Examining the feasibility of terrestrial lichen transplantation and seeding technology for woodland caribou habitat restoration

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Executive Summary

Lichens, despite their vital ecological functions, are often not included in reclamation efforts. Terrestrial lichens, especially reindeer lichens, serve as a winter food source for caribou, underpinning the entire ecosystem. However, the recovery of these lichen communities is severely hampered by habitat disturbance, their inherently slow growth rates, and limited natural dispersal capabilities. While studies have demonstrated that artificial dispersal and transplanting of lichen fragments are promising techniques for restoring these communities, significant challenges remain regarding the complexity of transplantation, large-scale feasibility, and the effects of different substrates. Therefore, dedicated research and innovative approaches are essential to bridge these gaps and successfully incorporate lichen restoration into broader ecological recovery strategies.

This study aimed to 1) conduct a comprehensive examination of lichen establishment studies, evaluating their long-term outcomes, methodological approaches, and the lessons for contemporary restoration efforts, 2) investigate how substrate and fragment size affect transplant success of common boreal terrestrial lichens in a greenhouse environment over a 26-month duration, 3) assess the effects of terrestrial lichen fragment size on establishment across different substrates within harvest forest harvested area, and 4) examine the feasibility of hydroseeding lichens for restoring harvested forest areas.

Chlorophyll fluorescence analysis (F_v/F_m) was used to assess the survival and health of the lichen. Lichen cover at historical sites and within field plots was evaluated using percentage covers, while hydroseeding trials measured the number of lichen fragments within 10 x 10 cm quadrats. In greenhouse experiments, the length of lichen fragments, biomass, and visual health were recorded. The covers of woody plants, forbs, graminoids, and mosses were documented in the field plots, and ecological site data were collected for all sites.

Reindeer lichen transplantation has shown promising results in long-term field assessments, with transplanted lichens demonstrating resilience and growth in reclaimed areas. Greenhouse experiments revealed that larger lichen fragments stayed viable longer, though they were more prone to breaking apart. Field trials confirmed this pattern: larger fragments had significantly higher survival rates than smaller ones, while medium-sized fragments achieved the best coverage. Lichens grown on soil and pine needle substrates showed better health than those on moss substrates, though all four substrates used in the study resulted in a similar survival rate. Lichen dispersal rates were significantly higher in plots adjacent to transplanted lichens, indicating that proximity drives establishment. Hydroseeding trials proved effective for distributing lichen material, though initial survival rates remained low. While the tackifier did not significantly affect growth during the experiment, it did produce higher initial fragment densities. Together, these findings offer practical insights into management strategies that can improve lichen restoration outcomes.

In conclusion, successful lichen restoration, especially within caribou ranges, is achievable with careful attention to substrate, fragment size, and effective competition management. Long-term

monitoring and further research are needed to optimize restoration methods and improve transplant viability. Based on the results of these studies, the following are recommended:

- Initiate larger-scale reindeer lichen transplantation projects to support caribou habitat recovery and to improve understanding of factors affecting transplant success.
- Optimal transplantation success is anticipated in burn sites, which are preferred over forest harvested blocks because of their diminished natural regeneration.
- The size of lichen fragments can affect their survival, with larger, less fragmented pieces generally doing better. However, medium-sized fragments might offer greater overall coverage
- The recommended transplant species for this application include *Cladonia arbuscula ssp. mitis*, *C. rangiferina, C. uncialis*, and *Stereocaulon tomentosum*, while *C. stygia* is not recommended.
- Lichens should be spread more evenly and over a broader area, rather than in dense groups, to decrease competition among individual lichen fragments and consider their naturally limited dispersal abilities.
- Habitats more likely to succeed are those with less feather moss, forbs, and dense trees, and not in or near areas where water might pool.
- Open sites with poor nutrient regimes and mesic to xeric moisture regimes have been tested most extensively over the long term, although richer and moister sites may also be successful.
- Hydroseeding can spread reindeer lichen fragments, but further research is advised to enhance fragment survival.
- Investigating lichen collection techniques that cause minimal damage to source populations is an important next step, and has not been studied in the boreal forest to our knowledge

Reassessment of Historical Reindeer Lichen Transplantation Sites in Alberta and British Columbia

Abstract

Lichens play a vital role in many ecosystems and are a key food source for caribou during the winter when other food sources are limited. Currently, in forest harvested block reclamation, the focus tends to be on re-establishing trees and shrubs, which frequently leads to lichens being overlooked due to their small size, slow growth, and a lack of understanding of their growth and dispersal requirements. We used greenhouse experiments, field trials, and assessments of historical lichen transplant sites to gain insights into lichen restoration methods.

We reassessed three historical lichen transplanting sites. The first site, initiated by West Fraser Timber 24 years ago in west-central Alberta, involved a trial across nine harvested blocks with three treatments: control, hand transplant, and broadcast transplant. This study focused on three lichen species: *Cladonia arbuscula* ssp. *mitis, C. rangiferina, and C. uncialis.* The second site was established in a burned area in northern British Columbia 8 years ago. It included the transplantation of *C. mitis*, *C. rangiferina*, and *C. uncialis* using three methods: mat, fragment, and hybrid. The third site was the Tweedsmuir project, initiated in west-central British Columbia 6 years ago, which explored three different transplant techniques in burned areas.

Historic transplant sites indicated promising long-term viability of the lichen transplants, with healthy lichen mats observed in many treatment plots. To effectively promote the recovery of lichen populations, which serve as a winter food source for caribou, it is important to understand the various elements that contribute to their establishment and proliferation. By identifying optimal light exposure, moisture levels, suitable substrates, and the presence and intensity of competition from other lichens, mosses, or vascular plants, practitioners can prescribe targeted strategies to cultivate thriving lichen habitats. These factors are interconnected, dictating where and how effectively lichens can colonize and thrive within an ecosystem (Armstrong 2014).

Introduction

Lichens comprise a significant portion of the diet of woodland caribou in winter when their forage is most limited (Bergerud 1972; Danell et al. 1994). Terrestrial lichens, such as species of *Cladonia* subgenus *Cladina*, have been shown to comprise 60-83% of their winter food source in west-central Alberta (Thomas et al. 1996). In boreal Alberta, these lichens include species such as *Cladonia arbuscula* (Wallr.) Flotow., *C. rangiferina* (L.), *C. stellaris* (Opiz.), *C. stygia* (Fr.) Ruoss, and *C. uncialis* (L.) Weber ex Wigg, here collectively referred to as “reindeer lichens”. In northern and west-central Alberta, these lichens are predominantly found in dry locations, such as sandy sites and bogs (Beckingham and Archibald 1996; Beckingham et al. 1996).

Reindeer lichens are long-lived, perennial species, with ages estimated to be between 20 and 30 years for *C. rangiferina* (Wangerman 1965) and up to 100-120 years for *C. stellaris* (Andreev 1954).

The organisms have long been recognized as K-strategists, allocating more resources to non-reproductive activities than to reproduction (Ahti 1982; Longton 1992). They are well-suited to the cooler temperatures typical of northern latitudes and need a steady, though not excessive, supply of moisture from precipitation and humidity, while also showing resilience to drier conditions once established. Despite their ability to survive in places where many organisms cannot thrive, these lichens generally establish themselves later in ecological succession because of several specific ecological limitations (Kershaw 1977; Ahti and Oksanen 1990; Webb 1998; Roturier et al. 2017). First, they require a particular substrate that can support both initial colonization and their delicate long-term growth. Even after finding a suitable substrate, these lichens grow very slowly, taking a considerable amount of time to develop into mature, widespread populations. Their limited dispersal ability worsens this problem by restricting their capacity to spread to new, suitable areas.

As poikilohydric organisms, lichens rely on water supplied through precipitation and atmospheric moisture. Most lichen species grow very slowly (Karenlampi 1971; Helle et al. 1983; Brodo et al. 2001; den Herder et al. 2003). On average, reindeer lichens grow about 3 to 6 mm per year (Scotter 1963; Vasander 1981; Helle et al. 1983; McMullin and Rapai 2020; Duncan 2011), although this varies by latitude and forest cover (Scotter 1963; Helle et al. 1983). Vasander (1981) estimated the combined annual production of *C. rangiferina* and *C. arbuscula* at 2.8 g/m^2 in southern Finland.

Forest management, climate change, and wildfire impact to the natural habitats of lichens (Pykala 2004; Richardson and Cameron 2004; Reinhart and Menges 2004; Johansson and Reich 2005; Ray et al. 2020). It takes considerable time to re-establish reindeer lichens after disturbance; for example, after a forest fire, their re-establishment takes about 40 years in peatlands and 50 to 100 years in upland woodlands (Morneau and Payette 1989; Coxson and Marsh 2001; Dunford et al. 2006). Accordingly, fires have a strong influence on reindeer lichen distribution, and therefore on the spatial distribution of foraging habitat for boreal caribou (Dunford et al. 2006; Ray et al. 2020).

Many studies have examined the use of transplanting whole or fragmented reindeer lichens for restoring reindeer and caribou habitats in areas that have been heavily disturbed (Crittenden 2000), Sweden (Roturier et al. 2007; Roturier and Bergsten 2009; Roturier et al. 2017), and Canada (Enns 1998; Campeau and Blanchard 2010; Duncan 2011; Hugron et al. 2013; Duncan 2015; Ronalds 2018; Rapai et al. 2018). Fragmentation is thought to be the main reproductive strategy in reindeer lichens (Ahti 1977), although some may produce sexual or asexual spores, particularly under ideal conditions (Jahns et al. 2004). However, most fragments disperse within only 1 m of their source (Roturier et al. 2007), likely at least partially explaining their slow ingress after disturbance.

Terrestrial lichen transplantation studies often follow the establishment and growth of lichen for the first few years, making the assessment of the long-term efficacy of lichen transplants difficult. Historical lichen transplantation sites provide a unique opportunity to assess changes in lichen communities over time and address questions that cannot be answered in short-term field trials.

Recognizing this knowledge gap, this study employed a multi-faceted approach that integrated historical data analysis with contemporary field assessment, creating a temporal perspective that spans from the initial transplantation events to the present day. By examining sites where transplantation occurred years or even decades ago, the study captures the long-term trajectory of community development rather than merely documenting short-term survival. The evaluation identified which lichen communities successfully survived and established by comparing them to natural reference sites. The study compared historical data on lichen establishment with their current establishment status, assessed the long-term persistence of transplants using various methods, documented key habitat variables such as moisture, nutrient regimes, and soil types, and carefully recorded the species composition, cover, and overall biodiversity of lichen communities on the treated sites. These detailed records allowed for comparisons among transplant sites and with natural communities. Ultimately, this assessment provides vital data for understanding the success and ecological integration of transplanted lichen populations.

Historical Study 1: Terrestrial lichen enhancement of forest harvested areas in west-central Alberta

Project Summary

This study was established in 2000 by K. Kranrod and E. Anderson and funded by West Fraser Timber Co. Ltd. (Kranrod and Anderson 2001). The initial design of the study is detailed in the private report "Terrestrial lichen enhancement of second growth stands in west-central Alberta". A follow-up study was conducted in 2016 by Alder Owl Ltd. (performed by S. Bonar and T. Kathol) and presented in the private report "Report on the Re-visitation of Lichen Woodland Enhancement Trial Plots, 2016" (Alder Owl 2016).

The study was initiated in September and October 2000 within the Berland 1 and 21 Compartments of Weldwood of Canada, Hinton Division's Forest Management Agreement area, which is just north of the Berland River, north of Hinton, Alberta (Kranrod and Anderson 2001). Berland 1 is within the Subalpine Natural Subregion of the Rocky Mountain Natural Region of Alberta and was harvested in 1994. Berland 21 is within the Upper Foothills Natural Subregion of the Foothills Natural Region of Alberta and was harvested in 1998.

The study design consisted of ten transects arrays. Seven were within seven separate harvest blocks in the Berland 1 compartment, chosen to have "identical" characteristics, while three were within a single large harvest block in the Berland 21 compartment. Arrays were placed at least 100 m from adjacent forest stand edges.

Each transect array included three 40 m long transects, placed at 0°, 120°, and 240° from the array center (**Fig. 1.1.1**). Along each transect, five 0.5 m × 0.5 m quadrats were placed at 5 m intervals, starting 20 m from the array center. Along each transect, all quadrats were treated the same. The 0° transect quadrats were treated as controls (CP), and no lichens were introduced (**Fig. 1.1.2 a**). The 120° transect quadrats were treated as hand-transplants (HTP), where three clumps of lichen

(**Fig. 1.1.2 b**), including one each of *Cladonia arbuscula* ssp. *mitis*, *C. rangiferina*, and *C. uncialis* were placed to provide approximately 10% cover of each species. The clumps were set into the duff layer to avoid movement and desiccation. The 240° transect quadrats were treated as broadcast transplants (BTP) (**Fig. 1.1.2 c**), where approximately the same amount of each of the three species as in the HTP quadrats was crushed into smaller pieces and evenly scattered in the quadrat.

Before transplanting, the percent cover and number of fragments of *C. arbuscula* ssp. *mitis*, *C. rangiferina*, and *C. uncialis* naturally found within the quadrats were assessed. The data is presented as the mean percent cover, and the mean number of fragments, for the five quadrats in each transect. None of the three target species were found in the Berland 21 Compartment quadrats. In the Berland 1 Compartment, *C. arbuscula* ssp. *mitis* had the highest initial covers, ranging from 0-0.6% cover and 0-8.4 fragments. For *C. rangiferina*, the mean covers were all below 0.1%, and the mean number of fragments ranged from 0-1.2. *Cladonia uncialis* also had below 0.1% mean covers in all transects and ranged from 0-0.6 fragments.

The 2016 site reassessment (Alder Owl 2016) found a high success rate for the transplanted lichens, but there were challenges as well. One entire array in Berland 1 was lost to a pipeline installation, and some plots could not be located due to the loss of markers over time. Lost control quadrats were re-created in the approximate areas where they had previously been. Four BTP quadrats were also not able to be located. While the markers for some HTP quadrats had also been lost, they were all re-located by combining information from the GPS coordinates and the distinctive presence of the three lichen clumps.

In the 2016 reassessment, the mean percent cover of *C. arbuscula* ssp. *mitis* was found to be about 10% in both HTP and BTP quadrats, while only 1% in the control quadrats. When averaged by each transect, it ranged from a mean of 3-15% and trace-25% per transect in HTP and BTP respectively, and 0-3% in the controls. Similarly, *C. rangiferina* had 10% mean cover in both HTP and BTP quadrats, and only trace cover in the controls. The transect means ranged from 8-20%, 1-30%, and 0-3% for HTP, BTP, and control plots, respectively. The mean cover of *C. uncialis* was a bit lower at 10% and 8% in the HTP and BTP, respectively, with transect means from 5-15% and 1-15% cover. However, there seems to be a discrepancy in the data for the controls for *C. uncialis*: while the figures and summary chart show a mean of about 3% cover, the transect means each only range from 0% to trace (see Table 1 in Alder Owl 2016), which cannot both be correct.

A data analysis using paired t-tests performed by Alder Owl (2016) found significant differences between control and both BTP and HTP treatments for both *C. mitis* and *C. rangiferina*, but no significant difference between BTP and HTP treatments, at P=0.05. However, for *C. uncialis*, their analysis showed a difference between the control and HTP, and between HTP and BTP, but not between the control and BTP. However, this seems inconsistent with the transect means in Table 1 of the Alder Owl report. We performed our own unpaired t-tests assuming unequal variances (which seems the more appropriate statistical test) on the data from Table 1 of the report, using 0.5% for “trace cover” values and 0% for “absent” values. Our analysis found a significant

difference between the controls and both the HTP and BTP treatments for all three species, and no significant difference between the HTP and BTP treatments for any species.

Methods

In 2023, ecological site information was collected for the arrays, conducted at each array center. This included the Alberta Vegetation Inventory (AVI) code for estimating crown closure and tree height, site characteristics (slope, aspect, surface expression, surface shape, and slope position), soil information (depths of LFH, Ah, Ae, and total A horizons, surface and effective textures, coarse fragment percent, contrast of mottles, effective rooting depth, and the depths to mottles, gleying, water table, and bedrock). Using this, the humus form, parent material, drainage, moisture regime, and soil type were determined. The percent surface substrate was also recorded and was categorized into decaying wood, bedrock, cobbles/stones, mineral soil, and organic material. The AVI code was determined following Alberta Sustainable Resource Development (2005), soil classification followed Soil Classification Working Group (1998), and other information was determined following Beckingham et al. (1996). Note that while some arrays were within the Upper Foothills Natural Subregion, others were technically within the Subalpine, all ecosystems were classified using Upper Foothills Ecological Area classifications for easier comparisons, and because all ecosystems fit more closely with these descriptions.

In 2023, many plots were unable to be located due to a lack of time and information regarding the original experimental design. However, complete vegetation assessments, estimating the percent cover of each plant and lichen species, were performed at each located plot, using 1 m x 1 m square quadrats. This data was not analyzed because the 2024 data had more plots located and more closely replicated the original experimental design.

In 2024, new assessments were performed using 0.5 x 0.5 m plots to more closely align with the original experimental design. Within these plots, the percent cover of the three target species were recorded. As well, additional ecological information was explicitly recorded for each transect, to account for some of the variability that had been observed within the transects of some arrays. This information was collected beside each transect's middle plot and included the Alberta Vegetation Inventory (AVI), slope, aspect, surface shape, and percent surface substrate. The percentage of surface substrate was further classified into decaying wood, bedrock, cobbles/stones, mineral soil, feather moss, and pine needles to describe the dominant substrate types (Alberta Biodiversity Monitoring Institute 2015). Lichen dispersal was evaluated in two directions, 90° and 270° of the transect, using the middle plot of each transect as the center point. A 0.5 m x 0.5 m quadrat was flipped ten times in each direction, and the covers of *C. arbuscula* ssp. *mitis*, *C. rangiferina*, and *C. uncialis* were documented (**Fig. 1.1.3**).

Statistical analysis

The statistical software package R was used for all statistical analyses and graphical presentations (R Core Team, 2024). Statistical analyses were conducted using a generalized linear mixed model (GLMM) with the glmmTMB package. Data on lichen cover for each species assessed in 2016

and 2024 were combined. The percent cover was modeled using the glmmTMB function with an ordbeta family function, which is appropriate for proportional data (Brooks et al. 2017). Blocking served as a random factor variable. The gamma distribution was used to model the different dispersal distances observed for each species with the glmmTMB package. Model assumptions were checked using a histogram of residuals and diagnostic plots of both fitted and residual values. Pairwise comparisons between treatments were conducted using least-squares means with the R package "emmeans" (Lenth et al. 2021). Letter codes indicating significant differences in groupings for the pairwise tests were assigned using the "cld" function from the R package "multicomp" (Bretz et al. 2011). Scatter plots were generated using the "ggplot" function from the R package "ggplot2" (Wickham 2016). Pearson correlations between the covers of the three target lichen species within each of the three treatments and for all the treatments combined assessed in 2024 were performed using the "cor" function in R.

Results

Ecological Site Information

In the 2023 assessment using array centers, all sites had dense lodgepole pine stands (D density, 71-100% cover) ranging from 6 to 8 m tall (Table 1.1.5). Moisture regimes were mesic to hygric. Most arrays were nutrient-medium with mor or raw moder humus forms, while Berland 1 Block 104's array was nutrient-rich with a mull humus form. Additional site information is summarized in **Table 1.1.5**.

Plot location

In the 2024 assessment, all plots were first located based on flags or stakes that were either placed initially or installed in 2016. Twenty-seven of the 135 plots were missing flags or stakes, but almost all transects had at least three plots with flags or stakes. Seventeen unmarked plots were relocated by measuring the distances between other located plots, and/or based on the presence of recognizable clumps of the three lichen species in some HTP plots. However, CP transects in Block 104 of the Berland 1 Compartment and Block 7 East of the Berland 21 Compartment had no plots found with flags or stakes. These ten plots were relocated to the exact approximate locations as previously, using the found markers within other transects in the array as guidance.

The effect of treatment, lichen species, year, and their interactions

When the data for lichen cover by species collected in 2016 and 2024 were combined, the analysis revealed that treatment, year, the interaction between treatment and species, and the interaction among treatment, species, and year all had a significant effect (**Table 1.1.1**). The lichen cover assessed in 2024 was lower than that evaluated in 2016, while the covers of *C. arbuscula* ssp. *mitis*, *C. rangiferina*, and *C. uncialis* in HTP, BTP, and CP were not significantly different between the assessments in 2016 and 2024 (**Fig. 1.1.4**). The BTP and HTP treatments showed substantially higher lichen covers than the control in three lichen species across both years. The cover of *C. uncialis* and *C. rangiferina* was substantially higher in the HTP treatment assessed in 2016 than

the cover of *C. arbuscula* ssp. *mitis* and *C. uncialis* in the BTP treatment evaluated in 2024. The effect of species was not significant on lichen cover per species (**Table 1.1.1**). There was no significant difference in cover among the three lichen species in all treatments across two years, except that the cover of *C. arbuscula* ssp. *mitis* was significantly higher than that of *C. rangiferina* and *C. uncialis* in the control plots assessed in 2024 (**Fig. 1.1.4**).

