Leng	University of Windsor	BSc	2019	2023
Hannah Porter	University of Calgary	MSc	2022	2024

\* Travel restrictions associated with the COVID-19 pandemic prevented Dr. Hartsock from travelling to Canada and resulted in his leaving the program at the end of August 2020.



## Research Collaborators:

The following collaborators indicated their willingness to participate in the program as envisioned during the proposal phase of the research plan. The timing and extent of collaboration will vary according to the stage of research and the individuals' expertise. New collaborations have developed since the start of the program. These collaborators are marked with an \*.

Name	Institution or Company	Role/Expertise
Greg McDermid	Geography, University of Calgary	Remote sensing (BERA program)
Laura Chasmer	Geography, University of Lethbridge	Wetland ecosystem change detection
Kevin Devito	Biological Sciences, University of Alberta	Landscape controls on boreal ecohydrology
Alice Grgicak-Mannion	Earth Sciences, University of Windsor	Disturbance mapping and analysis
Bernhard Mayer	Geosciences, University of Calgary	Stable isotope analyses
Leland Jackson	Biological Sciences, University of Calgary	Nutrient and water chemistry analyses
Jean Birks	InnoTech, Alberta, Calgary	Isotope techniques to quantify water balance
Christopher Weisener	School of Environment, University of Windsor	Microbial controls on wetland biogeochemistry
Dale Vitt	Biological Sciences, S. Illinois University	Wetland succession and biogeochemistry
Rebecca Rooney	Biological Sciences, University of Waterloo	Bioindicator development; Fuzzy Cognitive Mapping; State and transition modelling
Lee Foote	Renewable Resources, University of Alberta (emeritus)	Community structure and bioindicators
Colin Daniel	Apex Resource Management Solutions	Wetland state and transition modelling
Leonardo Frid	Apex Resource Management Solutions	Wetland state and transition modelling
Jabed Tomal	Thompson Rivers University	Statistical modelling
Erin Bayne*	Biological Sciences, University of Alberta	Cumulative ecological impacts of human activities on biodiversity
Peter Dunfield*	Biological Sciences, University of Calgary	Aquatic Microbial Ecology
Virgil Hawkes*	LGL Ltd.	Wildlife & habitat assessment of reclaimed landscapes
John Headley*	Nati'l Hydrol. Res. Ctr (NHRC) Envir. Climate Change Canada	Analytical Chemistry of acid extractable organic compounds
Felix Nwaishi*	Earth & Environmental Sciences, Mount Royal University	Role of plant-soil processes in regulating ecosystem functions
Faramarz Samavati*	Computing Science, University of Calgary	Computer/spatial modelling of wetland persistence

## Evaluating the Success of Fen Creation — Phase 2

**COSIA Project Number:** LJ0098

**Mine**

**Research Provider:** University of Waterloo

**Industry Champion:** Suncor

**Industry Collaborators:** Teck, Imperial

**Status:** Year 5 of 5 (field) and Year 5 of 7 (lab)

### PROJECT SUMMARY

The overall goals of Phase 2 of the Evaluating the Success of Fen Creation Project are i) to evaluate the longer-term trajectory of the constructed Nikanotee Fen (NF) watershed; and ii) to develop alternate wetland watershed designs and strategies suitable for a closure landscape. This project will provide an ongoing assessment of ecosystem function and development, using empirical manipulation experiments as well as develop conceptual and numerical models of the system performance under the constraints of the current design for various climate cycles and trends. These conceptual and numerical models will be used to test and recommend new fen wetland designs for integration with other constructed landscape units at the scale of closure landscapes.

The Phase 2 Project has three objectives:

1. Ongoing assessment of NF ecosystem functions: Under a range of climatic conditions, evaluate the NF performance relative to natural reference ecosystems, and provide a database to demonstrate its suitability for reclamation certification.
2. Assess how changes to soil and vegetation form and function affect system trajectory: To project the trajectory of the NF it is important to understand how placed materials have evolved over the first five to 10 years (per the above objective). The rates and processes observed over time are needed to parameterize the numerical modelling of hydrology and solute transport, the output of which is needed to apply conceptual models of biogeochemical and ecological functions including carbon dynamics and plant community development.
3. Use numerical and conceptual models to evaluate alternative design applicability to closure landscape scales. Numerical models of NF hydrology and solute transport validated using field data will be used to understand how design modifications to the closure landscape can improve system function and performance. Design optimization will involve consideration of improvements to contaminant management and water use by different landscape elements.



## PROGRESS AND ACHIEVEMENTS

### Evaluate soil development and runoff from reclaimed slopes and upland at NF, since inception

Sodium ( $\text{Na}^+$ ) concentrations in ponded surface water were found to be more responsive to rainfall-induced dilution and evapo-concentration, whereas in the fen subsurface vadose zone, they were insensitive to meteorological conditions. Surface water discharge controlled the mass efflux of  $\text{Na}^+$  from the system — calculated daily seasonal average for sodium efflux was  $4 \text{ kg day}^{-1}$  between the peak growing season of June to August. Measurements of ponded surface water and sub-surface ( $< 35 \text{ cm}$  below ground surface) at the fen found  $\text{Na}^+$  was  $277 \pm 131 \text{ (mg L}^{-1}\text{)}$ . Given the large mass of sodium present within the tailings sand aquifer that is migrating towards the fen, compared to the amount exported from the fen in surface water outflow, elevated sodium concentrations in the fen will be sustained for several decades in line with modelling predictions.

### Characterize plant functions in relation to solute concentrations in the rooting zone

Greenhouse experiments investigating effects of salinity, water table and competition on *Carex aquatilis* and *Juncus balticus* growth and function were completed and found that water table and salinity interactions were dominant in determining growth and function outcomes. There was evidence of additional salinity stress on *Juncus* when water tables were near surface ( $\sim 5 \text{ cm}$ ) at sodium concentrations more than  $2,300 \text{ mg/L}$ . Analyzed data from 2021 field trials found no measurable effect in measured sodium concentrations in the NF ( $113 \text{ mg/L}$  to  $238 \text{ mg/L}$ ) on growth, including above and belowground tissue production on *Juncus* vegetation.

### Solute and soil interactions

Analysis completed on prior field measurements of hydraulic conductivity in the tailings sand of the NF, demonstrated the largely homogeneous nature of the upland aquifer. Hydraulic conductivity measurements were conducted using a variety of methods (laboratory cores, single-well response tests, and tracer test), across several spatial scales. Across sample volumes that varied five orders of magnitude, average permeability varied by less than 15%.

A field-scale tracer test showed that the dispersivity (an intrinsic property of porous media that describes the spreading of a solute), functions like other sand aquifers. However, it also indicated that the asymptotic dispersivity (the stable dispersivity that no longer increases with further displacement) was obtained in a far shorter transport distance ( $< 20 \text{ m}$ ). A further indication of the homogeneity of coarse tailings sand deposits.

Given that coarse tailing sand experiences homogenization during the bitumen extraction process (due to particle-size segregation), and again when it is placed at a reclamation site with heavy earth-moving equipment, other coarse tailings sand landforms could be expected to have similar dispersivity and asymptotic distances.

### Controls on water use efficiency and biogeochemical cycling

Analysis of seven years of measured  $\text{CO}_2$  and  $\text{H}_2\text{O}$  fluxes in the NF watershed demonstrates that the ecosystem function during early development was driven by plant establishment. The fen evolved from a carbon source to sink within the first three years following construction. Net ecosystem exchange (NEE)  $70 \text{ g C m}^{-2}$  in 2013 to  $-244 \text{ g C m}^{-2}$  in 2015. Ranged between  $-123$  to  $-244$  from 2015 to 2019. Upland:  $519 \text{ g C m}^{-2}$  in 2013, decreased to  $46 \text{ g C m}^{-2}$  in 2019.



Stable water use efficiency (WUE) in dry years has been indicative of ecosystem resilience to periodic water stress with the overall ecosystem performance comparable to natural, undisturbed sites within the region.

Assessed multiyear peak growing season variability of WUE at four fens (poor treed, poor open, treed moderate-rich, open saline) near Fort McMurray along with a set of environmental variables. Freshwater fens were characterized by WUE values within the range reported from other boreal wetlands while a saline fen had significantly lower values of WUE. Moisture conditions were responsible for interannual differences in WUE, whereby increasing WUE under wetter conditions was observed. However, such a pattern was offset by decreased air temperature resulting in moisture oversupply.

### Nutrient sources for plant productivity

The reclaimed fen is located within an active industrial site susceptible to nitrogen (N) deposition. Areas of highest N deposition coincide with the spatial pattern of deposition loads — i.e., closer to a haul road from where haul truck emissions originate. Dominance of fast-growing graminoids in the NF suggest that N loading could limit vegetation diversity and modify the ecological trajectory of the wetland. Although the fen received approximately 8.5 kg N ha<sup>-1</sup> yr<sup>-1</sup> and the upland received approximately 6.2 kg N ha<sup>-1</sup> yr<sup>-1</sup> (greater-than-background N deposition levels for the region, approximately 1 kg N ha<sup>-1</sup> yr<sup>-1</sup>), N deposition does not appear to currently be increasing the N availability or dynamics of the soil-water-vegetation continuum of either landscape unit. Ecosystem indicators within the soil-water-vegetation continuum suggest that the fen-upland is currently N-limited.

### Variations in dominant plant cover

Field measurements of *Juncus* productivity on the new peat deposit areas indicated that the species had established and was doing well. Samples of plants, soils, and water were collected from NF in 2021 and replicated in the saline fen in 2022 to assess the effects of intra- and interspecific competition on growth with a focus on *Carex aquatilis*, *Juncus balticus*, *Triglochin maritima*, *Typha latifolia*.

Post-doctoral fellow Goud investigated whether variation in dominant plant cover could be caused by competition or environmental stress (water and salt). This is being achieved via a paired greenhouse and field experiment to determine the relative roles of inter- and intraspecific competition and stress responses.

The field study was expanded to include seaside arrowgrass (*Triglochin maritima*) and cattail (*Typha angustifolia*). Researchers identified focal plants growing either with members of their own species (intraspecific competition treatment) or in diverse communities (interspecific competition treatment) and measured their growth and traits (described below). To identify mechanisms underlying variation in growth across treatments, they measured leaf and root stable isotopes of carbon ( $\delta^{13}\text{C}$ ), oxygen ( $\delta^{18}\text{O}$ ), and sulphur ( $\delta^{34}\text{S}$ ) to integrate physiology, as well as tissue ion concentrations (e.g., Na<sup>+</sup>) and morphological traits (e.g., height, leaf area).

Results from the greenhouse experiments found species-specific responses, with *Carex* growing best under wet, low-salt conditions, displaying strong intraspecific competition. *Juncus* grew best in dry, low salt conditions where it was able to outcompete *Carex*. *Juncus* was able to maintain similar growth across variable treatments, except when it was outcompeted under wet, low-salt conditions where *Carex* had the largest rates of growth. These results fit with the observed growth patterns at the NF where the *Juncus* is dominant at the drier edges, and *Carex* dominates the wetter areas of the fen.





Both species showed the lowest growth rates under high salt conditions, regardless of water availability. Growth variation was driven by metabolic tradeoffs related to water use and salt tolerance, as measured by stable isotopes, and salt avoidance traits measured by tissue chemistry. *Carex* appears to respond to water and salt stress by adjusting leaf physiology to limit water loss while *Juncus* adjusts allocation to root biomass and salt sequestration. Although *Carex* may be a superior competitor under favourable conditions, increasing salinity and water limitation reduce *Carex*'s growth and competitive ability. High salt concentrations may continue to limit productivity at constructed fens, although *Juncus* is likely to persist and possibly increase over time. These results highlight the importance of species choice and competitive interactions in restoration outcomes in the face of dynamic environmental stress.

### Microbial communities and biogeochemical functions in upland and fen

Soil cores collected in the summer of 2021 from across the constructed fen and the poor and saline fen reference sites were 16S rRNA sequenced to describe the microbial community. Metagenomic analyses were completed on the same cores to provide additional detail on community composition and function.

Current work is focusing on the methane cycling community and will link to field fluxes and environmental conditions. There was a low relative abundance of methane producers at the NF compared to the reference fens, but an abundant methane oxidizer community. Many methane oxidizers were also capable of sulphate reduction, supporting the links between high sulphate conditions in Nikanotee and the low methane fluxes. This provides a dataset that can be used to assess the whole microbial community composition and focus on other functions (e.g., nitrogen cycling). A phylum *Desulfobacterota* was present (approximately 10% of the total community) in the peat soils from 30 cm to 35 cm depth lined above either *Typha* or *Carex* vegetation. *Desulfobacterota* was characterized by heterotrophic growth capacity, which means they are utilizing sulphate compounds (i.e., thiosulfate etc.), and thus low sulphate concentrations were reported in the areas where *Typha* or *Carex* type of vegetation is growing well. Larger differences were observed when comparing above the water table (WT) and below the water table peat samples, whereas no such differences were found between constructed and reference sites. However above water samples were characterized by stronger dominance of members of the phylum *Proteobacteria* (approximately 60% of the total community) and *Bacteroidota* (approximately 20% of the total community), but desulfobacteria were almost absent. These results demonstrate the linkage between microbial community structure, geochemical gradients, and dominating vegetations (i.e., *Juncus balticus*, *Typha latifolia*, *Carex aquatilis* was established at two depths in the peat profile).

### Simulations incorporating modifications of the initial design, including the addition of layering, altered system geometry

Groundwater flow and sodium transport were simulated for 2013 to 2050 at the NF watershed using MODFLOW-SURFACT. Model validation demonstrated good performance when compared to observed flux, head, and sodium concentration data. Simulations indicate that the constructed fen will maintain a high, stable water table, even under dry climatic realizations. Sodium concentrations at the surface of the fen will rise for 12 years to 22 years post-construction, depending on the prevailing climatic conditions.

Surface sodium concentrations in the fen exceeded salt stress thresholds for mosses after only three to four years post-construction. These geochemically inhospitable conditions will continue for several decades. Average surface



concentrations across the fen will peak between 600 mg/L to 900 mg/L, which means that the majority of the fen will likely remain amenable to the growth of salt-tolerant grasses and sedges like *Carex aquatilis* or *Juncus balticus*.

This predictive model strongly suggests that the Nikanotee Fen will continue to function in a manner supportive of fen eco-hydrological processes. Salinity that arises from the inclusion of process-affected tailings sand will not prevent representative fen vegetation species from proliferating, although mosses will be inhibited in the short to medium term.

### Energy and moisture exchanges during seven years post-construction

Surface conditions evolved from bare ground to robustly vegetated plant communities resulting in altered biophysical surface properties such as albedo, surface roughness and increased plant-mediated shading, ultimately affecting energy partitioning and evapotranspiration (ET) rates. Results from the simplified land cover classification found:

Surface water covered 47% and 41% of the fen surface in 2013 and 2014, respectively. The remainder of the surface was dominated by wet, dark, bare peat (53% in 2013) or a mix of bare peat and juvenile vegetation (18% bare, 41% mixed bare + veg in 2014). By 2015, fen vegetation had become widespread (62%). From 2016 onwards, the fen surface is dominated by full plant coverage (ranging from 53% to 68%) which fluctuated depending on environmental and meteorological conditions (e.g., wetter (2018) versus drier (2017) years).

The NF upland saw similar trends. Bare ground decreased over time from greater than 95% in 2013, to 34% in 2016. From 2014 to 2016, upland surface conditions consisted of regions with varying degrees of mixed bare ground and plant coverage. By 2017 there were regions of full plant coverage (22%), followed by an increase in 2018 to 2019 to almost complete vegetation coverage (77% and 87% respectively).

These surface changes due to plant emergence and growth were mirrored in measured albedo values with the fen and upland exhibiting contrasting values during bare ground conditions (0.9 [dark-coloured peat] and 0.25 [light-coloured sand] respectively). As vegetation cover expanded, mean seasonal albedo values shifted in both landscapes and ranged between 0.12 - 0.14 (fen) and 0.16 - 0.18 (upland) from 2017 to 2019.

Adjusted values for total seasonal evapotranspiration in the fen ranged between 296 mm and 452 mm and decreased over time as the system developed. The highest seasonal rates occurred in 2014 and 2015 with 424 mm and 452 mm, respectively, while the lowest season total occurred in 2019 (296 mm). In the early years (2013 to 2015) ET exceeded or was equal to potential evapotranspiration (PET), whereas, from 2017 onwards, ET was less than PET. Fen total ET exceeded total seasonal precipitation in six out of seven study years. Temporally, there was a decrease in daily ET rates, with mean daily rates in the early years (2013 to 2015) ranging between 3.6 mm to 3.8 mm day<sup>-1</sup>, in contrast to later years (2016 to 2019) with 2.5 to 2.9 mm day<sup>-1</sup>. Within the upland, adjusted values for seasonal ET remained relatively constant throughout the study period, with only a slight increase from 2013 to 2019. However, although seasonal totals were similar across all years intra-seasonal/diurnal trends changed as the system developed. For example, early season (May) ET totals increased from an average of 25 mm (2013 to 2015) to 37 mm (2017 to 2019). Mean daily ET rates ranged from 1.2 mm day<sup>-1</sup> (2016) to 2.2 mm day<sup>-1</sup> (2018). In contrast to the fen, upland ET was consistently less than PET suggesting strong surface controls.



Initially in the fen, due to the near-surface water table and high soil moisture content, and the dark nature of bare peat (albedo 0.09), surface evaporation was high, and the majority of energy was partitioned to latent heat ( $Q_e$ , 91%; Bowen ( $\beta$ ) < 1) driven largely by abiotic factors (available energy and water availability) with a high degree of decoupled surface-atmosphere conditions ( $\Omega = 0.49$ ). These results agree with the literature regarding decoupled systems, where  $\Omega$  is closer to 1 and available energy controls evapotranspiration (McNaughton and Jarvis, 1991) and where wetter environments are typically more uncoupled (Knauer et al., 2018; Runkle et al., 2014). As the plant community developed and stabilized,  $Q_e$  remained the dominant energy flux (50% to 70%), but a larger proportion of available energy was partitioned to sensible heat flux ( $Q_h$ ) than in previous years. Effects of plant-mediated sheltering and shading decreased surface evaporative losses and there was an increase in surface-atmosphere coupling ( $\Omega = 0.32$ ), particularly during drier periods (2015, 2017).

The NF upland environment is dry, and plant emergence and establishment were slower than in the fen. Initially, bare ground conditions in the upland resulted in higher albedo (0.20 to 0.25), and  $Q_e$  and  $Q_h$  made up almost equal parts of energy partitioned (45 % to 55%,  $\beta > 1$ ). Once understory and treed species matured,  $Q_e$  increased with seasonal trends that mirrored plant phenology. Unlike the fen, the drier upland continually exhibited a higher degree of surface-atmosphere coupling ( $\Omega$  ranging between 0.23 to 0.3), where  $\Omega$  values closer to 0 indicates a well-coupled ecosystem, where evapotranspiration is typically constrained by stomatal controls on transpiration and affected by vapour pressure deficits. Like the fen, these findings agree with existing literature. The upland is a water-limited environment, and aerodynamically rougher ecosystems (treed vegetation), both of which in the literature tend to exhibit a higher degree of coupling (Knauer et al., 2018; Runkle et al., 2014).

While mean seasonal  $\beta > 1$  there was a decrease in magnitude compared to earlier measurement years as well as seasonal trends that mirrored plant phenology (e.g., lower  $\beta$  during peak leaf out)

Notes on vegetation-atmosphere decoupling ( $\Omega$ ): values range between 0 and 1, calculated using  $g_a$ ,  $g_s$ , slope of the vapour pressure curve and psychrometric constant.

## LESSONS LEARNED

- Ecosystem-scale remote sensing techniques such as land use/land classification (e.g., bare ground versus vegetated surface cover and enhanced vegetation index [EVI]), hold potential as valid, inexpensive monitoring tools for reclamation monitoring.
- When considering water use efficiency and carbon dioxide fluxes, the constructed fen watershed's ecosystem performance is comparable to natural, undisturbed sites within the Athabasca Oil Sands Region that were measured as part of the natural reference fen network.
- Groundwater flow and sodium transport simulations to 2050 show that the Nikanotee fen will maintain high, stable water tables, even under dry climate scenarios. Surface sodium concentrations at the fen will rise for 12 years to 22 years post-construction, depending on the prevailing climatic conditions and have exceeded salt stress thresholds for mosses after only three to four years post-construction. Bryophyte geochemically inhospitable conditions will continue for several decades. Average surface concentrations across the fen will peak between 600 mg/L to 900 mg/L, meaning the fen will remain amenable to the growth of salt-tolerant grasses and sedges like *Carex aquatilis* or *Juncus balticus*. Fen ecohydrological processes should therefore continue. Salinity that arises from the inclusion of process-affected tailings sand will not prevent representative fen vegetation species from proliferating, although mosses will be inhibited in the short-medium term until sufficient flushing has occurred. This offers industry some direction on delaying moss-transfer salvage and monitoring surface sodium concentrations if bryophytes are desired in reclaimed wetland areas.



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## **SOILS AND RECLAMATION MATERIAL**



## Topsoil Replacement Depth Study

**COSIA Project Number:** LJ0335

**In Situ**

**Research Provider:** NAIT Centre for Boreal Research

**Industry Champion:** ConocoPhillips

**Industry Collaborators:** CNOOC Petroleum North America ULC

**Status:** Year 2 of 5

### PROJECT SUMMARY

Approvals issued for in situ facilities under the Alberta Environmental Protection and Enhancement Act typically require operators to place a minimum of 80% of site pre-disturbance topsoil to ensure that the entire area has a uniform placement of topsoil during reclamation. The topsoil depth target of 80% relative to pre-disturbance native soil depth is also part of the 2010 reclamation criteria for well sites and associated facilities.

The application of approaches developed under an agricultural context often results in reclaimed areas being uniformly capped with topsoil, however, heterogeneity in placement depth is more desirable and similar to a natural forest system. Though heterogeneity of both site and plant community targets are acceptable and even desirable goals in the 2010 reclamation criteria — and the guidelines allow for stratification during certification assessments — no guidance is provided on how to achieve these goals. The ability to vary soil-cover design depths also has implications for optimizing the placement of available topsoil where the objective is to achieve the best reclamation outcomes across multiple sites where some may be locally deficient in available topsoil. In addition, depending on the target forest ecosystem and plant community desired, a thick placement of topsoil may be counterproductive as individual species may be more, or less well suited to richer soil conditions. Ongoing work on an interim reclamation study of subsoil and topsoil supports this notion ([LJ0226 Interim Reclamation](#)).

There has been significant interest in cover soil placement depths in mining (i.e., Farnden et al., 2013), but less attention has focused on the evaluation of recommended topsoil capping depths (80% threshold target) for smaller-scale industrial disturbances such as those at in situ and conventional oil and gas sites. This study encompasses four trials aimed at investigating both the effect of limited capping depths on forest establishment (Trial 1), as well as alternative approaches to mitigate for potential limitations associated with shallow topsoil capping on industrial disturbances in the boreal region (Trials 2 to 4). The study site is a former gravel pit, approximately 15 hectares in size, which allowed for sizeable plot installations with replication of treatments.

The specific context and objectives of each trial are described below:



### **Trial 1: Effects of topsoil replacement depth on forest establishment**

The purpose of this investigation is to evaluate the effect of capping depth on forest regeneration and soil properties. This trial comparatively evaluates three topsoil capping depth targets (no topsoil, shallow [5 cm] and standard [15 cm]) in a randomized complete block design. Recognizing that there will be variation around these targets, a ground survey was also conducted to confirm realized placement depths.

A lack of native seed propagules as well as early invasion by non-native species are two potential constraints with limited (or no) topsoil placement. This trial will attempt to mitigate these concerns using two approaches.

First, numerous plant species were planted (at a density of 4,800 stems per hectare) to evaluate species-specific survival and growth responses. In addition, intentional planting of native forbs specifically was accomplished through hitchhiking with jack pine (*Pinus banksiana*) or white spruce (*Picea glauca*), since this planting prescription may be beneficial to increasing the initial coverage and diversity of native understory species. Hitchhiking, in the plant context, is a nursery stock production treatment whereby two plant species are co-grown in the same nursery container. This approach has been tested previously with white spruce and multiple native forbs (Mathison 2018, Hudson 2020) and this study applied the same principles in terms of seedling production (timing of sowing the forb after the conifer) to hitchhiking jack pine with native forbs.

The second approach was an operational-scale test of a pre-emergent herbicide that was applied in strips as a split-plot treatment within each capping depth plot replicate. This treatment is anticipated to create growing space for nursery stock seedlings, thereby potentially speeding the development of woody plant cover while concurrently reducing the cover and dominance of non-native species.

The following questions will be answered through this trial:

1. In an operational setting with placement of soil under frozen conditions, how closely does the resultant topsoil depth match the planned topsoil depth and how does this change through time? As measured later in the same year after placement and thawing, and again after one and four years.
2. What is the impact of topsoil depth placement on native understory species?
  - a. Does the absence of topsoil preclude development of a forest plant community?
3. What are the impacts of topsoil placement depth on the;
  - a. Survival of planted woody species?
  - b. Natural ingress of woody species?
  - c. Growth of woody species?
4. How does the application of a pre-emergent herbicide affect;
  - a. The ingress of non-native species, particularly where no topsoil has been placed?
  - b. Survival and growth of planted woody species?

### **Trial 2: Nutrient loading with organic forms of nutrition to improve early growth following field outplanting (i.e., giving seedlings a lunchbox before their field trip)**

Another often cited motivation for utilizing topsoil is the soil nutrition present in this medium. In a separate investigation ([LJ0226 Interim Reclamation](#)), researchers have observed some evidence, in some species, that



supports this assertion. While broadcast application of fertilizers or other forms of organic amendments is possible, there are often unintended consequences. Namely, there may be increased competition from the grasses and other herbaceous species that are quick to capitalize on the abundant nutrient availability. An alternative approach could be to provide a more localized source of nutrition to the seedlings, thereby reducing the site-wide flux in soil nutrients.

Recently at the NAIT Centre for Boreal Research (CBR) in Peace River, a preliminary study was conducted to examine the concept of creating a ‘lunchbox’ for seedlings by incorporating different rates of alfalfa pellets in containers planted with two deciduous tree species (aspen and paper birch). This study found that alfalfa pellets applied at 10% and 20% of the total planting container cavity volume led to a 50% increase for all measured plant growth parameters — including seedling height, root collar diameter, leaf biomass and stem biomass — when compared to plants grown in containers with lower rates of alfalfa pellet incorporation.

Although the pilot study confirmed that it was possible to grow these seedlings, a field test to validate real-world growth is still required. Utilizing the positive results from this bench scale test, a plot-scale field study was initiated to further validate this ‘lunchbox’ approach to seedling growth against conventionally grown seedlings, as well as against nutrient loaded seedlings developed with inorganic fertilizers (following Schott et al., 2013; Schott et al., 2016). Four commonly occurring tree species were evaluated including white spruce (*Picea glauca*), balsam poplar (*Populus balsamifera*), trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*).

The objectives of this trial are to:

1. Compare the post outplanting growth and survival of nursery tree seedlings that are nutrient loaded through conventional means (inorganic fertilizer) versus those grown with the inclusion of an organic form of nutrition (alfalfa pellets).
2. Evaluate these ‘improved’ stock types under contrasting reclamation conditions;
  - a. the span of soil placement depths (no topsoil, shallow or standard); and
  - b. high versus low competition (no topsoil treatment only).

### **Trial 3: Hitchhiking native forbs with contrasting woody species: using the principle of hitchhiking forbs for varying purposes**

Hitchhiking multiple plant species in the same nursery plug has two key benefits including a direct increase in on-site species diversity and a cost reduction associated with planting. Even though larger containers are utilized, raising the per seedling cost and concurrently reducing the rate at which planters can plant these seedlings, the overall cost is still approximately 20% lower due to increased efficiency (i.e., shared plug, two plants established in one planting hole). There is also added logistic simplicity in reducing the number of individual plant orders made and coordinated. Incorporating or ‘hitchhiking’ native forbs into the same container as a shrub or tree is a potential means of efficiently establishing native forbs on a disturbed site. This concept was previously explored with white spruce (*Picea glauca*) and two different native forbs (*Chamerion angustifolium* and *Eurybia conspicua*), where these mixed-species plugs were successfully grown and established in a variety of reclamation sites (Mathison, 2018). Three different deciduous species (*Betula papyrifera*, *Alnus viridis* and *Salix discolor*) were also hitchhiked with fireweed with some success — though the interspecific competition was more challenging (Hudson, 2020). While



these studies have provided a framework from which to provide guidance on the use of this type of nursery stock, much of this research had focused on hitchhiking with fireweed and additional research is still required to examine other woody plant-forb mixtures and optimize their production.

This trial will evaluate three deciduous species, each representing different growth forms or growth strategies, in combination with one of three native forbs that also vary in their growth morphology as well as in known rates of spread and egress. These seedlings were established within contrasting condition types within the Trial 1 study design to evaluate the utility of these stock types across the span of capping depths (no topsoil, shallow, or standard) and in high versus low competition.

#### **Trial 4: Hitchhiking ericaceous shrubs with conifers**

As described above, a concern with not placing topsoil is the potential lack of seed propagules of native species. As in Trial 3, the concept of planting additional species is one approach to mitigate for this concern. Trial 4 will examine another configuration of hitchhiker seedlings. It will combine low-growing ericaceous shrubs (bog cranberry [*Vaccinium vitis-idaea*], common blueberry [*Vaccinium myrtilloides*] or Labrador tea [*Rhododendron groenlandicum*]) with coniferous tree species (jack pine [*Pinus banksiana*] or white spruce [*Picea glauca*]). Although, NAIT Centre for Boreal Research has previous experience growing these mixtures of species the logistics of combining two slow-growing species are quite distinct from the constraints and challenges found in Trial 3 using deciduous species.

The ericaceous shrubs must be started six to eight weeks ahead of the conifers due to their extremely slow growth. These shrubs can either be grown in trays or mini-blocks and then transplanted into the primary container with the emerging conifer, or they can potentially be grown in this cavity from the beginning thereby reducing the number of handling times required. A potential issue with this second approach is the development of mosses or liverworts that may inhibit seed emergence of the conifer which will be sown many weeks after sowing the shrub. This trial utilized the former approach as it was more practical from the perspective of seed emergence and in reducing issues with mosses and liverwort colonization.

The goal of this trial is to comparatively evaluate ericaceous shrubs grown singly, as well as hitchhiked shrubs (co-grown with one of two conifer species) and will also test the effect of soil inoculation on plant survival and growth. These seedlings will be outplanted across the range of capping depths to evaluate the conditions that are conducive to the healthy growth and persistence of these combinations of species.

#### **Relevance of Study to Industry**

The product of this work can be used as the basis to support soil cover design for both in situ and conventional operations that incorporate varying topsoil depths. This study should also provide evidence to support increasing flexibility for operators to move topsoil between dispositions to focus on reclamation outcomes rather than following a prescriptive approach to topsoil placement (i.e., use the topsoil where it is most needed). In addition, this study will also provide practical tools that operators will be able to employ to mitigate potential concerns with areas of limited topsoil placement. Overall, the results of this study are envisioned to support improved reclamation outcomes across the boreal forest.



## PROGRESS AND ACHIEVEMENTS

The results presented below relate to 2022 (two growing seasons) findings from Trial 1. Readers are encouraged to review the experimental layout (Figure 1) as it will facilitate a stronger understanding of the experimental design and discussion of findings.

### Understory vegetation responses

Experiment-wide, understory vegetation development is progressing slowly, and this was visually apparent across all experimental treatment levels (Figures 2 to 4). In contrast with many recently reclaimed industrial sites, there has been relatively low non-native forb growth (approximately 5% to 6% cover on average) after two growing seasons. There are two suggested explanations for this site-wide observation. Firstly, both 2021 and 2022 were extremely dry and warm summers. As earthworks activities occurred in May 2021 this would have further dried the surface soils, exacerbating the already drought-like conditions of the entire growing season and inhibiting seed emergence. Secondly, the soil stockpiles that were the source of subsoil and topsoil for the entire site were strategically handled to reduce introduction of non-native seed and rhizomes. This was accomplished by scraping the upper 5 cm to 10 cm of the stockpiles, and rather than indiscriminately spreading this rhizome and seed-rich material across the site, it was consolidated to a single discrete area (outside of the main study area). The underlying stockpiled soil was spread across the study area following the experimental design summarized in Figure 1. The potential downside to this approach is that it may have also reduced the quantity of viable native seed propagules into the study site. It is believed that this second treatment is likely to be strongest driver contributing to reduced coverage of non-native forbs.

Despite slower than anticipated vegetative cover development, there were some notable treatment effects worth mentioning:

1. The only observed differences amongst vegetation classes as it related to the topsoil depth treatments as a slight (though not statistically so) reduction in non-native forb cover in the no-topsoil treatment compared with standard and shallow placements (Figure 5c). This is not necessarily a surprising result as the presence of topsoil is likely to contain a greater quantity of seeds in the soil seed bank (both desirable and undesirable species) and provides a better substrate for germination. Moreover, the lower nutrient status of the no-topsoil treatment is expected to result in slower rates of growth, which could also influence the pace of vegetation cover development.
2. Utilization of pre-emergent herbicide was associated with a significant decline of non-native forbs and grasses (Figure 5b, 5d), though notably no difference was observed for native forbs and woody vegetation (Figure 5e to 5h). This distinction is likely because both the grasses and non-native forbs originated from the soil seed bank with seed emergence largely inhibited by the herbicide treatment while a high proportion of the woody and native forb vegetation present originated from rooted seedlings established in 2021. Three native forb species were hitchhiked with the conifers and all three of these species were observable across the study site (Figures 2 to 4).



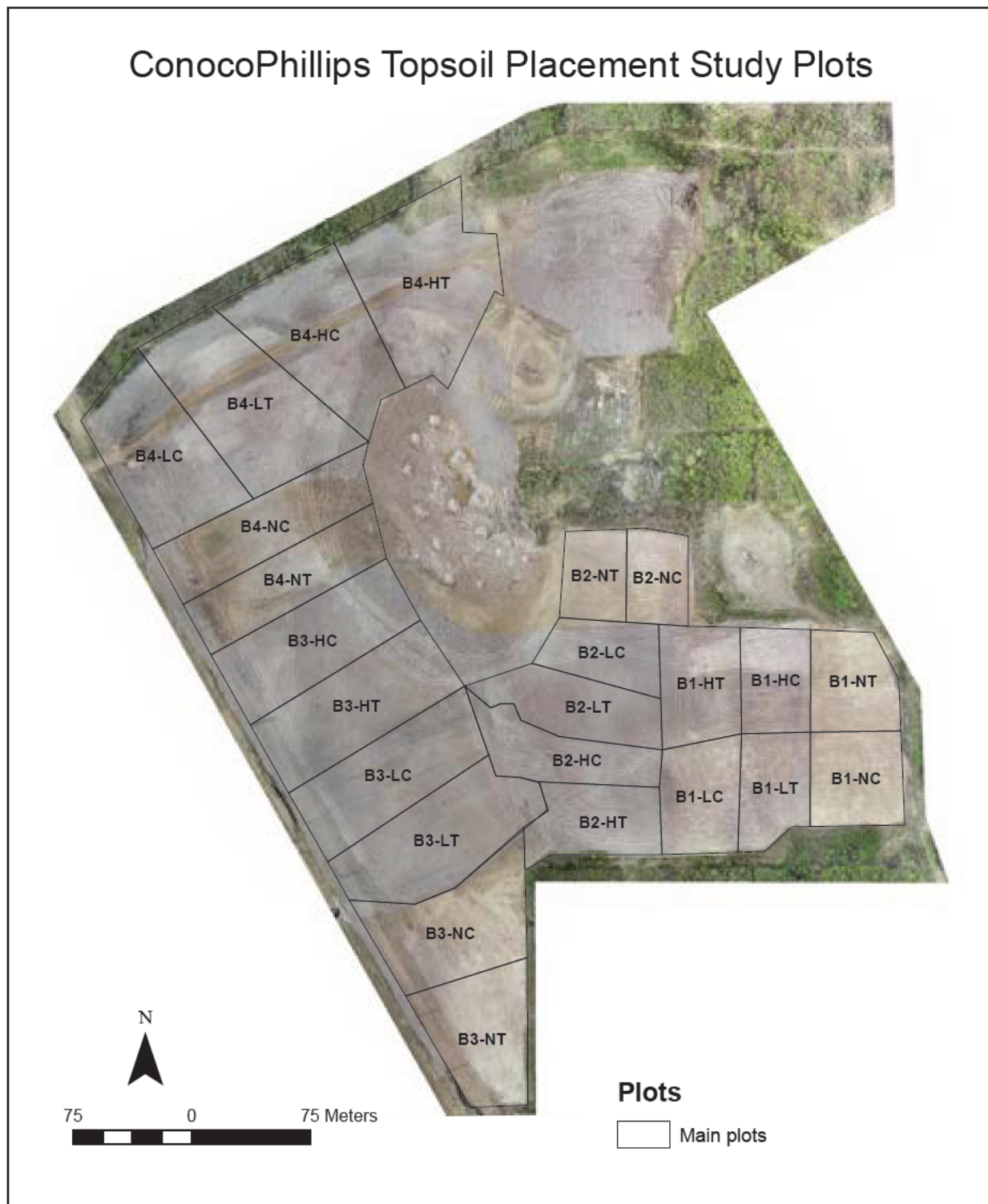
## Woody vegetation responses

A total of 4,800 stems per hectare (stems ha<sup>-1</sup>) of trees and shrubs were planted in spring 2021 at this study site. After two growing seasons, the observed stem densities of tall shrubs and deciduous trees consistently exceeded the initial establishment targets (Figure 6 and 7), both because of high survival (Figure 8) as well as natural ingress which was particularly evident with respect to ingress of *Populus tremuloides* and *P. balsamifera*. For conifers, all treatment levels showed a decline in stem density relative to the planting target (Figure 6a, 6b). In terms of the influence of topsoil depth and herbicide application, the following observations were noted:

1. The no-topsoil treatment was associated with a significant decline in conifer density at 1,298 stems ha<sup>-1</sup> relative to the standard and shallow topsoil treatments which averaged approximately 1,500 stems ha<sup>-1</sup> (Figure 6a). This appeared to be largely driven by lower and more variable survival in jack pine and white spruce to a lesser extent (Figure 8a, 8b). However, the pre-emergent herbicide treatment was associated with significantly higher stem densities of conifers at 1,536 stems ha<sup>-1</sup> compared with 1,368 stems ha<sup>-1</sup> in the control (Figure 6b).
2. Tall shrub density was not influenced by topsoil or herbicide treatment (Figure 6c, 6d) though medium shrub density (which originated wholly from natural regeneration) was significantly lower without the presence of topsoil (Figure 6e) as well as in the pre-emergent herbicide treatment (Figure 6f). This finding was not surprising given that most of this natural recovery would have originated from the soil seed bank, which would have been reduced with only subsoil present coupled with pre-emergent herbicide inhibiting seed emergence.
3. While there was a statistically significant interaction between topsoil and herbicide treatment with respect to deciduous tree density, the individual treatment levels were highly variable and without a logical significant difference detectable amongst them (Figure 7). This variation was likely due to the spatially variable nature of natural regeneration, which is strongly influencing treatment-level means at this early stage of the trial.

Despite two years of drought-like conditions, growth of trees and shrubs was largely positive with particularly vigorous growth observed visually throughout the site (Figures 2 to 4) across treatments. The growth response in the presence or absence of topsoil was species specific with no statistical differences amongst treatments observed in jack pine, raspberry, or paper birch (Figure 9). White spruce, however, did show a significant height growth benefit in the standard topsoil treatment compared with both the shallow and no-topsoil treatment (Figure 9e). Green alder also responded positively with progressively taller seedlings as the depth of topsoil increased (Figure 9c). While green alder is able to fix its own nitrogen, it is possible that other macro or micronutrients anticipated to be available in larger quantities with topsoil were also beneficial to this species.

Pre-emergent herbicide was not associated with growth differences for most species except for the deciduous tree species (Figures 9 and 10). Paper birch showed a significant increase in total height with pre-emergent herbicide (Figure 9j). Both *Populus tremuloides* and *P. balsamifera* had significant interactions between topsoil and herbicide treatments, suggesting pre-emergent herbicide was beneficial in some situations (Figure 10) — though this interpretation needs to be taken with some caution as it is speculated that some of this variation may also be due to natural regeneration from root suckers or seeds.



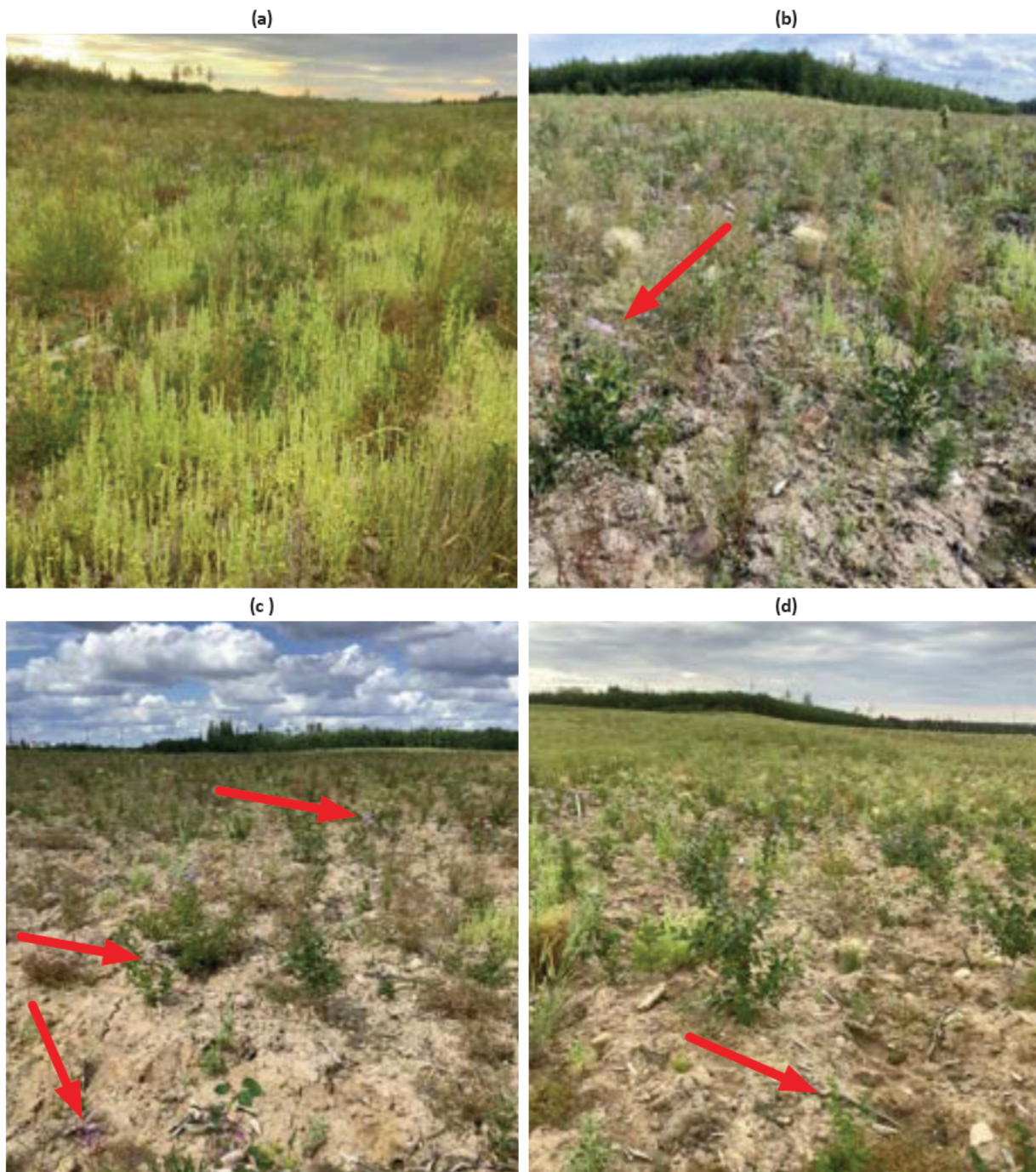
**Figure 1:** Layout of Trial 1 experimental treatment plots. The seedling stock Trials (2 to 4) are nested within these larger experimental units and are not shown to reduce crowding of the image. Treatment notations are as follows: B1-B4 refers to the replicate study blocks, H/L/C refer to the standard (H), shallow (L) or no-topsoil treatment (C) and T/C refer to treatment with pre-emergent herbicide (T) or untreated (C).





**Figure 2:** Treatment images from the second growing season in early August 2022 for the standard topsoil treatment and grouped by herbicide treatment — (a-b) no herbicide and (c-d) herbicide. Pink arrows denote observation of hitchhiked native forbs.



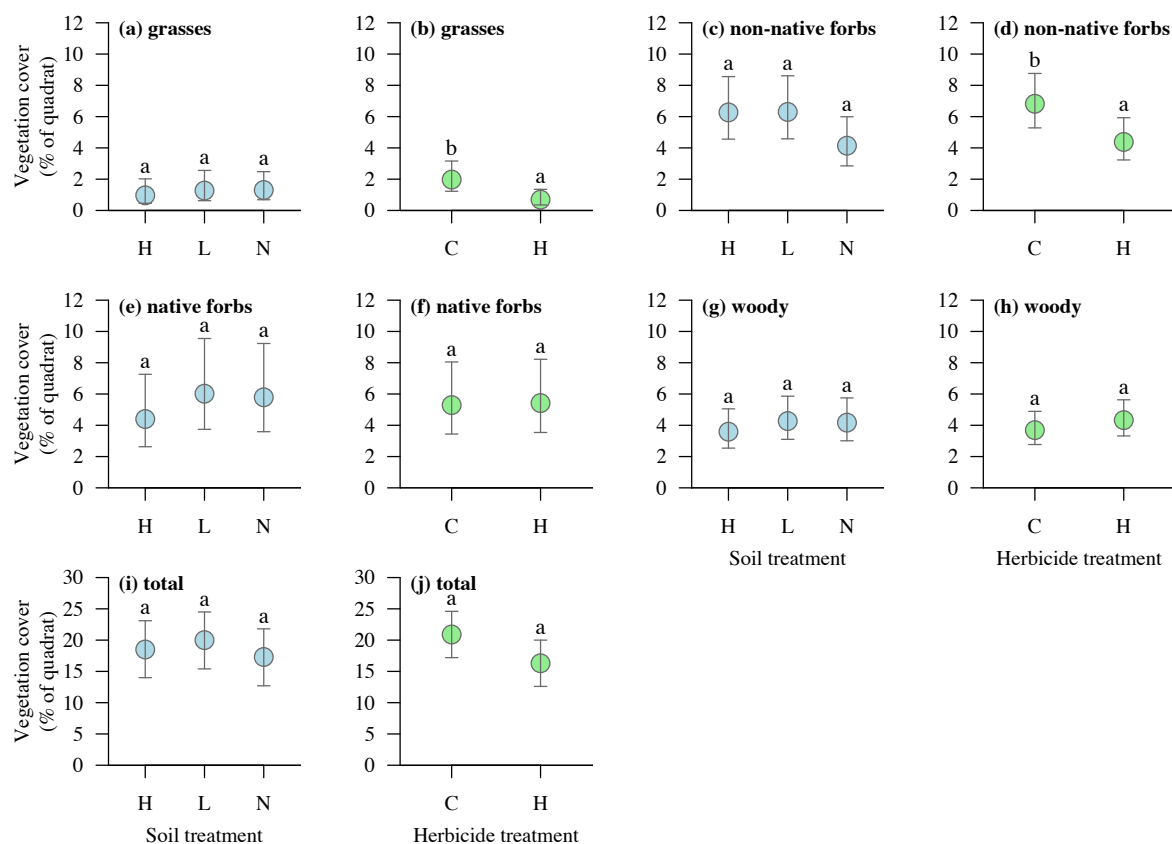


**Figure 3:** Treatment images from the second growing season in early August 2022 for the shallow topsoil treatment and grouped by herbicide treatment — (a-b) no herbicide and (c-d) herbicide. Pink arrows denote observation of hitchhiked native forbs.

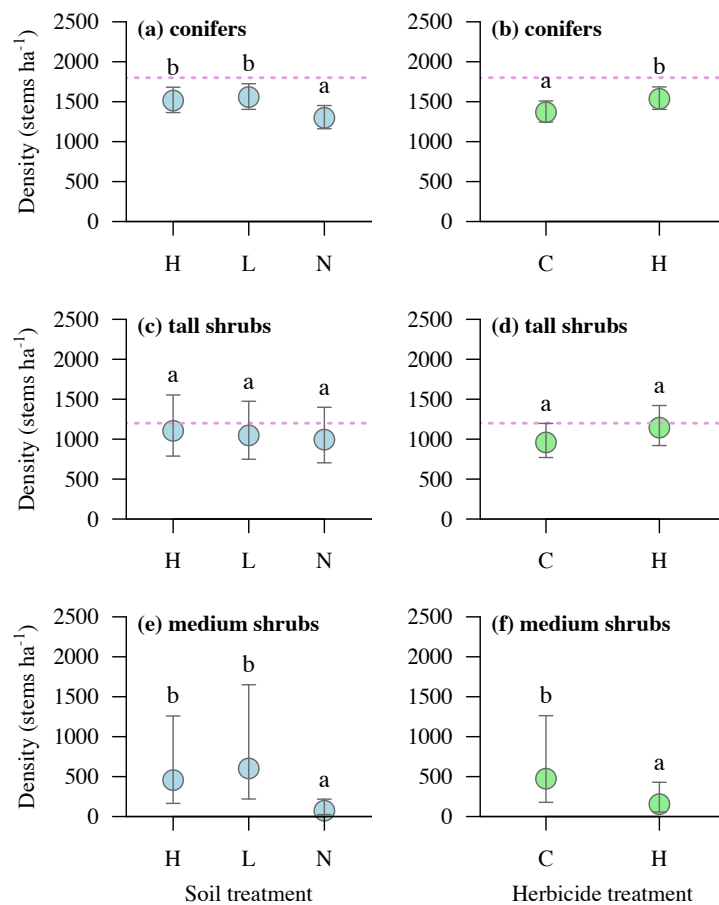




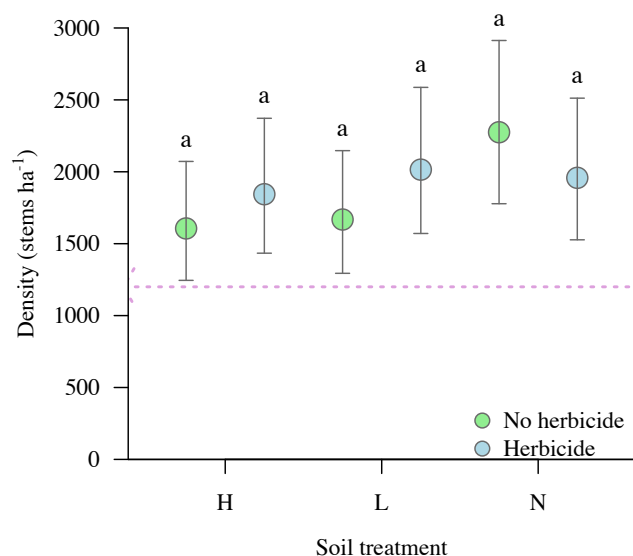
**Figure 4:** Treatment images from the second growing season in early August 2022 for the no topsoil treatment and grouped by herbicide treatment — (a-b) no herbicide and (c-d) herbicide. Pink arrows denote observation of hitchhiked native forbs.



