



2021 COSIA LAND EPA

In Situ Research Report

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INTRODUCTION

This report is funded by members of Canada's Oil Sands Innovation Alliance (COSIA) 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)
Teck Resources Limited (Teck)

COSIA LAND EPA publishes two reports, 2021 COSIA Land EPA – Mine Research Report and 2021 COSIA Land EPA – In Situ Research Report. This report summarizes progress for projects related to in situ reclamation of the COSIA Land EPA.

The project summaries included in this report do not include all projects completed under the Land EPA. As in 2020, some projects in 2021 were significantly impacted by the COVID-19 pandemic and consequently no summary is provided this year. Please contact the Industry Champion identified for each research project if any additional information is needed.

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*In 2020, Cenovus acquired Husky Energy Inc. All COSIA Land EPA projects previously supported by Husky Energy Inc. were transferred to Cenovus.

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WETLANDS

Removing the Wellsite Footprint (iFROG)

COSIA Project Number: LJ0216

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 5 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 2021, three projects were supported: Japan Canada Oil Sands Limited (JACOS) Road Reclamation, From Dirt to Peat, and Pad TT Road Construction Best Practice.

JACOS Road Reclamation Study (Year 3 of 5)

The JACOS 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 (Year 3 of 3)

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 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 3 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 or not 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

JACOS Road Reclamation Study

Bog species have established on the organic section of the reclaimed road, but many competitive undesirable species such as tickle grass (*Agrostis scabra*) and alsike clover (*Trifolium hybridum*) have also established — likely originating from the seed bank within the stockpiled peat. In 2021, woolgrass (*Scirpus cyperinus*) and water sedge (*Carex aquatilis*) became dominant, replacing both tickle grass and alsike clover in the organic section. The overall vegetation community differs between two subsections of the organic section. This is believed to be based more on the timing of bog species propagule inoculation than on substrate depth. Both of these communities differ from the adjacent bog reference. A subsection of the road which had a one growing season delay between construction (excavation and refilling with peat) and inoculation with propagules, was characterized by an abundance of grass and early successional forbs. In contrast, a subsection of the road where there was no delay before propagule inoculation had an abundance of clover, sedges, and rushes. The apparent ecological drivers on the delayed inoculated subsection were soil nutrients and organic matter depth, while the principal driver for the non-delayed inoculation subsection was distance to the subsurface drainage channels used to connect water between upstream and downstream sides of the road. The organic section as a whole is still going through rapid changes in vegetation in response to variable weather conditions (e.g., very wet 2020 growing season and relatively dry 2021 growing season). While it is too early to conclude the outcome of vegetation succession, the expectations are that prevailing moisture conditions and soil chemistry will favour the applied bog species and the undesirable species will diminish over time.

Fen species including sedges (*Carex* spp.) and brown mosses (e.g., *Tomenthypnum nitens*) established on the mineral section of the road, and fen vegetation cover increased from the first to second year of the study. Fen species were significantly more abundant on inoculated plots than non-inoculated controls. Compared to 2020, drier growing season in 2021 led to higher cover of both fen species (particularly *Carex* spp.) and undesirable species such as alsike clover. True mosses (e.g., *Drepanocladus aduncus*) that were abundant in wet areas in 2020 are showing stress due to drought in 2021. Primary environmental drivers of vegetation occurrence were soil pH, electrical conductivity, and magnesium concentration. Subsurface drainage channels were more responsible for soil water chemistry changes in the adjacent peatland than surface flow, but changes were limited to two metres from the road in either case. While the road still impedes water flow from the upstream side to the downstream side, the increased effect on adjacent water chemistry near the drainage channels indicates the channels are assisting in reducing the impediment to water flow caused by the road.

From Dirt to Peat

Peatland vegetation has established at all of the sites studied within this project and peatland species are generally more abundant than non-peatland species. Nevertheless, all vegetation communities on reclaimed sites remain different from their adjacent peatland references. However, the two sites that were inoculated with peatland propagules are more similar to reference communities than sites that were not inoculated. Greatest peatland species development was observed on sites with mixed soil substrates, but peat vegetation development on mineral substrate was similar to that found on peat substrate.



All sites are accumulating peat, but depths vary widely among sites depending on vegetation type, soil chemistry, moisture, and site age. Further analysis of data is required to understand which sites are most successful in carbon sequestration and storage. The organic substrate section of the reclaimed JACOS road sequestered more carbon in the form of CO₂ (carbon dioxide) via photosynthesis than it released through respiration, with net carbon gain similar to adjacent peatlands. However, the reclaimed organic section released more carbon in the form of methane (due to anaerobic decomposition) than the adjacent peatland, particularly on deeper peat substrate. There was very little carbon flux on the mineral section due to sparser vegetation, particularly during the first year of the study (2019). GHG data from 2020 and 2021 are being processed. Continued change in carbon flux is expected as vegetation continues to develop in coming years.

Pad TT Road Construction Best Practice

Preliminary results from measurements of water table elevation immediately adjacent to culverts, log bundles, and HDPE (high density polyethylene) pipe bundles on both the upflow and downflow sides of the road demonstrated differing drainage patterns among the conduits as the overall water table dropped over the season. There was no difference in water table elevations between sides of the road adjacent to culverts that were embedded into the peat to a depth at least 50% of their diameter, indicating that the culverts maintained water flow through the road over the range of water table conditions during 2021. Both log and pipe bundles rested more or less on the peat surface, rather than being more deeply embedded. The water table did not differ between sides of the road adjacent to pipe or log bundles early in the season when the overall water table was higher. However, the water table remained higher on the upflow side of the road than the downflow side when the water table subsided later in the season, indicating that the bundles facilitate flow well when the water table is near or above the peat surface, but do not contribute as much to flow when the water table is below the peat surface. The opposite pattern was observed adjacent to corduroy sections where no additional conduits were installed, indicating that the corduroy does not contribute as much to movement of surface water through the road, but does facilitate movement of subsurface water through the road.

LESSONS LEARNED

JACOS 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. However, 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, initial vegetation established, climate, and other factors. Alternatively, bog vegetation can immediately be established on a peat substrate of appropriate pH. However, 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 establishing 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. 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 sufficiently delayed by competition from undesirable vegetation.

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 also 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.

Pad TT Road

Study results to date indicate that increasing the number and type of drainage conduits over a given length of road improves drainage and reduces road-caused flow impediment. A range of conduit types are available, giving operators flexibility in designing systems to reduce flow impediment — the key being to address both surface and subsurface flows, as well as peak and non-peak flows. Culverts alone might accomplish addressing surface and subsurface flow, provided a sufficient number are installed, they are large enough, and embedded to a depth that accommodates both surface and subsurface flow. Alternatively, multiple smaller culverts could also be embedded at varying depths to ensure both surface and subsurface flow. Using materials available on hand, such as timber or surplus pipe liner to make bundles as surface flow conduits, is an economical way to increase surface flow, allowing more judicious use of expensive culverts. Using corduroy as a road foundation over peatland appears to facilitate flow under the road, thereby reducing the overall flow that would be required to be transported via culverts alone, while also reducing the amount of earthen fill required.

PRESENTATIONS AND PUBLICATIONS

Published Theses

Guérin, P. 2020. Restoration of fen plant communities on the mineral substrate of a peatland impacted by a road. MSc Thesis. University of Laval.



Conference Presentations/Posters

Guérin, P., Rochefort, L. and Xu, B. 2021. Restoration of fen plant communities on the mineral substrate of a peatland impacted by a road. Colloque annuel du Centre d'études nordiques – 60e anniversaire du CEN (virtual), Québec, Québec, 11-12 February 2021. (Poster)

Guérin, P., Rochefort, L. and Xu, B. 2021. Restoration on a mineral substrate of a peatland impacted by a mineral road. SER2021 – 9th World Conference on Ecological Restoration (virtual), USA, 24 June 2021.

Isabel, C., LeBlanc, M. -C. and Rochefort, L. 2021. Partial removal of an access road in a *Sphagnum*-dominated peatland: does peat amendment thickness and fertilization matter? Colloque annuel du Centre d'études nordiques – 60e anniversaire du CEN (virtual), Québec, Québec, 11-12 February 2021. (Poster)

Isabel, C., LeBlanc, M. -C. and Rochefort, L. 2021. Restoration of a *Sphagnum*-dominated peatland disturbed by a road: do peat amendment thickness and fertilization matter? Society of Wetland Scientists (SWS) 2021 Annual meeting (virtual), USA, 8 June 2021.

Isabel, C., LeBlanc, M. -C. and Rochefort, L. 2021. Restoration of a *Sphagnum*-dominated peatland disturbed by a road: plant establishment on introduced peat substrate is driven by restoration techniques. SER2021 – 9th World Conference on Ecological Restoration (virtual), USA, 24 June 2021.

Osko, T., and Albricht, R. 2021. Industry Research Collaboration for Advancement of Ecological Knowledge and Practices in Alberta's *In Situ* Oil Sands. SER2021 – 9th World Conference on Ecological Restoration (virtual), USA, 23 June 2021.

Pouliot, K., Rochefort, L., Guérin, P. and Beauchemin, A. 2021. Contrasting restoration methods for peatland impacted by roads: Burial Under Peat Method vs. peatland initiation on mineral substrate. SER2021 – 9th World Conference on Ecological Restoration (virtual), USA, 22 June 2021.

Pouliot, K., Rochefort, L., Isabel, C., Guérin, P., Beauchemin, A., Gagnon, J. and Jean, P. -O. 2021. Access roads in pristine and extracted peatlands: Restoration techniques. The 16th International Peat Congress (virtual), Tallinn, Estonia, 4 May 2021.

Albricht, R. and Osko, T. 2020. Research Collaboration for Advancing Best Practices in the *In Situ* Oil Sands. Presentation at World Wetlands Day, Mount Royal University, Calgary. 4 February 2020.

Guérin, P., Rochefort, L. and Xu, B. 2020. Restoration on a mineral substrate of a peatland impacted by a mineral road. 26e Symposium du Groupe de recherche en écologie des tourbières et rencontre annuelle de l'équipe Carbone McGill / 26th PERG Symposium and McGill Carbon team Annual Meeting, U. McGill, Montréal, Québec, 21 February 2020. (Poster)

Guérin, P., Rochefort, L. and Xu, B. 2020. Restoration on a mineral substrate of a peatland impacted by a mineral road. 40e colloque annuel du Centre d'études nordiques, UQAM, Montréal, Québec, 13-14 February 2020. (Poster)

Isabel, C., LeBlanc, M.-C. and Rochefort, L. 2020. Restauration d'une tourbière à sphaignes perturbée par une route d'accès en argile dans le nord de l'Alberta. 26e Symposium du Groupe de recherche en écologie des tourbières et rencontre annuelle de l'équipe Carbone McGill / 26th PERG Symposium and McGill Carbon team Annual Meeting, U. McGill, Montréal, Québec, 21 February 2020. (Poster)



Isabel, C., LeBlanc, M.-C. and Rochefort, L. 2020. Restauration d'une tourbière à sphaignes perturbée par une route d'accès en argile dans le nord de l'Alberta. 40e colloque annuel du Centre d'études nordiques, UQAM, Montréal, Québec, 13-14 February 2020. (Poster)

Osko, T. 2020. Research Collaboration Advances Best Practices and Ecological Outcomes for In Situ Oil Sands. CLRA Alberta Chapter Annual General Meeting and Conference (presentation), Red Deer, Alberta, 26-28 February 2020.

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	FPIInnovations	Senior Researcher		
Christine Isabel	Université Laval	MSc	2019	2021
Pascal Guérin	Université Laval	MSc	2019	2021

Research Collaborators: Dr. Maria Strack, University of Waterloo

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

COSIA Project Number: LE0037

Research Provider: University of Calgary

Industry Champion: Suncor

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

Status: Year 2 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 biodiversity.

BWRAP intends to answer 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 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 ≥ 100 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., 2009). 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 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 wet 10% to 17% 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', 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, 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 characteristic 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

Database architectural design and security protocols were approved by the University of Calgary, allowing hardware purchase, data compilation, and database development to begin. Water quality and selected biological data collected from a suite of natural and reclaimed wetlands between 2000 and 2015 were compiled and interpreted by three honours undergraduate thesis students. As well, remote sensing imagery of reclaimed lease areas and reference areas was shared by Syncrude and Suncor partners, which facilitated the search for suitable wetlands to sample during the 2021 field season. The success in locating suitable study sites was greatly aided by recommendations from Syncrude and Suncor field staff and other local experts.



Phase 2 - Field Investigations

In 2021, four 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. Lab members located and sampled the open water, emergent vegetation, and wet meadow zones of 40 young wetlands, ranging in age from two to 40 years, with open water area between 0.05 ha and 13.5 ha, and with specific conductance ranging from 250 $\mu\text{S}/\text{cm}$ to over 7,000 $\mu\text{S}/\text{cm}$. Soil texture composition ranged from sandy soils to a sandy loam, with a few silty loam sites. The specific conductance of water in the wet meadows was consistently around 20% higher than measurements taken in the open water zone. Young, ephemeral wetlands situated in reclaimed areas had considerably higher specific conductance than semi-permanent marshes or shallow open water wetlands.

Preliminary analyses of surface and groundwater radon concentration and stable isotopes of oxygen and deuterium are in progress. Together, these data will be used to infer the proportion of water in each wetland derived from surface water (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.

Data from field loggers (recording light intensity, temperature, conductivity, dissolved oxygen concentration and water level at 30-min intervals) are presently being analyzed to document day/night and seasonal trends within and among wetlands of each class. Logger records were consistent with spot readings taken at the time of biological sampling. Aerial drones and an autonomous water vessel were successfully deployed to provide imagery of topography and surface features, and bathymetry, respectively.

Surveys of vegetation provided estimates of community composition, biomass, and relative cover of the three zones. In all, 140 species were identified in the wet meadow and emergent zones across the 40 wetlands (submergent vegetation data are still being compiled). 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 and are awaiting processing and enumeration. Avian surveys were not conducted in 2021.

Preliminary results of two sets of benthic macroinvertebrate data analyses provided an intriguing contrast in the composition of communities sampled from young constructed and opportunistic wetlands versus a suite of pools in a naturally saline fen complex. Threshold Indicator Taxon analysis (Titan, King and Baker 2010) was performed on data from 39 ponds and opportunistic wetlands sampled between 1998 and 2012 (Kovalenko et al., 2013). Threshold values above which multiple (sensitive) taxa were absent were evident for two parameters: conductivity (1,200 $\mu\text{S}/\text{cm}$) and pH (8.1; Moore 2021). Similar thresholds were observed following analysis of published data of Preston et al. (2018) on benthic macroinvertebrates collected from brine-contaminated prairie pothole wetlands adjacent to oil and gas wells. In contrast, Vercruyse and Ciborowski (2021) who sampled macroinvertebrates from 52 pools in a naturally saline fen complex (conductivity range 3,757 $\mu\text{S}/\text{cm}$ to 20,170 $\mu\text{S}/\text{cm}$) observed greatest richness (24 families) at 9500 $\mu\text{S}/\text{cm}$. In this case, Titan identified 21 indicator taxa, 12 of which decreased in relative abundance along the conductivity gradient and nine of which increased. Community composition changed at an estimated threshold of 9,000 $\mu\text{S}/\text{cm}$ to 11,000 $\mu\text{S}/\text{cm}$.



LESSONS LEARNED

This program is still in early stages, with only the first field 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. Current field work has also found that many aquatic invertebrate taxa can occupy local natural wetlands with much greater salinity ranges than previously thought.

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PRESENTATIONS AND PUBLICATIONS

Published Theses

Gillis, E. 2021. Wet meadow community succession in constructed and opportunistic wetlands of the Alberta Oil Sands region. Hon. BSc Thesis, Biological Sciences, University of Calgary, Calgary, AB.*

Mebesius, L. 2021. Variation in wetland submerged plant community composition in relation to water salinity in boreal wetlands of the Alberta Oil Sands region. Hon. BSc Thesis. Biological Sciences, University of Calgary, Calgary, AB.*

Moore, E. 2021. Successional and disturbance controls on macroinvertebrate community composition in young boreal wetlands. Hon. BSc Thesis, Biological Sciences, University of Calgary, Calgary, AB.*

*Available upon request.

Conference Presentations/Posters

Ciborowski, J. J. H. 2021. Formalizing the Reclamation Assessment Approach (RAA) to evaluate wetland condition in reclaimed oil sands watersheds. COSIA Wetland Technical Sharing Session, Calgary, AB, November 5, 2021 (Virtual).

Ciborowski, J. J. H. 2021. Formalizing the Reclamation Assessment Approach (RAA) to evaluate wetland condition in reclaimed oil sands watersheds. Canadian Ecotoxicity Conference, Halifax, NS, October 2-5, 2021 (In person and virtual).

Ciborowski, J. J. H. 2021. Raising the bar on sustainable wetlands. Invited Presentation for World Water Day Lunchtime Symposium, University of Calgary, AB, March 25, 2021 (Virtual).

Moore, E. and J. J. H. Ciborowski. 2021. Successional and disturbance controls on macroinvertebrate community composition in young boreal wetlands. Entomological Society of Alberta Annual General Meeting, Calgary, AB, October 15-16, 2021, (Virtual).

Vercruyse, B. and J. J. H. Ciborowski. 2021. Variation of Aquatic Macroinvertebrate Community composition along a gradient of salinity in a boreal saline fen. Entomological Society of Alberta Annual General Meeting, Calgary, AB, October 15-16, 2021, (Virtual).

Vercruyse, B. and J. J. H. Ciborowski. 2021. Variation of aquatic macroinvertebrate community composition along a gradient of salinity in a boreal saline fen. Canadian Ecotoxicity Conference, Halifax, NS, October 2-5, 2021 (In person and virtual).



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		
Zach Wang	University of Calgary	Research Assistant (Drones)		
Ian Perry	University of Calgary	Research Assistant (Drone pilot)		
Erik Biederstadt	University of Calgary	MSc	2021	2023
Amanda Lizardo	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 Vercruyse	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

* 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.

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



SOILS AND RECLAMATION MATERIAL

Topsoil Replacement Depth Study

COSIA Project Number: LJ0335

Research Provider: NAIT Centre for Boreal Research

Industry Champion: ConocoPhillips

Industry Collaborators: CNOOC Petroleum North America ULC

Status: Year 1 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 in order 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 sph) in order 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 *Eryngia 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 singly-grown ericaceous shrubs 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 in order 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 in order 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 main trial (Trial 1) was laid out as a randomized block design with topsoil placement as a main effect and the use of pre-emergent herbicide as a split-plot factor (Figure 1). Trials 1 to 3 were established in spring 2021 with Trial 4 to be planted in spring 2022 — as such, all findings to date are preliminary.

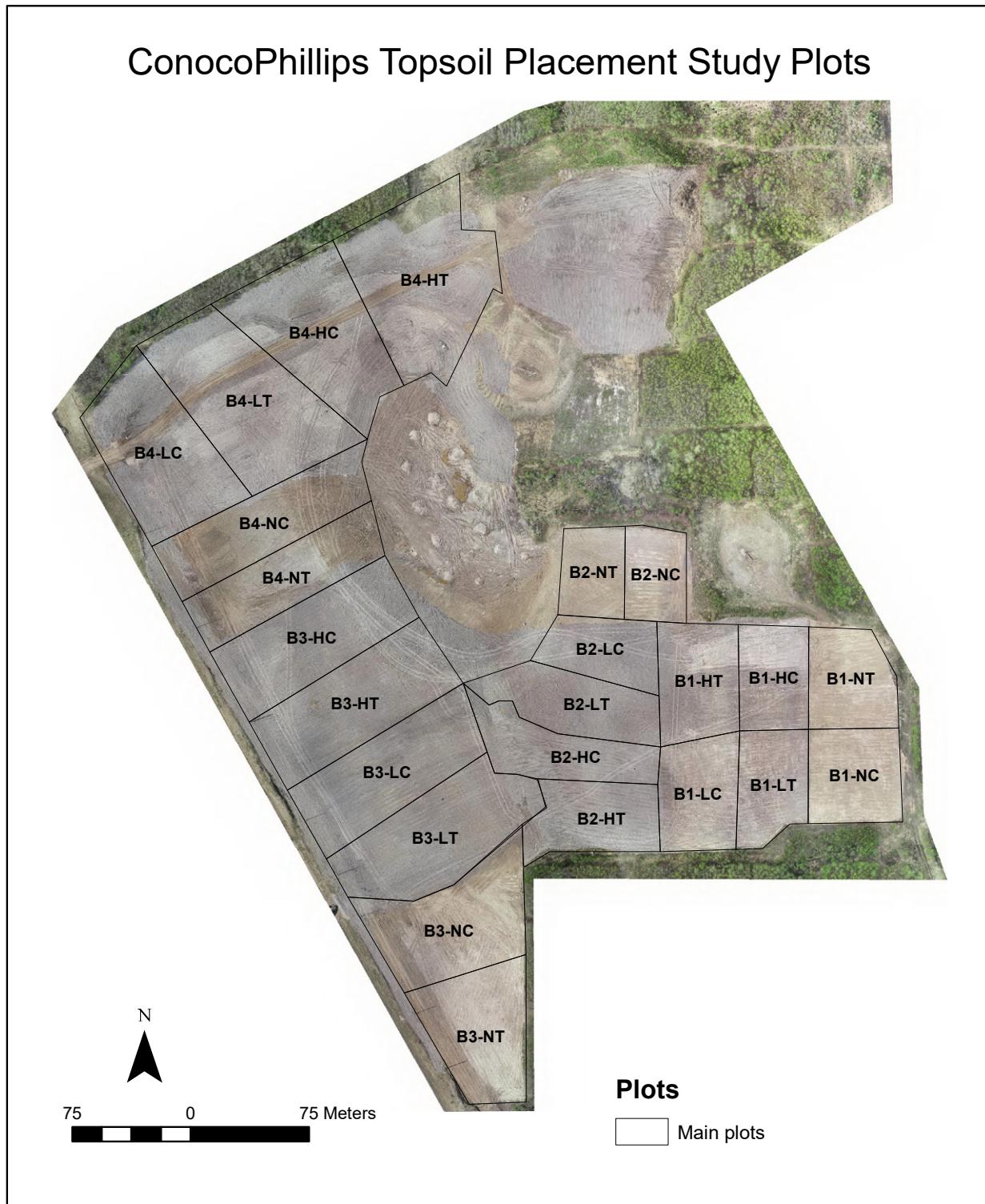


Figure 1: Layout of Trial 1 experimental treatment plots. The seedling stock Trials (2 to 4) are nested within these larger experimental units and are not shown to reduce crowding of the image. Treatment notations are as follows: B1 to B4 refers to the replicate study blocks, H/L/C refer to the standard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



Over seven hundred soil pits were excavated following earthworks, but prior to site decompaction activities, in order to validate that the topsoil placement treatments (shallow and standard) were in fact distinct. Although there was variation within treatments, the mean topsoil depth in the standard treatment was 12.3 cm (3.8 cm standard deviation [SD]) and in the shallow treatment was 6.0 cm (1.6 cm SD). Figure 2 provides visual examples of site conditions before and after decompaction, and Figure 3 illustrates the median values and spread amongst observations.



Figure 2: Images from June 2021. RipPlows™ were utilized to conduct site-wide decompaction of the study area following site recontouring and topsoil and subsoil placement. As these activities occurred sequentially and without time for soil settlement, it is anticipated that some soil admixing would have occurred.