The correlation between the covers of target lichen species

The pairwise correlations between the covers of the target lichen species assessed in 2024 were all positive (**Table 1.1.2**). Correlations were highest in the controls, ranging from 0.948 to 0.965, followed by the hand-transplant treatments, where they ranged from 0.615 to 0.666, and were lowest in the broadcast-transplant treatments, ranging from 0.139 to 0.237. When all treatments were combined, the correlations ranged from 0.303 to 0.465.

The effects of site characteristics on lichen cover

The 2024 data analyses also included information about site characteristics. The effects of site characteristics were significant on the covers of all three lichen species (**Table 1.1.3**). The slope had a significant effect on the cover of *C. arbuscula* ssp. *mitis* and *C. uncialis*, while other site characteristics showed no significant effect on any species cover. A negative correlation was observed between the slope and the cover of *C. arbuscula* ssp. *mitis* and *C. uncialis* (**Fig. 1.1.5 a, b**). There was more variability in *C. arbuscula* ssp. *mitis* and *C. uncialis* cover when the slope was gentle. While there was no significant effect between pine needle substrate and lichen cover (**Table 1.1.3**), we often had to clear some pine needles to view the lichen cover properly, and plots that had dense regenerating pine trees in or directly beside them, and thus a thicker needle cover, usually had little to no lichen present, even if they had been transplanted.

Lichen dispersal

The dispersal of lichens beyond the experimental plots was evaluated for all three transects within each array in 2024. There were significant differences between the covers of the three lichen species in all treatments (**Table 1.1.4**). The distance from the center plot, and the interaction between species and distance, both had significant effects on lichen covers in both transplant treatments, but not in the control. In the control treatment, the difference in the three lichen species cover was significant, with *C. arbuscula* ssp. *mitis* having the highest abundance, followed by *C. rangiferina*, and then *C. uncialis* (**Fig. 1.1.6 a**). Most data points remained at low cover values, but there were a few extreme outliers for *C. arbuscula* ssp. *mitis*, with some cover exceeding 50% (**Fig. 1.1.6 b**). Distance from the center plot did not strongly influence lichen abundance in the control plots.

In the hand transplant and broadcast transplant treatment dispersal assessments, overall *C. arbuscula* ssp. *mitis* had a significantly higher cover than the other two species, followed by *C. rangiferina*, and with *C. uncialis* showing the lowest cover (**Fig. 1.1.7 a, Fig. 1.1.8 a**). All three species showed a general trend where lichen cover decreased as distance from the center plot increased (**Fig 1.17 b, Fig 1.18 b**).

Discussion

Overall, this study indicates that both hand- and broadcast-transplanted lichens including *Cladonia arbuscula* ssp. *mitis, C. rangiferina*, and *C. uncialis* have survived 24 years within harvested pine forest area. The transplantation of lichen significantly increased lichen cover compared to the control plots throughout the study period, and similar covers were produced regardless of lichen species or transplanting techniques. This supports the concept that transplanting may be a viable option for practitioners aiming to restore terrestrial lichen communities and caribou forage.

After the initial transplanting in 2000, lichen cover was less than 1% in CP, 30% in HTP, and 80% in BTP (Kranrod and Anderson 2001). In 2016 and again in 2024, the mean lichen covers in both the HTP and BTP plots were close to 30%. This indicates that some of the original lichen may have died or been dispersed in the BTP plots, while the hand-transplanted lichens successfully maintained their original cover. However, it should be noted that there was considerable variation in success with both treatments, with some plots containing no lichen, and others far surpassing the original covers. This variation was observed both within and between transects, indicating that microsite characteristics beyond what we measured may have impacted success.

These results agree well with those of Roturier and Bergstern (2009), who found that transplanted reindeer lichens (primarily *Cladonia stellaris* with some *C. rangiferina*) produced similar increases in cover whether dispersed in patches or scattered fragments, although the patches did have higher absolute values for the cover increases. This was assessed six years after being transplanted in a pine forest in northern Sweden, but the area was also significantly affected by reindeer grazing and forestry. In contrast, Rapai et al. (2023) found that lichen cover was significantly higher with fragment transplanting compared to entire mat transplanting for *C. rangiferina*, but not for *C. arbuscula* ssp. *mitis*, as assessed five years after being transplanted in a post-wildfire environment in British Columbia, Canada. Differences may be due to the shorter timescale, different habitat, and different experimental design used in the study.

Cladonia arbuscula ssp. *mitis* cover in the control plots was significantly higher than that of *C. rangiferina* and *C. uncialis* in 2024, indicating some ability to re-establish on its own, though its cover was still significantly higher in transplanted plots (**Fig. 1.1.4**). In contrast, *C. rangiferina* and *C. uncialis* had very little cover in controls, so did not effectively become established without being transplanted. This difference may be attributed to varying life-history strategies among these species. Among reindeer lichens, *Cladonia arbuscula* ssp. *mitis* is known as an early successional species that can quickly re-establish after logging from very small to larger propagules (Ahti 1961); however, its growth slows relative to other reindeer lichen species as time progresses (Webb 1998). This finding is consistent with the results reported in 2001, which noted that *C. arbuscula* ssp. *mitis* was more prevalent in some plots, while very little *C. rangiferina* or *C. uncialis* were present (Kranrod and Anderson 2001). It contrasts, however, with Rapai et al. (2023), who found negligible cover of both *C. arbuscula* ssp. *mitis* and *C. rangiferina* in their control plots after five years. This may be due to the shorter timescale of the study, but it may also be because it was performed within a forest fire burn site rather than in a forest-harvested area. The evidence suggests

that areas affected by forest fires may benefit more from reindeer lichen transplantation than sites subjected to forest harvesting. This higher potential for success in post-fire landscapes probably arises from several factors: the unique substrate conditions, decreased competition from vascular plants, and changes in nutrient availability commonly found in burned environments. Unlike harvested forest areas, where soil disturbance and residual vegetation pose different challenges for lichen establishment, fire-affected regions may provide a more exposed and less competitive niche, which is particularly suitable for the initial colonization and growth of these slow-growing organisms. In our study, the covers of both *C. arbuscula* ssp. *mitis* and *C. uncialis* were negatively correlated with slope. This could be due to flatter areas having more soil moisture and thus being more favourable for lichen growth. Lichen growth is positively correlated with moisture and light, and more specifically the amount of light received while wet (Harris and Kershaw 1971; Kershaw and Rouse 1971; Palmqvist and Sundberg 2000; Sulyma and Coxson 2001; Čabrajič et al. 2010).

The negative correlation could also be due to lichens dispersing more readily out of the plots on steeper slopes. The apparent paradox of lichens having difficulty establishing on slopes yet dispersing effectively from them highlights two different processes: initial colonization and the release and movement of propagules. Steep or unstable slopes create challenging conditions for lichen establishment—rapid water runoff causes drying out, frequent erosion dislodges young thalli, and unstable substrates prevent secure attachment. Still, these problems don't stop mature or established lichen communities from spreading. Mature lichens, even those located in more stable microhabitats on a slope or nearby flat terrain, produce large amounts of reproductive structures like soredia, isidia, spores, or thallus fragments that are easily carried downslope by wind and water currents. In this way, the challenges to starting growth on unstable or exposed inclines don't prevent reproductive fragments from dispersing successfully, allowing the species to continue spreading across the landscape.The positive correlations observed between all pairings of the three transplanted lichens (**Table 1.1.2**) indicate that a plot that was good for one lichen's growth was good for them all, and vice versa. Lechowicz and Adams (1974b) found that these three species had very similar habitat requirements, and they are widely known to occur together in nature. The fact that the correlations were strongest in our control plots, all in the range of 0.948-0.965, supports the idea that the co-occurrence of these species occurred naturally in this study as well. It is interesting, however, to note that the correlations declined to 0.615-0.735 in the HTP plots and further declined to 0.139-0.237 in the BTP plots.

Given that the initial lichen correlations were close to 1.0, indicating equal abundances, the varying success rates in lichen establishment are notable. We do not have a definitive understanding of what drives these differences in establishment rates, which often leads to speculation about interspecific competition. It is plausible that some lichen species possess inherent physiological or structural traits that make them more difficult to establish through transplantation, regardless of the presence of other species. Alternatively, various factors may influence the success of each species, including specific microhabitat requirements, differing

tolerances to environmental stressors, or unique growth rates that confer a natural advantage under certain conditions.

The dispersal of transplanted lichens within this experiment was limited. Only in the plots immediately beside the transplanted plots were coverages higher than controls, with the BTP plots having higher lichen cover here than the HTP (**Fig. 1.1.7 b, Fig 1.1.8 b**). Field observations suggest that this was likely more due to expansion of the original transplants, which occurred more often in the BTP plots because the lichens were originally placed closer to the plot edges. Furthermore, the relative abundances of the three lichen species found beside transplanted plots (**Fig. 1.1.7 a, Fig.1.1.8 a**) were the same as those of the control plots (**Fig. 1.1.6 a**). Because the transplants were roughly equally distributed between *C. arbuscula* ssp. *mitis, C. rangiferina*, and *C. uncialis*, one would expect a more even distribution of these species beside transplant plots unless the species have different dispersal rates. Of course, the use of rather small 50 x 50 cm quadrats in this study provided limited material for dispersal. Heinken (1999) showed that animals disturbed and removed several fragmented reindeer lichen cushions, resulting in maximum dispersal distances ranging from 9 to 70 m in closed old-growth pine forests; we did not observe any evidence of animal-related dispersal in our study. In a study with much larger treated areas, Roturier et al. (2024) found that after 10 years, fragmented reindeer lichens had dispersed by at least 20 m from the treated plots, and in some areas up to 60 m, suggesting that they can disperse more noticeably over larger areas and when larger quantities are present.

Overall, this study has shown that transplantation of *C. arbuscula* ssp. *mitis, C. rangiferina*, and *C. uncialis* can have long-term success, producing significantly more cover than controls after 24 years. Based on our findings, recommendations for larger-scale transplant projects would be that dispersal of lichens can occur as broken-up fragments or larger clumps, that lichen propagules be relatively widely spread out to reduce competition and increase overall cover, and that they occur in areas with lower slopes. Since plots within transects were quite variable in lichen coverages, further study is recommended to determine microsite characteristics that result in higher success.

Table 1.1.1 Analysis of deviance for covers of *Cladonia arbuscula* ssp. *mitis*, *C. rangiferina,* and *C. uncialis* in broadcast-transplant plots (BTP), hand-transplant plots (HTP), and control plots (CP) assessed in 2016 and 2024.

Source	df	Chisq	Pr(>Chisq)
Treatment	2	259.32	<.001***
Species	2	4.71	0.095
Year	1	15.28	<.001***
Treatment*species	4	31.96	<.001***
Treatment*year	2	1.74	0.419
Species*year	2	0.12	0.940
Treatment*species*year	4	12.58	0.013*

* Significant at P<0.05 and *** significant at P<0.001.

Table 1.1.2 Pearson correlation coefficient for the covers of *Cladonia arbuscula* ssp. *mitis* (*C. mitis*), *C. rangiferina,* and *C. uncialis* within individual plots of broadcast-transplant (BTP), hand-transplant (HTP), control (CP) treatments, and all treatments combined, as assessed in 2024.

Treatment	*C. mitis*-*C.rangiferina*	*C. mitis*-*C. uncialis*	*C. rangiferina*-*C. uncialis*
CP	0.965***	0.949***	0.948***
HTP	0.666***	0.735***	0.615***
BTP	0.220ns	0.237ns	0.139ns
[a]Combined	0.465**	0.433**	0.303*

*Significant at P<0.05, ** Significant at P<0.01, *** significant at P<0.001, and ns=not significant.

[a]Cover means across treatments were used for correlation analysis. n=45.

Table 1.1.3 Analysis of deviance for covers of *Cladonia arbuscula* ssp. *mitis*, *C. rangiferina,* and *C. uncialis* with broadcast-transplant plots (BTP), hand-transplant plots (HTP), and control plots (CP) in 2024 using crown closure, tree height, slope, surface shape, and the cover of decaying wood, feather moss, and pine needles as covariates.

Source	df	*C. mitis*		*C. rangiferina*		*C. uncialis*	
		Chisq	Pr(>Chisq)	Chisq	Pr(>Chisq)	Chisq	Pr(>Chisq)
Treatment	2	24.57	<0.001***	41.84	<0.001***	34.22	<0.001***
Crown closure	2	2.53	0.282	0.86	0.651	2.03	0.362
Tree height	1	0.06	0.806	0.06	0.806	2.17	0.141
Slope	1	4.08	0.043*	3.01	0.083	8.53	0.003**
Surface shape	2	1.46	0.481	4.88	0.087	0.10	0.952
Decaying wood	1	0.00	0.949	0.41	0.520	0.81	0.367
Feather moss	1	0.05	0.815	1.68	0.195	0.11	0.739
Pine needles	1	0.55	0.458	1.41	0.236	0.63	0.436

* Significant at P<0.05, **significant at P<0.01, and *** significant at P<0.001.

Table 1.1.4 Analysis of deviance for covers of *Cladonia arbuscula* ssp. *mitis*, *C. rangiferina,* and *C. uncialis* within the measured distance in broadcast-transplant plots (BTP), hand-transplant plots (HTP), and control plots (CP) assessed in 2024.

Source	df	Control plots		Hand-transplant plots		Broadcast-transplant plots	
		Chisq	Pr(>Chisq)	Chisq	Pr(>Chisq)	Chisq	Pr(>Chisq)
Species	2	99.07	<0.001***	58.20	<0.001***	13.46	0.001**
Distance	1	2.25	0.133	28.17	<0.001***	17.77	<0.001***
Species*distance	2	0.83	0.661	10.76	0.005**	16.37	<0.001***

* Significant at P<0.05, **significant at P<0.01, and *** significant at P<0.001.

Table 1.1.5 Ecological site information summary for arrays assessed in 2023. Terminology and procedures follow Beckingham and Archibald (1996) and Soil Classification Working Group (1998). Array titles are abbreviated as B1 (Berland 1) BL (block) number, with block 7 additionally having north, east, and south arrays (N, E, and S respectively).

	B1Bl33	B1BL22	B1BL30	B1BL34	B1BL7N	B1BL7E	B1BL7S	B1BL26	B1BL104
AVI Code	D6Pl10	D7Pl10	D6Pl10	D8Pl10	D8Pl10	D8Pl10	D7Pl10	D7Pl10	D6Pl10
Ecosite	UF h1.1	UF e1.5	UF e1.5	UF e1.3	UF h1.2	UF e1.1	UF e1.1	UF e1.1	UF e1.1
Slope (%)	12	5	3	19	4	9	3	1	0
Aspect (degrees)	245	50	65	215	240	180	110	270	n/a
Surface Expression	Slope	Slope	Hummocky	Slope	Slope	Slope	Slope	Level	Level
Surface Shape	Straight	Convex	Convex	Straight	Straight	Straight	Straight	Straight	Straight
Slope Position	Midslope	Upper Slope	Upper Slope	Midslope	Midslope	Midslope	Midslope	Level	Level
Drainage	Poor	Mod. Well	Mod. Well	Imperfect	Imperfect	Imperfect	Imperfect	Mod. Well	Mod. Well
Moisture Regime	Hygric	Mesic	Mesic	Subhygric	Hygric	Subhygric	Subhygric	Mesic	Mesic
Nutrient Regime	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Rich
Total Organic Thickness (cm)	6	5	3	4	4	5	2	3	2
Soil Surface Texture	Sandy Clay Loam	Sandy Loam	Sandy Clay	Sandy Clay	Sandy Clay Loam	Sandy Clay	Sandy Clay	Sandy Clay	Sandy Clay
Soil Effective Texture	Sandy Clay Loam	Loamy Sand	Sandy Loam	Sandy Clay	Clay	Clay	Clay	Loamy Sand	Sandy Clay Loam
Water Table Depth (cm)	>60	>60	>60	>60	>60	>60	>60	>60	>60
Humus Form	Mor	Mor	Raw Moder	Mor	Mor	Mor	Raw Moder	Moder	Mull
Parent Material	Till	Till	Till	Till	Till	Till	Till	Till	Till
Soil Type	SWm	SM1	SM2	SM4	SWm	SM4	SM4	SM1	SM4
Percent Surface Substrate[a]	10 DW, 0.5 CS, 89.5 OM	5 DW, 95 OM	7 DW, 93 OM	10 DW, 0.5 CS, 0.5 MS, 89 OM	4 DW, 96 OM	15 DW, 85 OM		4 DW, 96 OM	5 DW, 95 OM

[a]Percent Surface Substrate codes: DW=decaying wood, CS=cobbles/stones, MS=mineral soil, OM=organic matter

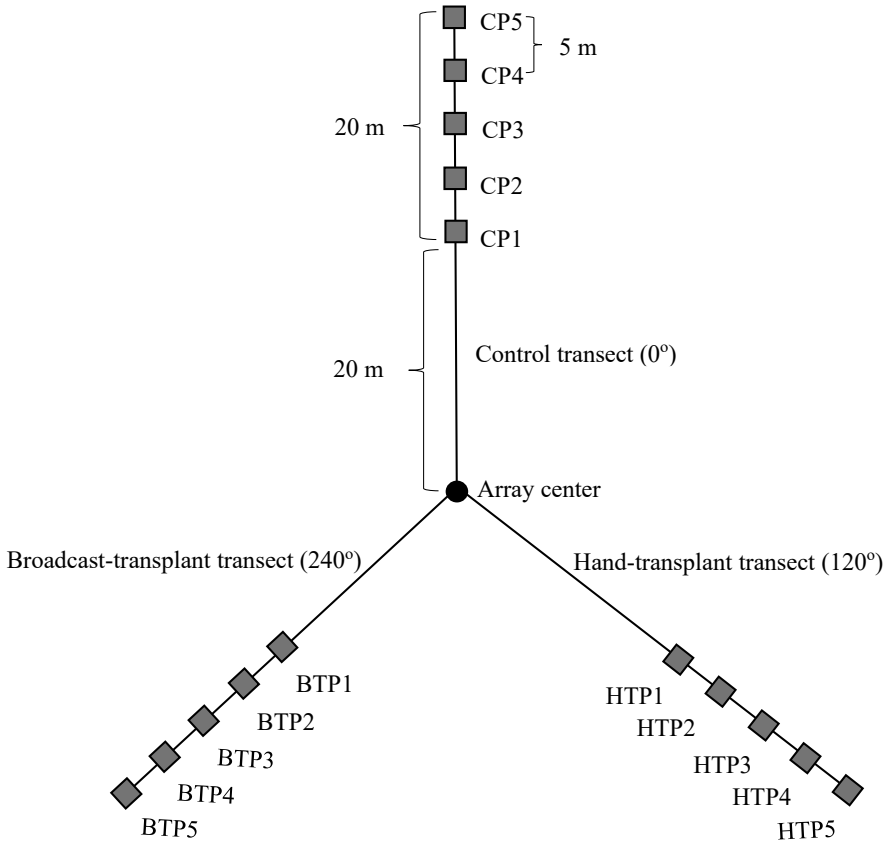

Fig. 1.1.1 A diagram of the layout of plots and transects at a study site (modified from Bonar and Kathol 2016

Fig. 1.1.2 Representative examples of control (**a**), hand-transplant (**b**), and broadcast-transplant (**c**) plots, photographed in 2024.

Fig.1.1.3 Assessment of lichen dispersal in 2024.