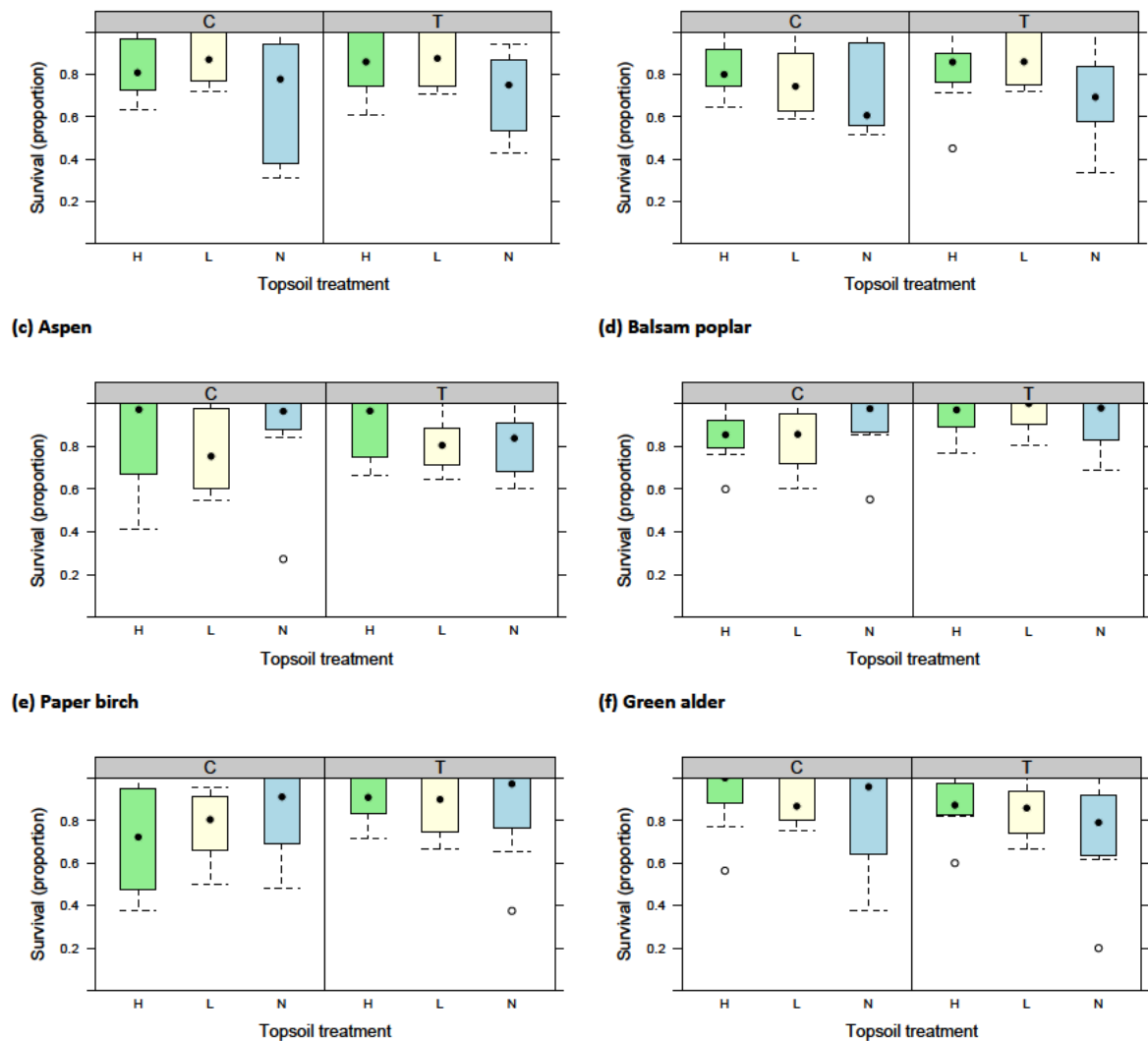
**Figure 5:** Estimated marginal mean vegetation cover of (a-b) grass, (c-d) non-native forb, (e-f) native forb, (g-h) woody, and (i-j) total cover by topsoil depth treatment (H [standard], L [shallow] or N [none]) and pre-emergent herbicide (T) or no-herbicide (C) after two growing seasons. Treatments not sharing the same letters indicate a significant ( $p < 0.05$ ) difference in means. Error bars represent 95% confidence intervals on the treatment mean ( $n = 4$  replicate blocks). Estimated means were generated from a split-plot two-factor generalized linear mixed effects model (fitted with a beta distribution). Note that there was a significant interaction detected for the horsetail vegetation group though the data are not shown (mean values approximately 1%).



**Figure 6:** Estimated marginal mean stem density of (a-b) conifers [white spruce and jack pine], (c-d) tall shrubs [green alder and willows] and (e-f) medium shrubs [raspberry and roses] by topsoil depth treatment (H [standard], L [shallow] or N [none]) and pre-emergent herbicide (T) or no-herbicide (C). Treatment means not sharing the same letters indicate a significant ( $p < 0.05$ ) difference in means. Values in brackets represent 95% confidence intervals on the treatment mean ( $n = 4$  replicate blocks). Estimated means were generated from a split-plot two-factor generalized linear mixed effects model (fitted with a negative binomial distribution) on plot-level stem counts that were back-transformed to represent stem densities. The purple dotted line indicates the target planting density at the time of establishment in 2021.

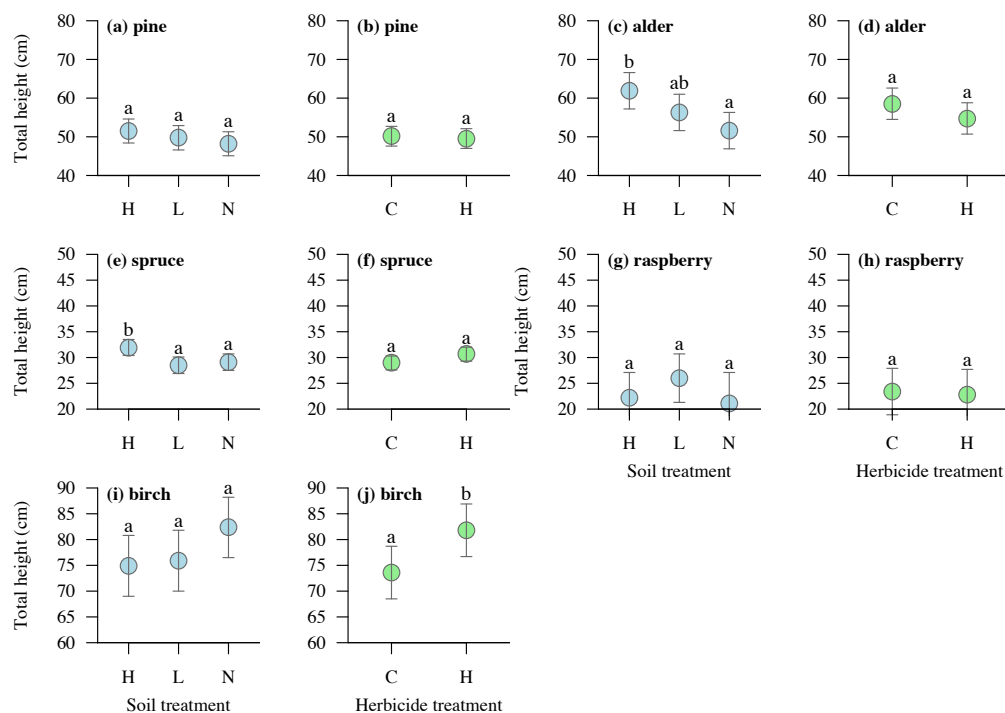


**Figure7:** Estimated marginal mean stem density of deciduous tree species shown as an interaction of topsoil depth treatment (H [standard], [shallow] or N [none]) and pre-emergent herbicide (T) or no-herbicide (C) after two growing seasons. Treatment means not sharing the same letters indicate a significant ( $p < 0.05$ ) difference in means. Values in brackets represent 95% confidence intervals on the treatment mean ( $n = 4$  replicate blocks). Estimated means were generated from a split-plot two-factor generalized linear mixed effects model (fitted with a negative binomial distribution) on plot-level stem counts that were back-transformed to represent stem densities. The purple dotted line indicates the target planting density at the time of establishment in 2021.

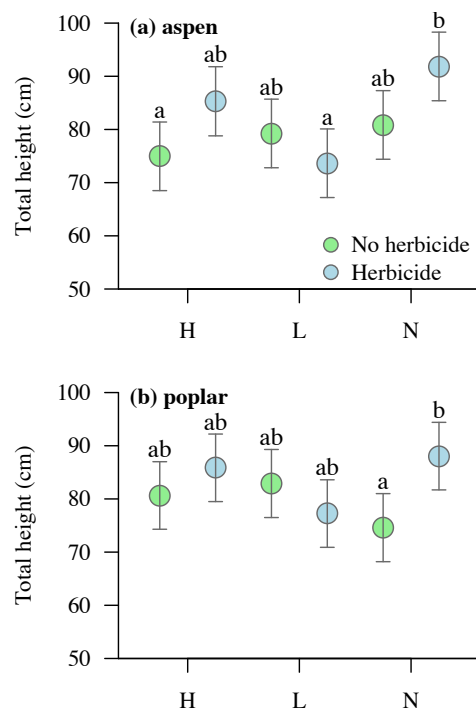


**Figure 8:** Box and whisker plots showing proportional survival from year one to year two by topsoil depth treatment (H [standard], L [shallow] or N [none]) and pre-emergent herbicide (T) or no-herbicide (C) for all species planted in the trial. Results are based on two 15 m x 15 m permanent sampling plots per soil and herbicide treatment level per replicate block (n = 4 replicate blocks). No formal statistical analyses are presented due to modeling constraints with high number of plots with nearly 100% survival.





**Figure 9:** Estimated marginal mean total height of jack pine [*Pinus banksiana*], green alder [*Alnus viridis*], white spruce [*Picea glauca*], raspberry [*Rubus idaeus*] and paper birch [*Betula papyrifera*] with topsoil depth treatment (H [standard], L [shallow] or N [none]) and pre-emergent herbicide (T) or no-herbicide (C) after two growing seasons. Treatments not sharing the same letters indicate a significant ( $p < 0.05$ ) difference in means. Error bars represent 95% confidence intervals on the treatment mean ( $n = 4$  replicate blocks). Estimated means were generated from a split-plot two-factor generalized linear mixed effects model (fitted with a gaussian distribution).



**Figure 10:** Estimated marginal mean total height of aspen [*Populus tremuloides*], and balsam poplar [*Populus balsamifera*] shown as an interaction with topsoil depth treatment (H [standard], L [shallow] or N [none]) and pre-emergent herbicide (T) or no-herbicide (C) after two growing seasons. Treatments not sharing the same letters indicate a significant ( $p < 0.05$ ) difference in means. Error bars represent 95% confidence intervals on the treatment mean ( $n = 4$  replicate blocks). Estimated means were generated from a split-plot two-factor generalized linear mixed effects model (fitted with a gaussian distribution).

## LESSONS LEARNED

As this study is only two growing seasons along, no conclusive statements can be reported for 2022.

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## PRESENTATIONS AND PUBLICATIONS

No public presentations or publications in 2022.

## RESEARCH TEAM AND COLLABORATORS

Institution: NAIT Centre for Boreal Research

Principal Investigator: Dr. Amanda Schoonmaker

Name	Institution or Company	Degree or Job Title	Degree Start Date (Students Only)	Degree Completion Date (Students Only)
Dr. Mark Baah-Acheamfour	NAIT Centre for Boreal Research	Research Associate		
Dr. Chibuike Chigbo	NAIT Centre for Boreal Research	Research Associate		
Marie-Pierre Ouellet-Pariseau	NAIT Centre for Boreal Research	Student Research Assistant	2020	ongoing
Athena Henderson	NAIT Centre for Boreal Research	Student Research Assistant	2019	2022
Tacy Wilkes	NAIT Centre for Boreal Research	Student Research Assistant	2020	2022

Research Collaborators: Dr. Dani Degenhardt and Dr. Jaime Pinzon Northern Forestry Centre, Canadian Forest Service

# DNA-Based Technologies to Evaluate Reclamation Success

**COSIA Project Number:** LJ0294

**In Situ**

**Research Provider:** Imperial

**Industry Champion:** Imperial

**Status:** Final Cumulative Summary

## PROJECT SUMMARY

Different reclamation practices have been implemented over the years at Imperial's Cold Lake in situ Operations in northern Alberta. Most current practices (i.e., newer reclamation practices) are focused on planting target ecosite tree and shrub species with the application of woody debris and the use of rough and loose soils. Older reclamation practices often resulted in compacted soils and included the planting of non-native grasses, herbaceous species and monocultures.

The status of a reclaimed site and its trajectory are evaluated through continuous monitoring. Thus, it is worth exploring emerging technologies for environmental assessment that have the potential to be cost effective and to improve reclamation monitoring. DNA-based approaches, such as environmental genomics, require very small environmental sampling and can obtain comprehensive biological information from organisms living in a given environment. Thus, genomics can help to assess the status and trajectory of reclaimed sites through analysis of soil DNA. Soil biological communities including bacteria and fungi are involved in important ecological functions such as cycling of nutrients, soil structure, and decomposition of organic matter.<sup>(1, 4)</sup> These soil processes are also necessary for the establishment of plant communities and ecosystem functions.<sup>(2, 3)</sup> The presence and abundance of soil microbial species or communities (e.g., bacteria, fungi, metazoan) have shown to reflect the status of the environment in which they are found.<sup>(5)</sup>

This project aimed to explore the application of environmental genomics to evaluate reclamation trajectory and success at multiple reclaimed sites at Cold Lake Operations using next generation sequencing (NGS) and targeting soil biological communities including fungi, metazoans and plants. The main objectives of this work included:

- Conduct genomic sequencing analysis of soil samples collected from upland reclaimed sites undergoing different reclamation practices and from undisturbed sites (reference) at Cold Lake Operations over time (2016, 2018 and 2021).
- Identify biological trends and metrics that can be used to infer reclamation trajectory and success at selected sites.
- Assess physicochemical variables in soils from selected reclaimed and reference sites to understand what environmental factors are driving the trends and biological communities' composition in the selected sites.

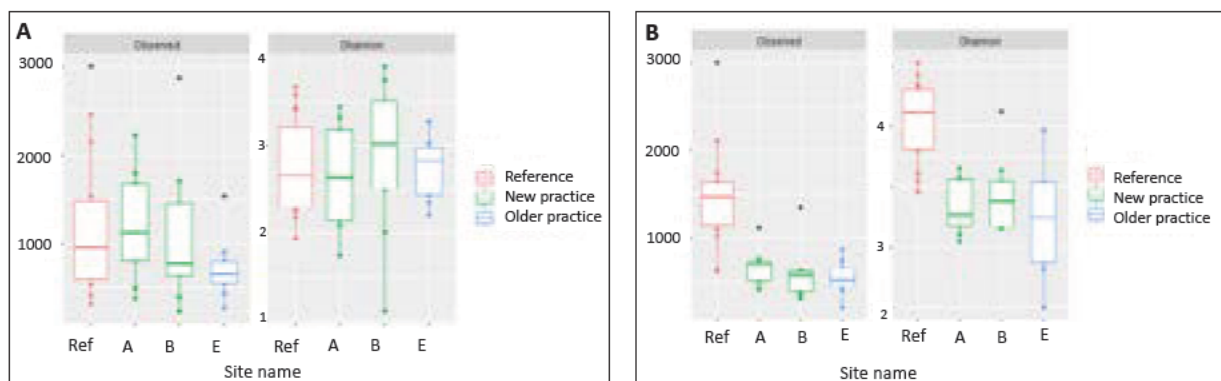


## PROGRESS AND ACHIEVEMENTS

In 2016 and 2018, soil samples were collected in four reclamation sites (A, B, C, D). Site A and Site B are considered to be reclamation sites with newer practices, while Site C and Site D are considered to have older reclamation practices. Samples from two undisturbed (reference) sites were also collected in those years. In 2021 reclaimed Site A and Site B were sampled again. Soil samples from three reference sites were also collected in addition to the samples collected from the two reference sites in previous years, aiming to capture the range of natural variability found in the undisturbed sites. Site C and D were not sampled due to budget limitations, instead additional samples were collected from a legacy grassy site (Site E) to include a location with “older reclamation” practices. As was done in 2016 and 2018, sequencing data from soil samples collected in 2021 were received and analyzed based on taxonomic abundance, biodiversity, and dissimilarity metrics using R v4.0.3<sup>(6)</sup> packages including vegan 2.5.5, phyloseq 1.26.1, and indicspecies 1.7.6.<sup>(7,8,9)</sup> Data were analyzed using anova analysis of variance and Tukey HSD for comparison of means. In 2021, soil samples were also collected to measure physicochemical parameters such as total organic carbon (TOC), organic matter (OM), pH, Total Kjeldahl Nitrogen (TKN), texture, and salinity.

The main trends observed in data from samples collected in 2021 aligned with results obtained from sites in 2016 and 2018. Although a direct comparison was possible between data from 2016 and 2018, qualitative differences in data from 2021 relative to previous years were taken with caution because samples thawed during shipment (despite no quality control concerns). Main trends described here are focused on differences between sites rather than differences between years.

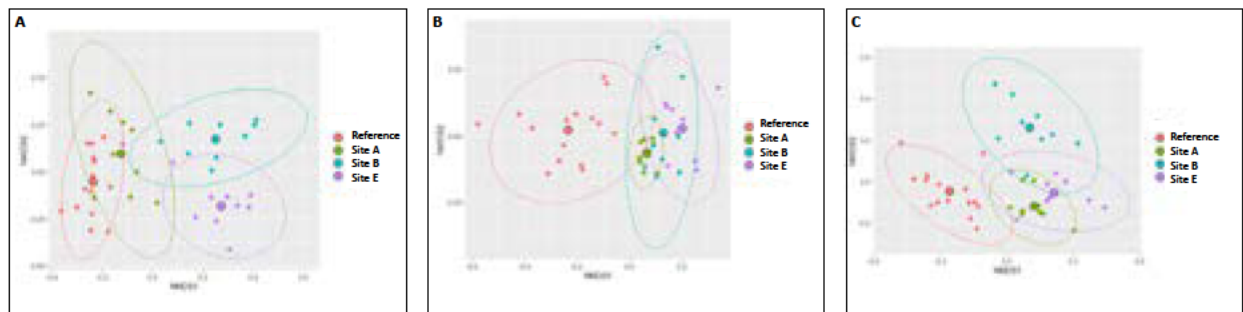
Overall, alpha diversity in communities of fungi and plants — measured as observed index and Shannon index — was not statistically different ( $P > 0.05$ ) between reclaimed sites and reference sites. However, sites with older reclamation practices applied had generally lower alpha diversity relative to undisturbed sites (example of this trend is shown in Figure 1A for plants). In contrast, in the metazoan community, observed and Shannon indexes were significantly different for all reclaimed sites (A, B, E) and the reference sites (Figure 1B). Physicochemical data suggest that the soil pH (which range from 4 to 7) measured in the reclaimed and reference sites was driving the alpha diversity in metazoan communities indicated by the strong negative correlations observed between both observed and Shannon indexes relative to soil pH (R: -0.72 for log observed and pH correlation; R: -0.6 for log Shannon and pH correlation). No significant correlations were seen for alpha diversity indexes in plant and fungi communities relative to measured soil properties.



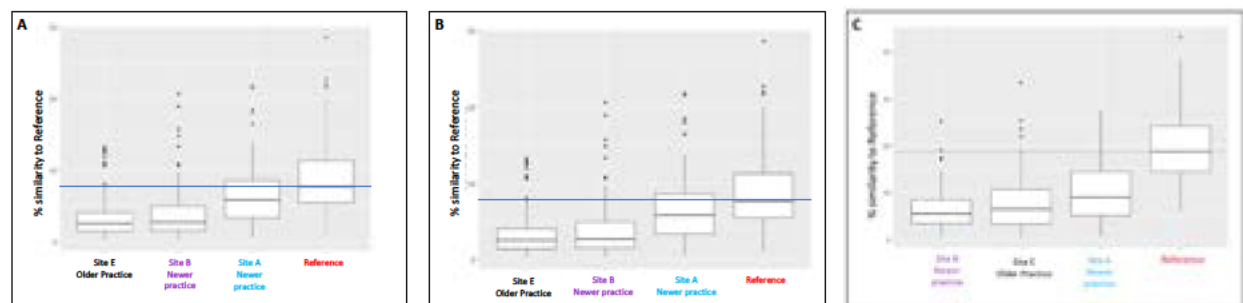
**Figure 1:** Alpha diversity measured as Observed and Shannon indexes in samples from 2021 for A) plants and B) metazoans communities.

**Note:** In metazoans, *P* value for Shannon index between Site A, B, and C relative to reference sites was 0.003, 0.001, 0.00004, respectively, and *P* value for Observed index between Site A, B, and C relative to reference sites was 0.0002, 0.00006, 0.00002, respectively.

Species composition and betadiversity data from 2021 were also consistent with what was observed in previous years. Generally, communities of fungi and plants in the sites that had newer practices applied were closer to communities in the reference sites, while the communities from sites from older reclamation practices showed dissimilar species composition and were generally more statistically distant relative to reference sites (Figure 2 and Figure 3). Plant community distribution represented on a non-metric multidimensional (NMDS) plot indicated that while no significant differences were observed between Site A and reference sites (*P*: 0.65), communities of Site B and Site E were significantly different relative to communities in undisturbed sites (*P*: 0.002 and *P*: 0.01, respectively) (Figure 2A). Similarly, fungi community from Site E, “older reclamation” was more dissimilar to the community in undisturbed sites (Figure 2B). In the metazoan community (Figure 2C), the species composition and betadiversity in all reclaimed sites were more distant to the reference sites. Based on Bray-Curtis similarity distance, Site A (“newer reclamation”) was repeatedly observed to have a community composition more closely similar to the community in undisturbed (reference) sites for the plants, fungi and metazoan communities (Figure 3). Soil pH and clay content were the main soil properties that were driving the species composition in plants, fungi and microbes along the reclaimed and reference sites. For instance, a strong correlation between pH and NMDS scores, as well as clay content and NMDS scores were observed for the three ecological markers (plants: pH, *R* = 0.77 and clay content, *R* = 0.84; fungi: pH, *R* = 0.93 and clay content, *R* = 0.72; metazoans: pH, *R* = 0.86 and clay content, *R* = 0.77).



**Figure 2:** Non-metric multidimensional scaling (NMDS) representing communities in A) plants, B) Fungi, and C) metazoans from 2021 data.



**Figure 3:** Percentage similarity to reference calculated with Bray-Curtis dissimilarity distance for A) plants, B) Fungi, and C) metazoans from 2021 data. Blue lines depict the "target" median similarity for the reference samples.

In accordance with data from previous years, indicative species analysis for samples collected in 2021 suggested there may be key fungi taxa with great potential to be indicators of successful trajectory. Table 1 shows the top taxa that were associated to reference sites from samples collected in 2021. Detected taxa in undisturbed sites included Saprotrophic and ectomycorrhizal fungi known to have important ecological functions in forest development.<sup>(10, 11)</sup> Some of these taxa were also identified as indicator species in the reclaimed sites indicating key biological organisms have emerged in soils from reclaimed areas (Table 1). Site A which showed a high similarity to reference sites, had the highest number of common indicator taxa (8) with the reference sites, including members of the family *Umbelopsidaceae*, *Cladosporiaceae*, *Mucoraceae*, and *Atheliaceae* (Table 1). Many metazoan taxa were also associated to reference sites, and some were identified in reclaimed sites in 2021 (Table 2). Main taxa that were shown to emerge in reclaimed sites included members of the phylum Arthropoda such as *Oppidae*, a known soil decomposer and key organism in nutrient cycling.<sup>(12)</sup> Nematoda members were also observed in both reference and reclaimed sites including the plant feeding *Tylenchus*, *Malenchis* and *Filinchus*, and bacterial feeding *Teratocephalus*, *Prodesmodora*, and *Prismatolaimus*. The "newer reclamation" Site A had six metazoan taxa members that were also associated to reference sites.



**Table 1: Fungi taxa identified as indicative species associated to reference sites, which were also detected as indicative species in reclaimed sites**

Family or genus	Phylum	Trophic function <sup>(11)</sup>	Site A	Site B	Site E
Pseudeurotiaceae	Ascomycota	U	X	X	X
Nectriaceae		EP/PP	X	X	X
Aspergillaceae (Penicillium sp)		PGP/PP			X
Hypocreaceae		P	X		
Herpotrichiellaceae		ST			
Cladosporiaceae		ECM	X	X	
Melanommataceae		U			
Cortinariaceae	Basidiomycota	ECM			
Atheliaceae		ECM	X		
Lycoperdaceae		ST			
Tricholomataceae (Leucopaxillus sp)		SP			X
Tremellaceae		U			
Umbelopsidaceae	Mucoromycota	ST	X		
Mortierellaceae		ST	X	X	X
Mucoraceae		ST	X		

Trophic function: ST, saprotroph; ECM, ectomycorrhizal; EP, endophyte; P, parasite; PP, plant pathogen; PGP, plant growth promoting; U, unknown.

**Table 2: Metazoan taxa identified as indicative species associated to reference sites which were also detected as indicative species in reclaimed sites**

Family or genus	Phylum	Trophic function <sup>(12)</sup>	Site A	Site B	Site E
Tylenchus	Nematoda	Plant feeding	X	X	
Teratocephalus		Bacterial feeding	X		
Prodesmodora		Bacterial feeding	X		X
Prismatolaimus		Bacterial feeding	X	X	X
Malenchus		Plant feeding		X	
Filenchus		Plant feeding	X	X	
Bastiania		Bacterial feeding			
Aphelenchoides		Fungi feeding			
Amphidelus		Unknown			
Cocceupodes	Arthropoda	Unknown			
Filieupodes		Unknown			
Neoliochthonius		Decomposers			
Oppidae		Soil Decomposers	X		





## LESSONS LEARNED

This work has demonstrated that genomic analysis of selected soil biological communities can support monitoring efforts in reclamation areas. For the selected sites at Cold Lake Operations, sequencing data showed that the biological communities in sites considered with newer reclamation practices were generally more similar to the communities in reference sites. Trends observed in the fungi community analysis generally aligned with what was observed in the plant community. Nevertheless, the indicative species analysis showed that specific individuals of both fungi and metazoans can be potential bioindicator species, as key taxa from these biological groups were consistently observed in undisturbed (reference) sites and seemed to emerge over time in reclaimed sites. The addition of new reference sites to the analysis in 2021 resulted in lower variability in the data relative to previous years. Finally, one of the selected reclaimed sites (Site A) was consistently showing a higher similarity to reference among the communities of plants, fungi and metazoans, strongly indicating this site is going to the right trajectory. Continuous sampling and monitoring are still needed to confirm these trends. This work has shown that the information obtained through DNA-based tools such as next generation sequencing of selected biological markers can be useful for monitoring the status of reclaimed sites and reclamation trajectory.

A manuscript detailing this project's research findings is currently in preparation.

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<sup>11</sup> Nagati M., Roy M., DesRochers A., Bergeron Y., Gardes M. (2020). Importance of Soil, Stand, and Mycorrhizal Fungi in *Abies balsamea* Establishment in the Boreal Forest. **Forests** 11, no. 8: 815. Available at: <https://doi.org/10.3390/f11080815>

<sup>12</sup> Kitamagi Y., Kawai Kiyosada, Ekino Taisuke. 2021. Soil physicochemical properties shape distinct communities in serpentine ecosystems. *Pedobiologia*. Vol 85–86

## PRESENTATIONS AND PUBLICATIONS

### Conference Presentations/Posters

International Workshop on Environmental Genomics (IWEG). Held virtually on June 24 to 25, 2020. Presentation Title: Environmental Genomics to assess reclamation success.

## RESEARCH TEAM AND COLLABORATORS

Institution: Imperial

Principal Investigator: Dr. Carolina Berdugo-Clavijo

Research Collaborators: Dr. Mehrdad Hajibabaei, Centre for Environmental Genomics Applications (CEGA); Dr. Greg Singer, CEGA; Dr. Jordan Angle, ExxonMobil; and Dr. Lucie N’Guessan, ExxonMobil.

## Shrub and Perennial Outplanting Study

**COSIA Project Number:** LJ0322

**Mine**

**Research Provider:** University of Alberta

**Industry Champion:** Syncrude

**Industry Collaborators:** Suncor, Imperial

**Status:** Year 4 of 4

### PROJECT SUMMARY

The rationale for this study stems from historic uncertainties around outplanting success for a number of boreal forest understory species. Successful deployment of these species in oil sands reclamation areas may be required to meet stakeholder and regulatory expectations to realize forest compositions that resemble locally common boreal forest ecosystems. This includes characteristic target species for forest land reclamation and specific species utilized and identified by Indigenous communities listed in *Guidelines for Reclamation to Forest Vegetation in the Athabasca Oils Sands Region* Alberta Environment, 2010.

For some species there is insufficient operational experience to gauge outplanting success, while for others the existing anecdotal evidence suggests that survival rates are poor or inconsistent. Overall, there is a substantial lack of quantitative and observational evidence on the magnitude of the problem and on the potential causes of poor establishment success such as seedling quality, environmental conditions, and their interactions.

While height, root collar diameter and root system size (plug volume) are seedling characteristics useful for evaluating seedling quality in tree species, shrubs and perennials might require additional evaluation measures to characterize seedlings, such as branch numbers and the number of leaves in the case of evergreen species. Other characteristics that are more difficult to obtain might also be considered and include measures such as root volume and root mass, root-to-shoot ratio (RSR), state of dormancy, carbohydrate reserves, and other physiological measurements. The relevancy of these indicators might be species-specific and are as of yet not clarified for the species of interest here.

The overarching objectives of this study were to:

1. Characterize early outplanting success (growth and mortality) of shrub and perennial forb species for which oil sands operators have observed low historical survivorship or have a limited understanding.
2. Develop hypotheses on potential factors of influence related to outplanting success for each understory species that could guide subsequent controlled experiments.

As suggested in objective 2, ecological understanding of these understory species is generally poor, and it is currently unclear what contributes to their performance under reclaimed land field conditions. Therefore, this study was primarily inductive rather than deductive, as the intention was to develop meaningful and testable hypotheses that can be addressed and explored in the future.



Two discrete components of this research were:

1. To collect data describing the seedling characteristics of nursery stock of nine common understory shrub species and explore the variability of seeding characteristics between two production years for four of those species. In a controlled study, these identified shrub seedling quality characteristics were then used to test whether they can predict potential seedling survival and growth under relatively ideal (non-stressful) conditions.
2. To subsequently test outplanting success (mortality and growth), the understory species (four in 2019 and nine in 2020), were planted on a range of different reclamation sites in a large field study. Further, the effects of different site and annual weather conditions were explored on the survival of these seedlings over a two- or three-year period.

The results from this research will add to the fundamental understanding of what, where and when to introduce understory species to forest reclamation areas. Working in parallel with a new proposed operational monitoring protocol for understory species, this project was a first step toward fully identifying the magnitude of challenges in propagating each species and has provided useful information to guide future research.

## PROGRESS AND ACHIEVEMENTS

Progress in 2022 included analysis and reporting. Results are summarized below.

## LESSONS LEARNED

### **1. Describe seedling nursery stock characteristics and correlate them to seedling survival and growth under ideal conditions.**

The uniformity of planting stock characteristics is a sign of consistency of seed quality, seed germination, as well as growing and storage conditions during seedling production. In this study, measured values for morphological characteristics often varied between 30% and 50% from the mean, indicating relatively high variability. Physiological characteristics, measured as non-structural carbon (NSC) reserve concentrations, were generally less variable within species (between 10% and 30%) but varied significantly in some species by production year. Genetic and phenotype variability is an unknown factor for these species and may also have contributed to this observed variability. However, seed collection protocols for these species are highly regulated through selected seed zones and may minimize the genotypic and phenotypic impact. Therefore, further studies that help to refine protocols are needed to improve the homogenizing of seedling characteristics during nursery production in matters such as the timing of germination, general growing and hardening conditions, and seedling dormancy and storage.

Assigning seedlings into quality categories based on semi-objective measures of height, root collar diameter (RCD) and general vigour did not lead to a successful separation of the assigned quality categories when growing in near-optimal conditions. This result is not surprising, given that most seedlings (unless of truly poor quality (see more below) are generally able to survive and grow under such conditions. However, it is unclear how these quality categories translate into the field where seedlings generally are exposed to additional stresses. To explore these relationships in more depth controlled and carefully executed field and greenhouse studies need to be undertaken.



Based on the knowledge gained from research on deciduous tree planting stock of trembling aspen, one can expect that some of these measured characteristics (e.g., RSR and reserve status) might play a significant role as indicators of early establishment success in the field for some of these species. Some of these measures, such as RSR and leaf area, appear to be critical indicators of appropriate seedling conditioning and could provide a “balance” between the need of for an establishing plant to assimilate carbon and access soil resources.

Obvious signs of poor quality such as mould (i.e., *Cornus canadensis*) and poor plug fill (i.e., *Linnaea borealis*) lead to significantly reduced establishment success. This has also been noted for *Vaccinium myrtilloides* for operational plantings. While poor plug fill (low RSR) is a condition that can be addressed during the growing phase of the seedling stock by using different fertilizer and watering regimes, mould is a problem that most likely relates to the hardening and/or storage of seedlings. Mould in particular was an issue with most evergreen and wintergreen shrub species used in this study; however, in *Cornus canadensis* it appeared to have the greatest effect on survival, indicating that current plant hardening procedures and/or storage conditions prior to outplanting will likely need to be adjusted for this species.

Apart from specific exceptions mentioned above, overall growth and establishment of seedlings were good in this controlled study, and mortality was generally low. Apart from *Cornus canadensis* and *Linnaea borealis*, all seedlings added new roots (approximately three to five times their initial mass) to their existing root systems with *Lonicera involucrata* adding more than 10 times its initial mass. A much more subdued growth response was observed above ground, resulting in overall increases in RSR for these species, potentially indicating that maximizing initial RSR in planting stock might be a target for improving seedling quality.

While initial seedling size played little role overall, it was noted that a few shrub species that were older and more mature performed particularly well. Interestingly, *Symphoricarpus albus* and *Viburnum edule* were three-year-old planting stock, suggesting that the age of planting stock (or associated effects on physiological conditioning) might play a role in outplanting performance.

In contrast, some species such as *Vaccinium spp.* (*Vaccinium myrtilloide* and *Vaccinium vitis-idaea*), *Ledum groenlandicum*, and *Empetrum nigrum*, showed little egress from the original plug. This potentially indicates that seedlings might be poorly conditioned for field planting where active root growth and egress into the surrounding soil are critical for accessing resources, increasing stress tolerance, and competing with other vegetation.

Year-to-year variation in morphological and physiological conditions for *Lonicera involucrata* and *Vaccinium vitis-idaea* was relatively low or only specific to a very few characteristics, while in *Vaccinium myrtilloides* and *Cornus canadensis* it was considerable.

## 2. Evaluate outplanting success on a wide range of reclamation sites

As expected, overall mortality was higher across all species when planted in the field as compared to the pot study (see part 1 above). The breadth of these differences was indicative of the occurrence and magnitudes of different stresses (including resource availability) experienced by the newly planted seedling, in combination with the physiological conditioning of the seedlings to tolerate those stresses. For example, the high mortality and overall poor performance of *Cornus canadensis* (2019 and 2020) and *Linnaea borealis* (2020) stock were most likely a reflection of the quality of the seedling stock rather than an effect of different reclamation site types. In the case of *Cornus canadensis*, the most likely issue was poor tolerance to overwintering cold storage and the associated growth



of mould in the seedling boxes. Regardless of the planting site, field mortality of both these species was extremely high (> 90%) and therefore it was not useful to explore site differences in more detail. Differences in mortality of the remaining species were most likely in response to differences in site conditions, which also interacted with the annual weather conditions.

Two sites stood out to be extremely challenging for the establishment of all planted shrub species. One site was very dry, and had sandy soils, no tree cover and sparse ground vegetation, while the second site had moderately dry soils, a very open canopy, and a very well-developed grass/forb layer. On the first site, poor overall colonizing vegetation performance after 11 years since initial vegetation establishment, indicates a high degree of inherent drought stress, similar to that found on the driest eco sites in the region. On the second site, the established colonizing vegetation of sod-forming grasses likely posed a very competitive environment that was detrimental to planted seedlings. Likely, neither of these sites would generally be considered for future understory species planting, although the first site could potentially be planted with a selection of other species that are more adapted and appropriate for this site type.

The other seven sites appeared to provide much better establishment conditions. Particularly, the newly established reclamation sites with organic-dominated cover soil that initially had low populations of colonizing and competitive vegetation. These sites appear to provide adequate growing conditions such as moisture and growing space for shrub establishment, even during the drier conditions of 2020. Sites with an existing tree canopy (mature off-site forest; 30-year-old, closed canopy pine; and 30-year-old mixedwood) also provided acceptable conditions for understory species establishment, possibly moderating stressful conditions associated with exposure. However, across all sites, the mortality of most species within the study was well over 20%.

Differences in mortality of planted species are confounded by site and year, making it difficult to identify underlying variables for mortality responses. For example, *Lonicera involucrata* planted in 2019 had three-year mortality of 51% on the worst-performing site (moderately dry grassy site mentioned above) and two-year mortality for 2020 stock of 76%. However, since the first growing season conditions in 2019 (average year) and 2020 (dry year) were very different, the mortality of *Lonicera involucrata* seedlings planted in 2019 was 15% in 2020, while it was 61% in the seedlings newly planted in 2020, indicating that the initial establishment moisture conditions likely played a role. Such conclusions can be confounded by planting stock quality and other factors that changed between the two years. For example, *Vaccinium myrtilloides* seedlings planted in 2019 performed as poorly in 2020 (58% mortality) as those planted in 2020 (51%), potentially indicating the root systems of the 2019 planted seedlings had not yet sufficiently developed to resist the drier 2020 soil conditions. Overall, these potentially confounding effects make it difficult to evaluate the effects of stock quality, planting year and site conditions individually. To address such issues, special attention should be given to the connection between seedling characteristics and stress tolerance in future controlled studies.

Beyond the very dry and grassy sites having high mortality, very few species in this study showed preference for particular site conditions, suggesting there is at least a short-term tolerance for broadly defined ecological niches for most of these species. A potential exception might be *LG* and *EN*, which appear to prefer soils with high organic content. Longer-term monitoring might shed light on the persistence of species on different sites.



The rate of mortality was the only consistently reliable indicator of seedling performance in this study. Given the different growth forms present (upright versus laterally spreading; single versus multiple stems), metrics of growth (size increase) were difficult to interpret and would be particularly difficult to interpret outside of an intensive research study (for example, in an operational monitoring program). Root growth offers some promise, but requires destructive sampling.

### Potential solutions and/or knowledge gaps:

- The ability of planted seedlings to quickly grow roots and access site resources is a key quality parameter but is not yet reliably measured as a screening tool; indirect measures may include RSR and root reserves.
- Many of the species in the study successfully increased their root mass and spread out after outplanting (up to 500%), with notable exceptions being the *Vacciniums*, *Empetrum nigrum* and *Linnaea borealis*. However, root growth responses were greatly impacted by site growing conditions.
- Homogenization of seedling crop metrics likely requires improvements to stratification or other germination treatments and standardized growing conditions that are repeatable to ensure increased uniformity of emergence and early growth.
- Use of older planting stock, possibly including nursery transplants, may be beneficial for some species.
- Improved culling standards are likely feasible.
- Improvements to methods of hardening evergreen seedlings, and/or alternatives to traditional cold storage for species such as *Cornus canadensis*, show promise for improved outplanting performance; fall planting may be an alternative to improving cold hardening techniques.
- Alternatives to container stock such as bareroot seedlings or live cutouts (sods) may be a viable approach for laterally spreading species such as *Linnaea borealis* and *Cornus canadensis*.
- Establishment of permanent monitoring plots for longer-term observations of operational shrub and understory plantings is strongly advised.

## LITERATURE CITED

Alberta Environment, 2010. Guidelines for reclamation to forest vegetation in the Athabasca oil sands region. Prepared by the Terrestrial Subgroup of the Reclamation Working Group of the Cumulative Environmental Management Association, Fort McMurray AB, December 2009. Available at: <https://open.alberta.ca/publications/9780778588252>

## PRESENTATIONS AND PUBLICATIONS

### Published Theses

Hynes, Brittany 2023. Evaluating the quality of boreal shrub seedlings and their out-planting performance for forest reclamation. M.Sc. Thesis, University of Alberta (in prep.)



## RESEARCH TEAM AND COLLABORATORS

Institution: University of Alberta

Principal Investigator: Dr. Simon Landhäusser

Name	Institution or Company	Degree or Job Title	Degree Start Date (Students Only)	Degree Completion Date (Students Only)
Brittany Hynes	University of Alberta	MSc	2019	2023
Serena Farrugia	University of Alberta	Research Assistant		
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Caren Jones	University of Alberta	Technician		
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Industry collaborator: Robert Nemeth, Smoky Lake Forest Nursery





## REVEGETATION

# Restoration of Native Tree and Shrub Species on Reclaimed Grassy Sites

**COSIA Project Number:** LJ0291

**In Situ**

**Research Provider:** Natural Resources Canada, Canadian Forest Services

**Industry Champion:** Imperial

**Status:** Year 7 of 8

## PROJECT SUMMARY

The objective of the study is to determine the most effective site treatment for oil sands legacy sites (20 to 30 years old) in the boreal forest that were reclaimed using non-native grass and herbaceous species. These sites were reclaimed to the standards of the day (standards in place at the time the reclamation was completed) and have grass as the only or predominant vegetation growing on the site. The intent is to establish desirable boreal tree and shrub species so that these sites can be placed on trajectory to becoming fully functioning forest ecosystems. Restoration of forest ecosystems on these sites will reduce the area of disturbed forest and forest fragmentation impacting woodland caribou and other wildlife species.

In the spring of 2016, a field study was designed to test a range of mechanical and chemical site preparation treatments on the establishment and growth of tree and shrub seedlings. The field study was established at D63 Borrow which is located at Imperial's Cold Lake Operations. The soil is a compacted sandy clay loam with a 2 cm to 5 cm thick LFH layer. Soil pH ranged between 6.4 and 7.8 and sodium adsorption ratio (SAR) between 0.5 and 1.1. Soil nutrient concentration was approximately 4 ppm of nitrogen, less than 4 ppm of phosphorus and 77 ppm to 110 ppm of potassium. The site was divided into forty, 9 m x 30 m plots in two rows of twenty and oriented north/south along the short axis of the site. Treatments were assigned randomly to each plot.

The treatments (four site preparation techniques and an untreated control) being tested are:

1. Non-selective herbicide (glyphosate) 1 m x 2 m spot spray followed by planting the next year, installation of a 40 cm tall biodegradable waxed paper tree shelter supported by a wooden stake after planting, and an additional application of a non-selective herbicide (glyphosate) around the tree shelter if required;
2. Excavator mounding of soil (mounds 30 cm wide x 25 cm long), followed by planting;
3. Excavator mounding of soil followed by the application of a non-selective herbicide (glyphosate) only over the mound area in the year of treatment, followed by herbicide before planting if needed;
4. High-speed soil mixing (160 cm wide x 140 cm long patches) followed by the application of a non-selective herbicide (glyphosate) only over the mixed area in the year of treatment, followed by herbicide before planting if needed; and
5. Untreated control.



Given the poor nutrient availability, fertilizer tablets (Forestry Suppliers 20-10-5, 21 gram) were placed by each seedling/cutting in half of the plots (randomly selected) on each site treatment. There were four replications of each site treatment/fertilizer combination.

Eighteen seedlings of white spruce (*Picea glauca* Moench Voss.) and green alder (*Alnus viridis* [Chaix] DC.), and eighteen 20 cm long balsam poplar cuttings (*Populus balsamifera* L.) were planted in June of 2017 in each of the treatments. Seedlings/cuttings were planted on the top of the mounds or in the middle of the mixed bed. The planting spot was randomly assigned to each seedling.

In 2018, two additional study sites were developed at Cold Lake Operations (J10, P3). Treatments at these sites incorporated learnings from the D63 Borrow. In J10 and P3, the pre-emergent herbicide Torpedo™ was added to the tank mix with glyphosate in the treatments where herbicides were used. In addition, the fertilizer application protocol was modified. The fertilizer tablets were placed no closer than 10 cm from the stem of the seedling/cutting. Plants were site prepared in 2018 and planted in the spring of 2019.

## PROGRESS AND ACHIEVEMENTS

The study sites J10 and P3 were assessed in the fall of 2022. The results below are a preliminary (non-statistical) analysis of the four-year combined data from the two study sites.

Green alder mortality increased between 2021 and 2022 for all treatments. The greatest increase was for the mixing with herbicide and fertilizer treatment at 22%. The mortality increases for the remaining treatments ranged between 8% and 17%. After four growing seasons, the lowest green alder mortality is in the mounding with herbicides and no fertilizer treatment (73%). All other treatments had mortality rates between 77% and 95%. The significance of these differences will be determined. The highest mortality was in the controls, with and without fertilizer at 96% and 98% respectively.

Balsam poplar mortality after four years was also highest in both control treatments, at 96% and 99% for the fertilizer and no fertilizer treatments, respectively. The lowest mortality was in the mounding with herbicide and no fertilizer treatment (62%). The greatest increase in mortality between 2021 and 2022 occurred in the herbicide with shelter and fertilizer treatment (13%). Mortality increases for all other treatments ranged between 2.1% and 10%.

White spruce has the lowest mortality rate of all the species evaluated after four years. The highest mortality occurred in the herbicide with shelter and no fertilizer treatment (51.4%). The lowest mortality was in the mounding with herbicides (both with and without fertilizer) and the mounding with no fertilizer treatments, all at 9%. Mortality in the controls was 28% and 32% without and with fertilizer respectively. The highest mortality increases between 2021 and 2022 was 3% for the control with no herbicide, herbicide with shelter and herbicide and the mounding with fertilizer treatments.

Balsam poplar and green alder mortality was 57% and 34% greater in the control when compared to the mounding with herbicide treatment. For White spruce, mortality in the control was 283% greater than in the mounding with herbicide treatment; albeit, mortality in the control was only 32%. From these preliminary results, we have learned that some form of site preparation is required to improve survival when trying to establish tree or shrub species on legacy grassy sites. This is especially true for the deciduous species.



Total average height measurements follow similar patterns as mortality. For green alder, total average height was greatest on the two mounding treatments (with and without herbicides), and with and without fertilizer, and the mixing with herbicide and no fertilizer treatment. Heights ranged between 43 cm to 47 cm. Total average height in the controls was 17 cm and 30 cm with and without fertilizer respectively. Balsam poplar height was greatest on the mounding with herbicide treatment, with and without fertilizer. The heights were 85 cm and 77 cm, respectively. Balsam poplar heights in the controls were 6 cm and 8 cm with and without fertilizer respectively; however, sample sizes are extremely small due to the high mortality. For white spruce, the mounding with herbicide and the mixing with herbicide treatments, with and without fertilizer, were the best treatments. Heights ranged between 41 cm and 47 cm. Average total height for white spruce in the controls was approximately 30 cm for both fertilizer treatments.

## LESSONS LEARNED

From these preliminary results, we have learned that some form of site preparation is required to improve survival when trying to establish tree or shrub species on legacy grassy sites. This is especially true for the deciduous species. White spruce can tolerate the grass competition, but height growth is impacted as compared to planting on some form of site preparation.

## PRESENTATIONS AND PUBLICATIONS

No publications or presentations available for 2022.

## RESEARCH TEAM AND COLLABORATORS

Institution: Natural Resources Canada, Canadian Forest Service, Canadian Wood Fibre Centre, Edmonton

Principal Investigator: Richard Krygier

Name	Institution or Company	Degree or Job Title	Degree Start Date (Students Only)	Degree Completion Date (Students Only)
Ryan James	Canadian Wood Fibre Centre	Research Technician		
Martin Blank	Canadian Wood Fibre Centre	Research Technician		
Danielle Perri	University of Alberta	BSc	September 2020	December 2024

## Hitchhiker Field Trial at Kearl Operations

**COSIA Project Number:** LJ0324

**Mine and In Situ**

**Research Provider:** Paragon Infinity General Partner Ltd.

**Industry Champion:** Imperial

**Status:** Final Cumulative Summary

### PROJECT SUMMARY

Hitchhiker planting has been proposed as a means to introduce early successional herbaceous species and facilitate the growth and survival of planted woody species simultaneously (Dosite et al., 2016). This method involves sowing two species in the same plug — a shade tolerant, slower growing woody plant along with an early-successional pioneer herbaceous plant. Co-planting in this way provides later successional species with important shade and protection, potentially increasing growth and survival while promoting early herbaceous cover. Alternatively, separate plugs for the woody and herbaceous plants can be planted at the same planting site (companion planting), to achieve the same effect.

A hitchhiker planting trial (the Trial) was established at Imperial's Kearl Oil Sands Mine (Kearl) in July 2018 along an east-facing temporary reclamation area on the East Tailings Area (ETA). The Trial included shrub species (green alder [*Alnus viridis*] and willow [*Salix* spp.]), partner forb species (common fireweed [*Chamerion angustifolium*] and bunchberry [*Cornus canadensis*]) and planting methods (hitchhiker planting and companion planting) as treatments in a modified split-plot design. Control plots where green alder (alder control) or willow shrubs (willow control) were planted without partner forbs were included. The soil prescription was consistent across treatments and fertilizer was not applied.