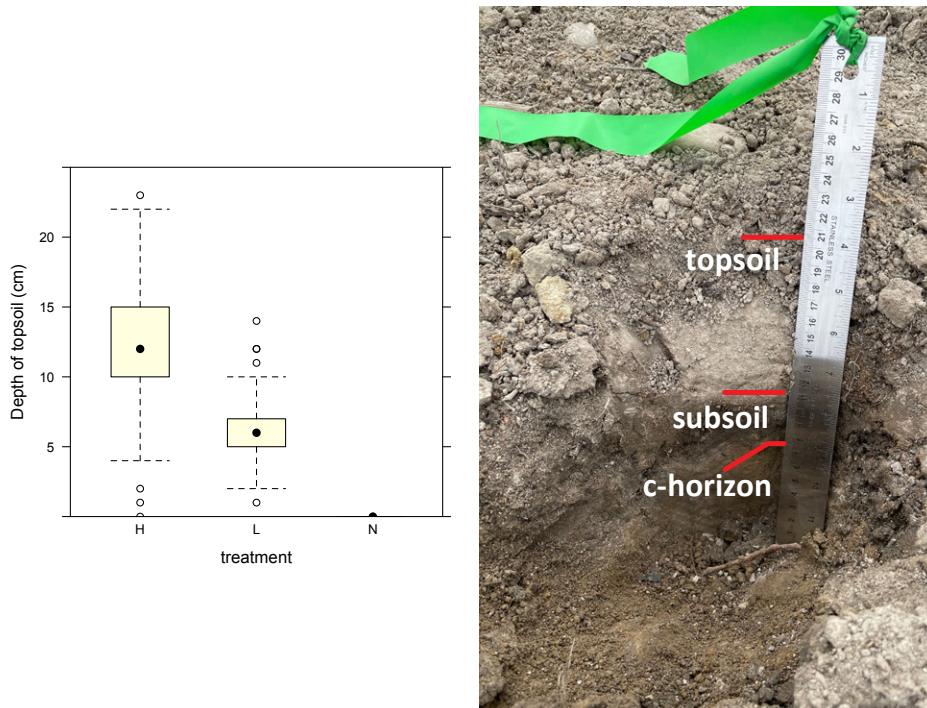


Figure 3: Box and whisker plot illustrating measured topsoil depth following placement in May 2021. Sampling was conducted less than three weeks following soil placement. Topsoil treatments were as follows: H (standard treatment), L (shallow treatment) and N is the no-topsoil treatment. N = 235 to 302 observations per treatment level. Note the image on the right of the figure shows an example of a soil pit and illustrates placed topsoil, subsoil (target was 5 cm experiment-wide) and underlying C-horizon (which tended to be coarser in texture).

The 2021 growing season was extremely dry throughout the region and initial herbaceous vegetation growth was extremely low experiment-wide (Figure 4). The overall dryness may have been further exacerbated by site decompaction activities, which occurred into early June as opposed to late fall of the prior year. The soil was worked after spring snow melt and therefore there was reduced soil water stored at the time of planting. Despite the limited herbaceous development, there were observable differences amongst soil depth treatments as well as with the pre-emergent herbicide treatment — plant species richness was consistently lower in the absence of topsoil and tended to be lower with pre-emergent herbicides (Figure 5). Conversely coverage of non-native forbs increased with the presence of topsoil and without herbicide (Figure 6).

There were no consistent patterns in woody coverage, stem densities or total height across the five tree and shrub species planted.

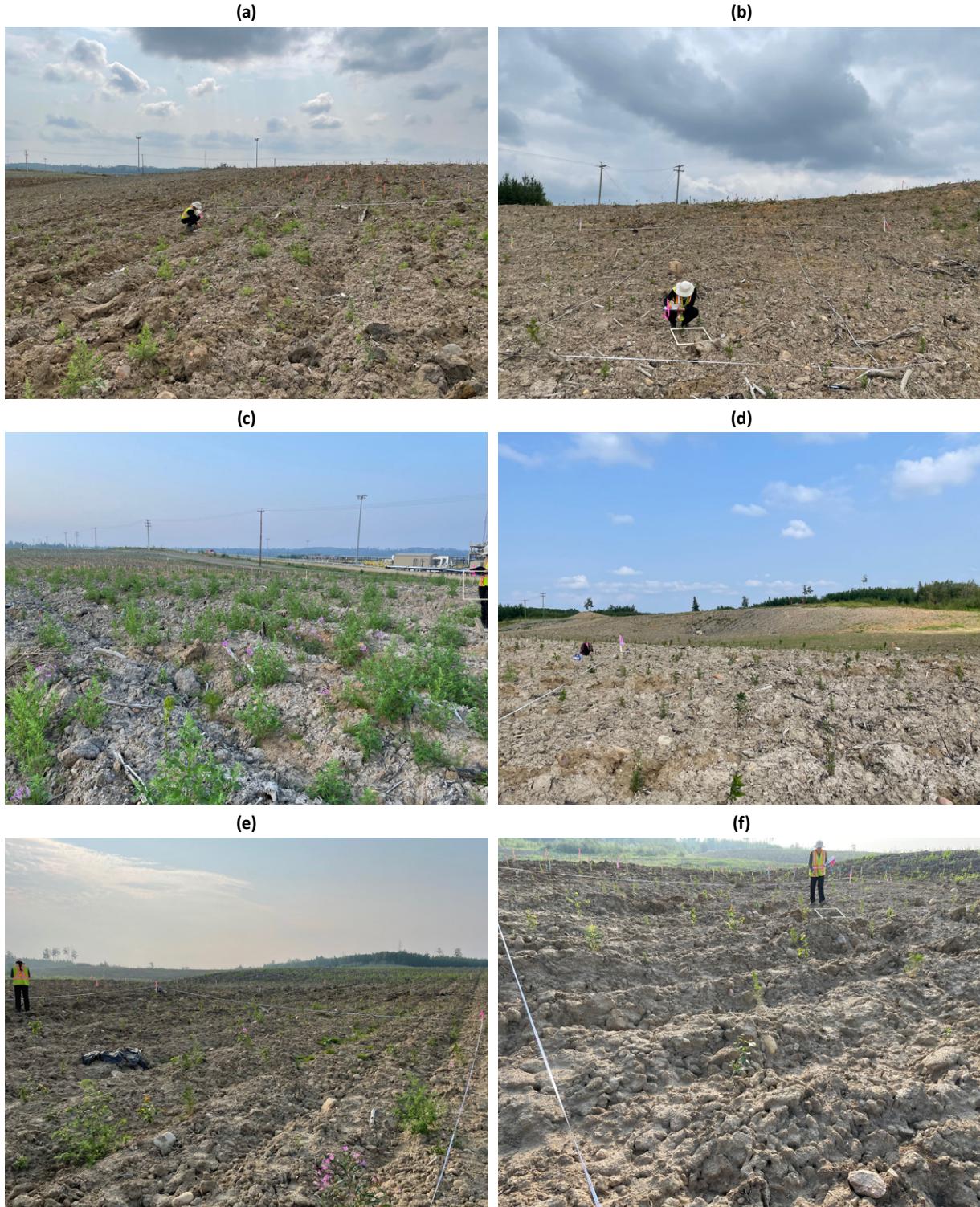


Figure 4: Illustrations of the study treatments in early August 2021 with: (a-b) standard topsoil treatment; (c-d) shallow topsoil treatment; and (e-f) no-topsoil treatment. Images on the left show no-herbicide application, and images on the right show the treatment with pre-emergent herbicide of adjacent split-plots.

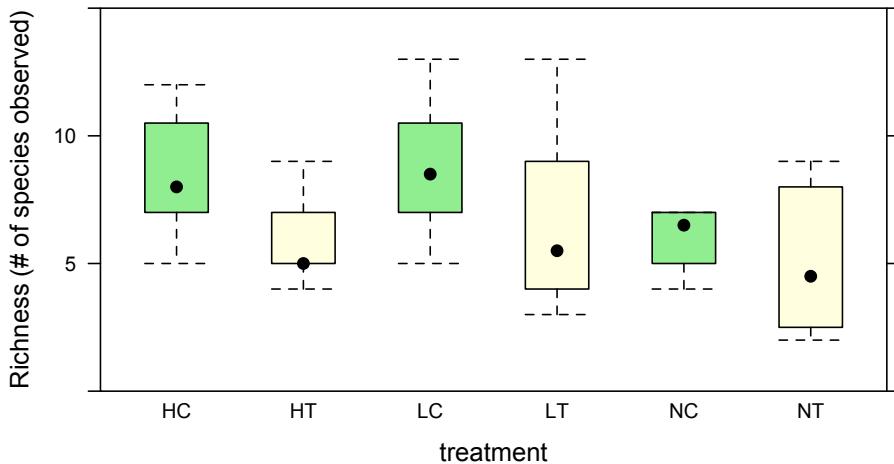


Figure 5: Box and whisker plot illustrating total species richness (per sampling plot) in August 2021 by topsoil depth treatment and pre-emergent herbicide (T) or no-herbicide (C). Results are based on two 15 m x 15 m permanent sampling plots per soil and herbicide treatment level per replicate block. Each sampling plot contained 11 quadrat measurements.

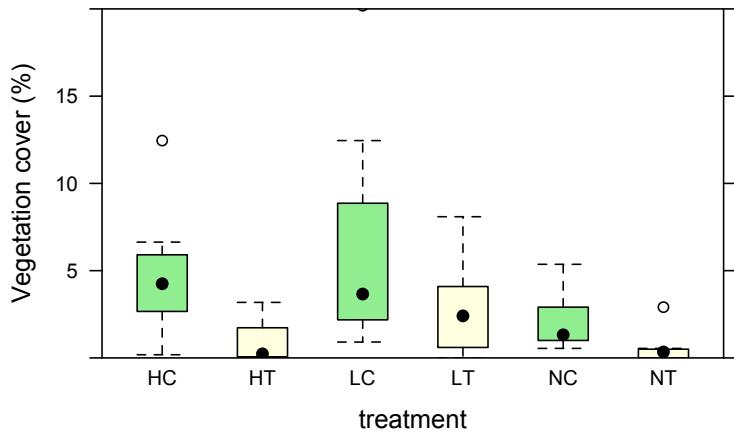


Figure 6: Box and whisker plot illustrating percentage cover of non-native forbs in August 2021 by topsoil depth treatment and pre-emergent herbicide (T) or no-herbicide (C). Results are based on two 15 m x 15 m permanent sampling plots per soil and herbicide treatment level per replicate block. Each sampling plot contained 11 quadrat measurements.

LESSONS LEARNED

This project is in early stages so there are no lessons learned to report for 2021.



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Schott, K. M., Pinno, B. D., Landhäusser, S. M. 2013. Premature shoot growth termination allows nutrient loading of seedlings with an indeterminate growth strategy. *New Forests* 44: 635-647

PRESENTATIONS AND PUBLICATIONS

No public presentations or publications in 2021.

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		
Kaela Walton-Sather	NAIT Centre for Boreal Research	Research Assistant		
Sofia Toledo	NAIT Centre for Boreal Research	Research Assistant		2021
Marie-Pierre Ouellet-Pariseau	NAIT Centre for Boreal Research	Student Research Assistant	2020	
Carlos Avila Jimenez	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
Katelyn Grado*	NAIT Centre for Boreal Research	Student Research Assistant	2019	2021



Emma Miller*	NAIT Centre for Boreal Research	Student Research Assistant	2019	2021
Derek Alcorn*	NAIT Centre for Boreal Research	Student Research Assistant	2018	2020
Carrie Pawlyna*	NAIT Centre for Boreal Research	Student Research Assistant	2018	2020

*Students assisted with the greenhouse propagation work at various stages in 2020.

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

DNA-Based Technologies to Evaluate Successful Reclamation

COSIA Project Number: LJ0294

Research Provider: Imperial

Industry Champion: Imperial

Industry Collaborators: ExxonMobil

Status: Year 5 of 6

PROJECT SUMMARY

Different reclamation practices have been implemented over the years at Imperial's Cold Lake in situ operations in Northern Alberta. 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 and monocultures.

Cost-effective tools to monitor reclamation trajectories are lacking. DNA-based tools, such as genomics, can be used to obtain comprehensive biological information from soil and potentially assess progress and trajectory in sites that have been reclaimed. 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.^(1, 4) These soil processes are necessary for the establishment of plant communities and ecosystem functions.^(2,3) The presence and abundance of soil microbial species or communities (e.g., bacteria, fungi, mesofauna) have shown to reflect the status of the environment in which they are found.⁽⁵⁾

The main goal of this project is to explore the application of genomics to evaluate reclamation trajectory at various reclaimed sites at Cold Lake operations by monitoring biological communities in soil at different ecological levels including plants (RBCL), fungi (ITS), and soil bacteria/mesofauna (18S).

The main objectives for 2021 were as follows:

- Apply genomics and statistical analyses to sequencing data collected in 2016 and 2018 to identify trends and potential biological indicators that can be used to infer reclamation progress and trajectory at selected sites;
- Assess physicochemical parameters in soils from selected reclaimed and natural sites and correlate with genomic sequencing data;
- Collect soil samples for genomic sequencing and physicochemical analysis in selected sites at Cold Lake Operations.

PROGRESS AND ACHIEVEMENTS

In 2016 and 2018 soil samples were collected at four selected reclamation sites, as well as two undisturbed (natural) sites at Cold Lake Operations. In 2019, soil was collected in the same site locations for physicochemical analysis including total organic carbon (TOC), organic matter (OM), pH, total Kjeldahl nitrogen (TKN), soil texture, and salinity. In 2021, soil samples were collected for genomic analyses, as well as for soil physicochemical properties. This year, three additional sites from undisturbed areas were added to the study. Sequencing data from samples collected this year are expected in early 2022. Bioinformatics and data analyses continued on samples collected in 2016 and 2018. Statistical analyses performed during this study are based on taxonomic abundance, biodiversity, and dissimilarity metrics using R v3.5.1⁽⁶⁾ packages, including vegan 2.5.5, phyloseq 1.26.1, and indicspecies 1.7.6.^(7,8,9)

For this study, sites that are considered under the “older reclamation practices” are those where grasses and monocultures were planted (at least during first years of reclamation) and resulted in compacted soils with sandy loam textures. Sites considered under “newer reclamation practices” are characterized as having higher number of native-species (tree, shrubs, and grasses) planted, application of woody debris, and use of loose and rough soils (sandy loam).

The main findings for 2021 included:

Based on community similarity (Bray-Curtis) in a non-metric multi-dimensional scale analysis (nMDS), using samples collected in 2016 and 2018, the sites where newer reclamation practices were applied resulted in more similar microbial communities to those from the natural sites. Along the axis 1 (NMDS1), microbial communities from new reclamation sites were closer to the natural sites than communities from older reclaimed sites (Figure 1). Data also show that the microbial communities found in new reclaimed sites are becoming more distinct than the communities present in the sites where old practices occurred (Figure 1). Similarly, for plants and fungi, data suggest that differences in community composition are becoming more evident between sites where new and old practices were adopted (data not shown). More sampling is needed over time to confirm this trajectory.

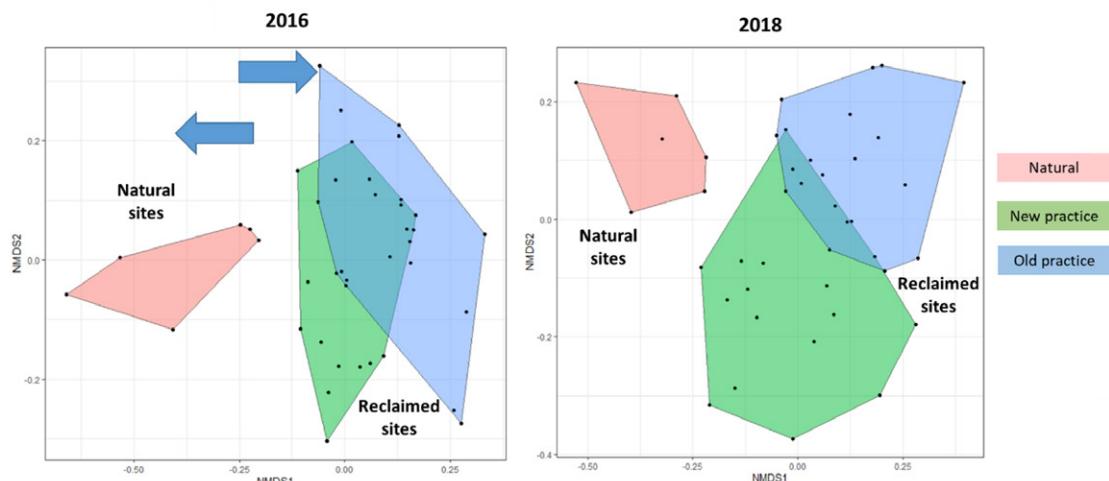


Figure 1: Community assemblage of microorganisms detected in samples collected in 2016 and 2018. nMDS plot based on the dissimilarity index (Bray-Curtis).



Indicator species analyses and relative species abundance from data collected in 2016 and 2018 shows there are specific taxa from fungi and microbes that are associated to natural sites, which are emerging in the reclaimed sites. For instance, ectomycorrhizal fungi (EM) including Atheliaceae, Thelephoraceae, Inocybaceae, and Pyronemataceae associated to natural areas were also shown to be associated with some of the reclaimed sites both with new and older practices (Table 1). In addition, from 2016 to 2018 there was an increase in relative abundance of EM and saprotrophic taxa such as Pyronemataceae, Thelephoraceae, Sebacinaceae in the reclaimed sites (Table 2). EM and saprotrophic fungi are known to form symbiotic associations with boreal tree species including spruce, aspen, and pine.^(10, 11) Soil bacteria (Bradirhizobium) and nematodes (Tylenchidae) were also taxa associated to natural sites which have emerged in samples from newer reclamation sites (data not shown), and these taxa are known to be involved in soil's nitrogen and nutrient cycles.⁽⁴⁾ The identified fungi and microbial taxa from this analysis can be potential indicator species of reclamation progress and they will be further explored.

Table 1: Summary of fungi taxa that have shown high association to natural sites and which were also associated to reclaimed sites in 2018 (data generated from *indicspecies* analysis).

Fungi (family)	New practice		Old practice	
	Site A	Site B	Site C	Site D
Thelephoraceae	X	X	X	X
Inocybaceae				X
Atheliaceae		X	X	X
Mortierellaceae			X	X
Herpotrichiellaceae			X	
Cortinariaceae	X	X		X
Pyronemataceae	X			X
Leotiaceae		X		
Russulaceae				
Gloniaceae	X			
Serendipitaceae		X	X	



Table 2: Relative abundance percentage of fungi (family) identified in soil samples from 2016 and 2018 within the natural sites and reclaimed sites.

Fungi (family)	Natural	New practice (%)		Old practice (%)	
		2016	2018	2016	2018
Thelephoraceae	24.94	0.92	3.75	5.75	14.46
Russulaceae	9.80	0.00	0.00	0.00	1.92
Clavariaceae	9.46	17.51	3.57	35.14	14.58
Cortinariaceae	9.23	0.11	6.49	0.01	2.16
Atheliaceae	7.52	5.21	4.53	1.97	13.44
Inocybaceae	6.57	13.16	4.38	5.92	5.98
Pyronemataceae	5.21	8.77	18.58	4.59	14.53
Herpotrichiellaceae	5.17	0.43	0.81	0.48	1.57
Hygrophoraceae	1.92	4.52	18.29	3.78	2.55
Sebacinaceae	0.48	11.79	15.01	0.77	7.94

In sites where new practices have been implemented, carbon- and nitrogen-cycle properties were not significantly different than in the natural sites (Table 3), while in those sites where old practices were applied, organic matter and total organic carbon were significantly different than in the natural sites (Tukey HSD, $p = 0.00254$, $p = 0.0125$, respectively) (Table 3). When looking at the carbon and nitrogen ratio, there were no significant differences observed between any of the tested groups (ANOVA, $p = 0.229$). pH and clay content were shown as the two properties that are distinctly different in the reclaimed sites relative to the natural sites ($p = 0$ for pH, $p = 0.00004$ and $p = 0.00001$ for clay content) (Table 3).



Table 3: Results from ANOVA and Tukey HSD analyses of the difference of mean values for total Kjeldahl Nitrogen (TKN), Organic matter (OM), total organic carbon (TOC), carbon and nitrogen ratio (C:N), pH and clay content from collected soils in natural sites and reclaimed sites (from old and new practices).

ANOVA Results			
Metric	F	p	df
TKN	3.234	0.0563	2
OM	4.653	0.0192	2
TOC	5.245	0.0125	2
C:N	1.567	0.229	2
pH	65.75	1.1E-10	2
Clay content	20.46	5.45E-06	2

ANOVA post hoc test (Tukey HSD)			
Metric			p
TKN	New Practice	Natural	0.7091
	Old Practice	Natural	0.0588
	Old Practice	New Practice	0.1594
OM	New Practice	Natural	0.7690
	Old Practice	Natural	0.0255
	Old Practice	New Practice	0.0545
TOC	New Practice	Natural	0.6236
	Old Practice	Natural	0.0144
	Old Practice	New Practice	0.0518
C:N	New Practice	Natural	0.999
	Old Practice	Natural	0.355
	Old Practice	New Practice	0.249
pH	New Practice	Natural	0
	Old Practice	Natural	0
	Old Practice	New Practice	0.1175
Clay content	New Practice	Natural	0.00004
	Old Practice	Natural	0.00001
	Old Practice	New Practice	0.5527



LESSONS LEARNED

In 2021, soil samples were collected from three additional natural (undisturbed) sites. Researchers expect that adding these sites in the analyses will account for natural variability in the undisturbed areas and improve their characterization of the undisturbed sites. This will ultimately help assess the trajectories of the reclaimed sites.

A few trends have been observed in the community of plants, fungi and microorganisms from soil samples collected in undisturbed and reclaimed sites. For instance, microbial community composition in reclaimed sites is becoming closer to the community in natural (undisturbed) sites. Also, the community composition for plants, fungi and microbes is becoming more distinct between the sites where newer reclamation practices were applied and those with older reclamation practices. At least one additional time point will be added to the analyses to confirm these trends.

Project research has also identified taxa from fungi and microbial markers that are associated to natural sites and which are emerging in the reclaimed sites. Correlation analyses and other statistical approaches will be used to confirm whether these identified taxa can be used as indicator species for monitoring reclamation trajectory.

Further statistical analysis (i.e., correlations) between physicochemical properties and genomic sequencing data from soils of selected sites will be conducted to assess whether tested soil properties can predict community composition of biological communities from fungi, plants, and microbes.

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PRESENTATIONS AND PUBLICATIONS

No presentations or publications available for 2021.

RESEARCH TEAM AND COLLABORATORS

Institution: Imperial

Principal Investigator: 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



REVEGETATION

Restoration of Native Tree and Shrub Species on Reclaimed Grassy Sites

COSIA Project Number: LJ0291

Research Provider: Natural Resources Canada, Canadian Forest Service

Industry Champion: Imperial

Status: Year 6 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

Preliminary (non-statistical) analysis of three-year results shows that, for the J10 and P3 sites, heat and drought in 2021 had an impact on the percent of vegetative cover on the treatments. The mounding treatments, with and without herbicide had the lowest plant cover when compared to the other treatments but not when compared to the control. This contrasts with 2020, a much wetter year, where only the mounding with herbicide treatment had the lowest percent cover. In better growing years, this treatment appears to provide better control of competition, especially clover. Interestingly, regardless of year, the mounding with herbicide treatment had the highest percent cover of the planted seedling (all species combined) compared to all the other treatments. Seedlings on this treatment produced greater leaf area than on all other treatments.

Seedling survival varies between the J10 and P3 site. After three years, percent survival of green alder was highest on the mounding with herbicide treatment on the P3 site (42% to 50%) but not on the J10 site (30% to 33%). The highest survival on P3 occurred on the mounding with no herbicide treatment. The reverse was observed for poplar. Survival on the P3 site was 32% to 33% whereas on J10 it was 40% to 55%.

For green alder, the change in survival percent between 2020, a wet year, and 2021 a dry, hot year was the lowest for the mounding with herbicide treatment for both sites. For poplar, the change in survival percent was lowest for the mounding with herbicide with no fertilizer treatment in J10 and for both the fertilized and unfertilized mounding with herbicide treatment in P3.

For the deciduous species, mounding appears to be the best treatment for survival, ranging between 30% to 45% for alder and 32% to 40% percent for poplar after three years depending on site. In the control, alder survival ranged between 1% and 5%, and poplar survival ranged between 2% and 10% after three years depending on the block.