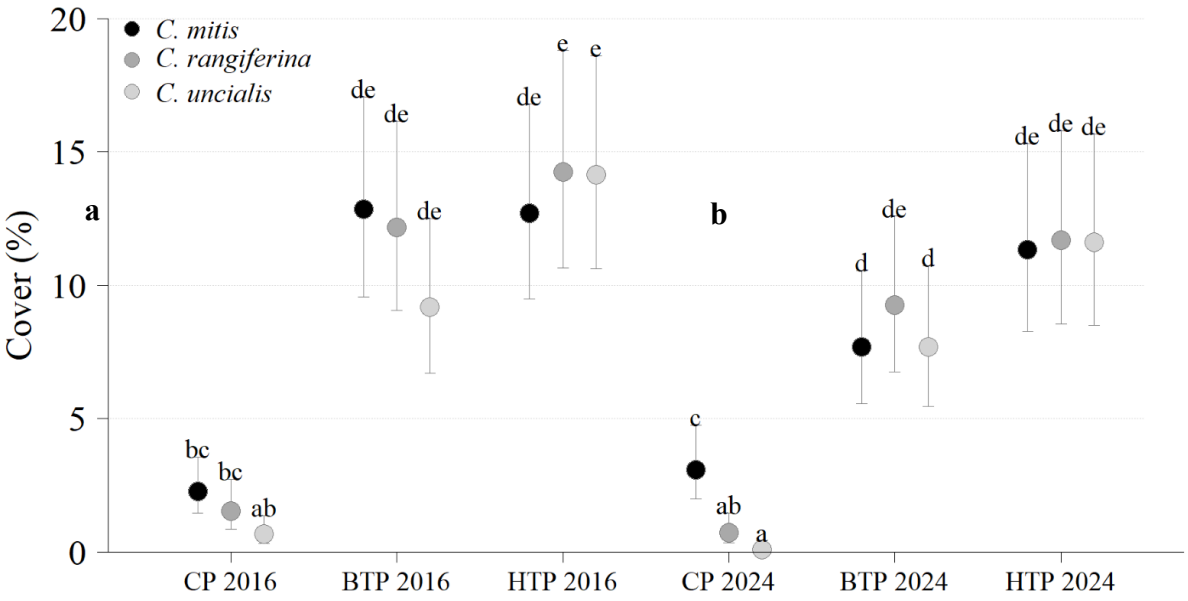

Fig. 1.1.4 Cover (%) of *Cladonia arbuscula* ssp. *mitis* (*C. mitis*), *C. rangiferina*, and *C. uncialis* in broadcast-transplant plots (BTP), hand-transplant plots (HTP), and control plots (CP) assessed in 2016 and 2024. The dots indicate the means, and the horizontal bars indicate asymptotic lower and higher confidence limit. Treatments with different letters are significantly different at $P<0.05$.

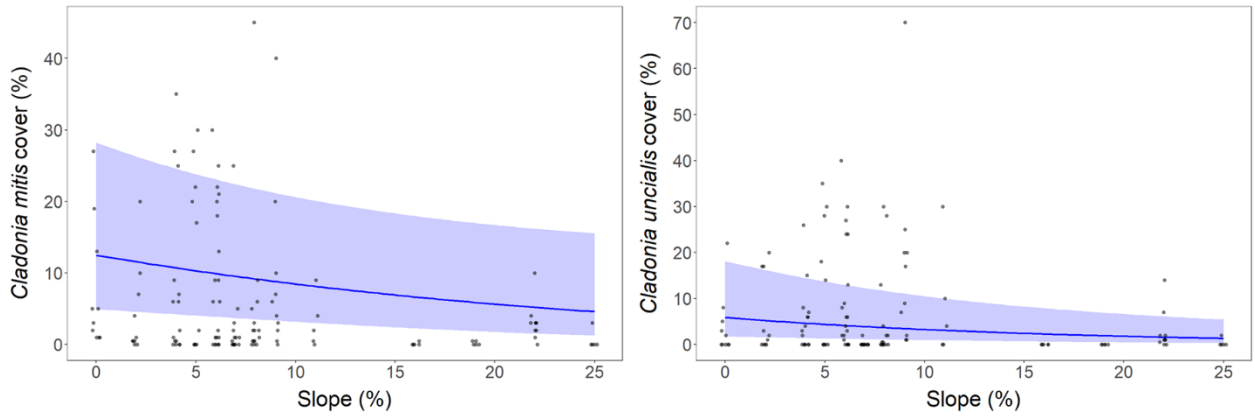

Fig. 1.1.5 Scatter plot between (**a**) slope (%) and *Cladonia arbuscula* ssp. *mitis* (*C. mitis*) cover (%) (**b**) slope (%) and *C. uncialis* cover (%) of broadcast-transplant plots (BTP), hand-transplant plots (HTP), and control plots (CP) assessed in 2024.

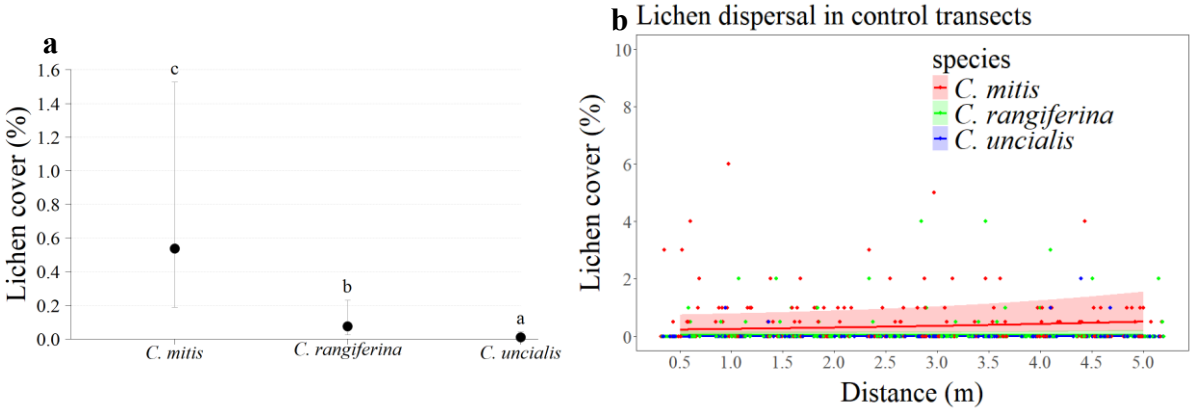

Fig. 1.1.6 (**a**) Cover (%) of *Cladonia arbuscula* ssp. *mitis* (*C. mitis*), *C. rangiferina*, and *C. uncialis* in the transect of control plots assessed in 2024. The dots indicate the means, and the horizontal bars indicate asymptotic lower and higher confidence limit. Treatments with different letters are significantly different at P<0.05. (**b**) Cover of *Cladonia arbuscula* ssp. *mitis* (*C. mitis*), *C. rangiferina*, and *C. uncialis* compared to the distance from the center plot, assessed in 2024.

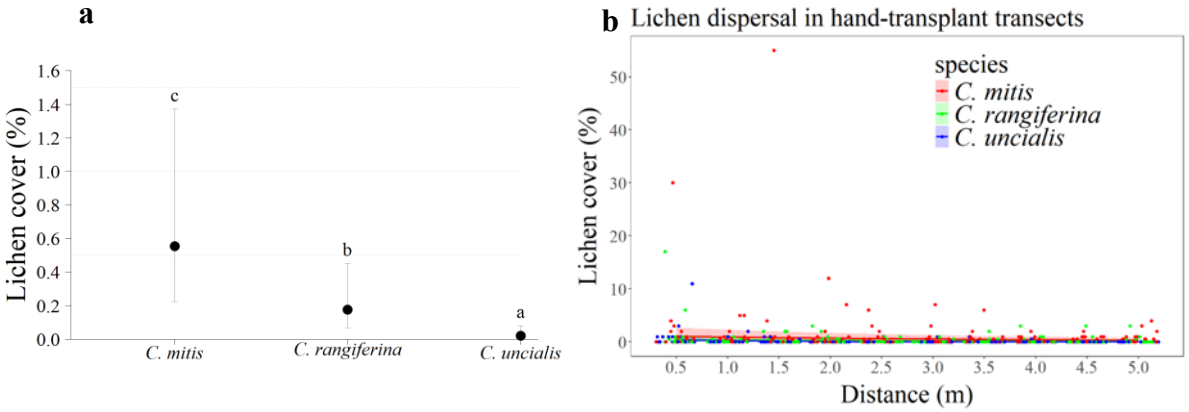

Fig. 1.1.7 (a) Cover (%) of *Cladonia arbuscula* ssp. *mitis* (*C. mitis*), *C. rangiferina*, and *C. uncialis* in the transect of hand-transplant plots assessed in 2024. The dots indicate the means, and the horizontal bars indicate asymptotic lower and higher confidence limit. Treatments with different letters are significantly different at P<0.05. (**b**) Cover of *Cladonia arbuscula* ssp. *mitis* (*C. mitis*), *C. rangiferina*, and *C. uncialis* compared to the distance from the center plot, assessed in 2024.

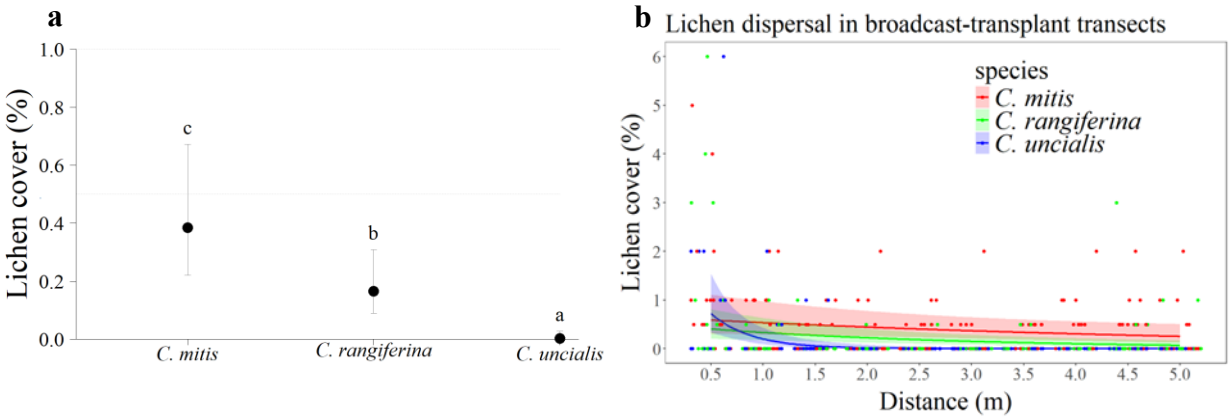

Fig. 1.1.8 (a) Cover (%) of *Cladonia arbuscula* ssp. *mitis* (*C. mitis*), *C. rangiferina*, and *C. uncialis* in the transect of broadcast-transplant plots assessed in 2024. The dots indicate the means, and the horizontal bars indicate asymptotic lower and higher confidence limit. Treatments with different letters are significantly different at P<0.05. (**b**) Cover of *Cladonia arbuscula* ssp. *mitis* (*C. mitis*), *C. rangiferina*, and *C. uncialis* compared to the distance from the center plot, assessed in 2024.

Historical Study 2: Northern mountain caribou post-fire habitat restoration program (Mesilinka River area, British Columbia)

Project summary

This study was established in 2015 and 2016 in an area burned by a large-scale 2014 wildfire, and the initial study is presented in "Examining the role of terrestrial lichen transplants in restoring caribou winter habitat" (Rapai et al. 2017). Located adjacent to the Melsilinka River and Chase Provincial Park in northern British Columbia, the site is within the Chase and Finlay northern mountain caribou herd ranges and the traditional territory of the Tsay Keh Dene First Nation. Funded by the Society for Ecosystem Restoration in northcentral British Columbia, and in cooperation with the Tsay Keh Dene First Nation, Chu Cho Environmental and associates performed a series of reindeer lichen transplants. Twenty replicates, each including four different treatments, were performed within the burn site, with several site-selection criteria such as being away from areas where water might pool, having coarse, well-drained soil, and being over 50 m from wetland/riparian habitats, forest roadways, or remnant lichen communities. The four treatments were each performed in 10 m x 10 m square plots, which were either treated with 100 L worth of lichen mats only ("mats"), 100 L of lichen fragments only ("fragments"), 50 L each of lichen mats and fragments combined ("hybrid"), or as controls with no lichen applied. The mats were intact lichen clumps "the size of a clenched fist to an outstretched palm" that were "planted" into the substrate and intentionally placed near sheltering objects like logs and stumps. The fragments were lichens hand-shredded into 2-7 cm pieces, broadcast by hand, and brushed to the ground if they fell on rocks, stumps, or logs.

In Rapai et al. (2017), the target lichen species used were *C. arbuscula* ssp. *mitis*, *C. stellaris*, *C. stygia*, and *C. uncialis*. The initial percent cover of these species within the plots was estimated, and the mean for each treatment is presented in the report. No target species were found in the controls, and in the other treatments, *C. arbuscula* ssp. *mitis* ranged from 1.27-3.05%, *C. stellaris* ranged from 0.00-0.01%, *C. stygia* ranged from 0.38-1.03%, and *C. uncialis* ranged from 0.35-2.27%. The mean total covers of the target species were 0.00% in the controls, 2.01% in the mats-only treatments, 3.78% in the mats-plus-fragments treatments, and 4.78% in the fragments-only treatments.

A follow-up study was conducted in 2021 and published as "Terrestrial lichen caribou forage transplant success: year 5 and 6 results" (Rapai et al. 2023). In this study, the "operational" plots set up by Rapai et al. (2017) were re-assessed, as well as those from an additional "experimental" field trial that were established in 2015. We will focus on the 2016 operational field trial in this summary, since these are the plots that we also revisited in the current study. It should be noted that the *C. stygia* from Rapai et al. (2017) was presumably re-identified as *C. rangiferina* in Rapai et al. (2023), as the former species was not utilized in any of the analyses in the latter study.

Rapai et al. (2023) used only 11 of the original 20 operational field trial replicates, since nine had been destroyed by a 2021 wildfire. The mean total percent cover of target lichens in the control

plots remained at zero, while the mean percent covers in the mat, fragment, and hybrid treatments were 3.5-4%, and did not significantly differ by treatment method. Samples of *C. arbuscula* ssp. *mitis*, *C. rangiferina*, and *C. uncialis* were also sent for chlorophyll fluorescence analysis to assess their health. These analyses found that all of the lichens within all of the treatments were healthy (i.e. had chlorophyll fluorescence (F_v/F_m) values above 0.7), and there were no significant differences in F_v/F_m values between the three treatments for any of the three lichen species.

Methods

For the current study, we revisited 12 of the field trial replicates established by Rapai et al (2017) in 2022 (**Fig. 1.2.1**). We gathered ecological site information, including the following:

- Site characteristics including slope, aspect, surface expression, surface shape, and slope position
- Percent surface substrate of decaying wood, bedrock, cobbles/stones, mineral soil, organic matter, water, or other substrate
- Soil information including:
    - Total organic thickness, and further divided into the depths of L, F, H/H_i, Of, Om, and Oh layers
    - For both the 0-20 cm and 20-60 cm layers: the soil texture (as determined by hand texturing), percent of coarse fragments, coarse fragment type, and contrast of mottles if present
    - Thickness of Ah, Ae, and total A horizons
    - Depth to gleying, mottles, water table, bedrock or frozen, and bottom of pit, as well as effective rooting depth
    - Drainage, humus form, parent material, soil type, moisture regime, and nutrient regime

AVI codes (recording the height and density of trees or other tallest vegetation) were not collected at these sites, because minimal vegetation cover was present. Because plots within each replicate were fairly widely spaced, some information, such as percent surface substrate, surface shape, and slope position, were recorded for each plot. However, the soils and related information was only assessed once per replicate, unless there were significant site differences apparent between the plots within a replicate. The replicates visited included four replicates within Site 2 (4, 5, 6, and 7), five within Site 3 (4, 5, 6, 7, and 8), and three within Site 9 (1, 2, and 3). Note that this is one more than Rapai et al. (2023) had utilized because we were able to relocate a replicate that was previously thought destroyed by the 2021 wildfire.

In addition to the site information, at each of the four plots, target lichens were randomly selected and scored with a visual assessment of vitality as outlined above. This was done by placing a 1 m

x 1 m square quadrat within the plot, which was further divided into 100 10 x 10 cm divisions (**Fig. 1.2.2**). A random specimen of each of the three target species (*C. arbuscula* ssp. *mitis*, *C. rangiferina*, and *C. uncialis*) present in the quadrat was scored, and notes were taken on other health-related details for each specimen. This was repeated twice per plot. Photos were taken of each quadrat, which could later be used for estimating total lichen covers. At least one specimen of each lichen species within each plot was also collected, dried, and sent for chlorophyll fluorescence analysis. Once the analyses were performed, these specimens were sent back and their identifications were confirmed using microscopic examination and chemical spot tests as needed.

Visual assessment of lichen vitality

Visual assessment of the lichen vitality in the historical sites was done visually using a modified classification class used by Liden et al. (2004). Lichen mats and fragments were classified according to a scale ranging from 0 to 5 (**Table 1.2.1**).

Chlorophyll fluorescence analysis

All collected fragments were measured for chlorophyll fluorescence Measurements were conducted by the University of Northern British Columbia, Coxon Research Group. All thalli were preconditioned by spraying them with de-ionized water until they rehydrate fully. The samples were kept in a container under saran wrap sitting on a damp paper towel in the light at moderate illumination of 200 µmol m^{-2} s^{-1} and temperature of 15 °C for 24 h. Immediately after the preconditioning, the F_v/F_m was recorded with a pulse-modulated chlorophyll fluorescence unit (Hansatech, Norfolk, United Kingdom) with a 6 mm measurement disc after a 5-min period of dark adaptation following the methods of Gauslaa et al. (2012).

Statistical analysis

The mean F_v/F_m values for each species within each plot was used for data analysis. The effect of treatment and species on lichen vitality and F_v/F_m were analyzed using the "lmer" functions within the lme4 package in R (R Core Team, 2024). In the model, the treatment and species served as fixed factors, and the block and the plot within the block served as random factors. Model assumptions were checked with a histogram of residuals and diagnostic plots of fitted and residual values.

Results and Discussion

Ecological Site Information

The ecological site information is summarized in Tables 1.2.3a and 1.2.3b. Site slopes ranged from 0-20%, and had rapid to moderate-well drainage. The moisture regimes of most sites were xeric, although two were mesic, and one was subhygric. Nutrient regimes were very poor to poor. The percent of coarse fragments was high in both the 0-20 cm and 20-60 cm layers, from 20-90% but mostly closest to 60%, and was composed of gravels and cobbles (data not shown). Because sites

all had similarly high success regarding lichen cover and health, using this data in analyses was not possible.

Lichen Health

Statistical analyses were not conducted on the visual assessment of vitality due to the insufficient number of samples of some species at some sites, and lack of variability in the data. All lichen samples scored either 4 or 5 for lichen vitality, and the mean lichen vitality was between 4.29 and 5.00 (**Table 1.2.2**). Across all sites, *C. rangiferina* had the lowest mean vitality score (4.68), followed by *C. arbuscula* ssp. *mitis* (4.79), and *C. uncialis* scored the highest (4.93). In particular, *C. rangiferina* scored particularly poorly in the fragment and hybrid treatments in Sites 2 and 3. This could be at least partly explained by the fact that *C. rangiferina* is a paler species that lacks the yellowish usnic acid found in *C. arbuscula* and *C. uncialis*, which may make it appear less healthy, especially when not in larger clumps.

There were no significant differences in the F_v/F_m values when analyzed by treatment, lichen species, or their interaction. The mean F_v/F_m value among treatments and species ranged from 0.79 to 0.86 (**Fig. 1.2.3**). The results are consistent with Rapai et al. (2023), where F_v/F_m values were similarly over 0.7 for all three species, and also where treatment had no significant effect on F_v/F_m results.

Table 1.2.1 Transplanted lichen vitality criteria modified from Liden et al. (2004).

Levels	Description
0	Late stage of decay
1	Showing signs of decay (light brown, pink or moulded)
2	Lacking green pigment
3	Fragmented but otherwise vital
4	Moderate level of green pigment (pale pear green)
5	Full levels of green pigment

Table 1.2.2 Mean visual assessment of vitality scores of *Cladonia arbuscula* ssp. *mitis* (*C. mitis*), *C. rangiferina*, and *C. uncialis* for each treatment within each site for Historical Study 2 near Mesilinka River, BC. In brackets are the number of samples (n) used.

Site	Treatment	*C. mitis*	*C. rangiferina*	*C. uncialis*
2	Fragment	4.87 (8)	4.37 (8)	5.00 (6)
	Hybrid	4.62 (8)	4.29 (7)	5.00 (8)
	Mat	5.00 (6)	5.00 (5)	5.00 (6)
3	Fragment	4.80 (10)	4.56 (9)	5.00 (10)
	Hybrid	5.00 (10)	4.44 (9)	4.86 (7)
	Mat	4.87 (8)	5.00 (4)	4.50 (4)
9	Fragment	4.67 (6)	4.75 (4)	5.00 (3)
	Hybrid	4.67 (6)	4.67 (3)	5.00 (1)
	Mat	4.60 (5)	5.00 (1)	5.00 (1)

Table 1.2.3a Ecological site information summary for Northern Mountain caribou post-fire habitat restoration program plots, plots within project LIMA-01 and LIMA-22. Plot number codes are site number-plot number-plot direction, with north plots being controls, east being mat transplants, west being fragment transplants, and south being hybrid transplants. Terminology and procedures follow Beckingham and Archibald (1996) and Soil Classification Working Group (1998).