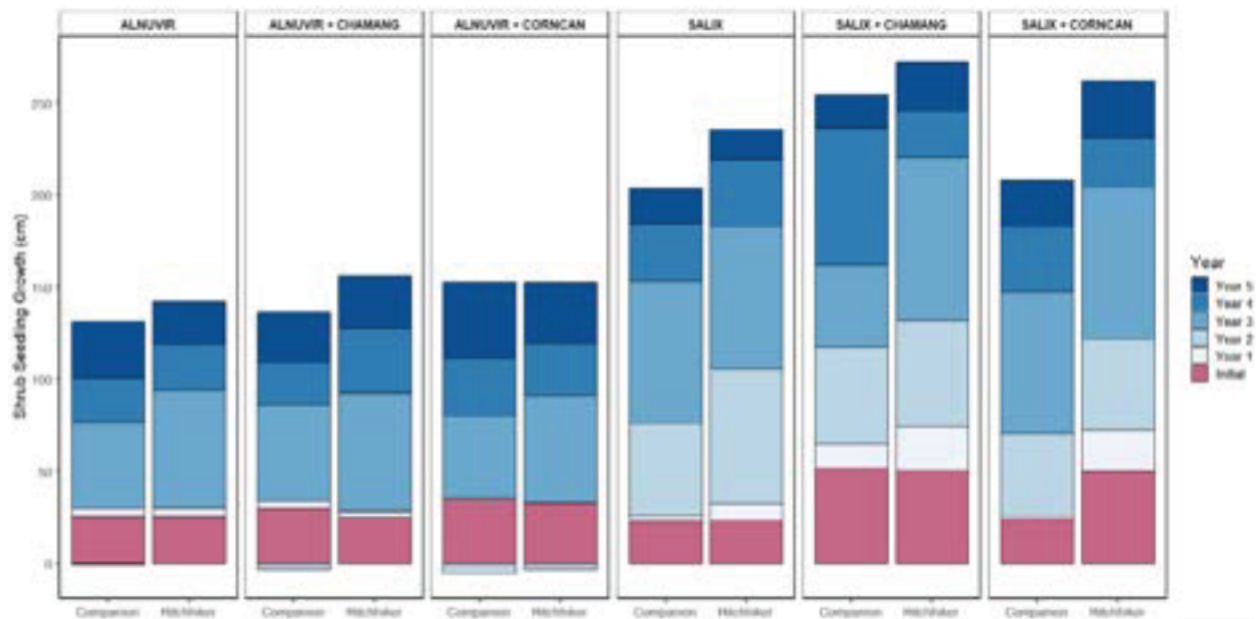
The main objectives of the Trial were to determine whether:

1. Survival and growth of woody shrubs (green alder and willow) are facilitated by co-planting with locally common forb species (common fireweed and bunchberry).
2. Similar survival and growth rates can be achieved by co-planting two plugs in one planting site (companion planting) as opposed to true hitchhiker planting (i.e., two plants in one plug).
3. Co-planting methods provide additional vegetative cover in plots planted with woody shrubs and herbaceous species compared to sites planted with woody shrub seedlings only, providing additional erosion control in newly reclaimed areas.



## PROGRESS AND ACHIEVEMENTS

The trial plots were monitored in September 2022 at the end of the growing season. Performance metrics including seedling height (Figure 1), health (Figure 2), survivorship (Figure 3), percent shrub cover (Figure 4) and percent herbaceous cover (Figure 5) were measured. A summary of the results is provided in Table 1. Outcomes for each objective are described below

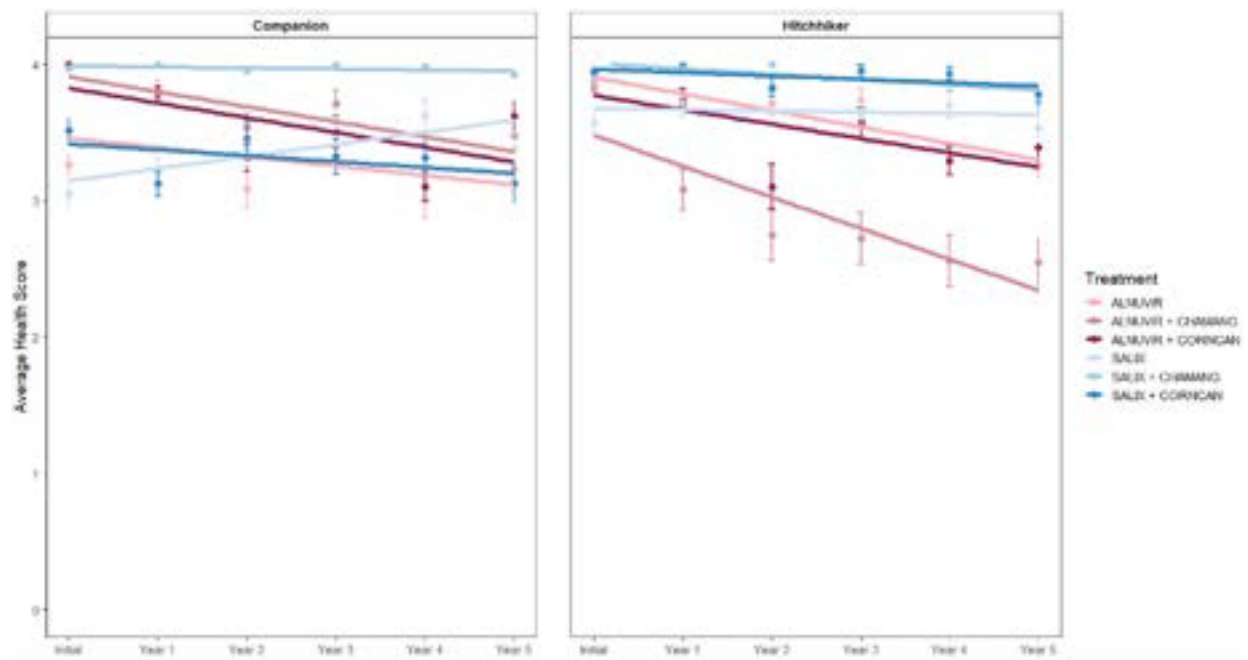


**Figure 1:** Average Growth of Shrub Seedlings Over Five Growing Seasons.

Notes:

<sup>1</sup> ALNUVIR = green alder (*Alnus viridis*), CHAMANG = common fire weed (*Chamarion angustifolium*), CORNCAN = bunchberry (*Cornus canadensis*), and SALIX = willow spp. (*Salix* spp).

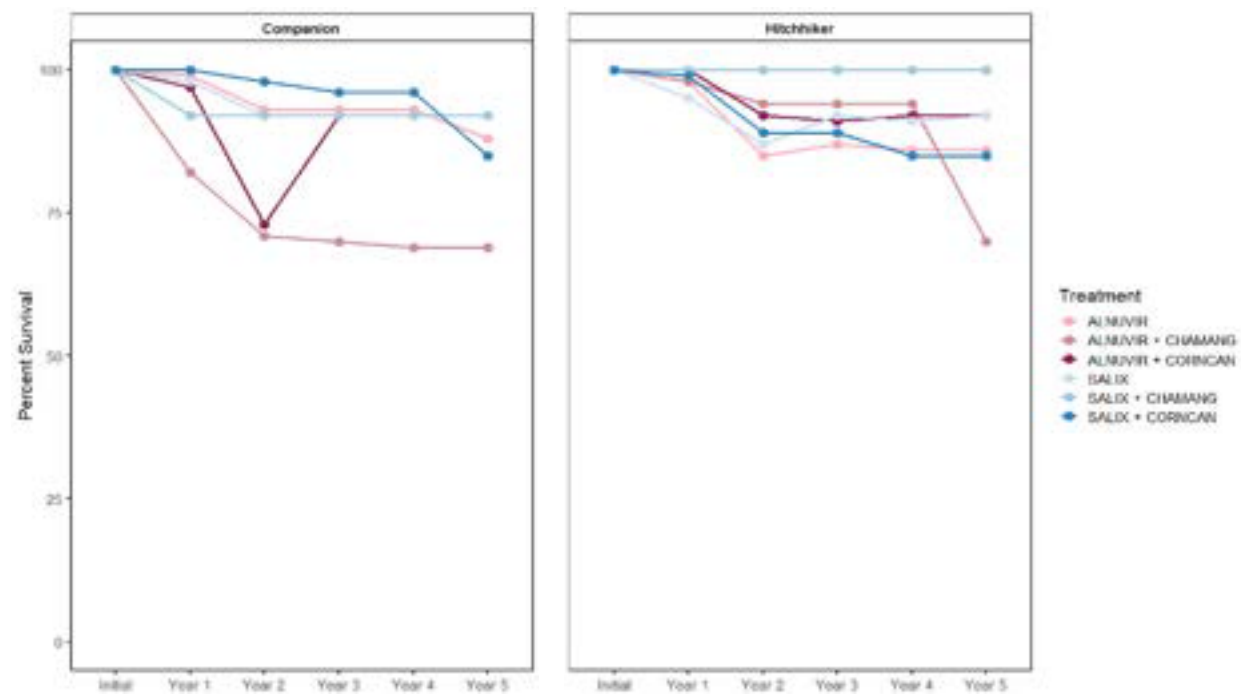
<sup>2</sup> Average height of green alder decreased in some treatments in Year 2 — depicted as negative average growth.



**Figure 2:** Average Health of Shrub Seedlings after Five Growing Seasons

Notes:

1 Health Score was measured on a scale of 0 to 4, where 0 = missing, 1 = dead, 2 = poor, 3 = good, and 4 = excellent



**Figure 3:** Percent Survival for Shrub Seedlings During the First Five Growing Seasons

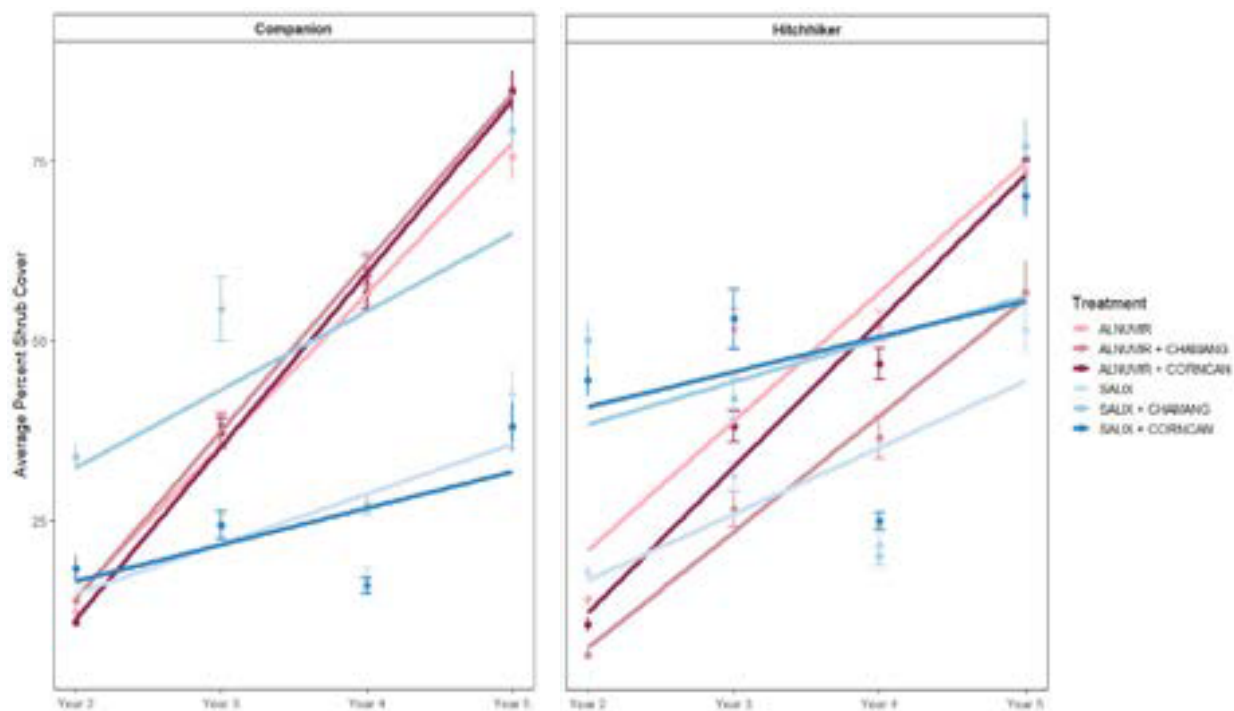


Figure 4: Average Percent Cover of Planted Shrubs after the First Five Growing Seasons

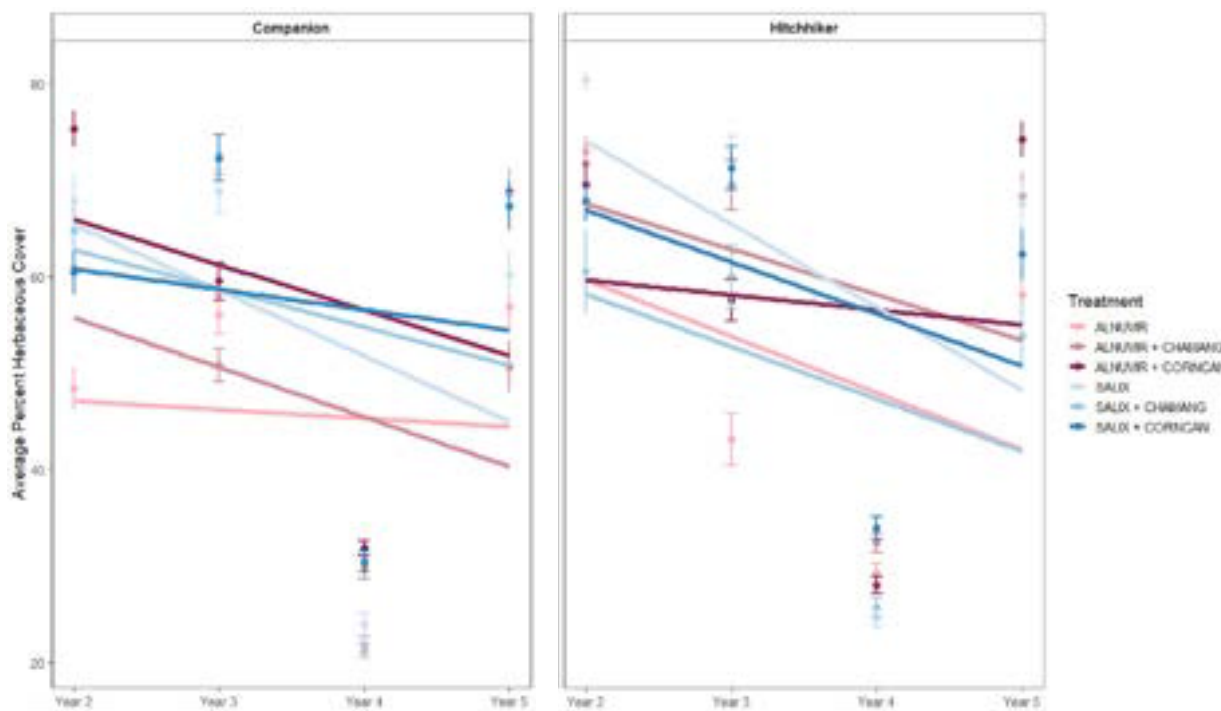


Figure 5: Average Percent Cover of Herbaceous Species after Five Growing Seasons





**Table 1: Hitchhiker Trial Results Summary**

Shrub	Measure	Partner Forb Trends <sup>1</sup>			Planting Method Trends <sup>1</sup>	
		None	CHAMANG	CORNCAN	Hitchhiker	Companion
Green alder	Shrub Height					
	Shrub Health					
	Shrub Survival <sup>2</sup>					
	Shrub Cover					
	Herbaceous Cover					
Willow	Shrub Height					
	Shrub Health					
	Shrub Survival <sup>2</sup>					
	Shrub Cover					
	Herbaceous Cover					

Notes:

<sup>1</sup> Significant differences are denoted with different colours across a row for forb trends and planting method trends — darker cells are significantly higher than lighter cells. No fill indicates that the effect was not significant.

<sup>2</sup> Shrub survival was not tested statistically — trends were identified from the data.

## Objective 1: Co-planting with Forb Species

Based on the results from Year 5 of the Trial, green alder and willow respond differently when co-planted with locally common forb species. The effects observed are likely driven by the different site conditions each species needs to thrive.

Willow seedlings planted with common fireweed were taller ( $P < 0.001$ ; Figure 1) and healthier ( $P < 0.001$ ; Figure 2) than those planted with bunchberry or those that were planted alone. Green alder seedling growth was not affected by the presence of partner forbs ( $P = 0.056$ ); all shrub:forb combinations had similar heights by the end of the fifth growing season, regardless of planting method (Figure 1). In terms of shrub health, green alder that were planted with common fireweed fared less well than other treatments, particularly in the later years of the Trial (Figure 2). This effect was not statistically significant despite health scores for green alder planted with common fireweed being markedly lower in 2022. A three-way interaction term between year:forb:method would likely have indicated significantly lower health and shrub cover in the latter years of the trial, but the model could not be run due to overdispersion.

Co-planting with partner forbs also affected the percent cover of planted shrub species differently (Figure 4). Green alder percent cover was lowest when planted with common fireweed ( $P = 0.041$ ), whereas willow percent cover was highest when planted with common fireweed ( $P < 0.001$ ). Average health (Figure 2) and survival (Figure 3) were also lower for green alder planted with common fireweed, particularly when using the hitchhiker-planting method. This suggests that green alder are more susceptible to competition with their partner forbs than willow are with their partner forbs.



## Objective 2: Planting Method

Planting method generally had a stronger effect on shrub performance than the species of partner forb planted. For green alder, growth ( $P < 0.001$ ; Figure 1) and survival (Figure 3) was highest in the hitchhiker-planted plots, but percent cover was higher in the companion-planted plots ( $P < 0.001$ ; Figure 4). Growth ( $P < 0.001$ ; Figure 1), health ( $P < 0.001$ ; Figure 2), and percent cover ( $P < 0.001$ ; Figure 4) of willow shrubs were higher in the hitchhiker-planted plots. These results support the possibility that the closer interaction between green alder and their partner forbs in the hitchhiker planting method is costly for the green alder seedlings, reducing their overall survivorship and canopy cover. This is supported by recent research which suggests that a “zone of net benefit” exists for seedling establishment in some environments, where the balance between positive (facilitation) and negative (competition) interactions changes with distance from a benefactor (Teste and Simard, 2008). The increased growth rates observed for green alder may therefore be the result of seedlings investing more resources into growing taller rather than bushier to escape competition with the herbaceous layer. Conversely, willow, which are stronger competitors, with a narrower, less-dense canopy, thrive with the close associations of the hitchhiker planting method.

## Objective 3: Vegetative Cover and Erosion Control

By the end of the fifth growing season, no obvious signs of erosion were observed at the Trial site. Shrub and herbaceous cover appear to be stabilizing the substrate adequately in all plots. However, herbaceous cover in green alder plots was greater when co-planted with partner forbs, particularly using the Hitchhiker planting method ( $P < 0.0001$ ; Figure 5). Herbaceous cover in willow plots was maximized when bunchberry was included as the partner forb species ( $P < 0.0001$ ; Figure 5).

Herbaceous cover in the Trial decreased each year in an inverse relationship with increasing shrub cover (Figures 4 and 5), which is an expected pattern associated with primary succession (Prach et al., 1999). Herbaceous cover in green alder plots and shrub cover in willow plots were higher when co-planted with partner forbs, particularly using the hitchhiker planting method. This suggests that green alder planted with partner forbs using the hitchhiker method are better able to encourage natural ingress via seed-trapping and the development of a favourable microclimate, while willow shrubs are better able to spread under the protection of partner forbs during the first five years of growth.

## LESSONS LEARNED

After five years of growth, there appear to be both benefits and drawbacks to co-planting green alder and willow shrub seedlings with partner forbs. Co-planting encouraged herbaceous cover but decreased shrub survival when the partner forb was common fireweed. Of the two planting methods, hitchhiker planting resulted in seedlings that grew taller and had higher survival rates than companion planting, but the performance of seedlings that were co-planted was similar to control plots without partner forbs in many cases. Co-planting, and particularly hitchhiker planting is a costly method of revegetating reclaimed areas. While there were benefits to both methods of co-planting, similarity between control plots and plots that were co-planted suggests that neither method is worth the cost at the end of the fifth year.



Lessons learned from this hitchhiker planting trial should be used to develop a new trial exploring alternative species pairings and different planting methods:

- Instead of using the hitchhiker or companion planting method, a newly reclaimed area should be seeded with a nurse crop of the partner forb. This would not only be less costly, but also more operationally feasible and more likely to provide the protection young shrub seedlings would benefit from.
- This Trial focused on only four species pairings. Reclamation planting prescriptions typically include many more species. Alternative species pairings should be investigated in the new trial, including difficult-to-establish species, such as common blueberry (*Vaccinium myrtilloides*).
- Incorporate remote imagery (drone or satellite) to quantify changes in vegetation health and cover over the full trial area.

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Prach, K., P. Pysek, P. Smilauer. 1999. *Prediction of Vegetation Succession in Human-Disturbed Habitats Using an Expert System*. Restoration Ecology 7: 15-23.

Teste F., and S. Simard. 2008. *Mycorrhizal Networks and Distance from Mature Trees Alter Patterns of Competition and Facilitation in Dry Douglas-fir Forests*. Oecologia 158: 193-203.

## PRESENTATIONS AND PUBLICATIONS

No public presentations or publications were released.

## RESEARCH TEAM AND COLLABORATORS

Institution: Paragon Infinity General Partner Ltd.

Principal Investigator: Paragon Infinity General Partner Ltd.

# Interim Reclamation

**COSIA Project Number:** LJ0226

**Mine and In Situ**

**Research Provider:** NAIT Centre for Boreal Research

**Industry Champion:** ConocoPhillips

**Status:** Year 8 of 10

## PROJECT SUMMARY

This program of research encompasses study topics of: (1) interim reclamation (also known as temporary reclamation); as well as (2) final reclamation. Even though there is a distinction between interim and final reclamation, it should be recognized that much of the interim reclamation research is applicable to final reclamation. Each study (and projects therein) is described below

### Study 1: Interim Reclamation of a Facility Soil Stockpile

Industrial site disturbances, whether in the mining or oil and gas sector, typically result in the clearing of forests and stockpiling of surface soils during the development and operational phases. Ongoing management of these stockpiles is required until the site is decommissioned, and final reclamation is undertaken. This is where the facilities are removed, the site is recontoured and stockpiled soils are redistributed. Historical and current practices include seeding stockpiled soils with grasses and the use of chemical herbicides to eradicate or control prohibited and noxious weeds. In principle, the temporary reforestation of soil stockpiles will provide; root and seed propagules; coarse woody materials; long-term soil erosion control; reduced use of chemical herbicides for noxious weed management; and increase biodiversity. Temporary reforestation of soil stockpiles is an alternative, though not widely used, practice that may better fit the fundamental long-term final reclamation goals in forested settings, which is to re-establish a self-sustaining functional boreal forest.

This temporary (or interim) reclamation project is situated on an eight-hectare topsoil and subsoil stockpile that is anticipated to be in place for several decades. The intent of this study is to advance interim reclamation a step beyond historical recontouring and seeding practices to include the establishment of woody species on non-active areas of an in situ project (e.g., soil stockpiles) during the life of the facility. It is hypothesized that this will speed establishment of forest cover and reduce the need for ongoing and repeated weed management. To date, this practice is not something that has been commonly implemented at in situ facilities in the oil sands region. This project provides an on-site demonstration of the effect of site preparation (dozer to create furrows and backhoe to mound soil on steeper slopes), varying planting densities (0, 2,500, 5,000 and 10,000 stems per hectare), and the use of coarse woody material as a reclamation material (present or absent). Rather than assessing one combination of interim reclamation techniques, the experimental trials have been structured to support the development of best practices that will have a high probability of success at final in situ specific reclamation and will be cost effective to implement.



This project is also designed to question assumptions about species suitability for use (in terms of the out-planting of different nursery stock species) in a reclamation context. Industrial disturbances do not necessarily follow the same early vegetation dynamic patterns found after fires or forest harvesting. Industrial disturbances require soil to be moved during construction and again during reclamation prior to final revegetation. This anthropomorphic soil redistribution forces the system into being a largely seed-based regeneration/revegetation system rather than root based. This has consequences for the native species being established and will favour those species that are able to tolerate competition as young seedlings. The project plots are situated on a big hill with soil and aspect variability which should help inform tolerance ranges for each of the planted species in a reclamation context.

While the long-term goal of this project is to initiate forest development, in the short term, three separate projects were initiated at this site to ask specific questions related to initial planting density, how to include desirable native herbaceous species, and alternative methods of planting deciduous trees.

Specific objectives and study questions for these projects are further described below.

***Project 1: Site preparation and establishment density***

1. Compare three densities of container stock planting (2,500, 5,000 and 10,000 stems per hectare) and monitor natural regeneration (within unplanted controls).
  - a. Which native tree and shrub species will provide speedy establishment, produce viable seed within the time frame of facility life and have capacity to regenerate aggressively through root fragments following reclamation activities?
  - b. Which species are best suited to different combinations of slope position and aspect on reclamation soils?
  - c. Is natural regeneration a viable approach for forest plant establishment?
  - d. How does the speed of canopy development and structure compare with different densities over time?
  - e. Does the overstory density impact development of understory vegetation?
  - f. Does aspect or slope position interact with plant establishment through these methods?
2. Compare use of soil adjustment to create a rough and heterogeneous soil surface against track-packed “smooth” reclamation approach.
  - a. Does soil adjustment impact the growth and production of planted woody species?
  - b. Does soil adjustment improve natural ingress and regeneration of desirable woody species?
3. Demonstrate the utility of coarse woody material in conjunction with soil treatments to create a rough and heterogeneous soil surface.
  - a. Does coarse woody material impact growth and production of planted woody species?
  - b. Does coarse woody material increase the stability of sloped soils and reduce erosion?
  - c. Does coarse woody material improve the natural ingress and regeneration of desirable woody species?
4. Examine the impact of wildlife browsing (and presence) on establishment and development of planted woody species.
  - a. Which species are preferentially browsed?
  - b. What is the impact of browsing on plant performance?
  - c. Does browsing significantly impact canopy development?



### **Project 2: Cover crop establishment through planting**

1. Evaluate two methods of planting native forbs including: individual planting of container stock and co-planting native forbs with a woody species (produce plants in same plug).
  - a. Does the forb develop (increase in vegetation cover) equally well with both approaches?
  - b. Is there a positive, neutral or negative impact for the woody species which shares the plug initially?
2. Compare the effect of the addition of native forbs during the early phase of forest development.
  - a. Do they facilitate ingress of other desirable species?
  - b. Do they reduce ingress of undesirable species?
  - c. Do they aid in soil stabilization?
  - d. What is incremental cost of planting native forbs?
3. Optimize production of mixed-species container stock for three different woody species (green alder, willow, and paper birch) each co-grown with fireweed.
  - a. What is the best time to sow the forb into container with woody species?
  - b. Is mixed-species container stock appropriate for all woody species or only for specific species?

### **Project 3: Aspen establishment through container stock, optimizing plant deployment through grouped planting**

This study was conducted as a pilot project to further the concept of cluster planting of deciduous trees ([see project Cluster Planting \(page 18\), 2018 COSIA Land EPA — In Situ Report](#)).

The objective of this project was to compare localized cluster planting of aspen with conventional planting at uniform spacing. In this project, the question of how many plants are required for a “cluster” to positively impact survival and growth of aspen container stock will be addressed.

### **Study 2: Vegetation Management Solutions for Final Reclamation**

Noxious weeds and other undesirable vegetation (e.g., sweet clover [*Melilotus* sp.], alsike clover [*Trifolium hybridum*], creeping red fescue [*Festuca rubra*], timothy [*Phleum pratense*] and smooth brome [*Bromus inermis*]) are transported to reclamation sites by a variety of mechanisms. These include historical presence in the soil seed bank from previous decades of utilization in cover crop mixes, contaminated equipment, wind, wildlife and in some cases intentional broadcasting. Collectively, these undesirable species present challenges to the development of forest plant communities. In northern Alberta, management of aggressive agronomic species is a significant issue to forest development and the certification of reclaimed wellsites (Bressler, 2008). Regulatory criteria and legislation clearly define the need to control and eradicate noxious weed species (Weed Control Act, 2010; Environment and Sustainable Resource Development, 2013), as well as undesirable species (Environment and Sustainable Resource Development, 2013). Site preparation, cultural control (cover crop establishment) and chemical management represent a range of approaches to control or eradicate undesirable species.

The objective of this study was to examine the ability of combinations of native plant cultural controls (cover crop) and herbicide-based approaches to reduce and eliminate undesirable plant ingress. In this study, approaches that are appropriate for use in the early stages of revegetation development following soil replacement will be evaluated.



Each of these approaches was initiated in the first year following reclamation with plans to monitor the study for three growing seasons. At the completion of the study, the following questions will be answered:

1. Which approaches are most effective at reducing the initial establishment of undesirable species?
2. By controlling ingress of undesirable plants, are there also differences in native plant establishment through natural ingress?
3. Is there a reduction in the growth and productivity of desirable native woody species when utilizing a treatment that is aimed at reducing undesirable plant development (i.e., a trade-off)?
4. What is the potential return on investment of the vegetation management approaches considering relative benefit/success at managing undesirable species?

## PROGRESS AND ACHIEVEMENTS

### Study 1: Interim Reclamation of a Facility Soil Stockpile

#### ***Project 1: Site preparation and establishment density***

This section will focus on the aspects of findings from Project 1 based on results collected during the sixth growing season (2021) towards objectives 1d and 1e. The results presented below will focus on two key components: comparisons between density treatments in terms of stem densities of woody species and understory vegetation development.

#### **Objective 1d: How does the speed of canopy development and structure compare with different densities over time?**

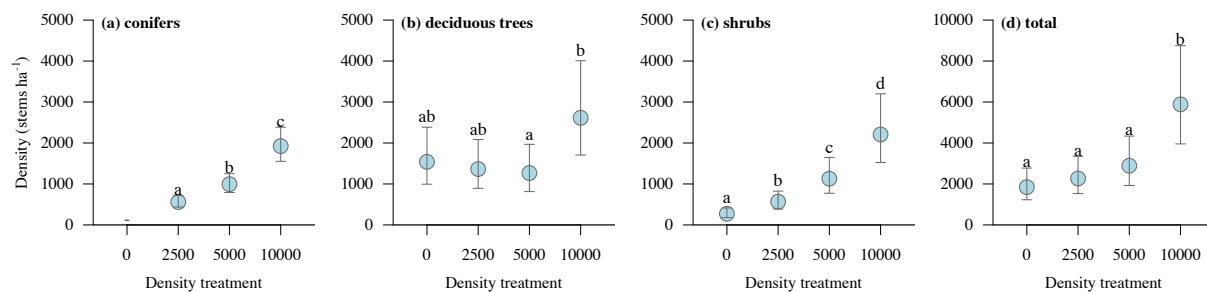
Both conifer and tall shrubs (*Salix* and *Alnus viridis*) showed a progressive increase in stem density with the lowest densities associated with the control (zero stems per hectare [stems ha<sup>-1</sup>] treatment) and highest densities in the 10,000 stems ha<sup>-1</sup> treatment (Figure 1); this is not an unexpected finding as this follows the pattern of initial densities established for these species. Due to natural regeneration observed in *Populus tremuloides* and *P. balsamifera* (Table 1) coupled with variable survival of planted individuals of these species, there was no difference in deciduous tree density amongst the control, 2,500 and 5,000 stems ha<sup>-1</sup> treatments though the 10,000 stems ha<sup>-1</sup> treatment had approximately double the density of the other treatment levels (Figure 1b). Despite differences in stem densities, most species maintained similar mean heights experiment-wide with two notable exceptions (Figure 2). *P. balsamifera* was shorter, on average in the control relative to 5,000 and 10,000 stems ha<sup>-1</sup> treatments (Figure 2a) with this difference representing a larger proportion of shorter trees from natural recovery compared with planted seedling treatments. The 10,000 stems ha<sup>-1</sup> treatment was also associated with taller *R. idaeus* compared with the control and 2,500 stems ha<sup>-1</sup> treatments though the stem densities of this species were similar — and very high — experiment-wide (Table 1). It is possible this difference was an indication of greater intraspecific competition of woody vegetation, which may have exerted a stronger influence towards height growth in this species.





### Objective 1e: Does the overstory density impact development of understory vegetation?

Total understory vegetation cover was not significantly different amongst planting density treatments and the unplanted control though total cover was lower, on average, by 7% to 10% in the control treatment (Figure 3f). Both grass and horsetail cover were low experiment wide (< 5%) (Figure 3a, 3b). Non-native forb cover was similar, regardless of planting density treatment and notably at its lowest value since study inception with mean values at 5% to 10% (Figure 3c). Surpassing non-native forbs, mean cover values of approximately 20% for non-native forbs were observed in all density treatments except for the 10,000 stems  $\text{ha}^{-1}$  treatment at 13% (Figure 3d). It is notable, that despite the lack of statistical differences in grasses, horsetails, non-native and native forbs, it was the 10,000 stems  $\text{ha}^{-1}$  treatment that was typically associated with the lowest cover on average (Figure 3). This subtle response suggests a progressive shift in understory community, likely because of the closing overstory canopy in this treatment. Planted stem density treatments exhibited a strong influence on the cover of woody vegetation in the understory with an average of nearly 20% in the control and 27%, 43% and 50% in the 2,500, 5,000 and 10,000 stems  $\text{ha}^{-1}$  treatments, respectively (Figure 3e). While coverage of raspberries represented a large proportion of the understory woody cover, individually planted tree and shrub species each contributed 1% to 3%, which invariably contributed to the pattern observed.



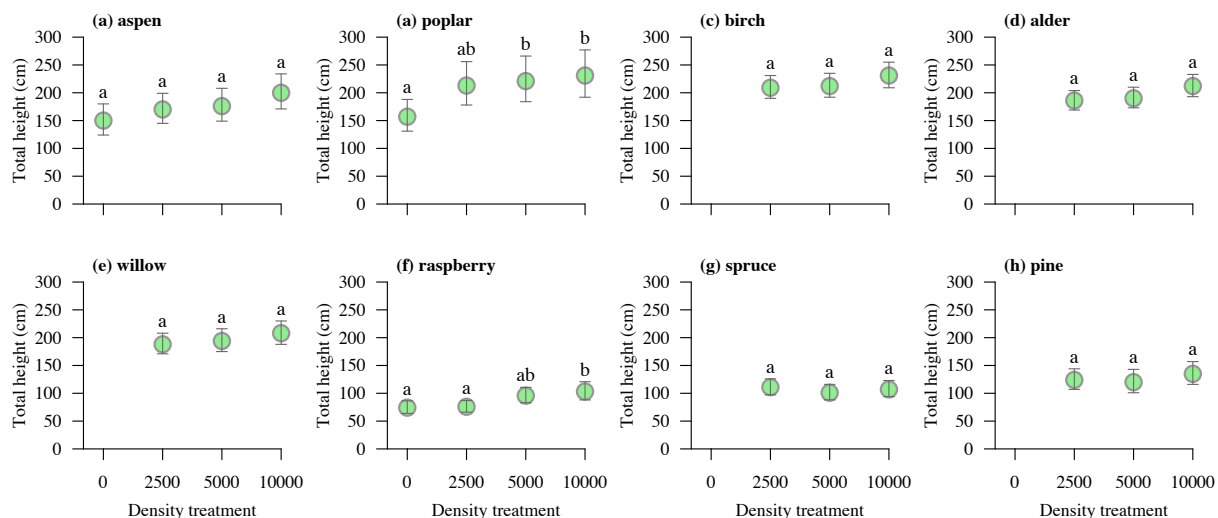
**Figure 1:** Estimated marginal mean stem density of (a) conifers, (b) deciduous trees, (c) shrubs and (d) total woody vegetation within each density treatment; control (0), low density (2,500 stems  $\text{ha}^{-1}$ ), moderate density (5,000 stems  $\text{ha}^{-1}$ ), and high density (10,000 stems  $\text{ha}^{-1}$ ) in the sixth (2021) growing season. The total woody and shrub densities excludes raspberry stem counts which were exceptionally high throughout the study site and tended to mask density treatment effects (refer also to Table 1 below). Treatments not sharing the same letters indicate a significant ( $p < 0.05$ ) difference in means. Values in brackets represent 95% confidence intervals on the treatment mean ( $n = 6$ ). Estimated means were generated from a two-factor generalized linear mixed effects model (fitted with a negative binomial distribution) on plot-level stem counts that were back-transformed to represent stem densities.



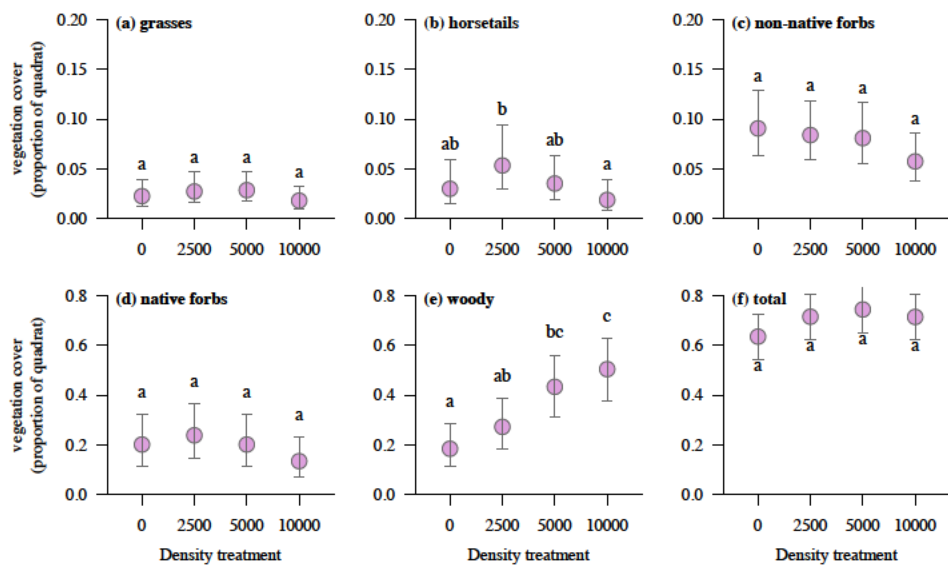
**Table 1: Estimated marginal mean stem density for deciduous trees** (balsam poplar [*Populus balsamifera*], aspen [*Populus tremuloides*], paper birch [*Betula papyrifera*]), conifers (jack pine [*Pinus banksiana*], white spruce [*Picea glauca*]), and shrubs (green alder [*Alnus viridis*], willow [*Salix* sp.], common raspberry [*Rubus idaeus*]) across four density treatments; control (0), low density (2,500 stems ha<sup>-1</sup>), moderate density (5,000 stems ha<sup>-1</sup>), and high density (10,000 stems ha<sup>-1</sup>). Where too few observations were present, no statistical comparisons were made with respect to stem densities in the control treatment. Treatments not sharing the same letters indicate a significant ( $p < 0.05$ ) difference in means. Values in brackets represent 95% confidence intervals on the treatment mean ( $n = 6$ ). Estimated means were generated from a two-factor generalized linear mixed effects model (fitted with negative binomial distribution) on plot-level stem counts that were back-transformed to represent stem densities.

Species	0*	2,500		5,000		10,000	
	measured	target	measured	target	measured	target	measured
aspen	536a (289-994)	250	346a (187-642)	500	268a (139-519)	1,000	702a (375-1,311)
birch	-	250	235a (180-309)	500	342a (270-434)	1,000	661b (549-796)
poplar	942a (553-1,602)	375	766a (469-1,245)	750	642a (389-1,060)	1,500	1,219a (748-1,989)
pine	-	500	118a (61-228)	1,000	120a (58-247)	2,000	390b (212-717)
spruce	-	375	169a (109-264)	750	267ab (177-402)	1,500	394b (266-584)
alder	-	375	170a (114-252)	750	304a (214-431)	1,500	541b (388-753)
willow	-	375	322a (232-449)	750	645b (480-867)	1,500	1,248c (949-1,641)
raspberry	8,712a	-	8,360a	-	5,632a	-	7,568a
	(4,171-18,172)		(4,039-17,336)		(2,680-11,880)		(3,608-15,928)

\*Note that one of the six replicate plots exhibited exceptionally high natural regeneration of aspen (1,400 stems ha<sup>-1</sup>) and balsam poplar (2,300 stems ha<sup>-1</sup>).



**Figure 2: Estimated marginal mean total height of deciduous tree** (balsam poplar [*Populus balsamifera*], aspen [*Populus tremuloides*], paper birch [*Betula papyrifera*]), conifers (jack pine [*Pinus banksiana*], white spruce [*Picea glauca*]), and shrubs (green alder [*Alnus viridis*], willow [*Salix* sp.]) across four density treatments; control (0), low density (2,500 stems ha<sup>-1</sup>), moderate density (5,000 stems ha<sup>-1</sup>), and high density (10,000 stems ha<sup>-1</sup>). Treatments not sharing the same letters indicate a significant ( $p < 0.05$ ) difference in means. Error bars represent 95% confidence intervals on the treatment mean ( $n = 6$ ). Estimated means were generated from a two-factor generalized linear mixed effects model (fitted with a gamma distribution).



**Figure 3:** Estimated marginal mean vegetation cover of (a) grass, (b) horsetails, (c) non-native forb, (d) native forb, (e) woody, and (f) total cover within each density treatment; control (0), low density (2,500 stems ha<sup>-1</sup>), moderate density (5,000 stems ha<sup>-1</sup>), and high density (10,000 stems ha<sup>-1</sup>) in the sixth (2021) growing season. Treatments not sharing the same letters indicate a significant ( $p < 0.05$ ) difference in means. Error bars represent 95% confidence intervals on the treatment mean ( $n = 6$ ). Estimated means were generated from a two-factor generalized linear mixed effects model (fitted with a beta distribution).



**Figure 4:** Replicate block 2 (subsoil) where (a) 0 stems ha<sup>-1</sup>, (b) 2,500 stems ha<sup>-1</sup>, (c) 5,000 stems ha<sup>-1</sup> and (d) 10,000 stems ha<sup>-1</sup> density treatments in August 2021.





**Figure 5:** Replicate block 5 (topsoil) where (a) 0 stems ha<sup>-1</sup>, (b) 2,500 stems ha<sup>-1</sup>, (c) 5,000 stems ha<sup>-1</sup> and (d) 10,000 stems ha<sup>-1</sup> density treatments in August 2021.



## LESSONS LEARNED

Emerging lessons: This project has demonstrated that there are benefits to actively planting nursery stock seedlings on a previously disturbed reclamation area (in this case a soil stockpile). High-density planting (10,000 stems ha<sup>-1</sup> treatment) is clearly the candidate for the speediest development of forest canopy vegetation, and after only six growing seasons, it is showing the strongest shifts in terms of progression towards a vegetation community with typical forest attributes. At lower planting densities, the differences between unplanted and the 2,500 or 5,000 stems ha<sup>-1</sup> treatments are still rather subtle though there was a notable increase in understory woody vegetation coverage as the planting treatment density increased. Without intentional planting, natural plant establishment is at the mercy of site conditions and chance events, resulting in high variation in recruitment levels. Moreover, tree species recruitment, thus far, has been largely restricted to wind-dispersed species in the current study.

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Bressler, A. 2008. Weed management in Alberta's oil and gas industry. Proceedings of the Weeds Across Borders Conference, Banff, Alberta. May 27-30, 2008.

Environment and Sustainable Resource Development 2013. Update Report on Alberta Environment and Sustainable Resource Development's Upstream Oil and Gas Reclamation Certificate Program. Edmonton, Alberta: Government of Alberta.

Weed Control Act 2010. Weed Control Regulation, Alberta Regulation 19/2010. Edmonton, Alberta.

## PRESENTATIONS AND PUBLICATIONS

### Conference Presentations/Posters

Schoonmaker, A.L., Chigbo, C., Albricht, R., Pinno, B. (2022). Boreal tree and shrub development on stockpiled subsoil and topsoil. Canadian Land Reclamation Association Meeting, Red Deer, Alberta. Abstract and oral presentation.



## RESEARCH TEAM AND COLLABORATORS

Institution: NAIT Centre for Boreal Research

Principal Investigator: Dr. Amanda Schoonmaker

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Baah-Acheamfour	NAIT Centre for Boreal Research	Research Associate		
Dr. Chibuike Chigbo	NAIT Centre for Boreal Research	Research Associate		
Kaela Walton-Sather	NAIT Centre for Boreal Research	Research Assistant		
Sofia Toledo	NAIT Centre for Boreal Research	Research Assistant		
Katelyn Grado	NAIT Centre for Boreal Research	Student Research Assistant	2019	2021
Tacy Wilkes	NAIT Centre for Boreal Research	Student Research Assistant	2020	2022
Adam Feldberg	NAIT Centre for Boreal Research	Student Research Assistant	2019	2021
Carlos Avila	NAIT Centre for Boreal Research	Student Research Assistant	2019	2021

Research Collaborators: Dr. Brad Pinno, University of Alberta (formerly Canadian Forest Service); Dr. Derek MacKenzie, University of Alberta



## Oil Sands Vegetation Cooperative

**COSIA Project Number:** LE0014

**Mine and In Situ**

**Research Provider:** Wild Rose Consulting Inc.

**Industry Champion:** Canadian Natural

**Industry Collaborators:** Cenovus, ConocoPhillips, Imperial, Suncor, Syncrude

**Status:** Ongoing

### PROJECT SUMMARY

Thirteen years ago, the Oil Sands Vegetation Cooperative (OSVC) was established to enable collaborative harvesting and banking of native boreal forest seed for use in revegetation and research. In 2014, the OSVC became a project led by Canada's Oil Sands Innovation Alliance (COSIA) Land Environmental Priority Area (EPA). The OSVC supports seed collection initiatives in the northern Athabasca Oil Sands (NAOS), Southern Athabasca Oil Sands (SAOS) and Cold Lake (COLK) regions.

The OSVC's strategic objectives include working with industry to identify knowledge gaps, and to propose and support research programs to optimize seed harvest, storage, propagation, and final field establishment. In addition, the OSVC provides open communication regarding our project with COSIA members and to a wider audience.

The scope of work for this project includes preparation of seed harvest needs, coordination of the annual seed harvest program, management of records for the OSVC seed inventories in the provincial seed bank, provision of technical expertise on identification, collection, storage and deployment of native seed, technical guidance to the OSVC regarding research needs, coordination and record keeping for ongoing discussions related to research project development, preparation of support documents such as literature reviews and data summaries, and preparation of a bi-annual newsletter.

### PROGRESS AND ACHIEVEMENTS

#### Seed Banking

In 2022 (13<sup>th</sup> season) the OSVC harvested 176 litres (L) of fruit. Seeds were harvested from two seed zones in northeastern Alberta for the NAOS and SAOS divisions based on requests from member companies. The following were extracted and registered:



**Table 1: Species harvested**

40 L <i>Alnus alnobetula</i> (green alder) SAOS
2 L <i>Alnus incana</i> (river alder) NAOS
30 L <i>Betula papyrifera</i> (paper birch) SAOS
73 L <i>Populus tremuloides</i> (trembling aspen) NAOS
12 L <i>Rhododendron groenlandicum</i> (Labrador tea) NAOS
5 L <i>Vaccinium myrtilloides</i> (dwarf blueberry) both
8 L <i>Viburnum edule</i> (lowbush cranberry) SAOS

In 2022, activities supporting the OSVC seed collection initiatives included:

- Annual updates to the cross-company record keeping system and administration of the cooperative bank continued in 2022.
- Seed testing was conducted on seed harvested in 2021 to evaluate seed quality. (Seed harvested in 2022 will be tested in January 2023.)

### Progress on Strategic Objectives

- OSVC members held eight meetings in 2022 to discuss knowledge gaps and propose research.
- A Knowledge Gap Context document was initiated in 2020 to further identify specific gaps and propose hypotheses and research questions to address these concerns. In 2022, a detailed analysis was completed for four species: chokecherry, Labrador tea, dwarf blueberry and lowbush cranberry.
- Work on the Operational Monitoring program (initiated in 2018) to determine survival of outplanted shrubs over the oil sands area continued. This monitoring program examines survival by species on various reclamation materials. Two species (red-osier dogwood and green alder) are approaching a threshold of data points to allow for rigorous analysis.
- In 2021 a trial (in cooperation with Smoky Lake Forest Nursery) was initiated to improve operational production methods for *Viburnum edule* (lowbush cranberry), a particularly difficult species to produce. In 2022, the first emergence was observed but continues slowly. Results will be analyzed and presented in 2023.

### Communications

- Two editions of the Oil Sands Vegetation Cooperative Newsletter (May and November 2022) were published.
- An overview of the OSVC was presented at the 34<sup>th</sup> Alberta Native Plant Council Annual Conference.

## LESSONS LEARNED

- Researchers are working towards filling numerous identified knowledge gaps, particularly in the areas of seed handling practices, shrub mortality, vegetative and seed propagation of shrubs. It was determined that the best way to proceed was to focus on a few (four) species. Collaboration with growers continues.
- For the seed bank to be successful, it is important that the banked seeds are of the highest quality possible. The harvest and handling of native seed is expensive and labour intensive. Boreal shrub species exhibit strong dormancies and require excessive time periods for germination testing. Moreover, standard protocols for



testing do not exist. Therefore, the OSVC is having all seedlots assessed by a third-party, accredited seed testing laboratory using a standardized TZ viability test. Seed quality will be invaluable in determining seeding rates, will assist in improving seed harvest and handling operations, and be useful for evaluating longevity.

- Little is known about the survival of seedlings once planted onto reclaimed sites. Operational level monitoring plots are being installed and monitored but a wider variety of sites and species is needed.

## PRESENTATIONS AND PUBLICATIONS

### Newsletters

[Wild Rose Consulting Inc. 2022. Oil Sands Vegetation Cooperative Newsletter. May 7\(1\). 4 pages.](#)

[Wild Rose Consulting, Inc. 2022. Oil Sands Vegetation Cooperative Newsletter. November 7\(2\). 3 pages.](#)

### Conference Presentations/Posters

Wild Rose Consulting 2022. Growing Native Shrubs: Seed harvest, handling, storage, and propagation. Growing Native Plants, 34<sup>th</sup> annual Alberta Native Plant Council conference. March 12, 2022.

## RESEARCH TEAM AND COLLABORATORS

Institution: Wild Rose Consulting, Inc.