There were fewer differences with white spruce survival between treatments. After three years, all treatments but the herbicide with shelters appear to perform better than the control on the P3 site. In J10, only the mounding treatments performed better than the control. The lowest spruce survival after three years was in the herbicide with shelter ranging from 47% to 53%. Spruce survival in the control was 70% for both blocks. The lower spruce survival



on the herbicide with shelter treatment may be due to the removal of the shelter and the exposure of the trees to harsher growing conditions (i.e., lower humidity, full sun).

Height increment for green alder on P3 was negative for all but the herbicide with shelter and no fertilizer treatment. This was not observed on J10 where alder height increment was positive for a majority of the treatments. Alder on P3 experience greater rodent damage and browsing than J10 resulting in a reduction in height of the seedlings. More work is required to tease out treatment effects for alder growth.

Poplar height increment for J10 was greater for all site preparation treatments than the control. The greatest height increment in 2021 occurred in the mounding with herbicide treatments and the mixing with herbicide treatments. In P3, poplar height increment was greater than the controls for all but the herbicide with shelter and no fertilizer treatment. The greatest height increment occurred in the mixing with herbicide and mounding treatments.

LESSONS LEARNED

Over time, differences between treatments and a difference between blocks (J10 vs P3) are being observed. After five years of monitoring J10 and P3, project researchers will be able to recommend the optimum treatment for seedling establishment and growth leading to the transition of the legacy grass dominated sites to forest tree and shrub species. After three years, the mounding with herbicide treatment appears to be the best. Full statistical analyses will determine if it is significantly better than other treatments and the control.

PRESENTATIONS AND PUBLICATIONS

No publications or presentations available for 2021.

RESEARCH TEAM AND COLLABORATORS

Institutions: 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		

Interim Reclamation

COSIA Project Number: LJ0226

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 in order 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

5. 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?
6. 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?
7. 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

Site occupancy with native plant species is a key objective of reclamation and reforestation of industrial sites. However, noxious weeds and other undesirable vegetation (e.g., sweet clover [*Melilotus* sp.], alsike clover [*Trifolium hybridum*], creeping red fescue [*Festuca rubra*], timothy [*Phleum pretense*] 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 is focused on findings from Project 1 and is based on results collected during the sixth growing season towards objectives 1b and 1c. During the data collection in 2021 it became clear that there was marked differentiation in woody vegetation development occurring between the topsoil and subsoil stockpiles across the study area. The results presented below focus on two key components of these differences; survival and growth of planted seedlings; and quantifying the natural recovery observed across these contrasting soil conditions. It is important to bear in mind that the configuration of the study site, where the subsoil pile was placed adjacent to the topsoil pile and there was a small road that bisected the two piles. The topsoil pile received the SW-W-NW-N aspects while the subsoil pile received all aspects except for the W aspect.

For six out of seven planted species, there was a consistent and often significant decline in survival in the topsoil compared with the subsoil — regardless of overall density treatment (Figure 1). The willow species planted, *Salix discolor*, was the single exception to this general pattern. Jack pine (*Pinus banksiana*) and white spruce (*Picea glauca*) suffered substantially experiment-wide, in part due to winter-desiccation after the first growing season which resulted in terminal stem dieback and likely put these seedlings at a competitive disadvantage. At the same time, the topsoil was highly competitive with herbaceous species by the second growing season and this likely exasperated the compromised condition of these seedlings. After six years in the topsoil, less than 10% of jack pine and only 20% of white spruce survived. In comparison, 50% of jack pine and 67% of white spruce survived in the subsoil after the same period of time (Figure 1).

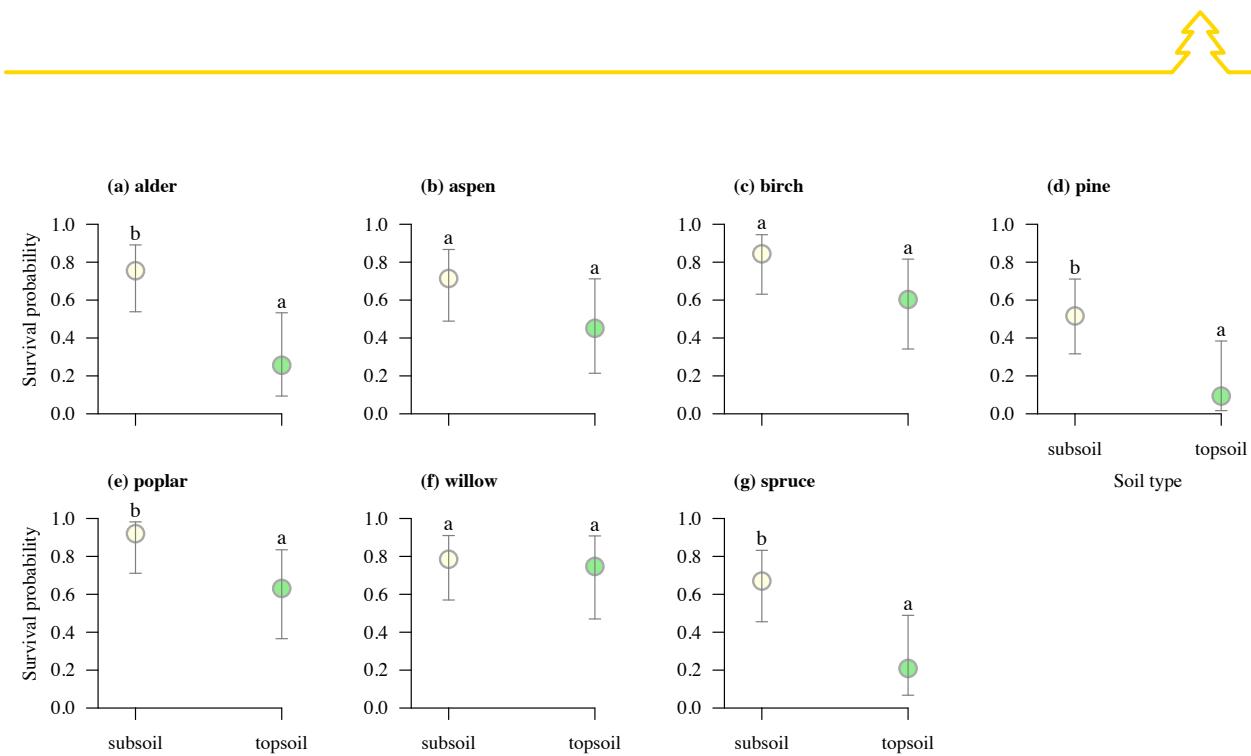


Figure 1: Study 1: Effect of stockpile soil type (topsoil or subsoil) on the probability of survival for alder (*Alnus viridis*), aspen (*Populus tremuloides*), birch (*Betula papyrifera*), pine (*Pinus banksiana*), poplar (*Populus balsamifera*), willow (*Salix discolor*) and spruce (*Picea glauca*) after six growing seasons. Measurements were based on stem counts taken within 15 m x 15 m permanent sample plots with the difference between observed and predicted (based on planted stem densities) as an estimate of survival; when values exceeded 1 (this occurred when natural regeneration was observed) the value was reduced to 1. Values are means and 95% confidence intervals on the mean (n = 24 plots in subsoil and 14 plots in topsoil).

Conversely, for these species total height was often significantly higher in topsoil, with the exception of green alder (*Alnus viridis*) and jack pine, which were similar in height between subsoil and topsoil (Figure 2). It is likely that the observed tradeoff in survival versus growth is at least partly due to increased herbaceous competition in the topsoil, which benefits individual tree or shrub growth, provided those individuals can survive the forbs and graminoids in the early years of establishment. Balsam poplar, paper birch and pussy willow demonstrated the highest levels of survival (> 60%) of the seven species planted, suggesting these species may have a greater level of tolerance for competing vegetation.

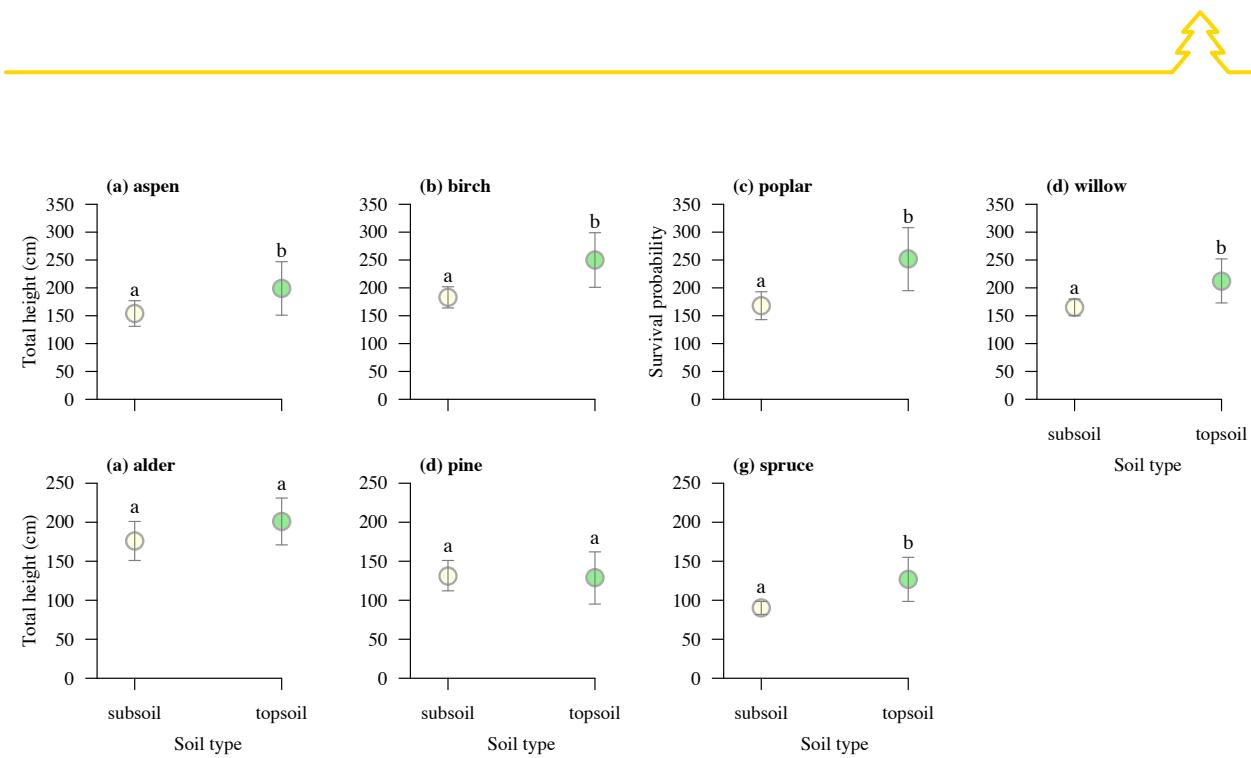


Figure 2: Study 1: Effect of stockpile soil type (topsoil or subsoil) on total height for aspen (*Populus tremuloides*), birch (*Betula papyrifera*), poplar (*Populus balsamifera*), willow (*Salix discolor*), alder (*Alnus viridis*), pine (*Pinus banksiana*) and spruce (*Picea glauca*) after six growing seasons. Measurements were based on assessment of all trees observed within 15 m x 15 m permanent sample plots. Values are means and 95% confidence intervals on the mean (n = 24 plots in subsoil and 14 plots in topsoil).

Measurable natural regeneration was observed for raspberry (*Rubus idaeus*), aspen, balsam poplar and willows (*Salix* spp.), both within the unplanted treatment replicates as well as within the planted density treatments. There was also a small quantity of chokecherries (*Prunus virginiana*), green alder and paper birch observed — though there were too few to analyze statistically. Regeneration across density treatments was consolidated in order to detect broader patterns in natural recovery as a function of soil type (topsoil or subsoil). The probability of observing natural regeneration (regardless of density) was strongly associated with subsoil over topsoil in balsam poplar and to a lesser extent (non-significant) in aspen and willow (Figure 3). Interestingly within measurement plots that contained natural regeneration, the stem counts did not differ significantly between subsoil and topsoil for these species. Common raspberry did tend to have higher densities in topsoil compared with subsoil, though both soil types had notable quantities of stems exceeding 15,000 stems ha⁻¹ (Figure 3). This finding suggests that even within the subsoil, there appears to be some quantities of raspberry seed that lay dormant until site preparation treatments disturbed the soil in fall 2015. The difference in the occurrence of natural woody species regeneration is also likely a function of the relative differences in herbaceous vegetation coverage in the early years of the trial.

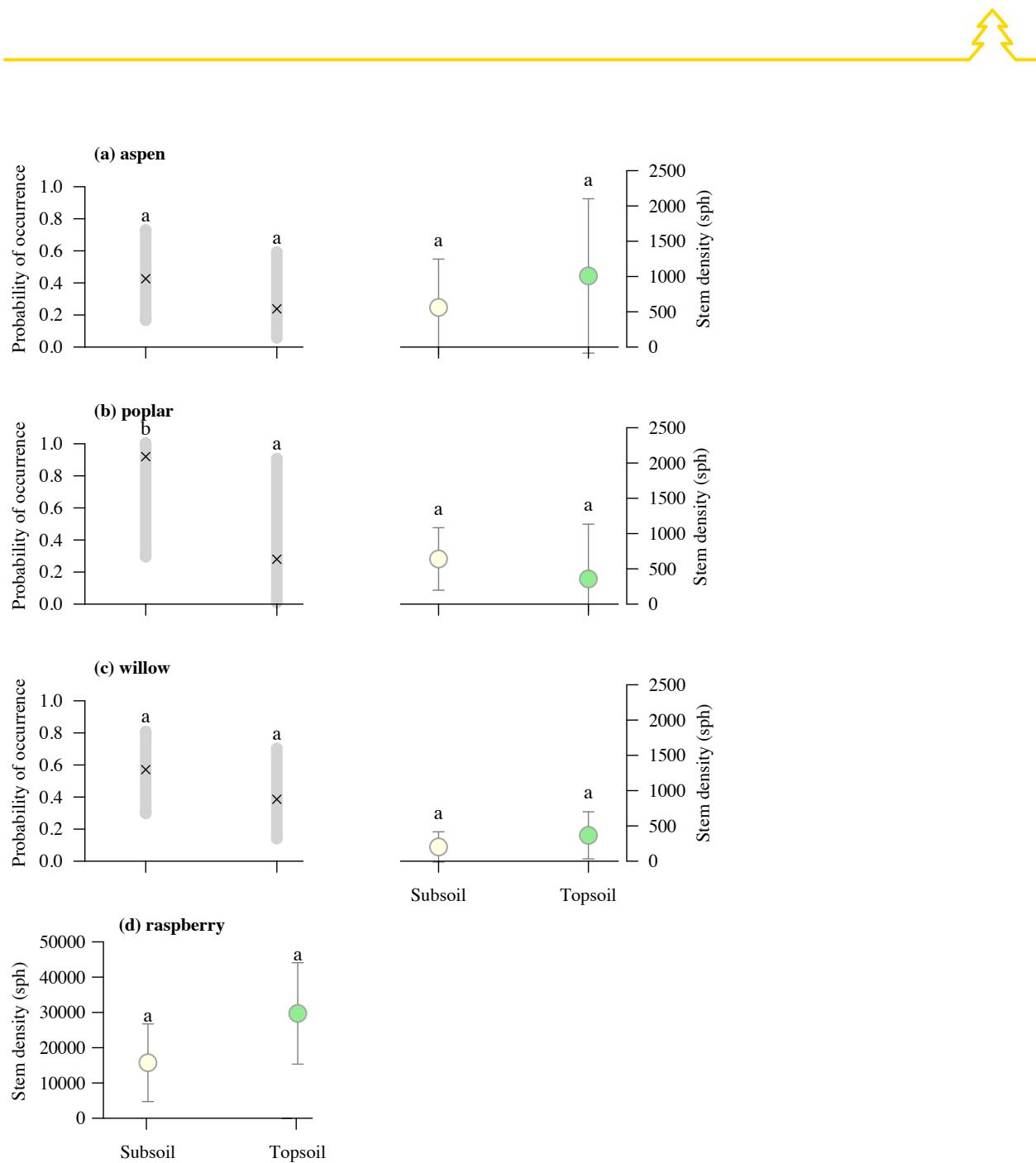


Figure 3: Study 1: Natural ingress of aspen (*Populus tremuloides*), poplar (*Populus balsamifera*), willow (*Salix* spp.) and raspberry (*Rubus idaeus*) across the density trial after six years. The probability of occurrence refers to whether natural regeneration was observed within a 15 m x 15 m measurement plot and the stem density (stems per ha) represents the measured density within the plots that contained natural regeneration. Values are means and 95% confidence intervals on the mean (n = 30 plots in subsoil and 18 plots in topsoil).



LESSONS LEARNED

Key lessons learned to date:

1. Establishing tree and shrub species on stockpiled subsoil is achievable. However, although reasonable levels of growth can be expected, the growth rates will be slower than those anticipated with topsoil.
 - Jack pine and green alder (both pioneer species) appear to be particularly adept at surviving on subsoil, moreover, these species did not show an improvement in growth rate when planted into topsoil relative to subsoil. In addition, low survival rates for these two species in topsoil suggest they may not be good candidates in reclaimed areas where richer soil conditions are expected; whether this is driven by a lack of tolerance for higher levels of competing vegetation or directly by the properties of the soil remains unclear.
2. When developing planting prescriptions, consideration for soil quality should be incorporated on the front end. Study 1 illustrated that there are substantial differences in survival associated with topsoil versus subsoil and this is likely indirectly due to the level of competing herbaceous vegetation — though other factors related to physical or chemical differences in soil properties may also contribute. Balsam poplar, paper birch and pussy willow demonstrated the highest levels of survival (> 60%) of the seven species planted.
3. Planting diverse mixtures of tree and shrub species on areas to be reclaimed is recommended. This study illustrated that even species expected to be relatively tolerant of competition (white spruce) can be at risk for high rates of mortality when extreme environmental factors occur (such as the winter desiccation observed in Study 1).
4. Natural recovery of woody species was more consistent in subsoil compared with topsoil — presumably this is due to differences in initial competing herbaceous vegetation, though this study did not specifically test this hypothesis.
 - This suggests that developing surface soil treatments that create a more heterogenous range of surface conditions (exposed mineral soil coupled with patches of more organic rich topsoil), may better facilitate more consistent and dependable rates of natural regeneration.
 - For example, balsam poplar was extremely dependable in terms of occurrence in subsoil with a rate of 90%, though where it occurred, the mean density was only 640 stems ha^{-1} . Aspen, on the other hand, showed an average stem density of 1,000 stems ha^{-1} in topsoil but the rate of occurrence was extremely low at 23%.
 - Together these results suggest that natural recovery on sites similar in size and terrain to this study area are unlikely to succeed to a functional forest within a reasonable time frame given the spatial variation (uneven/clumpy distribution) in natural regeneration coupled with the relatively low densities observed to date.



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

PRESENTATIONS AND PUBLICATIONS

No public presentations or publications in 2021.

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		
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

Research Provider: Wild Rose Consulting, Inc.

Industry Champion: Canadian Natural

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

Status: Ongoing

PROJECT SUMMARY

Twelve 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 2020 and 2021 (12th season) the OSVC harvested 225 litres (L) and 116 litres (L) of fruit respectively. Seeds were harvested from two seed zones in northeastern Alberta for the NAOS division. Harvest was based on requests from member companies. The following were extracted and registered:



Table 1: Species harvested

<i>Alnus alnobetula</i> (green alder) 2020 and 2021	<i>Dasiphora floribunda</i> (shrubby cinquefoil) 2021
<i>Alnus incana</i> (river alder) 2021	<i>Populus tremuloides</i> (trembling aspen) 2020
<i>Betula pumila</i> (dwarf birch) 2020	<i>Populus balsamifera</i> (balsam poplar) 2021
<i>Betula papyrifera</i> (paper birch) 2020	<i>Prunus pensylvanica</i> (pin cherry) 2021
<i>Calamagrostis canadensis</i> (Canada reed grass) 2020	<i>Shepherdia canadensis</i> (buffaloberry) 2021
<i>Carex aquatilis</i> (water sedge) 2020	<i>Vaccinium vitis-idaea</i> (lingonberry) 2020
<i>Cornus sericea</i> (red-osier dogwood) 2020 and 2021	<i>Viburnum edule</i> (lowbush cranberry) 2020 and 2021

In 2020 and 2021, activities supporting the OSVC seed collection initiatives included:

- In 2020, following a quick review of production effectiveness, routine seed testing was initiated to provide qualitative information regarding purity and viability of each stored seed lot.
- In 2020, a business case and cost analysis of seed orchards for individual shrub species was completed.
- Annual updates to the cross-company record keeping system and administration of the cooperative bank continued in 2020 and 2021.
- In 2021, a harvest site audit was undertaken to ensure healthy populations and to maintain an active inventory of suitable harvest sites in each seed zone for individual species.
- The 10-year seed requirement projections were completed in 2020 and will be used to plan future harvests.

Progress on Strategic Objectives

- OSVC members held eight meetings in 2020 and 11 in 2021 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. Refinements to this document continued in 2021.
- A review of viability, germination and production gaps was prepared for priority species as a starting point for continued research (2020).
- In 2020, an initial meeting was held with representatives of the Wetland Working Group to determine if the OSVC could assist with accessing required seed.
- In 2020, the OSVC members were included in discussions with nursery producers to improve communication and streamline plant production methods.
- The pilot vegetative propagation trial with *Corylus cornuta* (beaked hazelnut) and *Viburnum edule* (lowbush cranberry) was completed and a report submitted.
- 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, and information may be used to begin to assess stock quality.
- 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.



Communications

- Three editions of the Oil Sands Vegetation Cooperative Newsletter (May 2020, May and November 2021) were published.
- An overview of the OSVC was presented at the World Conference on Ecological Restoration in June 2021 and was well received and generated much interest.
- A meeting between Wild Rose Consulting and Federal MP James Cummings to discuss the overall state of revegetation in Alberta, provided an opportunity to introduce and explain the effectiveness of the COSIA-OSVC seed banking system.

LESSONS LEARNED

- The vegetative propagation pilot project completed by OSVC and NAIT Centre for Boreal Research in 2020 determined stem cuttings of both *Corylus cornuta* (beaked hazelnut) and *Viburnum edule* (lowbush cranberry) could be rooted, but additional work was needed to refine rooting methods. This led to further investigation into the production of both species by Canadian Forest Service.
- Researchers are moving towards filling the numerous identified knowledge gaps. Particularly in the areas of; seed handling practices, shrub mortality, vegetative and seed propagation of shrubs, and the potential for establishment of seed orchards and stooling beds for specific 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 standard TZ viability test. Seed quality testing will be invaluable in determining seeding rates, will assist in improving seed harvest and handling operations, and be useful for evaluating longevity.

PRESENTATIONS AND PUBLICATIONS

Wild Rose Consulting, Inc. 2020. Vegetative Propagation of *Corylus cornuta* and *Viburnum edule* from softwood stem cuttings. In cooperation with NAIT Centre for Boreal Research. Prepared for the Oil Sands Vegetation Cooperative and Canadian Oil Sands Innovation Alliance.

Newsletters

Wild Rose Consulting Inc. 2019. Oil Sands Vegetation Cooperative Newsletter. May 4(1). 3 pages. <https://www.cosia.ca/sites/default/files/attachments/OSVC%204%281%29.pdf>

Wild Rose Consulting Inc. 2019. Oil Sands Vegetation Cooperative Newsletter. November 4(2). 3 pages. <https://www.cosia.ca/sites/default/files/attachments/OSVC%20Newsletter%204%282%29.pdf>

Wild Rose Consulting, Inc, 2021. Oil Sands Vegetation Cooperative Newsletter. November 6(2). 4 pages. <https://cosia.ca/sites/default/files/attachments/Oil%20Sands%20Vegetation%20Cooperative%20November%202021%20Newsletter.pdf>



Conference Presentations/Posters

COSIA 2021. A Collaborative Initiative for Sustainable Plant Community Establishment. Society for Ecological Restoration – A New Global Trajectory, 9th World Conference on Ecological Restoration. June 23, 2021.