Project No.	LIMA-01						LIMA-22	
Plot No.	2-4-E	2-4-S	2-4-W	2-5-E	2-5-S	3-6-S	2-6-E	2-7-S
Slope (%)	20	5	0	0	0	5	3	2
Aspect (degrees)	325	40	n/a	n/a	n/a	330	205	150
Surface Expression	Undulating	Undulating	Undulating	Undulating	Undulating	Incline	Undulating	Undulating
Surface Shape	Convex	Straight	Convex	Straight	Straight	Straight	Straight	Straight
Slope Position	Upper Slope	Lower Slope	Crest	Level	Crest	Mid-slope	Mid-slope	Mid-slope
Drainage	Rapid	Mod. Well	Very Rapid	Very Rapid	Very Rapid	Very Rapid	Very Rapid	Very Rapid
Moisture Regime	Xeric	Subhygric	Xeric	Xeric	Xeric	Xeric	Xeric	Xeric
Nutrient Regime	Very Poor	Poor	Very Poor	Very Poor	Very Poor	Very Poor	Very Poor	Very Poor
Total Organic Thickness (cm)	1	1	0	1	2	1	0	0
Soil Surface Texture	Loamy Sand	Sandy Loam	Sandy Loam	Loamy Sand	Sand	Sandy Loam	Sandy Loam	Sand
Soil Effective Texture	Sand	Sand	Sand	Loamy Sand	Sand	Sand	Sand	Sand
Water Table Depth (cm)	>60	>60	>60	>60	>60	>60	>60	>60
Humus Form	Mor	Mor	Mor	Mor	Mor	Mor	Mor	Mor
Parent Material	Till	Till	Till	Till	Till	Till	Till	Till
Soil Type	SV1	SM1	SV1	SV1	SV1	SV1	SV1	SV1
Percent Surface Substrate[a]	5 DW, 15 CS, 5 MS, 75 OM	5 DW, 5 MS, 90 OM	5 DW, 15 CS, 15 MS, 65 OM	20 DW, 15 CS, 20 MS, 45 OM	2 DW, 4 CS, 20 MS, 74 OM	2 DW, 50 CS, 10 MS, 38 OM	1 DW, 1 CS, 5 MS, 93 OM	4 DW, 3 CS, 23 MS, 70 OM

[a]Percent Surface Substrate codes: DW=decaying wood, CS=cobbles/stones, MS=mineral soil, OM=organic matter

Table 1.2.3b Ecological site information summary for Northern Mountain caribou post-fire habitat restoration program plots: additional plots within project LIMA-22. Plot number codes are site number-plot direction, with north plots being controls, east being mat transplants, west being fragment transplants, and south being hybrid transplants. Terminology and procedures follow those of Beckingham and Archibald (1996) and the Soil Classification Working Group (1998).

Plot No.	3-4-W	3-5-S	9-2-S	9-1-S	9-3-S	3-7-S	3-8-S	3-8-W
Slope (%)	3	4	5	4	0	3	1	3
Aspect (degrees)	210	340	140	170	n/a	340	175	30
Surface Expression	Undulating	Incline	Undulating	Undulating	Undulating	Incline	Undulating	Undulating
Surface Shape	Straight	Straight	Convex	Concave	Straight	Straight	Convex	Concave
Slope Position	Mid-slope	Mid-slope	Mid-slope	Mid-slope	Mid-slope	Mid-slope	Mid-slope	Mid-slope
Drainage	Mod. Well	Very Rapid	Very Rapid	Very Rapid	Very Rapid	Very Rapid	Very Rapid	Mod. Well
Moisture Regime	Mesic	Xeric	Xeric	Xeric	Xeric	Xeric	Xeric	Mesic
Nutrient Regime	Very Poor	Very Poor	Very Poor	Very Poor	Very Poor	Very Poor	Very Poor	Very Poor
Total Organic Thickness (cm)	0	0	0	0	0	0	0	0
Soil Surface Texture	Sandy Loam	Loamy Sand	Loamy Sand	Loamy Sand	Sandy Loam	Sandy Loam	Sandy Loam	Sandy Loam
Soil Effective Texture	Sand	Sand	Sand	Sand	Sand	Loamy Sand	Sand	Sand
Water Table Depth (cm)	>60	>60	>60	>60	>60	>60	>60	>60
Humus Form	Mor	Mor	Mor	Mor	Mor	Mor	Mor	Mor
Parent Material	Till	Till	Till	Till	Till	Till	Till	Till
Soil Type	SM1	SV1	SV1	SV1	SV1	SV1	SV1	SM1
Percent Surface Substrate[a]	5 DW, 2 CS, 5 MS, 88 OM	3 DW, 5 CS, 15 MS, 77 OM	10 DW, 15 CS, 15 MS, 60 OM	3 DW, 7 CS, 45 MS, 45 OM	3 DW, 27 MS, 70 OM	2 DW, 10 CS, 5 MS, 83 OM	5 DW, 5 CS, 40 MS, 50 OM	5 DW, 3 CS, 25 MS, 70 OM

[a]Percent Surface Substrate codes: DW=decaying wood, CS=cobbles/stones, MS=mineral soil, OM=organic matter

Fig. 1.2.1 Northern mountain caribou post-fire habitat restoration site revisited in 2022.

Fig. 1.2.2 Field data collection using a 1 m x 1 m square quadrat divided into 100 10 x 10 cm divisions.

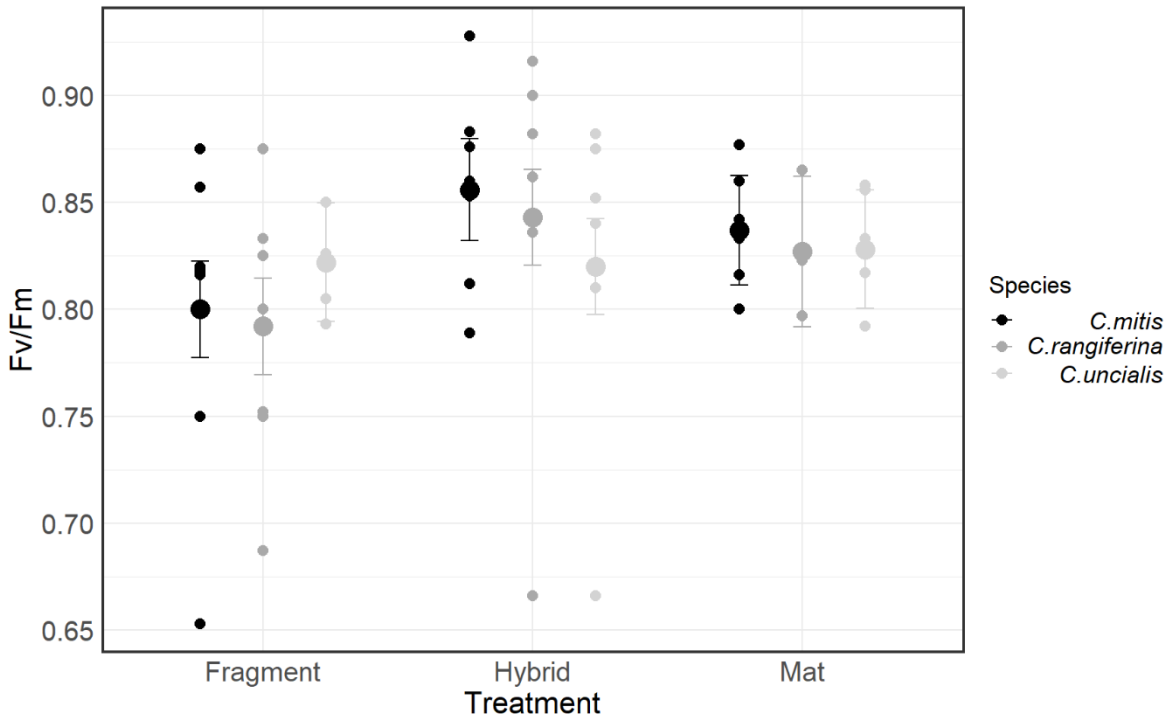

Fig. 1.2.3 Mean F_v/F_m values of *Cladonia arbuscula* ssp. *mitis* (*C. mitis*), *C. rangiferina*, and *C. uncialis* collected from fragment, hybrid, and mat treatments from Historical Study 2 near Mesilinka River, BC. The black dots indicate the means, and the horizontal bars indicate ± SE.

Historical Study 3: Tweedsmuir post-fire restoration trial (Tetachuk Lake area, British Columbia)

Project summary

The Tweedsmuir project was initiated in response to a 2014 wildfire that spread along the Chelaslie River in west-central British Columbia, near Tetachuk Lake, and within the winter home range of the Tweedsmuir-Entiako caribou herd and the traditional territory of the Cheslatta Carrier Nation. The initial setup is described in the private report "Tweedsmuir Lichen Restoration Trial: Year 1 Report" (Ronalds 2018), presented to Skeena Region, Ministry of Forests, Lands, Natural Resource Operations, and Rural Development. The major ("target") lichen species collected in this study included *C. arbuscula* ssp. *mitis, C. uncialis, C. rangiferina,* and *Stereocaulon* species. Lichens were collected from a pine site near Fort St. James, British Columbia, in June and September 2017, and fragmented either manually or with a weed whacker into 2-4 cm long fragments. The researchers explored three different transplant techniques to deploy fragments at the burned sites, using primarily a ratio of 40 L lichen per 100 m^2. This included manual dispersal, using a modified leaf blower, and using a helicopter with a bucket that had a hydraulic aperture, rotary disc, and blower. Lichens were applied along transects that were either 20 x 100 m or 40 x 50 m. There were also smaller 100 m^2 circular treatment areas established, nested within the general transect areas, where either 40 L or 80 L lichen per 100 m^2 were manually dispersed. For both types of treatment areas, 1 × 1 m plots were randomly set up to assess the covers of the lichens and other vegetation. There were also control plots established in nearby untreated areas.

Detailed information is provided in Ronalds (2018) as to the stand structure, fire severity, coarse woody debris, mineral soil cover, and other ecological information of the sites where lichen was applied. Additional ecological information was also collected by Ronalds that is not presented in the report but was made available to us. In assessments performed after the treatments, no target lichens were found in the control transects or plots, although some naturally regenerating *Cladonia* subgenus *Cladina* lichens were observed near some sites. After treatment, the percent cover of target lichens ranged from 1-4% (average 2.4%) within the manually dispersed and leaf-blower treatment sites, and ranged from 1% to 1.7% (average 1.2 %) within the aerial treatment transects. While statistics were not applied, it appears that the manually treated transects had roughly double the lichen cover than the aerially treated transects. Ronalds (2018) hypothesizes that this may have been due to the lower volume by weight of the lichens dispersed aerially, since they were fragmented with a weed-whacker versus the others which were manually fragmented. Ronalds (2018) also notes that only small amounts of aerially dispersed lichen fell outside of the target transects.

A follow-up assessment was performed a year later and presented in the private report "Tweedsmuir Lichen Restoration Trial: Year 2 Report - Project Monitoring", prepared for the Society for Ecosystem Restoration in Northern British Columbia (SERNbc) and Skeena Ecosystems, Ministry of Forests Lands and Natural Resource Operations (Ronalds 2019). In this

assessment, the plots established in Ronalds (2018) were re-visited, but it appears that only those within the main transects were assessed, not including those in the 100 m^2 circular treatment areas. An additional control transect was also established.

Within the plots, the percent coverage of all plants and lichens was recorded, and samples of the target lichen species were collected and sent for chlorophyll fluorescence analysis to assess vitality and vigour. Note that in this study, "manual" dispersal seems to include both the manual and leaf-blower dispersal treatments. Also, due to the difficulty in their identifications, all small acrocarpous mosses were lumped together in the category "fire moss" during the assessments, and presented as "Cerapur" in the report, after the presumably most abundant species *Ceratodon purpureus* (I. Ronalds, pers. com.). As well, only fruticose lichens were included in the assessment, and they were not separated by species.

The chlorophyll fluorescence analyses found the collected lichens to have overall high viability, with most F_v/F_m values between 0.7-0.8. There was no significant difference between the F_v/F_m values of lichens in the transects dispersed manually versus aerially. With regards to cover, the total mean lichen cover dropped about 50% between 2017 and 2018 in the two manually dispersed transects, which was suspected to be due to differences in lichen hydration in the assessments: they were assessed moist in 2017, but dry in 2018.

In the aerially treated transects, the mean lichen cover increased by about 30% in two of the transects but decreased by about 40% in the other. This was suspected to be due to the fact the lichens were compressed by the weed whacker fragmentation, and in the transects where the cover increased, the fragments had become more spread out and upright over time. For the transect where the cover decreased, it was suspected to be due to wind moving the fragments, because the transect was on a more exposed east-facing esker. However, when plots in this transect were grouped by aspect and slope position on the esker, the losses to lichen cover did not appear to correlate with either the proximity to the ridge of the esker or their aspect.

Methods

In August 2023, the plots established by Ronalds (2018) were re-assessed by a team including the original study author Irene Ronalds, a field assistant, and a member of our team. Some ecological site information had already been gathered by Ronalds in 2017. As well, lichen covers within the plots had all been previously assessed for the target species combined, but no estimations had been done for each constituent species, nor for other lichen species that may naturally be present in the plots. Due to a very limited timeframe because of the remoteness of the site (only accessible by float plane), the reassessment tasks were divided. Ronalds and the assistant performed the vegetation covers and surface substrate estimations as per the previous reassessment, and the member of our team estimated the covers of all lichen species within each plot, gave each lichen species a visual assessment of vitality as described above, as well as gathered additional site information. This information included the AVI code, slope, aspect, and slope position for each

plot. Samples of lichens within each plot were also collected for more accurate identification later, using microscopic examination and chemical spot tests as required.

For this assessment, lichen samples were not sent for chlorophyll fluorescence analysis. By this time, the chlorophyll fluorescence data had already been obtained from the previous two historical studies, showing that virtually all lichens remaining on the historical sites were healthy, and demonstrating no correlations between F_v/F_m scores and any site or species variables. In addition, Ronalds (2019) performed chlorophyll fluorescence analyses on the same plots and found healthy lichens with no significant differences between the treatments. For these reasons, it was determined that chlorophyll fluorescence data would be unlikely to provide any useful additional information for this study.

In the revisit, we were unable to re-assess all plots due to time and access limitations. The transects assessed (with the plot numbers in brackets) included transects 1 (9-18), 2 (19-28), 3 (37-46), 4 (47-56), 5 (57-66), and 6 (71-80). We also re-assessed the small circular treatments including treatments 101 (1-4), 102 (5-7; plot 8 could not be found), 201 (33-36), 501 (67-70), and 601 (81-84). Data from treatment 102 was not included in our analyses, because it was the only treatment visited where the lichens were applied at the higher rate of 80 L/100 m^2. In all, 79 plots were re-assessed, 76 of which were used in our analyses. Of these, 10 were leaf-blower dispersed (Transect 2), 20 were aerially dispersed (Transects 5 and 6), 20 were controls (Transects 3 and 4), and 26 were manually dispersed (Transect 1, plus small circular treatment areas 101, 201, 501, and 601).

Results and Discussion

The ecological site information is provided in **Table 1.3.1**. Much of the information is missing because Ronalds did not collect site data for transects 1-6 in 2017, and some cards had fields that were not readable. However, while the area was quite variable in its topography, field observations lead us to believe that the transplant sites were fairly uniform, being performed in generally mid-slope to crest areas that presumably had similar nutrient and moisture characteristics. The information provided indicates that the sites had generally rapid drainage, xeric moisture regimes, and poor to very poor nutrient regimes (**Table 1.3.1**).

Due to limitations with the original study design, and low numbers of replicates for some treatments, statistical analyses could not be performed with this data. The visual assessment of lichen vitality showed almost all species scoring a healthy score of "5", with only a few specimens scoring lower, which were all either damaged or naturally darker species of *Peltigera* and non-target species of *Cladonia* (data not shown).

In all, 27 lichen species were identified within the plots, including 18 species of *Cladonia* and two species of *Stereocaulon* (data not shown). The mean percent cover of all lichen species combined, using our data, was similar between the manually dispersed, leaf-blower dispersed, and aerially dispersed plots, with mean percent covers of 4.60, 4.55, and 5.53 respectively (**Fig. 1.3.1**). This is consistent with the previous findings in Ronalds (2019), which similarly found little difference between the percent cover of *Cladonia* covers by transect. The one transect that had a lower lichen

cover in Ronalds (2019) was Transect 7, which was not included in our reassessment. The total lichen cover in the treated plots contrasts strongly with the controls, which had a mean of only 0.75% total lichen cover (**Fig. 1.3.1**).

To better compare with the findings of Ronalds (2018) and Ronalds (2019), the mean total cover of *Cladonia* species for each transect was also calculated from our data (**Fig. 1.3.2**). Estimating from the data provided in Figure 4 of Ronalds (2019), the mean percent cover of *Cladonia* species in Transect 1 (manually dispersed) was about 2.7% in 2017, 2.2% in 2018, and we had nearly 4% in 2023. The results from Transect 2 (leaf-blower dispersed) were about 2.0% in 2017, 1.3% in 2018, and we had 3.3% in 2023. The controls (Transects 3 and 4) are not presented in Ronalds (2019), but our data had a mean of 0.05% cover in both. For Transects 5 and 6 (both aerially dispersed) the respective mean covers were about 1.4 and 1.2% in 2017, 1.7% and 1.5% in 2018, and 5.3% and 3.9% in our 2023 data. Overall, then, all transects have shown a substantial increase in lichen cover since 2018. However, the results may be partly because species may overlap in cover, so our summed totals may not fully correspond with what the total covers in the field would be if done as a whole. As well, different surveyors may estimate differently. Reynolds did collect her own data for total fruticose lichen cover during this reassessment, which will be presented in a future report authored by her, and will likely shed better light on the total changes in cover. Nonetheless, the overall increase in cover deems well for future applications of reindeer lichen transplantation.

The species composition of the lichens was similar between the manually dispersed, leaf-blower dispersed, and aerially dispersed plots (**Fig. 1.3.3**). *Cladonia arbuscula* ssp. *mitis* was by far the most common species encountered, with a mean of 5.9% cover between all treatments, followed by *C. rangiferina*, *C. uncialis*, and *Stereocaulon paschale* with 1.8%, 1.7%, and 1.5% cover respectively. Unfortunately, it is not possible to determine if differences in species coverages represent differences in survival or growth because we do not know the initial percentages of each species applied to each transect. However, this data can provide a baseline if future studies are performed on this site.

None of the target species were found in the controls, where the most common species present was *Peltigera didactyla*, with a mean of 1.1% cover. This species, together with small amounts of other *Peltigera* and non-target *Cladonia* species, was the only species found in the control plots.

Table 1.3.1 Ecological site information summary for Tweedsmuir post-fire restoration trial. Plot number codes are transect number with plot numbers in brackets for our data. When measurements were made at each plot at a site, the mean value is provided with the range following in brackets. AVI Code, slope, aspect, and percent surface substrate from our data in 2023, with terminology and procedures following Beckingham and Archibald (1996) and Soil Classification Working Group (1998). Other data provided by I. Ronalds and collected in 2017, following Government of British Columbia (1998).