Principal Investigator: Ann Smreciu

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Research Collaborators: Natural Resources Canada; Peggy Popel, Smoky Lake Forest Nursery

# The Use of Carbon Nanomaterials to Improve Seed Germination Seedling Vigour and Growth

**COSIA Project Number:** LE0067

**Mine and In Situ**

**Research Provider:** NAIT Centre for Boreal Research

**Industry Champion:** Canadian Natural

**Industry Collaborators:** Cenovus, ConocoPhillips, Imperial, Suncor, Syncrude

**Status:** Final Cumulative Summary

## PROJECT SUMMARY

Seed priming techniques are applied to improve seed germination and seedling vigour by rehydrating seeds and triggering metabolic processes during the early phase of germination (Paparella et al., 2015). This technique has been widely used as one of the most effective and inexpensive propagation methods (Hasanuzzaman and Fotopoulos, 2019). Nanopriming is a recent seed priming technique with nanoparticles that have been used extensively on crop plant species (Pawar and Laware, 2018) yet hasn't been tested on native boreal forest species, particularly in improving seed germination and growth performance. A recent study on two dormant boreal forest species, green alder (*Alnus viridis*) and buffaloberry (*Shepherdia canadensis*), using nanopriming with non-functionalized and functionalized (with carboxylic acid (COOH) or hydroxyl (OH)) multi-walled carbon nanotubes (MWCNT), combined with cold stratification, was successful in improving the seed germination and seedling vigour (Ali et al., 2020). However, the need remains to investigate the potential of using nanopriming with carbon nanotubes (CNT) to improve the germination of other key native boreal species used in the reclamation of industrially disturbed sites.

The primary objective of this project was to evaluate the effects of MWCNTs in improving seed germination in select native boreal plant species ideally suited for forest reclamation following oil sands mining and in-situ development. To achieve this objective, project researchers identified key reclamation species to evaluate in this study, including chokecherry (*Prunus virginiana*), bearberry (*Arctostaphylos uva-ursi* [L.]), common blueberry (*Vaccinium myrtilloides*), low-bush cranberry (*Viburnum edule*), and redcurrant (*Ribes triste*).

The effect of MWCNTs and their concentrations were tested separately and combined with other seed pre-treatments, including scarification, cold stratification, and use of gibberellic acid and smoke water. Seed scarification involved using sulfuric acid (5%) to soften the seed coat to facilitate water imbibition. Seed stratification consisted of a moist and chill treatment for zero (no stratification), two, four, eight, and 12 weeks to promote seed germination. Three MWCNT types (non-functionalized MWCNT, MWCNT-COOH, and MWCNT-OH) were used at 10 µg/L, 20 µg/L, and 30 µg/L concentrations. The gibberellic acid (GA<sub>3</sub>) was used at a concentration of 1,000 mg/L.



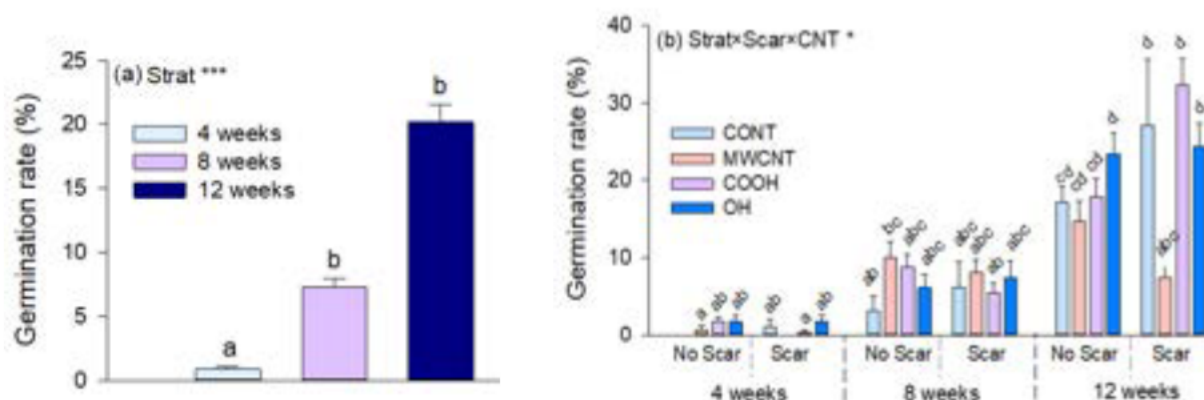
## PROGRESS AND ACHIEVEMENTS

### Chokecherry (*Prunus virginiana*) - Seed germination test

Cold stratification time significantly increased the seed germination rate in chokecherry (Figure 1). There was an interactive effect of cold stratification length, scarification, and carbon nanotube (CNT) type. The seed germination rate increased with increasing cold stratification time in all CNT types except for seeds scarified and treated with MWCNT. The scarified seeds that were treated with MWCNT showed no improvement in germination rate after 12 weeks of cold stratification. The highest germination rate (33%) was observed in scarified seeds, treated with MWCNT-COOH, and cold stratified for 12 weeks (Figure 1).

The mean germination time (MGT) was significantly affected by the interactive effect of cold stratification time, scarification, and CNT concentration. Overall, MGT was significantly shortened for eight and 12 weeks of cold stratification, whereas further treatment by scarification and CNT type did not significantly affect MGT when combined with the eight- and 12-week stratification treatments. Significant differences were observed between the scarification and CNT type treatments under the four weeks of cold stratification treatment. However, this difference is likely due to the low germination rate preventing the MGT from being measured.

Due to the low germination rate, the synchronization index (SYN) was not estimated for the four-week stratification treatment. SYN was significantly affected by the interactive effect of cold stratification length, scarification, and CNT type. However, the difference between the two treatments (cold stratification time and scarification) was marginal except for scarified seeds treated with MWCNT and stratified for eight and 12 weeks.



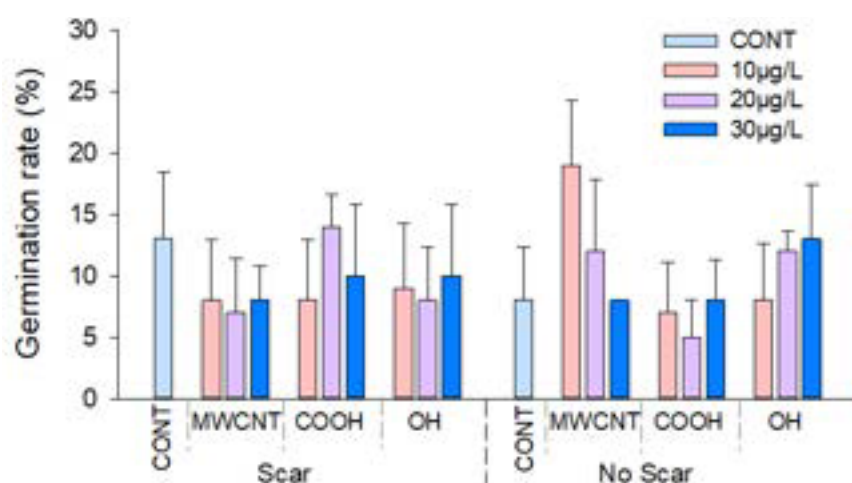
**Figure 1:** Mean (+SE) germination rate (%) of chokecherry seeds treated with three levels of cold stratification time (four, eight, 12 weeks), two levels of scarification (no scarification and 5% sulfuric acid), four levels of CNTs (control [deionized water], functionalized hydroxyl [OH], carboxylic acid [COOH] multi-wall nanotubes, and non-functionalized multi-wall nanotubes [MWCNT]), and three levels of CNT concentration (10 µg/L, 20 µg/L, and 30 µg/L). Means with different letters are significantly different from each other. Significance levels: \* $p < 0.05$  was indicated on the top.



### Low-bush cranberry (*Viburnum edule*) - Seed germination test

Seed Germination for low-bush cranberry was conducted in two trials.

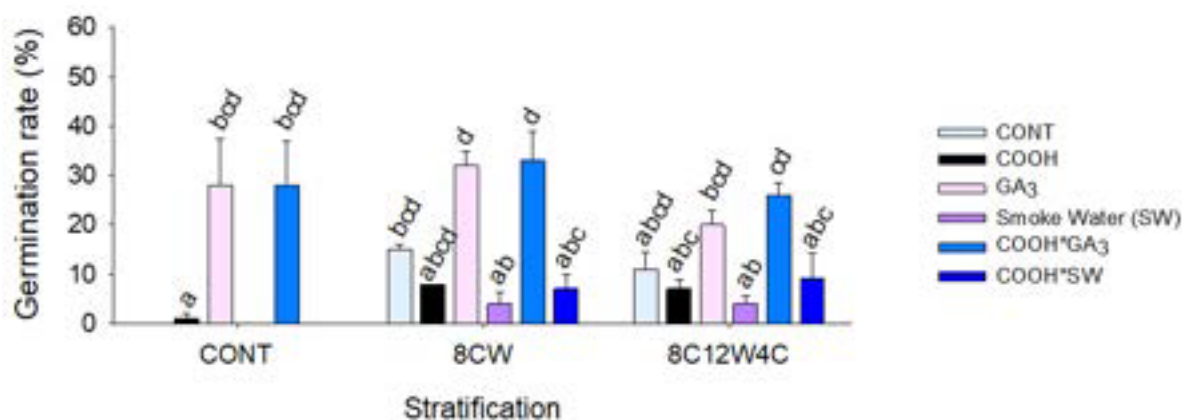
In the first trial the effects of MWCNTs in improving seed germination by scarifying the seed with 5% sulfuric acid, priming them with CNT at a concentration of 10 µg/L, 20 µg/L, 30 µg/L, and then stratifying for zero, two, four, eight and 12 weeks were evaluated. During this test, low-bush cranberry did not germinate during the observation period of 50 days. With a longer incubation, the best germination rate came from seeds treated with 10 µg/L of MWCNT, without prior scarification and stratified for 12 weeks (Figure 2). Whether scarified or not, seeds treated with functionalized CNT (COOH and OH) did not provide significantly higher germination rates when compared to the control.



**Figure 2:** Mean (+SE) germination rate (%) of low-bush cranberry seeds treated with two levels of scarification (no scarification [No Scar] and 5% sulfuric acid [Scar]), four levels of CNTs (control [deionized water; CONT], functionalized hydroxyl [OH], carboxylic acid [COOH] multi-wall nanotubes, and non-functionalized multi-wall nanotubes [MWCNT]), and three levels of CNT concentration (10 µg/L, 20 µg/L, and 30 µg/L). All seeds underwent 12-week cold stratification (5 °C).

### Trial 2 of low-bush cranberry seed germination

To improve this result from Trial 1, project researchers assessed the effect of seed priming with MWCNT-COOH, gibberellic acid (GA<sub>3</sub>), and smoke water (SW) separately and in combination. The following priming treatments were applied: (1) Control (Deionized Water) (2) COOH (20µg/L), (3) GA<sub>3</sub> (1,000mg/L), (4) SW (1:20), (5) COOH + GA<sub>3</sub>, and (6) COOH + SW. The treated seeds were then submitted to a single stratification or to multiple stratifications (Cold-warm-cold). The following treatments were applied: (1) Control (20 °C /10 °C), (2) eight weeks cold (5 °C) + warm (20 °C /10 °C) (8CW), (3) eight weeks cold (5 °C) + 12 weeks warm (20 °C /10°C) + four weeks cold (5 °C) (8C12W8C). GA<sub>3</sub> application was the most effective treatment among other seed priming treatments for low-bush cranberry. GA<sub>3</sub> application helped to increase the germination of low-bush cranberry seed from 18% in the first trial above to 35% (Figure 3).

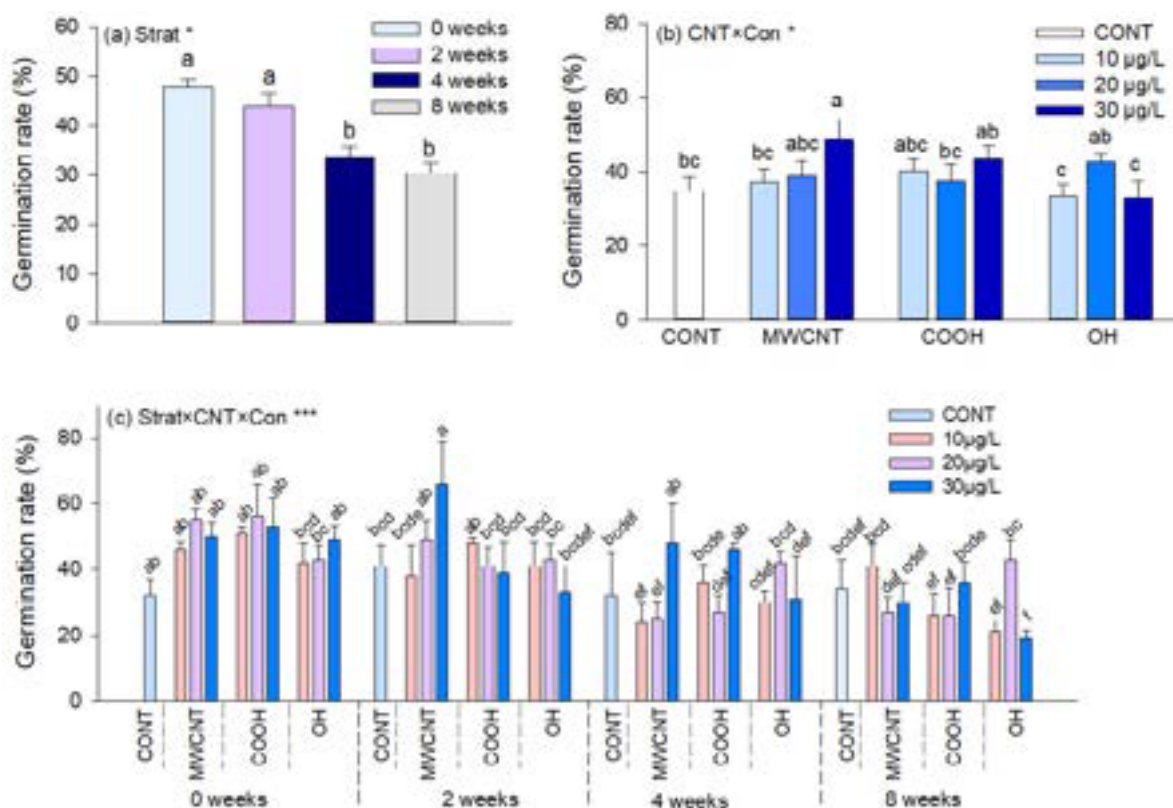


**Figure 3:** Mean (+SE) germination rate (%) of low-bush cranberry seeds treated with three levels of cold stratification time (1: No stratification, 2: eight weeks of cold stratification, and 3: eight weeks of cold stratification + 12 weeks of warm stratification + four weeks of cold stratification), and six levels of seed priming treatments (control [deionized water], functionalized carboxylic acid [COOH] multi-wall nanotubes, gibberellic acid [GA<sub>3</sub>], smoke water [SW], COOH\*GA<sub>3</sub>, and COOH\*SW). Means with different letters are significantly different from each other.

### Common blueberry (*Vaccinium myrtilloides*) - seed germination test

Unlike other species tested in this project, seed germination of common blueberry significantly decreased with an increase of the cold stratification length (zero weeks: 47.7%; two weeks: 43.9%; four weeks: 33.41%; and eight weeks: 30.3%) (Figure 4a). The germination rate was significantly higher only with the highest concentration of MWCNT (Figure 4b). The germination rate also increased with an increase of MWCNT concentrations (CONT: 34.9%; MWCNT-10 µg/L: 37.25%; MWCNT-20 µg/L: 39.0%; and MWCNT-30 µg/L: 48.57%). However, other CNT type did not show such trend or a significant effect on seed germination rate.





**Figures 4a and 4b:** Mean (+SE) mean germination rate (%) of common blueberry seeds treated with four levels of cold stratification time (zero, two, four, eight weeks), four levels of CNTs (control [deionized water], functionalized hydroxyl [OH], carboxylic acid [COOH] multi-wall nanotubes, and non-functionalized multi-wall nanotubes [MWCNT]), and three levels of CNT concentration (10 µg/L, 20 µg/L, and 30 µg/L). Means with different letters are significantly different from each other. Significance levels: \* $p < 0.05$ , \*\*\* $p < 0.001$  was indicated on the top.

## LESSONS LEARNED

The lesson learned from this project is that select carbon nanoparticles (functionalized multi-walled carbon nanotube) improved seed germination in two native boreal species (low-bush cranberry and blueberry) that have challenging seed dormancy issues and low germination rates.

Gibberellic acid ( $GA_3$ ) separately or in combination with carbon nanotubes showed to be a better source for improving seed germination. General benefits of this technology to the industry include, a new approach to improve plant propagation for land reclamation, more efficient use of limited native seed resources through an improvement in germination, and improved cost efficiency as project researchers reduced the amount of seed wasted during the germination. Very low amounts (microgram quantities) of nanoparticles and/or gibberellic acid conferred beneficial effects to two of the four tested species; indicating this is a promising technology with low inputs.

Forest nurseries and reclamation companies will be interested in the project learnings including: (1) identification of specific nanoparticles and protocols for use on seeds, and (2) data on germination improvement for each species.

Based on the poor results recorded on bearberry and redcurrant seeds, further research is required to induce seed germination for these species.



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Pawar, V. A., and Laware, S. L. (2018). Seed priming a critical review. *Int. J. Sci. Res. Biol. Sci*, 5, 94–101.

## PRESENTATIONS AND PUBLICATIONS

### Conference Presentations/Posters

Inoue S. and Sobze J. M. (2022) The Use of Carbon Nanomaterials to Improve Seed Germination Seedling Vigor and Growth — Industry Overview, Presentation, and Discussion. *Canada's Oil Sands Innovation Alliance* Upland, Soils, and Vegetation Technical Sharing Session, November 2022 (Canada).

## RESEARCH TEAM AND COLLABORATORS

Institution: NAIT Centre for Boreal Research

Principal Investigator: Dr. Jean-Marie Sobze

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# NSERC — Industrial Research Chair in Terrestrial Restoration Ecology

**COSIA Project Number:** LE0034

**Mine**

**Research Provider:** University of Alberta

**Industry Champion:** Syncrude

**Industry Collaborators:** Canadian Natural, Cenovus, ConocoPhillips, Devon Canada Corporation, Imperial, Suncor, Teck

**Status:** Final Cumulative Summary

## PROJECT SUMMARY

After mining, some landforms are reconstructed with oil sands that do not meet the criteria for processing yet contain petroleum hydrocarbons. In the same region of boreal forest where oil sands mining and reclamation occurs, soils containing bitumen naturally occur and forests have developed on these deposits over thousands of years post-glaciation. Lean oil sand differs from naturally occurring bitumen deposits, however, in that it has not undergone similar amounts of weathering. As part of reclamation, lean oil sand (LOS) may be in contact with the rooting zone of forests. Though forests have developed on naturally occurring bitumen deposits, there are concerns that the disruption and placement of LOS in a new environment may pose an environmental risk and negatively impact the growth of vegetation on reclaimed lands. Specifically, LOS may act as a barrier to root growth with subsequent effects on the aboveground functioning of trees, shrubs, and herbaceous plants established on sites reconstructed with this material. Currently there is a lack of science-based evidence guiding reclamation involving LOS and the approvals framework for how these activities are regulated.

The focus of the research program is to build knowledge on belowground features (roots and their fungal symbionts) that support self-sustaining boreal forests. The overarching research question is whether LOS acts as a barrier or medium to root growth of plants comprising a typical boreal forest that have developed in naturally-occurring hydrocarbons (NOHCs) in the region. Several objectives were addressed through the IRC program to:

1. Determine natural dimensions and mechanisms underlying rooting zones of boreal plants;
2. Understand the effects of LOS on root structure and function; and
3. Investigate whether root fungal symbionts mediate tree survival on soils and substrates containing NOHCs.

## PROGRESS AND ACHIEVEMENTS

In the last year of the industrial research chair (IRC), much of the research addressed objectives 2 and 3 above. As root function cannot be understood in isolation of the mycorrhizal fungi that mediate resource uptake, their role in influencing seedling performance was specifically addressed. Fungi play pivotal roles in boreal forests as they



influence organic matter decomposition, carbon and nutrient cycles, and plant performance. Similar to other forests, the vast majority of plants in the boreal forest interact with mycorrhizal fungi, symbionts that colonize fine roots. Mycorrhizal fungi are known for facilitating nutrient uptake, primarily of phosphorus and nitrogen, in exchange for sugars from photosynthesis (Smith and Read, 2008). These fungi have also been found to increase plant tolerance to stressors such as soil salinity (Muhsin & Zwiazek, 2002), heavy metals contamination (Hildebrandt et al., 2007), and petroleum hydrocarbons (Krzmaric et al., 2009; Kuo et al., 2014). Although not fully understood, there are a variety of hypotheses for how mycorrhizal fungi could increase plant tolerance to hydrocarbons. Fungi may form a physical barrier between plant roots and hydrocarbons, and/or hydrocarbon molecules may be sequestered in fungal tissues (Gunderson et al., 2007; Gao et al., 2010). Alternatively, non-specific fungal enzymes produced for the degradation of organic material may also degrade petroleum hydrocarbons (Robertson et al., 2007; Harms et al., 2011). Ectomycorrhizal fungi, those that colonize the roots of the majority of boreal forest trees, are capable of taking up nutrients from organic matter, which are released by specific enzymes such as polyphenol oxidases. These enzymes have been shown to break down organic pollutants including petroleum hydrocarbons (Braun-Lülleemann et al., 1999; Meharg & Cairney, 2000; Robertson et al., 2007).

Exploring the interaction between trees and mycorrhizal fungi in native soils containing NOHCs provided a unique opportunity to investigate whether mycorrhizas promote plant tolerance to NOHCs in LOS. In his PhD studies, James Franklin surveyed soil fungi across sites varying in hydrocarbon concentrations and fractions to prospect for soil fungi that may increase tolerance of seedlings to NOHCs present in reclaimed LOS landforms. The locations of the sites included: 1) NOHC ‘shallow-bituminous’ soil (n = 3) identified in soil surveys of the area (Leskiw et al., 2005, 2006); 2) soils with no NOHCs, ‘bituminous-free’ (n = 3); and 3) a site of historic oil sand operation (Bitumount) with an unprocessed oil sand stockpile, Bitumount ‘ore pile’. The shallow bituminous sites were chosen because fungi found in these soils may be a promising source of inoculum for tree seedlings, however, these soils are more weathered than LOS. This means lighter fractions of hydrocarbons may have volatilized and are no longer present in shallow-bituminous soils. Recently exposed bitumen at the Bitumount Historic site may contain fungi experiencing hydrocarbon concentrations and fractions more similar to those present in reclaimed LOS, thus fungi found at this site may also be candidates for seedling inoculation.

The field survey confirmed that there were differences in fungal community composition based on site. Fungal community composition in bulk soil differed among the three site types. Thus, using soils from different sites could be a simple way to inoculate seedlings with different fungal communities. With this information in hand, Franklin then used small amounts of soil from the field sites as inoculum for two tree species commonly used to revegetate reclaimed sites, *Populus tremuloides* (aspen) and *Pinus banksiana* (jack pine). For four to six months in a growth chamber, seedlings of the two tree species were grown from seed in different reclamation soils and LOS inoculated with soils from the three site types (see above for site types). The effect of inoculum varied by origin and tree species. Pine seedlings grown in LOS (3.67% hydrocarbons) were 64% and 49% larger when inoculated with ore pile-soil and bituminous-free soil, respectively than those that received sterile inoculum from the same origin. Inoculum from shallow-bituminous field sites had no effect on pine seedling biomass grown in LOS. Conversely, aspen seedlings grown in LOS did not respond positively to any soil inoculum and were insensitive to the origin of the inoculum.

While these results show some promise for inoculating seedlings with field soil to increase their growth in the presence of LOS, several caveats must be acknowledged. First, mycorrhizas establish on newly planted seedlings in reclamation soils (Hankin et al. 2015; Pec et al. 2019). Thus, further research would be required to demonstrate inoculation is necessary given that some reclamation soils harbour mycorrhizal fungi and that some fungi can



disperse to reclaimed sites. Second, in just two tree species tested, only one responded positively to inoculation. Therefore, inoculation cannot be assumed to be positive for all plant species. Also noteworthy is that when grown in some reclamation soils (bituminous-free and shallow-bituminous soils), inoculation depressed the biomass of aspen. While easy to collect and apply, soil inoculation may pose some risks because though they may contain mycorrhizal fungi, they may also include pathogens. Finally, not all mycorrhizal fungi tolerate hydrocarbons. Species differ in their response to LOS. This result is demonstrated by an in vitro experiment performed by Franklin testing the response of six species of ectomycorrhizal fungi grown on agar compared to agar mixed with LOS (1.5% hydrocarbons). Soil inoculation may thus have highly context-dependent effects based on origin of collection, microbes present, receiver plant species, timing, and soil. Much more research is needed before this method could be adopted into reclamation practices.

Though much uncertainty exists around the use of soil inoculation, that LOS limits seedling growth was clear across several studies performed by Franklin. When grown for 35 days in pots containing 0.88% (Low LOS) or 3.67% (High LOS) hydrocarbons, compared to field soil, Low and High LOS reduced total biomass of *Lactuca sativa* (lettuce) by 66% and 77%, respectively. In a different pot study, the biomass of aspen decreased by 39% and 23% in Low LOS and High LOS, respectively compared with growth in field soil. Similar results were observed for jack pine in this same pot study. However, when pine seedlings were grown in microcosm plates on agar mixed with LOS (1.5% hydrocarbons), they had higher biomass than those grown on agar free of LOS. It remains unclear how LOS decreases plant growth. Further experiments are required to conclusively determine whether LOS directly affects plant growth or whether its influence is indirect through nutrient and/or water availability.

Despite not pinpointing the underlying mechanism, the negative plant growth responses to LOS found in most experiments performed by Franklin are consistent with those reported in a recent review on the effects of crude oil and refined petroleum products on plant growth across a variety of species (Haider et al., 2021). Furthermore, Franklin's results are similar to a recent study on *Lactuca sativa*, showing that plant biomass was 38% and 47% less when the soil was contaminated with 10% and 20% crude oil, respectively (Ilyas et al., 2021). Finally, Franklin's results are also consistent with the few reports that investigated the direct effect of LOS on plant growth across a variety of plant species. For example, Brown (2020) found that when petroleum hydrocarbon concentrations in LOS increased from 1.95% to 4.54%, this decreased *Pinus banksiana* and *Populus tremuloides* seedling growth. Additionally, Visser (2008), reported that the growth of *Hordeum vulgare*, *Pinus banksiana*, *Populus tremuloides*, and *Picea glauca* seedlings significantly decreased with increasing petroleum hydrocarbon concentrations up to 5.3%. Taken together, these results indicate that LOS can limit plant growth and current reclamation practices to limit contact between this substrate and establishing vegetation should continue. Soil reclamation cover is a key mitigation measure required to alleviate potential negative impacts that originate from LOS reclamation.

## LESSONS LEARNED

At its core, this IRC relied on sampling and identifying roots in soils. However, excavating soils to observe roots is an arduous and destructive process. Two approaches were developed that may serve as alternatives to excavating soils to collect roots. First, a DNA-based tool was developed to identify plant species from soils where reliable morphological traits are not available. Identifying roots to species typically requires tracing roots to a stem that can be identified. Coring soils to collect roots is a less destructive process, however identifying roots is challenging. Fortunately, DNA sequencing holds much promise in solving this problem. Fluorescent amplified fragment length



polymorphisms can distinguish species within a mixed sample, are high throughput, and are inexpensive. DNA size profiles were created for 193 species common to the western Canadian boreal forest. This DNA tool could be used by practitioners to identify roots in reclaimed or natural soils. Second, with strong analytical methods and good reference conditions, it was found that dendrochemistry may be a feasible methodology for monitoring root growth over time in NOHC soils with V and Ni acting as potential signal elements. Practitioners can sample tree rings and analyze their chemistry to infer the chemistry of soils surrounding the roots. This presents reclamation practitioners and researchers with a useful tool to monitor plant growth across both constructed landforms and natural landscapes along with data against which to compare future values.

To identify whether LOS is a barrier or medium to root growth, it is necessary to eliminate or account for factors that may confound interpretations of the effects of LOS on root behaviour. Various abiotic and biotic factors influence root distributions of boreal forest plants and may be confounded with LOS placement. Two such factors were explored: soil texture and stand age. It was found that roots decrease in abundance with depth, and soil texture did not strongly influence these trends (up to 40 cm in depth) for medium and coarse-textured soils. With respect to stand age, after about 15 years, roots in jack pine stands were reaching similar depths (sampled to 90 cm) as to those in mature stands (> 65 years old). These results suggest that should reclamation sites be sampled in the early years of vegetation development; the placement of LOS may appear to be a barrier because not enough time has passed for roots to grow to depth. Research from the IRC demonstrates the importance of holding other factors constant when evaluating the effects of LOS on rooting behaviour.

Finally, LOS appears to limit plant growth. In one reclamation site, researchers found that roots rarely penetrated the LOS overburden (50 cm from the surface), although the mechanism of limitation was not investigated (i.e., the presence of hydrocarbons and/or physical root restrictions of the parent material). Further, across many lab experiments performed under the IRC, plant growth was limited in the presence of LOS. To explore ways to mitigate the putative effects of LOS on plant growth, researchers tested the ability of roots to enhance the degradation of hydrocarbons. At the seedling stage, the presence of aspen or jack pine did not enhance hydrocarbon degradation under controlled conditions. However, at low concentrations (> 5% by volume), the native microbial community appeared to be stimulated by the presence of hydrocarbons such that the hydrocarbons were incorporated into new microbial biomass. The other approach explored was to increase plant tolerance to LOS through interactions with root symbionts. Researchers prospected for naturally occurring root-associated fungi that exist under relatively high hydrocarbon concentrations and found some promise in inoculating seedlings with small amounts of field soil. However, before inoculating seedlings destined for areas where interactions between plants and LOS are likely, further research is needed to test its effectiveness in reclaimed sites (see caveats listed in the above section). Taken together, given that LOS can limit plant growth, current reclamation practices and regulatory requirements of an appropriate soil cover design and depth for LOS reclamation are warranted to create an adequate root zone to achieve the targeted vegetation growth outcomes.

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## **PRESENTATIONS AND PUBLICATIONS**

### **Published Theses**

Metzler P. 2018. Molecular identification of boreal forest roots: an expansion of techniques and investigation of limitations and biases. MSc thesis, Department of Renewable Resources, University of Alberta

La Flèche M. 2019. Tree cores for root bores: Exploring tree rooting behaviour in bituminous soils. MSc, University of Alberta, Department of Renewable Resources

Wasyliw J. 2019. Ectomycorrhizal functional diversity parallels fine root and leaf abundance with forest stand age. MSc, University of Alberta, Department of Renewable Resources

Brown A. 2019. Cutin and suberin in mixed-wood boreal forest plants and their use as markers for origin of soil organic matter. MSc, University of Alberta, Department of Renewable Resources

Brown, Nicholas. 2020. Biodegradation of hydrocarbons in bitumen: exploring plant-assisted and microbial stimulation techniques. MSc thesis, University of Alberta, Department of Renewable Resources

Franklin, J. 2022. Exploring how soil fungi can be used to restore native trees on reclaimed substrates containing petroleum hydrocarbons. PhD thesis, University of Alberta, Department of Renewable Resources

### **Journal Publications**

Metzler P., La Flèche M., and J. Karst. 2019. Expanding and testing fluorescent amplified fragment length polymorphisms for identifying roots of boreal forest plant species. *Applications in Plant Sciences* doi/pdf/10.1002/aps3.1236

La Flèche M., Cuss C. W., Noernberg T., Shotyk W., and J. Karst. 2020. Trace metals as indicators of tree rooting in bituminous soils. *Land Degradation & Development* <https://doi.org/10.1002/ldr.3848>

Wasyliw J., and J. Karst. 2020. Shifts in ectomycorrhizal exploration types parallel leaf and fine root area with forest age. *Journal of Ecology* <https://doi.org/10.1111/1365-2745.13484>

### **Conference Presentations/Posters**

Metzler P. and J. Karst. 2017. Revealing the belowground diversity of boreal forests with molecular tools. Poster at Ecological Society of America, August 6-11, Portland, USA

La Flèche M., Shotyk W., and J. Karst. 2018. Using trace metals as indicators of tree rooting behaviour in bituminous soils. Canada Oil Sands Innovation Summit, Calgary, Canada



La Flèche M., Shotyk W, and J Karst. 2018. Trace metals as indicators of rooting behaviour in bituminous soils. International Conference on Heavy Metals, Athens, USA July 22-July 26

Franklin, J, Antunes, P., and J. Karst. 2019. Do microbes mediate plant growth on natural bituminous soils? Canada Oil Sands Innovation Summit, Calgary, Canada

Franklin J., Antunes P., and J. Karst. 2019. Do microbes mediate plant growth on natural bituminous soils? Poster at International Conference on Mycorrhizas 10 Merida, Mexico June 30-July 5

Wasyliw, J. and J. Karst. 2019. Do ectomycorrhizal fungi compensate for decreasing fine root area in old forests? Poster at International Conference on Mycorrhizas 10 Merida, Mexico June 30-July 5

Franklin J., Antunes P., and J.Karst. 2021. Can fungal communities restore native trees on reclaimed substrates containing hydrocarbons? Society for Ecological Restoration 9th World Conference on Ecological Restoration June 19 — 24 (Virtual oral presentation)

## AWARDS

James Franklin's, PhD thesis was nominated for Department of Renewable Resources Outstanding Thesis Award in 2022

## RESEARCH TEAM AND COLLABORATORS

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Principal Investigator: Dr. Justine Karst

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Ariel Brown	University of Alberta	MSc	2017	2019
Nicholas Brown	University of Alberta	MSc	2018	2020
James Franklin	University of Alberta	PhD	2017	2022
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Chloe Christenson	University of Alberta	Summer Undergraduate Technician		
Dana Hopfauf	University of Alberta	Summer Undergraduate Technician		
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Joshua Wasyliw	University of Alberta	Summer Undergraduate Technician		



Nicholas Brown	University of Alberta	Summer Undergraduate Technician		
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Andrea Simeon	University of Alberta	Summer Undergraduate Technician		
Jason Eerkes	University of Alberta	Summer Undergraduate Technician		
Serena Farrugia	University of Alberta	Summer Undergraduate Technician		
Alison Wilson	University of Alberta	Field Technician		
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# Syncrude Upland Vegetation Monitoring Program

**COSIA Project Number:** LJ0343

**Mine**

**Research Provider:** Syncrude

**Industry Champion:** Syncrude

**Status:** Ongoing

## PROJECT SUMMARY

This project is a long-term vegetation monitoring initiative, started in 1980, on Syncrude's reclaimed lands at the Mildred Lake mine site. The program consists of remeasured plots distributed across a diverse set of reclaimed starting conditions and reflecting a wide range of reclaimed landforms and reclamation treatments. Each monitoring plot combines a forestry-style growth and yield plot and a vegetation community assessment where the presence and abundance (percentage cover) of every species are tallied. Almost 200 sampling locations have been established, with roughly 125 currently active as of 2021. Each plot is planned for reassessment at a five-year interval.

Objective 1: Quantify diversity and trends by age for species composition in reclaimed plant communities for the purposes of (i) validating that reclaimed communities are converging with those of locally common boreal forest ecosystems, (ii) validating metrics and assumptions that are proposed for use in reclamation certification, and (iii) providing an empirical basis for continuous improvement of best reclamation practices that impact plant community composition and diversity.

Objective 2: Validate the achievement of timber yields comparable to those found on natural landscapes, and provide an empirical basis for continuous improvement of best reclamation practices that impact timber yields.

Objective 3: Develop and implement reporting structures for effectively communicating learnings from this program with respect to variations in plant community development and potential causal factors, and de-risking achievement of lease-level expectations for reclaimed plant community composition.

In 2021 a major project milestone was reached, consisting of the analysis and reporting on 39 years of plant community trends observed at the Mildred Lake mine site. In 2022 this information was contributed to COSIA.

## PROGRESS AND ACHIEVEMENTS

Annual monitoring activities were completed in 2022.



## LESSONS LEARNED

This project offers comprehensive empirical validation related to the success of reclamation treatments. Specifically, it quantifies the development of plant community composition and commercial timber production, offering meaningful opportunities to make defensible assertions regarding the achievement of end land uses of wildlife habitat, biodiversity, and commercial forestry.

The synthesis report summarizes up to 39 years of plant community development trends on Syncrude's reclaimed mine sites near Fort McMurray, Alberta. These trends are contrasted with a target condition, defined here as the natural range of variability for species composition on older (60-plus years) closed canopy forests having similar mesic and sub-mesic site conditions within 200 km of the mine sites.

The primary outcome of the study is that patterns of plant community change on reclaimed sites are consistent with Alberta's objectives for reclamation, which require increasing similarity between reclaimed and reference plant community structures over time. Evidence is also provided demonstrating the strong influence of a developing tree canopy on these patterns, where native forest-dependent species gain an increasing competitive advantage over time as compared to early-arriving ruderal or weedy species. Overall, it is concluded that expected natural processes, consistent with conventional ecological theory, are leading to reclaimed sites demonstrating substantial convergence with locally common boreal forest ecosystems.

Additional learnings include:

1. The initial pace at which new target species are observed on reclaimed sites (species per year), and the rate of increase in that pace, appears to be higher for newer reclaimed areas than for older ones. While multiple explanations may exist, the most likely appears to be a move away from intensive agronomic approaches to reclamation, to practices which better conserve plant propagules in reclamation soils and better facilitate native species immigration.
2. With the possible (and speculated) exception of seeded and fertilized agronomic cover crops, there is no evidence that ruderal species and particularly noxious weeds are negatively impacting immigration rates of target species on reclaimed sites.
3. Consistent with many forested communities, there may be some degree of interruption to the rates of forest-dependent species arrival and proliferation on reclaimed sites as they reach canopy closure and undergo a period of maximum canopy density. In many cases, the percent cover of all understorey species will be temporarily reduced, including that for target species.
4. Trends observed in this study provide validation to the concept of using characteristic species counts, measured at a relatively early age, as an indicator of potential target species diversity within a criteria and indicators framework for reclamation certification. However, analyses to validate the proposed thresholds for this indicator have not yet been completed.
5. At the current stage of development, characteristic species and reclaimed community composition appear to be relatively weak indicators for discriminating between ecosystem classes (ecosites). It is speculated that exposure to a wider range of seral stages (and possibly even disturbance cycles) over many decades will be required in order for characteristic species to become stronger indicators of eco site phase on reclaimed lands.



## PRESENTATIONS AND PUBLICATIONS

### Reports & Other Publications

Farnden C. 2021. Reclaimed upland vegetation community trends on Syncrude's mine sites. Syncrude Canada Ltd, Edmonton, Alberta. 61 p. <https://era.library.ualberta.ca/items/e4af0e03-e6f8-4fd5-8838-21a26541c66a>

## RESEARCH TEAM AND COLLABORATORS

Institution: Syncrude

Principal Investigator: Dr. Craig Farnden



## **WILDLIFE RESEARCH AND MONITORING**



## Regional Industry Caribou Collaboration (RICC)

**COSIA Project Number:** LJ0155

**In Situ**

**Research Provider:** Alberta Biodiversity Monitoring Institute (ABMI)

**Industry Champion:** Canadian Natural

**Industry Collaborators:** Alberta-Pacific Forest Industries, Athabasca Oil Corporation, Cenovus, CNOOC Petroleum North America ULC, Imperial, MEG Energy, Suncor

**Status:** Ongoing, Annually

### PROJECT SUMMARY

Population declines of boreal caribou have been ultimately attributed to direct and indirect effects of landscape disturbance and climate change. The potential mechanisms linking these ultimate causes to caribou declines are varied. However, understanding these mechanisms and their relative importance is critical for developing effective conservation strategies for caribou. The primary objectives of this project are to:

- Comprehensively review the existing literature to identify current hypothesized mechanisms for explaining caribou declines; and
- For each mechanism, determine whether demographic effects (e.g., lowered adult survival or juvenile recruitment) have been reported and whether such effects have been explicitly linked to population growth rates of caribou.

The main cause of caribou declines across most of their ranges is excessive predation, mostly by wolves. The currently high predation rates are a result of many complex and interacting factors, including landscape level habitat changes (both natural and human caused). For any caribou recovery program to be successful, it must address the full range of habitat and population factors impacting caribou, and it must be implemented at the broad-range scale to spur caribou population growth over time.

The Regional Industry Caribou Collaboration (RICC) is a group of resource companies operating in the oil sands region of northeast Alberta that are working together across their project boundaries to:

- Restore caribou habitat on legacy seismic lines
- Conduct research on caribou ecology and their relationships with other parts of the landscape
- Lead trials on restoration methods, effectiveness and how wildlife respond to restoration

Reversing the decline of caribou requires a focused, science-based strategy that involves multiple partners, including industry, government, academia, and non-profit organizations. RICC brings these parties together to contribute to the recovery of boreal woodland caribou and their habitat.

More information about RICC can be found at: <https://cosia.ca/initiatives/land/projects/regional-industry-caribou-collaboration>.



## PROGRESS AND ACHIEVEMENTS

RICC is a multi-year program that includes many individual projects that also span multiple years. Achievements in 2022 include the following:

### Large-Scale Habitat Restoration

Linear features, including legacy seismic lines, have been implicated in caribou declines mostly through their facilitation of predator movement and increasing predator access to — and overlap with — caribou. Restoration of seismic lines has been identified as a key management tool to support caribou recovery in Alberta. Restoration treatments, such as planting and mounding, aim to return forest cover and reduce predator use of these features, ultimately reduce predation on caribou. Member companies recognize our role in habitat restoration and have assessed or treated over 2,300 km of legacy seismic lines since the inception of RICC. In 2021, RICC member companies treated 254 km of seismic lines.

Using silvicultural tools like mounding, coarse woody material placement, and planting over one million seedlings, RICC companies have initiated habitat recovery across an area of approximately 1,050 km<sup>2</sup> in Cold Lake, 545 km<sup>2</sup> in ESAR and 20 km<sup>2</sup> in WSAR caribou ranges [3]. RICC also has an implementation-ready plan to address 268 km in ESAR.

### Wildlife Monitoring

The study continues to monitor and evaluate how moose, caribou, bears, and wolves use two areas, one without habitat restoration (“business as usual”), and one with extensive restoration treatments (“restored landscape”). The study uses both camera traps and GPS collars to monitor animals as they used seismic lines and the surrounding habitat within these two areas.

## LESSONS LEARNED

### Predator and Prey Responses to Caribou Habitat Restoration

Habitat restoration is increasingly being implemented to recover caribou habitat. Given the extent of the disturbance and the cost to conduct restoration, evaluating the effectiveness of restoration treatments is needed for effective recovery of caribou populations. A multi-scale and multiple-lines-of-evidence approach is being used to evaluate the response of wildlife to restoration treatments in a 378 km<sup>2</sup> area — one of the largest studies of its kind.

### Resource Exploitation Collapses the Home Range of Wolves

Previous work suggests that restoration of linear features, such as seismic lines, pipelines, and roads, can reduce wolf hunting efficiency, but project research results suggest that restoration can also reduce regional wolf density, especially in less productive areas favoured by caribou. Linear features enable wolves to travel more efficiently and provide easy access into woodland caribou habitat. It is hypothesized that these changes in wolf behaviour leads them to encounter caribou more frequently, increasing predation pressure on declining caribou. However, the effect of increased hunting efficiency on wolf space-use at a larger scale is not well understood.



It was found that wolf home range size was influenced by not only prey density, but also how the landscape influenced access to those prey. Home range size decreased as linear density increased because movement within the forest by the wolves was easier.

This study demonstrates how an understanding of animal movement and space use can assist conservation. Linear features not only facilitate wolf travel, but can shrink the area wolves need to survive, especially where resources are scarce. This could mean that areas with more linear features have higher wolf density, which may also increase predation on caribou.

### **Implications of Wildfire for Woodland Caribou**

Wildfire is the dominant natural disturbance in the boreal forest and can influence food availability and disrupt predator-prey relationships for species like boreal woodland caribou. Understanding how caribou use burned habitat is important for informing caribou conservation and recovery. Land use and economic decisions depend on appropriately defining and managing critical habitat. Because fire is currently considered a disturbance to caribou habitat, understanding how caribou respond to these disturbances is necessary.

There is uncertainty around whether unburned residual patches should be included in disturbed habitat. To better understand the implications of wildfire on caribou ecology, project researchers mapped residual patches within fires occurring on six boreal woodland caribou ranges in northeastern Alberta.

#### **Management Implications**

- No support was found for considering unburned residual patches separately from the larger burn area when assessing disturbance thresholds under recovery strategy guidelines.
- Instead of focusing on managing the effect of wildfires on caribou, recovery efforts should focus on reducing the effect of human-caused habitat alteration.
- This research highlights the need to differentiate fire disturbance and human-caused habitat alteration when assessing critical habitat.

### **Trophic Consequences of Terrestrial Eutrophication**

Climate change and landscape transformation are having a combined, enriching effect on ecosystems through “terrestrial eutrophication”, i.e., an increase in habitat productivity. For example, forest cutting leads to an increase in the production of early seral vegetation. How changes in productivity influence food webs is unclear because these changes can result in positive outcomes for some species, and negative outcomes for others. Understanding the response of different species to this habitat alteration is crucial to inform caribou recovery management decisions.

The data suggests that increased forage from habitat alteration resulting from resource extraction increases the density of moose, and thereby wolves, which incidentally prey on caribou. Caribou recovery will therefore likely require management actions that operate across these various trophic levels (predator reductions, prey reductions and habitat restoration). This work provides insight into the cascading effects of forest harvest, shining light on how altering habitat can influence natural predator-prey dynamics.



From a management perspective, understanding the relative roles of natural disturbances (e.g., fire), climate effects, and human-caused disturbance will inform future effectiveness of efforts to recover human-caused habitat disturbance. This work therefore has the potential to influence future decisions regarding habitat restoration and activating other population management levers.

## PRESENTATIONS AND PUBLICATIONS

### Reports & Other Publications

Dickie, M., McNay, R. S., Sutherland, G. D., Sherman, G. G., Cody, M., 2021. Multiple lines of evidence for predator and prey responses to caribou habitat restoration. *Biol. Conserv.* 256, 109032. <https://doi.org/10.1016/j.biocon.2021.109032>

Konkolics, S., Dickie, M., Serrouya, R., Hervieux, D., Boutin, S., 2021. A burning question: What are the implications of forest fires for woodland caribou? *J. Wildl. Manage.* <https://doi.org/10.1002/jwmg.22111>

Serrouya, R., Dickie, M., Lamb, C., Van Oort, H., Kelly, A. P., Demars, C., McLoughlin, P. D., Larter, N.C., Hervieux, D., Ford, A.T., Boutin, S., 2021. Trophic consequences of terrestrial eutrophication for a threatened ungulate. *Proc. R. Soc. B Biol. Sci.* 288. <https://doi.org/10.1098/rspb.2020.2811>

Dickie M., In press. Resource exploitation efficiency collapses the home range of an apex predator. *Ecology*. <https://doi.org/10.1002/ecy.3642>

Dickie, M., 2021. Disentangling the influence of anthropogenic habitat alteration from climate on expanding white-tailed deer populations in western Canada. *Caribou Ecology and Webinar Series*.

Dickie, M., McNay, S.R., Sutherland, G.D., Cody, M., Avgar, T., 2020. Corridors or risk? Movement along, and use of, linear features varies predictably among large mammal predator and prey species. *J. Anim. Ecol.* 89, 623–634. <https://doi.org/10.1111/1365-2656.13130>

Laurent, M., Dickie, M., Becker, M., Serrouya, R., Boutin, S., 2020. Evaluating the mechanisms of landscape change on white-tailed deer populations. *J. Wildl. Manage.* 85, 340–353. <https://doi.org/10.1002/jwmg.21979>



## RESEARCH TEAM AND COLLABORATORS

Institution: Alberta Biodiversity Monitoring Institute (ABMI), Caribou Monitoring Unit

Principal Investigator: Dr. Rob Serrouya

Name	Institution or Company	Degree or Job Title	Degree Start Date (Students Only)	Degree Completion Date (Students Only)
Melanie Dickie	Alberta Biodiversity Monitoring Institute (ABMI), Caribou Monitoring Unit	Research Analyst — PhD Student	2020	
Natasha Crossland	Alberta Biodiversity Monitoring Institute (ABMI), Caribou Monitoring Unit	Field Operations Coordinator		
Craig DeMars	Alberta Biodiversity Monitoring Institute (ABMI), Caribou Monitoring Unit	Research Analyst		
Liam Horne	University of Alberta	MSc Student	2021	

Research Collaborators: University of Alberta; Government of Alberta, Department of Environmental Protection; University of Calgary; Wildlife Infometrics Inc.

# A Portable Testing Device for Wildlife Conservation

**COSIA Project Number:** LJ0334

**Mine and In Situ**

**Research Provider:** McMaster University, University of Calgary

**Industry Champion:** ConocoPhillips

**Industry Collaborators:** Imperial, Teck

**Status:** Year 3 of 4

## PROJECT SUMMARY

The goal of this project is to develop an affordable (less than CAN\$1.00 per assay), simple to use, paper-based device, capable of extracting and identifying DNA from biological samples, in the field, in real time. Once developed, the device can be employed by non-specialist users without the need for access to laboratory facilities. Important applications of this technology include the detection of pathogenic bacteria in food and the analysis of biological samples (feces, skin, and mucus) for real-time wildlife detection. For example, it can be used to identify species from fecal remains in the wild, which will assist in wildlife monitoring activities and in the detection of illegal trafficking of wildlife parts.

This project builds on several technologies that have been and are continuing to be developed by the research team from McMaster University (McMaster) and the University of Calgary. These technologies have proven to be effective in; extracting DNA directly onto paper; concentrating the DNA and linking it to a simple colour change; and the ability to print, dry and therefore stabilize reagents at any temperature. The challenge for this project is to integrate these technologies into a simple-to-use paper-based device that can detect species-specific DNA from non-invasively collected samples.

For proof-of-concept, the research team is using caribou (as a test species), an elusive animal that can be difficult to survey and whose fecal pellets are sometimes indistinguishable from those of other ungulate species with which it shares its range. Caribou are considered a Species at Risk (Environment Canada, 2011) and are therefore highly relevant in the Canadian context, particularly for areas with a high development interest. However, the approach is easily transferrable to the identification of other species of elusive wildlife or to other species of conservation concern. This device offers a non-invasive and potentially cost-effective technology to monitor wildlife in reclamation areas in Northeast Alberta where oils sands development occurs.

The overall objective of the proposed research is to engineer an all-in-one paper-based device for the detection of animal DNA in the field. Researchers will pursue this objective with the following specific aims:

**Aim 1: Paper-based DNA extraction method** - to establish a simple and effective paper-based method to extract genomic DNA from fecal samples of caribou.