RESEARCH TEAM AND COLLABORATORS

Institution: Wild Rose Consulting, Inc.

Principal Investigator: Ann Smreciu

Name	Institution or Company	Degree or Job Title	Degree Start Date (Students Only)	Degree Completion Date (Students Only)
Kimberly Gould	Wild Rose Consulting, Inc.	Field Ecologist		

Research Collaborators: Jean-Marie Sobze, NAIT Centre for Boreal Research; Dani Dagenhardt, Canadian Forest Service, Natural Resources Canada; Peggy Popel, Smoky Lake Forest Nursery

Hitchhiker Field Trial at Kearl Operations

COSIA Project Number: LJ0324

Research Provider: Paragon Infinity General Partnership

Industry Champion: Imperial

Status: Year 4 of 5

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 was set up with species (shrub species [green alder and willow] and partner forb species [common fireweed and bunchberry]) and planting methods (Hitchhiker planting and Companion planting) as treatments in a modified split-plot design. Control plots where only green alder (Alder Control) or willow shrubs (Willow Control) were planted (no partner forb) were included. The soil prescription was consistent across treatments and fertilizer was not applied.

The main objectives of the Trial are to determine whether:

1. Survival and growth of woody shrubs (green alder [*Alnus viridis*] and willow [*Salix* spp.]) are facilitated by co-planting with locally common forb species (common fireweed [*Chamerion angustifolium*] and bunchberry [*Cornus canadensis*]).
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 co-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

As in previous years, the 2021 trial plots were monitored in September at the end of the growing season. Performance metrics including seedling survivorship, height, health, and percent vegetative cover were measured.



Objective 1: Co-planting with Forb Species

Based on the results from Year 4, green alder and willow appear to 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.

Green alder seedling growth was not affected by the presence of partner forbs; all shrub:forb combinations had similar heights by the end of the fourth growing season, when planting method was excluded (Figure 1). In terms of shrub health, green alder seedlings that were Hitchhiker-planted with common fireweed fared the worst ($P < 0.0001$; Figure 2). Conversely, willow seedlings planted with common fireweed were taller ($P < 0.0001$) and healthier ($P < 0.0001$) than those planted with bunchberry, and both treatments were generally taller than willow that were planted alone (Figure 2). Notably, both green alder and willow seedlings were taller in the Hitchhiker-planted Control plots than in the Companion-planted Control plots ($P < 0.0001$), where partner forbs were not planted (Figure 1). Seedling heights would be expected to be similar in the Control plots under consistent site conditions.

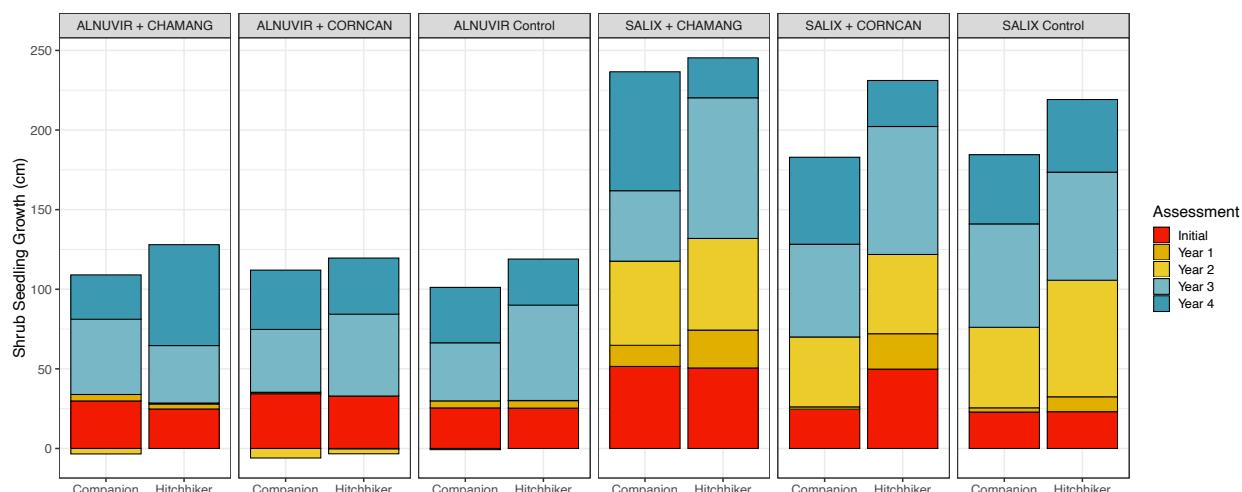


Figure 1: Average Growth of Shrub Seedlings During the First Four Growing Seasons.

Notes:

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

² Average height of green alder decreased in some treatments in Year 1 – depicted as negative average growth

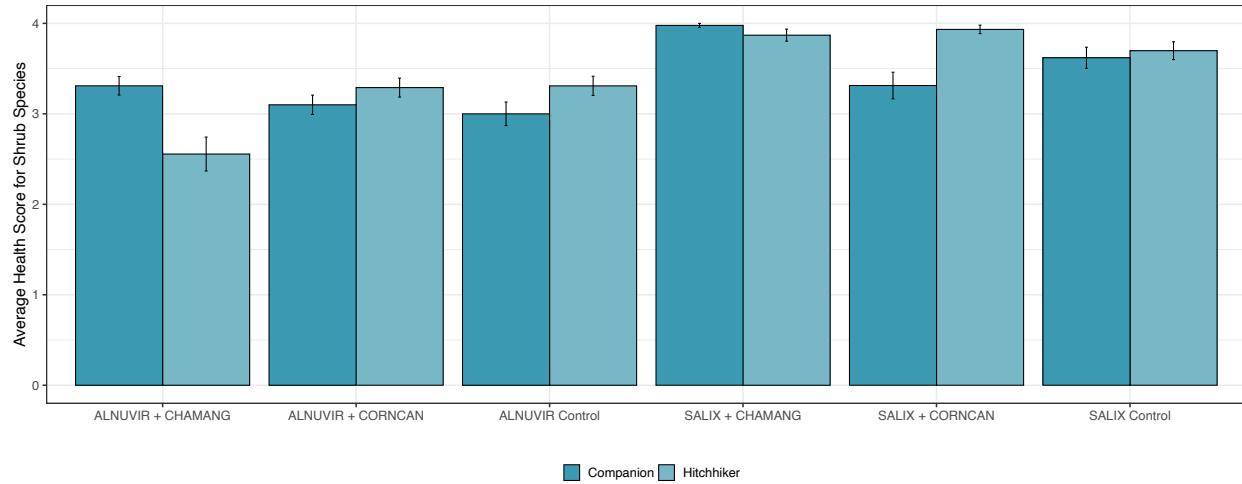


Figure 2: Average Health of Shrub Seedlings after Four Growing Seasons.

Note:

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

Co-planting with partner forbs also affected the percent cover of planted shrub species differently (Figure 3). Green alder percent cover was maximized when planted alone ($P < 0.0001$), whereas willow percent cover was maximized when planted with common fireweed ($P < 0.0001$; Figure 3). Generally, trends observed in green alder performance were more pronounced in Hitchhiker-planted plots than in Companion-planted plots ($P = 0.0004$).

Decreases in the percent cover and health of shrubs in Year 4 may have been a result of a relatively hot and dry summer, which caused necrosis and early leaf drop, particularly in green alder shrubs.

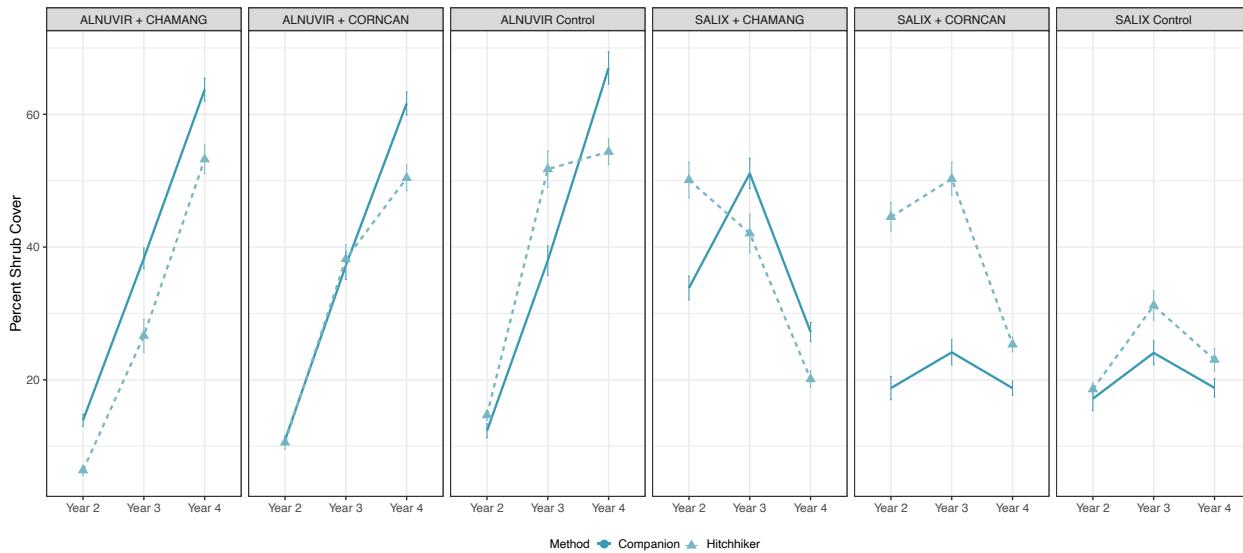


Figure 3: Average Percent Cover of Planted Shrubs after Four Growing Seasons.



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 was highest in the Hitchhiker-planted plots ($P < 0.0001$; Figure 1), but shrub survival (Figure 4) and percent cover ($P = 0.0004$; Figure 3) was maximized in the Companion-planted plots. Growth, health, and percent cover of willow shrubs were maximized in the Hitchhiker-planted plots ($P < 0.0001$ for all contrasts).

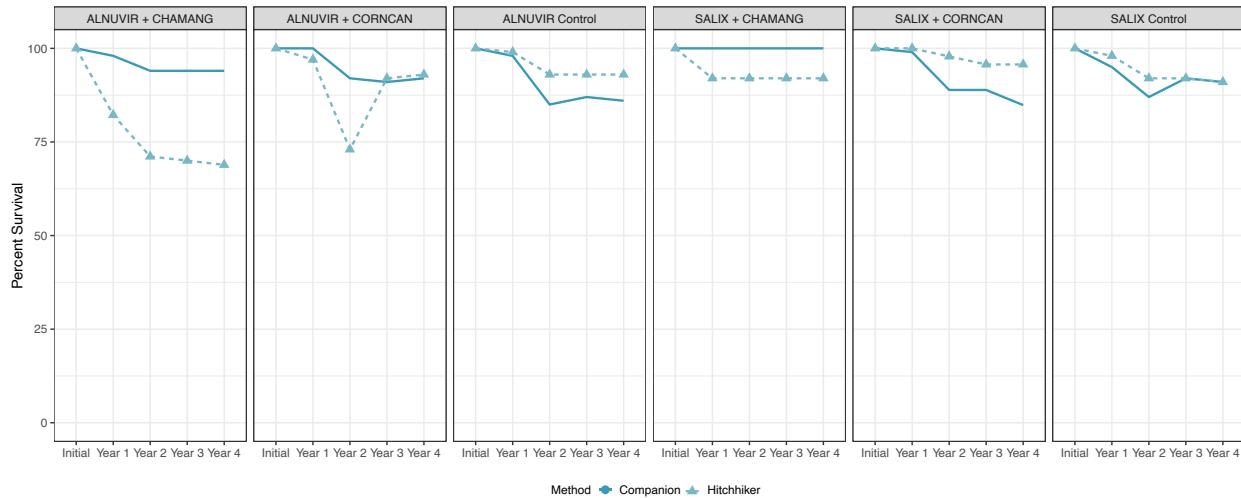


Figure 4: Percent Survival for Shrub Seedlings During the First Four Growing Seasons.

Objective 3: Vegetative Cover and Erosion Control

By the end of the fourth 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 ($P < 0.0001$).

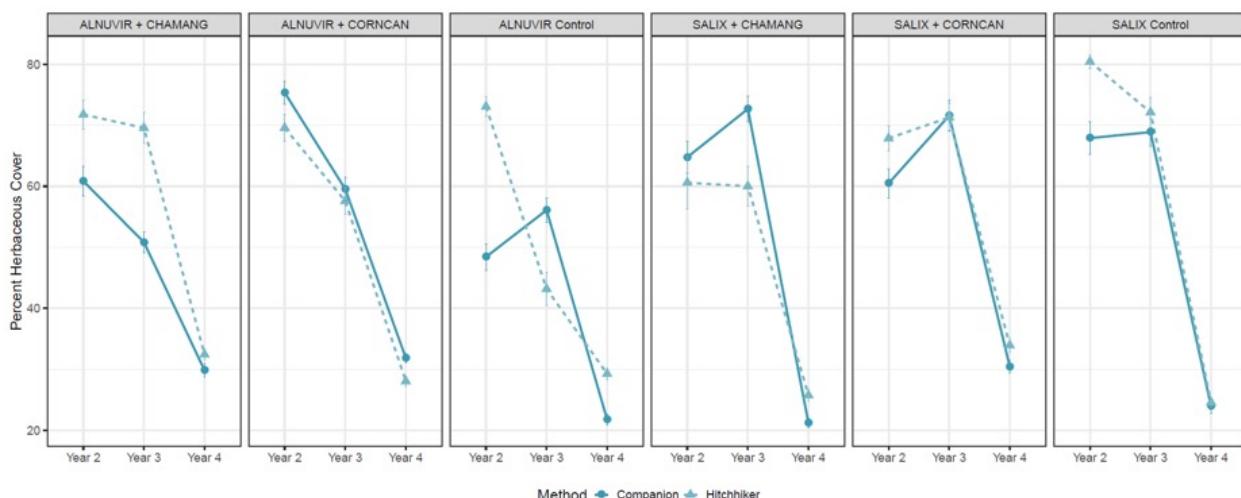


Figure 5: Average Percent Cover of Herbaceous Species after Four Growing Seasons



LESSONS LEARNED

Lessons learned will be summarized after the final year of the program (2022).

LITERATURE CITED

Dosite, J., Floreani, T., and Schoonmaker, A. 2016. Hitchhiker planting: *Development of combination container stock of target woody and herbaceous plants*. NAIT Boreal Research Institute. Oral Presentation ASSW.

PRESENTATIONS AND PUBLICATIONS

No public presentations or publications were released in 2021.

RESEARCH TEAM AND COLLABORATORS

Institution: Paragon Infinity General Partnership

Principal Investigator: Paragon Infinity General Partnership

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

COSIA Project Number: LE0067

Research Provider: NAIT Centre for Boreal Research

Industry Champion: Canadian Natural

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

Status: Year 1 of 2

PROJECT SUMMARY

Seed priming techniques are applied to enhance seed germination and seedling vigour and are especially useful in treating seed to become more resistant to abiotic stresses. Nanopriming is a technique based on the combination of seed priming and nanoparticle treatment. Nanopriming methods have been used extensively on agricultural crops but have not been widely tested on native boreal forest species, particularly in the context of improving seed germination and growth performance. Recent work using nanopriming with multi-walled carbon nanotubes (MWCNTs) and functionalized with carboxylic acid (MWCNT-COOH) or functionalized with hydroxyl (MWCNT-OH), combined with cold stratification techniques, was successful at improving the seed germination and seedling vigour for two dormant boreal forest species, green alder (*Alnus viridis*) and buffaloberry (*Shepherdia canadensis*) (Ali et al., 2020). However, the need remains to investigate the potential of using nanopriming with carbon nanoparticles to improve the germination of key native boreal species used in the reclamation of industrially disturbed sites.

The primary objective of this project is to evaluate the effects of MWCNTs in improving seed germination, seedling vigour and growth in select native boreal plant species ideally suited for forest reclamation following oil sands mining and in-situ development.

To achieve this objective, NAIT and COSIA members have 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*).

Seed scarification involved using 5% sulfuric acid to soften the seed coat to facilitate water imbibition. Seed stratification consisted of breaking seed dormancy through a moist and chill treatment to promote the germination. The effect of stratification, scarification, and carbon nanotubes on the germination of three native seeds (chokecherry, low-bush cranberry, and redcurrant) is being investigated. The tested stratification times were for zero, two, four, eight and 12 weeks. For scarification, 5% sulfuric acid was used. Three carbon-based nanotube types (MWCNT, COOH, and OH) were used at concentrations of 10 µg/L, 20 µg/L, and 30 µg/L.

Chokecherry was the only species that germinated during the observation period (50 days), while the germination of low-bush cranberry was completed in 130 days. No germination of redcurrant was observed.



Germination was monitored every two days for a period of 50 days and recorded when the radicles grew to approximately 3 mm. For chokecherry, the germination rate (%), mean germination time (MGT), and synchronization index (SYN), which measures asynchrony associated with the distribution of the relative frequency of germination, were estimated through observation. The germination rate is the percentage of seeds that complete the germination process. MGT indicates the mean average time required to germinate one seed from a seed lot. The germination synchrony (SYN) was initially proposed to evaluate the degree of overlap of flowering individuals in a population (Primack, 1985). When SYN is equal to one, seed germination occurs simultaneously — whereas a SYN closer to zero indicates that at least two seeds complete the germination process at different times. For low-bush cranberry, only seeds stratified for 12 weeks had estimated germination rates.

In 2021, the membrane integrity test was conducted on three species, chokecherry (*Prunus virginiana*), low-bush cranberry (*Viburnum edule*), and bearberry (*Arctostaphylos uva-ursi*). The membrane integrity test has been determined to be the most promising method for the assessment of seed vigour (Ramos et al., 2012).

PROGRESS AND ACHIEVEMENTS

Chokecherry (*Prunus virginiana*) - Seed germination test

Cold stratification time significantly increased the seed germination rate in chokecherry. For the seed germination rate, there was an interactive effect of cold stratification time, scarification, and carbon nanotube (CNT) type. The seed germination rate increased with increasing cold stratification time in all CNT types with the exception of 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 rates were observed in scarified seeds, treated with COOH, and cold stratified for 12 weeks.

MGT was significantly affected by the interactive effect of cold stratification time, scarification, and CNT concentration.

Overall, MGT was significantly shortened through the use of 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, 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 with the exception of scarified seeds treated with MWCNT and stratified for eight and 12 weeks.

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

Since low-bush cranberry did not germinate during the observation period, the only results acquired were for the germination rate of seeds that were stratified for 12 weeks. The best germination rate came from the seeds treated with 10 µg/L of MWCNT, without prior scarification and stratified for 12 weeks. Whether scarified or not,



seeds treated with functionalized CNT (COOH and OH) did not provide significantly higher germination rates when compared to the control.

Membrane integrity test

The membrane integrity test was conducted on three species including chokecherry, low-bush cranberry, and bearberry. The germination test for bearberry is ongoing.

CNT type affected the electrical conductivity measurements for chokecherry. The leakage from the seeds treated with MWCNT was significantly higher compared to the control.

LESSONS LEARNED

Overall, chokecherry and low-bush cranberry reacted differently to treatments with carbon nanotubes. The functionalised CNT treatment improved chokecherry seed germination after a long stratification period, and the non-functionalised (MWCNT) treatment improved the germination of low-bush cranberry after 12 weeks of stratification.

Study results indicate the significant value of cold stratification when using nanoparticles to improve seed germination rate, MGT, and SYN. The highest germination rates were found within the longest cold stratification period (12 weeks). However, depending on the combination of scarification and CNT treatments, germination could be negatively affected by a longer stratification period. In the membrane integrity test, a weakening of cell membranes was found in MWCNT treated seeds. This may be a reason why 12 weeks of cold stratification did not improve the seed germination rate in treatments that combined scarification and longer treatment with MWCNT.

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Primack, R. B. (1985). Patterns of Flowering Phenology in Communities, Populations, Individuals, and Single Flowers. In J. White (Ed.), *The Population Structure of Vegetation* (pp. 571–593). Springer Netherlands. https://doi.org/10.1007/978-94-009-5500-4_24

Ramos, K. M. O., Matos, J. M. M., Martins, R. C. C., and Martins, I. S. (2012). Electrical Conductivity Testing as Applied to the Assessment of Freshly Collected *Kielmeyera coriacea* Mart. Seeds. *ISRN Agronomy*, 2012, 1–5. <https://doi.org/10.5402/2012/378139>

PRESENTATIONS AND PUBLICATIONS

No public presentations or publications available for 2021.



RESEARCH TEAM AND COLLABORATORS

Institution: NAIT Centre for Boreal Research

Principal Investigator: Dr. Jean-Marie Sobze

Name	Institution or Company	Degree or Job Title	Degree Start Date (Students Only)	Degree Completion Date (Students Only)
Sahari Inoue	NAIT Centre for Boreal Research	PhD		
Raymond Thomas	Memorial University	PhD		
Katie Bartman	NAIT Centre for Boreal Research	Research Assistant		



WILDLIFE RESEARCH AND MONITORING

Regional Industry Caribou Collaboration (RICC)

COSIA Project Number: LJ0155

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

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 has to 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://www.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 2021 include the following:

Large-Scale Habitat Restoration

In 2021, RICC members conducted restoration treatments on approximately 213 km of seismic lines. An additional 54 km of seismic lines have been assessed as advanced regeneration and on a trajectory towards recovery. RICC members also identified approximately 10 km of seismic lines that were assessed in previous years as advanced



regeneration and have included this information in RICC's linear feature database. Cumulatively, over 1,800 km of seismic lines have been treated or assessed as advanced regeneration to date, advancing RICC's goal of restoring caribou habitat on legacy seismic lines.

In addition, RICC members supported the development of restoration treatment plans across 1,217 km in the Birch Mountains East and Central, and 385 km in Birch Mountains West.

Ecosystem Monitoring Camera Program

In partnership with the Alberta Biodiversity Monitoring Institute's (ABMI) Caribou Monitoring Unit, the Government of Alberta and the University of Alberta, RICC continued deployment of wildlife cameras in Cold Lake, East Side Athabasca River (ESAR), West Side Athabasca River (WSAR), Richardson, and Saskatchewan boreal plains caribou ranges (Figure 1). The project began in 2017 with the goal of monitoring mammal (deer, moose, caribou, wolf, bear and mesocarnivores) response to i) wolf reduction and restoration programs in Cold Lake and ESAR, advancing RICC's goal of leading trials on restoration effectiveness and how wildlife respond to restoration, and ii) the relative influence of anthropogenic habitat alteration and climatic factors, advancing RICC's goal of research on caribou ecology and their relationships with other parts of the landscape. The project also expanded in 2020 to include the Richardson caribou range to collect baseline ungulate and carnivore data prior to white-tailed deer establishment into the Richardson range and to address the relative influence of human disturbance (WSAR, ESAR and Cold Lake) in comparison to predominately fire disturbance (Richardson) on mammalian densities. In 2021, a total of 275 cameras in 11 clusters were serviced to continue this program.

Influence of wolf reductions and habitat restoration on prey and predator densities over time

Preliminary results from 2021 found that moose, deer, caribou, black bear and wolf densities did not significantly change over time, nor did they change over time differently in the wolf reduction area (ESAR) relative to the low habitat-alteration reference area (Saskatchewan) or the business-as-usual range (i.e., WSAR which has no wolf control and no habitat restoration). Animal densities were estimated based on a 'time in front of camera' method. Caribou densities were higher on average in 2020, though this did not differ across ranges with different wolf reductions or habitat restoration. Moose density was significantly higher in the business-as-usual range than the wolf reduction range in 2018.