Plot No.	1 (09-18)	2 (19-28)	3 (37-46)	4 (47-56)	5 (57-66)	6 (71-76)	101 (1-4)	201 (33-36)	501 (67-70)	601 (81-84)
AVI Code	A3Pl10	A3Pl10	?	A2Pl10	A3Pl5Aw5	A3Pl10	A3Pl10	A2Pl10	SO7HG3	A3Pl10
Slope (%)	11 (2-20)	4 (0-7)	8 (0-25)	4 (0-15)	6 (0-12)	7 (1-12)	5 (2-10)	8 (2-15)	4 (2-7)	8 (4-12)
Aspect (degrees)	182 (30-290)	173 (130-220)	129 (20-200)	281 (175-350)	137 (14-235)	177 (150-210)	208 (120-270)	190 (180-200)	199 (150-220)	243 (220-310)
Surface Expression	?	?	?	?	?	?	Hummocky	Terrace	Undulating?	Undulating?
Surface Shape	?	?	?	?	?	?	Convex	Straight	?	?
Slope Position	?	?	?	?	?	?	Crest	Mid-Slope	Crest	Upper Slope
Drainage	?	?	?	?	?	?	Rapid	?	Rapid	?
Moisture Regime	?	?	?	?	?	?	Xeric	Xeric	Xeric	Xeric
Nutrient Regime	?	?	?	?	?	?	Very Poor	Very Poor	Poor	Poor
Total Organic Thickness (cm)	?	?	?	?	?	?	0	0	2.5?	?
Soil Surface Texture	?	?	?	?	?	?	Sand	Sand	Sand	?
Soil Effective Texture	?	?	?	?	?	?	Sand	Sand	Sand	?
Water Table Depth (cm)	?	?	?	?	?	?	n/a	n/a	n/a	n/a
Humus Form	?	?	?	?	?	?	n/a (Mor)	n/a	?	?
Parent Material	?	?	?	?	?	?	Till	Till	Till	Till
Percent Surface Substrate[a]	1 (0-5) DW, 2 (0-15) CS, 11 (0-35) MS, 85 (50-100) OM	5 (0-35) DW, 1 (0-6) CS, 27 (0-95) MS, 67 (5-100) OM	1 (0-6) CS, 18 (0-50) MS, 81 (50-100) OM	7 (0-35) DW, 4 (0-10) MS, 90 (55-100) OM	1 (0-13) DW, 1 (0-2) CS, 15 (0-50) MS, 83 (50-100) OM	10 (0-60) DW, 0.2 (0-2) CS, 11 (0-35) MS, 80 (40-100) OM	13 (0-30) DW, 13 (0-40) MS, 75 (60-100) OM	5 (0-10) DW, 4 (0-15) CS, 10 (3-15) MS, 82 (70-97) OM	6 (0-15) DW, 4 (0-14) CS, 23 (7-45) MS, 68 (40-90) OM	23 (0-65) DW, 6 (0-25) MS, 71 (35-100) OM

[a]Percent Surface Substrate codes: DW=decaying wood, CS=cobbles/stones, MS=mineral soil, OM=organic matter

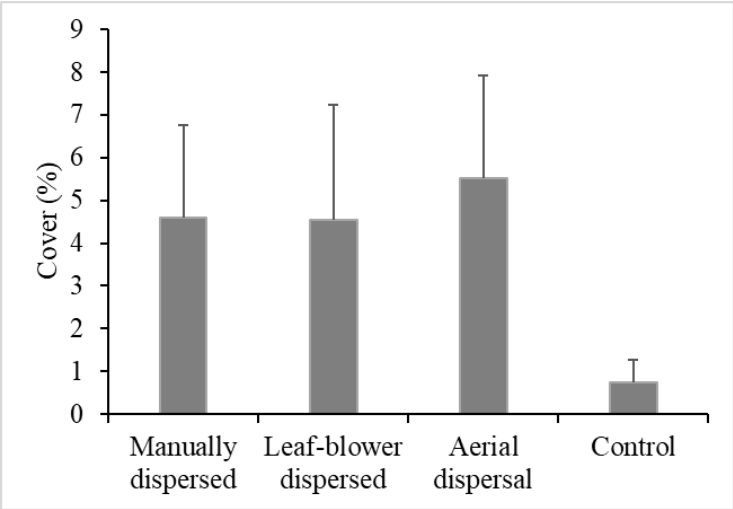

Fig. 1.3.1 Mean total cover (%) of all lichen species in manually dispersed, leaf-blower dispersed, aerially dispersed, and control plots from the Tweedsmuir post-fire restoration trial near Tetachuk Lake, BC. The horizontal bars indicate ± SD.

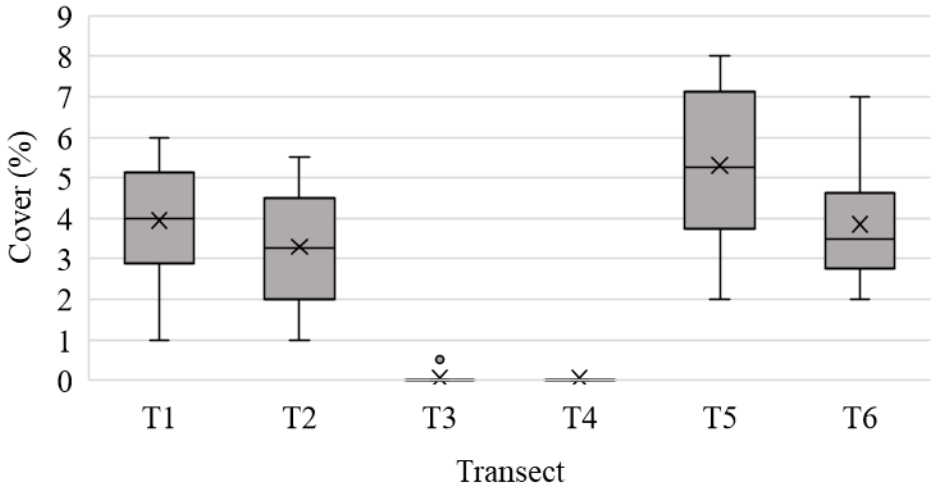

Fig. 1.3.2 Mean total cover (%) of all *Cladonia* lichen species by transect in the Tweedsmuir post-fire restoration trial near Tetachuk Lake, BC. Transect 1 (T1) was manually dispersed, Transect 2 (T2) was leaf-blower dispersed, Transects 3 and 4 (T3 and T4) were controls, and Transects 5 and 6 (T5 and T6) were aerially dispersed. The horizontal bars indicate minimum and maximum values.

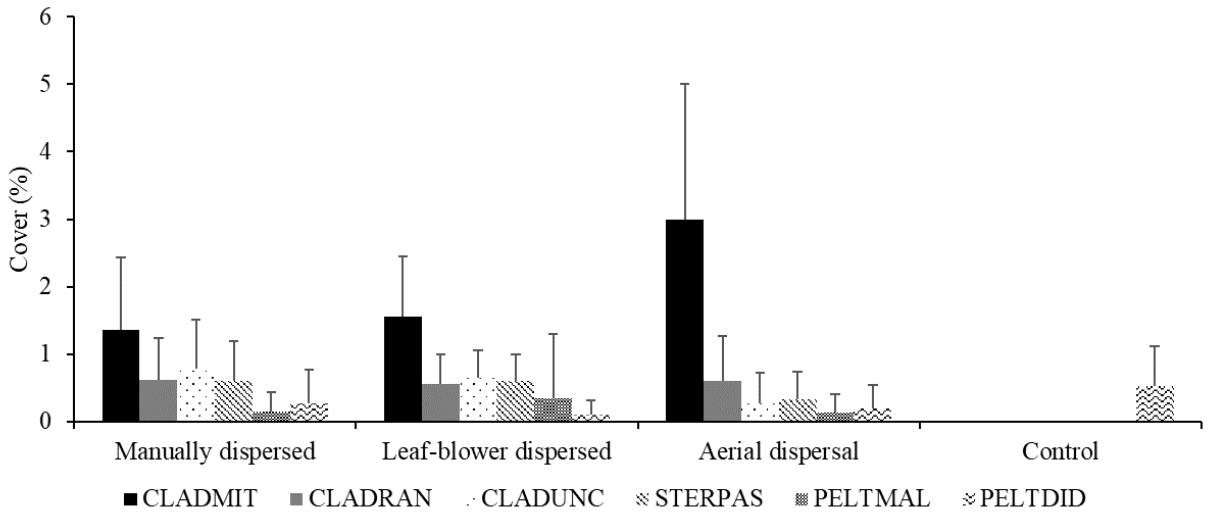

Fig. 1.3.3 Lichen cover (%) of the six most common lichen species in manually dispersed, leaf-blower dispersed, aerially dispersed, and control plots from the Tweedsmuir post-fire restoration trial near Tetachuk Lake, BC. CLADMIT=*Cladonia arbuscula* ssp. *mitis*, CLADRAN=*Cladonia rangiferina*, CLADUNC=*Cladonia uncialis*, STERPAS=*Stereocaulon paschale*, PELTMAL=*Peltigera malacea*, and PELTDID=*Peltigera didactyla*. The horizontal bars indicate ± SD.

Historical Report Summary

The three historical studies conducted all provide strong support for the long-term efficacy of reindeer lichen transplantation. At twenty-three, eight, and six years respectively, the reindeer lichens transplanted by Kranrod and Anderson (2001), Rapai et al. (2017), and Ronalds (2018) have all been successful at re-establishing themselves and in good health. The different transplantation techniques, using either planted clumps or dispersed fragments, provided a similar percent cover with the same material in both the Kranrod and Anderson (2001) and Rapai et al. (2017) reassessments, which were significantly higher than the controls. There did not appear to be any significant difference in the percent lichen cover whether fragments were dispersed aerially by helicopter, by leaf blower, or manually, according to the Ronalds (2018) reassessment results.

The research conducted by Kranrod and Anderson (2001) offers important insights into the ecological behaviour of reindeer lichen, especially regarding interspecific competition and dispersal limitations. A correlation analysis between different reindeer lichen species revealed that competition becomes more intense when they are found in dense concentrations. The study of the dispersal plots showed that lichen species did not spread significantly beyond 50 cm. This finding is notable despite the relatively small size of the plots used in the study. It suggests that, to improve the effectiveness of source material and promote better establishment, lichen fragments should be dispersed more broadly and evenly across an area instead of being placed in compacted clumps.

Data from the Kranrod and Anderson (2001) reassessment may indicate that some reindeer lichens, particularly *C. arbuscula* ssp. *mitis*, may somewhat re-establish on its own in forest harvest areas. This was not, however, the case in the burn site reassessments of Ronalds (2018) and Rapai et al. (2017), which had virtually no cover of reindeer lichens including *C. arbuscula* ssp. *mitis*, *C. rangiferina*, *C. uncialis*, *Stereocaulon paschale*, and others, unless they were transplanted. This aligns well with studies such as Webb (1998), who similarly found virtually no reindeer lichens surviving forest fire, but many surviving logging, in Ontario. This indicates that burns may benefit even more than harvested forest area from larger-scale lichen transplantation efforts.

The ecological site information gathered at these sites has shown that reindeer lichen transplantation can be successful in a wide range of site conditions. Due to the initial experimental designs, the impacts of most of the site conditions could not be statistically analyzed except for some factors in the Kranrod and Anderson (2001) study. However, it is clear that transplantations were successful in all of the sites, including those that were very rapidly to poorly drained, xeric to hygric in moisture regime, and very poor to rich in nutrients. Surface expression, surface shape, and soil factors did not appear to affect transplant success, nor did the surface substrates present. Lichen fragments performed well on slopes from 0-20%, although higher slopes did result in slightly lower percent cover in the Kranrod and Anderson (2001) reassessment.

However, there was considerable variation in the success of transplants in different locations that could not be correlated with any of the site features gathered. Some transplanted plots had no lichens remaining, while others grew profusely, even within short distances from one another. Field

observations suggest that plots in shadier sites performed more poorly, particularly if they were located directly under regenerating conifers, and that plots in wet, depressional microsites also performed more poorly. Conversely, plots in more open, sunny areas appeared to be the most successful. However, additional studies need to be performed to confirm and detail these observations.

Another potential avenue for future research would be to compare the biomass, rather than just the percent covers, of the lichens in the reassessed plots. As mentioned above, reindeer lichens planted as clumps or dispersed as fragments had similar covers in the Kranrod and Anderson (2001) and Rapai et al. (2017) reassessments. However, since the lichens dispersed as fragments had a higher percent cover when originally dispersed, this means that a larger proportion of the fragments died, while the clumps grew in area, as detailed in the discussion of the Kranrod and Anderson (2001) reassessment results. It would be useful to know if the larger percent covers accomplished by dispersing fragments actually translates to an increase in caribou forage, or if it only serves to spread a smaller amount of biomass over a larger area. Being spread over a larger area could help to decrease competition and allow for faster reindeer lichen growth, but there may also be benefits to growing in larger clumps, such as less exposure to wind and desiccation. Unfortunately, biomass estimations would be difficult to do without altering or destroying the assessed treatments.

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Growth of Terrestrial Lichen Transplants of Different Sizes on Various Substrates in a Greenhouse

Abstract

Lichens are important components in ecosystems but have traditionally not been a focus of forest reclamation efforts. There is little information regarding the best methods of transplanting lichens and long-term greenhouse studies are currently lacking. We conducted a greenhouse study to provide information on possible reclamation techniques and methods in the field. In a greenhouse environment, we investigated the effects of substrate (mineral soil, moss, pine needles, and wood) and fragment size (large, medium, small) on the growth of terrestrial lichens commonly found in boreal ecosystems. Lichens were assessed for dry biomass and length over 16 months, and chlorophyll fluorescence measurements (F_v/F_m) were used to determine health and survival. Lichens on the wood substrate had the lowest survival at the end of the experiment, and lichens on moss had the highest reductions in both length and biomass. Smaller fragments were less likely to break apart, but their photosynthetic health was dependent on species and substrates. Greenhouse studies offer valuable insights on potential reclamation options for transplanting lichens. The transplantation of lichens will help to restore ecosystem structure and function on disturbed lands.

Implications for Practice:

- Specific characteristics of the substrates influenced both the survival rates and overall health of lichen colonies. Lichen fragments attempting to establish themselves on a mossy surface were likely exposed to persistent moisture, a condition attributed to the moss's ability to retain standing water. Those same lichen fragments, when situated on wooden substrates, experienced rapid desiccation.
- The use of small fragments in the field may be appropriate, and the collection of small pieces would presumably be less destructive to communities than large fragments.
- Species differences should also be carefully considered. The responses by species were not consistent. For example, small fragments of *C. arbuscula* had high F_v/F_m relative to large pieces, while small fragments of *Peltigera* sp. had lower F_v/F_m in soil relative to large pieces.
- We recommend watering the lichens more frequently for proper hydration and minimizing the handling of lichens to break apart. These greenhouse studies will help guide the design of reclamation field experiments.

Introduction

Industrial activities can have significant effects on lichen communities in boreal ecosystems. For example, the adverse effects of silviculture may occur when moving heavy harvesting equipment or when pulling cut trees out of a forest during clearcutting, which can directly harm the lichen thalli (Harris 1996). Indirect effects of forest management on lichen communities may also occur from a change in ground-level microclimate conditions (changes in surface air temperature,

irradiance, and wind conditions) in response to canopy openings during harvesting (Harris 1996). However, reclamation efforts in response to human disturbances, including forestry, have often been focused on the return of trees and shrubs into the ecosystem. Lichens have often been neglected because of their small size and slow growth; "reindeer" lichens in the genus *Cladonia* subgenus *Cladina* (e.g., *Cladonia arbuscula* (Wallr.) Flot., *Cladonia rangiferina* (L.) Weber, *Cladonia stellaris* (Opiz) Pouzar & Vězda, and *Cladonia stygia* (Fr.) Ruoss) are estimated to grow only 4–5 mm per year in Alberta (Scheidegger et al. 1995; Sillett and McCune 1998; Campeau and Blahchard 2010; Duncan 2011). However, lichens play a vital role in boreal ecosystems by fixing nitrogen and carbon (Henry 2011), supporting soil formation and stability (Leddy et al., 2019), and providing food for large wildlife populations, including boreal caribou (*Rangifer tarandus caribou* Gmelin), which is currently listed as threatened under Canada's federal Species at Risk Act (SARA, 2002) There is currently a lack of understanding regarding the environmental conditions that best promote the re-establishment of lichen on disturbed sites through fragment transplants. Some studies have indicated that substrate may have significant impacts on the survival and growth of transplants. The growth of *C. arbuscula* fragments was greater on moss compared to mineral soil, twigs, and bark in clearcuts; however, different substrates had no impact on growth in pine stands (Roturier et al. 2007). Many reindeer lichens do not appear to grow well when placed directly onto mineral soil (Webb 1988; Roturier 2009; Duncan 2015), and mosses, twigs, and pine bark may assist with the attachment of lichen fragments to the ground and help facilitate their growth (Roturier 2009). Likewise, Duncan (2011) found that moss and leaf litter were better at retaining lichen fragments in 12-year-old forests (Duncan 2011).

Fragment size may play an important role in transplant success in the field (Coxson and Stevenson 2007). Larger lichen fragment sizes were more likely to stay within a plot than smaller fragments, resulting in greater lichen cover over time (Roturier et al. 2007). *Cetraria islandica* (L.) Ach. lichens planted in mats had higher growth over time relative to those dispersed by fragments (Zarabska-Bożejewicz et al. 2015). However, Rapai et al. (2023) found no significant difference in percent cover or the health of lichens including *C. arbuscula* ssp. *mitis*, *C. stygia*, and *Cladonia uncialis* (L.) F.H. Wigg. when transplanted as fragments, mats, or a hybrid between the two.

Few studies have investigated growing lichens in a greenhouse environment (Stewart 2019), but greenhouse studies can provide us with valuable information regarding the best techniques to apply in the field. The growth of lichen in a greenhouse environment was first observed and documented by Culberson (1963). In an unpublished informal study, Stewart (2019) grew *Usnea* and *Ramalina* species on sterilized branches in a greenhouse, and the lichen were watered with tap water or deionized water. Stewart (2019) found that the lichens died within 2 weeks and 19 months, respectively, possibly due to the differences in water pH or overwatering (Stewart 2019). On the other hand, Henry (2011) grew *Parmelia sulcata* Taylor, *Umbilicaria hyperborea* (Ach.) Hoffm., *Usnea perplexans* Stirton, and *Xanthoparmelia coloradoensis* (Gyelnik) Hale in the greenhouse for 12 weeks. The lichens were watered 3 times a week and no mortality was reported, though the duration of the experiment was relatively short.

Here, we investigated the effects of substrate and fragment size on transplant success of common boreal terrestrial lichens in a greenhouse setting. This study was conducted to provide information on the most effect methods for reclamation in the harvested blocks. The findings will also benefit researchers focused on lichen biology and those involved in storing lichens. We hypothesized that substrate would impact lichen growth, with moss facilitating greater growth because it can retain moisture more effectively than pine needles, wood, or mineral soil. Larger fragments were expected to grow more than smaller ones because they were less disturbed and had higher growth potential.

Methods and materials

Lichen and substrate collection

Lichen samples were collected in northern Alberta near Peace River and Lac La Biche (**Table S1**). Collection occurred by laying out 1 × 1 m quadrats, identifying and determining the percentage covers of all the lichen species, and removing all terrestrial lichen within the quadrats. The lichens were air-dried and fragmented into small, medium, and large fragments about 0-2 cm, 2-4 cm, and 4-6 cm in diameter, respectively.

Four substrates (mineral soil, moss, pine needles and woody debris) were collected in the field around the Peace River sites. Mineral soil was collected from within 20 cm of the surface and all visible plant material was removed. Moss was comprised of a mix of species but was dominated by the feather moss *Pleurozium schreberi* (Brid.) Mitt. Pine needles were collected from the ground under jack pine (*Pinus banksiana* Lamb.) trees and cleaned of extraneous material. Woody debris was a combination of large logs (up to 20 cm in diameter) down to smaller wood chips. All material was sterilized using a Sterilmatic autoclave (Market Forge Industries, Everett, MA, USA) to remove any existing lichen propagules and reduce the potential for disease or other contamination.

Experimental setup

The experiment was performed in square black plastic trays (56 cm × 56 cm with a 7 cm high edge), each containing one of the three lichen fragment sizes placed on one of the four different substrates, creating 12 unique treatment combinations (**Fig. 2.1**). Each treatment combination was replicated 6 times, for a total of 72 trays. For the mineral soil treatment (hereafter "soil"), a layer approximately 2 cm thick of soil was spread evenly on the tray. For pine needles (hereafter "needles"), a layer approximately 3 cm deep was used, and for the moss, a layer about 5 cm deep was used. With the woody debris (hereafter "wood"), the layer was variable in thickness due to the varying sizes of the pieces used, but the pieces were packed tightly to reduce the chances of lichen fragments falling in between cracks. Trays were randomly arranged on two large metal tables in the Northern Alberta Institute of Technology Centre for Boreal Research greenhouse in Peace River, Alberta.

To each tray, 40 g of dry lichen of a given size was applied in the greenhouse in October 2021. Within each tray, 20 lichen fragments were selected for more detailed monitoring in January 2022.

A flagged and numbered wooden dowel was placed at the tip of each selected fragment, and three additional wooden dowels were placed at the base and each side of the fragment to mark its location clearly and help prevent movement.