**Aim 2: Paper-based amplification and detection method** - to develop a simple method capable of amplifying DNA and generating a visual signal in the presence of caribou-specific DNA sequence.



**Aim 3: Device integration** - to combine the paper-based extraction and amplification systems above into a one paper device.

**Aim 4: Device testing in lab setting** - to test the device in the laboratory using caribou fecal samples that have been collected from the field and archived at University of Calgary.

**Aim 5: Device optimization** - to test the device in the field through consultation with COSIA stakeholders and optimize its field usability by non-specialists.

The device will provide researchers, environmental managers, indigenous communities, citizen scientists and industries with a cost-effective tool capable of producing real-time presence/absence data for species without the need for complex analytical processes. The direct output will be a highly useful device for targeted monitoring of a highly sensitive flagship species. The broader outcome will be a novel platform technology with the potential to make a transformational contribution to the field of conservation biology internationally. As an example, researchers were contacted by the South African government, as they are interested in the potential for using these tools to detect illegal trade of wildlife parts.

When the device is closer to deployment, workshops will be organized with COSIA members and public stakeholders to discuss the collaborative testing and implementation of this technology. These activities will serve two purposes; to verify the technology can be easily used by both experts and non-experts alike; and to ensure it can be implemented in the real-world

## PROGRESS AND ACHIEVEMENTS

For the research team, 2022 was a productive year. Advances were made towards the development of a portable, affordable, and easy-to-use biosensor technology for wildlife detection (specifically focused on caribou). A highly specific lab-based test was developed which will serve as the foundation for creating a field-based assay. This was the most substantial challenge researchers envisioned at the start of the project and overcoming it was a critical step. The challenges with recruitment created by the COVID-19 pandemic were resolved in 2022 and there is now a full team complement.

The research team's progress in 2022 towards the five project aims are as follows.

### Identify Caribou Specific Markers

Researchers are testing a combination of mitochondrial and nuclear markers identified in the scientific literature and designed within the lab from the alignment of caribou DNA, with that of closely related cervids. Mitochondrial markers are very important as these often show the highest rate of recovery from degraded samples compared to nuclear markers.

PhD student Letizia Dondi (McMaster) had success with a primer set (DI\_15588 & DI\_15829 — Table 1) that she designed from the alignment of cervid mitochondrial sequences. The primer set is specific for caribou and amplifies well for both tissue and fecal samples. This discovery is significant as there are currently no markers in the literature that amplify so effectively and specifically for fecal DNA in caribou. Dondi is developing a manuscript which describes the process of marker design for caribou species detection. This is an important process not just for this technology, but for use in other portable DNA detection devices such as qPCR machines and for the detection of other cervid species nationally and internationally.





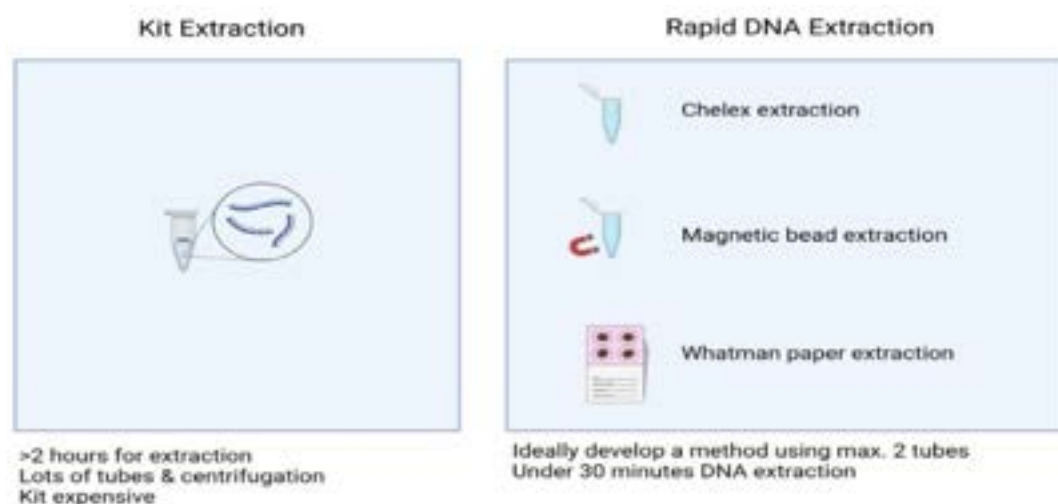
**Table 1: Primer sequences specific to caribou amplifying a 193bp region of the mitochondrial genome (D-loop).**

Primer	Nucleotide sequence (5' - 3')
DI_15588	TTTATAAACGTACATATATGGTCCTGTACGGT
DI_15829	TTGGCGAGAAAGGATTTGACTTAATGTGCTATG

### Sample Preparation and DNA Extraction Method

Obtaining viable DNA from fecal samples can be challenging. In addition to issues with low quantity and quality of DNA, feces also carry inhibitors from soil, diet, or the digestive system that can interfere with the ability to amplify the DNA downstream. Therefore, considerations need to be made regarding sample preparation and DNA extraction needing to be simple and efficient for non-experts, and including ways to remove inhibitors from extraction methods. The research team has identified and successfully tested methods for DNA extraction that yield highly reproducible results when analyzing fecal samples using the primer set DI\_15588 & DI\_15829. .

Researchers are collaborating with the Ancient DNA Centre at McMaster — specialists in extracting quality DNA from difficult and degraded sample types — to further simplify the methods for field-application (Figure 1).



**Figure 1:** Methods of DNA extraction from fecal samples being trialed for rapid diagnostic testing in the field compared to traditional extraction methods. Traditional kit DNA extraction requires at least two hours, extensive pipetting, multiple tubes, as well as a centrifuge step. For this technology to be truly useful for non-experts in the field, we are trialing rapid DNA extraction which requires a maximum of two tubes and a 30-minute time frame.

### DNA Amplification and Detection Methodology

Prior to the COVID-19 pandemic, project researchers were focused on Rolling Circle Amplification (RCA) over other isothermal amplification methods. The rationale for focusing on RCA was; RCA works at room temperature; is highly specific and sensitive; can be performed on paper with high efficiency; and can be customized to produce functional nucleic products that can be easily used to elicit a simple colour change response (Ali et al., 2014; Li & Macdonald 2015; Notomi et al., 2000). However, learnings from the research team's work on SARS-CoV-2 detection methods are being incorporated into this project. One important discovery was the challenge around the use of rolling circle



amplification (RCA) to concentrate DNA from biological samples. RCA, because of its sensitivity in recognizing and amplifying low quantities of DNA/RNA, can also have a tendency for non-specific amplification, producing false positive results (Bialy et al., 2022). This discovery saved considerable time and motivated the teams to adjust their approach and to trial a different form of RCA that uses a padlock probe, along with two other amplification methods: recombinase polymerase amplification (RPA), and loop-mediated isothermal amplification (LAMP). All three approaches have shown considerable promise recently for rapid diagnostic tests (Huang et al., 2020; McConnell et al., 2021; Tsou et al., 2021). RPA has been preferred above other isothermal methods due to its rapid amplification speed, isothermal temperature ranging from 25°C to 42°C and its ability to be multiplexed. Studies outside of McMaster have shown considerable progress in simple and effective downstream detection methods coupled with one of the above amplification methods (e.g., CRISPR; Lau et al., 2020).

McMaster researchers will be trialing some of these methods alongside RCA for comparison — it is these amplification methods that the team believes have the greatest potential for success for wildlife DNA detection.

In addition to the progress towards the five aims the following progress and achievements in 2022 are worth noting.

### New team member

Dr. Rahul Chaudhari, a new postdoctoral fellow, joined the McMaster team. Chaudhari is a specialist in biotechnology from the Indian Institute of Technology Bombay (IIT-Bombay) and the Tata Institute of Fundamental Research Mumbai (TIFR-Mumbai). He was the Chief Technology Officer for two clinical diagnostic start-ups and has a wealth of expertise in molecular biology, biochemistry, nanotechnology, and four years of experience in rapid, low-cost diagnostic product development. Chaudhari will be instrumental in applying the methods that have come out of COVID detection to the detection of caribou fecal samples and developing a paper-based platform for the easy extraction of DNA.

### Partnerships and Collaboration

**Earth Biogenome Project (EBP)** - is an initiative that aims to sequence and catalogue the [genomes](#) of all of Earth's currently described [eukaryotic](#) species over a period of ten years. The initiative would produce an open [DNA database](#) of biological information that provides a platform for scientific research and supports environmental and conservation initiatives. The EBP has reached out to project researchers to express interest in helping to expand the technology to other species of interest to conservation. Despite being a future partnership and beyond the scope of this project, it highlights the global interest and enthusiasm for this technology for monitoring and addressing biodiversity loss.

**Ancient DNA Centre at McMaster University** — this organization uses state-of-the-art techniques to extract and sequence DNA from archaeological, paleontological, and forensic remains — discerning origins and population histories of a wide range of species, both extinct and extant. Professor Hendrick Poinar has offered to collaborate on this work (at no cost) to find novel and effective ways to deal with difficult samples like feces, which is the most challenging component of this technology.



## LESSONS LEARNED

One of the most valuable lessons learned in 2022 was to ensure that all available caribou specific markers in the literature are thoroughly tested for effectiveness when using fecal samples, rather than assuming their effectiveness. None of the markers currently published in the scientific literature work effectively and specifically for caribou fecal DNA. This finding has implications beyond this study, not just for caribou detection but also for the detection of other cervid species in field and lab-based studies.

Caribou fecal DNA is challenging to work with due to the high variation in fragmentation and a mitochondrial genome that is 89% similar to four other ungulate species. It is crucial for this technology that project researchers have markers that are highly specific to reduce detection error. Letizia Dondi has developed a marker set that has been proven to work effectively and consistently for caribou fecal DNA and she is currently testing further markers from the alignment of cervid mitochondrial sequences. This is a unique and very important contribution on its own, as these markers will also be hugely valuable to industry and other stakeholders for the monitoring of caribou outside the use of our technology. Project researchers are committed to publishing these marker sets in 2023.

The available scientific literature is inconsistent with regards to the best practices for preparing fecal pellets for optimal DNA extraction, and as to whether the entire pellet should be used or just the external epithelial layer. The initial experiments have been inconclusive, but this is an essential step to resolve to ensure optimal consistency in the DNA obtained whilst ensuring the process is simple for non-experts. This finding will be important not only for this technology but also for the extraction of DNA in the lab for other purposes such as individual caribou identification.

Finally, discoveries made by the McMaster lab on COVID detection methods during the pandemic, have highlighted issues with RCA for concentrating the DNA from caribou once it is extracted. Assays have been found to suffer from high false positive rates and require prolonged incubation for signal detection. As such, project researchers have expanded their isothermal amplification methodology, as outlined above, to include other methods that show great promise in being very effective for this technology.

## LITERATURE CITED

<sup>1</sup>Environment Canada. 2011. Recovery Strategy for the Woodland Caribou, Boreal population (*Rangifer tarandus caribou*) in Canada [Proposed]. Species at Risk Act Recovery Strategy Series. Environment Canada, Ottawa. vi + 55 pp.

<sup>2</sup>Ali et al., and W. Zhao. 2014. Rolling circle amplification: a versatile tool for chemical biology, materials science and medicine. *Chem Soc Rev* 43:3324-3341.

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<sup>4</sup>Notomi et.al., and Hase. 2000. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research* 28:7.

<sup>5</sup>Bialy et al., and Brennan. 2022. Functional nucleic acid biosensors utilizing rolling circle amplification. *Chemical Society Reviews*.



<sup>6</sup>Huang et al., and Ji. 2020. RT-LAMP for rapid diagnosis of coronavirus SARS-CoV-2. *Microbial biotechnology* 13:950-961.

<sup>7</sup>McConnell et al., and Li. 2021. Biosensing with DNazymes. *Chemical Society Reviews*.

<sup>8</sup>Tsou et al., and Jiang. 2021. Rapid and sensitive detection of SARS-CoV-2 using clustered regularly interspaced short palindromic repeats. *Biomedicines* 9:239.

<sup>9</sup>Lau, A., C. L. Ren, and L. P. Lee. 2020. Critical review on where CRISPR meets molecular diagnostics. *Progress in Biomedical Engineering*.

## PRESENTATIONS AND PUBLICATIONS

### Scientific publications

Cavedon et al., and Musiani (2022). Population structure of threatened caribou in western Canada inferred from genome-wide SNP data. *Conservation Genetics*. 23(6), 1089-1103. <https://doi.org/10.1007/s10592-022-01475-1>

Cavedon et al., and Musiani (2022). Selection of both genes and habitat in specialized and endangered caribou. *Conservation Biology*, e13900. <https://doi.org/10.1111/cobi.13900>

Cavedon et al., and Musiani (2022). Genomic legacy of migration in endangered caribou. *PLOS Genetics*, 18(2), e1009974. <https://doi.org/10.1371/journal.pgen.1009974>

This last paper resulted in the following media coverage and interviews (date):

- “A reindeer’s yearning to travel can be read in its genes” Research Highlight of paper, *Journal Nature* (2022/02/10).
- “Whether caribou migrate or stay put is determined by genes that evolved in the last ice age” *The Conversation: Academic rigour, journalistic flair* (2022/03/20).
- “Dozens of Genes Tied to Caribou’s Seasonal Migration” *The Scientist Magazine* (2022/05/02).
- “Genes reveal caribou are specialized to unique habitats” *The Wildlife Society* (2022/05/27).

### Conference Presentations/Posters

Letizia Dondi presented at the Canadian Chemistry Conference and Exhibition 2022 in Calgary. She additionally has obtained a distinction grade in her Ph.D. qualifying exam.

Maria Cavedon presented at the North American Congress for Conservation Biology (NACCB) 2022, and at the North American Caribou Workshop (NACW) 2022. Also note public outreach generated by academic papers (below)



## AWARDS

### Recognitions and public outreach

Dr Natalie Schmitt was chosen as “the Explorers Club 50 people changing the world that the world needs to know about” in January of 2022.

Since then, she has presented in New York at the Explorers Club Annual Dinner, the Azores at the Global Exploration Summit, was a plenary speaker at the Lowell-Thomas Awards honoring lifetime achievements in conservation genetics at Harvard University, presented virtually at the Global Biodiversity Festival, and Explorers Club podcasts.

Dr. Schmitt has been invited to reapply for the ROLEX Awards for Enterprise for 2023 and is currently in the second of four rounds for the \$270,000 prize.

Dr. Schmitt has been nominated for the National Geographic Wayfinder Award for 2023 which honors “groundbreaking work in new technology, research, photography, and impactful storytelling among other techniques to advocate for and protect the wonder of our world.”

The technology was recognized in a CITES report this year on jaguar illegal trade. “.... endorsing the development of a paper-based biosensor for the detection of jaguar genetic remains.... This could provide a fast and cheap mechanism to facilitate law enforcement actions concerning the jaguar, by rapidly identifying jaguar body parts and derivatives like bone, teeth, fat or meat. This could be particularly useful for testing the hypothesis that jaguars may be replacing tiger products in the Chinese wildlife market.” The potential scope of this technology for conservation is clearly expansive and there is incredible global interest.

## RESEARCH TEAM AND COLLABORATORS

Institution: McMaster University<sup>1</sup> and the University of Calgary<sup>2</sup>

Principal Investigator: Dr. Carlos Filipe<sup>1</sup>

Co-Principal Investigators: Dr. Marco Musiani<sup>2</sup> and Dr. Yingfu Li<sup>1</sup>

Name	Institution or Company	Degree or Job Title	Degree Start Date (Students Only)	Degree Completion Date (Students Only)
Natalie Schmitt	McMaster University	Post-Doctoral Fellow		
Maria Cavedon	University of Calgary	Post-Doctoral Fellow		
Rahul Chaudhari	McMaster University	Post-Doctoral Fellow		
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## Johne's Disease in Bison

**COSIA Project Number:** LJ0342

**Mine**

**Research Provider:** University of Calgary

**Industry Champion:** Syncrude

**Status:** Year 2 of 4

### PROJECT SUMMARY

The Beaver Creek Wood Bison Ranch started operations in 1993 when a herd of 30 wood bison arrived at Syncrude's Mildred Lake site from Elk Island National Park near Edmonton. The original wood bison herd has grown into a larger herd of approximately 300 animals, living on more than 300 hectares of reclaimed land on Syncrude's former Base Mine about 50 kilometres north of Fort McMurray.

A joint venture between Syncrude and Fort McKay First Nation manages the herd which has maintained tuberculosis and brucellosis disease-free status since inception. However, these bacterial diseases are present in free-ranging bison in other locations, including those found in Wood Bison National Park. Being free of these two reportable diseases is important for most producers since there is a risk of these infections spreading to other livestock and to humans (zoonotic).

Other diseases known to infect bison can be classified as production limiting, such as *Mycobacterium avium* subspecies *paratuberculosis* (*Map*). This is a bacterial pathogen that causes chronic intestinal inflammation and wasting in ruminants known as Johne's Disease (JD) (Barkema et al., 2018) many regional and country-wide control programmes for Johne's disease (JD). Although the study of Johne's Disease in domestic ruminants (primarily cattle) provides some guidance, the disease transmission, pathophysiology and environmental persistence of *Map* strains as present in bison is unknown (Forde et al., 2013) a number of wood bison populations (Bison bison athabasca). The presence of *Map* has implications for the health of bison populations, both farmed and wild. Syncrude's current Johne's Disease management strategy includes sampling, testing for Johne's Disease and culling animals that show signs of the disease. The information from this research program will provide guiding information to support the long-term viability of managed bison and wood bison species in general.

The overall objective of this project is to understand the current dynamics of *Map* infection in the bison herd, the impact on the health of the animals, and to develop a herd health strategy to reduce the prevalence and transmission of *Map*.

Specifically, project researchers will:

**Objective 1:** Refine diagnostic tools for *Map* detection in bison. Current efforts to refine diagnostic tools include optimizing real-time polymerase chain reaction (qPCR) — a test for the presence of genetic material — and culture of *Map* bacteria from bison. Culture will allow for genetic characterization of the strain infecting bison and comparison to existing *Map* strains.



**Objective 2:** Investigate the pathogen’s epidemiology (prevalence, transmission, maintenance, environmental persistence) within the herd.

**Objective 3:** Describe the course of disease (pathophysiology) in clinically affected bison.

**Objective 4:** Investigate comorbidities, such as worm burden and viral diseases. Successful herd management must consider the diverse array of pathogens that a herd is exposed to for two reasons. First, co-infections can alter the susceptibility of an animal to other pathogens, positively or negatively, and thus influence the epidemiology and impacts at the herd level. Understanding these interactions is critical. Second, many pathogens have a similar transmission route, thus controlling one pathogen through improved management may improve control of another one.

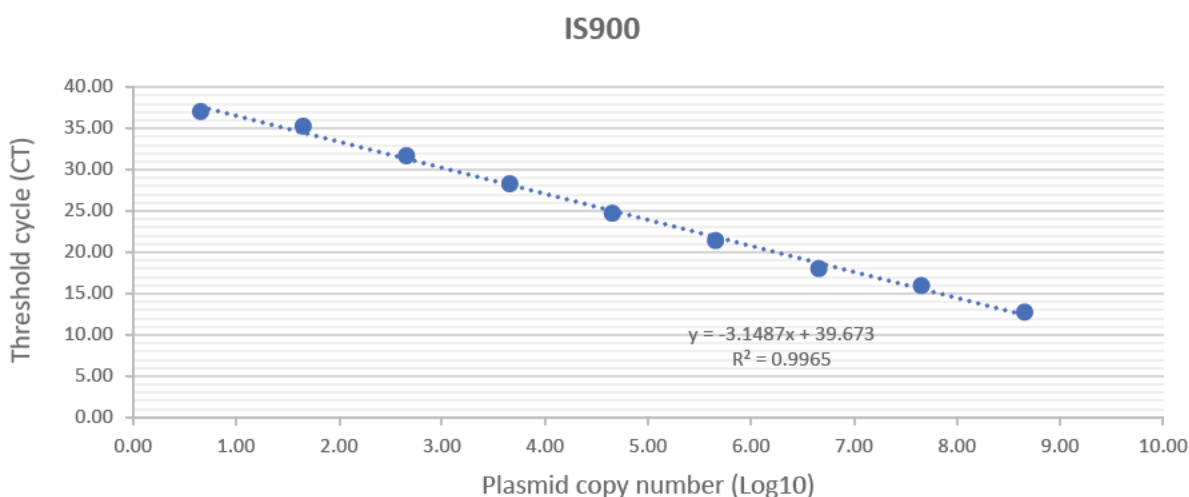
## PROGRESS AND ACHIEVEMENTS

### Objective 1: Refine diagnostic tools for *Map* detection in bison.

#### a. Optimization of qPCR of *Map*

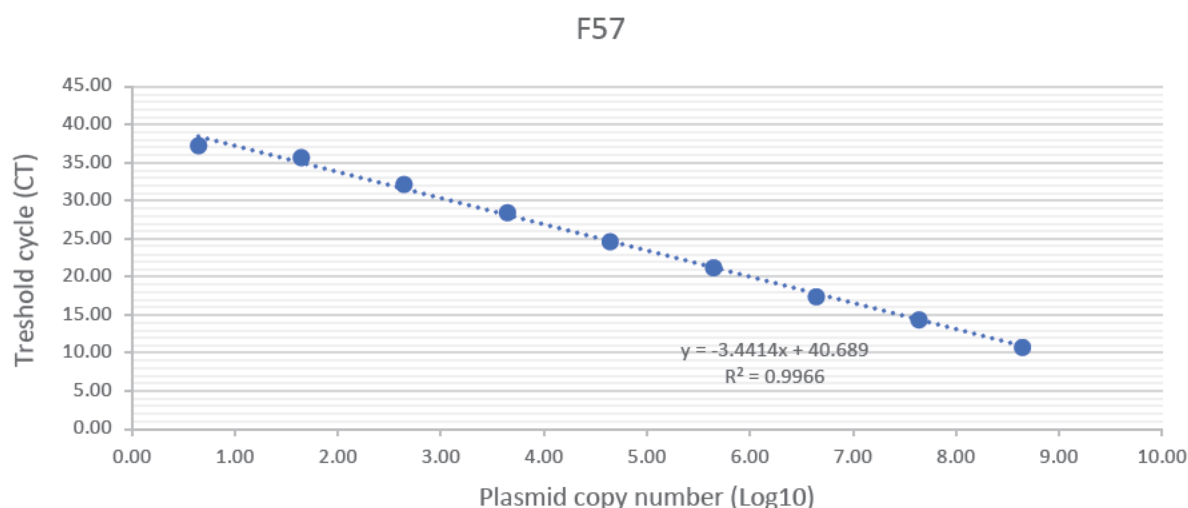
Two target genes are being used to identify *Map* DNA in fecal samples from the bison herd. Insertion sequence 900 (IS900), is one of the most common genes used in the diagnosis, with 15 to 20 copies in the *Map* genome (Timms et al., 2011; Vary et al., 1990). However, several studies have found the presence of IS900-like sequences in other environmental mycobacterial species, affecting the specificity of IS900-PCRs (Englund et al., 2002; Taddei et al., 2008). Therefore, a second more specific target gene, F57, with one copy in the *Map* genome is also being used (Vansnick et al., 2004).

To adjust the polymerase chain reaction (PCR) for bison, the first step was to standardize the qPCR. A standard curve was created for each gene to assess the efficiency of the primers selected. The standard curves for IS900 and F57 are shown in Figure 1 and Figure 2 respectively.



**Figure 1:** Standard curve of IS900 gene, generated with serial dilutions of a plasmid.





**Figure 2:** Standard curve of F57 gene, generated with serial dilutions of a plasmid.

Standard curves provide information on the capability and range of the test to detect *Map* bacteria following a stringent fecal sample decontamination procedure. As the performance of the (PCR) test can vary based on *Map* strain and target gene under investigation, each laboratory must define a standard curve. The standard curve also provides information on the reliable detection limit (i.e., cut-off to be used in the lab). Additionally, next steps in the qPCR test would allow for quantification of the *Map* bacteria present, and this provides information about the risk of transmission and disease progression.

### Objective 1b: Culture/strain typing

To obtain the strain of *Map* that affects this herd, all PCR positive fecal samples were cultured. A liquid culture (para-JEM®) was used, with 49 days of incubation, followed by qPCR confirmation.

Table 1 shows the number of positive cultures that have been obtained for each sampling to date (December 2022).

**Table 1: *Map* culture results from PCR positive fecal samples (as of December 2022)**

Sampling Number	Date	Number of Samples	Prevalence (positive for two gene)
1	October 2021	12	75% (9/12)
2	February 2022	18	77% (14/18)
3	September 2022	In progress	
4	October 2022	In progress	

Culturing the *Map* bacteria is valuable to confirm that viable bacterium is present in the sample as well as to grow enough of the bacterium to allow exploration of *Map* strain. Researchers have shown that almost 75 percent of PCR positive fecal samples were successfully cultured.



## Objective 1b: Next Step

To better understand the characteristics of the strain cultured from this herd, a comparison will be made with the most common strains circulating in the bovine population. Using the isolates obtained after culture, a molecular technique known as single nucleotide polymorphism PCR (SNP-PCR) is currently in progress to classify the strain. The SNP-PCR test recognizes multiple SNPs and can be used to differentiate the four *Map* subtypes most common to livestock in Canada. It is important to understand the differences that exist between *Map* strains and how they influence both development (pathogenicity) and transmission of disease.

## Objective 2: Investigate the pathogen's epidemiology (prevalence, transmission, maintenance, environmental persistence) within the herd.

### a. Prevalence of *Map* within herd determined by qPCR from fecal samples.

To have a better understanding of the epidemiology of *Map* within the herd, identifying the prevalence is important. Since the project started (October 2021), four sampling events have occurred. The majority of animals were sampled in two sampling events and the description and prevalence results are shown in Table 2. Animals are characterized as positive when both genes (IS900 and F57) are detected via PCR.

**Table 2: Prevalence of *Map* in Beaver Creek Wood Bison herd (2021-2022)**

Sampling	Date	Number of Samples	Prevalence (positive for two gene)
1	October 2021	181	12/181 (6.63%)
2	October 2022	194	7/137 (5.10%)

Although the total number of samples is comparable between 2021 and 2022, the sampled population was not identical. Some animals were removed from the herd after the first sampling event and therefore not included in the 2022 sampling.

Two additional sampling events (February 2022 and September 2022) were subsamples of the herd and individuals of interest (target group).

Sampling in February 2022 targeted animals that were found to be either positive or inconclusive for *Map* in October 2021. Eighteen of the 59 samples collected from this targeted sampling event provided a sample prevalence of approximately 31 percent.

A target group of bison from the herd that were grazing with a diseased animal were sampled in September 2022. Seventy-nine samples were collected resulting in a sample prevalence of approximately five percent.

Due to slow disease progression, and despite the removal of test positive animals from the herd between the two herd-level samplings in October 2021 and October 2022, no decline in herd prevalence was anticipated nor detected. Only management changes will prevent new infections from occurring and therefore herd prevalence reduction can only be anticipated over a five-to-ten-year management strategy adjustment period.



### Objective 3 Describe the course of disease (pathophysiology) in clinically affected bison.

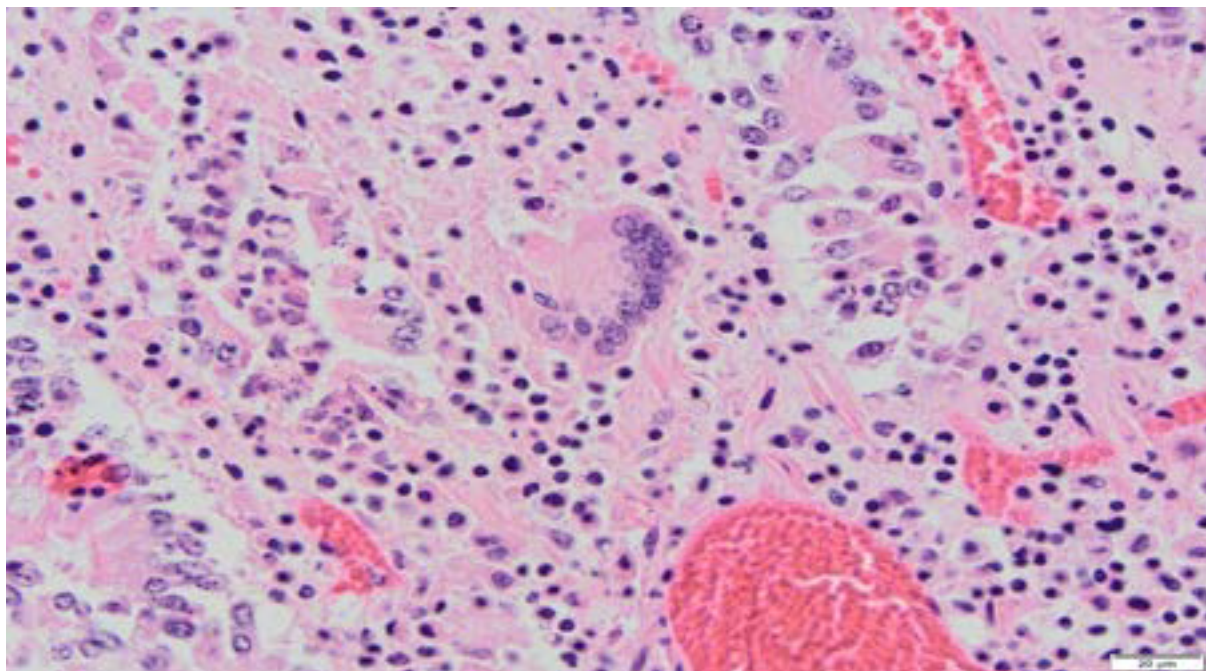
#### Clinical description of Johne's Disease in wood bison

Clinical signs such as diarrhea and weight loss as reported in domestic ruminants have also been reported in wild ruminants (Manning and Collins, 2001). However, there are some exceptions. For example, in cervids *Map* infection is different from that observed in other ruminant species. Clinical evidence of disease shows earlier in cervids than in other ruminants and is more focused on loss of body condition over presence of diarrhea (Mackintosh and Koets, 2006).

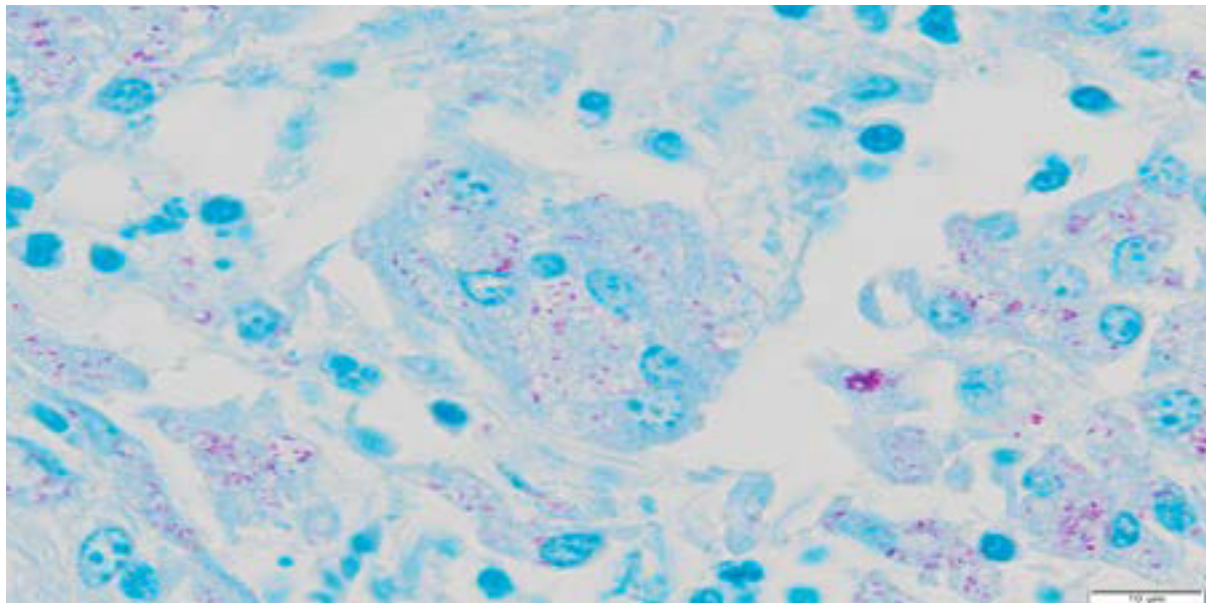
Although pathologists can easily characterize microscopic changes related to advanced clinical Johne's Disease of domestic ruminants, these morphological changes and target tissues can differ with wild ruminants. Specifically in wood bison, where only anecdotal information exists regarding Johne's Disease's clinical presentation and macroscopic pathological findings. Therefore, one of the main objectives of this study is to describe the clinical and pathological findings of Johne's Disease in wood bison.

As of December 2022, two bison carcasses (G671 and C282) were received and fully processed to perform the necropsy, followed by culture of several tissue sections. In December 2022 another four carcasses were presented to the University of Calgary but results from those animals are not yet available.

**G671:** In October 2021, the carcass of a two-and-a-half-year-old female wood bison was received. The bison was reported to have had diarrhea and weight loss and was suspected of having Johne's Disease. Samples (n = 26) taken for histopathology included those from the liver, kidney, spleen, and at various sections of the intestine. Tissues were fixed in 10 percent buffered formalin for 24 hours, processed routinely, and slides were stained with hematoxylin and eosin (HE) and Ziehl-Neelsen (ZN). Moreover, one part of each section was taken for *Map* detection by liquid culture. In Figures 3 and 4 the histopathology evidence of *Map* is shown.



**Figure 3:** Ileum; wood bison G671. Photomicrograph demonstrating macrophages and multinucleated giant cells expanding the lamina propria consistent with granulomatous enteritis. HE. Bar = 20 µm.



**Figure 4:** Ileum, wood bison G671. Photomicrograph demonstrating intracellular acid-fast bacilli within macrophages and multinucleated giant cells consistent with *Mycobacterium* species. ZN. Bar=10  $\mu$ m.

Morphological changes as presented in Figure 2 aligned with microscopic changes in beef and dairy cattle. The location of intracellular bacteria also confirms the systemic infection of the animal with *Map*. Overall comparisons of intestinal sections positive for *Map* will be made with published literature on plains bison and dairy cattle. Preliminary PCR results from G671, show that 17 of 23 tissues processed contained *Map* bacteria.

Providing detailed information on predilection sites will guide sample collections by veterinarians and pathologists to confirm a diagnosis.

**C282:** In September 2022, the carcass of a seven-year-old female wood bison was received. The bison was reported to have had diarrhea and weight loss and previous *Map* positive results in fecal samples taken in October 2021 and February 2022. Laboratory diagnostics are pending.

#### **Objective 4: Investigate comorbidities, like worm burden and viral diseases.**

##### **Comorbidities: Parasitology**

During annual handling of nearly all individuals of the herd (late October 2021 and 2022), we performed the fecal egg count of fecal samples obtained (including calves). All adult bison are regularly dewormed. Therefore, monitoring fecal egg counts will provide information on animals with anthelmintic resistance or animals that missed a proper treatment (including calves). Future work will identify the most likely reason.

In addition to the fecal egg counts parasite eggs were microscopically identified and classified into four distinct egg morphologies, Strongyle-type, *Trichuris*, *Nematodirus*, and *Moneiza* (tapeworm). Finally, protozoa, such as *Eimeria*, were counted. However, it should be noted that these do not respond to common anthelmintic treatments.



Table 3 shows the median egg count and the range of eggs found within the age and or sex groups of animals for each sampling location. When sample volume allowed, these samples represent the same adult individuals described in Table 2.

In summary, results are showing that deworming treatments for adult bison are managing overall worm burdens. Continued monitoring of fecal egg counts will provide information on the proportion of outliers and, if required, a discussion for alternative treatment options.

**Table 3: Fecal egg counts presented for the sampling years 2021 and 2022 per age group and location**

<b>Main Ranch October 2021</b>					
	<b>Strongyle Median (min, max)</b>	<b>Nematodirus Median (min, max)</b>	<b>Trichuris Median (min, max)</b>	<b>Moniezia Median (min, max)</b>	<b>Eimeria Median (min, max)</b>
<b>Cows (n = 77)</b>	1.12 (0, 24.26)	0 (0, 0)	0 (0, 9.92)	20.19 (0, 509.68)	10 (0, 239.39)
<b>Bulls (n = 24)</b>	6.49 (0, 35.19)	0 (0, 0.29))	0 (0, 2.38)	0 (0, 88.51)	64.09 (2.37, 566.56)
<b>Heifers (n = 36)</b>	18.84 (0, 110.64)	0 (0, 3.61)	0 (0, 45.28)	4.85 (0, 307.44)	29.59 (1.1, 289.32)
<b>Calves (n = 37)</b>	7.67 (0, 36.39)	2.87 (0, 24.53)	17.07 (0, 53.51)	0 (0, 71.3)	20.13 (0.78, 542.22)

<b>East Toe-Berm October 2021</b>					
	<b>Strongyle Median (min, max)</b>	<b>Nematodirus Median (min, max)</b>	<b>Trichuris Median (min, max)</b>	<b>Moniezia Median (min, max)</b>	<b>Eimeria Median (min, max)</b>
<b>Cows (n = 38)</b>	0.86 (0, 35.02)	0 (0, 0)	0 (0, 0)	0 (0, 98.53)	3.72 (0, 210.64)
<b>Bulls (n = 6)</b>	1.86 (0, 4.29)	0 (0, 0)	0 (0, 0)	6.49 (0, 73.01)	2.79 (0, 46.01)
<b>Calves (n = 16)</b>	18.02 (6.21, 96.65)	2.98 (0.32, 4.58)	0 (0, 0)	39.15 (0, 259.21)	68.25 (13.62, 344.72)



Main Ranch October 2022					
	Strongyle Median (min, max)	Nematodirus Median (min, max)	Trichuris Median (min, max)	Moniezia Median (min, max)	Eimeria Median (min, max)
Cows (n = 58)	0 (0, 1.03)	0 (0, 0.32)	0 (0, 13.67)	5.5 (0, 174.14)	12.5 (0, 101.61)
Bulls (n = 10)	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0.67)	37 (8.67, 223.33)
Heifers (n = 21)	0 (0, 0)	0 (0, 0)	0 (0, 0.32)	0 (0, 0.65)	20.36 (1.03, 109.68)
Yearlings (n = 7)	0 (0, 2)	0 (0, 0)	0 (0, 7.81)	25.31 (0, 178)	32.58 (8.75, 67.67)
Calves (n = 44)	0 (0, 1.3)	3.23 (0, 41.25)	1.91 (0, 88.06)	0 (0, 270)	161.29 (4, 500)

East Toe-Berm October 2022					
	Strongyle Median (min, max)	Nematodirus Median (min, max)	Trichuris Median (min, max)	Moniezia Median (min, max)	Eimeria Median (min, max)
Cows (n = 39)	0 (0, 0.71)	0 (0, 0.32)	0 (0, 0)	0 (0, 42.67)	17.42 (0, 249.31)
Bulls (n = 3)	0.32 (0, 1.61)	0 (0, 0)	0 (0, 0)	0 (0, 9.35)	64.52 (4.84, 81.29)
Calves (n = 22)	0.68 (0, 6.07)	0.34 (0, 1.72)	0 (0, 0)	42.68 (0, 332)	37.41 (0, 142.41)

## LESSONS LEARNED

There are no formal results available for 2022 as tools are in development and continued sample analysis is underway. However, as they become available, research results are shared with teams managing the bison herd to help inform the herd-management decision process.

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## PRESENTATIONS AND PUBLICATIONS

### Conference Presentations/Posters

July 2022. International Bison Convention 2022. Poster presentation: A case report on Johne's disease in wood bison (*Bison bison athabasca*) in Alberta.

### Awards

Second prize for Poster Presentation (see above) by Ana Hernandez at the International Bison Convention 2022, SK, Canada.





## RESEARCH TEAM AND COLLABORATORS

Institution: University of Calgary

Principal Investigator: Dr. Karin Orsel

Name	Institution or Company	Degree or Job Title	Degree Start Date (Students Only)	Degree Completion Date (Students Only)
Ana Hernandez Reyes	University of Calgary	MSc	2021	2023
Jeroen De Buck	University of Calgary	Professor		
John Gilleard	University of Calgary	Professor		
Susan Kutz	University of Calgary	Professor		
Frank van der Meer	University of Calgary	Professor		

# Early Successional Wildlife Dynamics Program

**COSIA Project Number:** LJ0013

**Mine**

**Research Provider:** LGL Limited environmental research associates

**Industry Champion:** Canadian Natural

**Industry Collaborators:** Canadian Natural Upgrading Limited, Suncor Energy Oil Sands Limited Partnership (Suncor and Fort Hill Operations), Imperial

**Status:** Final Cumulative Summary: Supplemental Year 1

## PROJECT SUMMARY

Wildlife use of naturally occurring upland and wetland habitat in the Athabasca Oil Sands Region is relatively well-understood; however, the ability for reclaimed upland habitats to provide habitat for wildlife in a manner consistent with those naturally occurring habitats requires further study.

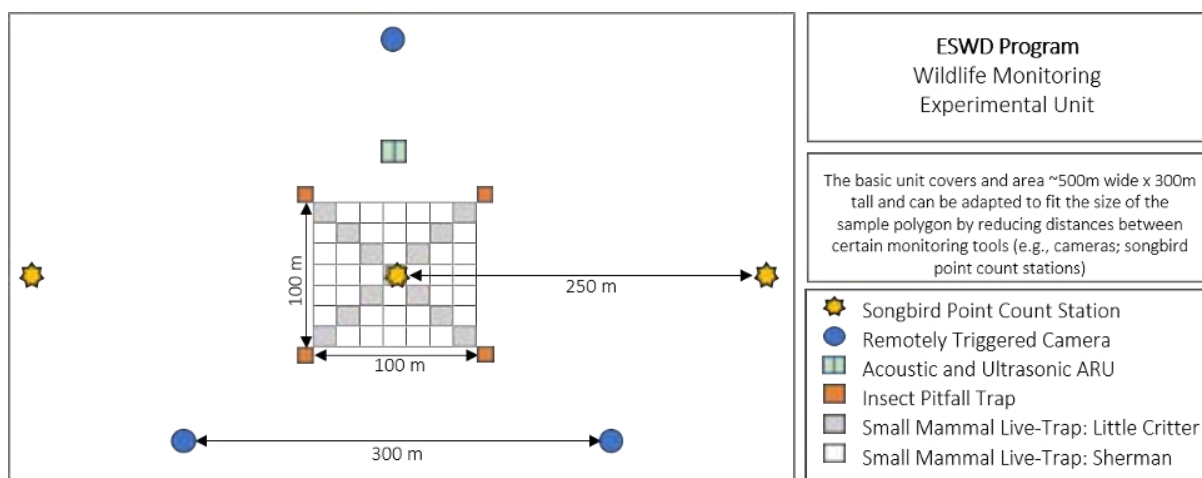
To address this deficiency, a five-year program was implemented to fulfil various objectives including:

- Addressing requirements for reclamation certification;
- Evaluating wildlife use of reclaimed habitats and areas adjacent to the development;
- Assessing return and re-establishment of wildlife on reclamation areas; and
- Evaluating effectiveness of practices and principles applied in reclamation areas to improve biodiversity.

Focal taxa representing terrestrial, and avian species were selected for annual monitoring of reclaimed habitats, mature forest, cleared, burned, and logged juvenile stands on leases operated by Canadian Natural (Horizon Oil Sands), Suncor (Oil Sands Base), Canadian Natural Upgrading Limited (Albian Sands), Fort Hills Operations (Fort Hills), and Imperial (Kearl Oil Sands). Annual sampling was used to generate a baseline dataset that can be used to assess how different species of wildlife are distributed relative to reclaimed habitats, and to assess whether reclaimed habitats are on a developmental trajectory similar to other juvenile stands in the region. Data collected from reclaimed and juvenile stands are compared to data collected from mature forest reference sites that represent the desired endpoint of upland reclamation in the Athabasca Oil Sands Region. Data collected from habitats reclaimed to an upland forest type were also compared to data collected from sites recovering from other human or natural disturbance (logging, clearing, forest fire). Results obtained from the wildlife program will be used to quantify the successful re-establishment of wildlife habitat on each operator's lease and will ultimately demonstrate to stakeholders and regulators that wildlife habitat is being successfully established and maintained within operational footprints. These data will also be used to ensure that oil sands operators comply the terms and conditions of their EPEA approvals. The design of the program is flexible enough to ensure expandability and adaptability over time. Further, wildlife sampling protocols are aligned with other regionally relevant and accepted methods and are part of a "living document" — one that will be updated as new information becomes available or adapted to changing goals and objectives.



Wildlife sampling is occurring in habitats representing several distinct types of sites: (1) reclaimed (REC); (2) reclaimed habitat adjacent to compensation lake (COMP); (3) mature forest (MF); (4) cleared habitats (CLR); (5) logged (LOG); and (6) burned (BRN). A standardized sample unit (Figure 1) is used that includes a small mammal live-trapping grid, songbird point count stations, and remotely triggered cameras. Winter-active animal usage is extracted from the wildlife camera data. Focal taxa include small mammals (deer mouse [*Peromyscus maniculatus*], meadow vole [*Microtus pennsylvanicus*], and southern red-backed vole [*Myodes gapperi*]), bats (with autonomous recording units [ARUs]), winter-active animals (with data collected by remotely triggered wildlife cameras); songbirds (point counts); and terrestrial arthropods (spiders and beetles). Other taxa considered for some or all leases include (Canadian Toad [*Anaxyrus hemiophrys*]), mammals (Canada lynx [*Felis canadensis*], North American beaver [*Castor canadensis*], common muskrat [*Ondatra zibethicus*], American moose [*Alces alces*], American black bear [*Ursus americanus*], snowshoe hare [*Lepus americanus*]), and various groups of birds (waterfowl, owls, and raptors [diurnal and forest-nesting]), specific species of birds (Ruffed Grouse [*Bonasa umbellu*], Yellow Rail [*Coturnicops noveboracensis*], and Pileated Woodpecker [*Dryocopus melanoleucus*]). All other wildlife observed on each lease that are not the focus of systematic surveys are recorded as incidental observations. These data often provide important insights regarding the use of an area by all wildlife species. In addition to sampling wildlife taxa, vegetation data collected are used to assess species to habitat relationships for the various plot types.



**Figure 1: Ideal experimental unit used to sample for various wildlife species and groups targeted by the Early Successional Wildlife Dynamics Program in the Athabasca Oil Sands Region.** The distance between certain monitoring tools (e.g., remote cameras, songbird point counts) and the size of the small mammal trapping grid may be changed to accommodate the size and shape of a plot.

Annual sampling occurs in all months, with most work occurring during the snow-free period. Survey methods include the use of qualified and proficient biologists to; (1) document songbird species occurrence and distribution; (2) capture and identify amphibians; (3) capture and identify epigeic beetles and spiders; (4) live trap and identify small mammal species; (5) deploy remote sensing equipment (remotely triggered cameras and autonomous recording units); (6) assess vegetation species composition, cover, and height at all sample sites; and (7) make reliable observations of all wildlife species during all seasons of the year. Autonomous passive recording devices such as Wildlife Acoustics Song Meters are being used for bats, amphibians, and some species of birds (e.g., Yellow Rail, owls). Wildlife cameras are deployed throughout each lease to track the presence and distribution of medium- and large-sized mammals. All data are collected in a standardized manner so that appropriate statistical tests can

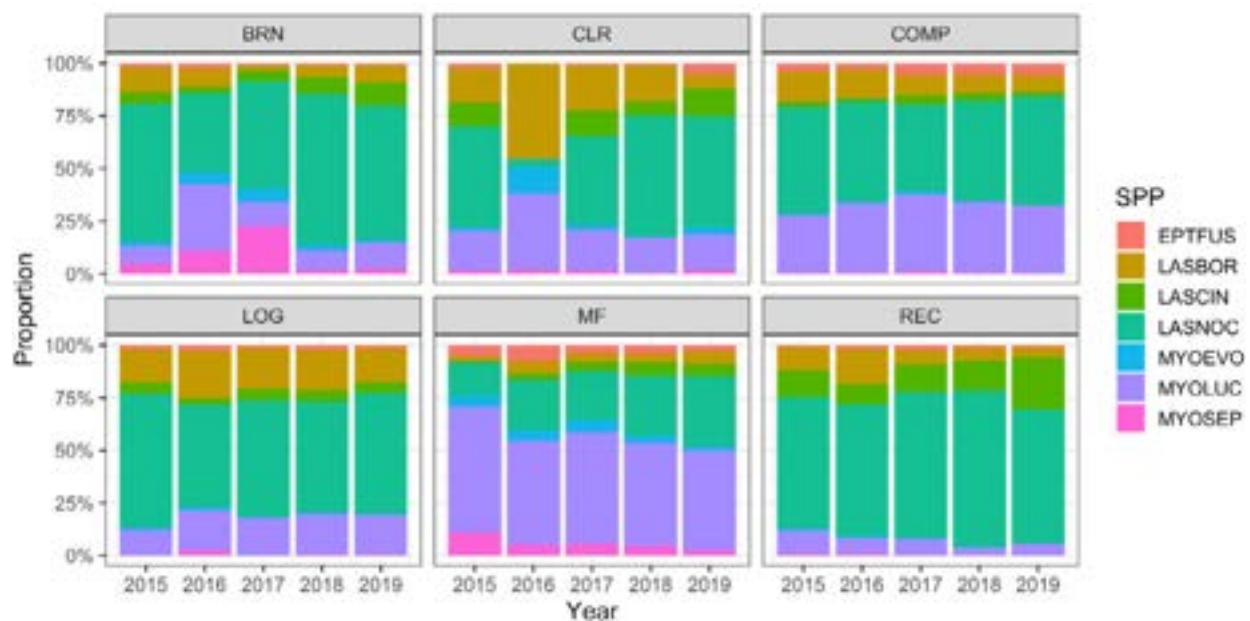


be applied. A comprehensive report summarizing the results of this program was made available in 2021. To fill several data gaps and further assess the utility or revised sampling methods to sample wildlife, a supplemental year of sampling was completed in 2022.