Relative influence of human habitat alteration on deer densities

Preliminary results from 2021 found deer densities were better explained by habitat productivity (i.e., remotely sensed forage availability) than by human habitat alteration or climate metrics. When controlling for habitat productivity, metrics of climate better explained deer density than did human habitat alteration. Deer density significantly increased as habitat productivity increased, and decreased as winters became longer, colder and snowier.

Baseline ungulate and carnivore densities in Richardson

Preliminary results from 2021 found that the most commonly observed species were black bears and moose. While white-tailed deer were observed, their densities were low (< 0.04 animals/km²). Caribou densities in the southern cluster (1.08 animals/km²) were higher than in the northern (0.07 animals/km²) and mid (0.01 animals/km²) clusters.

Relative influence of human habitat alteration and fire disturbance

Preliminary results from 2021 appear to show that white-tailed deer and moose were more abundant in camera clusters with less fire. In contrast, caribou densities did not appear to be influenced by fire. Additional data are needed to confirm preliminary assessments.

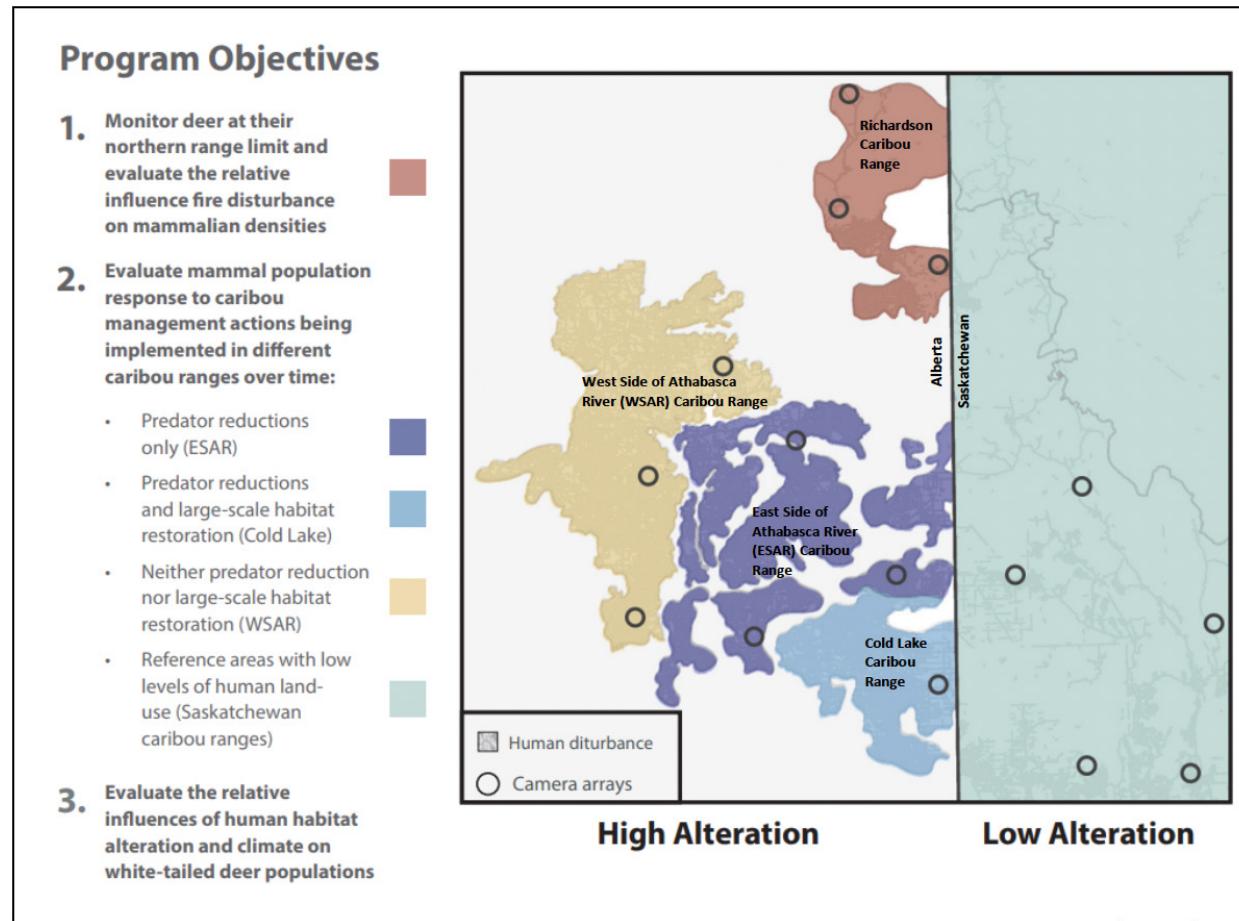


Figure 1: Scope of Ecosystem Monitoring Camera Program (source: modified from Regional Industry Caribou Collaboration 2020 Annual Report)

Caribou Ecology and Recovery Webinar Series

Following the success of the webinars started in 2020, RICC continued to sponsor the Caribou Ecology and Recovery Webinar Series to facilitate sharing of information and maintaining communication between academics, government and industry (<https://cmu.abmi.ca/about/caribou-ecology-and-webinar-series/>). The series is hosted by the Caribou Monitoring Unit and National Boreal Caribou Knowledge Consortium. Within the webinar series, Melanie Dickie, supported by RICC, presented preliminary results from the Ecosystem Monitoring Camera Program. The series has successfully engaged the community, with 50 to 100 participants from across Canada frequently attending webinars. Because of the success, the series will continue into the spring of 2022.



Multiple lines of evidence for predator and prey responses to caribou habitat restoration

In 2021 RICC supported the development of a peer-reviewed manuscript to evaluate the response of wildlife to restoration treatments. The work built off RICC-member habitat restoration and data collection in previous years. The study used a multi-scale and multiple-lines-of-evidence approach to evaluate the response of wildlife to restoration treatments in a 378 km² area, one of the largest studies of its kind. Moose, caribou, black bears and wolves were all less likely to be present at treatment sites monitored with camera traps, particularly those with higher intensity treatments, though effect sizes were small. In addition, moose, bears and wolves monitored with GPS collars also showed a decline in use of treated linear features, though the response was non-significant.

LESSONS LEARNED

Ecosystem Monitoring Camera Program

Influence of wolf reductions and habitat restoration on prey and predator densities over time

Preliminary results suggest that there has not been a short-term increase in moose, or white-tailed deer as a result of wolf reductions. There may be an indication that caribou densities are increasing as a result of wolf reductions. The consequences of species management strategies across the food web needs to be understood to inform future management decisions, especially when these management strategies are socially polarizing. Wolf reductions are intended to increase caribou populations in the short-term, because wolf predation is a primary source of caribou mortality. However, if wolf reductions also increase the densities of moose and deer, the area could ultimately support a higher abundance of wolves - making phasing out wolf reductions in the future more difficult.

Preliminary results further suggest that there has not been a short-term change in the mammal community within the habitat restoration area. Knowing if (or when) restoration results in decreased abundances of large ungulates and predators, and increased caribou abundance is key to an understanding of habitat restoration effectiveness.

We caution that analyses to date have only measured short-term responses, and additional monitoring is required to understand the long-term trends in population densities.

Relative influence of human habitat alteration on deer densities

Deer densities were primarily explained by habitat productivity, suggesting that forage availability is the primary predictor of local deer densities. When habitat productivity was controlled for, deer densities were primarily influenced by climate, such that deer densities declined in longer, colder and snowier winters. While an interaction between habitat alteration and climate on productivity and herbivore density is evident, preliminary study results do not support that white-tailed deer populations at the northern range limit in western Canada have increased due to habitat-alteration alone. Understanding the drivers behind white-tailed deer expansion in Canada's northern boreal forests will help with an understanding of the mechanisms behind increased predation on woodland caribou populations and inform caribou management actions. While management actions can directly reduce human habitat-alteration via habitat restoration, if climate is the primary driver of deer expansion these management actions are unlikely to be effective on their own. In such cases, alternative management strategies such as predator or prey reduction programs will need to be considered. If the effect of climate has a strong influence on white-tailed deer densities, restoration may be less effective in areas where climate or productivity are supporting high deer densities. Restoration programs should be designed to account for this information, particularly when restoration programs are planned across caribou ranges.



Baseline ungulate and carnivore densities in Richardson

Additional data are needed to draw meaningful conclusions.

Relative influence of human habitat alteration and fire disturbance

Additional data are needed to draw meaningful conclusions.

Multiple lines of evidence for predator and prey responses to caribou habitat restoration

The study found more evidence than not to support the assessment that animals reduced their use of the restoration sites. While no single test showed clear responses to restoration, the accumulation of evidence within the study suggests that habitat restoration may be effective at reducing the use of seismic lines by the large-mammal community. Moreover, the variation in responses observed is informative.

PRESENTATIONS AND PUBLICATIONS

Journal Publications

Dickie, M., McNay, R. S., Sutherland, G. D., Sherman, G. G., and Cody, M. 2021. Multiple lines of evidence for predator and prey responses to caribou habitat restoration. *Biological Conservation*. 256, 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>.

Cenovus Contributed Project

Murray, K. R., Bird, M., Strack, M., Cody, M., and Xiu, B. Restoration approach influences carbon exchange at in-situ oil sands exploration sites in east-central Alberta. *Wetlands Ecol. Manage* 29, 281–299 (2021). <https://doi.org/10.1007/s11273-021-09784-x>

Conference Presentations/Posters

Dickie, M. Serrouya, R., DeMars, C., Becker, M., Boutin S. and Ford, A. 2021. Disentangling the influence of anthropogenic habitat alteration from climate on expanding white-tailed deer populations in western Canada. *Caribou Ecology and Recovery Webinar Series*. <https://cmu.abmi.ca/about/caribou-ecology-and-webinar-series/>



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.

Assessment of Relevant Indicators for the Monitoring of Reclaimed Sites in Peatlands

COSIA Project Number: LJ0328

Research Providers: LOOKNorth, NAIT Centre for Boreal Research, InnoTech Alberta, Natural Resources Canada (Canadian Forest Services), Rankine Geospatial

Industry Champion: Canadian Natural

Status: Final Cumulative Summary

PROJECT SUMMARY

The peatland monitoring framework will help industry develop cost-efficient, relevant and effective monitoring and reporting programs that support reclamation objectives. Understanding reclaimed peatland recovery in comparison to natural peatland areas will help identify whether current reclamation practices are successful at achieving reclamation targets both on regional and site levels. It also allows for adaptive management approaches that will address problems in a timely manner for long-term ecosystem sustainability. Identifying indicators that are relevant for monitoring recovery, as well as those that replace conventional methods, may reduce the labour requirements for monitoring footprint disturbances and the financial burden of annual monitoring programs.

Five research providers have teamed up to bring together the right expertise to achieve a whole-ecosystem approach to wetland monitoring and assessment. Together, they will develop an integrated, scientifically robust and financially sustainable monitoring pilot program to assess the ecological recovery of physical, chemical and biological indicators for vegetation, soil, water and biodiversity at reclaimed and natural peatland sites across the Athabasca oil sands region.

The intent of the monitoring framework is to increase the feasibility and efficiency of in-field monitoring; identify indicators that are the most relevant (in terms of peatland recovery); identify technologies that can streamline and remove labour-intensive conventional sampling; and increase the consistency of sampling across sites. LOOKNorth anticipates that this will provide industry with the ability to communicate and report more efficiently with regulators on peatland recovery and compare results between sites (within company and between companies, if warranted) for landscape integration planning. The early identification of indicators that are not performing well will also allow early intervention to help ensure reclamation success. The expected benefit from the project is more efficient allocation of reclamation budgets and resources (i.e., more area monitored with less or similar effort), which will eventually lead to a better performance of the aggregate total reclaimed areas.

This project scope includes several in situ oil and gas sites (well pads and linear features) reclaimed to peatland status in the last decade. A series of natural and reclaimed sites have been selected and instrumented to establish benchmarks for monitoring and to assess site progress towards meeting provincial criteria. Observations on regional hydrology, soil, vegetation – particularly ground layer bryophytes – and soil/water chemistry were collected at multiple spatial scales using field-based methods, unmanned airborne vehicles (UAV) and satellite remote sensing.



The multi-scale data will be analyzed and evaluated for contributions and insights related to progress from disturbed areas towards fully functional boreal wetlands. While wildlife surveys are not within the scope of the activity, the project will consider wildlife use of the study areas as a habitat value indicator.

The main objectives of this project are to:

1. Consolidate ground-truthed field data from a series of reclaimed in-situ peatland sites;
2. Identify key indicators for field monitoring;
3. Pilot and field test ground based and remote sensing techniques appropriate for monitoring peatland indicators; and
4. Develop a monitoring framework integrating indicators across temporal and spatial scales to inform planning and reclamation decision making.

Achieving the goals listed above will require execution of the following major tasks over two years (2020-2021):

1. Selection of peatland indicators through survey and consultation, involving industry, consultants, government, and academia;
2. Design of a two-year pilot monitoring plan. Use of existing field sites with options to add additional sites, and conducting both field and remote sensing monitoring; and
3. Execution and analysis of the two-year pilot plan, with annual review and updates.

The project aims to advance and optimize the manner in which wetland reclamation is monitored. This project builds on the existing state of knowledge regarding reclamation of wetlands in the oil sands region, as gathered and established through multi-stakeholder discussions and presented in 2007 as reclamation guidance by the province of Alberta. The guidelines presented to the province through the Reclamation Working Group under the now defunct Cumulative Environmental Management Association (CEMA) do not consider recent advancements in harmonization of wetland monitoring by industry via COSIA and by Alberta Environment and Parks (AEP). The recent advancements have created an opportunity to establish a framework for assessing reclamation success against these indicators. In addition to producing the reclamation monitoring framework, which is missing from the developed monitoring system, this project provides an opportunity to advance best practices by utilizing novel methods to quantify changes in indicators diagnostic of wetland function. Assessing these indicators efficiently can significantly reduce monitoring costs related to the assessment of reclamation success beyond the operational phase of assets. The envisioned framework is expected to leverage data generated by provincial policies and frameworks to support planning, design, construction and monitoring of reclaimed wetlands in an integrated approach as outlined by existing provincial wetland policies and objectives.

PROGRESS AND ACHIEVEMENTS

This project, which was completed in December 2021, is the first of its kind to apply UAV and remote sensing technologies in the assessment of reclaimed peatland sites. Accurate models were developed for vegetation and microtopography classification, surface terrain, and water table estimation. These parameters are among the most common indicators for peatland health and part of routine reclamation assessment requirements. Learnings from this study can be used to make decisions at the regional scale using UAV and remote sensing techniques. More importantly, these models used for monitoring peatland indicators can greatly streamline field assessment, reduce risks, and increase confidence.



Overall, work towards the four main project objectives has been completed including: The completion of the consolidation of ground-truthed field data from six peatland sites over two years; key indicators for peatland monitoring (hydrology, water quality, vegetation, and topography) were identified; remote sensing techniques were tested and validated on a subset of the indicators including vegetation, hydrology, and topography; and a monitoring framework to aid in planning and reclamation decision making was developed and submitted in January 2022 (InnoTech, 2022).

Due to project constraints, remote sensing techniques for monitoring water chemistry were not assessed in this project. Additionally, due to the limitation of the study sites, some of the parameters in the original plan were not included in the final remote sensing outputs. These include vegetation, such as broad-leaved herbs and grasses, which are common weeds in reclamation trials. The study sites chosen did not have enough representative vegetation present. It was hoped that the ground layer bryophytes could be estimated by association with visible vegetation layers such as shrubs and trees. The estimation of sphagnum moss cover with reasonable accuracy was achieved using such methods, but true mosses in fens remain a significant challenge. Densely treed areas and areas with high herb and shrub cover generally have lower accuracy for estimating water table and topography.

A description of the processing steps and assessment of accuracy of the remote sensing vegetation classification, microtopography, and water table depth are presented below.

Vegetation Classification: The processing methodology for vegetation classification from the UAV data is shown in Figure 1 and the overall accuracy assessment of the classes in Table 1 (from C-CORE, 2021; InnoTech, 2022). The accuracy was evaluated using a user's and producer's method, a common method for measuring accuracy in remote sensing applications. These statistics provided over 70% accuracy in all vegetation classes.

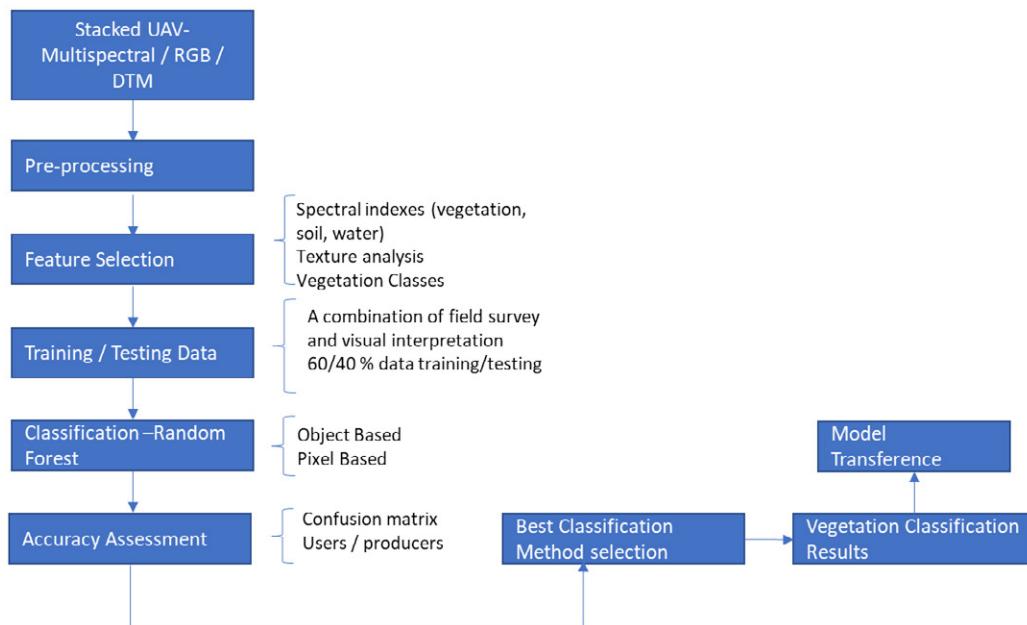


Figure 1: Vegetation classification workflow.



Table 1: Overall vegetation classification accuracy from all study sites

Class	Class Name	Class #	Colour	Accuracy
SPHGNM	Sphagnum	1	Yellow	87.98%
NLHERB	Narrow leaved herb	2	Light Yellow	96.22%
EVGSHB	Evergreen shrub	3	Teal	83.11%
CNFRTFpm	Evergreen tree	4	Dark Teal	72.96%
LICHEN	Lichen	5	Light Green	84.13%
CNFRTLL	Conifer large tree	6	Light Green	91.23%
DCDSHB	Deciduous shrub	7	Orange	91.45%
AQUATIC	Aquatic herb	8	Cyan	93.83%
DCDTR	Deciduous tree	9	Red	95.48%
OP	Open Water	10	Blue	100.00%
DEADTR	Dead tree	11	Black	98.04%
LITTER	Litter	12	Yellow	84.69%
Overall				89.93%

Figure 2 shows the red-green-blue (RGB) UAV imagery collected over a study site (left image). The multispectral data with relevant indexes calculated as additional channels is shown in the centre image and the classification results are shown in the right image. This site shows the prevalence of narrow leaved herbs and litter across most of the site compared to the natural peatland in the upper right which is dominated by shrubs and trees. Similarly, narrow leaved herbs such as sedges dominate open seismic lines and winter roads.

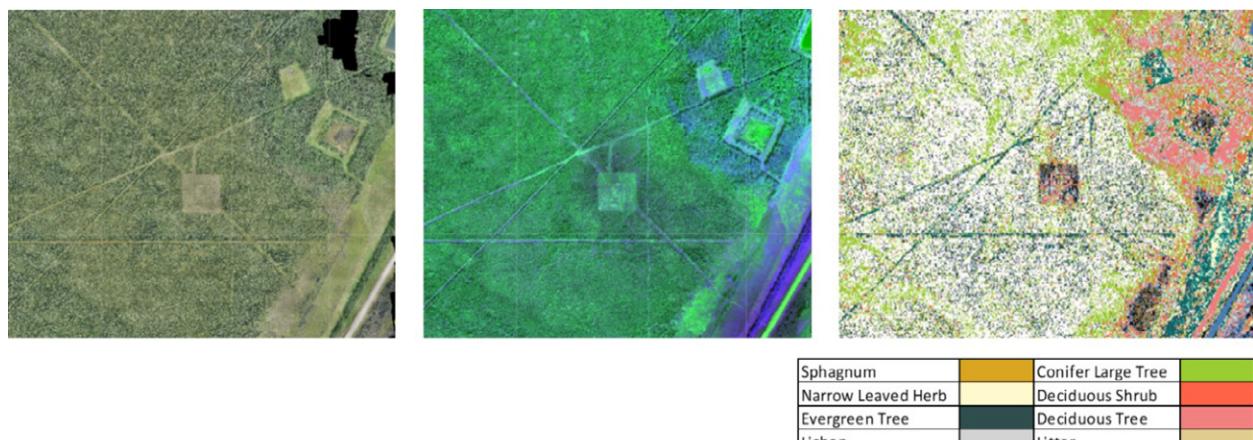


Figure 2: RGB image (left), multispectral imagery (centre) and classification results.



Microtopography

Peatlands microtopography is dominated by a hummock, hollow, and lawn complex that plays a major role in hydrologic, ecologic, and biochemical processes. The primary influence of microtopography is associated with water table depth. The position of the water table controls how decomposition occurs in the peat column. A detailed, dense, and highly accurate source of elevation data is essential to perform microforms mapping.

The dense point clouds collected during the UAV surveys were suitable to characterize peatland microtopography. Figure 3 shows the developed microtopography workflow. Overall, the model maintains the representative gradients of the study sites and characterizes their relative fine scale changes in elevation using small neighborhoods of local variations.

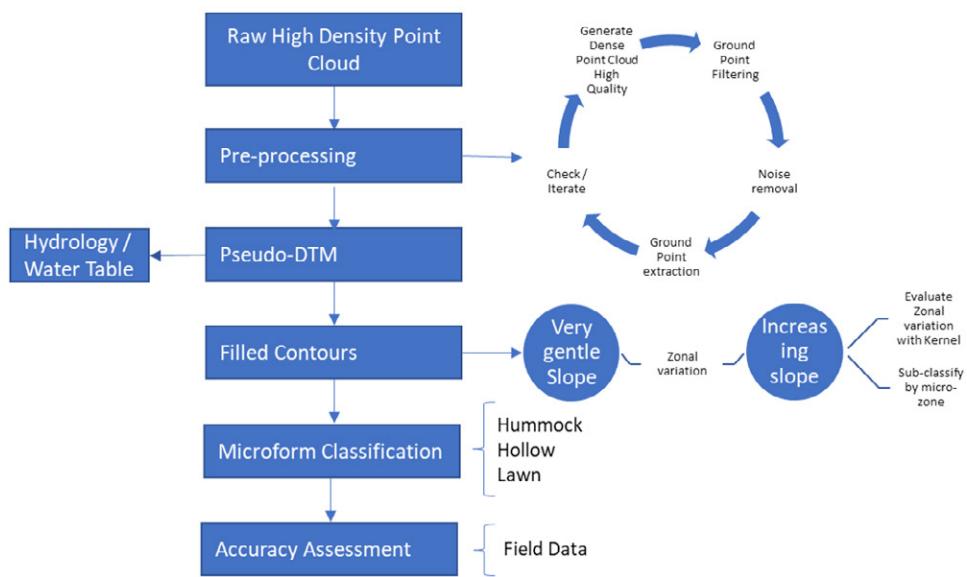


Figure 3: Microtopography workflow.