The selected lichens included branching species of *Cladonia* as well as foliose lichens including species of *Peltigera* (including *Peltigera aphthosa* (L.) Willd., *Peltigera didactyla* (With.) Laundon, *Peltigera leucophlebia* (Nyl.) Gyelnik, *Peltigera malacea* (Ach.) Funck, and *Peltigera kristinssonii* Vitik.), *Cetraria ericetorum* Opiz, *C. islandica*, and *Flavocetraria nivalis* (L.) Karnefelt & Thell. Fragments were selected based on them (1) being relatively healthy in appearance and texture, (2) representing all target lichen species present in the tray, (3) appearing to be within the intended size range for the tray, (4) being well-spaced enough to be distinguished from other marked fragments, and (5) being relatively evenly spaced on the tray. Species determinations for each fragment were performed at the end of the experiment, when all measurements were completed, using a combination of morphological features and chemical spot tests.

Greenhouse conditions

Temperature and humidity in the greenhouse were monitored daily using Titan Omni-Sensors (Argus Controls, Surrey, BC, Canada) placed directly above the greenhouse benches. The greenhouse was set to 25 °C / 20 °C in the spring and summer (March to September), and cooled down to 5 °C in the fall and winter (October to February), and observed temperatures ranged from 4.8 to 25.0 °C (**Fig. S2**). The temperature was deliberately set to simulate thermal cycling in the field, thereby optimizing lichen growth and management. This intentional thermal cycling was implemented because the higher daytime temperature of 25 °C, combined with a nightly drop to 20 °C, replicates ideal conditions for active photosynthesis and strong physiological development during the main growing seasons, ensuring plants receive the warmth needed for vigorous growth. Conversely, lowering the temperature to 5 °C during the colder months is essential for triggering dormancy, allowing the plants to rest and conserve energy. SolarSystem 1100 lights (California LightWorks, CA, USA) were placed above 2.3 m above the benches to supplement natural lighting in the spring and summer. Humidity was set to 75 % but observed levels ranged between 47.5 % to 55.7 % (**Fig. S2**). In general, lichens were misted once a week from November–March, twice a week from March–May and September–November, and three times a week from June–August. Type 2 de-ionized water was used (MilliporeSigma Canada Ltd., Oakville, ON, Canada) to prevent potential impacts from chemicals in tap water

Measurements

The experiment was established in October 2021 (T_0). Before each set of measurements, the lichen material was air-dried for 2 weeks. Baseline measurements of each marked lichen fragment for length and biomass were conducted in March 2022 (T_1). The length of each marked fragment, measured as the longest distance, was measured using 0-150 mm digital calipers. The biomass was weighed using a scale (Sartorius, Goettingen, Germany) after cleaning the lichen from any debris.

Subsequent measurements were conducted approximately 16 months after T_1 in July 2023 (T_2). The experiment was concluded 21 months after T_1 in December 2023 (T_3). The visual health of lichen fragments at T_1 and T_3 was scored based on a modified ranking table (**Table S2**) used by Lidén et al. (2004).

At T_3, chlorophyll fluorescence measurements were conducted on all marked fragments. Measurements were conducted by the University of Northern British Columbia, Coxson Research Group. A pulse-modulated chlorophyll fluorescence unit (Hansatech, Norfolk, United Kingdom) with a 6 mm measurement disc was used. The lichen samples were sprayed with distilled water until rehydrated and kept in containers under saran wrap on damp paper towel. The samples were kept under a moderate illumination of 200 μmol m^{-2} s^{-1} and a temperature of 15 °C for 24 h. Subsequently, a 15-minute dark adaptation period was followed by measurement. **Table 2.1** provides a summary of the timeline and the measurements taken on the fragments.

Statistical analysis

To assess whether the proportion of a given species significantly differed amongst treatments, we used generalized linear mixed models (glmm) with "treatment" as a fixed factor and specified the family ordbeta. A separate model was run for the abundant lichen species in the experiment (proportion > 0.05). Histograms at T_1 and T_2 were created to observe the change in visual health of fragments.

At T_3, fragments were assessed as dead or alive, with an F_v/F_m value of zero indicating that the lichens were not alive, and we calculated the proportion of fragments that were alive regardless of the species. We also assessed the fluorescence of lichen fragments that were alive ($F_v/F_m > 0$), which indicated their stress status and overall health. In addition, we calculated relative biomass and length changes using the formula $(X_{T3} - X_{T1}) / (X_{T1})$. The mean (1) survival, (2) fluorescence, (3) relative change in length, and (3) relative change in biomass for each tray replicate was calculated regardless of species, and a generalized linear mixed model with "Substrate" and "Size" as fixed factors was run with an ordbeta family. In addition, separate models were run for each abundant species in the study using the same methods described above.

The statistical software package R was used for all statistical analyses and graphical presentations (R Core Team, 2024). The analyses were carried out using the function *glmmTMB* from the glmmTMB package. (Brooks et al. 2017). Post-hoc multiple comparisons of the estimated marginal means were conducted using the "emmeans" package of R to complete EMMEANS testing using the Tukey P-value adjustment method. (Lenth et al. 2021). Residuals and model fit were assessed using the package "DHARMa" (Hartig et al. 2024). An alpha value of 0.05 was used to determine significance.

Results

Proportion of species

Cladonia arbuscula ssp. *mitis* (hereafter "*C. arbuscula*") was the most abundant and represented 40.5 % of the fragments, while *C. uncialis* (10.6 %), *C. stellaris* (9.8 %), *Peltigera* sp. (8.5 %), *C. stygia* (7.1 %), and *C. rangiferina* (6.6 %) were lower in abundance (**Table S3; Fig. S1**). The proportion of *C. arbuscula*, *C. stellaris*, *C. stygia*, *C. rangiferina* and *Peltigera* sp. did not significantly differ across the 12 treatments. The proportion of *C. uncialis* was significantly greater in the soil-small fragment treatment (21.8 % ± 3.5 %) compared to the moss-large treatment (8.1 % ± 2.1 %) but did not differ between other groups. *C. arbuscula*, *C. uncialis*, *C. stellaris*, *Peltigera* sp., *C. stygia*, *C. rangiferina* and *Cladonia gracilis* ssp. *turbinata* (Ach.) Ahti (hereafter "*C. gracilis*") were used for species analyses; these species had proportions > 5 %.

Visual health

At T_1, the majority of fragments were classified as fully healthy with a score of 4 (68.5 %); however, 59 % of *Peltigera* sp. showed significant bleaching with a score of 1 (**Table S4**). At T_3, the majority of fragments were moderately healthy with a score of 2 (74.2 %), and no fragments scored 4. At this time, 82 % of *Peltigera* sp. showed significant decay with a score of 0, and 15 % scored 1.

Survival

For the pooled species data, there was a significant substrate effect (**Table S6**), where survival was significantly lower on wood when compared to each of the other three substrates based on model means (**Fig. 2.2A**). There was a significant substrate effect for *C. arbuscula* (**Table S6**), and survival was significantly lower on wood (58.7 % ± 3.7 %) when compared to needles (76.0 % ± 3.6 %) and soil (77.3 % ± 4.3 %) but not moss (68.3 ± 3.9 %). There was a significant substrate effect for *C. stellaris* (**Table S6**), where survival was significantly higher on moss (77.0 % ± 6.0 %) over wood (53.9 % ± 7.5 %). For *C. rangiferina,* there was a significant size effect (**Table S6**), and large fragments (69.8 % ± 4.0 %) had significantly greater survival than small (49.5 % ± 5.9 %) or medium (42.7 % ± 4.3 %). There were no significant substrate or size effects for *C. uncialis*, *C. gracilis*, *Peltigera* sp., or *C. stygia* (**Table S6**).

Photosynthetic stress and health

For the pooled species data, there was a marginally significant interaction between size and substrate (**Table S7**); higher F_v/F_m values were observed when small fragments were on moss or wood relative to soil. The model means for the moss-small and moss-medium were significantly higher than those of soil-small (**Fig. 2.3**). There was significant interaction for *Peltigera* sp. (**Table S7**); F_v/F_m on soil-small (0.32 ± 0.05) was significantly lower than all other groups except soil-medium (0.56 ± 0.06). For *C. arbuscula*, there was a marginally significant substrate effect and a significant fragment size effect (**Table S7**). The wood substrate (0.78 ± 0.009) had significantly lower F_v/F_m values than moss (0.82 ± 0.010), while small fragments (0.82 ± 0.008) had

significantly higher values than large fragments (0.78 ± 0.009). For *C. stellaris*, there was a significant substrate and size effect (**Table S7**), though no significant differences were observed amongst means based on the Tukey tests. For *C. stygia*, there was a significant substrate and size effect (**Table S7**). Medium fragments (0.85 ± 0.01) had significantly greater F_v/F_m values than large fragments (0.76 ± 0.02), but multiple comparisons did not indicate any significant differences amongst substrates. For *C. rangiferina*, there was a marginally significant substrate effect, but no significant size effect or interaction (**Table S7**). With *C. uncialis*, there was a marginally significant substrate effect (**Table S7**); lower F_v/F_m values were observed on pine needles but there were no significant differences based on Tukey tests. For *C. gracilis*, there were no significant substrate or size effects (**Table S7**).

Relative change in length

For the pooled data of relative change in length, there was no significant interaction, but there was a significant substrate and size effect (**Table S8**). The model mean for wood (-19.1 ± 1.8 %) was significantly higher than for moss (-26.4 ± 1.8 %) **(Fig. 2.4A**), and small fragments (-18.4 ± 1.6 %) were significantly higher than large (-25.4 ± 1.6 %) and medium fragments (-24.1 ± 1.6 %) (**Fig. 2.4B**). All changes in relative length were negative (**Fig. 2.4**). For *C. arbuscula*, there were both significant substrate and size effects (**Table S3**). The model mean for moss (–26.1 ± 2.4 %) was significantly lower than for soil (–16.9 ± 2.5 %), and large fragments (–23.4 ± 2.0 %) had significantly more negative declines in length than small fragments (–15.3 ± 2.0 %). All means were negative as with the pooled species data. We observed a significant substrate effect for *C. stellaris* (**Table S8**), but no differences were observed based on Tukey tests. No significant substrate, size or interactions were observed for the remaining species.

Relative change in biomass

As opposed to the changes in length, the relative changes in biomass were, on average, positive (**Fig. 2.5**). With the pooled data, there was no significant interaction, but there was a significant substrate effect (**Table S4**). The change in biomass on moss (-10.0 ± 6.0 %) was significantly lower than on soil (18.55 ± 6.0 %) (**Fig. 2.5A**), but there were no differences between fragment sizes (**Table S4**). For *C. stellaris* there was a significant substrate and size effect (**Table S9**). The decline in length of fragments on moss (–24.90 ± 7.76 %) was significantly lower than wood (19.13 ± 8.88 %). In addition, declines in length for small fragments (–16.60 ± 7.51 %) were significantly lower than medium fragments (14.10 ± 9.3 %). No interaction was observed for *C. stygia*, but there were significant substrate and size effects (**Table S4**). The model mean for soil (28.46 ± 12.10 %) was significantly higher than that of moss (-20.66 ± 11.4 %). The model mean for small fragments (24.6 ± 11.40 %) was significantly higher than that of medium (-21.0 ± 10.0 %) and large fragments (-13.5 ± 8.35 %). No significant treatment effects were observed for the remaining species.

Discussion

Substrate

Our results demonstrate the complex responses of lichens on various growing substrates. Lichens on wood and moss did not perform well in the greenhouse, which contradicts our hypothesis that moss would be the optimal substrate. In particular, the lichens on wood exhibited the lowest health, as indicated by their survival rates at the end of the experiment; this response was driven primarily by *C. arbuscula* and *C. stellaris*. Photosynthetic health was also lower on wood and was driven by *C. arbuscula*. The low survival of wood could be explained by the relatively quick drying that occurred here because the wood held relatively little moisture. Lichen on moss had significant declines in length, driven by *C. arbuscula*. Other studies have suggested that moss is a good substrate in nature for reindeer lichens (Roturier et al. 2007), but in this study, moss was sterilized, which may not have provided the same moisture and nutrient conditions as living moss in the field. However, Ficko et al. (2023) found no differences between responses on autoclaved and non-sterilized substrates.

Fragment size

Overall, fragment size did not impact survival, except for *C. rangiferina*, where large fragments had higher survival. However, responses to fragment size were varied. For example, small fragments of *C. arbuscula* had greater photosynthetic health than large fragments. In contrast, small fragments of *Peltigera* sp. had lower photosynthetic health on soil relative to large fragments. Fragment size effects on length were driven by *C. arbuscula*, and smaller fragments were less likely to fragment during handling. A decline in health or the handling of lichen and the movement of greenhouse benches could result in fragmentation. Due to their often widespread and abundant branching, larger fragments seemed to be more likely to break during handling. Duncan (2011) also found that tracked reindeer lichen fragments in plots were observed to break apart over the study, and roughly 50 % of the fragments were lost after two years. Overall, this may suggest the use of small fragments in reclamation may be sufficient. This is promising as transporting large lichens is difficult and hard to maintain without fragmentation. In addition, the collection of small lichen fragments in communities may be less destructive overall to donor communities.

Lichen measurements

It is important to note that biomass increased in most of the fragments, as demonstrated by the positive changes in relative biomass. This suggests that the fragments were either storing more carbohydrates, or that substrates were adhering to the lichen surface. While attempts were made to remove extraneous materials, the tight adhesion of small particles to the dry, brittle lichens made it impossible to remove them completely without damaging the fragments. In future studies, fragments could also be washed prior to weighing, but this would require additional handling that could result in more fragmentation.

Growing lichen in controlled environments

Results from growth chamber and cabinet studies can be used to guide greenhouse research. Pearson (1970) found that lichen was able to grow for 4.5 months under controlled growth chambers, and variation in humidity was a strong factor affecting the growth of *Parmelia physodes* and *Xanthoria parietina*. Drying down periods are beneficial for growth of *Lobaria pulmonaria* in growth chambers (Gauslaa et al. 2016). *Peltigera membranacea* grown for 28 days in growth chambers grew better under a light/dark cycle and organic substrates (Almer and Werth 2024). High (25 °C/20°C) and low (6°C /1°C) temperatures were not ideal when *Lobaria* lichen were wet in the day (Bidussi et al. 2013).

Greenhouse experiments with lichen are challenging, and simulating their natural environment is difficult. Our data indicates that by the end of the experiment, most of the lichen fragments in the study were becoming stressed; this was especially true for *Peltigera* species. We recommend the use of de-ionized water and perhaps finding a way to simulate the dew in natural environments. However, a watering regime of 3 days may be appropriate (Ficko et al. 2023). This study provides valuable information about lichen growing in a greenhouse over 2 years and can be used to help guide future lichen studies.

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Table 2.1 Dates and activities during experiment.

Time	Date	Description
T0	October 2021	Experiment established
T1	March 2022	Dry length and biomass, visual health scores
T2	July 2023	Dry length and biomass, visual health scores
T3	December 2023	Chlorophyll fluorescence (F_v/F_m), Species identification, experiment concluded

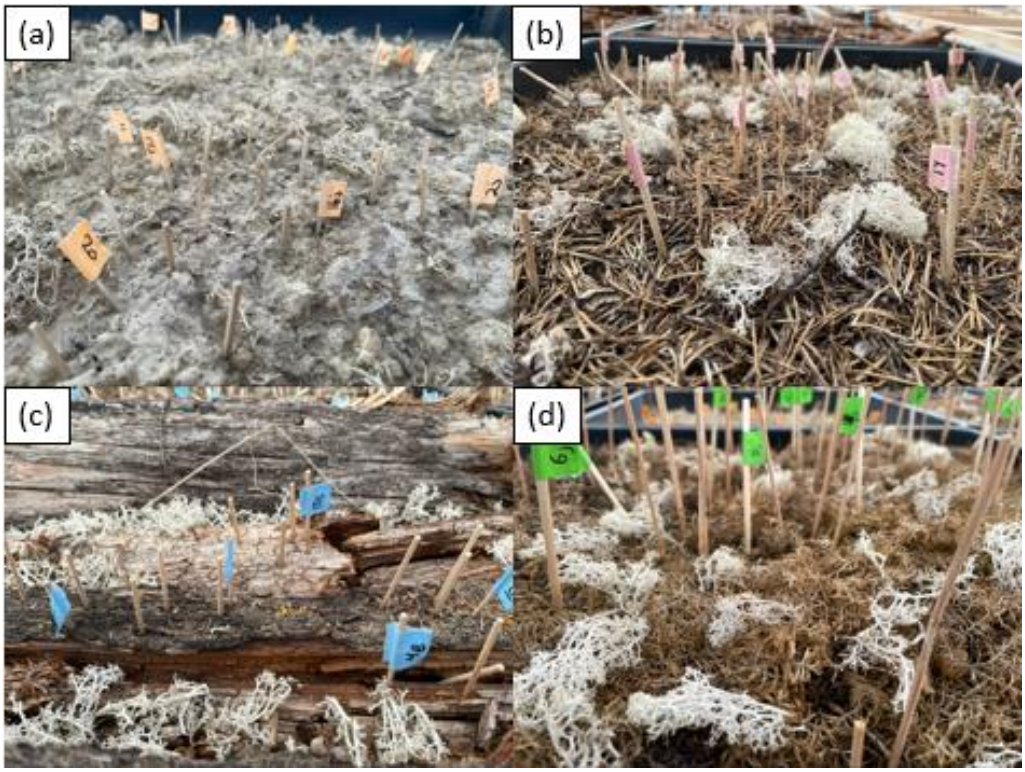


Fig. 2.1 Examples of large lichen fragments on mineral soil (a), pine needle (b), wood (c), and moss (d) substrates. Flags and dowels were used to mark and stabilize selected fragments.

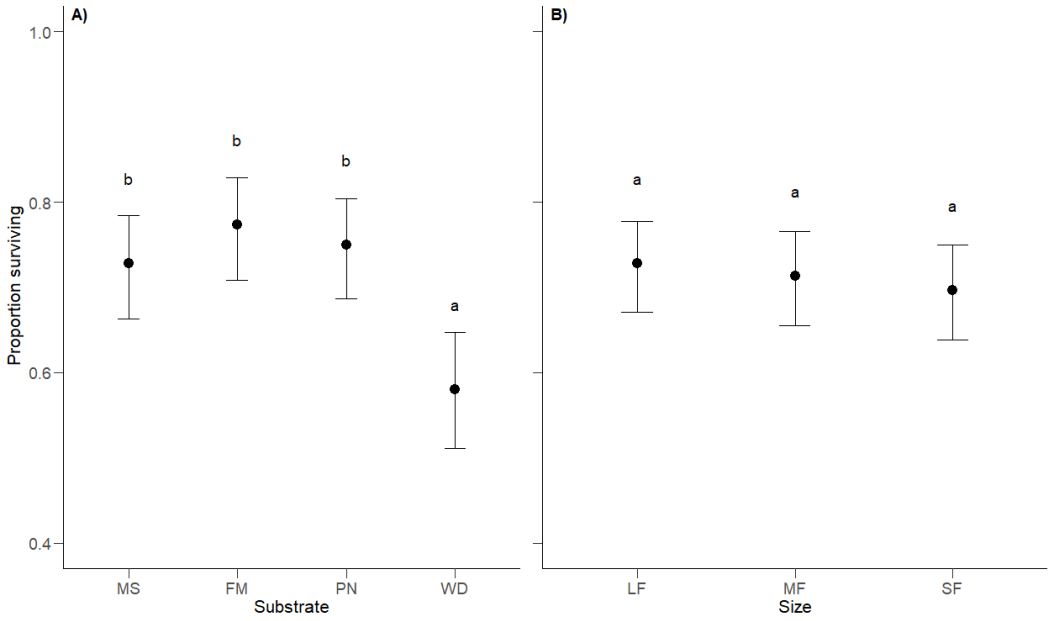


Fig. 2.2 The mean and standard error for the proportion of lichen fragments surviving on (A) different substrates and for (B) different fragment sizes based on the pooled species data and glmm model. Large (LF), medium (MF) or small (SF) fragments were placed on either mineral soil (MS), moss (FM), pine needles (PN) or woody debris (WD). The twenty marked fragments within a replicate tray were used to calculate survival and were classified as dead if $F_v/F_m = 0$, and alive if $F_v/F_m > 0$.