## PROGRESS AND ACHIEVEMENTS

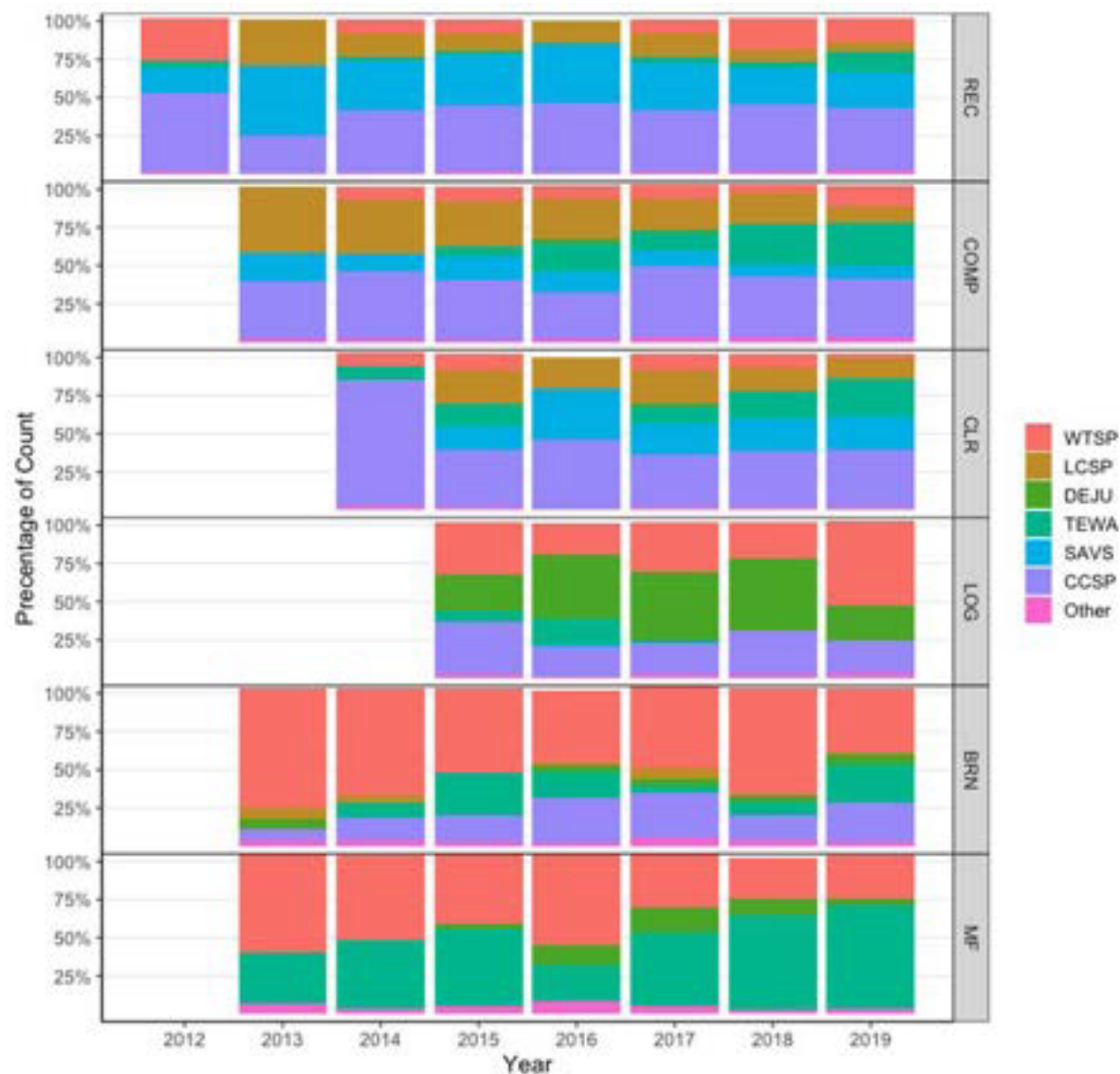
Sampling In 2022 occurred across 37 sites on Suncor's Base Plant (n = 12 sites); Canadian Natural's Horizon Oil Sands (n = 12 sites); Canadian Natural's Albion Sands Muskeg River and Jackpine Mines (n = 5 sites); Fort Hills (n = 3 sites); and Imperial's Kearl Oil Sands (n = 5 sites). Sampling was distributed among six types of habitats: 17 reclaimed to an upland forest type (REC; 12 mature forest sites (MF); four sites adjacent to compensation lakes (COMP); two sites recovering from stand-replacing fires in 2011 (BRN); one site cleared during initial mine development but left to regenerate naturally (CLR); and one site replanted following clearcut logging (LOG). Sampling of select groups of wildlife was accomplished using remote sensing (remotely triggered cameras [mammals, some birds] and acoustic autonomous recording units [owls, songbirds, amphibians]), small mammal live traps, pitfall traps (arthropods), bird point counts, as well as vegetation surveys across all five partner leases in 2022. All data collected in 2022 was combined with data collected in previous years to evaluate the potential of using revised (reduced) sampling methods to collect data on wildlife occurrence and distribution. Data collected in 2022 will be combined with all other data to update results and produce revised sampling protocols for wildlife sampling on reclaimed habitats. These data are also being used in various analyses that serve as the foundation for technical reports and publications.

The data collected to date were used to develop species profiles for each treatment sampled, one of the key outcomes of the Phase 1 program. For example, of the seven species of bat known to occur, silver-haired bat, little brown myotis, and eastern red bat had the greatest relative proportion of detection rates in most years and treatments ([Figure 2](#)). Silver-haired bat had the greatest relative proportion of detection rates in all treatments (excluding mature forest [MF]) each year except for cleared (CLR) habitat in 2016. Little brown bat had the greatest proportion of detection rates in MF habitats each year. Bat species associated with MF (long-eared bat and Northern myotis) had greater proportions of detection rates in MF and burned (BRN) treatments than other treatments. The data collected to date contribute to the development of a 'data profile' for bats using the various treatment types sampled. More importantly, these data provide an indication of bat usage patterns of mature forest types. As habitat reclaimed to an upland forest type matures, the data profile of bats on those sites should start to resemble those of mature forests, providing an indication of reclamation success.



**Figure 2: Relative proportion of detection rates of each of the seven bat species for each year and treatment sampled in the Athabasca Oil Sands Region.** Detection rate is equal to the number of classifications to species in a given treatment. Treatment codes are: MF = mature forest; BRN = burned forest; CLR = cleared; REC = reclaimed; COMP = compensation lake; LOG = logged. EPTFUS: *Eptesicus fuscus* (big brown bat); LASBOR: *Lasiurus borealis* (Eastern red bat); LASCIN: *Lasiurus cinereus* (hoary bat); LASNOC: *Lasionycteris noctivagans* (silver-haired bat); MYOEVO: *Myotis evotis* (long-eared myotis); MYOLUC: *M. lucifugus* (little brown bat); MYOSEP: *M. septentrionalis* (Northern myotis).

Similar results are available for birds. The data collected to date suggest that white-throated sparrow (WTSP) is a good indicator for BRN, though it is also present in a relatively high proportion in MF and logged (LOG) treatments, with a relatively low proportion of detections in early successional habitats such as reclaimed (REC) and CLR (Figure 3). Clay-colored sparrow (CCSP) shows the opposite trend, being an indicator for REC, and found in relatively high proportions in early successional habitats of REC, CLR and compensation lake (COMP), but with few to no observations for BRN and MF (Figure 3). Many species were detected in multiple treatment types, but at varying levels of occupancy. In general, the proportional distribution of certain indicator species of songbirds relative to each other and to all other songbird species, provides an indication of the songbird fauna that should be supported by reclaimed plots when they eventually mature (Figure 3).



**Figure 3: Proportion of songbird species detections within each treatment type sampled in the Athabasca Oil Sands Region, 2012 to 2019.** Only select indicator species are specifically shown. BRN = burned reference; CLR = cleared; COMP = compensation lake; LOG = logged; MF = mature forest; REC = reclamation. WTSP = White-throated Sparrow; LCSP = LeConte's Sparrow; DEJU = Dark-eyed Junco; TEWA = Tennessee Warbler; SAVS = Savannah Sparrow; CCSP = Clay-coloured Sparrow.

These data profiles provide guidance with respect to what to expect for each taxonomic group sampled in each treatment and provide an indication of what the 'target' wildlife species profiles on landforms reclaimed to an upland forest type should resemble when they have reached mature forest age.

Additional analyses of the terrestrial arthropod data are reported below. For a review of results associated with all other taxa, see the most recent comprehensive report (Hawkes et al., 2021) and publications (Hawkes et al., 2019, 2021). The potential to use epigeic arthropod assemblages as indicators of ecosystem shift is the focus of a current manuscript and the results are compelling; species habitat preferences for spiders and beetles and the ease of sampling makes them ideal taxa for assessing reclamation success.



When working with large datasets comprised of many species it is helpful to identify a subset of species that are associated with a given habitat type. Indicator Species Analysis (ISA; Dufrêne and Legendre, 1997) was used to quantify the value of each species' relationship to treatment types and/or sites of interest. ISA is a useful method for identifying biological indicators for any combination of habitat types or sites of interest and has been routinely applied in monitoring studies (Dufrêne and Legendre, 1997; McGeoch and Chown, 1998; McGeoch et al., 2002). Indicator species may be associated with more than one treatment type if some treatments are similar in terms of their habitats, or due to differences in the niche breadth of species. To account for this, researchers employed the Multi-level Pattern Analysis ('multipatt') routine of the indicpecies package (De Cáceres and Legendre, 2009), which measures the association between a species and an individual treatment, and between combinations of treatments (De Cáceres et al., 2010).

An indicator value (IndVal) ranging from zero (no indication) to one (perfect indication) was calculated for each species in each group treatment type. A species was considered an indicator for a given habitat when its IndVal differed significantly from random after a Monte Carlo test based on 9,999 permutations (using the IndVal value of Dufrêne and Legendre, 1997). To be conservative in the analyses, a threshold level of 0.50 was chosen for designating 'strong' indicator species (significant at  $\alpha = 0.10$ ). Only strong and significant indicator species were included in results.

Following analyses, there were ten species of carabid beetles and 28 species of spiders unique to a single treatment type (Table 1). All species identified were characteristic of a single habitat; no species were identified that were associated with a combination of treatment types. There were associations between carabid beetles and five of the six treatment types, excluding LOG sites. Spiders showed species associations between all treatment types. CLR and MF sites had the highest number of unique species associations with carabid beetles ( $n = 3$ ). MF and COMP sites had the highest number of unique species associations with spiders ( $n = 7$ ). The species listed in Table 1 can be the focus of future monitoring efforts.

**Table 1: Potential indicator ground beetle (Carabidae) and spider (Araneae) species for individual treatments based on IndVal statistics and p-values. Sampling occurred from six treatments in the Athabasca Oil Sands Region between 2015 and 2019. BRN = Burned reference; CLR = Cleared; COMP = Compensation Lake; LOG = Logged; MF = Mature Forest; REC = Reclamation.**

Taxonomic group	Treatment	Species	IndVal Stat	p-value
Carabidae	BRN	<i>Agonum gratiosum</i>	0.573	0.011
	CLR	<i>Syntomus americanus</i>	0.596	0.018
		<i>Pterostichus adstrictus</i>	0.596	0.049
		<i>Cicindela longilabris</i>	0.510	0.076
	COMP	<i>Harpalus somnulentus</i>	0.561	0.020
	MF	<i>Platynus decentis</i>	0.663	0.004
		<i>Pterostichus pensylvanicus</i>	0.554	0.037
		<i>Carabus chamissonis</i>	0.516	0.024
	REC	<i>Poecilus lucublandus</i>	0.721	< 0.001
		<i>Carabus serratus</i>	0.492	< 0.001





Taxonomic group	Treatment	Species	IndVal Stat	p-value
Araneae	BRN	<i>Pardosa xerampelina</i>	0.638	0.008
		<i>Arctosa rubicunda</i>	0.524	0.026
		<i>Agyneta olivacea</i>	0.473	0.032
		<i>Arctosa alpigena</i>	0.382	0.100
	CLR	<i>Zelotes fratris</i>	0.576	0.008
		<i>Pardosa concinna</i>	0.573	0.007
		<i>Antistea brunnea</i>	0.400	0.087
	COMP	<i>Pardosa fuscula</i>	0.584	0.012
		<i>Gnaphosa parvula</i>	0.562	0.024
		<i>Phlattothrata parva</i>	0.521	0.018
		<i>Thanatus formicinus</i>	0.485	0.038
		<i>Islandiana longisetosa</i>	0.464	0.054
		<i>Islandiana flaveola</i>	0.444	0.062
		<i>Sciastes truncates</i>	0.435	0.084
	LOG	<i>Callilepis pluto</i>	0.669	0.004
		<i>Haplodrassus signifler</i>	0.582	0.007
		<i>Micaria medica</i>	0.497	0.017
		<i>Xysticus montanensis</i>	0.490	0.019
		<i>Gnaphosa muscorum</i>	0.489	0.045
	MF	<i>Agroeca ornata</i>	0.726	0.001
		<i>Cybaeopsis euopla</i>	0.688	0.002
		<i>Amaurobius borealis</i>	0.667	0.002
		<i>Pardosa mackenziana</i>	0.662	0.002
		<i>Xysticus obscurus</i>	0.632	0.002
		<i>Trochosa terricola</i>	0.482	0.063
		<i>Diplocentria bidentata</i>	0.376	0.082
	REC	<i>Micaria rossica</i>	0.651	0.006
		<i>Alopecosa aculeata</i>	0.624	0.002

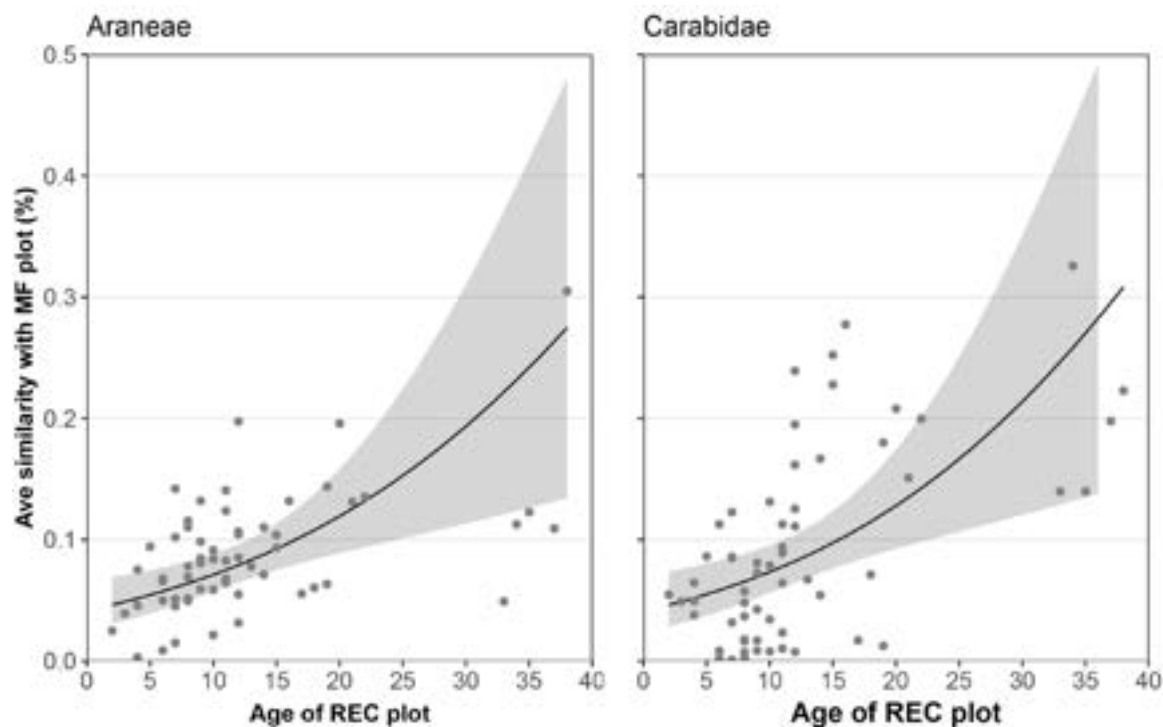
To assess the progression of habitat development on reclaimed sites, spider and beetle assemblages on sites reclaimed to an upland forest type were compared to mature forest sites. In 2022, a total of 28,537 individual terrestrial arthropod specimens were collected. These data were combined with data collected starting in 2015. Three new spider species (*Hypsosinga pygmaea*, *Improphantes complicatus* and *Sisicottus montanus*), and two new carabid species (*Carabus maeander* and *Carabus taedatus*) were identified from the 2022 sample, increasing the respective species lists to 165 Araneae (spider) species and 64 Carabidae (beetle) species.

Specimen identification for 2022 collections has just recently been completed. However, new analysis (generalized linear mixed models [GLMM; Zuur et al., 2009]) of arthropod catch data from 2015 to 2021 demonstrates how epigaeic arthropod assemblages associated with reclaimed landforms are, over time, beginning to mimic assemblages indicative of undisturbed habitat (Figure 4). While numerous species are present on reclamation plots of various



ages ranging from two to 37 years post-reclamation, the presence of *Amaurobius borealis*, *Platynus decentis*, and *Pterostichus pensylvanicus*, as well as other mature forest specialists at various reclamation plots greater than 10 years post-reclamation suggests that these aging reclaimed habitats are gradually starting to develop habitat characteristics of older forests relevant for epigaeic arthropods (Buddle et al., 2000; Richardson and Holliday, 2012; Werner and Raffa, 2000). Carabid species assemblages were mainly explained as a product of grass litter and fine woody debris coverage based on the environmental parameters included in the analysis. Higher diversity observed amongst spider assemblages allowed for a stronger correlations relative treatment type as opposed to other environmental variables, though coarse woody debris coverage and soil temperature were identified as important variables impacting species composition.

While arthropod assemblages continue to be highly variable, the general trend on reclamation plots regarding loss of open habitat specialists and re-colonization of mature forest specialists over time, leads to assemblages which shadow that of pre-disturbance fauna (Buddle et al., 2006). The maximum percent similarity in species composition of *Araneae* and *Carabidae* assemblages observed on REC plots compared to neighboring mature stands was 30% and 33%, respectively (Figure 4). A positive regression between age of reclamation plot and percent similarity of species composition compared to mature stands was observed for both spider and carabid beetle assemblages. This positive correlation was stronger in Carabid beetle assemblages compared to spiders Figure 4.



**Figure 4:** Similarity (%) in species composition between REC and MF plots over time according to the age of the REC plot for *Araneae* (left panel) and *Carabidae* (right panel). The grey dots represent raw similarity (1-D14) and the fitted line and shading represent predicted values and confidence interval based on a GLMM model fitted with beta regression and a random effect for plots to account for the repeated nature of the sampling.



The identification of epigaeic arthropods as bioindicators of ecosystem condition on habitats, on and adjacent to, active oil sands mines in AOSR represents a large step forward in ecological monitoring and evaluation of reclamation efforts (Kremen et al., 1993; Work et al., 2004). The abundance, fecundity, limited dispersal capabilities, and short generation time of these taxa make for ideal bioindicators (Kremen et al., 1993). Moreover, epigaeic arthropod assemblages react faster and more dramatically to localized disturbances compared to longer-lived species with potentially large home ranges or significant migration capabilities (Buddle et al., 2000; Koivula et al., 2002). Additionally, arthropod collection methods are often cheaper and easier to use than many conventional wildlife sampling techniques (Blanchet et al., 2016). As a result, the use of spiders and carabid beetles as bioindicators can provide operators in the AOSR region with a method for quantifiable assessments of the effectiveness of reclamation activities on a timescale spanning years as opposed to decades.

## LESSONS LEARNED

Current results indicate that wildlife is returning to and using reclaimed upland habitat, with the return being a function of time since reclamation, proximity to intact mature forest, and vegetation composition of the reclaimed habitats. In general, the species of wildlife encountered at each treatment type were as expected (based on known habitat associations). The following lessons learned/recommendations for future consideration are provided. Some of the lessons learned affirmed expectations (e.g., well-established wildlife survey methods are appropriately applied to study wildlife use of upland reclaimed sites) while others (e.g., consideration of habitat function and productivity) have developed as the Early Successional Wildlife Dynamics Program has been implemented.

1. Commonly used wildlife survey methods (small mammal live-trapping, songbird point counts, use of remote-sensing equipment, autonomous recording units and remotely triggered wildlife cameras) provide a standardized dataset upon which appropriate statistical analyses can be performed. The application of these survey methods contributes to the development of a time-series dataset that can be used in trend assessments and community ecology analyses.
2. Insect sampling (pitfall trapping) is providing data by which ecological shift can be assessed. Preliminary results reveal the presence of species-specific habitat associations with some species occurring in only a single habitat type. With time, it should be possible to better characterize the species-habitat associations and use the presence and abundance of a suite of species to discuss reclamation efficacy. As project researchers review the data, the ability to use epigaeic arthropods as bioindicators of ecosystem shift is becoming more compelling as a viable tool with which to assess reclamation success.
3. The use of remotely triggered wildlife cameras to sample winter-active animal use of reclaimed habitats and other treatments sampled, is proving to be more reliable than snow-tracking.
4. The inclusion of incidental data (i.e., data not collected using standardized data collection techniques) provides a more robust understanding of wildlife occurrence and distribution on sites reclaimed to upland habitats when combined with data collected using standardized methods. These incidental observations are an important part of the Early Successional Wildlife Dynamics Program.
5. Preliminary analyses suggest that an indicator species approach could be used to focus surveys on a subset of species in each taxonomic group. More data are required to fully test this hypothesis, and this is currently being investigated through targeted analyses on insect data and bird data.



6. The current focus is on developing a baseline against which future comparisons can be made, which necessitates the collection of species occurrence, distribution, and abundance data relative to each site sampled. Although informative, species presence and abundance data are only telling part of the story. In addition to knowing which species occur on upland reclaimed sites relative to the various analogs (burned, logged, cleared, compensation lake, and mature forest) an understanding of the function and productivity of upland reclaimed habitats is required. It will be necessary to determine if upland reclaimed habitat provides the habitat attributes necessary for wildlife to fulfill their life requisites in a manner consistent with (but not necessarily identical to) existing mature forest in the region. This will ensure a comprehensive assessment and understanding of reclamation efficacy and success.
7. Existing mature forests are being used as the reference point for upland reclamation. These habitats provide one possible outcome of upland reclamation; however, it is unknown if reclaimed habitats will develop into mature forests characterized using existing accepted methodology (e.g., Beckingham and Archibald 1996), or if they will simply resemble a currently described mature forest with a different species assemblage. As such, the utility of mature forest points as desired outcomes of upland reclamation may need to be reconsidered or at the very least, put into the context of one of several to many possible outcomes.

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### Journal Publications

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Online video: Early Successional Wildlife Dynamics Program: <https://pathwaysalliance.ca/biodiversity/>

## RESEARCH TEAM AND COLLABORATORS

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Principal Investigator: Virgil C. Hawkes

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# Canadian Toad (*Anaxyrus hemiophrys*) Monitoring on Canadian Natural's Horizon Oil Sands

**COSIA Project Number:** LJ0325

**Mine**

**Research Provider:** LGL Limited environmental research associates

**Industry Champion:** Canadian Natural

**Status:** Year 4 of 5

## PROJECT SUMMARY

The Canadian Toad (*Anaxyrus hemiophrys*) is known to occur in the Athabasca Oil Sands Region (AOSR), including in ponds and wetlands on Canadian Natural's Horizon Oil Sands. This has been documented by both Canadian Natural and LGL Limited staff, and during work associated with the Early Successional Wildlife Dynamics Program ([COSIA Project LJ0013](#)). Their current status in Alberta is "May be at Risk," though robust data are lacking. Despite the confirmed presence of this species on the Horizon lease, there remains considerable uncertainty regarding (1) annual variation in wetland occupancy; (2) the occurrence and characterization of suitable overwintering habitat; and (3) whether Canadian Toads can be relocated from areas likely to be impacted by mine development into suitable receptor ponds.

To address the above uncertainties, and to reduce impact on Canadian Toads during mine expansion, a Canadian Toad monitoring and mitigation plan was developed in 2017 and implemented starting in 2018. The primary objective of this work was to relocate toads from wetlands within the mine footprint, which required studying the efficacy of mitigation translocations (i.e., moving toads that would otherwise be destroyed or adversely affected by mining-related activities) to lessen the effects of habitat loss on toads. In pursuit of this objective, the following tasks were assigned:

Task 1: Determine wetland occupancy of breeding Canadian Toads and other amphibians on Canadian Natural leases.

Task 2: Test Canadian Toads for chytridiomycosis (*Bd* infection) at collection and receiving sites prior to relocation.

Task 3: Translocate toads and/or egg masses with subsequent monitoring to determine success of relocations.

Task 4: Identify, characterize, and monitor suitable overwintering habitat on Canadian Natural leases.

Task 5: Validate (and, if needed, refine) the updated Canadian Toad habitat suitability index model ([see COSIA Study LJ0326](#)).

Tasks 1 to 3 have been an ongoing effort since the project's inception in 2018. Task 1 requires conducting nocturnal calling surveys, performed in person or via autonomous recording units (ARUs), and daytime visual encounter surveys (Task 1 also involved the development of a [Canadian Toad eDNA assay in 2019 under COSIA project LJ0327](#)).



To determine wetland occupancy, acoustic data are collected from ARUs and analyzed using a pattern-recognition software to detect species-specific vocalizations. Hits identified by the software are validated by a trained biologist who then targets those sites with recent calling activity for follow-up visual encounter surveys. This improves survey efficiency by focusing on sites with confirmed calling activity — an indication of breeding site occupancy. During the follow-up field surveys, Canadian Toads are captured, morphometric data recorded, and photographs taken. Dermal swabs are also collected at this time, pursuant to Task 2: testing for *Batrachochytrium dendrobatidis* (*Bd*), the causative fungal pathogen of chytridiomycosis. Because *Bd* is readily transmissible and can persist in the environment between hosts, testing toads prior to translocation is essential to avoid introducing *Bd* into uninfected ponds or watersheds (Carey et al., 2006). Mitigation translocations (Task 3) can then be carried out, while ensuring toads are moved only between wetlands with *Bd* infection statuses that are alike (i.e., collection and receiving sites are either both positive or both negative). Translocations alleviate the immediate threat of habitat loss due to encroaching mine activities, but further study is needed to determine their long-term efficacy (Randall et al., 2018). Receiving sites will remain a focus of the monitoring program to evaluate translocation outcomes. Success is measured in the near-term as initial translocation survivorship, then over subsequent years as annual recruitment, with the ultimate goal of establishing or augmenting self-sustaining populations.

As a frost-intolerant amphibian species near the northern end of their range, Canadian Toads in the AOSR have adapted life-history traits to help them endure the harsh winters. They spend much of the year underground in upland areas, beneath the frostline but above the water table, to insulate themselves from freezing temperatures (Hamilton et al., 1998; Russell et al., 2000). Thus, their overwintering habitats must possess specific thermal and hydrological properties while being accessible to a low-vagility species with a limited capacity for digging. Recent declines in Canadian Toad populations are believed to be caused in part by a lack of habitats on the landscape with this particular confluence of biophysical properties (Eaton et al., 2005; Browne 2009). For this reason, identifying and characterizing overwintering habitat has become a priority for Canadian Toad ecologists. LGL has made efforts to integrate new knowledge of Canadian Toad habitat requirements into the current project by: (1) analyzing soil sensor data from a putative Canadian Toad hibernaculum on Horizon South, (2) revising a Canadian Toad habitat suitability index (HSI) model to consider both breeding and wintering habitat along with the distance between them, (3) field-validating the new model, and (4) preparing initial plans for a Canadian Toad habitat creation project.

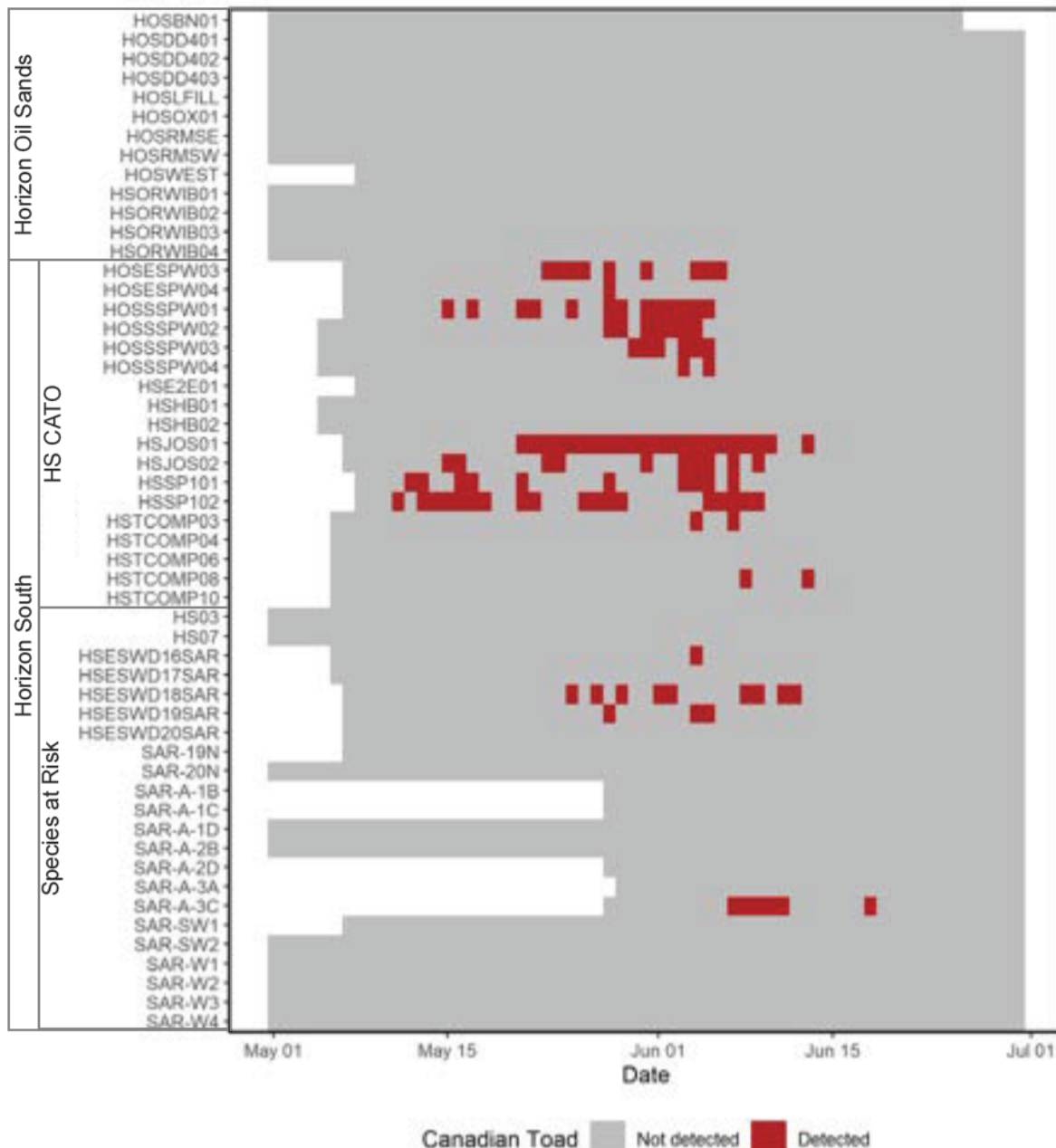
This work will assist in determining the efficacy of translocations as a strategy to mitigate the effects of habitat loss on Canadian Toads. If deemed effective, it will provide a template for similar projects to be carried out elsewhere in the AOSR. It will also contribute to an increased understanding of behaviour, population trends, and habitat use of Canadian Toads that will be applicable to other developments in the AOSR.

## PROGRESS AND ACHIEVEMENTS

In 2022, LGL made significant progress on all five tasks listed above. For Task 1, wetlands on the Horizon and Horizon South leases were monitored by 31 ARUs, which recorded acoustic activity on a nightly basis for the duration of the active season (roughly May to August; [Figure 1](#)). The ARU data were used to guide a survey effort totalling 130 surveyor-hours. Surveys were conducted nocturnally and during the day depending on the season: breeding and calling activity generally occurs at night in late May and early June while juveniles and recent metamorphs are more likely to be encountered during daylight hours in July and August (Breckenridge and Tester 1961; Eaton et al., 2005). Recordings from an array of 22 additional ARUs targeting other species at risk (SAR) in remote areas of Horizon



South were scanned for toad vocalizations. Four of these were associated with positive identifications of Canadian Toad, for a combined total of 19 ARU-nights with detections. These sites were not available to biologists during the active season for surveying but were analyzed in the fall to broaden the scope of the monitoring program.



**Figure 1: Time series of detections by ARUs stationed on Canadian Natural's Horizon Oil Sands and Horizon South leases.** Positive detections were made at the Athabasca River sites (HOSESPW03, 04), Sandpit South (HOSSSPW01, 02, 03,04), Joslyn Road (HSJOS01, 02), SP1 (HSSP101, 02), Toad Complex (HSTCOMP03, 08), and at several ARUs in the Species at Risk array. The SAR units occupy remote locations on Horizon South and were not surveyed as part of the mitigation translocation project. Non-detections outside of May 1 to July 1 have been truncated to maintain scale.



Task 2, testing toads for chytridiomycosis (at the University of Guelph's Animal Health Laboratory), was also partly addressed in 2022. Dermal swab samples were collected from 12 adult Canadian Toads and six frogs (four Wood Frogs *Lithobates sylvaticus* and two Boreal Chorus Frogs *Pseudacris maculata*) in May and June. Swabs were then tested for the presence of *Bd* in preparation for translocations, Task 3. A total of 119 individuals were then translocated out of the mine footprint to a nearby wetland (Figure 2). All toads were collected from two opportunistic wetlands (i.e., not naturally occurring) near mine hazards including the main pit and active haul roads. All toads survived the initial move, appearing active and in good condition upon release. The receiving site will be monitored in 2023 to assess overwinter survivorship of the translocation cohort.



**Figure 2: Juvenile Canadian Toads patiently awaiting their turn to be processed.** All individuals were weighed, measured, and photographed before being relocated away from the mine footprint to a wetland off lease.

Recent studies have emphasized the availability of suitable and proximal overwintering habitat as a major factor limiting populations of Canadian Toads. Tasks 4 and 5 address the need to integrate new knowledge of seasonal habitat requirements into applied ecological studies such as this. Specifically, this research contributed to Task 4 by describing a series of sandy berms believed to house Canadian Toad hibernacula in the winter of 2020 and 2021. This involved recording GPS tracks along the perimeter, mapping the topography (e.g., height and slope of berms and depressions), and assessing biophysical/habitat features (e.g., vegetation community, soil attributes).



The data will provide a standard of comparison useful for identifying suitable habitat elsewhere on lease and in other parts of the AOSR. It has also contributed to early designs for a new habitat creation project wherein one nearby wetland complex would be engineered to provide all the seasonal habitat requirements for Canadian Toads. The project would provide compensation habitat for toads and other wildlife species in the area. Moreover, the ability to manipulate habitat features offers compelling opportunities for in situ experiments to further our understanding of Canadian Toad habitat use.

The Canadian Toad HSI model was updated in 2019 (COSIA project LJ0326; Hawkes et al. 2020a, b) to reflect the importance of suitable and accessible overwintering habitat. Due to delays encountered during the height of the pandemic, LGL is still in the process of ground-truthing the model. Further information on this project is provided in the summary report for [LJ0326](#). In 2022, LGL biologists addressed Task 5 by validating model outputs in the field. They visited over half of the selected sites and evaluated substrates in terms of their ability to harbour overwintering toads. This involved inspecting soil texture, consistency, accessibility, drainage, and other attributes. The remaining validation sites on Horizon leases and those at Albion Sands are scheduled for inspection in 2023.

LGL was able to contribute to all five tasks in 2022; however, more work remains. In particular, the efficacy of translocations as a viable mitigation strategy for habitat loss on active oil sands mines remains in question.

## LESSONS LEARNED

Several lessons related to Canadian Toad monitoring and relocation on the Horizon Oil Sands leases can be shared at this point:

1. Results from this project will yield valuable insights applicable to studies of Canadian Toad habitat use throughout the AOSR:
  - a. The updated HSI model will be a useful resource for mapping potentially valuable habitats on other industrial developments, e.g., to locate extant populations and candidate relocation sites.
  - b. Detailed habitat descriptions from LGL's report, including those produced during HSI model field validation and from the translocation collection site, will contribute to the growing body of literature characterizing Canadian Toad seasonal habitats.
  - c. Depending on overwinter survivorship at the receiving site, the translocation process described here may provide a protocol to follow for future mitigation translocation efforts.
  - d. Prescriptions for the habitat creation project, though still in preliminary stages, may provide a model for other industry stakeholders seeking to build effective compensation habitat.
2. Telemetry monitoring can be a useful tool to determine post-translocation movements of individuals fitted with transmitters. In this context, movement data can provide valuable insights into (a) the suitability of habitat at translocation receiving sites (e.g., by determining the fate of relocated toads) and (b) the location of overwintering sites (e.g., by tracking toads to their hibernacula). Unfortunately, since most toads relocated in 2022 were juveniles, none in the translocation cohort were large enough to support attachment of VHF radio transmitters. In fact, no toads encountered this year had enough body mass to support VHF transmitters, although several from previous years of the project met the size requirement. Efforts to track Canadian Toad movements will continue in the future.



3. The two most active breeding populations of Canadian Toads in 2022 were located directly adjacent to sites with persistent, loud mine activity (the main pit and a haul road near active cells, respectively). Industrial disturbance is apparently not an effective deterrent to Canadian Toads.
4. Annual variation in wetland occupancy has been evident throughout this project. Each year, some sites with previously robust breeding populations go quiet, while others are newly colonized. Breeding site fidelity seems to be low in Canadian Toads, perhaps partly due to the constant environmental perturbations inherent to the natural resource industry. More research is needed to determine fidelity to overwintering sites.
5. Determining the outcome of the 2022 translocation will present its own research challenges: acoustic monitoring targets breeding male toads only. Considering males take one to three years to reach sexual maturity, some in the small translocation cohort may have to survive multiple winters in their new habitat before they begin vocalizing and thus become detectable (Breckenridge and Tester 1961; Eaton et al., 2005).

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## PRESENTATIONS AND PUBLICATIONS

No presentations or publications in 2022.

## RESEARCH TEAM AND COLLABORATORS

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# Canadian Toad (*Anaxyrus hemiophrys*) Habitat Suitability Index Model Update and Validation

**COSIA Project Number:** LJ0326

**Mine**

**Research Provider:** LGL Limited environmental research associates

**Industry Champion:** Canadian Natural

**Industry Collaborators:** Suncor

**Status:** Ongoing

## PROJECT SUMMARY

In 2019, the Canadian Toad (*Anaxyrus hemiophrys*) habitat suitability index (HSI) model was updated to address regulatory requirements associated with Canadian Natural's *Environmental Protection and Enhancement Act* (EPEA) approvals (Horizon Lease: 149968-01-00; Albion Sands Lease: 20809-02-00 [Muskeg River Mine] and 00153125-01-00 [Jackpine Mine]) and to inform recent initiatives regarding Canadian Toad relocations on Canadian Natural's Horizon Oil Sands (Hawkes and Papini 2020a, b). HSI models use a rubric to score environmental and biophysical traits on their suitability as habitat for a taxon of interest. Mapping the model output produces a prediction of the occurrence of suitable habitat ([Figure 1](#)).

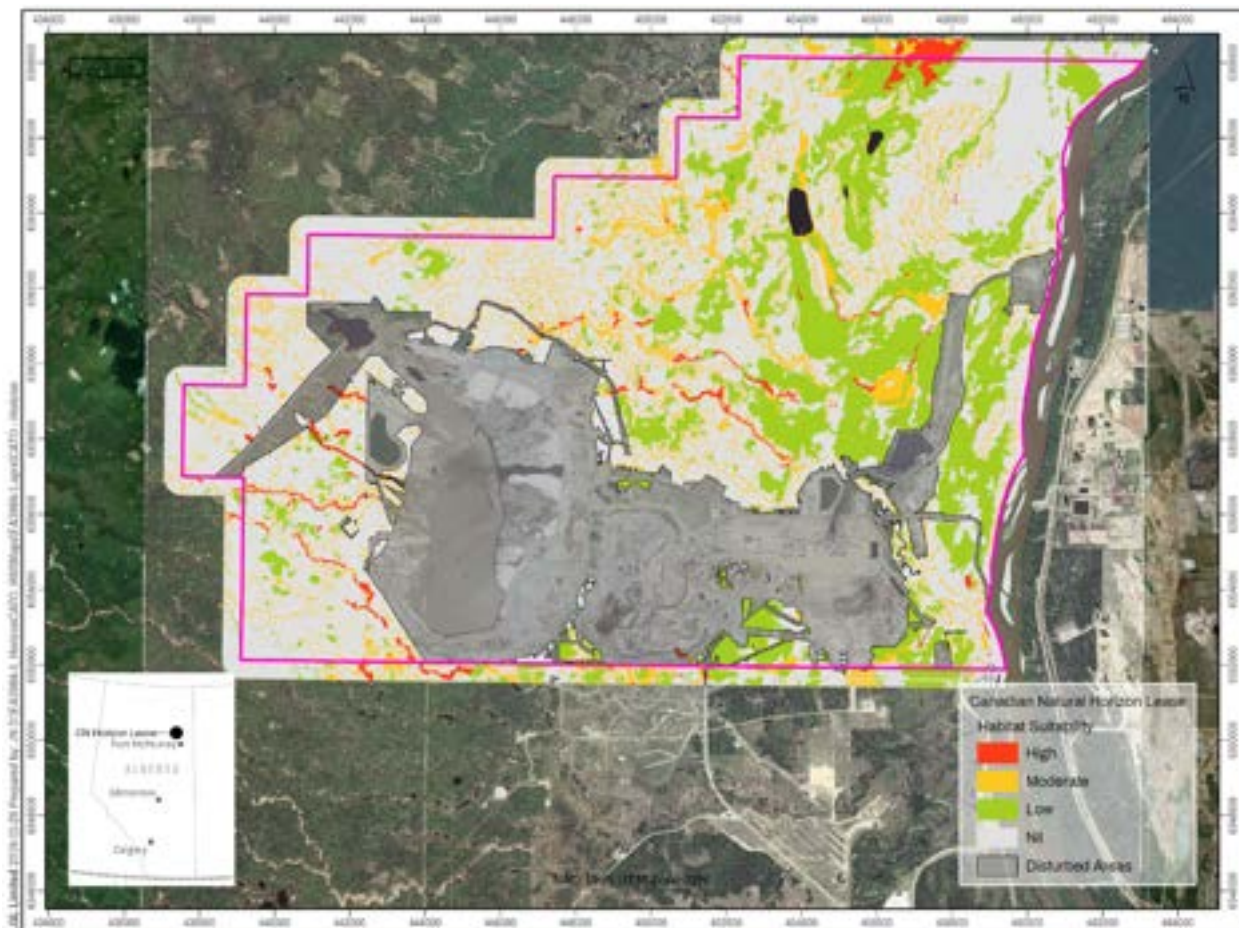
The updated HSI model considered habitat attributes used in previous models (i.e., 1999, 2005 and 2007) but placed increased weight on overwintering habitat, while reducing the relative importance of breeding habitat. The update emphasizes the relationship between suitable overwintering habitat (friable soils up to 1.25 m in depth in the a and b ecosites), breeding habitat (certain shallow water ponds and wetland habitats), and — importantly — the distance between them. To sustain populations year-round, wintering and breeding habitats must co-occur within the range of Canadian Toad seasonal movements. As a frost-intolerant amphibian species near the northern end of their range, Canadian Toads in the Athabasca Oil Sands Region (AOSR) have adapted life-history traits to help them endure the harsh winters. They spend much of the year underground in upland areas, beneath the frostline but above the water table, to insulate themselves from freezing temperatures (Hamilton et al., 1998; Russell et al., 2000). Thus, their overwintering habitats must possess specific thermal and hydrological properties while being accessible to a low-vagility species with a limited capacity for digging.

A base layer comprised of the derived ecosite phase (DEP) dataset, which is based on Alberta Vegetation Inventory (AVI) and LiDAR-derived datasets, was used to display the results of the model. Maps were produced for Canadian Natural's Horizon Oil Sands, Horizon South, and Albion Sands leases. A comparison between updated HSI mapping and the original model produced for the mine expansion environmental impact assessments (EIAs) from 1999, 2005, and 2007 suggests that the current distribution of highly and moderately suitable habitat on Canadian Natural leases is significantly less than previously thought. The updated suitability maps are based on more recent information regarding Canadian Toad ecology and are a better representation of the occurrence and distribution of



Canadian Toad habitat on each lease. They also align with recent observations of Canadian Toads on the Horizon and Albian leases. For model specifics see Hawkes and Papini (2020a, b) or [COSIA project LJ0326](#) in the 2019 edition of this report.

Habitat models require several internal and external validation measures to confirm model assumptions are reasonably met (Muir et al., 2011). Assumptions are inherent in the design of every model; validation procedures are used to ensure the assumptions being made accurately reflect reality. Internal validation involves reviewing model outputs, sensitivity testing, and calibrating variable weightings and model equations (see Van Horne and Wiens 1991). External validation is concerned with comparing model predictions to data collected in the field, i.e., ground-truthing. Ideally, model developers will have access to a test variable that is indicative of habitat quality. For example, species abundance/density or a measure of population success such as body condition or annual recruitment. In the absence of a convenient test variable, or for species that are difficult to survey, an alternate method may be used whereby habitat quality is independently assessed by expert opinion (Brooks 1997). Due to delays caused by the COVID-19 pandemic, field validation of the updated model was postponed in 2020 and 2021.



**Figure 1: Distribution of high, moderate, low, and nil suitability Canadian Toad habitat on Canadian Natural's Horizon Oil Sands.** A 500 m buffer was added to the lease boundary to indicate that suitable toad habitat occurs in habitats adjacent to the lease.



External model validation began in earnest in 2022. Polygons were sampled using a generalized random-tessellation stratified (GRTS) design, which is a spatially-balanced sampling method that can accommodate variable inclusion probabilities (Stevens and Olsen 2004). At each polygon LGL biologists conducted qualitative assessments of the habitat features relevant to overwintering Canadian Toads, focusing on soil characteristics. This involved digging pits to appraise soils in terms of their ability to support effective hibernacula ([Figure 2](#)). Researchers focused on the texture, consistency, and accessibility of mineral soils — all key components of overwintering habitat. To support toad hibernacula over the winter, soils should be loose, friable, well-drained, and accessible to a depth of 1 m to 1.5 m (Hamilton et al., 1998).

Recent declines in Canadian Toad populations are believed to be caused in part by a lack of habitats on the landscape with the hydrological and thermal properties required by overwintering toads (Eaton et al., 2005; Browne 2009). For this reason, locating and characterizing overwintering habitat has become a priority for Canadian Toad ecologists. The updated habitat model described here will be an important resource for identifying and conserving suitable Canadian Toad habitat throughout the AOSR, including at Suncor’s Base Plant and Fort Hills oil sands leases. Suncor has recently committed to joining the Canadian and Western Toad Monitoring Joint Industry Partnership ([project LJ0325 of this report](#)) in 2023. The updated HSI model will therefore be adapted to Suncor leases in the near future, followed by the field validation procedures described above.

## PROGRESS AND ACHIEVEMENTS

Although final results are not yet available, initial validation findings have generally corroborated model predictions. Sites rated as highly suitable tended to have dry, loose substrates with shallow organic horizons, if present at all. Mineral soils were, for the most part, readily accessible. Polygons rated as moderately suitable had variable habitat profiles. Some had horizons that appeared hospitable to overwintering toads but lacked access; they were restricted by the presence of roots or coarse fragments or buried beneath impenetrable mineral or organic horizons. Accessibility, though, is unlikely to be uniform throughout a polygon. It may be improved in certain areas by erosion or secondary cavities made by other burrowing animals. Other sites with moderate suitability were accessible but siltier or had higher clay content, resulting in substrates too cohesive for toads to penetrate. Low-suitability sites were often characterized by thick organic layers, sometimes with standing water. Mineral horizons were sometimes inaccessible even to surveyors, for example, in peat wetlands. Most low-suitability sites showed little or no potential as overwintering habitat.

Work completed in 2022 contributed to the external validation of the updated Canadian Toad HSI model on Canadian Natural’s Horizon and Horizon South leases. LGL biologists conducted habitat assessments at 50 of the 70 field sites selected for validation ([Figure 2](#)). The remaining sites, including those at Albion Sands, are scheduled for validation in 2023. Additionally, Suncor has recently committed to joining the Canadian Toad Habitat Suitability Model Update and Validation work, increasing the spatial scope from, Canadian Natural’s Horizon, Horizon South, and Albion Leases to include their Fort Hill operations and Suncor Base Plant. In 2023, the habitat model will be adapted and applied to their Base Plant and Fort Hills projects. Field sites will then be selected for validation following the GRTS approach described above. Habitat assessments are tentatively scheduled for the summer of 2023.



**Figure 2: Soil pit excavated at Canadian Natural's Horizon Oil Sands as part of the external validation process for the updated Canadian Toad habitat model.** Mineral soils were assessed for their suitability as overwintering habitat by qualified biologists.

## LESSONS LEARNED

Some early findings related to the updated Canadian Toad habitat suitability maps for Horizon and Albion leases and their validation will be of interest to other stakeholders in the AOSR, even before results from this project are finalized:

1. The updated model revealed that significantly less Canadian Toad habitat exists on the landscape than previously believed. Models produced during the EIA process were based on data available at the time; however, more recent data describing habitat associations or species occurrences should be used to update





habitat models. This will result in a more accurate depiction of the current distribution of suitable habitat. Outdated information may obscure the actual state of local Canadian Toad habitat, which could prevent effective conservation action and affect reclamation planning. Updating models with current data to reflect new information regarding Canadian Toad habitat use in Northern Alberta is recommended.

2. The updated model described here is applicable to other developments in the AOSR. Extending the model into new areas is relatively straightforward provided that the same environmental datasets are available, which is indeed the case. The DEP dataset is based on Alberta Vegetation Inventory and LiDAR-derived data and is available for much of the AOSR. This makes for a seamless integration of new areas into the study.
3. Field validation serves to ground-truth several assumptions inherent to the model: (1) that environmental inputs (i.e., DEP data) accurately represent current conditions, and (2) that model predictions are tightly correlated with actual habitat suitability. In short, that the information in both the inputs and outputs of the model is reliable.

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## PRESENTATIONS AND PUBLICATIONS

No presentations or publications in 2022.

## RESEARCH TEAM AND COLLABORATORS

Institution: LGL Limited environmental research associates

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Name	Institution or Company	Degree or Job Title	Degree Start Date (Students Only)	Degree Completion Date (Students Only)
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Joanne Hogg	Canadian Natural	Lead, Research		

# Alberta Biodiversity Conservation (ABC) Research Program – Phase 2

**COSIA Project Number:** LE0056

**In Situ**

**Research Provider:** University of Alberta

**Industry Champion:** Canadian Natural

**Industry Collaborators:** Cenovus, ConocoPhillips, Imperial, Suncor, Syncrude

**Status:** Year 1 of 5

## PROJECT SUMMARY

Phase two of the Alberta Biodiversity Conservation (ABC) Research Program consists of a five-year plan to address a set of complementary themes related to biodiversity conservation and management in the oil sands region of Alberta. Some of these thematic areas are a single research question unto themselves, while others are comprised of multiple research questions within a taxa. This works builds on the first phase of the ABC program by addressing research gaps and aligning with the COSIA Biodiversity Working Group priorities.