Field data using RTK GPS (centimetre level positioning) was used to evaluate the accuracy of the derived microtopography. In general, the algorithm performs well in reclaimed areas and shrub free zones. In areas where the ground layer was not visible in the imagery, the method was not able to determine terrain height. Figure 4 show the values and the general elevation trend of the microtopography at a study site. The trend verifies that elevations in the field and in the image are comparable, and that the spikes in the digital terrain model (DTM) values represent trees and shrubs that block the direct measurement of the elevation in treed areas.

Predicted values at the fen in both linear features and natural areas have a good match against the measured values, likely due to the absence of large trees and the spread of shrub cover in these areas. As shown Figure 5 it is possible to get a reasonable estimate of the elevation at the complete study site when vegetation does not interfere.

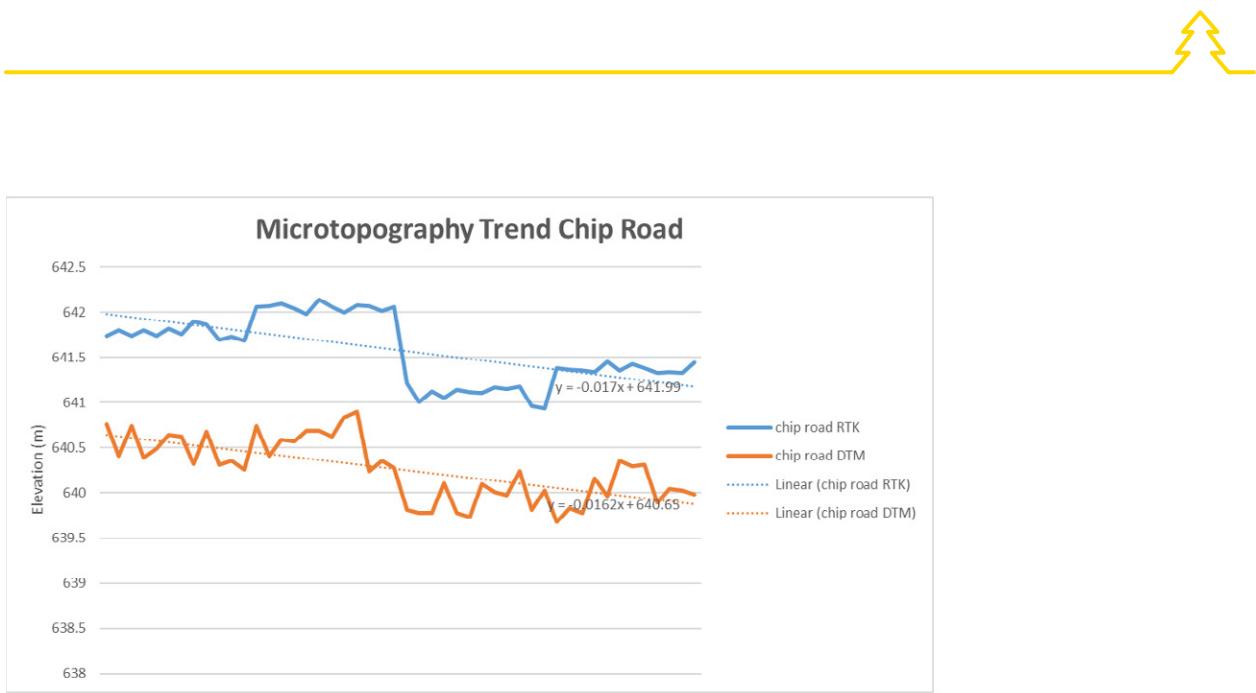


Figure 4: Chip Road microtopography field elevation versus image elevation..

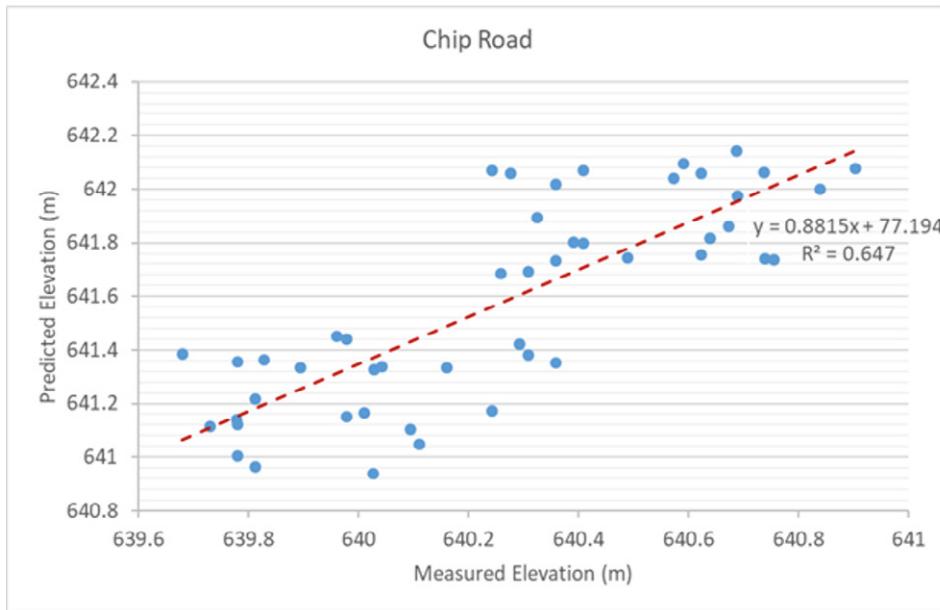


Figure 5: Predicted and measured elevation of fen in linear features and natural area.



Water Table Depth

Figure 6 shows the water table workflow. The source data is the same as used for microtopography, but in this case the colour of the point cloud data is used to select visible water features. These features represent the height of the ground level water. A Kriging interpolation is used to generate a ground water level model and an estimation of depth to water levels are calculated following a method modified from (Rahman et al., 2017).

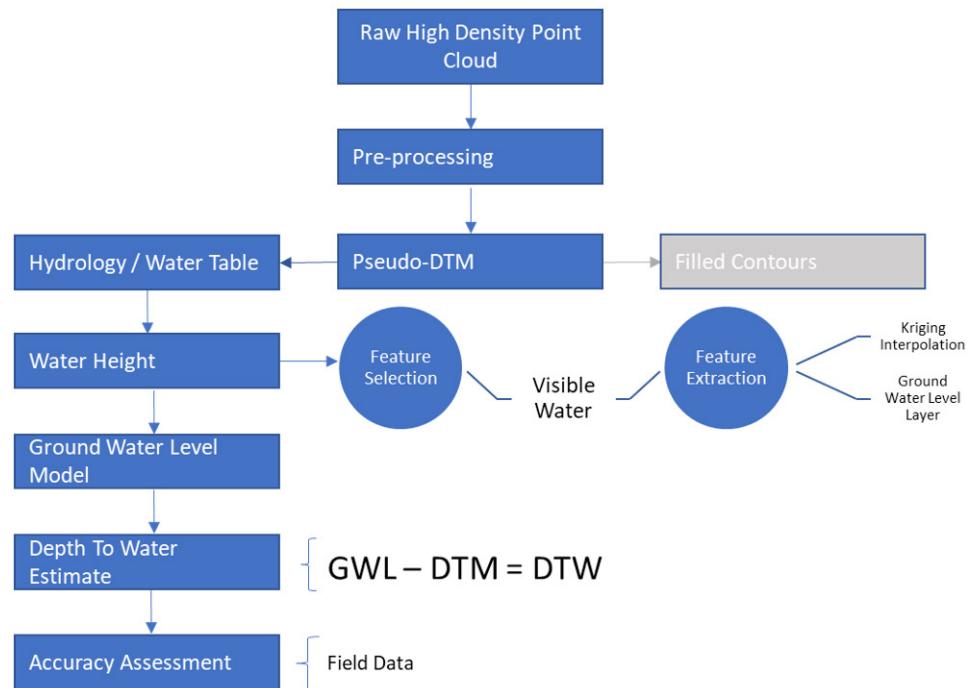


Figure 6: Water table workflow.

Similar to the accuracy assessment in microtopography, field data captured using RTK GPS was used to assess the calculated depth to water level. A relative misalignment or invisibility of water in the position of the wells was corrected by buffering 20 cm around the field point location and selecting the lowest pixel available in the DTM. This procedure allowed for corrected point locations where water pockets were not visible in the data.

Figure 7 shows the water well elevation measured with RTK GPS, and the elevation extracted from the DTM. The spikes in DTM represent where the water wells weren't visible in the DTM from overhead trees. The high model accuracy is shown in Figure 8, and the predicted values align well with the measured values on the reclaimed well pad. In open areas without heavy tree/shrub cover (e.g., reclaimed well pad or open linear features), it was possible to get a reasonable estimate of the water table position relative to the ground surface.

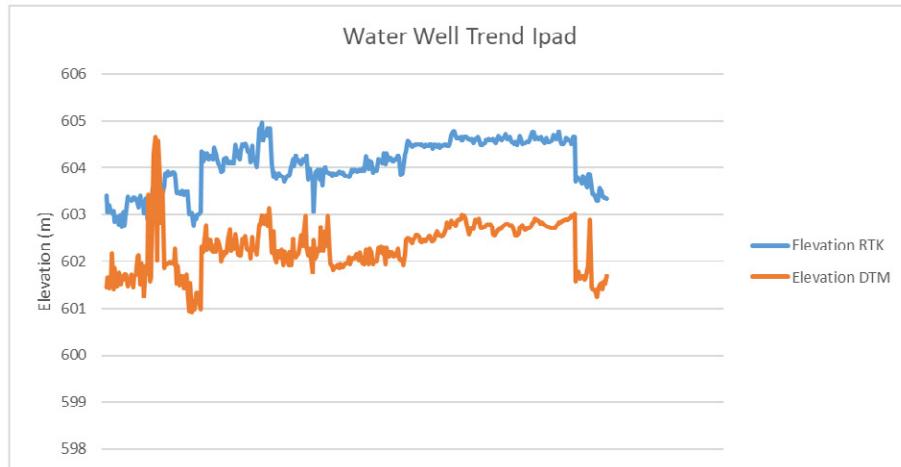


Figure 7: Water well field elevation vs image elevation (IPAD site).

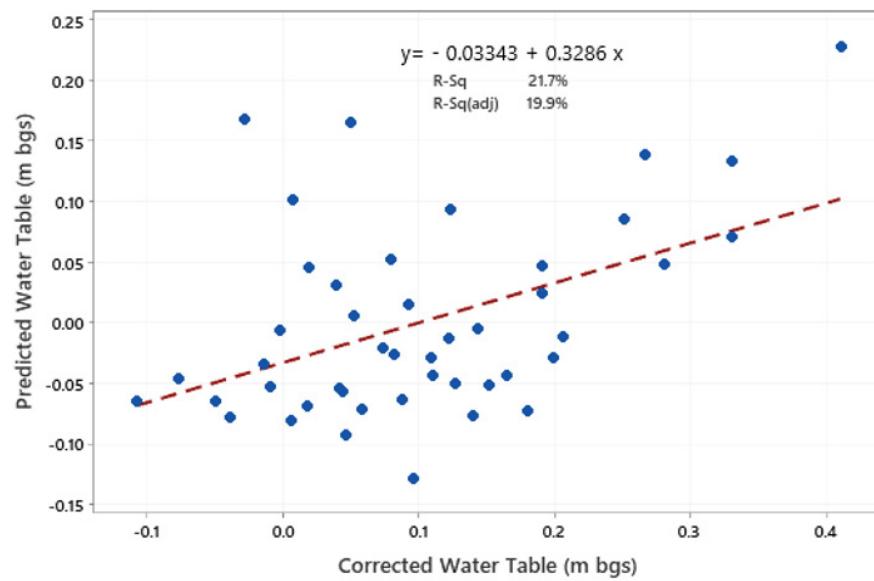


Figure 8: Fitted line plot of predicted and measured water table at the reclaimed well pad (IPAD).

Three case studies were developed (InnoTech, 2022) to demonstrate how the vegetation classification (Case Study 1), topography (Case Study 2), and hydrology (Case Study 3) can be used to assess peatlands.



Case Study 1

Objective

This study aims to assess vegetation cover of a reclaimed well pad using the vegetation classification model following the procedures outlined in the “Reclamation Criteria for Well Sites and Associated Facilities for Peatlands” (Alberta Environment and Parks (AEP) 2017). Typically, an in-situ well pad, (e.g., 120 m x 120 m IPAD) is delineated into nine 40 m x 40 m grids (Figure 9). A landscape survey is first conducted both on-site within each grid and off-site in four locations in the surrounding peatlands. This survey determines the general hydrological conditions and the presence of open water, upland areas, erosion, industrial debris, and gravel/rock. Within each 40 m x 40 m grid, the site characteristics, such as moisture regime, pH, and electrical conductivity (EC), are measured to determine the peatland types on-site.

Both species richness and percent cover (desirable and undesirable species) are assessed for each grid. The species richness assessment is completed using a timed (15 min) meandering assessment to record species encountered from all strata. For the field, shrub, and tree layers, a 10 m² vascular plant plot is centered on a representative area of each grid. Both desirable and undesirable vascular species percent coverage is calculated. Tree stem count for desirable species (e.g., black spruce and larch) within the plot is also recorded. Shrubs (including willows) is captured using percent coverage. For the ground layer vegetation, primarily peat-forming bryophytes, a 1 m x 1 m quadrat is used to assess percent cover by species when feasible.

Methods

In 2021, a detailed field survey was conducted at the IPAD (AOI 1) using the provincial criteria. Model outputs were then extracted at two scales – the whole pad, and 40 m x 40 m grids. This allows comparison of the survey results (i.e., measured) to model outputs (i.e., predicted).

Whole Pad: In Google Earth Engine, a large, square polygon was drawn encompassing the entire reclaimed pad (Figure 9). The model generates predicted area (m²) covered by each of the 12 vegetation classes – Sphagnum moss, lichen, narrow leaf herbs, evergreen shrubs, deciduous shrubs, spruce, larch, deciduous trees, dead trees, aquatics, open water, and litter. For each vegetation class, the predicted area was divided by the total area of the polygon to determine the predicted percent cover. The predicted percent cover for each vegetation class was compared to the measured percent cover determined by the field survey.

40 m x 40 m Grids: Nine square polygons, each 40 m x 40 m, were drawn on the reclaimed pad mimicking the grids used in the field survey. The model was run on each polygon, and the predicted percent cover of each vegetation class was determined using the same method as above. The model predictions for each polygon were compared to the measured percent cover from the corresponding grid in the field survey.

Sphagnum moss calculation: To predict ground layer bryophytes masked by shrubs, herbs, and tree layers, a regression approach was taken to associate bryophytes (Sphagnum mosses and brown mosses separately) with other vegetation classes as predictors. Several multiple linear regressions were undertaken by pooling 2020 field data by site types:

1. All areas, including natural bogs and fens, disturbed and reclaimed features
2. Natural areas, including only bogs and fens
3. Disturbed areas, including linear features and reclaimed well pads
4. Individual areas by bog, fen, linear disturbances, and reclaimed features



Option three was chosen to recalculate Sphagnum moss cover using the following equation: Sphagnum % = 43.2 – 0.651 * (narrow leaf herb) % + 0.481 * (evergreen shrub) % (R² adjusted = 39.38%, p < 0.01). None of the equations were significant for brown mosses.

Results and Discussion

Table 2 shows the model output for the entire pad and the calculation of percent cover for each vegetation class. The total area is 12646.35 m². Litter is the most abundant class, covering over half of the entire site (55.5%), followed by narrow leaf herb (25.2%) and deciduous shrubs (15.4%). None of the other vegetation classes exceed 1% in cover. Sphagnum moss cover is estimated at 0.12%. However, recalculated Sphagnum moss cover is 27.2% using the regression method.

Table 3 shows the model outputs (predicted values) for both the pad and the nine grids with the 2021 field survey results (actual values) listed as comparisons. Figure 10 shows that there is a significant correlation ($r^2 = 68.6\%$, $p < 0.001$) between the predicted and actual values:

$$\text{Actual Cover \%} = 5.04 + 0.5979 \text{ Predicted Cover \%}$$

Areas above the identity lines are underestimate (predicted < actual), and areas below are overestimate (predicted > actual). The model accuracy varies with vegetation classes. The model underestimates larch and evergreen shrub cover and overestimates narrow-leaf herb and litter cover. It does a good job of predicting *Sphagnum* moss and deciduous shrub cover, although there is a wide dispersion of data for both (Figure 10).

Based on the results of Case 1, the model can provide a reliable summary of the vegetation communities at both the entire pad, and 40 m x 40 m grid scale. It can even estimate vegetation abundance (percent cover) with reasonable accuracy.

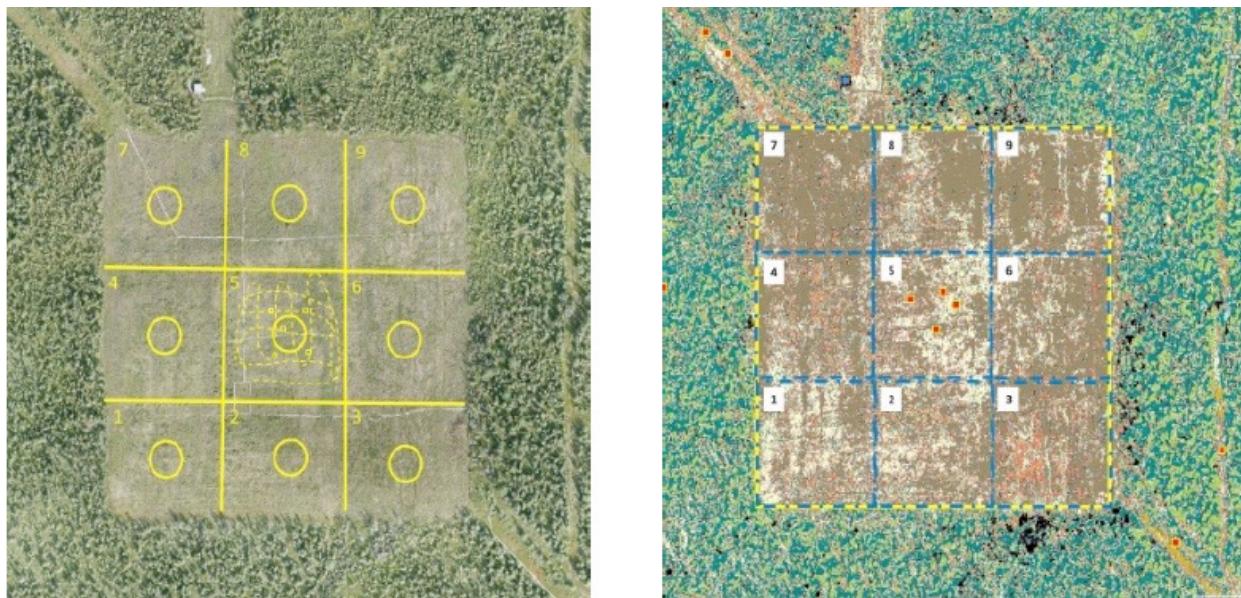


Figure 9: Left: Layout of the Peatland Criteria field survey at IPAD in 2021. Right: Layout of polygons in the vegetation model. Yellow dashed line outlines the whole pad; blue dashed lines divide the whole pad into nine 40 m x 40 m grids.



Table 2: Case study 1 – Model predicted area by vegetation class for the whole IPAD. *Sphagnum moss cover is re-calculated using regression equation: Sphagnum = 43.2 – 0.651 * (narrow leaf herb) + 0.481 * (evergreen shrub).

Species	Predicted Area (m ²)	Total Area (m ²)	Predicted % Cover
Sphagnum	15.28	12,646.35	0.12 (27.22*)
Narrow Leaf Herb	3,191.91		25.24
Evergreen Shrub	117.86		0.93
Spruce	5.79		0.05
Lichen	97.86		0.77
Larch	83.05		0.66
Deciduous Shrub	1,944.31		15.37
Aquatics	118.51		0.94
Deciduous Tree	0.00		0.00
Open Water	6.85		0.05
Dead Tree	50.25		0.40
Litter	7,014.69		55.47

Table 3: Predicted versus actual vegetation percent cover by whole pad and by 40 m x 40 m grids. Recalculated Sphagnum moss covers are used.

Vegetation	Pad		Grid 1		Grid 2		Grid 3		Grid 4		Grid 5		Grid 6		Grid 7		Grid 8		Grid 9	
	Pred.	Act.	Pred.	Act.	Pred.	Act.	Pred.	Act.	Pred.	Act.	Pred.	Act.	Pred.	Act.	Pred.	Act.	Pred.	Act.	Pred.	Act.
Sphagnum moss	27.2	30.0	20.0	45.0	21.1	40.5	32.3	11.5	27.2	37.5	14.9	30.5	26.0	27.5	37.2	33.0	24.1	24.0	28.5	21.0
Narrow Leaf Herb	25.2	15.1	37.0	13.3	35.0	15.4	17.0	15.0	25.8	14.0	43.8	17.7	26.5	20.2	10.0	16.1	29.7	12.1	22.7	12.0
Evergreen Shrubs	0.9	3.3	2.0	7.6	1.5	5.2	0.4		1.7	4.1	0.5	10.2	0.2	0.4	1.0	0.1	0.5	0.4	0.1	2.1
Spruce	0.0	2.3	0.0	2.0	0.0	5.0	0.0		0.0	1.0	0.0	5.0	0.0	1.0	0.0	5.0	0.0	0.5	0.0	1.0
Lichen	0.8	0.0	2.4		1.5		0.5		1.3		0.9		0.5		0.6		0.2		0.3	
Larch	0.7	2.6	0.2	2.0	0.3	2.0	0.3		0.4	5.0	0.1	2.0	0.1		0.3	2.0	0.2	5.0	0.0	5.0
Deciduous Shrubs	15.4	15.2	15.1	12.0	14.4	10.0	26.4	20.0	18.4	35.0	7.6	5.0	8.3	9.0	12.7	15.0	14.7	12.0	11.1	19.0
Aquatics	0.9		0.5		0.5		1.0		0.8		0.4		0.6		1.9		1.2		0.5	
Deciduous Tree	0.0	2.0		1.0		2.0				1.5		1.6				11.0		1.1		
Open Water	0.1				0.1		0.0		0.1		0.2		0.0		0.1		0.0			
Dead Tree	0.4		0.1		0.1		0.4		0.3		0.1		0.1		0.8		0.4		0.3	
Litter	55.5	39.3	42.6	28.3	46.5	34.2	53.9	35.8	50.9	40.8	46.3	41.7	63.6	56.7	72.4	47.5	53.0	31.1	64.9	37.9

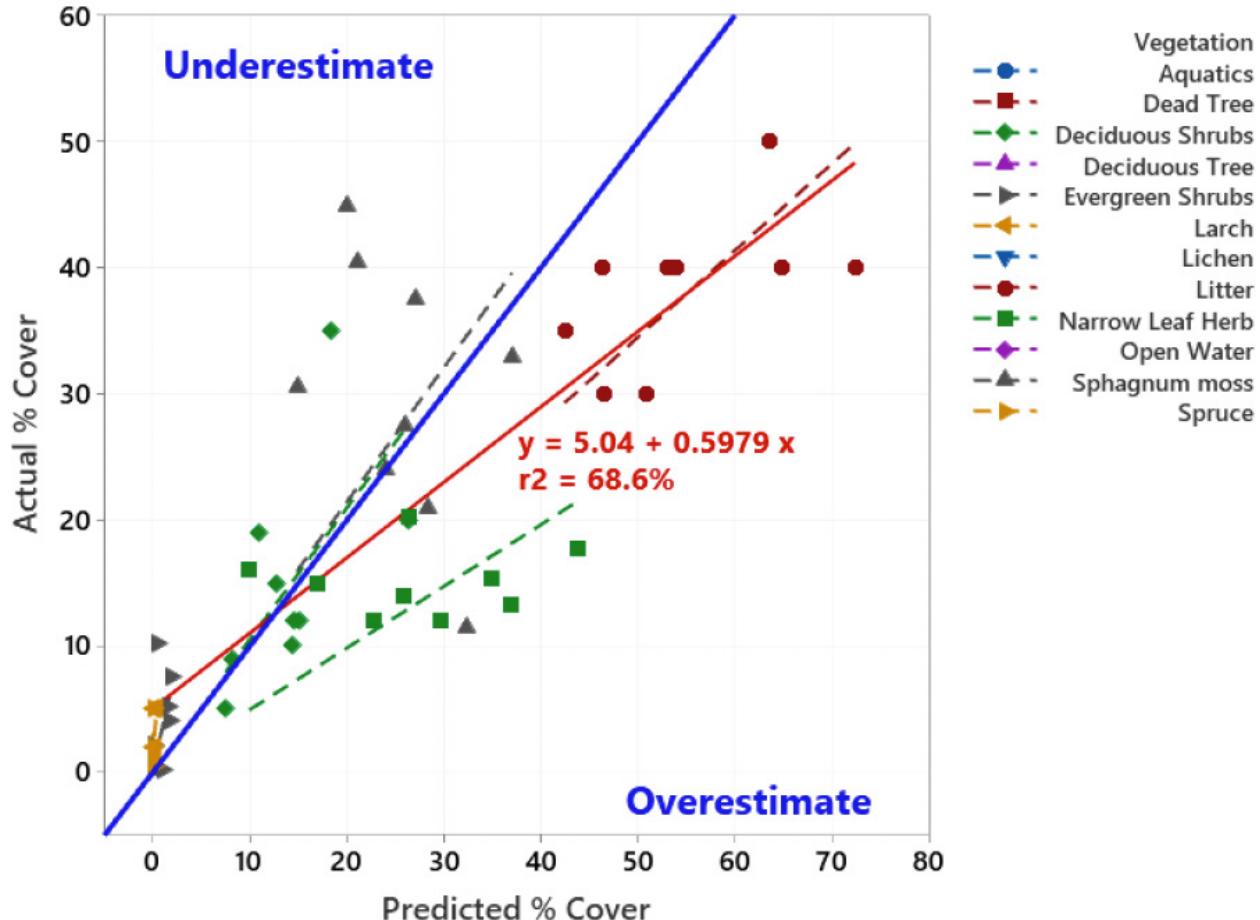


Figure 10: Predicted versus actual percent cover of the 12 vegetation classes on IPAD. Solid blue line is the identity line (predicted = actual). Area above the identity line is underestimate while area below is overestimate. Solid red line indicates the significant regression fit between predicted and actual cover ($p < 0.001$). Coloured dash lines are trend lines by vegetation classes.