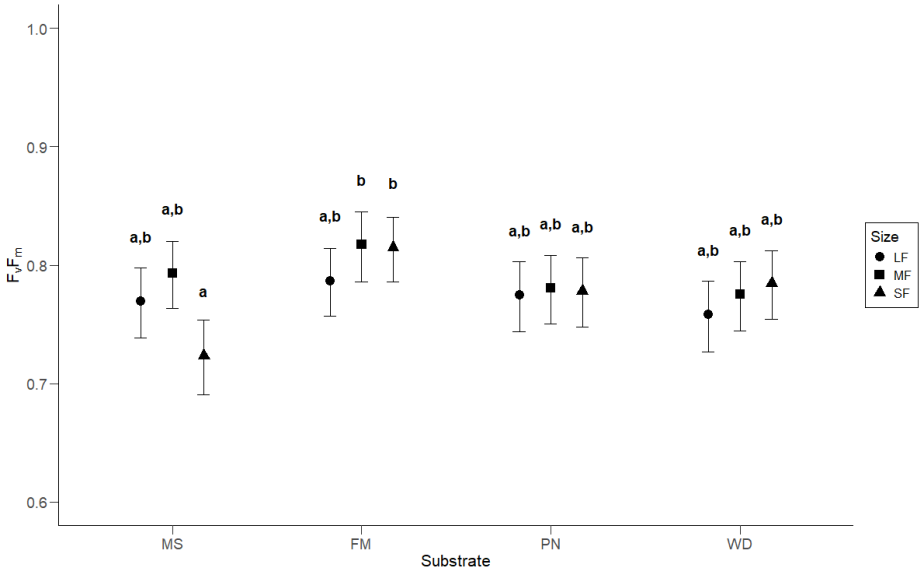


Fig. 2.3 Means and standard errors for the chlorophyll fluorescence (F_v/F_m) for living fragments based on the pooled species data and glmm model. Large (LF), medium (MF) or small (SF) fragments were placed on either mineral soil (MS), moss (FM), pine needles (PN) or woody debris (WD). At the end of the experiment, F_v/F_m was measured, and lichen were classified as dead if $F_v/F_m = 0$, and alive if $F_v/F_m > 0$.

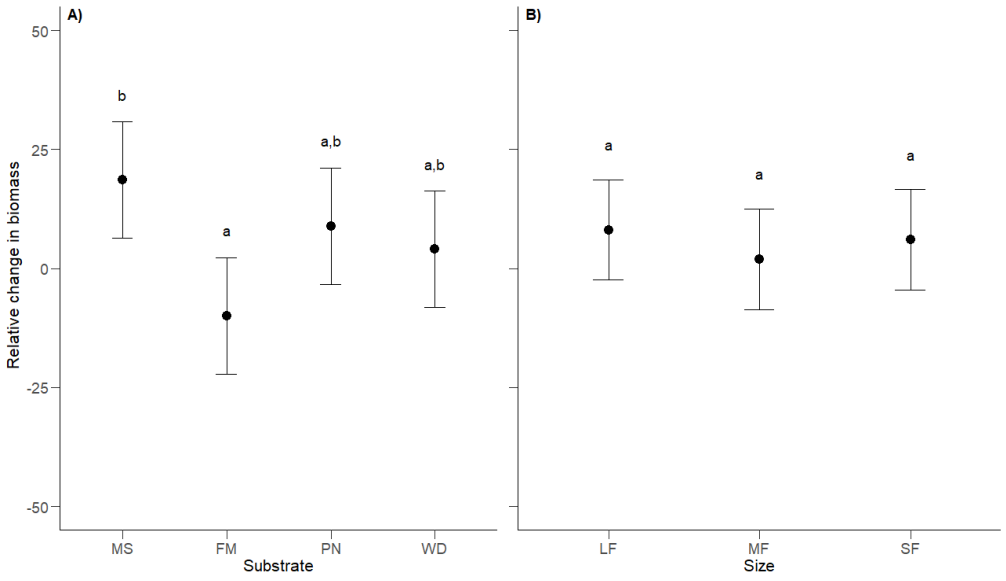


Fig. 2.4 Relative change in length from T_1 and T_3 using pooled species data. Differences were standardized by initial length, and the model means and standard errors are presented. Large (LF), medium (MF) or small (SF) fragments were placed on either mineral soil (MS), moss (FM), pine needles (PN) or woody debris (WD).

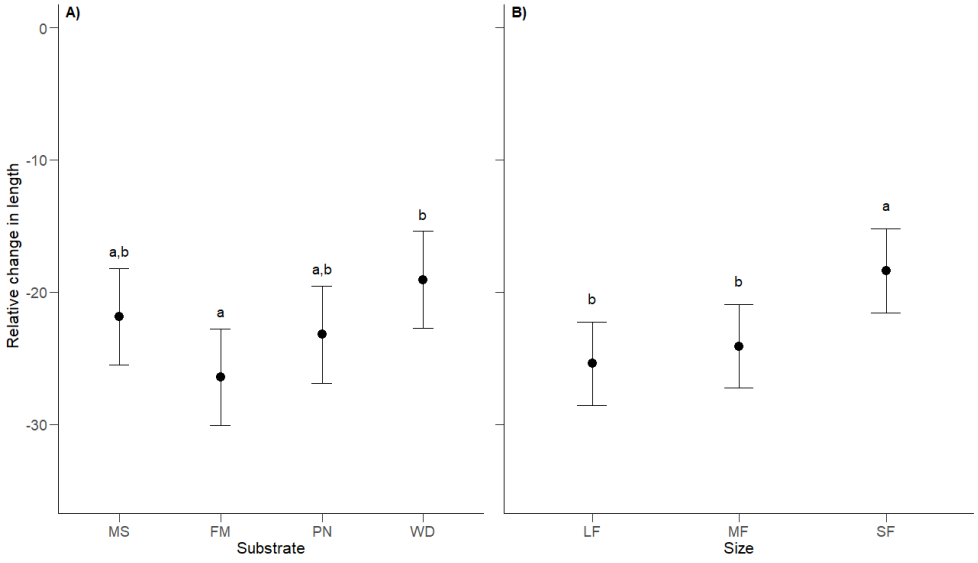


Fig. 2.5 Relative change in biomass from T_1 and T_3 using pooled species data. Differences were standardized by initial biomass, and the model means and standard errors are presented. Large (LF), medium (MF) or small (SF) fragments were placed on either mineral soil (MS), moss (FM), pine needles (PN) or woody debris (WD).

Supplementary material

Table S1 Location, ecosite, and major lichen species from lichen source plots. Ecosites in Peace River based on Beckingham et al. (1996), and those in Lac La Biche based on Beckingham and Archibald (1996). For major species, genera are as follows: *C.* = *Cladonia*, *S.* = *Stereocaulon*, *P.* = *Peltigera*, *F.* = *Flavocetraria*, *Cet.* = *Cetraria*.

Plot	Location	Latitude	Longitude	Ecosite	Major Species
01-A	Peace River	56.1672355	-116.8081983	LF-a1.1	*C. arbuscula, S. tomentosum*
01-D	Peace River	56.1672355	-116.8081983	LF-a1.1	*C. arbuscula, C. rangiferina*
02-B	Peace River	56.1682797	-116.8110884	LF-a1.2	*C. arbuscula, C. rangiferina*
03-A	Peace River	56.6681	-118.06181	LF-h1.1	*C. arbuscula, C. crispata, P. aphthosa, F. nivalis*
04-C	Peace River	56.66726	-118.25926	LF-k2.1	*C. stygia*
05-GR	Lac La Biche	54.760412	-112.398064	BM-b1.1	*C. arbuscula, C. uncialis, P. malacea*
02-GR	Lac La Biche	54.761458	-112.399737	BM-a1.1	*C. arbuscula, C. uncialis, Cet. islandica*
05-CO	Lac La Biche	54.994399	-111.780138	BM-i1.1	*C. arbuscula, C. deformis*
03-CO	Lac La Biche	54.976353	-111.841736	BM-i1.1	*C. stygia*

Table S2 Visual health scores used for lichen fragments using a modified method from (Liden et al. 2004).

Levels	Description
0	Showing signs of decay (light brown, pink or moulded)
1	Significant bleaching
2	Lacking green pigment
3	Moderate level of green pigment (pale pear green)
4	Full levels of green pigment

Table S3 Proportions of lichen fragments by species calculated with pooled data from all replicates.

Species	Code	Proportion
C. arbuscula ssp. *mitis*	CLADMIT	0.405
C. uncialis	CLADUNC	0.106
C. stellaris	CLADSTE	0.098
Peltigera sp.	PELT	0.085
C. stygia	CLADSTY	0.071
C. rangiferina	CLADRAN	0.066
C. gracilis	CLADGRA	0.064
F. nivalis	FLAVNIV	0.042
S. tomentosum	STERTOM	0.032
C. islandica	CETRISL	0.022
C. multiformis	CLADMUL	0.006
C. sulphurina	CLADSUL	0.003

Table S4 Counts of visual health scores across all fragments that were identified at T_1 or T_3 (16 months after T_1). Refer to Table S3 for species codes and Table S2 for score descriptions.

	T_1					**T_3**				
Score	**0**	**1**	**2**	**3**	**4**	**0**	**1**	**2**	**3**	**4**
CETRISL	0	1	0	14	12	8	16	2	0	0
CLADDEF	0	0	0	0	1	0	1	0	0	0
CLADGRA	0	5	5	13	52	0	10	61	4	0
CLADMIT	0	18	34	71	362	2	53	413	14	0
CLADMUL	0	0	1	2	4	0	0	6	1	0
CLADRAN	0	8	3	15	51	1	9	64	3	0
CLADSTE	0	3	5	24	89	0	10	111	1	0
CLADSTY	0	4	7	16	61	0	19	65	3	0
CLADSUL	0	0	0	0	3	0	1	1	1	0
CLADUNC	0	2	2	15	111	2	10	103	11	0
FLAVNIV	0	1	0	18	28	5	20	21	1	0
PELT	0	59	9	10	22	81	15	3	0	0
STERTOM	0	1	0	11	24	0	5	32	0	0
Total	0	102	66	209	820	99	169	882	39	0

Table S5 Summary statistics for glmm models for pooled species data using survival, F_v/F_m, and relative change in biomass and length as responses. An asterisk indicates significance for terms.

Response	Term	Estimate	SE	*z*	*P*	
Proportion surviving	Intercept	1.05768	0.19472	5.432	5.57e-08	*
	Moss	0.24714	0.23257	1.063	0.28795	
	Needles	0.11321	0.22111	0.512	0.60865	
	Wood	-0.65878	0.21156	-3.114	0.00185	*
	Medium	-0.07131	0.19263	-0.370	0.71124	
	Small	-0.15065	0.19089	-0.789	0.42999	
Chlorophyll Fluorescence (F_v/F_m)	Intercept	1.20608	0.08521	14.154	<2e-16	*
	Moss	0.1001	0.12215	0.82	0.4125	
	Needles	0.02891	0.12094	0.239	0.8111	
	Wood	-0.06434	0.11949	-0.538	0.5902	
	Medium	0.1391	0.12285	1.132	0.2575	
	Small	-0.24429	0.11707	-2.087	0.0369	*
	Moss–Medium	0.05244	0.1818	0.288	0.773	
	Needles–Medium	-0.10486	0.17312	-0.606	0.5447	
	Wood–Medium	-0.04234	0.17174	-0.247	0.8053	
	Moss–Small	0.42064	0.17283	2.434	0.0149	*
	Needles–Small	0.26398	0.16889	1.563	0.118	
	Wood–Small	0.39581	0.16833	2.351	0.0187	*
Relative biomass change	Intercept	21.284	7.486	2.843	0.004468	*
	Moss	-28.509	8.644	-3.298	0.000974	*
	Needles	-9.65	8.644	-1.116	0.264264	
	Wood	-14.554	8.644	-1.684	0.092253	
	Medium	-6.142	7.486	-0.82	0.411935	
	Small	-2.051	7.486	-0.274	0.784134	
Relative length change	Intercept	-24.643	2.241	-10.998	< 2e-16	*
	Moss	-4.554	2.587	-1.76	0.07839	
	Needles	-1.355	2.587	-0.524	0.60036	
	Wood	2.799	2.587	1.082	0.27928	
	Medium	1.31	2.241	0.585	0.55865	
	Small	7.029	2.241	3.137	0.00171	*

Table S6 Survival analysis of deviance tables for the pooled species data and each individual species.

Response	Term	X^2	*df*	*P*		Comments
Species Pooled	Sub	21.4	3	<0.0001	*	
	Size	0.6	2	0.74		
	Sub * Size	2.6	6	0.85		
C. arbuscula	Sub	14.5	3	0.002	*	
	Size	1.5	2	0.48		
	Sub * Size	3.1	6	0.79		
C. uncialis	Sub	4.2	3	0.24		Insufficient replication in the wood-small treatment
	Size	3.5	2	0.18		
C. stellaris	Sub	8.5	3	0.04	*	Insufficient replication in the soil-medium
	Size	0.3	2	0.88		
Peltigera sp.	Sub	1	3	0.8		Issues with model fit in interactive model
	Size	0.9	2	0.63		
C. stygia	Sub	7.4	3	0.06		Issues with model fit in interactive model
	Size	1.8	2	0.41		
C. rangiferina	Size	20.2	2	<0.0001	*	Issues with model fitting, single factor models were run
C. rangiferina	Sub	0.4	3	0.94		Issues with model fitting, single factor models were run
C. gracilis	Sub	6.9	3	0.08		Insufficient replication in the soil-large treatment
	Size	3.7	2	0.15		

Table S7 Chlorophyll fluorescence (F_v/F_m) analysis of deviance tables for the pooled species data and each individual species.

Response	Factor	X^2	*df*	*P*		Comments
Species Pooled	Sub	14.2	3	0.003	*	
	Size	3.8	2	0.15		
	Sub * Size	11.4	6	0.08		
C. arbuscula	Sub	7.6	3	0.05		
	Size	12.3	2	0.002	*	
	Sub * Size	4	6	0.68		
C. uncialis	Sub	6.7	3	0.08		Insufficient replication in the wood-small treatment
	Size	2.9	2	0.23		
C. stellaris	Sub	13.1	3	0.004		
	Size	7.1	2	0.03	*	
	Sub * Size	10.2	6	0.12		
Peltigera sp.	Sub	35.8	3	<0.0001	*	
	Size	7.6	2	0.02	*	
	Sub * Size	14.8	6	0.02	*	
C. stygia	Sub	11.1	3	0.01	*	
	Size	23	2	<0.0001	*	
	Sub * Size	10	6	0.13		
C. rangiferina	Sub	6.7	3	0.08		
	Size	3.1	2	0.21		
	Sub * Size	8.5	6	0.2		
C. gracilis	Sub	3.4	3	0.33		Insufficient replication in the soil-large treatment
	Size	4.6	2	0.1		

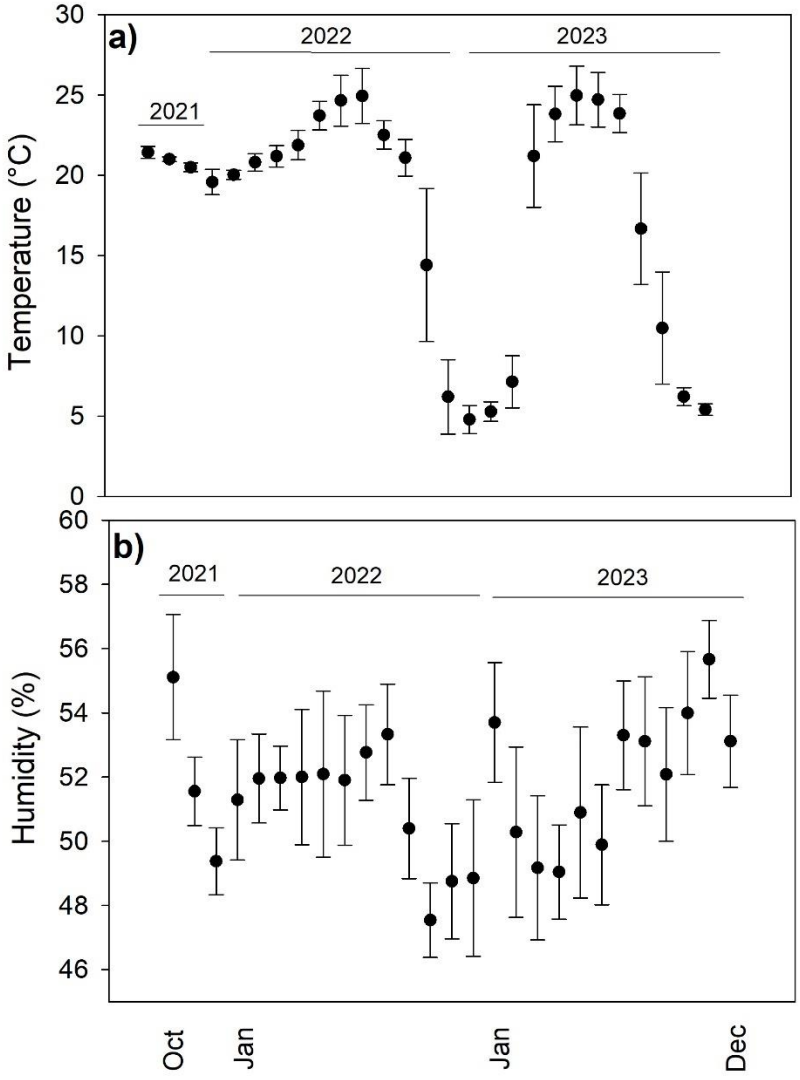
Table S8 Relative change in length analysis of deviance tables for the pooled species data and each individual species.

Response	Factor	X^2	*df*	*P*	
Species Pooled	Sub	8.6	3	0.04	*
	Size	11.5	2	0.003	*
	Sub * Size	2.1	6	0.91	
C. arbuscula	Sub	8.6	3	0.04	*
	Size	9.5	2	0.009	*
	Sub * Size	1.3	6	0.97	
C. uncialis	Sub	5.5	3	0.14	
	Size	1.8	2	0.4	
	Sub * Size	6.4	6	0.38	
C. stellaris	Sub	4	3	0.26	
	Size	6.1	2	0.048	*
	Sub * Size	7.3	6	0.29	
Peltigera sp.	Sub	4.2	3	0.24	
	Size	3.1	2	0.21	
	Sub * Size	5.8	6	0.45	
C. stygia	Sub	3.6	3	0.31	
	Size	4.6	2	0.1	
	Sub * Size	5.6	6	0.47	
C. rangiferina	Sub	3	3	0.4	
	Size	3	2	0.22	
	Sub * Size	1.3	6	0.97	
C. gracilis	Sub	3.8	3	0.29	
	Size	0.9	2	0.62	
	Sub * Size	4.2	6	0.65	

Table S9 Relative change in biomass analysis of deviance tables for the pooled species data and each individual species.

Response	Factor	X^2	*df*	*P*	
Species Pooled	Sub	11.6	3	<0.01	*
	Size	0.7	2	0.7	
	Sub * Size	1.7	6	0.94	
C. arbuscula	Sub	2.6	3	0.46	
	Size	2.7	2	0.26	
	Sub * Size	1.7	6	0.94	
C. uncialis	Sub	3.4	3	0.34	
	Size	5.1	2	0.08	
	Sub * Size	6.1	6	0.41	
C. stellaris	Sub	17.1	3	<0.0001	*
	Size	10	2	<0.01	*
	Sub * Size	6.4	6	0.38	
Peltigera sp.	Sub	3	3	0.39	
	Size	0.6	2	0.74	
	Sub * Size	5.5	6	0.49	
C. stygia	Sub	10	3	0.02	*
	Size	11.4	2	<0.01	*
	Sub * Size	6.8	6	0.34	
C. rangiferina	Sub	5.8	3	0.12	
	Size	2.9	2	0.24	
	Sub * Size	4.3	6	0.63	
C. gracilis	Sub	4	3	0.26	
	Size	1.6	2	0.45	
	Sub * Size	1.5	6	0.96	

Fig. S1 Stacked bar chart of the proportions of each species in each of the 12 treatments. Refer to Table S3 for species codes. Large (LF), medium (MF) or small (SF) fragments were placed on either feather moss (FM), mineral soil (MS), pine needles (PN) or woody debris (WD).