In the first phase of the program, Nielsen held a NSERC-CRD grant that supported work on responses of vascular plants and butterflies to seismic lines primarily as it was affected by edge effects, line width, and synergistic effects with wildfires.

This Phase 2 research program focuses on: (1) songbirds and bats via acoustic assessments; (2) wildlife (birds and caribou); and (3) plant phenology and fitness responses to in situ oil sands; as well as (4) an overall risk assessment for terrestrial biodiversity to landscape and climate change. A number of program elements will examine the synergistic effects of footprints and climate. This is important as it helps guide what is feasible for long-term conservation and restoration in the oil sands region in the presence of climate change and thereby assist with planning, mitigation, and restoration activities. The work will be done through a collaborative research team and graduate student projects at the University of Alberta led by Professor Nielsen.

Impacts to habitats and biodiversity from in situ mining can be separated into: (1) the active mining footprint consisting of active well pads and associated infrastructure (roads, pipelines, compressor stations, sometimes upgraders, etc.), or (2) the exploratory footprints of seismic lines and well pads that disturb habitats but are not further developed. Total habitat loss within the region is only 6% (Riva and Nielsen, 2021), but is much higher in the surface mineable region or at the in-situ lease scale. Seismic lines have average densities of 1.5 km/km<sup>2</sup> across the region (Lee and Boutin 2006) but can reach 40 km/km<sup>2</sup> in active leases (Stern et al., 2018). Many exploratory seismic lines, particularly older exploratory lines, are failing to regenerate to forests decades after their disturbance (van Rensen et al., 2015; Filicetti et al., 2019). This not only fragments forest habitats (Riva and Nielsen, 2021) but also alters movements of organisms (Tigner et al., 2014; Roberts et al., 2018; Riva et al., 2018), affecting population processes and the distribution of species (Fisher and Burton 2018). Given the existing disturbances there is potential





to affect interior habitats and movements of species, seismic lines, together with the exploratory well pads, may have a much greater effect on biodiversity in the region than what their total footprint suggests. Therefore, they are a priority for research, monitoring, and restoration to identify where impacts occur and what may be done for mitigation.

To date, a number these factors have been studied to better understand the impacts of seismic lines. Examples of factors studied include responses to the abiotic environment (Stern et al., 2018; Stevenson et al., 2019), vegetation (Filicetti and Nielsen 2020; Echiverri et al., 2020), invertebrates (Riva et al. 2018; Nelson et al. 2021), songbirds (Bayne et al., 2005a, 2005b; Mahon et al., 2019), and mammals (Tigner et al., 2014, DeMars et al., 2020). Some work has been done to understand localized edge effects to vegetation (Dabros et al., 2017; Echiverri et al., 2022), but this is often focused to one small region and/or a single forest type, particularly treed peatlands. More work is needed to understand how fragmentation is affecting other forest types and in particular the adjacent forests themselves (edge effects), as well as how this varies with size and age of oil sands footprints and changes in their recovery. This proposal will address, among other things, this gap providing insight into where impacts really occur and what conditions minimize their effects thus guiding future mitigation and restoration actions.

Beyond understanding the current state of biodiversity on these disturbances (habitat loss/alterations) or their changes in adjacent forests (edge effects), a second key threat to biodiversity within the region is climate change. As with many other regions, Alberta's boreal forest is expected to be warmer and drier over the 21st century (Stralberg et al., 2018). This will lead to more frequent, larger, and intense wildfires (Tymstra et al., 2007; Bergeron et al., 2010), as well as increase the physiological stress to boreal vegetation (Michaelian et al., 2011) and alterations to stand-level processes (Boulanger et al., 2018; Whitman et al., 2019). Climate change can also act in a synergistic way with disturbances whereby the combination of fire, climate stress, and footprints rapidly alter trajectories of biotic change more than expected from any individual stress or predicted additive effect. Indeed, parts of this have already been shown for plants and butterflies on seismic lines following fire (Riva et al., 2020), while different types of disturbances have been shown to be either additive or multiplicative for songbirds (Mahon et al., 2019) and multiplicative with climate change (Cadieux et al., 2020). More broadly, over longer time horizons the boreal biome associated with the oil sands region is predicted to retreat north with more Parkland-like vegetation replacing it (Stralberg et al., 2018). This will have massive effects on the biota that depend on boreal forests such as woodland caribou and other boreal-dependent species. Although some work has been done to examine these threats, no systematic framework for biodiversity and climate change risk of the oil sands has been developed as it relates to the combination of climate risk and alterations to boreal habitat from oil sands disturbances (habitat loss and fragmentation). A second key objective of this grant is to develop a risk assessment for a large suite of boreal species to these synergistic disturbances.

Finally, the oil sands industry through COSIA (Canada's Oil Sands Innovation Alliance) has identified a number of threatened wildlife species as research priorities to better understand their impacts and mitigation options. This includes woodland caribou, bats, and birds (yellow rail, sharp-tailed grouse, in general boreal birds, and woodpeckers). The impacts of oil sands disturbances have been well-studied for woodland caribou (e.g., Dickie et al., 2017; Nagy-Reis et al., 2021; Serrouya et al., 2020), but some key knowledge and management gaps remain, particularly around the interplay of bottom-up changes (including from climate change) in forage (lichens) for caribou (e.g., Silva et al., 2019; Hillman and Nielsen, 2020) and how scales of disturbances affect trends in caribou. Much has been done for ground and tree-nesting songbirds (e.g., Bayne et al., 2005a; Machtans, 2006), but a number of birds have new monitoring clauses including woodpeckers, bats, and yellow rails. This work would fill those gaps with a series of studies to understand impacts and mitigation strategies.



The scope of work provided here provides an opportunity to address a number of key biodiversity conservation challenges facing Alberta's oil sands using a collaborative set of research projects from industry, government, and academia, while engaging with Indigenous communities and training the next generation of scientists.

## PROGRESS AND ACHIEVEMENTS

In 2022 a post-doctoral fellow was hired and began a literature review of climate change on biological systems.

## LESSONS LEARNED

The work described above is in the initiation phase and lessons learned are not available at this time.

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## PRESENTATIONS AND PUBLICATIONS

No public presentations or publications for 2022.

## RESEARCH TEAM AND COLLABORATORS

Institution: University of Alberta

Principal Investigators: Dr. Scott Nielsen

Name	Institution or Company	Degree or Job Title	Degree Start Date (Students Only)	Degree Completion Date (Students Only)
Erin Bayne	U of A	Professor		
Andrew Crosby	U of A	Post-Doctoral Fellow		

Research Collaborator: Alberta Innovates



## **ENVIRONMENTAL RESEARCH AND MONITORING**

## Boreal Ecosystem Recovery and Assessment — Phase 2

**COSIA Project Number:** LJ0220

**In Situ**

**Research Provider:** University of Calgary

**Industry Champion:** ConocoPhillips

**Industry Collaborators:** Cenovus, Canadian Natural, Imperial, Alberta-Pacific Forest Industries Ltd.

**Status:** Year 2 of 5

### PROJECT SUMMARY

The Boreal Ecosystem Recovery and Assessment (BERA) program ([www.bera-project.org](http://www.bera-project.org)) is a multi-sectoral partnership of academic researchers and partners from industry, government, and non-government organizations. Its central goal is to understand the effects of industrial disturbance on natural ecosystem dynamics in the boreal forest, and to develop the knowledge and planning tools required to restore disturbed boreal landscapes.

This work targets knowledge gaps relevant to five strategic management priorities: (i) promoting a return to forest cover in disturbed areas, (ii) restoring natural carbon dynamics in disturbed peatlands, (iii) maintaining wildlife habitat, (iv) enhancing woodland caribou habitat, and v) remote sensing. The research is designed to provide knowledge and planning tools for researchers and resource managers engaged in boreal restoration, and to train the next generation of highly qualified personnel working in this space.

### PROGRESS AND ACHIEVEMENTS

#### Promoting Return to Forest Cover

##### **Let it grow: Recovery trajectories of passive restoration of seismic lines (project ongoing, year 1 of 4)**

Colleen Sutheimer, Department of Renewable Resources, University of Alberta

Best management practices have been widely adopted to reduce the footprint of seismic lines in the boreal forest, but there continues to be variability in seismic line recovery. Recovery status of seismic lines is dependent on spatial and temporal scales such that recovery at one scale does not necessarily translate to recovery at all scales. Appropriate scaling, both temporally and spatially, is essential to identify and assess where leaving disturbed areas for natural forest regeneration (passive restoration) leads to recovery of seismic lines. This project seeks to model recovery trajectories of natural regeneration in seismic lines at multiple spatial and temporal scales to provide insights to decision makers and resource managers on where and when passive restoration can be sufficient to meet recovery targets.

Researchers in BERA Phase I (2015 to 2020) provided many insights regarding natural regeneration on seismic lines. However, one gap in existing knowledge involved transitional areas like swamps. During the summer of 2022,



47 transitional sites were sampled across the eastern Athabasca Oil Sand region. Adding to work led by Angelo Filicetti, project researchers now have an extensive dataset of tree and shrub height, abundance, and growth for 462 sites across the 23,000 km<sup>2</sup> BERA study area. This data will be used to model growth trajectories of passive restoration in seismic lines across multiple spatial and temporal scales.

Researchers in BERA Phase I showed that natural tree regeneration on seismic lines is generally low in peatlands and is increased by fire (except in treed rich and poor fens where there were no detected increases in regeneration densities). Additionally, in most sites other than peatlands, passive restoration resulted in recovery that met provincial height and density targets. This information is useful for assessments of recovery and identifying what influences regeneration at the scale of a single seismic line but has limitations in projecting recovery across time and space. Decision-makers and resource managers need both assessments and projections to identify where and when passive restoration will be sufficient for recovery of seismic lines. This project will estimate recovery trajectories to provide an assessment of where passively restored seismic lines are recovering and provide estimates of how long that recovery is taking.

#### **Forest harvesting promotes seismic line regeneration in mesic upland forest (project ongoing, year 1 of 4)**

Leonardo Viliani, Department of Renewable Resources, University of Alberta

Seismic lines represent one of the largest contributors to forest fragmentation in western Canada's boreal forest. Active restoration treatments are therefore being used to restore habitat. Given the high cost of restoration and extensive network of seismic lines, identifying favourable conditions for 'leave for natural' reforestation is a priority. In some cases, other resource extraction, including forest harvesting, overlaps with seismic line disturbances. A key question is whether forest harvesting 'erases' seismic lines during regeneration of early successional trees within the overall harvest block. Forest harvesting results in several factors that positively affect tree regeneration (e.g., higher sunlight, less plant competition, scarification of the soil). Thus, with the re-establishment of viable seedbed and microsite heterogeneity we might expect a successful regeneration of trees in seismic lines. However, soil and peat compaction during seismic line establishment, possibly reinforced if lines were used as in-block harvest trails, may continue to restrict recovery despite silvicultural treatments in the overall harvest block. This project examines whether cut-blocks in mesic upland forests enhance the regeneration of seismic lines and thus 'erase' seismic lines from the need for future restoration actions.

Tree regeneration in 15 upland mesic forest harvest blocks ranging from one to ten years since harvest, between Fort McMurray and Lac la Biche, Alberta were assessed. Each site was represented by two 20 m<sup>2</sup> plots inside the cut-blocks (seismic line and adjacent recently harvested forest) and two 20 m<sup>2</sup> plots in the mature forest (seismic line and adjacent forest). In each plot, all acceptable, non-germinant and alive tree and shrubs stems by species and height category were counted. These variables were then compared between treatments (harvest on/off line; mature forest on/off line).

Ninety-five percent of seismic lines within cut-blocks reached the density target from The Provincial Restoration and Establishment Framework for Legacy Seismic Lines in Alberta, while 65% of seismic lines in the mature uncut forest reached the same benchmark standards. Moreover, regeneration density inside the line within harvests was 65% higher than in the uncut forest. Although the total reforestation process was mostly related to the aspen suckers' growth, these results suggest that harvesting does provide the conditions for the structural regeneration of seismic lines, even when natural regeneration does not occur in adjacent unharvested sites. This suggests that harvesting practices are facilitative and thus effectively 'erase' seismic lines, while factors, such as soil compaction,





do not appear to be limiting. Although forest harvesting is not an immediate solution for sensitive species like caribou (early seral habitat negatively impacts caribou), these results suggest that areas that have forest harvesting will contribute in the long-term towards landscape-level structural restoration of linear features without more directed active restoration needs.

#### **Planted seedlings and saplings have higher growth rates on actively restored seismic lines (project complete)**

Colette Shellian, Department of Geography, University of Calgary

Natural conifer tree regeneration may be slow or stagnant on seismic lines due to a range of growth-limiting factors. In lowlands, active restoration treatments (mounding and tree planting) may be prescribed to improve soil, hydrologic, and microsite conditions. However, these treatments are expensive and expose areas to additional disturbances. Knowledge is needed to assess how young conifer trees respond to treatments to better target limited restoration dollars. This study evaluates seedling growth rates among planted and naturally regenerating seedlings, and assesses the influence of ecosite and light on growth responses.

Field surveys were performed during summer 2021 and 2022 in the Canadian Natural Kirby and Cenovus LiDea 1 restoration project areas. For each of the 1182 seedlings sampled, height, leader growth, and growth from the previous five seasons was measured. On-line light conditions were predicted using a LiDAR-based (light detection and ranging) digital surface model, and results were modelled using generalized linear mixed-effects models.

For growth measured over five seasons (2016 to 2021), both black spruce and tamarack growth was influenced by the seedling height, ecosite type, light, and restoration treatments. The effect of restoration treatments depended on ecosite, with planted seedlings consistently having significantly higher growth than natural seedlings. In poor fens, planted black spruce grew 40% more (26.6 cm versus 16.0 cm) relative to untreated sites, and tamarack grew 41% more (26.3 cm versus 15.6 cm) compared to untreated sites. In moderate-rich fens, planted black spruce grew 32% more (33.8 cm versus 22.9 cm), and planted tamarack grew 35% more (34.1 cm versus 22.2 cm) than natural seedlings on untreated lines. Increasing seedling height and greater light availability had a positive effect on growth for both species under all conditions measured.

The results of this study indicate mounding and tree-planting in fens was effective for accelerating initial growth of black spruce and tamarack seedlings and saplings.

#### **Mounding effects on tree seedling survivability and growth along seismic lines (project ongoing — year 1 of 2)**

Jennifer Fliesser, University of Waterloo Department of Geography and Environmental Management

Previous studies of seismic lines have shown that boreal treed peatlands have little tree recovery, even decades after the initial disturbance. Seismic lines fragment the landscape with notably different environmental properties. The reasons behind the slow recovery have been attributed, at least in part, to the altered microtopography and hydrologic characteristics of seismic lines. However, the specific mechanisms of this have not been fully documented. This research will explore the complexities of tree establishment on treed peatland seismic lines by examining how mounding treatments (that alter microtopography) affect the rate of tree seedling growth. By identifying the effects of mounding treatments on tree establishment and growth, researchers can suggest which methods work best in which sites thus informing future restoration projects.



Five mounding treatments were examined across two study locations. At Site 1, near Brazeau Dam in Brazeau County, Alberta, the researcher examined black spruce and tamarack seedling planted within the (i) Rip & Lift (mounds created by dragging a plow along the line), (ii) Inline Mounding (mounds dug up from the line and placed veg-side up), (iii) Hummock Transfer (natural hummocks transplanted from undisturbed areas to the line), and (iv) Unmounded (no mounding treatment). At Site 2, off HWY 881 in Lac La Biche County, Alberta, black spruce seedlings planted on (v) Inverse Mounding (mounds dug up from the line and placed vegetation-side down) were examined.

Variables measured for each tree seedling included: seedling health, seedling height and first and second leader lengths, mound relative elevation, soil moisture, and vegetation percent cover of nine major land covers. Six to nine seedling samples were also collected for an ongoing analysis of root characteristics and ectomycorrhizal associations.

This study will inform the scientific community and industry partners about the effectiveness of different mounding treatments for seismic line restoration. As a part of the BERA research project researchers are looking for solutions to restore barriers along seismic lines and make them less navigable for predators. An important contributor to these barriers would be advanced conifer regeneration. This study will help determine the effects of mounding on tree establishment and growth. In turn, this will inform future seismic line restoration on the ideal mounding characteristics for optimal seedling survival and rapid growth.

**Linear disturbances may promote browse subsidy in peatlands and their ecotone with uplands (project ongoing, year 1 of 2)**

Spencer Quayle, Department of Renewable Resources, University of Alberta

Industrial activities and changing climate have contributed to the decline of the boreal woodland caribou population. The most widely accepted explanation for these declines is that early seral forests promoted by disturbance increases forage supply (particularly winter browse) for moose and white-tailed deer. This increase in ungulate populations leads to increase in predator numbers. This project is examining the connection between linear features and forage availability, specifically the concept of browse subsidy, to quantify how much these lines may be subsidizing the competitors (e.g., deer and moose).

In the summer of 2022, twenty legacy (2D) seismic lines sites were sampled between Fort McMurray and Lac La Biche, Alberta. Each site comprised four treatments based on the combinations of two main habitat types (upland forest versus peatland) and two positions within each habitat (edge or interface of peatland/upland versus interior habitat). For each of the four treatments (peatland centre, peatland edge, upland centre upland edge) two plots were installed — one on the seismic line and another in the adjacent forest. At each plot, the researcher measured the density of available browse and browse levels (extent and severity) for each shrub and tree species. Preliminary analyses show evidence for a relative increase in available browse on seismic lines in transitional habitats and peatlands, but not in uplands.

If seismic lines do provide browse subsidy for moose and deer in one or more habitat types, this research may help to further understand a mechanism contributing to caribou population declines. Statistical and spatial models will be developed predicting browse subsidy throughout the oilsands region. These products will provide information on the possible effects of disturbance on ungulates and provide strategic insights for restoration managers.



## Restoring Natural Carbon Dynamics

### Effect of seismic lines on peat accumulation rates in boreal peatlands (project complete)

Percy Korsah, Department of Geography and Environmental Management, University of Waterloo

Peatland management has been highlighted as an important nature-based climate solution. Carbon sequestration in peatlands occurs when primary productivity exceeds decomposition. Earlier studies have highlighted the effect of seismic line disturbances on vegetation and microbial community changes as well as microclimatic conditions. However, the cumulative effect of these changes on potential peat accumulation rates in boreal peatlands has not yet been investigated. This study assessed the effect of seismic line disturbances on net primary production (NPP) and decomposition rates in boreal peatlands and is the first study of its kind.

This study was completed across three peatlands associated with oil sands exploration/extraction near the town of Peace River. Four peatland subsites were selected based on the peatland type (bog, fen) and type of seismic line disturbance (legacy, low-effect). Vegetation surveys, production (biomass) and decomposition (litter bags) data were collected between 2018 and 2021 from disturbed and adjacent undisturbed areas. Measurements related to water table levels, physicochemical parameters, and meteorological conditions were also recorded.

Seismic line disturbances in boreal peatlands encourage saturated soil conditions, decrease tree recovery, and alter vegetation communities and microclimatic conditions. These changes alter peat accumulation rates and long-term carbon storage in boreal peatlands. Vegetation inputs after two years was significantly reduced at all disturbed sites in this study. Although understory NPP rates increased on seismic lines, it could not compensate for the overall NPP lost due to the absence of overstory woody vegetation, hence justifying the need for tree restoration in maintaining the carbon sink function of peatlands. Results from this research can serve as a foundation for new perspectives on designing and setting restoration targets in boreal peatlands.

### Seismic lines increase methane emissions from boreal peatlands (project complete)

Percy Korsah, Department of Geography and Environmental Management, University of Waterloo

Boreal peatlands serve as long-term carbon sinks and provide a significant source of methane ( $\text{CH}_4$ ) to the atmosphere. However, peatlands are altered by both natural and anthropogenic disturbances, resulting in potential release of large amounts of carbon to the atmosphere. The effect of seismic lines on peatland functioning, such as carbon cycling and hydrology, is not well understood. Previous studies have suggested that linear disturbances increase  $\text{CH}_4$  emissions, but little on-the-ground data exists to test this hypothesis. This study measured understory  $\text{CH}_4$  emissions from disturbed and surrounding peatlands, and assessed environmental controls (vegetation cover and productivity, water table, peat temperature) on  $\text{CH}_4$  dynamics.

Portable gas analyzers were deployed to measure fluxes of  $\text{CH}_4$  and the net ecosystem exchange (NEE) of carbon dioxide, from 48-paired plots distributed across a poor fen and two wooded bogs near Peace River, Alberta. Data was collected over two summers (2018 and 2019) together with environmental variables such as water table, peat temperature and vegetation cover.

Sections of the peatland effected by seismic lines were significantly warmer and wetter, providing ideal conditions for increased  $\text{CH}_4$  emissions at all sites. Methane emissions relative to undisturbed plots were: 176% (fen, legacy), 261% (bog, low effect) and 308% (bog, legacy) over the two studied growing seasons. The persistence of the seismic lines and the elevated  $\text{CH}_4$  emissions is a cause for concern, since  $\text{CH}_4$  has a much higher global warming



potential than carbon dioxide. Results from this study will contribute to accurate greenhouse gas (GHG) reporting for anthropogenic disturbance in boreal peatlands, which is lacking for many disturbance types, as well as provide a scientific foundation for integrated land management practices and policies related to boreal peatland restoration.

**Assessing changes in peatland plant community function following seismic line disturbance (project ongoing — year 1 of 2)**

Christina Bao, University of Waterloo Department of Geography and Environmental Management

This study aims to determine if plant communities in peatlands function differently on the seismic lines versus undisturbed areas, and whether newly disturbed areas differ from recovering lines. Previous research has shown that plant community composition changes following seismic line disturbances, but it is not known how the changes of composition affect the community functions, such as their ability to cycle nutrients or store carbon. The changes in plant community functions can be assessed by measuring plant traits. Plant traits are the characteristics of a plant, such as height, colour, and leaf chemistry. Plant traits are also called plant functional traits because they affect plant functions, such as their ability to take up carbon. However, plant functional traits for peatland species are not well-represented in trait databases. Researchers also do not know the extent of trait variation within a species or whether disturbance affects this. This study examines species that represent a full range of peatland functional types, including mosses, shrubs, forbs, sedges, horsetails, and trees. Results of the study will indicate how and if plant community functions have changed on seismic lines. Additionally, this study measures how plant communities change over 20 years following disturbance. This will enable researchers to determine if communities are recovering towards the undisturbed areas or if human intervention is needed for functional restoration.

The study is being carried out at Canadian Natural's Kirby South project in a rich fen with sparse tree cover. Sample plots were distributed across four different site types: undisturbed, disturbed 20 years ago, disturbed recently, disturbed twice (once 20 years ago and once recently). Five replicates of each site type were established, and plant community composition was determined in each plot. The plant functional traits were measured on 18 dominant plant species, including plant height, leaf masses, leaf chemistries and leaf areas. At the end, the species composition and plant traits of each site type will be compared to assess the differences.

This project will provide better understanding of how plant communities in peatlands respond to anthropogenic disturbances in oil-sand regions in Alberta and whether this effects ecosystem functions. It will allow us to assess the regeneration status one year and 20 years after disturbance. Knowing more about the regeneration processes allows us to determine whether human interventions are advantageous to accelerate recovery towards undisturbed functions. While the primary focus has been on returning tree cover, restoring ground layer plant communities in peatlands is also an important step in restoring ecosystem functions that was often overlooked in previous studies. Thus, the results will inform whether more investment in understory regeneration is needed in future management.

**Effects of seismic lines and their restoration on peatland carbon cycle (project underway, year 1 of 4)**

Nazia Tabassum, Department of Geography and Environmental Management, University of Waterloo

Many peatlands in Alberta are crossed by seismic lines. Restoration attempts to re-establish the self-regulatory mechanisms of these ecosystems are underway. One such technique is "mounding", which involves restoration by replicating the topography of natural peatlands on seismic lines. Given the potential importance of restored sites for helping to sequester atmospheric carbon in the long term, this research examines the ecological and



biogeochemical functions of mounded lines to assess how restoration affects carbon dioxide and CH<sub>4</sub> fluxes. Results will allow managers to assess benefits of restoration for returning peatland carbon exchange.

Using data gathered from the field in 2022, the key factors responsible for differences in GHG exchange between mounded seismic lines, non-mounded seismic lines and the interior of natural peatlands are being investigated. Fluxes were measured with closed chamber techniques on undisturbed plots, new seismic lines, un-mounded lines, and lines mounded in 2016. Plant community composition and soil and hydrological conditions were measured at all plots.

Peatland management has been highlighted as a key nature-based climate solution. Currently, the effect of seismic line disturbance on peatland carbon and greenhouse gas exchange, along with recovery trajectories and the benefits of restoration remain uncertain. Results from this study will be included in a larger database of carbon flux measurements on peatland affected by seismic lines to provide information to managers on the benefits or trade-offs of mounding on peatland seismic lines from a carbon exchange perspective.

#### **Synthesis of Soil Properties on Seismic Lines in Northern Alberta (project ongoing, year 1 of 2)**

Marissa Davies, Department of Geography and Environmental Management, University of Waterloo

Restoring seismic lines to their adjacent reference systems requires assessing all abiotic and biotic aspects that contribute to ecosystem function. Basic soil properties are associated with water and nutrient retention and biogeochemical cycling and effect vegetation growth and subsequent ecosystem recovery. This project aims to collect new and compile previously published data on three major soil properties at both reference and on-line localities across the various ecosites of northern Alberta: (i) bulk density, (ii) water content, and (iii) organic-matter content. The dataset will provide a summarized baseline for land managers to quantitatively evaluate seismic line recovery and the efficacy of restoration techniques.

Soil samples were collected across the BERA study region in the summer of 2022. Each study site had soil samples taken on, and adjacent to, a seismic line. Bulk density (g/cm<sup>3</sup>) was measured by taking a known soil volume from the upper approximately 10 cm (soup can — 284 cm<sup>3</sup>) and was dried to quantify the soil weight (g). Weight lost during drying was the water content (percentage). Organic matter content (percentage) was estimated by igniting a subsample at 550 °C for four hours and measuring the weight lost. Samples from the BERA 2022 field season were combined with data from previous studies across northern Alberta for a total of 122 sites and 755 individual samples to date.

This data compilation emphasizes what has been previously established: soil on seismic lines generally has higher bulk density and reduced organic-matter content. In lowland regions, higher bulk density also leads to increased water content in soil that may persist until sufficient peat is built up to re-establish microtopography. Comparison of inverted mounds across a wide variety of untreated sites confirms previously published conclusions: that inverted mounds have higher soil bulk density than natural mounds in adjacent reference areas. Further, the data suggests that inverted mounds have comparable water content to untreated lines and therefore may not promote drier conditions. These are preliminary findings and not yet fully reported. This data suggests that forest harvesting in upland regions combined with seismic line creation does not further alter soil properties. However, more observations are required.

NOTE: The researchers for this project are interested to connect with others collecting similar soil samples in the 2023 summer field season. Please connect through the BERA or COSIA websites.



### **Snow accumulation and melt patterns on seismic lines (project ongoing, year 1 of 2)**

Lelia Weiland, Department of Geography, University of Calgary

Seismic lines may alter the hydrology of ecosystems. Water and soil properties are being studied with respect to vegetation growth, but little is known about how snow accumulation and melt patterns are affected by seismic lines. The removal of canopy vegetation during seismic line construction may change the nature of over-winter snow accumulation and the snowpack properties (depth and density); however, these effects have yet to be considered. Differences in snowpack and snowmelt on lines may have an outsized effect, since snow is frequently the largest influx of precipitation for the season.

In March and April 2022, a three-week field study was completed on one seismic line at Stony Mountain, and four lines at Kirby South. At each seismic line, transects were set up perpendicular to line direction, and snow depth. Every three days, density and snow water equivalent (SWE) were measured along these transects at the center and extending out into the natural area on either side of the line. Ablation (snowmelt) rates were also measured on a regular basis and were based on the distance to the snow surface from a level string tied across the seismic line between trees. A similar setup was completed in the natural area on one side of the seismic line. This provides a comparison of snow accumulation and melt patterns on and off the seismic line.

Snowmelt is a major component of the water balance of a northern boreal ecosystem. The effect of this is twofold: (i) seismic lines have the potential to affect downstream processes, potentially increasing runoff when snowmelt occurs. This work will quantify changes to hydrologic inputs from snowmelt on seismic lines. While this project is limited to the specific lines that are being sampled, the long-term goal is to provide initial insights on how seismic lines alter water balance at the watershed level as future work is scaled up. (ii) Melting snowpacks may also affect the thermal properties of the underlying soil, with further implications for vegetation growth.

### **The influence of seismic lines on soil properties and water table dynamics (project ongoing, year 1 of 2)**

Lelia Weiland, Department of Geography, University of Calgary

Much research has been completed on vegetation regrowth on seismic lines; however, little is known about the underlying mechanisms that control this growth. Previous work has identified changes to soil properties in wetlands, where soils on seismic lines can be more compacted than in adjacent undisturbed areas. By investigating the hydrology of seismic lines, researchers hope to answer the question: How do seismic lines affect water availability and movement? Project researchers are working collaboratively to pair these studies with projects addressing vegetation metrics to further understand this link.

In the summer and fall of 2021 and 2022, researchers conducted fieldwork at five intensive research sites. Two of the sites are located at Stony Mountain (near Fort McMurray) and three at Kirby (near Conklin). The Stony Mountain research site has eleven transects — six on an East-West seismic line and five on a North-South seismic line — which bisect the untreated lines over multiple ecosystem types. The Kirby sites have two transects at each site for a total of six transects, with one transect having been treated with mounding. These transects extend approximately 25 m into the adjacent forest on one side of the seismic line. The sites at Kirby were selected in collaboration with existing research sites associated with the remote sensing team. Researchers installed wells and piezometers to quantify water table levels and hydraulic conductivity at the center of the seismic line, the edge, and 25 m into the adjacent forest. Corresponding soil samples were taken at the surface (0 cm to 5 cm) and at 10 cm to 15 cm below surface



to understand the effects on soil properties at the surface and within the rooting zone. Researchers completed modified vegetation surveys and paired this study with LiDAR flights to quantify the microtopography of the area.

Understanding how increased bulk density of the soil affects the hydrology, specifically with respect to other soil properties and water table variability, will allow researchers to understand key processes controlling the growth of vegetation on seismic lines. By investigating the relationship between compaction and porosity, hydraulic conductivity, and water storage, paired with vegetation data (through working with the remote sensing/vegetation teams), researchers aim to understand how plant water availability is affected. These are the underlying mechanisms which affect vegetation growth on seismic lines. By understanding these processes, there is potential to remediate the soil and water conditions which will in turn benefit the goal of restoring forest cover and carbon dynamics/storage.

#### **Effect of seismic lines on boreal ecosystem water balance: Evapotranspiration (project ongoing, year 1 of 4)**

Maryam Bayatvarkeshi, University of Waterloo Department of Geography and Environmental Management

Seismic lines are common anthropogenic disturbances in Alberta's boreal region. It is likely that these disturbances affect hydrological processes, particularly water balance, but little data is available to understand these changes. Alberta's boreal plain is often in a moisture deficit, so understanding effects on water balance is important for water resource management.

Numerical modelling will be used to simulate how the presence of seismic lines in a catchment affects components of the water balance from local to watershed scales. Since accuracy of parameters in the model are pivotal to model outcomes, measuring them with high accuracy is the first priority of this project. In summer 2022, the focus was on collecting ground truth data that can be used for model parameterization and validation. Meteorological stations collected data in 30-minute intervals from May 28 to Aug 17 for net radiation, air temperature, relative humidity, wind speed and direction, precipitation, soil temperature, and photosynthetically active radiation (PAR) both on and off seismic lines. Measures of ground layer evaporation, soil moisture, and local precipitation were measured across 57 plots.

Data from the summer of 2022 revealed that the net radiation, soil temperature, wind speed, and PAR increased considerably on the seismic line. Air temperature and relative humidity both increased on seismic lines; however, the difference was insignificant. Surprisingly, the rainfall reaching the ground layer on the seismic line was lower than the adjacent forest which might be related to heterogeneity of rainfall. Therefore, seismic lines may alter local weather variables and subsequently change hydrological drivers. Potential effects on hydrological function should be incorporated into future management and restoration plans in terms of maintaining equilibrium among hydrological components.

#### **Maintaining Wildlife Habitat / Enhancing Caribou Habitat**

##### **Response of boreal songbird communities to linear features of varying width (project ongoing, year 1 of 3)**

Tharindu Kalukapuge, University of Alberta Department of Biological Sciences

Alberta's boreal forest is heavily fragmented by linear features such as seismic lines, pipelines, transmission lines, and roads. Vegetation recovery and gap width make linear features different from one another. In provincial bird models used for regulatory decision making, all linear features are treated as being the same width. The goal of





this study is to understand how different linear features of varying width influence bird communities in the boreal forest. Bioacoustic, vegetation, and spatial data were used to answer the primary research question “how do boreal songbird communities respond to linear features?” with special emphasis on the influence of gap width and vegetation recovery.

During the 2021 and 2022 field seasons, thousands of different linear features including seismic lines, pipelines, transmission lines, and roads in north and northeast Alberta and other parts of the province were surveyed. Bird communities associated with linear feature gap and edge habitats were assessed using fixed Autonomous Recording Units (ARUs) and digital mobile point count surveys. In addition, footprint and edge vegetation and recovery data were collected for each study site.

In this study, the researcher is trying to recognize the threshold linear feature widths that support high species diversity, richness and evenness and create new bird habitat attracting early seral, open habitat, and grassland associated birds that are among the fastest-declining bird species in North America. This knowledge will help researchers to understand true linear feature width-species relationships and determine which linear features should be prioritized for reclamation and which should be left to natural regeneration.

#### **Linear feature edge effects on songbird communities in the boreal forest of Alberta (project ongoing, year 1 of 3)**

Tharindu Kalukapuge, University of Alberta Department of Biological Sciences

It is crucial to understand the cumulative effects of linear feature width and vegetation recovery on edge effect and its influence on songbird communities to inform effective conservation and restoration-planning decisions. This project will investigate the effects of linear feature width on boreal songbirds with the goal of determining if linear features simply cause habitat loss or whether there are additional edge effects that result in a greater effect. The effects of vegetation recovery on linear features will also be assessed to determine if restoration of linear features can mitigate edge effects and habitat loss.

During the 2021 and 2022 field seasons, acoustic and vegetation data were collected at over 150 linear feature edge sites using Autonomous Recording Units (ARUs) transects that provide bird data along edge-to-interior habitat gradients as a function of linear feature width. In this project, data was collected from seismic lines, pipelines, transmission lines, roads, and other non-linear human footprints such as well pads. In addition, researchers were able to survey linear feature edges situated in various ecosites in coniferous, broadleaf and mixedwood forests and wetlands.

Preliminary results indicate that there is a change in bird community characteristics including diversity, richness, and evenness as the distance from linear feature edge to interior forests increases. However, how these changes occur as a function of width and vegetation recovery is yet to be assessed. Results of this project will help researchers, managers and government and private sector organizations to assess edge effects of different linear features and their influence on bird communities using linear feature gap size and the vegetation recovery stage. This will aid in the implementation of restoration practices to minimize the influence of edge effects where they are detrimental on associated wildlife communities.



### **Small Mammal Feedback on Linear Features and Well Pads Recovery (project ongoing, year 1 of 4)**

Alessandro Franceschini, University of Alberta Department of Biological Sciences

Small mammals are critical components of terrestrial ecosystems. They can significantly alter forest regeneration and succession and affect their predator species assemblage. Therefore, disturbance in small mammal communities introduced by seismic lines will likely have ramifications for linear features reclamation and boreal food web structure. This project will be the first to assess the effect of small mammals on forest regeneration along seismic lines. It will contribute knowledge meant to assist more efficient restoration, indicating how to avoid some causes that prevent or delay regeneration along linear features and well pads.

During the 2022 field season, 60 sites located in an area between Fort McMurray and Conklin were surveyed. Sites were distributed along a gradient of upland to lowland forest stratified by linear feature width (low-effect seismic, conventional seismic, and pipelines). Each site was paired with a control sampling site 50m inside the forest to avoid edge effects. In each sampling location, two camera traps were deployed (a total of 240 camera traps) for five consecutive nights and tested the efficiency of using two different methods of data collection.

Findings will help determine how seismic line features affect the community of small mammals present and how the resulting community affects regeneration. Thus, this study could lead to policies that outline the seismic line characteristics that need to be achieved to promote regeneration. For example, if it is found that wider lines are associated to a higher abundance of meadow voles and if this higher abundance is correlated to a decreased regeneration rate, future management can focus on reducing seismic line width with the goal of a more cost-efficient line regeneration. Moreover, with this study, specific thresholds for seismic line widths can be provided, after which the resulting community of small mammals starts exerting significant negative effects on seismic line regeneration. This information can be used to inform policies that will set different limits to the width of seismic lines depending on the forest type.

### **LiDAR and Landsat spectral change metrics predict avian response to harvesting (project complete)**

Brendan Casey, Department of Biological Sciences, University of Alberta

Retention forestry aims to promote multifunctional landscapes that integrate wood production with the habitat needs of forest species through the maintenance of pre-harvest legacy structures. The availability of province-wide avian monitoring and remote sensing data provides opportunities to improve on existing research by enabling the study of large numbers of harvest blocks over wide spatial extents and providing novel covariates related to the structure and recovery of harvests.

The viability of using LiDAR and Landsat time-series to predict avian response to harvesting was explored. Expanding covariates beyond discrete harvest severity classes included in forest resource inventories to those derived directly from remote sensing, may reveal subtle relationships between the structure of harvest residuals and avian communities.

Point count data was gathered from forestry harvest blocks across 50,190 km<sup>2</sup> of the Boreal Central Mixedwood natural subregion of northern Alberta. Linear and generalized linear mixed models were used to evaluate the power of forest resource inventories, LiDAR, and Landsat spectral change metrics to predict bird abundance and community response to harvesting.



To adequately conserve federally listed species, wildlife managers need to understand the stand-level habitat conditions contributing to species declines. Remote sensing can help, but the costs and availability limit its adoption in ecological research. Wall-to-wall remote sensing products, cloud-based computing platforms such as Google Earth Engine, and long-term bird monitoring data provide opportunities to model species-habitat relationships using novel remote sensing metrics over large spatial extents. Here, it was found that LiDAR derived height and complexity metrics and spectral measures of harvest severity are more predictive of species abundance and diversity than the discrete harvest severity classes often used to model avian response. Supplementing forest resource inventory data with LiDAR and Landsat time-series can improve the accuracy of bird models while avoiding the costs of boots-on-the-ground vegetation surveys. This work may inform choice of covariates in future studies on the effects of forestry and oil and gas exploration in the boreal area. For example, the use of spectral change metrics to measure disturbance severity and recovery can be applied to work focused on habitat restoration assessment.

## Remote Sensing

### **Monitoring disturbance recovery using time series satellite data (project ongoing, year 1 of 2)**

Tanya Yeomans, Department of Geography, University of Calgary

Characterizing the state of forest recovery on anthropogenic disturbance features is a key element of restoration planning and monitoring programs. Satellite time-series analysis from high-resolution imagery like Planet Labs can provide metrics of forest recovery over large areas and complements existing airborne observations and field-based analyses. Previous research has shown that spectral-recovery metrics from medium-resolution sensors like Landsat can track vegetation recovery over forest harvest areas and burns. However, these strategies have yet to be applied to small- and medium-footprint disturbance features using very high-resolution satellite imagery. The change in resolution from Landsat with a 30 metre pixel size and an eight-day revisit time, to PlanetScope imagery with a three metre pixel size and near-daily imaging opens new possibilities for monitoring disturbance recovery.

Changing pixel values in satellite time series data as proxy indicators of recovery is being analyzed. Based on these data, disturbance features will be classified as “functionally recovered”, “recovering”, and “not recovering”. Predictions will be validated against canopy height and canopy cover metrics derived from LiDAR data and field measurements.

This summer, three field missions were conducted at the ConocoPhillips Surmont lease in northeastern Alberta to collect data on well sites and seismic lines distributed across a range of ecosite types.

The current analysis of PlanetScope time-series data will compare two different analytical approaches derived from the literature. Spatial and radiometric issues contained in the time-series data will have to be addressed.

The long-term goal of this research is to provide a method to assess disturbance recovery over large areas. Such methods would allow land managers to monitor ecosystem recovery and cumulative effects over vast operational areas. This research will help stakeholders in the decision-making process and allow managers to effectively direct resources to areas where they are most needed.



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### **Mapping seismic-line vegetation cover and species composition with remote sensing (project ongoing, year 1 of 2)**

Nicole Byford, Department of Geography, University of Calgary

The Provincial Restoration and Establishment Framework for Legacy Seismic Lines in Alberta uses the term advanced regeneration to describe seismic lines that are successfully regenerating naturally. These areas must demonstrate 70% coverage of acceptable vegetation species, with 50% coverage on either side of the seismic line. Seismic lines that do not meet these criteria are in a state of early or arrested succession and are potential candidates for active intervention. Restoration assessment strategies for distinguishing early/arrested succession lines from those that have reached advanced regeneration are needed. This requires information on seismic-line vegetation cover and species composition. Remote sensing from high-resolution airborne platforms is an attractive option for performing restoration assessments over large areas. This study aims to provide a remote assessment of seismic-line conditions (vegetation cover and species composition) using a remotely piloted aircraft system (RPAS) to assess the recovery status of seismic lines.

RPAS remote sensing (LiDAR, multispectral, and photogrammetry) and field validation data (vegetation surveys of canopy cover, crown diameter, species, and height) were collected for ten sites in the Surmont area and eight sites in the Richardson Caribou Range in July and August (2022). Deep-learning algorithms will be used to estimate vegetation cover and species composition for each site. An accuracy assessment will be performed against field validation data. The resulting information on vegetation cover and species composition will be used to estimate the recovery status of the line.

High-resolution remote sensing data have potential to increase the speed and efficiency of restoration assessment when compared with field-based efforts. Demonstrating coverage of acceptable species on seismic lines poses a challenge to remote sensing-based restoration assessments. This study will inform whether remote sensing methods can fulfill this requirement and further be used to report seismic-line recovery status (advanced regeneration, early/arrested succession). This information will allow government and industry stakeholders to focus field assessments and restoration treatments in areas where they are needed most. Alternatively, measures of seismic line vegetation regeneration that are better suited to assessment from remote sensing perspectives will be proposed.

### **Leaf-phenology and species influences seedling and sapling height predictions from remote sensing (project complete)**

Colette Shellian, Department of Geography, University of Calgary

Individual tree seedling height is an important parameter to assess the status of forest recovery on treated and naturally regenerating seismic lines. Digital Aerial Photogrammetry (DAP) can be used to measure seedling height remotely. However, there has been no assessment of the environmental conditions that are most conducive to accurate and reliable measurements. The goal of this project was to understand how leaf-phenology, seedling species and size, and nearby vegetation influenced the accuracy of point-cloud based height predictions of coniferous and deciduous tree seedlings and saplings.

The study was conducted in Kirby South on ten seismic lines sampled across gradients of ecosite (lowland and upland) and treatment status (treated and untreated). High resolution (0.50 cm pixel) leaf-off (spring) and leaf-on (summer) photographs were acquired with a remotely piloted aircraft system (RPAS). Seedling and sapling heights from point clouds were compared to reference measurements made in the field.



Seedlings occluded by other features or confused with their surroundings were not measured reliably in either season. Woody debris, neighbouring seedlings, overhanging canopy trees, and annual vegetation that grew over top of the seedling caused heights to be overestimated.

Point clouds derived from the summer photographs yielded the most accurate height predictions across all tree species (median absolute error [MEDAE] = 9.5 cm). For black spruce and pine, height prediction error was similar between seasons (MEDAE<sub>spring</sub> = 10 cm, 16 cm and MEDAE<sub>summer</sub> = 9 cm, 18 cm, respectively). For deciduous species, height predictions were more accurate in the summer point clouds (MEDAE<sub>summer</sub> = 11 cm). The median height error declined with increasing seedling height in the summer point clouds, indicating taller seedlings can be measured with less error. No such pattern was observed in the spring point clouds.

Using summer point clouds, DAP can be used to measure the height of regenerating seedlings and saplings. DAP point clouds capture the outer envelope of forest structure, and therefore do not work well for measuring seedlings that are shorter than understory vegetation, or that are growing in dense clusters with overlapping crowns. The most reliable estimates were obtained for coniferous species. Our work shows that high-resolution DAP point clouds are suitable for measuring the height of individual trees for establishment monitoring.

### **Artificial Intelligence for Coniferous Seedling Detection (project ongoing, year 1 of 3)**

Irina Terenteva, University of Calgary Department of Geography

Automatic processes for detecting regenerating seedlings in very high-resolution remote sensing data would greatly enhance the processes of forest-restoration planning and assessment. An emerging synergy between drones and artificial intelligence (AI) shows great promise in this respect. If developed effectively, this combination of technologies could provide end-to-end learning approaches, with no need for complicated pre-processing steps.

The goal of this project is to develop best practices for automatic seedling detection with AI. Specifically, researchers are documenting the environmental and technical controls of AI detection accuracy.

RetinaNet — a powerful one-stage architecture for object detection — to identify coniferous seedlings on seismic lines in Kirby South was used. The best model recalls located 82% of coniferous seedlings and 90% of those larger than 60 cm in height. While the results are promising, this project is best regarded as a case study due to limited geographic range.

Visual photo interpretation during the establishment survey remains labour-intensive which limits its applicability on a larger scale. AI provides an opportunity for accurate, and automatic, detection of regenerating seedlings — a key element of restoration planning and assessment. Combined with drones, AI can reduce project costs while providing sufficient accuracy of core metrics to evaluate the restoration success.

To achieve the best model performance, researchers need to train AI with a large, diverse reference dataset of regenerating seedlings — which is collected under BERA — and then test these models in a variety of settings. To fully exploit spatial patterns of the drone imagery, researchers are experimenting with the model fine-tuning by changing the tile size, sample distribution, input data and training set size.

A fully developed AI could not only detect seedling locations, but also make additional calls on species, health status, and define environmental controls of trees growth and survival. This project facilitates developing workflows for the operational assessment of the restoration success on seismic lines and other disturbance features.



### **A drone-based approach for mapping plant traits on seismic lines (project ongoing, year 1 of 2)**

Xue Yan Chan, University of Calgary Department of Geography

Peatlands are important carbon sinks where plants play a role in directly controlling carbon cycling through processes such as photosynthesis. The creation of seismic lines leads to changes in the plant community; for example, sedges and willows tend to outcompete mosses and lichens on seismic lines. These changes are accompanied by a shift in the composition of plant functional traits (characteristics such as height or greenness) and functional types (groups of plants that have similar effects on ecosystem processes due to sharing functional traits, such as shrubs or mosses). Carbon cycling and productivity of a region can be affected by these changes, possibly compromising a peatland's ability to be a carbon sink over time.

There is a need for efficient and non-destructive methods for monitoring and mapping changes in carbon dynamics in peatland ecosystems, which could be achieved using remotely piloted aircraft systems (RPAS). This study aims to link plant traits (and subsequently, carbon dynamics) to RPAS-based remotely sensed datasets.

This study focuses on Canadian Natural's Kirby South fen (near Conklin, AB), which covers an area of 30 hectares and comprises a grid of low effect seismic lines. There are 24 BERA standard plots in this area, about half of which are in undisturbed peatland and serve as reference sites. In 2022, plant samples and percent cover estimates were collected for 18 indicator species from all plots. The fen was surveyed using RPAS-based LiDAR and multispectral sensors in May, July, and August. The resulting point clouds and multispectral images can be used to derive structural information and spectral indices that can differentiate unique plant functional groups.

Technological advancements in RPAS-based remote sensing allow for monitoring at traditionally unattainable spatial and temporal scales, but more research is required before these datasets can be used reliably to map plant types or productivity in peatlands. Accurate baseline and ongoing monitoring are crucial for ensuring that our ecosystems recover according to predefined plans and ensuring that intervention can be adapted as necessary. By generating methods to map plant types and creating remotely sensed indicators of productivity status on seismic lines, this study hopes to make progress towards a systematic workflow that can be used to monitor plant traits related to productivity over large areas.

### **Modelling solar irradiance on seismic lines — where do trees have enough light to grow? (project ongoing, year 1 of 2)**

Marlis Hegels, Department of Geography, Ludwig-Maximilians University, Munich

The boreal ecosystem in Northern Alberta is strongly fragmented by seismic lines — linear corridors created for oil and gas exploration. Many of these lines are in a state of slow recovery or arrested succession and may require active intervention. With an aim of promoting a return of forest cover on these lines, it is beneficial for restoration assessments to recognize the site-limiting factors constraining tree growth. Previous research has shown that seismic lines alter abiotic conditions relative to the surrounding undisturbed forest. Nevertheless, there is a lack of tools designed to help characterize microclimatic effects for management purposes. The goal of this research project is to measure and model photosynthetically active radiation (PAR) in Alberta's treed wetlands using in situ measurements and remote sensing.

The project started in April 2022 and is currently underway. Accomplishments to date include (i) project specification and sampling design, (ii) procurement and calibration of in situ sensors, (iii) planning and completion of field work in the boreal forest, and (iv) the data acquisition of PAR and aerial light detection and ranging (LiDAR) data within Kirby South. Data preparation and processing is still in progress.



Given that there are more than 1.5 million kilometres of seismic lines in Alberta alone, development of tools that improve the effectiveness and efficiency of restoration are needed. Light (PAR) availability is a growth-limiting factor for tree growth and establishment. Applying treatment to lines with enough light decreases the cost for the restoration assessment through an increased seedling survival. Through the characterization of light conditions on seismic lines it is possible to detect unsuitable tree sites and therefore develop restoration practices which improve site conditions such as adjacent forest thinning. Regarding the light conditions, orientation and line width can be evaluated through the analysis of factors controlling the variability of PAR on seismic lines. The goal is to quantify the relationship between the light conditions and LiDAR point clouds to understand and map sites with light limitations and to provide tools for targeting thinning applications without the necessity of cost and labour intensive in situ measurements.