Case Study 2

Objective

The surface elevation and water table are critical for the successful establishment of peatland vegetation on reclaimed sites (Xu et al., 2021). Therefore, we wanted to apply the models to estimate differences in surface elevation and depth to water table between the natural peatland and the reclaimed well pad.

Methods

We divided the IPAD into the same nine grids and used the depth to water table model to estimate the mean water table for each grid (predicted WT). In 2020, we also measured the depth to water table in 50 water wells across the IPAD (Figure 11). Field measurements of the depth to the water table were corrected for the water table level on the day of the drone flight by using the change in water table data from continuous loggers. Each water well was marked with RTK and is assigned to one of the nine survey grids. The corrected mean water table (corrected WT) was then calculated for each grid and the entire pad.

We then randomly selected three transects (T4 to T6) along the edges of the IPAD. Each transect is about 30 m long, extending from the natural area onto the well pad (Figure 11). The topography and water table models were used to estimate the changes in surface elevation (metres above sea level [m asl]) and depth to water table (metres below ground surface [m bgs]) at 25 cm intervals along each transect.



Figure 11: Layout of transects across winter roads (T1 to T3) and the edges of IPAD (T4 to T5). Red dots represent installed water wells in each 40 m x 40 m grid (blue squares).



Results and Discussion

The model accurately predicted the depth to water table for the entire pad (predicted WT 10.7 cm versus corrected WT 10.9 cm below ground surface). also shows the predicted WT values by each 40 m x 40m grid. There is a wide range of values, some are overestimate and some are underestimate (Figure 12). Overall, there is a significant correlation ($r^2 = 25.3\%$, $p = 0.002$) between predicted and actual WT:

$$\text{Corrected WT (m bgs)} = 0.0938 + 0.678 \text{ Predicted WT (m bgs)}$$

Figure 13 shows a close-up view of Transects 4 and 6 drawn from natural peatland onto the IPAD. Along Transect 4, there is a 0.54 m mean elevation difference between the natural area and the IPAD. The natural area has highly variable elevation compared to IPAD's uniformly flat surface. Along Transect 6, the difference in mean elevation is much less at 0.24 m. Because the model generates elevation predictions as metres above sea level (m asl), different transects can be compared. In this case, there is a slight decrease (0.069 m) in mean elevation in the natural areas between Transect 4 to Transect 6. On the IPAD, mean elevation increases by 0.24 m from Transect 4 to Transect 6. The IPAD surface along Transect 6 is more variable than the IPAD area along Transect 4.

Along Transect 4, the mean water table changes from 0.546 m bgs in the natural area to 0.188 m bgs on IPAD. This indicates that the water table is much closer (by 0.358 m) to the ground surface on IPAD than in the natural area. Similarly, the water table is 0.277 m closer to the surface on IPAD than the natural area along Transect 6 (Figure 13). Areas along Transect 6 are wetter than areas along Transect 4.

This exercise shows that the models can reveal changes in microtopography and estimate the depth to water table with reasonable accuracy. The model outputs can be used to assess the conditions on-site compared to the surrounding peatland.

Table 4: Case study 2 – Predicted versus actual depth to water table (cm bgs) of the entire IPAD and by 40 m x 40 m grids.

Area	Corrected Depth to WT (cm bgs)	Predicted Depth to WT (cm bgs)
Entire Pad	10.9	10.7
Grid 1	25.8	24.2
Grid 2	23.7	11.9
Grid 3	2.9	15.1
Grid 4	12.8	15.1
Grid 5	7.5	2.3
Grid 6	10.4	2.3
Grid 7	8.1	12.4
Grid 8	2.1	10.1
Grid 9	6.4	6.0

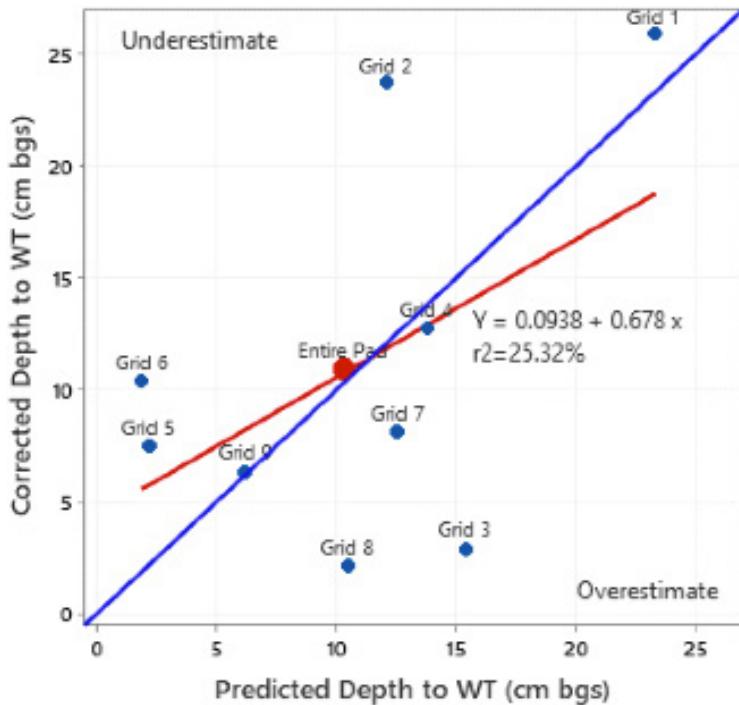


Figure 12: Corrected versus predicted depth to water table (cm) below ground surface (bgs) for the entire pad (red dot) and by 40 m x 40 m grids (blue dots). Positive values indicate a water table below ground surface (drier) and negative values indicate a water table above the ground surface (wetter). Solid blue line is the identity line (predicted = actual). Area above identity line is underestimate and area below is overestimate. Solid red line is the significant regression fit ($p = 0.002$).

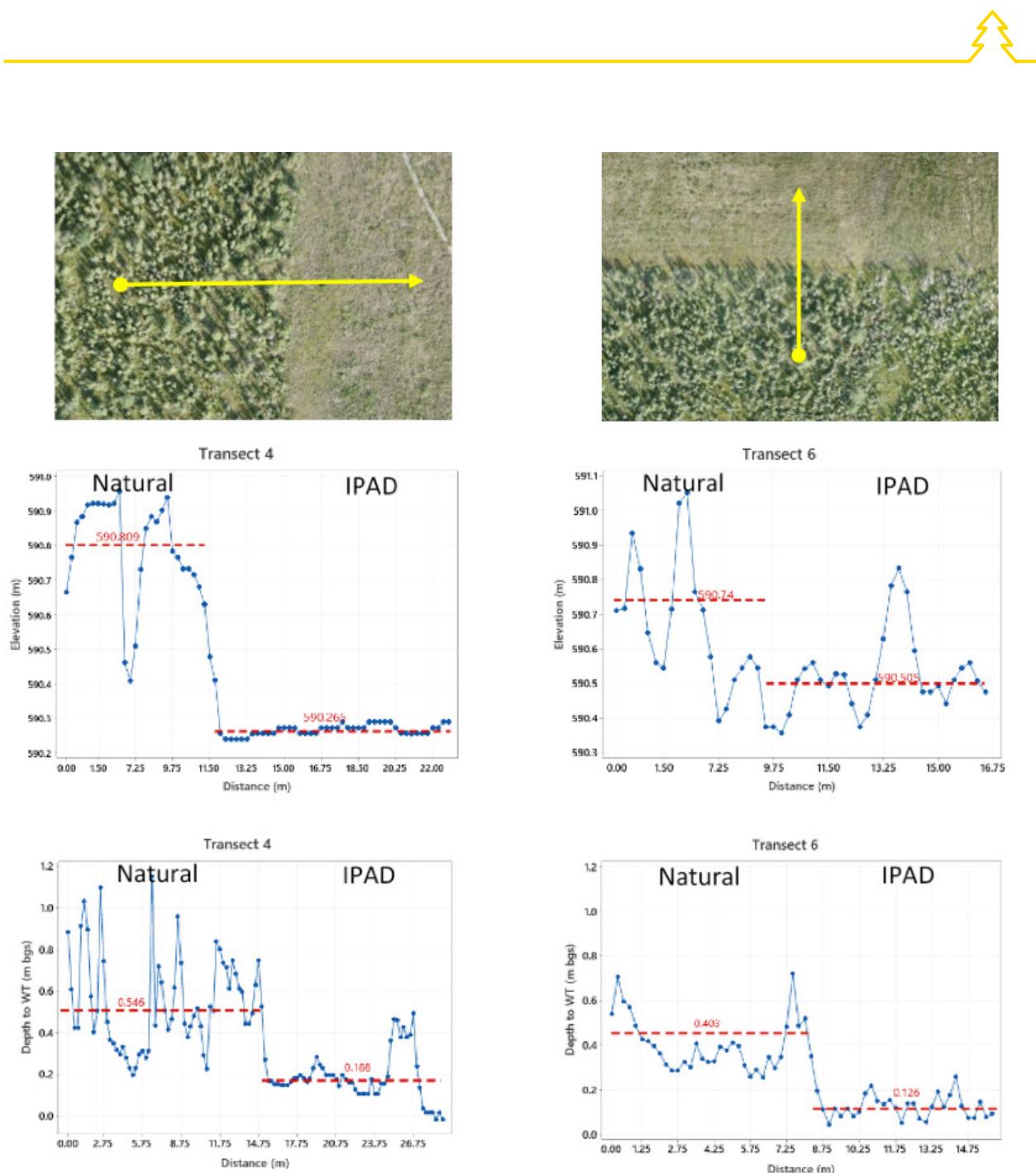


Figure 13: Top: RGB images of Transect 4 and 6 along the edges of IPAD. Middle: Estimated ground surface elevation (m) at 25 cm interval along Transect 4 and 6. Bottom: Estimated depth to water table (cm bgs) at 25 cm interval along Transect 4 and 6. Red dash lines and numbers are the means. Positive values indicate a water table below ground surface (drier) and negative values indicate a water table above the ground surface (wetter).



Case Study 3

Objective

Linear features through peatland often experience surface compression, which leads to changed soil thermal regime, nutrient cycling, vegetation composition, and greenhouse gas emissions (Strack et al., 2017; Davidson et al., 2020). The goal is to estimate the differences in ground elevation, an indication of compression, and depth to water table between natural areas and linear features.

Method

We selected two linear features around the IPAD to estimate microtopography and water table using the models. Three transects (T1 to T3) were randomly placed across two winter roads (Figure 14). Each transect was about 15 metres long, cutting across the entire road and extending into natural areas on both sides. The starting end of the natural area is referred as the “Upside” while the other end is referred to as the “Downside”. This naming scheme does not indicate the water flow direction. Elevation (metres above sea level or masl) and depth to water table (metre below ground surface or m bgs) points were generated at 25 cm intervals along each transect.

Results and Discussion

Along Transect 1, the mean road surface elevation is 590.405 m asl. It is 0.277 m lower than the upside and 1.085 m lower than the downside. In comparison, the mean road surface elevation along Transect 3 is 590.847 m, only 0.212 m, and 0.198 m lower than the upside and downside, respectively. The road along Transect 3 is 0.442 m higher in mean elevation than Transect 1 (Figure 14).

The mean water table of the road along Transect 1 is 0.355 m bgs. Water tables of the upside and downside are similar, both more than 0.4 m lower than the road. Along Transect 3, the mean road water table is 0.615 m bgs, only 0.16 m and 0.198 m higher than the upside and downside, respectively. The water table of Transect 1 road is 0.26 m closer to the ground surface than that of the road along Transect 3 (Figure 14).

The model outputs paint a clear picture of the conditions on the road versus the natural areas. They also allow us to compare the conditions of different linear features. The winter road along Transect 1 is flat and open with few woody species. It is much wetter (i.e., water table closer to the ground surface) than the surrounding peatland. In contrast, the road along Transect 3 has more trees and shrubs. It is less compressed and has a water table similar to the surrounding areas.

These results show that the models can reveal changes in microtopography and water table elevations along linear features. This can be very useful to prioritize linear features for restoration by assessing the degree of compression and water table. It can also be used to evaluate the recovery of linear features.

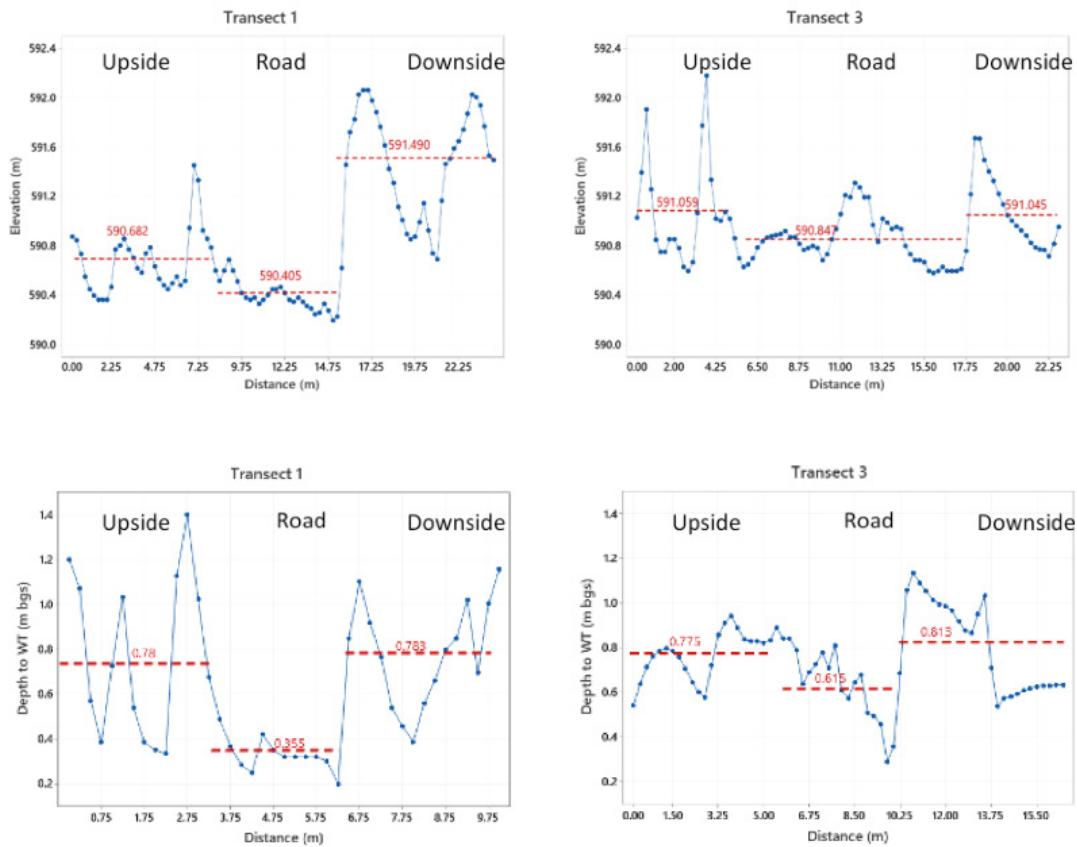


Figure 14: Top: RGB images of Transects 1 and 3 across two winter roads around IPAD. Middle: Estimated ground surface elevation at 25 cm interval along Transects 1 and 3. Bottom: Estimated depth to water table at 25 cm interval along Transects 1 and 3. Red dash lines and numbers are the means. Positive values indicate a water table below ground surface (drier) and negative values indicate a water table above the ground surface (wetter).



LESSONS LEARNED

Planning and correctly executing a site acquisition plan is an integral part of the success of a program such as this. The drone imagery acquired in this project had centimetre resolution at its finest scale and even the slightest shift in correcting can lead to poor classifications. There are many specifics to data acquisition and processing that are referenced in the technical document. Installed ground control points — which should be repainted for each acquisition — as well as the use of RTK GPS, increases the spatial accuracy of field data and helps to significantly decrease avoidable registration errors from the outset. The ultra-high resolution coupled with challenges of flying drones in remote areas, environmental conditions, limited sight to the ground layer, and sun angle are all essential considerations in creating a useful and accurate data set. This project utilized a team of experienced drone operators to oversee and deliver the survey.

The drone data produced many different types of products, each of which carries considerations. Vegetation can be masked by treed and high shrub areas as only the top of canopy is imaged. In some cases, conducting survey during the leaf off season can help. Selecting representative and visible vegetation classes and well distributed training data, plays a key role in the success of the vegetation classification model. This is also true for the water table products, as the drone needs visibility to the surface area in which there is surface water. Field RTK well locations used to produce accuracy measurements were under treed areas. In addition, treed areas and areas containing high shrub counts can also impact the accuracy of the microtopography layer. The level of detail obtained with photogrammetric point cloud UAS products is high enough to model microtopography in peatlands, although it does require significant processing power and is computationally expensive to produce.

The current study developed models for assessing vegetation, hydrology, and topography of peatland sites with reasonable accuracy validated against field data. With more training and tests, these technologies can be applied in the reclamation assessment as well as site planning at the regional scale. Areas with high disturbance density and significant changes in surface topography and vegetation can be prioritized for reclamation efforts. Standardized data acquisition can be repeated over time, which will become powerful tools for managers to understand the progression of site recovery (or lack thereof) in a timely and efficient manner. The use of UAV and remote sensing techniques can greatly reduce operational costs and health risks associated with accessing difficult terrains such as boreal peatlands. This project will pave the way for more studies like this. Ultimately, researchers should be able to include UAV and remote sensing as part of the monitoring and reporting system.



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PRESENTATIONS AND PUBLICATIONS

No public presentations or publications available.

RESEARCH TEAM AND COLLABORATORS

Institutions: LOOKNorth¹, NAIT Centre for Boreal Research², C-CORE³

Principal Investigators: Mark Kapfer¹, Bin Xu², Garrett Parsons³

Name	Institution or Company	Degree or Job Title	Degree Start Date (Students Only)	Degree Completion Date (Students Only)
Meyra Fuentes	C-CORE	Remote Sensing Engineer		
Rebekah Ingram	NAIT Centre for Boreal Research	Research Associate		
Melanie Bird	NAIT Centre for Boreal Research	Research Officer		
Frankie NcCorchuk	NAIT Centre for Boreal Research	Research Assistant		
Dani Degenhart	Natural Resources Canada	Research Scientist		
Jaime Pinzon	Natural Resources Canada	Restoration Ecology Scientist		
Neal Tanna	Innotech Alberta	Research Scientist		
Cassidy Rankine	Rankine Geospatial	Principal		

A Portable Testing Device for Wildlife Conservation

COSIA Project Number: LJ0334

Research Provider: McMaster University, University of Calgary

Industry Champion: ConocoPhillips

Industry Collaborators: Imperial, Teck

Status: Year 2 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 teams. 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 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 the Oil Sands region.

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.

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

COVID-19 pandemic restrictions continued to prevent both university teams from spending much time in the lab in 2021. The McMaster University (McMaster) labs were not accessible, and the University of Calgary labs were available only after a critical research designation for this project was successfully obtained. Despite this, researchers were still able to make good progress on the project and even benefitted from some of the insights yielded from McMaster's work around point-of-care COVID-19 detection methods (explained below). Below is a summary of project achievements for 2021:

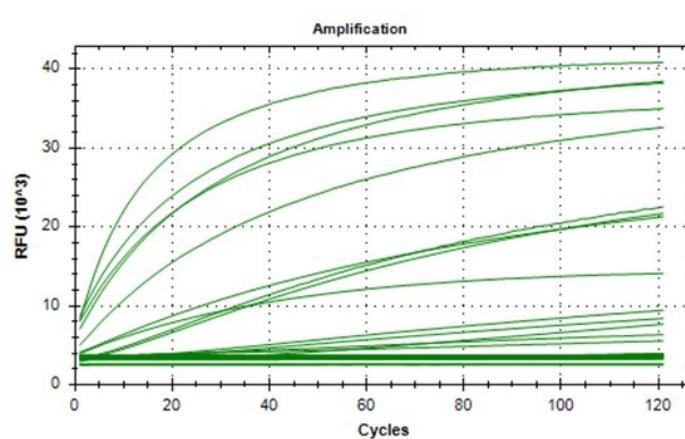
- In 2021, lab teams within and outside McMaster focused on developing SARS-CoV-2 detection methods that provided some valuable insights that research on this project will incorporate moving forward. One important discovery was the challenge around the use of rolling circle amplification (RCA) to concentrate 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. 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 method: 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). 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 (starting with the CRISPR Cas12a approach) once the isothermal amplification studies have generated sufficient data, as part of the project milestone goals.
- The McMaster team welcomed an exceptional PhD student in September from the UK, Letizia Dondi. Her previous experience in developing diagnostic assays for pathogen detection made her an ideal candidate for this project.
- Dondi and Natalie Schmitt (Post-Doctoral Fellow and McMaster project lead) identified four caribou specific regions from the mitochondrial genome to trial with the amplification methods. The first sequence was developed



from the Cytochrome b gene (51nt) (Mumma et al., 2016). The second from a consensus region identified from an alignment of haplotype sequences from the control region (34nt), which was provided by Maria Cavedon and Marco Musiani (University of Calgary). The third is a forward and reverse primer sequence from D-loop, and last is a small non-homologous region obtained from differences found between the mitochondrial genome of caribou and white-tailed deer. All four regions were found to be identical to *Rangifer tarandus* (caribou). However, to ensure absolute specificity to caribou, these sequences will need to be confirmed on this project's caribou and white-tailed deer fecal samples (stored at the University of Calgary). This next step is essential to determine the level of cross-reactivity of the target with closely related phylogenetic species.