#### **Measuring microtopography on seismic lines using LiDAR (project ongoing, year 1 of 2)**

Jasper Koch, Department of Geography, Ludwig-Maximilians University, Munich

The return to forest cover on seismic lines in peatlands is heavily influenced by the level of microtopography on these lines. The hummocks and hollows that are characteristic of peatlands, influence regrowth on seismic lines. Hummocks allow young seedlings to grow slightly above the shallow groundwater table. Artificial mounding has been one strategy to spur regrowth by creating artificial hummocks for seedlings to grow on. By identifying the level of microtopography on lines, treatment can be applied more efficiently to lines with a depressed microtopography. Most studies on microtopography in peatlands have been conducted using photogrammetry, which is limited in its ability to measure ground surfaces through canopy cover. Using the canopy penetration capabilities of LiDAR researchers hope to achieve accurate measurements of microtopography throughout a variety of ecosites and dense vegetation.

For this study high resolution UAV-LiDAR-Data with a point density of 150 points per square metre was collected for 28 sites in Stony Mountain, Kirby South and Surmount. To validate the quality of LiDAR derived ground models, a total of around 4,500 RTK-measurements were taken in three 15 m transects. The difference between LiDAR and RTK measurements was then determined for these sites in order to estimate the accuracy of the ground models. Microtopographic variability is then quantified by deriving the roughness of the ground model. Next researchers will test if it is possible to derive similar measurements of roughness through lower LiDAR point densities of 10 to 15 points per square metre which are available for large parts of the BERA study region.

#### **Effects of 4D seismic activity on a boreal fen: a case study (project ongoing, year 1 of 2)**

Xue Yan Chan and Lelia Weiland, University of Calgary Department of Geography

4D seismic activity, also termed repeated seismic or time-lapse seismic, describes recurrent seismic surveys that are conducted in the same area. The amount of vegetation regrowth on seismic lines between consecutive surveys will depend on multiple factors, such as the line condition and elapsed time between surveys. In instances where regrowth is significant, vegetation on pre-existing lines is required to be re-cleared before the next seismic survey can occur. The resulting feature, a 4D seismic line, describes a corridor that has been re-disturbed by repeated seismic activity. In boreal peatlands, repeated visits by vegetation-clearing equipment may lead to accumulated effects to soil compaction, groundwater, and microtopography in ways that are not yet well understood.

In Spring 2022, a boreal fen in northern Alberta was identified to comprise the following seismic line classes: one-time disturbance, new disturbance, and re-disturbance. This opportunistic case study aims to evaluate soil





compaction, groundwater, and microtopography across these line classes, which will provide insight on how these peatland health metrics are effected by 4D seismic activity.

The Kirby South Fen was surveyed using LiDAR and multispectral sensors aboard a remotely piloted aircraft system. The resulting point clouds and images can be used to derive microtopography and elevation of open-water surfaces. Ground-based measurements of elevation were measured with an RTK elevation survey and altimeter surveys on transects crossing seismic lines in upland, transition, and wetland ecosystems. The open water elevation data can be combined with surface elevation to create a water table map. Water table was measured across three sites in water level wells. Wells were strategically placed in the center of the line and 25 metres into the adjacent stand on either side of the line. Soil samples were taken at the surface (0 cm to 5 cm) and the rooting zone (10 cm to 15 cm), and of the mulched surface, if present, at each of the well locations. Bulk density and hydraulic conductivity values were obtained for each sample.

This case study provides a unique opportunity to reveal expected changes to peatland health over time (i.e., lines with one-time disturbance versus new disturbance) and as the result of repeated clearing (i.e., lines with one-time disturbance vs. re-disturbance). This will help to set an important baseline for growth trajectories following 4D seismic activity in boreal peatlands. If 4D seismic activity is found to result in accumulated peatland effects, then the return of 4D seismic lines to a pre-disturbance state may require a longer timeline or different restoration treatments.

## LESSONS LEARNED

BERA Phase 2 is in year two of five. As a result, most work is on-going. These lessons learned are for completed projects only.

### Promoting Return to Forest Cover

#### **Planted seedlings and saplings have higher growth rates on actively restored seismic lines**

Colette Shellian, Department of Geography, University of Calgary

For growth measured over five seasons (2016 to 2021), both black spruce and tamarack growth was influenced by the seedling height, ecosite type, light, and restoration treatments. The effect of restoration treatments depended on ecosite, with planted seedlings consistently having significantly higher growth than natural seedlings. In poor fens, planted black spruce grew 40% more (26.6 cm versus 16.0 cm) relative to untreated sites, and tamarack grew 41% more (26.3 cm versus 15.6 cm) compared to untreated sites. In moderate-rich fens, planted black spruce grew 32% more (33.8 cm versus 22.9 cm), and planted tamarack grew 35% more (34.1 cm versus 22.2 cm) than natural seedlings on untreated lines. Increasing seedling height and greater light availability had a positive effect on growth for both species under all conditions measured.

The results of this study indicate mounding and tree-planting in fens was effective for accelerating initial growth of black spruce and tamarack seedlings and saplings.



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## Restoring Natural Carbon Dynamics

### Seismic lines decrease peat accumulation rates in boreal peatlands

Percy Korsah, Department of Geography and Environmental Management, University of Waterloo

Seismic line disturbances in boreal peatlands encourage saturated soil conditions, decrease tree recovery, and alter vegetation communities and microclimatic conditions. These changes alter peat accumulation rates and long-term carbon storage in boreal peatlands. Vegetation inputs after two years were significantly reduced at all disturbed sites in this study. Although understory NPP rates increased on seismic lines, it could not compensate for the overall NPP lost due to the absence of overstory woody vegetation. Thus justifying the need for tree restoration in maintaining the carbon sink function of peatlands. Results from this research are novel and can serve as a foundation for new perspectives on designing and setting restoration targets in boreal peatlands.

### Seismic lines increase methane emissions from boreal peatlands

Percy Korsah, Department of Geography and Environmental Management, University of Waterloo

Sections of the peatland effected by seismic lines were significantly warmer and wetter, providing ideal conditions for increased CH<sub>4</sub> emissions at all sites. Methane emissions relative to undisturbed plots were: 176% (fen, legacy), 261% (bog, low effect) and 308% (bog, legacy) over the two studied growing seasons. The persistence of the seismic lines and the elevated CH<sub>4</sub> emissions is a cause for concern since CH<sub>4</sub> has a much higher global warming potential than carbon dioxide. Results from this study will contribute to accurate greenhouse gas reporting for anthropogenic disturbance in boreal peatlands — which is lacking for many disturbance types — as well as provide a scientific foundation for integrated land management practices and policies related to boreal peatland restoration.

## Maintaining Wildlife Habitat / Enhancing Caribou Habitat

### LiDAR and Landsat spectral change metrics predict avian response to harvesting

Brendan Casey, Department of Biological Sciences, University of Alberta

To adequately conserve federally listed species, wildlife managers need to understand the stand-level habitat conditions contributing to species declines. Remote sensing can help but the costs and availability limit its adoption in ecological research. Wall-to-wall remote sensing products, cloud-based computing platforms such as Google Earth Engine, and long-term bird monitoring data provide opportunities to model species-habitat relationships using novel remote sensing metrics over large spatial extents. Here researchers found that LiDAR derived height and complexity metrics and spectral measures of harvest severity are more predictive of species abundance and diversity than the discrete harvest severity classes often used to model avian response. Supplementing forest resource inventory data with LiDAR and Landsat time-series can improve the accuracy of bird models while avoiding the costs of boots-on-the-ground vegetation surveys. This work may inform choice of covariates in future studies on the effects of forestry and oil and gas exploration in the boreal. For example, the use of spectral change metrics to measure disturbance severity and recovery can be applied to work focused on habitat restoration assessment.



## Remote Sensing

### Leaf-phenology and species influences seedling and sapling height predictions from remote sensing

Colette Shellian, Department of Geography, University of Calgary

Seedlings occluded by other features or confused with their surroundings were not measured reliably in either season. Woody debris, neighbouring seedlings, overhanging canopy trees, and annual vegetation that grew over top of the seedling caused heights to be overestimated.

Point clouds derived from the summer photographs yielded the most accurate height predictions across all tree species (median absolute error [MEDAE] = 9.5 cm). For black spruce and pine, height prediction error was similar between seasons (MEDAE<sub>spring</sub> = 10 cm, 16 cm, and MEDAE<sub>summer</sub> = 9 cm, 18 cm, respectively). For deciduous species, height predictions were more accurate in the summer point clouds (MEDAE<sub>summer</sub> = 11 cm). The median height error declined with increasing seedling height in the summer point clouds, indicating taller seedlings can be measured with less error. No such pattern was observed in the spring point clouds.

Using summer point clouds, DAP can be used to measure the height of regenerating seedlings and saplings. DAP point clouds capture the outer envelope of forest structure, and therefore do not work well for measuring seedlings that are shorter than understory vegetation, or that are growing in dense clusters with overlapping crowns. The most reliable estimates were obtained for coniferous species. This work shows that high-resolution DAP point clouds are suitable for measuring the height of individual trees for establishment monitoring.

## PRESENTATIONS AND PUBLICATIONS

### Published Theses

Shellian, C. A. 2022. Assessing Seedling and Sapling Height and Growth Using Field and UAV Remote Sensing Data for Boreal Restoration. M.Sc. Thesis. University of Calgary.

### Journal Publications

Filicetti, A.T. and S.E. Nielsen. 2022. [Effects of wildfire and soil compaction on recovery of narrow linear disturbances in upland mesic boreal forests](#). Forest Ecology and Management 510, 120073

Gregoire, J., Hedley, R., and E. M. Bayne. 2022. [Canada Warbler response to vegetation structure on regenerating seismic lines](#). Avian Conservation and Ecology 17(2):26

Hird, J. N., Kariyeva, J. and G. J. McDermid. 2022. [Satellite Time Series and Google Earth Engine Democratize the Process of Forest-Recovery Monitoring over Large Areas](#).

Sánchez, N. V., Sandoval, L., Hedley, R. W., St Clair, C. C., and E. M. Bayne. 2022. [Relative Importance for Lincoln's Sparrow \(\*Melospiza lincolnii\*\) Occupancy of Vegetation Type versus Noise Caused by Industrial Development](#). Frontiers in Ecology and Evolution, 198.

Sánchez, N. V., E. M. Bayne, and B. Hilke. 2022. [Lincoln's sparrow \(\*Melospiza lincolnii\*\) increases singing rate in areas with chronic industrial noise](#). IBIS, doi.org/10.1111/ibi.13174.



Kleinke, K., Davidson, S. J., Schmidt, M., Xu, B., and Strack, M. 2022. [How mounds are made matters: Seismic line restoration techniques affect peat physical and chemical properties throughout the peat profile](#). Canadian Journal of Forest Research, 52(6): 963–976.

## Reports & Other Publications

BERA 2022. [The Edge — Pushing the Limits of Restoration Research](#). 2022 Edition of the annual synthesis of BERA research.

## RESEARCH TEAM AND COLLABORATORS

Institution: University of Calgary

Principal Investigator: Dr. Greg McDermid

Name	Institution or Company	Degree or Job Title	Degree Start Date (Students Only)	Degree Completion Date (Students Only)
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Dr. Erin Bayne	University of Alberta	Professor		
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Maverick Fong	University of Calgary	Geospatial Technician		
Claudia Maurer	University of Calgary	Admin. Coordinator		
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## EcoSeis Phase 1

**COSIA Project Number:** LJ0340

**In Situ**

**Research Provider:** OptiSeis Solutions Limited

**Industry Champion:** Suncor Energy

**Industry Collaborators:** Canadian Natural, Cenovus, ConocoPhillips, Imperial

**Status:** Final Cumulative Summary

### PROJECT SUMMARY

The Government of Alberta has proposed changes to seismic regulations for new seismic cut-lines, including reducing the line of sight to less than 100 m and decreasing the receiver line width to 0.75 m after 2024 (draft Cold Lake Sub-Regional Plan). Additionally, there is a push to reclaim existing seismic lines with industry actively testing various reclamation methods. These efforts are costly, so industry is also pursuing methods to minimize the footprint of seismic data acquisition activities, and consequently reduce the reclamation effort that may be required in future for new seismic programs.

The focus of this project was to develop the EcoSeis methodology and software implementation. EcoSeis is an innovative land footprint reduction technology that allows oil and gas operators to sustainably acquire high-resolution seismic images of their resources. The technology is a first-of-its-kind collaboration that successfully brings environmental conservation and greenhouse gas accounting practices into the design and execution of data collection in the field. EcoSeis utilizes proprietary algorithms to rank biological, ecological, and geographical surface information to automatically guide the creation of custom survey designs.

Field acquisition of subsurface data is often conducted in remote areas with varying terrain and habitat types. Historically, seismic exploration in forested regions required the clearing of cutlines for safe access and equipment deployment, resulting in numerous interconnected pathways. Decades of ecological research have uncovered the impact this work has on revegetation as well as the challenges it poses to sensitive species and habitats, such as woodland caribou in Canada's boreal forests. Innovative land stewardship combined with recording equipment miniaturization was drove OptiSeis Solutions Ltd. (OptiSeis) to envision and create EcoSeis, which has the potential to reduce the total amount of seismic line clearing by more than 50%.





## Technology Description

EcoSeis is a methodology to reduce the amount of line clearing on seismic programs. This method applies conventional and/or miniaturized seismic sources in a unique geometry focused on maintaining sensitive habitat and minimizing surface disturbances while also providing high quality seismic data and safe access for field personnel. This method will work with geometry variations and multiple source types, increasing the ability to minimize surface disturbance and making it possible to apply the method in many different types of terrains.

Goals of the project included:

- Developing an easy-to-implement solution for reducing the environmental impact of seismic acquisition in sensitive environments.
- Creating two algorithms to solve the problems associated with utilizing smaller, minimal-impact seismic sources and minimize the amount of line clearing by utilizing an irregular geometry.
- Validate the algorithms through processing trials using synthetic and real-world data

During the project, additional environmental analyses were conducted to enhance algorithm development and quantitatively compare both land footprint and potential greenhouse gas emissions reductions achieved with an EcoSeis program versus a conventional seismic acquisition program.

## Performance Metrics

The key metric for the Land and Biodiversity Program success was to achieve a 25% to 50% reduction in seismic line clearing with EcoSeis while also maintaining the required subsurface resolution needed for accurate assessments of the resources being developed. Additional success metrics included de-risking a miniaturized source for oil sands seismic imaging, developing an algorithm for combining miniaturized and conventional sources, and developing the concept, implementation, and code for an EcoSeis algorithm.

## PROGRESS AND ACHIEVEMENTS

Phase 1 of this project is now complete. This is the first year that this summary has been included in this report and so the cumulative progress and achievements are outlined below.

### EcoSeis Geometry Development and Validation

In this study, conventional and EcoSeis geometries were generated from a well-sampled existing seismic dataset after which various seismic processing tests and interpretation analyses were completed. The processing tests involved applying various decimations to the fully sampled dataset based on either conventional or EcoSeis geometries. Decimation involves removing stations/lines from the original geometry definition. Datasets can be decimated in a regular pattern (removing every second, third, etc. station or line) or in an irregular pattern to create more randomization. In seismic, we typically try to sample the seismic wavefield with at least two samples per wavelength (Nyquist sampling) and would ideally have the same sampling distance in all directions (i.e., receiver station = source station = receiver line = source line interval). When data is decimated to reduce environmental impact and/or cost, then the data must be reconstructed using appropriate seismic data processing algorithms. This is typically done via an interpolation algorithm. However, there are limits on how far a dataset can be decimated before the subsurface





image is negatively affected (i.e., the geoscientist can no longer identify important subtle subsurface features). Typically, interpolation of seismic datasets results in a four times (4x) increase in trace density. Larger increases (8x, 16x) can be applied, but results become less accurate when large interpolation factors are used on complex datasets (multiple dips, lower signal-to-noise).

Initially, the project was focused on generating synthetic data for testing various types of EcoSeis geometries. Various methods of synthetic data generation were examined and ultimately a flat model with overlapping diffraction curves was utilized. This model provided an opportunity to test complex, overlapping dipping events. Three geometries (one conventional and two potential EcoSeis) were tested with a synthetic dataset. This testing involved generating a well-sampled grid of data for a small survey area, decimating it into the three geometries (a conventional seismic geometry, one similar EcoSeis geometry, and one sparse EcoSeis geometry which had a 50% reduction in linear km), and then applying interpolation. Results from the interpolation were compared back with the well-sampled grid of data. However, during the project, one of the collaborators donated a small, well-sampled grid of seismic data enabling more advanced processing tests, which provided an opportunity to test EcoSeis simulations using real-world data.

The first step with this data was to process the well-sampled (grid) seismic data volume through AVO-compliant pre-stack time migration. Then, cost-effective pseudo-decimation interpolation tests were conducted. From these tests, the best-performing geometries were selected for more expensive processing tests with decimations applied to raw shots instead of to processed, pre-interpolation data. By approaching the testing in this manner, numerous geometries could be quickly evaluated in a cost-effective manner. In total, twelve geometries were tested through the pseudo-decimation processing tests with four plus the input dataset processed through full processing from raw shots.

During the synthetic data tests, two of the geometries (the conventional and the comparable EcoSeis geometry) had a 4x interpolation factor applied. The third geometry represented the sparsest possible EcoSeis geometry and required a 16x interpolation factor. Results from the synthetic testing showed a good match between the three interpolated datasets and the input data, although the sparser dataset struggled with the interpolation of some of the crossing events.

These three datasets were then applied as decimations of the real well-sampled seismic data volume and processed through the pseudo-decimation interpolation test. Unfortunately, the sparse dataset requiring 16x interpolation failed this test. It was concluded that the trace density of this volume was too low for accurate target imaging, and another geometry was developed with a similarly high level of land footprint reduction, but with a trace density equivalent to the conventional and comparable EcoSeis geometries. This dataset passed the pseudo-decimation interpolation test and was selected for full processing from raw shots. Results from this test provided good subsurface imaging of the deeper targets but were not ideal for shallow targets indicating that overall, the trace density of this dataset as well as the other two datasets (conventional and EcoSeis) that passed the pseudo-decimation interpolation tests may actually be too low. This was an interesting learning since the conventional geometry had been previously used for actual seismic data acquisition at this subsurface target level (see key learnings for further details).

The next steps were to develop a new base case conventional seismic geometry with slightly higher trace density. This geometry was based on a more recently utilized seismic acquisition geometry in the area that had improved shallow target imaging. From this, four more geometries were generated for pseudo-decimation testing, two



conventional geometries with similar trace density, but different land footprints (one slightly higher, one slightly lower), and two EcoSeis geometries, one with both station (equipment) and linear km (seismic cut line) reductions that had slightly lower trace density than the new conventional geometry, but not as low as the previous EcoSeis, and one with a similar footprint reduction to the previous EcoSeis geometry, but the same trace density as the new conventional geometry. The challenge here was to generate an EcoSeis geometry with similar land footprint reduction as the one from the pseudo-decimation tests, but with higher trace density. Due to the limitations of the input dataset, the ideal EcoSeis geometry could not be generated, but an acceptable alternative was generated for testing. All five of these geometries were tested through pseudo-decimation processing with all resulting in good subsurface imaging, although both the conventional and EcoSeis geometries that had more land footprint reduction (and therefore relied more heavily on interpolation in processing) were slightly inferior to the conventional baseline and other EcoSeis geometry.

Based on these successful results, the baseline conventional and two EcoSeis geometries were taken through full processing from raw shots. Results showed that the cost-effective EcoSeis geometry that had both a reduction in stations and linear km of cut-lines most closely matched the conventional baseline geometry. Interestingly, the EcoSeis geometry appeared to interpolate better than the conventional geometry across a small gap in the dataset. Based on this, these two geometries were taken through one more round of pseudo-decimation interpolation testing, but this time, multiple gaps were introduced into the dataset to mimic the effect of pipeline exclusions. Both datasets performed well with the EcoSeis dataset providing a slight advantage in some locations. This result has led to a new area of potential research where not all sources impacted by surface exclusions may need to be relocated and could instead be potentially excluded. The other EcoSeis geometry, which had a 51% reduction in linear km relative to the conventional seismic, required more interpolation and had slightly more percentage differences relative to the conventional seismic, but is still an acceptable geometry for imaging subsurface targets. Although not tested in this project due to the small survey area size, it is expected that a full-scale EcoSeis project would incorporate multiple geometries (conventional and various EcoSeis) and would assign the geometry with the greatest reduction in land footprint to the most sensitive habitats.

### **Impact of Utilizing Conventional vs. Miniaturized Sources with EcoSeis Geometries**

The EcoSeis geometries tested in this project can be acquired with conventional seismic sources, miniaturized seismic sources, or a combination of the two. Although the total linear km of the seismic cut lines (and the related interconnectedness of the lines or lack thereof) remains the same no matter which source type is used, the total area cut will vary depending on the source type. This is due to the need to cut seismic lines wider for conventional sources, which require larger equipment for deployment, than for miniaturized sources. Current seismic cutting practices use a cut line width of 2.75 m to 4.0 m for conventional source lines and 1.75 m for conventional receiver lines. Various miniaturized sources are in development with many of them being optimized for cut lines that are 1.75 m wide or less.

Miniaturizing a seismic source can lead to a decrease in signal strength. Additionally, comparisons of small versus large explosive sources have shown that although smaller sources can have a higher dominant frequency, they are often lacking in overall frequency content (Cordsen et. al., 2000). Also, in areas with poor source coupling such as over muskeg or when imaging through gravel-filled channels, smaller, low-energy sources may attenuate rapidly and have insufficient frequency content for pre-stack analyses such as quantitative interpretation.



At the beginning of the EcoSeis research project, two miniaturized source datasets were examined to determine if a combination of larger conventional sources with the smaller miniaturized sources would improve the miniaturized source datasets since earlier processing had indicated that miniaturized sources were lacking low frequencies. A series of processing tests were conducted to examine the signal strength and frequency content of the two previously acquired 2D seismic source tests. Both of these datasets compared the same small, miniaturized source, which was deployed at a depth of 1 m, with a larger conventional source. However, in one dataset, the conventional source was deployed at 3 m, and in the other dataset, the conventional source was deployed at 6 m.

In general, despite the difference in charge size and depth, the final processed datasets from the two different source types were very similar. As expected, processing results did show that the smaller, shallow source had lower energy than the conventional explosive sources. However, this decrease in energy could be compensated for with a good deconvolution operator and, unexpectedly, the overall frequency content between the large and small sources was very similar. Greater differences between the sources were observed on the dataset that had the larger variation in source depth. This is hypothesized to be due to near-surface effects rather than the effectiveness of the miniaturized source since traditionally, a deeper shot hole depth provides better source coupling.

These unexpected results indicate that shallow deployment of some miniaturized seismic sources can provide sufficient energy and resolution for imaging subsurface seismic targets, and as a result, an investigation into the impact of combining miniaturized and conventional sources for improved data quality was not further conducted. It should be noted that great care was taken in applying the processing algorithms and results may vary if different processing algorithms are applied. This is an important area for further research. Additionally, the reader is cautioned to **not** automatically assume all miniaturized sources are created equally. It is recommended that prior to utilizing a miniaturized source on a seismic program, it is thoroughly tested and compared with a conventional source. Finally, this research did not address potential operational issues associated with deploying miniaturized sources on narrower seismic lines.

## Development and Implementation of Software Algorithms

A primary goal of this research project was to incorporate the learnings from the geometry decimation and miniaturized source processing tests into an easy-to-apply software algorithm for implementing reduced land footprint seismic surveys. In order to achieve this goal, several different algorithms and tools were developed. This included algorithms to automatically generate EcoSeis geometries and optimize them for efficient field operations and the classification of various input layers such as habitat and species types for application of the different EcoSeis geometries. This work occurred throughout the entire project with updates as new developments and results were generated from the processing tests.

As part of the algorithm verification, two environmental datasets provided by project participants were analyzed and then used to develop the EcoSeis classification methods. Additionally, although not a direct goal in this phase of the research, one dataset was used in a preliminary analysis to quantify not only the land footprint reduction but also the potential reduction in greenhouse gas emissions associated with the seismic acquisition field operations. These calculations were conducted by a third-party contractor specializing in greenhouse gas emission reductions in preparation for determining potential future EcoSeis greenhouse gas emissions reductions. A conservative mix of EcoSeis geometries with similar or higher trace density than the conventional geometry was used in the calculation which showed the EcoSeis program had a 37.5% reduction in total hectares cut. Despite



utilizing a lower land footprint reduction EcoSeis geometry, the greenhouse gas emissions were calculated to be 54% lower than the conventional program. Given these promising results, further validation testing is recommended.

## LESSONS LEARNED

The project successfully met and exceeded the key success metric for the Land and Biodiversity Program, that is reducing seismic line clearing by 25% to 50% with 27% to 62% reductions achieved depending on the type and method of EcoSeis implementation.

Table 1 summarizes the percent reduction in land footprint achieved with three different EcoSeis geometries (A, B and C) as compared with a conventional seismic program. As mentioned previously, the linear km reduction is independent of the source type utilized, but the reduction in total hectares cut varies with the source type. In this case, the calculations were completed using a conventional source cut-line 2.75 m in width, and a miniaturized source cut-line 1.75 m in width. It is expected that the actual deployment of an EcoSeis program would utilize a combination of source types. In comparison, a conventional seismic program acquired with miniaturized sources would have no reduction in linear kilometres of cut-lines and would only have a 22% reduction in total hectares (Ha) cut, highlighting the importance of an EcoSeis type solution for land footprint reduction.

**Table 1: Seismic Intensity Reduction**

Parameter	EcoSeis Geometry		
	A	B	C
Seismic Trace Density	45%	26%	0%
Linear km of Cut-lines	50%	23%	51%
Area Cut (ha) - Conventional Source	39%	27%	40%
Area Cut (ha) - Miniaturized Source	61%	49%	62%

The additional success metric defined as de-risking a miniaturized source for oil sands seismic imaging was also achieved with results exceeding initial expectations. Due to careful signal processing, the small seismic source evaluated in the project did not require a combination with conventional seismic sources. As a result of this success, the development of an algorithm for combining miniaturized and conventional sources was not pursued. However, based on experience with other miniaturized sources, this is likely required for other types. Therefore, a framework for the incorporation of mixed sources into the EcoSeis algorithm was developed.

Finally, an EcoSeis software algorithm was successfully developed and implemented in OptiSeis' software package. This involved extensive processing tests to verify the EcoSeis geometries had a sufficient resolution for accurate subsurface imaging, as well as the development of methods for safely and efficiently implementing an EcoSeis seismic program.



## Project Learnings

The project was more successful than expected with EcoSeis geometries and methodologies developed that can reduce the planned total linear km of seismic cut-lines by up to 51%; total hectares cut by 40% to 62% (depending on the type of equipment utilized); and based on preliminary calculations (to be verified with a follow-up field test), up to a 55% reduction in greenhouse gas emissions.

The miniaturized source validation test showed that some miniaturized sources can provide sufficient data quality for near-surface imaging. Based on the results, further research is recommended to verify the use of miniaturized sources for deeper targets and to develop combination methods for miniaturized sources that may have lower signal strength, in order to obtain the environmental benefits of utilizing the sources without compromising subsurface image quality.

EcoSeis processing tests were successful with multiple geometries validated. Learnings included determining trace density limitations for applying interpolation in processing.

All results were incorporated into the EcoSeis algorithm and coded in OptiSeis' software platform.

## Broader Impacts of the Learnings

In addition to the EcoSeis geometry tests, the project provided an opportunity to accurately test and compare conventional geometries with different trace densities. Trace density decreases with increasing source and receiver line intervals, with lower trace density surveys resulting in lower resolution, particularly at shallower targets. Generally, it's only possible to examine this effect from a theoretical perspective, but in this case, we had four datasets to compare: the original fully sampled dataset; the pseudo-decimation comparison dataset; the first conventional dataset tested in pseudo-decimation processing; and the higher trace density conventional dataset that had better trace density for shallow targets. An abstract is currently being prepared to show the benefits of designing surveys with higher trace density.

A key project learning that goes beyond the project scope was that seismic geometries successfully tested with synthetic data should also be tested with real-world data as results from synthetic tests may be overly positive due to the lack of complex diffractors and realistic signal-to-noise levels. Furthermore, although pseudo-decimation tests are a cost-effective method for quickly evaluating multiple geometries, it is critical that final validation occurs on a fully processed dataset where the decimation occurs at the beginning of the processing (raw shot stage).

During the project, a new area of research was identified and is being investigated further. It was found that there is a potential to use EcoSeis geometries to reduce the need for seismic source relocation due to surface exclusions. Results from the processing tests indicated that some of the EcoSeis geometries appear to provide better interpolation results across gaps caused by surface exclusions such as pipelines, waterways, etc. This may be due to improved interpolation results with geometry irregularities.

## Next Steps: EcoSeis Phase 2

The next steps for the project are to complete a full-scale field demonstration of the EcoSeis technology. The EcoSeis Phase 2 Field Trial is a three-year project that commenced in February 2022. The project will involve extensive environmental assessments, the acquisition of an EcoSeis program in conjunction with a conventional



seismic program, and the analysis of both technical and operational data. As a result of the test design, direct comparisons between EcoSeis and conventional seismic methods will be possible enabling quantitative comparisons of imaging and environmental impact. Upon completion of the field trial, the EcoSeis method will be made available as a service and as a software solution for designing, modelling, and implementing seismic surveys.

Two publications that summarize the results of the research to date for both Phase 1 and Phase 2 are included below. Additional publications are in preparation and will be included in the Phase 2 research summary.

## **LITERATURE CITED**

Cordsen, A., Galbraith, M. and Peirce, J. [2000]. Planning Land 3-D Seismic Surveys. Society of Exploration Geophysicists, Tulsa.

## **PRESENTATIONS AND PUBLICATIONS**

### **Journal Publications**

2023 (January) The Leading Edge, Vol. 42, No. 1: 61-68. EcoSeis: A novel acquisition method for optimizing seismic resolution while minimizing environmental footprint.

2023 (January) First Break, Vol. 41, No. 1: 77-86. The use of miniaturised seismic sources for reduced environmental impact.

### **Conference Presentations/Posters**

2021 (October): EAGE 82<sup>nd</sup> Annual Conference & Exhibition, Session: Seismic Acquisition & Processing — High Resolution for Shallow Subsurface, Title: Case Study: The use of miniaturized seismic sources for reduced environmental impact.

2021 (September): GeoConvention, Title: Case Study: Testing a Miniaturized Source for Reduced Environmental Impact.

### **Reports & Other Publications**

2022 Alberta Innovates Public Report on Phase 1

2021 (September): Innovation Hacks, Interview with Andrea Crook

2021 (May): COSIA Land Challenge — Reducing Seismic Exploration's Footprint

2021 (May): CSEG Recorder: Interview with Andrea Crook

2021 (March): GUSSOW, Panel Discussion: Future of Heavy Oil and Oil Sands

2021 (March): GUSSOW, Seismic Acquisition Innovations for Oil Sands

2021 (February): Innovation Hacks: Interview with Andrea Crook

2021 (February): 1<sup>st</sup> CSEG Virtual Learning Series 2021: A Showcase of Canadian Innovation, Seismic



## AWARDS

Second Place at the [COSIA Land Challenge Competition](#), funded by Alberta Innovates.

## RESEARCH TEAM AND COLLABORATORS

Institution: OptiSeis

Principal Investigator: Andrea Crook

Name	Institution or Company	Degree or Job Title	Degree Start Date (Students Only)	Degree Completion Date (Students Only)
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Dr. Mostafa Naghizadeh	OptiSeis Solutions Inc	Geophysics Research		

\*Note: Not an exhaustive list; some researchers and collaborators are not included here.



# Edge Effects of Seismic Footprint in the Lowland Boreal Ecosystems of Alberta

**COSIA Project Number:** LE0081

**In Situ**

**Research Provider:** Canadian Forest Service — Natural Resources Canada

**Industry Champion:** Imperial

**Industry Collaborators:** Canadian Natural, Cenovus, ConocoPhillips, Suncor, Syncrude, Teck

**Status:** Year 1 of 3

## PROJECT SUMMARY

This study addresses the extent and nature of edge effects (Murcia, 1995) of seismic lines in lowland ecosystems of the northern Alberta landscape. Oil and gas development has resulted in a dense (up to 40km/km<sup>2</sup> [Filicetti et al., 2019]) network of seismic lines, combined with other industrial disturbances (e.g., roads, well pads, pipelines), leading to extensive landscape fragmentation. Persistence of seismic lines and lack of canopy regeneration in boreal ecosystems is particularly notable in boreal treed peatlands (van Rensen et al., 2015). Despite the potential ecological risks associated with edge effects caused by industrial disturbances such as seismic lines (e.g., loss of native biodiversity, establishment of invasive species [Bender et al., 1998; Haddad et al., 2014]), there is little information on their nature or how they might be mitigated in boreal forests and peatlands, nor the potential for restoration efforts to remove edge. This study builds on the research projects on edge effects in boreal ecosystems (including peatlands) already initiated and carried out by the Canadian Forest Service in the department of Natural Resources Canada (Dabros et al., 2017; Dabros et al., 2021).

Persistence of ubiquitous seismic lines in Alberta's boreal region, along with their edge effects can lead not only to change in native biodiversity, but also to altered habitat quality for native species of flora and fauna (Bender et al., 1998; Haddad et al., 2014). This in turn may change the resilience and successional trajectories of the disturbed ecosystem. Knowledge of the full extent of influence of industrial linear features such as seismic lines on a range of organisms (plants, lichens, arthropods, soil fungi) is important to inform questions of recolonization during site recovery. Further, characterization of recolonization by native biodiversity, when active treatments (e.g., restoration including silvicultural practices such as site preparation and tree planting) are employed is of value. The study of edge effects is valuable not only to help better understand the impact of seismic lines, but also for continual improvement of restoration practices. The results from this study will contribute to the understanding of whether restoration practices mitigate edge effects, which may help identify important indicators of restoration performance and thereby lead to continual improvement opportunities

The general objectives of this study are:



1. To assess edge effects on biotic and abiotic factors. The hypotheses are that edge effects are proportional to line width and degree of ground alteration. The degree of changes in environmental conditions associated with the disturbances (abiotic factors) will affect responses of biotic factors, e.g., diversity and abundance of various vegetation functional groups, lichens, arthropods and fungi.

In addressing this objective, researchers found synergy with another research project that is a part of the [Boreal Ecosystem Recovery Assessment \(BERA\)](#) collaborative research initiative. This allowed this research to address a question regarding potential edge effects on plant functional traits (e.g., do plant species on seismic lines possess traits that allow them to persist or thrive on the lines, and potentially prevent or inhibit other species from establishing? Do plant functional traits vary with proximity to forest edges?), as well as to explore the relationship between plant functional traits, their environment, and remotely sensed variables (and ultimately, to test the application of remote sensing as a cost-saving tool to detect edge effects and optimize restoration practices of seismic lines).

Research sites were set up in poor bogs of the Peace River region in NW Alberta (conventional seismic lines dating as far back as 1950s, Figure 1); moderate-rich fens in the Conklin region (near Christina Lake, Figures 1 and 2) in NE Alberta (conventional seismic lines dating back to 1990s, and low impact seismic lines from 2021); and for the BERA project, moderately rich fens in the Conklin region at Canadian Natural's Kirby South site (Figure 1).

2. To assess biotic and abiotic responses to edge over time. The hypotheses are that edge effects will diminish over time if the site is on the trajectory towards recovery. However, if succession is arrested, edge effects will persist or increase over time.

To address this objective, researchers plan to re-sample the research sites in Peace River that were initially sampled in 2015 to 2016. Additionally, resampling will occur at the upland research site in the Swan Hills region in NW Alberta (low impact seismic lines from 2011, which were sampled in 2014 and 2019, Figure 1). Potentially, researchers will also be able to determine the ages of seismic lines in the same area and assess if the age of the line is correlated to line recovery, or the extent and magnitude of edge effects.

3. To assess whether restoration treatment measures mitigate edge effects in adjacent boreal ecosystems, and to assess the interaction of time and treatment on edge effects. The hypothesis is that over time, successful restoration treatments will mitigate edge effects.

To address this objective, researchers plan to set up research sites in the summer of 2023 — either in the Woodlands CHRP Area A near Clyde Lake, that has received restoration treatment in the recent years, or in the CHRP Area near Winefred and Kirby Lakes (Canadian Natural lease), or potentially at the Kirby South sites in the moderately rich fens (all in the Conklin region of NE Alberta, Figure 1), where some lines have also received a mounding restoration treatment.

The identification of optimal treatment regimes to restore sites affected by an industrial footprint is one of COSIA's priorities and is also important in the implementation of Alberta's Caribou Habitat Recovery Program (CHRP). This is especially pertinent in Alberta, where over 200,000 km of conventional seismic lines need to be actively restored because they are located in threatened caribou habitat. Ultimately, this study will deliver knowledge on the extent of edge effects on baseline ecological components in boreal ecosystems (with a focus on peatland ecosystems), both biotic and abiotic, which may support the ability to model, predict, and plan for successful restoration of seismic line footprint. Science-based evidence about which methods are most effective in reducing the industrial footprint, including edge effects, can increase the effectiveness of restoration and reduce its costs. Furthermore,



while most research on edge effects focuses on plants only, in the present study, researchers will collect data on native assemblages of living organisms (biotic factors: percent cover and species diversity of plants and lichens, and species richness and diversity of arthropods and soil fungi) and their environmental conditions (abiotic factors: soil moisture, temperature, pH, electrical conductivity (EC), and light levels). As such, researchers will focus on the biodiversity of major drivers of ecosystem functioning, as well as their interactions with each other and their environment (i.e., abiotic factors), over time.



**Figure 1:** Map of all locations of current and potential future research sites in northern Alberta.



## PROGRESS AND ACHIEVEMENTS

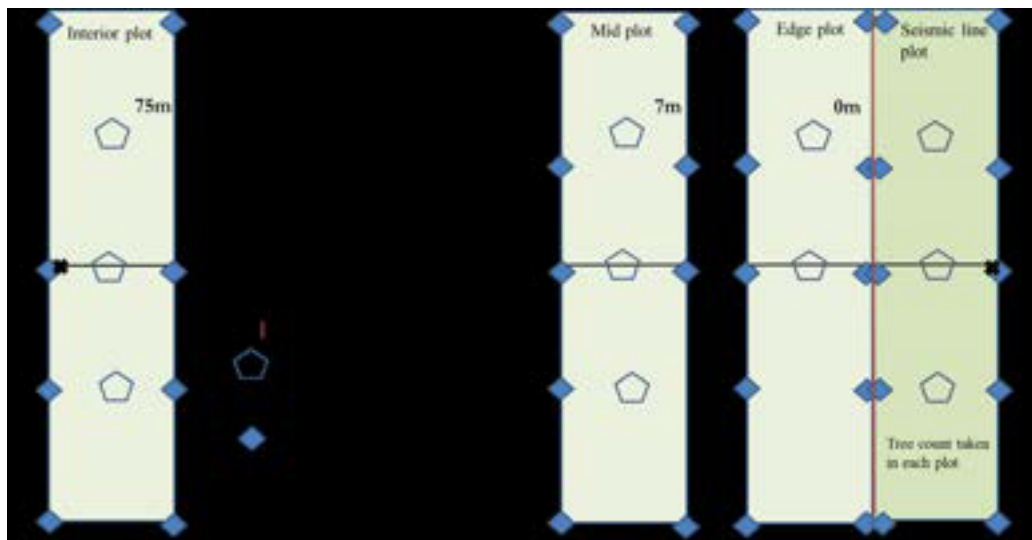
### Establishment of experiments and sampling

- The Conklin region was explored in spring 2022, and in summer 2022, new research sites were established near Christina Lake, in the moderate rich fens, on conventional seismic lines (approximately 8 m wide) found on MEG Energy's lease (Figure 2). To assess the extent of edge effects, researchers sampled on seismic lines as well as along ten replicate transects running 75 m from the line edge into the interior fen, in 1m<sup>2</sup> quadrants set up at various distances, by measuring environmental variables (soil temperature, moisture, pH and EC, as well as light reaching the ground) and vegetation percent cover by species. Density and height of the overstory were also measured, in 100 m<sup>2</sup> rectangular plots (20 m x 5 m) laid out on the seismic line, at 0 m to 5 m, 7 m to 12 m, and 75 m to 80 m, placed perpendicular to the transect (i.e., along the seismic line) (Figure 3).



**Figure 2:** Map of the transects established at the Christina Lake research site. CH1\_LIS and CH3\_LIS consist of ten transects established on low impact seismic (LIS) lines. The other sites (CH and NC) are on conventional seismic lines — five on East to West oriented lines, and five on North to South oriented sites.





**Figure 3:** Experimental design for sampling the overstory in Peace River and Christina Lake research sites in summer 2022. Within each plot, all trees were counted and separated into categories according to their size (seedling, sapling, pole, tree), density was measured in three spots with each measurement made in four directions using a densiometer and tree height and diameter at breast height (DBH) was measured for the eight trees at the corners and along the lengths of the plot (every 5 m).

- In the same general research area, 10 potential replicate sites were identified in two areas (five replicates per area: in the poor bog and in the moderate rich fen) along an approximately 3 m wide low impact seismic (LIS) line grid (with lines 80 m apart) constructed in 2021. At these potential sites researchers established loggers set to continuously measure soil temperature and light at 0 m (line edge), 5 m, and 40 m into the interior forested fen. These loggers were left in the fen over winter 2022 to 2023, and data will be collected and processed in the spring-summer of 2023 to determine the feasibility of these sites for future research on edge effects (in summer 2023).
- In the poor bogs of the Peace River region, the same methods were followed as those used in the Conklin region for assessing edge effects on environmental conditions, plants, and lichens along ten replicate transects on conventional seismic lines (approximately 8 m wide). These factors were already measured/sampled once before (2015 to 2016), so re-sampling will allow researchers to address the second project objective, assessing the impact of time on edge effects. Following the same methods used in the Conklin region, the overstory was measured for the first time.
- In addition, along five of the 10 transects at the Peace River site, arthropod pitfall traps were established to assess potential edge effects on arthropods. These traps were visited and collected every three weeks over the summer. Soil samples to assess edge effects on soil fungi were collected at these same locations.
- In the moderately rich fens at the Kirby South area, sample plots (100 m<sup>2</sup>, with ten 1 m x 1 m sub-plots) were distributed across four different site types; seismic lines constructed approximately 20 years ago; constructed in the spring of 2022; constructed 20 years ago and re-disturbed in the spring of 2022; and undisturbed interior fen 50 m away from the line edge. Five replicates of each site type were established, and plant community composition was determined for each plot, within the ten sub-plots. Researchers measured plant functional traits (height, leaf fresh and dry mass, leaf area, leaf nutrient composition) for 18 dominant plant species on the lines and in the interior fen. For the five sites with seismic lines constructed 20 years ago, samples and data were also collected on plant species composition at the edge and at 5 m, 10 m, 15 m, and 25 m from the edge,



to assess the extent of edge effects on plant functional traits. Remotely piloted aircraft systems based LiDAR surveys were collected in May, July, and August of 2022 (capturing both pre/post green-up conditions) over the same sites.

### Analyses progress and preliminary results

- For the Conklin site, soil moisture, pH and light were much higher on the seismic lines (approximately 30% for moisture and light, and 15% for pH), dropped sharply around 1 m away from the edge, and then went up again but remained lower than on the seismic line. The same pattern was observed for graminoid species and low/medium shrub cover. The abundance of forbs was lowest on the seismic line, relatively higher near the edges, and highest in the interior fen — approximately 35 m to 50 m away from the edge. Overall, plant species composition was distinctively different on the seismic lines compared to the adjacent fen.
- For the Peace River site, soil moisture and light were much higher on the seismic lines, followed by a sudden drop (approximately 20% for moisture and up to 50% for light) near the edge (dry zone with less light) to around 5 m away from the line edge. This was reflected by a lower abundance of herbaceous species and lichens in that zone. Overall, plant and lichen species composition was distinctively different on the lines compared to the adjacent bog.
- For the Peace River site, arthropod samples are currently being processed. So far around 5,000 spiders, 1,000 beetles, and 2,000 ants have been sorted and identified. The process of sorting and identifying may take another few months. Soil fungi samples are being processed in the lab (i.e., dried, broken up and mixed in a sterilized blender, freeze dried, and then DNA will be extracted). The diversity and abundance of the fungi present in the soil will then be assessed.
- For the Kirby South site, some plants functional traits are still being measured in the lab (over 1,000 samples were collected in the field), and statistical analysis will follow to determine the differences on the lines and in the interior fen, and then compared across different site types. For the seismic lines that were constructed 20 years ago, analysis will be performed to assess potential edge effects on plant functional traits. Remote sensing data is being currently processed to integrate drone-based structural data (LiDAR) and reflectance data (multispectral) to create maps of plant functional traits, and to evaluate differences in plant trait composition between seismic lines and the adjacent fen. Further analysis will be done to determine if edge effects influence plant functional traits, and if remote sensing can be used as a tool to detect the effects.

## LESSONS LEARNED

Preliminary results indicate that the conditions on the conventional seismic lines in peatlands (poor bogs and fens) are distinctively different than in the adjacent treed peatlands, even for lines up to approximately 70 years old. In all instances, the lines are much wetter, receive more light, and have a different plant species composition compared to the adjacent treed peatlands.

In poor bogs, there appear to be edge effects up to approximately 5 m away from the edge, with a lower abundance of herbaceous species and lichens — the conditions within that zone are also drier with less light, compared to further away from the edge. These patterns are similar to the results obtained from data collected in 2015 and 2016, indicating that edge effects did not change over that period.



In fens, there is evidence of edge effects within a 1 m zone away from the edge, where there is less light, and lower abundance of graminoids and low/medium shrubs.

Further monitoring over time is required to assess if the conditions on the seismic, and the associated edge effect will change.

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## PRESENTATIONS AND PUBLICATIONS

No public presentations or publications for 2022.

## RESEARCH TEAM AND COLLABORATORS

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Maria Strack	University of Waterloo	Professor		



## EQUIPMENT

# Restoration Equipment Modernization

**COSIA Project Number:** LJ0345

**In Situ**

**Research Provider:** FUSE Consulting

**Industry Champion:** Cenovus

**Status:** Year 2 of 3

## PROJECT SUMMARY

Forest habitat restoration frequently involves active silvicultural treatment to address site limiting factors that result in arrested succession or delayed return to forest cover. In Alberta, the amount of anthropogenic forest disturbance in need of active restoration treatment exceeds current capacity for treatment execution. To date, treatments have been based on various types of mechanical mounding or scalping with an excavator bucket or mounding rake. Although this approach can result in treatments of high quality, production is typically limited to about one kilometre per day per unit of equipment. Alternative approaches to mechanical treatment are available and are believed to have the potential to increase treatment speed, and thereby overall restoration capacity, significantly.

The scope of this project is to test various alternatives for mechanical site preparation on a range of site-types representative of legacy linear disturbance in the boreal forest. Alternatives tested include a range of drawn implements such as the 3-Row Bracke Forest moulder specifically designed for forest soil scarification (Bracke moulder), as well as excavator attachments and mounding techniques.

The key objectives of this work are to identify tools and methods for mechanical site preparation that offer improvements on the current status quo (inverted mounds created with an excavator bucket) in terms of both quality and efficiency.

Forest habitat restoration and in particular, linear restoration, are key priorities for the resource industry in Alberta as evidenced by requirements for caribou recovery, Sub-regional Plans under the Alberta Land Stewardship Act, and institutional commitments such as those made at COP 15 in Montreal. Unfortunately, industry has yet to find a reasonably efficient means of applying restoration treatments. With status quo linear treatment costs typically greater than \$12,500 per kilometre, active restoration treatments may cost more than one billion dollars. In addition, while there are great examples of high-quality treatment outcomes, there are also examples of failures, and the consistent application of high-quality treatments remains elusive.

## PROGRESS AND ACHIEVEMENTS

Mechanical treatments were applied in the fall of 2021 and early spring of 2022, followed by tree planting in July 2022. Treatments were established within a controlled and replicated (before-after-treatment-control) design. Operational efficiency was assessed by measuring speed and reliability. Quality parameters assessed included microsite height and volume, seedbed quality, plantability, mixing, persistence, soil moisture, soil temperature and vegetation ingress. Data were compiled and averaged for measurements taken between Fall 2021 and Summer 2022.



The drawn implements tested were; bottom plow, cultivator, disc, potato plow, box blade, disc, line drum, harrows, ripper shank and a Bracke mounder. The excavator methods tested included standard (inverted) mounds, upright mounds, and upright mounds using a tree scoop.

Three types of implements were eliminated from further work within the trial due to issues of either reliability or microsite quality: harrows (low treatment quality), cultivator with sweeps (prone to debris accumulation within the shanks), and ripper plow (prone to debris accumulation within the shanks).

Assessment of treatment speed revealed that, as expected, drawn implements present significant promise with respect to efficiency, with speeds generally in the range of two to 10 minutes per 100 metres. In contrast, methods employing an excavator bucket were in the range of 30 minutes to 33 minutes per 100 metres on uplands, and 43 minutes to 54 minutes per 100 metres on lowlands.

As expected, excavator mounds were of high quality and acceptable density. It was noted that upright techniques have the benefit of maintaining some intact vegetation including trees thus offering an apparent immediate advantage. Of the drawn implements, the Bracke mounder could produce mounds of similar height to excavator methods, although quality varied with varied soil conditions. In lowlands, which are sites of particular significance for site preparation, all the treatments significantly lowered the moisture content of the microsite (control approximately 80%) and treatments (40% to 60%). The ultimate measures of treatment quality will be measures of conifer stocking and growth rate and carbon storage, which will require long term evaluation.

## LESSONS LEARNED

Development of an effective alternative to conventional inverted mounds using an excavator has the potential to significantly reduce restoration treatment cost as well as reduce variability in treatment quality. Although adapted agricultural or construction implements have some potential, they are not yet capable of producing high quality microsites in a consistent, reliable fashion. The Bracke mounder has the most potential of the implements tested in the trial and significantly exceeded expectations — rapidly creating good quality microsites in a consistent fashion. Drawn mounders such as the Bracke mounder are purpose-built silviculture equipment that have benefited from decades of continuous improvement. However, in these tests they are being deployed in lowlands that are outside the envelope of typical site conditions where they are commercially deployed. It is believed that with increased testing and adaptation, the mounds created by the Bracke mounder can be further improved. Excavator-based methods continued to produce consistent mounds of the highest quality of all, albeit at low treatment speed. Choice of treatment equipment and method will involve a trade-off between speed and quality—although future technical innovation and change will be the key to making this trade-off more elastic. Ultimately, results from longer-term monitoring and statistical analysis are required before firm conclusions can be drawn about the effectiveness of these treatment approaches.



## RESEARCH TEAM AND COLLABORATORS

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Principal Investigators: Michael Cody and Jason Barrie

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