- Dondi began testing the RCA padlock probe approach with the synthetic caribou specific sequences and five different circular DNA templates and had immediate success (Figure 1). The circles recognized and bound to the caribou sequences and initiated an amplification response. It is unusual for experiments to work on first attempts, so this is a great achievement. Dondi will continue to optimize these reactions and test sensitivity limits.

RCA ligation reaction



Sample	Result
CytB ligation	+
CytB circle	-
Dloop fwd ligation	+
Dloop fwd circle	+
Alignment ligation	+
Alignment circle	+
Dloop rvs ligation	+
Dloop rvs circle	+
Haplotype ligation	+
Haplotype circle	-

Figure 1: qPCR reactions trialing RCA ligation of 5 different circular DNA templates with synthetic caribou specific sequences. Ligation and amplification was a success for all circle/sequence combinations.

- Maria Cavedon (Post-Doctoral Fellow) with the University of Calgary team, sourced fecal samples from 30 caribou individuals, which were captured in northern Alberta from across three different years (10 from 2002; 10 from 2005; and 10 from 2009), as well as samples from white-tailed deer. Cavedon also extracted DNA from these fecal pellets following a new protocol available as part of the University of Calgary's collaboration on other projects with Laval University (Brodeur, 2021). Preliminary results indicate a good concentration of DNA obtained with this extraction protocol. McMaster has also sourced caribou fecal samples from the Toronto Zoo to ensure researchers have a diversity of individuals and populations. The samples will be initially used to test DNA quantities at different sites on the pellet (half from the surface of the pellet and half within), to ensure the robustness of our methodology combined with a 'forgiving' method of collection. The DNA extracted from these samples using conventional methods, will act as a benchmark for our paper-based extraction approach and will be used to verify the effectiveness of our caribou-specific sequences.



- Schmitt began preliminary discussions with ConservationX and the Wildlife Conservation Society (WCS), around adapting their DNA extraction methodologies for difficult sample types such as fecal material, skin, and bone samples for this project's technology. It is believed that incorporating tried-and-tested methodologies will save time and expense. ConservationX has developed a simple sample preparation strategy that could work well with this project's paper platform (Holmes et al., 2020). Likewise, WCS has an extraction protocol that is able to yield viable DNA, even from 30-year-old bone samples, that amplifies well using downstream PCR analyses (Henger, 2021). Project researchers will be exploring these options further in the coming year.
- McMaster has approved employment of an additional post-doctoral fellow from Iran, Dr. Mehdi Dadmehr using external funding sources. He is expected to arrive in Canada to work with the McMaster team in February 2022. Dadmehr brings a wealth of experience in electrochemistry and will be partially under the guidance of Dr. Leyla Soleymani, also at McMaster, who specializes in the development of advanced materials for biosensing. It is anticipated that Mehdi will help accelerate progress on this research.
- Despite major setbacks caused by COVID-19 pandemic restrictions in 2021, research teams were able to make significant advances towards Aims 1 and 2. Researchers are overcoming the lost time by; the addition of a PhD student to the McMaster University team; taking advantage of progress in DNA amplification methodology around point-of-care diagnostics for COVID detection; and the incorporation of DNA extraction protocols developed in other labs.

LESSONS LEARNED

Research experiences in 2021 showed how important simplicity, specificity, and adopting a pragmatic approach are to this caribou detection technology. Industry field personnel and managers need the device to be both easy to use for non-genetic experts and specific to caribou to ensure management decisions can be made in a timely manner.

Just as it is difficult to distinguish between caribou and white-tailed deer fecal samples in the field, researchers have realized that the two species are also challenging to distinguish genetically. As such, considerable time and care has been taken to ensure the right target areas of the mitochondrial genome have been selected. This will ensure specificity and that these sequences can be readily detected in fecal samples. There are five sequences this project will be trialing for caribou and white-tailed deer with the goal of ensuring that only the presence of caribou DNA will initiate an amplification reaction and colour response. Choosing these sequences was essentially the most important and challenging component of Aim 2 (paper-based amplification and detection), and researchers were surprised at how difficult this task was given the available literature.

Through the use of literature searches on current DNA/RNA detection methodologies and discussions with other labs that have tested similar technologies in the field for field-readiness, project researchers have learned that incorporating fewer steps and simpler technology results in less chance for error and lower costs. Incorporating these findings will enable industry to reduce environmental monitoring costs and allow more people (non-experts) to be involved in the monitoring process.

The pandemic has accelerated progress in molecular diagnostics (e.g., Lau et al., 2020) providing project researchers with the unique opportunity to pivot research towards the use of potentially more advanced methods that require fewer steps (Aim 2). Project researchers were also able to learn from other labs with similar goals in simplifying species detection around DNA extraction (Aim 1) and plan to incorporate aspects of their methodology.



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Journal Publications

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AWARDS

Natalie Schmitt of the McMaster team has been selected for the Explorers Club, Explorers 50 program. An initiative aimed at recognizing and amplifying 50 remarkable explorers changing the world. The Explorers Club is a multidisciplinary, professional society dedicated to the advancement of field research, scientific exploration and resource conservation (<https://www.explorers.org>). Some of the most well-known members of this club have included Sir Edmund Hillary, Roald Amundsen, Neil Armstrong, and Buzz Aldrin. Every honoree is exploring, inspiring, and creating the future - the future of the planet, sustainability, of communication, of biology, what our communities should look like, and so much more. The EC50 was established to not only reflect the great diversity of exploration, but to give a voice to trailblazing explorers, scientists, artists, and activists doing incredible work.

RESEARCH TEAM AND COLLABORATORS

Institution: McMaster University¹ and the University of Calgary²

Principal Investigator: Dr. Carlos Filipe¹

Co-Principal Investigators: Dr. Marco Musiani² and Dr. Yingfu Li¹

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		
Letizia Dondi	McMaster University	PhD Student	2021	Estimated 2025



ENVIRONMENTAL RESEARCH AND MONITORING

Boreal Ecosystem Recovery and Assessment, Phase 2

COSIA Project Number: LJ0220

Research Provider: University of Calgary

Industry Champion: ConocoPhillips

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

Status: Year 1 of 5

PROJECT SUMMARY

The boreal region of Alberta contains extensive disturbances related to natural-resource extraction. Roads, seismic lines, well pads, forest harvest areas, and other elements of human activity exert cumulative environmental effects that influence vegetation communities, wildlife, hydrology, and carbon dynamics. The Boreal Ecosystem Recovery and Assessment (BERA) project (www.beraproject.org) is a multi-sectoral research partnership of academic institutions (University of Calgary, University of Alberta, University of Waterloo), private-sector companies (Alberta-Pacific Forest Industries Inc., Canadian Natural, Cenovus, ConocoPhillips, Imperial), a public-sector division (Canadian Forest Service's Northern Forestry Centre), and a not-for-profit organization (Alberta Biodiversity Monitoring Institute). Our 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

Our work targets knowledge gaps relevant to four strategic management priorities: (i) promoting a return to forest cover in disturbed areas, (ii) restoring natural carbon dynamics in disturbed peatlands, (iii) maintaining wildlife populations, and (iv) enhancing woodland caribou habitat. 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

- Angelo Filicetti modeled the time it took trees on seismic lines to grow to heights of three metres. The work took place across a 30,000 km² portion of the Lower Athabasca from Conklin to McClelland Lake and involved destructively sampling more than 1,800 trees of nine species. Age-height relationships were examined for nine ecosites and for six of those under recently burned (< 23 yrs.) or unburned (mature) conditions. Tree density on most seismic lines was found to exceed provincial restoration targets, but the main limiting factor was vertical growth (height). It takes an average of 20 years for trees to reach three metres if the lines are not re-disturbed, although trajectories varied widely between ecosites and burned and unburned conditions. There are several factors affecting recovery failures: (i) higher water tables on lines in peatlands decrease recruitment, survival, and growth; (ii) forests which are shade-intolerant and fire-dependent require wildfires to stimulate regeneration; (iii) animal trampling and herbivory can limit growth; and (iv) seismic line re-clearing and re-use can limit establishment.



- Kimberly Kleinke compared changes to soil properties from different mounding techniques on seismic lines in peatlands. Understanding changes to soil properties, which in turn promotes a return to forest cover, provides information that will help land managers better assess the use of different mounding techniques in peatland restoration projects. The effects of mounding on soil properties were determined through analysis of surface peat and peat cores from seismic lines crossing fens near Cold Lake and Brazeau, Alberta. An incubation study using surface peat samples examined the effectiveness of easily measured decomposition markers as indicators of carbon (C) loss from mineralization. Inverted mounding was associated with changes in soil properties that may influence forest recovery (e.g., increase in bulk density that maintain high soil moisture content despite elevated position). Additionally, inverted mounding altered vegetation communities with lower moss cover and higher bare ground cover. The two newer mounding methods – intact mounding and hummock transfer, which keep the soil profile intact – did not show these changes and were largely comparable to natural conditions. Intact mounding, also referred to as in-line mounding, is a similar technique to inverted mounding apart from keeping the peat profile intact. Hummock transfer uses a different method by moving natural hummocks onto the seismic line. Hummock transfer has the advantage of not creating water-filled holes on the seismic lines but creates some disturbances in adjacent natural areas. Longer term testing is required to determine how the two lower disturbance techniques (intact mounding and hummock transfer) support return to forest cover in comparison to inverted mounding.
- Laureen Echiverri examined the recovery of understory communities on mounded and unmounded seismic lines three years after the application of mounding, with recovery defined as similarity to the adjacent reference fens. Sampling was conducted at three microtopographic positions on the hummock: top of hummock, slope of the hummock, and level ground. We found that mounding treatments disturbed the initial recovering vegetation on lines leading to communities dominated by disturbance-associated mosses. In contrast, recovery of the unmounded seismic lines was evident, with the re-establishment of a peat-accumulating understory community and the natural development of hummock-hollow microtopography. The bryophyte layer was particularly affected by mounding, with significantly lower Sphagnum and total bryophyte cover on mounded seismic lines than in the reference fens. Instead, mounded seismic lines were characterized by higher cover of pioneer true moss species than both the reference and unmounded treatments. In contrast, Sphagnum cover and bryophyte composition were more similar between the unmounded and reference fens. Bryophyte communities at the top position showed the strongest differences among treatments, indicating recovery is slowest at this position.

Restoring Natural Carbon Dynamics

- Recent efforts to restore seismic lines involve mechanically creating microtopography to encourage pre-disturbance soil and vegetation dynamics. Understory vegetation influences tree microhabitat conditions and competition, as well as ecosystem productivity and nutrient cycling. Ellie Goud measured the relative abundance and evolutionary relationships among 82 vascular and non-vascular plant species across six study sites, including Foster Creek, Kirby and Clyde BERA study sites and additional seismic lines near Peace River, Alberta. Functional trait data for 36 species was obtained from publicly available plant trait databases for plant height (H), leaf dry matter content (LDMC), leaf nitrogen (N) and phosphorus (P) contents. Mounded sites differed from untreated lines and reference sites, with shifts towards higher community-weighted H, LDMC, N, and P. These changes are caused by turnover of species with different functional attributes. For example, species unique to the mounds were predominantly horsetails, herbs, and graminoids with relatively larger leaf N and P. It appears that mounding pushes plant community structure and its function further from the adjacent forested peatland reference system.



- Seismic-line disturbances create changes in both the vegetation communities and how these communities function. One such functional change is how the ground-layer vegetation communities green up over the course of the growing season and how this affects productivity. Scott Davidson investigated the greenness patterns of vegetation communities on two different peatland types (bogs and fens) impacted by seismic lines. His team collected digital photographs using smartphones, alongside vegetation surveys and carbon exchange measurements. Disturbances were found to significantly impact the greenness of these communities, with disturbed areas becoming more productive faster. This may offset some of the loss of productivity of the overstory. Further, the strong link between greenness and plant productivity indicates that this could be an inexpensive method to monitor recovery and carbon uptake of the understory on seismic lines in peatlands.

Maintaining Wildlife Habitat / Enhancing Caribou Habitat

- The Canada Warbler (CAWA) is a species at risk that relies on old-growth aspen forest for breeding habitat. By addressing knowledge gaps on how this species responds to seismic lines we can further our understanding of them and advise on restoration practices in upland forests that would be broadly beneficial for species in similar and adjacent habitats. Jocelyn Gregoire completed acoustic-localization surveys along seismic lines near Lac La Biche and Lesser Slave Lake. Canada Warbler (CAWA) presence and relative abundance was predominantly influenced by seismic lines with a high level of disturbance and little to no woody regeneration. When CAWA were present near a seismic line, they were more actively singing and more likely to cross the line with increased shrub cover on the line. Most lines we encountered did have some regeneration making it unlikely that there are large population effects of seismic lines on Canada Warblers, but more information on what proportion of seismic lines in old aspen forest have advanced regeneration is needed.
- When evaluating how energy sector activities influence biodiversity, we often focus on the change of vegetation structure and composition. However, the presence of people and the disturbance we make (e.g., noise, light) can also influence habitat selection by certain species. Natalie Sánchez completed a study on the relative importance of natural vegetation; footprint from energy sector disturbances, and the noise generated by roads and energy sector facilities on the occupancy of a common sparrow, the Lincoln's Sparrow. She deployed 280 acoustic recording units along a gradient of industrial development. Lincolns Sparrows were found to be largely tolerant to industrial disturbance, but the acoustic environment had a slight negative effect on occupancy and influenced their singing behaviour.
- Forest understory structure is a key driver of many relevant ecosystem processes including forest regeneration, wildlife movement, and carbon dynamics. However, this type of information is largely absent from existing inventories. Silvia Losada compared field and LiDAR observations of five understory structural attributes: understory mean height, percent understory cover, understory density, understory complexity, and understory volume. The effect of three error influencing factors – canopy cover, bole density, and canopy complexity – was quantified. There is very little bias in LiDAR-based estimates of percent understory cover. However, LiDAR tends to underestimate understory mean height and volume, and to overestimate understory density and complexity. The main bias-inducing factors among those we observed are canopy closure (understory measurements were better in open canopies) and bole density (understory measurements were worse in stands with many boles/stems). Canopy complexity had negligible effects on everything except understory volume, where there was a weak positive association.



LESSONS LEARNED

Promoting Return to Forest Cover

- (Filicetti) Once trees are established on seismic lines and not re-disturbed, it takes an average of 20 years for them to reach heights of three metres, but this average varies widely among ecosites from rapid growth in upland ecosites to slow growth in peatlands. This implies that tree establishment, survival, and/or seismic line disturbances (re-clearing/re-use) are the main factors affecting seismic-line recovery.

A key uncertainty is seismic line history, such as: (i) original seismic line creation dates; (ii) seismic line re-clearing and re-use occurrences/dates; (iii) machinery used; (iv) operator instructions; (v) recreational use; and (vi) animal usage, trails/trampling, and herbivory. Other geographic regions and forests may behave differently, but we expect patterns observed across this region to be similar as other parts of the western boreal forest.

- (Kleinke) Intact mounding or hummock-transfer techniques may result in less soil and vegetation disturbance than current methods of inverted mounding. In addition, easily measured soil properties, $\delta^{13}\text{C}$ and C/N ratios, could replace or support traditional carbon flux measurements. For hard-to-access sites, time and expenses in the field could be saved by using one-day soil sampling over weekly carbon fluxing.

Although soil properties are strongly linked to forest recovery, more research would be needed to confirm the direct effects of altered soil properties on the goal of returning to forest cover. More long-term studies would be beneficial to assess the effects of new mounding techniques on tree growth and survival. The relationship of soil $\delta^{13}\text{C}$ and C/N ratios with carbon loss needs to be confirmed in the field.

- (Echiverri) Understory communities, particularly the bryophyte layer, drive the development of the hummock-hollow topography characteristic of peatlands and are responsible for the peat accumulation (i.e., carbon storage) that defines peatland ecosystems. Since understory communities influence many peatland ecosystem functions, understanding how mounding affects the recovery of the understory is important for evaluating the impacts of mounding on peatland recovery. Inverted mounding alters understory communities on seismic lines in treed fens, at least in the early years after treatment. Actions that limit continued disturbance on seismic lines, including limiting where possible applications of mounding treatments, will help prevent further delays in plant-community recovery.

This study focused on moderate-rich fens, future studies should also explore recovery on poor fens and bogs. Further monitoring is required to understand how these communities may change over time. One important feature of mounding treatments on seismic lines are the deep pools between mounds. The impact and recovery of these pools over time should also be assessed.

Restoring Natural Carbon Dynamics

- (Goud) We may be trading off function of the understory for improved tree growth. Although these changes might be temporary as plant communities undergo succession, more information is needed to assess the long-term success of mounding treatments on restoring pre-disturbance vegetation dynamics. Directly measuring functional traits in the field within species, especially species that occur in seismic lines (treated and untreated) and reference sites would provide key information on plant and ecosystem function between untreated and restored seismic lines.



- (Davidson) Linear disturbances shift plant-community phenology in peatlands. Plant communities on lines “green up” faster than their surroundings, creating greater CO₂ uptake. Using smartphones to collect photographs provided a quick and easy method to collect greenness data without the need for expensive equipment or fixed infrastructure. Furthermore, our ability to link the easy-to-measure greenness indices with productivity measurements also shows promise as a way to monitor and map shifts in peatland carbon exchange in response to linear disturbances and recovery over time. This would be valuable for regional to national accounting of greenhouse gas emissions.

Maintaining Wildlife Habitat / Enhancing Caribou Habitat

- (Gregoire) Seismic lines appear to have limited population-level effects on Canada Warblers, however more information on what proportion of seismic lines in their habitat are vegetated is needed. Seismic lines do have strong effects on Canada Warbler behaviour that can be mitigated by ensuring the lines in older aspen forest reach a minimum average height of one metre at a density equivalent to that of the adjacent forest. This may not require active restoration but will require better access management in upland forests (i.e., ATV restrictions). This would not only be beneficial to Canada Warblers and upland territorial songbirds, but also help restrict predator movement into lowland habitat.
- (Sanchez) To maintain wildlife habitat for songbirds, the acoustic environment must be considered in monitoring and mitigation efforts. For terrestrial passersines and especially for Neotropical migratory birds, such as the Lincoln’s Sparrow, who find new conditions every year in the boreal forest, understanding the multiple environmental factors that could diminish quality of breeding territories is needed. Current mitigation efforts focus on vegetation recovery and restoration. However, alternative strategies like noise suppression will also improve habitat conditions for some species of birds.
- (Losada) LiDAR is an active form of remote sensing that has the capacity to penetrate forest canopies and generate signals from the understory. LiDAR-derived measures of forest understory structure are largely ready for operational deployment under many boreal conditions. Some biases remain, which we have documented. LiDAR tends to underestimate understory mean height and volume, and to overestimate understory density and complexity. The main bias-inducing factors are canopy closure (understory measurements were better in open canopies) and bole density (understory measurements were worse in stands with many boles/stems). Canopy complexity has negligible effects on everything except understory volume, where there was a weak positive association.

The Losada research took place within the Canadian Natural Kirby South study area. The results may be different in other parts of the boreal forest with different forest types. For example, we did not assess many upland deciduous sites.



PRESENTATIONS AND PUBLICATIONS

Journal Publications

Filicetti, A. T., LaPointe, R. A. and S. E. Nielsen. 2021. Effects of Fire Severity and Woody Debris on Tree Regeneration for Exploratory Well Pads in Jack Pine (*Pinus banksiana*) Forests. *Forests* 12 (10), 1330 <https://doi.org/10.3390/f12101330>

Goud, E. M., Touchette, S., Strachnan, I. B. and M. Strack. 2021. Graminoids vary in functional traits, carbon dioxide and methane fluxes in a restored peatland: implications for modeling carbon storage. *bioRxiv* doi: <https://doi.org/10.1101/2021.05.27.445980>

Davidson, S. E., Goud, M., Malhora, A., Estey, C. O., Korsah, P., and M. Strack. 2021. Linear disturbances shift boreal peatland plant communities toward earlier peak greenness. *JGR Biogeosciences*. <https://doi.org/10.1029/2021JG006403>

Hedley, R. W., Wilson, S. J., Yip, D. S.A., Li, K., and E. M. Bayne. 2021. Distance truncation via sound level for bioacoustic surveys in patchy habitat. *Bioacoustics* 30 (3), 303-323 <https://doi.org/10.1080/09524622.2020.1730240>

DeLancey, E. R., Brisco, B., McLeod, L. J. T., Hedley, R. W., Bayne, E. M., Murnaghan, K., Gregory, F. and J. Karyieva. 2021. Modelling, Characterizing, and Monitoring Boreal Forest Wetland Bird Habitat with RADARSAT-2 and Landsat-8 Data. *Water* 13 (17), 2327. <https://doi.org/10.3390/w13172327>

Franklin, C. M. A., Filicetti, A. T. and S. E. Nielsen. Seismic line width and orientation influence microclimatic forest edge gradients and tree regeneration. *Forest Ecology and Management* 492 (2021) 119216 <https://doi.org/10.3390/f12101330>

Published Theses

Echiverri, L. F. I., 2021. Edge influence from linear disturbances and recovery of understory communities in boreal forests. PhD Dissertation, University of Alberta.

Filicetti, A. T., 2021. Fire and tree recovery on seismic lines. PhD Dissertation, University of Alberta.

Gregoire, J., 2021. Canada Warbler (*Cardellina canadensis*) response to vegetation structure on regenerating seismic lines. MSc Thesis, University of Alberta

Losada, S., 2021. LiDAR Characterization of Boreal Understory Structure. MSc Thesis, University of Calgary.

Kleinke, K. 2021. Effects of restoring peatland seismic lines on soil properties in boreal Alberta, Canada. MSc Thesis, University of Waterloo.

Sánchez-Ulate N. 2021. Variation in song characteristics and responses to anthropogenic noise of Lincoln's Sparrow (*Melospiza lincolni*) in the boreal forest. PhD Dissertation, University of Alberta.



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)
Dr. Scott Nielsen	University of Alberta	Professor		
Dr. Erin Bayne	University of Alberta	Professor		
Dr. Maria Strack	University of Waterloo	Professor		
Dr. Scott Ketcheson	Athabasca University	Assistant Professor		
Dr. Julia Linke	University of Alberta	Science Coordinator		
Dr. Richard Zeng	University of Calgary	Geospatial Technician		
Maverick Fong	University of Calgary	Geospatial Technician		
Claudia Maurer	University of Calgary	Admin. Coordinator		
Jennifer Hird	University of Calgary	Remote Sensing Scientist		
Colette Shellian	University of Calgary	MSc Student	2020	2022
Tanya Yeomans	University of Calgary	MSc Student	2021	2023
Lelia Weiland	University of Calgary	MSc Student	2021	2023
Tharindu Kalukapuge	University of Alberta	PhD Student	2021	2025
Dr. Richard Hedley	University of Alberta	Post-Doctoral Fellow		
Brenden Casey	University of Alberta	PhD Student	2018	2022
Laureen Echiverri	University of Alberta	PhD Student (defended)	2017	2021
Jocelyne Gregoire	University of Alberta	MSc Student (defended)	2015	2021
Angelo Filicetti	University of Alberta	PhD Student (defended)	2016	2021
Dr. Richard Hedley	University of Alberta	Post-Doctoral Fellow	2017	2021
Natalie Sanchez Ulate	University of Alberta	PhD Student (defended)	2017	2021
Dr. Scott Davidson	University of Waterloo	Post-Doctoral Fellow		
Percy Erasmus Korsah	University of Waterloo	PhD Student	2017	2022
Kimberley Kleinke	University of Waterloo	MSc Student (defended)	2019	2021
Jennifer Fliesser	University of Waterloo	MSc Student	2021	2023
Nazia Tabassum	University of Waterloo	PhD Student	2021	2025
Maryam Bayat	University of Waterloo	PhD Student	2021	2025
Dr. Ellie Goud	University of Waterloo	Post-Doctoral Fellow		
Dr Guillermo Castilla	Canadian Forest Service	Research Scientist		
Dr. Ralf Ludwig	Ludwigs-Maximilian University	Professor		
Dr. Matthias Schubert	Ludwigs-Maximilian University	Professor		