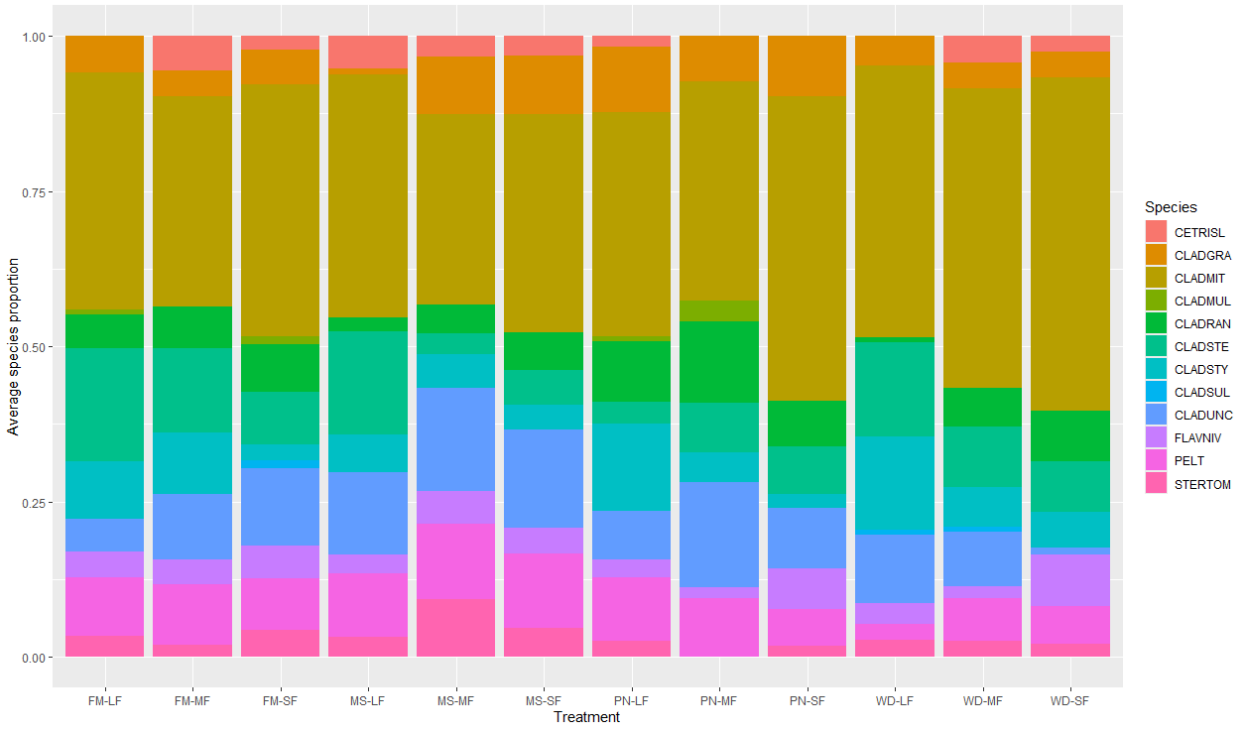


Fig. S2 Greenhouse conditions throughout experiment, including (a) mean monthly temperature and (b) humidity. Error bars are standard deviations.

Effect of Fragment Size and Substrate on the Survival and Health of Reindeer Lichen Transplants in Harvest Forest Areas

Abstract

Terrestrial lichens, particularly reindeer lichens (*Cladonia* spp.), are critical components of boreal forest ecosystems and an essential food source for caribou and reindeer. However, habitat disturbance, slow lichen growth, and limited natural dispersal impede the recovery of these lichen communities. This study investigates the viability of reindeer lichen fragment transplantation as a method for habitat restoration in forest harvested blocks in boreal Alberta. Specifically, we assessed the influence of substrate type (soil, moss, pine needles, and woody debris) and fragment size (small, medium, and large) on the survival and health of transplanted terrestrial lichen fragments. Field trials were conducted across five harvested blocks with various site treatments and ecological conditions. Chlorophyll fluorescence analysis (F_v/F_m) was used to evaluate the health and survival of lichen fragments over three years. Results indicated that larger lichen fragments exhibited significantly higher survival rates than small ones. Lichens grown on moss had significantly lower F_v/F_m values than those grown on soil or pine needles, although mean values were all within a healthy range. Species-specific responses demonstrated that *Cladonia stygia*. *Cladonia stellaris*, *Cladonia uncialis*, and *Stereocaulon tomentosum* had significantly different F_v/F_m values with different fragment sizes and/or substrates, while the most common species, *Cladonia arbuscula* ssp. *mitis* and *Cladonia rangiferina*, did not. Overall survival differed by species but was similar between the two years of the study and may indicate that some species may more effectively transplant than others. Additionally, moss and forb cover were negatively associated with lichen health, suggesting that these species may either indicate poorer lichen growth conditions, or compete with lichen fragments. These findings highlight the importance of substrate selection and fragment size in reindeer lichen restoration efforts and provide insights into strategies for restoring lichen communities in disturbed boreal forest habitats.

Introduction

Terrestrial lichens are important components of several ecological communities within the boreal forest (Beckingham and Archibald 1996; Beckingham et al. 1996). The main components of these lichen communities include species of *Cladonia*, *Stereocaulon,* and *Peltigera* (Brodo et al. 2001). Of these, reindeer lichens, including *Cladonia arbuscula, C. stellaris*, *C. rangiferina*, and *C. stygia* form a large component of the winter diets of caribou and reindeer (Scotter 1963a; Bergerud et al. 1972; Danell et al. 1994). Caribou in Canada have suffered serious declines: in particular, the Boreal population of woodland caribou (*Rangifer tarandus caribou*) was listed as threatened under the Species at Risk Act in 2003 (Environment and Climate Change Canada 2020). There are many ongoing efforts to reverse this trend, including predator control, habitat restoration, and many other techniques. To successfully restore caribou habitat to include the lichens they require, efforts need to be made, because the lichens do not naturally ingress for long periods of time, and they grow very slowly (Thomas 1996; Jandt and Meyers 2000; Brodo et al. 2001).

Reindeer lichens can reproduce both sexually and asexually, but they largely proliferate through asexual fragmentation (Ahti 1977). The natural dispersal distance of lichen fragments is short, with most fragments dispersed only within 1 m of their source (Roturier et al. 2007). The short distance of fragment dispersal seems to limit colonization of disturbances and regeneration of mats for the reindeer lichen (Duncan 2015). Accordingly, transplantation of fragmented thallus appears to be the most appropriate method for restoring reindeer lichen communities and has successfully been attempted in several studies (Roturier and Bergsten 2009; Duncan 2015; Rapai et al. 2017).

As reindeer lichens lack strong below-ground anchoring systems, the ability of thallus fragments to remain in place and become established is affected by the type of substrate present (Roturier et al. 2007). The substrate serves as a shelter to prevent lichen fragments from washing or blowing away (Duncan 2015; Roturier et al. 2007), and it retains and releases moisture for lichen growth (Topham 1977; Sillett and McCune 1998; Duncan 2015). The relative importance of different substrates for reindeer lichens may also vary depending on the type of site (Roturier et al. 2007; Duncan 2011). In boreal forests, reindeer lichens can be found growing on a large variety of substrates, including mineral soil, moss, wood, and litter (Brodo et al. 2001; Pope 2005; Tolpysheva and Timofeeva 2008). Mosses and decaying organic materials contribute to the stabilization of transplanted lichen fragments for continued growth (Brodo 1973; Webb 1998; Roturier and Bergsten 2009).

Similarly, fragment size has the potential to influence the establishment of lichen thalli, depending on the amount of disturbance experienced by transplanted fragments (Duncan 2015). Since lichen communities are slow-growing and sensitive to disturbance, making the most of harvested material is essential to large-scale lichen reclamation planning (Karenlampi 1971; Helle et al. 1983; den Herder et al. 2003). Moreover, the natural asexual dispersion of lichens presumably uses smaller propagules created when the lichens are dry, brittle, and disturbed (Kiss 1985; Honegger 1996; Webb 1998). Therefore, a good understanding of the ideal sizes of lichen fragments for establishment and growth on different natural substrates is essential to develop efficient lichen transplantation techniques.

Current information on the re-establishment of terrestrial lichen communities is limited. Relatively few lichen transplantation studies have been conducted (Duncan 2011; Roturier et al. 2017). Many previous studies used lichen mats transplants or at least large pieces of lichen (Duncan 2015). In this study, field trials were carried out in boreal Alberta to evaluate the viability of different lichen fragment sizes on different naturally occurring substrates. The objectives of the study were to 1) assess the viability of terrestrial lichen fragments of different sizes to establish growth on different substrates; 2) identify the most suitable substrates for promoting the re-establishment of terrestrial lichens in areas disturbed by forestry management; and 3) examine the relationships between success and cover of lichens, mosses, forbs, woody plants, and graminoids.

Materials and Methods

Lichen materials

Lichen samples were collected from bog, swamp, and pine forests around Peace River in 2021 and Fox Creek in 2022 (**Table 3.1**). Collection occurred by laying out 1 × 1 m quadrats in high-lichen density sites, selected to represent the diversity of lichen species present in each area. At the time of collecting, the percent cover of each species (including vascular plants, bryophytes, and terrestrial lichens) in each plot was assessed, and ecological site information such as soil types, moisture and nutrient regimes, and ecological site classifications were determined. All lichen material within each plot was collected by hand, placed in paper bags, and allowed to air dry completely. They were then stored in paper bags at room temperature in the dark at the Centre for Boreal Research (CBR) in Peace River.

Experimental field trial design

The experimental field trial was conducted using a randomized complete block design. Sites were established in June 2022 within seven conifer forest harvest forest area between Fox Creek and Whitecourt, AB, including Alberta News Print (ANP) (B), Block 690-Unit 2031 (F), Block 690-Unit 338 (G), Block 690-Unit 347 (ES), Block 690-Unit 347 (EM), Block 270-Unit A (D), and Block 270-Unit 1482 (C) (**Fig. 3.1**). ES and EM were on the same harvested block, but were treated with different site preparations; hence, there are only 6 locations on Fig. 1.These sites were chosen to represent a diversity of forest harvested block treatments: sites B and G had been modified with a RipPlow, sites D and ES had been modified through slashing, Site EM was modified by mounding, and sites C and F were untreated. In the spring of 2023, wildfire burned through sites C and D. As a result, the number of sites in 2023 and 2024 was reduced from seven to five. Ecological site information was collected in August and September of 2023 for all sites except for Site C (**Table 3.2**).

In each site, two transects were placed, each with twelve 1 x 1 m square plots. Within each transect, each plot had a different treatment combination of four different substrates (soil, moss, pine needles, and woody debris) treated with one of the three fragment sizes (small, medium, and large) (**Fig. 3.2**). In sites B, C, EM, F, and G, the transects were laid out with plots 5 m apart in a single straight line, and the two transects were placed in parallel to one another 20 m apart. In order to fit the plots into areas of similar site treatments, this had to be modified in sites D and ES, where plots were placed in four rectangular groupings of 2 plots wide by 3 plots long, with each plot 5 m apart, and the groupings at least 20 m apart.

The soil treatment included only the base layer of soil with the litter (LFH) layer manually removed. In some cases, they were mineral soil and in others they were peat, due to the types of soil present on the sites (see **Table 3.2**). The pine needle substrate consisted of a layer approximately 2 cm deep of lodgepole pine (*Pinus contorta*) needles spread as evenly as possible in the plot. For the moss treatments, a layer of live moss approximately 5 cm deep was transferred to the plots, consisting primarily of feather mosses (*Pleurozium schreberi*, *Hylocomnium*

splendens, and *Ptilium crista-castrensis*) and species of *Sphagnum*. The woody debris treatments consisted of downed woody material approximately 5-15 cm in diameter placed tightly within the plot. All added substrate materials were collected from the local vicinity of the plots.

To process the lichens for the field trials, bags were selected that best represented the diversity of lichen species present in the collections. The dried lichens were fragmented by hand into categories of small (0-2 cm), medium (2-4 cm), and large (4-6 cm). The lichen fragments were dispersed evenly within each plot by hand, and approximately 40 g of air-dried thallus fragments were placed into each plot. Plots were marked with a wooden stake in each corner.

Field data collection

Lichen fragments were collected in August 2022, August/September 2023, and July/August 2024 for fluorometry analysis. In 2022, one fragment of each fruticose lichen species that could be found within each plot was collected for analysis. In 2023 and 2024, our selection of lichen fragments was modified to reduce sampling biases. To randomly select the fragments to collect, a 10 x 10 cm square quadrat was tossed into each plot from a short distance away. The fragment of each identifiable species closest to the center of the quadrat that was within the target size for the plot was collected, regardless of whether it was inside or outside the quadrat. If the fragment was large enough, only a portion, selected to be representative of the overall fragment, was broken off and collected. This was done to allow for more of the larger fragments to remain in the plots for future analysis. As needed, dry fragments were moistened by spraying them with distilled water before collecting them to reduce fragmentation. A maximum of five samples were collected in each plot. The collected samples included *C. arbuscula* ssp. *mitis* (hereafter "*C. mitis*"), *C. arbuscula* ssp. *arbuscula*, *C. rangiferina*, *C. stellaris*, *C. stygia*, *C. uncialis*, *Stereocaulon tomentosum,* and potentially other species. During field collection, thalli were stored in envelopes, labeled, and placed in paper bags or envelopes for transportation to the laboratory. In 2024, an additional estimation of total lichen cover was performed. This was assessed by randomly tossing a 10 x 10 cm square quadrat into each plot and estimating the total lichen cover. This was repeated three times for each plot, with the quadrats re-thrown if they overlapped with previous tosses. At this time, the percentage of cover of all living plant species within each plot was also recorded and later categorized into woody (trees and shrubs), forbs, graminoids (grasses, sedges, and rushes), and mosses. This was not recorded in earlier years because the vegetation cover was not as well developed.

Chlorophyll fluorescence analysis

To obtain an indication of lichen fragment survival and health, all collected fragments were measured for chlorophyll fluorescence. Measurements were conducted by the University of Northern British Columbia, Coxon Research Group. All thalli were preconditioned by spraying them with de-ionized water until they rehydrated fully. The samples were kept in a container under saran wrap sitting on a damp paper towel in the light at moderate illumination of 200 µmol m^{-2} s^{-1} and temperature of 15 °C for 24 h. Immediately after the preconditioning, the F_v/F_m was recorded

with a pulse-modulated chlorophyll fluorescence unit (Hansatech, Norfolk, United Kingdom) with a 6 mm measurement disc after a 5-minute period of dark adaptation, following the methods of Gauslaa et al. (2012).

Lichen species identification

Lichen species were identified in the field based on morphological characteristics using a hand lens. After fluorescence analyses were performed, these identifications were confirmed or modified using a dissection microscope and as needed, chemical spot testing.

Statistical analysis

The statistical software package R was used for all statistical analyses and graphical presentations (R Core Team, 2024). For lichen samples collected in 2022, 2023, and 2024, the survival of lichen fragments collected from each plot was calculated as the number of lichen fragments having F_v/F_m values over zero divided by the total number of samples with available F_v/F_m values. Mean F_v/F_m values used in the report were the mean of all F_v/F_m values over zero within plots, indicating the health of the surviving lichen fragments. Differences in the survival of lichen fragments and mean F_v/F_m of alive lichen fragments were analyzed using the glmmTMB package with the "ordbeta" family distribution with year, substrate, fragment size as fixed factors and plot nested within transect and transect nested within site as random factors.

Differences in survival of lichen fragments collected in 2024 were also analyzed using the glmmTMB package and "gaussian" distribution, and the mean F_v/F_m of alive lichen fragments were analyzed using the glmmTMB package with "ordbeta" family distribution. Substrate, fragment size, the interaction between substrate and fragment size, and the cover of woody, forbs, graminoids, mosses, and lichens served as fixed factors. Transect nesting in site served as a random factor.

Data on mean F_v/F_m values from 2023 and 2024 were analyzed independently for each lichen species in each year. The mean F_v/F_m values were the mean of all F_v/F_m values over zero per treatment combination. Differences in mean F_v/F_m of lichen fragments were analyzed using the "glmmTMB" package with "ordbeta" family distribution in R with the substrate, fragment size, and their interaction as fixed factors and site as a random factor. Due to a limited number of samples of *C. uncialis* and *C. stygia* collected in 2024, and *S. tomentosum* collected in 2023 and 2024, the interaction between substrate and fragment size could not be included in the model for these species in these years.

Model assumptions were checked with a histogram of residuals and diagnostic plots of fitted and residual values. Pairwise comparisons between treatments were conducted using least-squares means with the R package "emmeans" (Lenth et al. 2021). Letter codes indicating significant differences in groupings for the pairwise tests were assigned using the "cld" function from the R package "multicomp" (Bretz et al. 2011). Scatter plots were generated using the "ggplot" function from the R package "ggplot2" (Wickham 2016). The effect of substrate and fragment size on percent lichen cover in 2024 was determined using a two-way ANOVA in R.

Results

Lichen survival

When data from all three years was combined, year and fragment size significantly affected the survival of lichen fragments collected in the field trials (**Table 3.3**). However, pairwise comparisons did not indicate a significant difference between years (**Fig. 3.3 a**). The survival rates of lichen fragments collected over the three years varied between 65.40% and 69.60%. Large lichen fragments had a significantly higher survival rate than small ones (**Fig. 3.3 b**), but neither significantly differed from the medium fragments.

Survival rates for each species differed much (**Table 3.4**). Using pooled data from 2023 and 2024, high survival rates of 92.0-98.3% were observed in *S. tomentosum*, *C. uncialis*, and *C. mitis*. Survival rates for *C. stellaris* and *C. rangiferina* were more moderate at 80.4% and 73.0% respectively, while *C. stygia* had low survival at 55.2%. Survival was similar in 2023 (85.1%) and 2024 (86.1%).

While survival rates for each species by fragment size and substrate could not be statistically analyzed due to insufficient replication in some treatments, there was sufficient replication within *C. mitis* to provide rates within each combination. Using the 2024 data, by fragment size, small fragments had the lowest survival rate of 89.3% (n=75), followed by medium with 95.7% (n=70) and large with 95.8% (n=72). By substrate, survival was lowest on wood with 90.4% (n=52), followed by soil (92.6%, n=54), pine needles (92.7%, n=55), and moss (98.2%. n=56).

Lichen health

The mean F_v/F_m values of alive lichen fragments also had a significant differences between years, by substrate, and in the interaction between size and year (**Table 3.3**). When the analysis was divided by fragment size, the mean F_v/F_m values of all lichen fragments measured in 2023 were significantly higher than those recorded in 2022 and 2024, except for small-sized fragments in 2022 (**Fig. 3.4**). In 2022, the mean F_v/F_m values of the small lichen fragments were significantly higher than those of large fragment sizes, but there were no significant differences between the different fragment sizes in 2023 or 2024. When pooled by substrate, lichen fragments exhibited significantly higher mean F_v/F_m values on soil and pine substrates than moss (**Fig. 3.5**), with mean F_v/F_m values ranging between 0.80 and 0.83.

Six lichen species, including *C. mitis*, *C. rangiferina*, *C. uncialis*, *C. stellaris*, *C. stygia*, and *S. tomentosum*, were identified from lichen fragment samples collected in 2023 and 2024. The effects of substrate, fragment size, and their interaction were not significant for the mean F_v/F_m values of alive lichen fragments of *C. mitis* and *C. rangiferina* in both years (**Table 3.5**). Substrate had a significant effect on the mean F_v/F_m values of alive lichen fragments for *C. uncialis* collected in 2024 (**Table 3.5**), with fragments on wood showing significantly higher mean F_v/F_m values than those on moss (**Fig. 3.6**).

For *C. stellaris*, there were significant differences between the mean F_v/F_m values of alive lichen fragments between the substrates, fragment sizes, and their interaction between fragment size and substrate in 2024 (**Table 3.5**). Only the interaction was significant in 2023. The data analysis for 2024 showed that small *C. stellaris* fragments on moss had significantly lower F_v/F_m values than all but three of the other size and substrate combinations (**Fig. 3.7**). On average, *C. stellaris* fragments on soil had the highest F_v/F_m values (0.848 ± 0.029, n=8), followed by pine needles (0.843 ± 0.048, n=13), wood (0.836 ± 0.052, n=7), and moss (0.770 ± 0.081, n=13). By size, medium fragments had the highest F_v/F_m (0.836 ± 0.053, n=13), followed by large (0.827 ± 0.040, n=16), and small (0.793 ± 0.098, n=12).

For *C. stygia*, substrate and the interaction between substrate and fragment size had a significant effect on the mean F_v/F_m values of alive lichen fragments collected in 2023 (**Table 3.5**). While pairwise comparisons did not show a significant difference across substrate or fragment size, the highest mean F_v/F_m values were present on mineral soil (0.884 ± 0.104, n=7), followed by pine needles (0.0867 ± 0.067, n=5), wood (0.861 ± 0.078, n=5), and moss (0.809 ± 0.068, n=9). By fragment size, the means were highest in small fragments (0.881 ± 0.068, n=8), followed by medium (0.0875 ± 0.048, n=5) and large (0.822 ± 0.095, n=13).

The effect of size was significant on the mean F_v/F_m values of alive lichen fragments of *S. tomentosum* collected in both 2023 and 2024 (**Table 3.5**). Pairwise comparisons indicated that large *S. tomentosum* fragments had significantly higher mean F_v/F_m values than small ones in 2023 (**Fig. 3.8**), although it did not show the difference in 2024.

The relationship between lichen survival or health and the cover of other plant groups

In 2024, we assessed the cover of all plant species in each plot, categorizing them into woody, forbs, graminoids, and mosses. The cover of forbs and mosses had significant effects on the mean F_v/F_m values of alive lichen fragments, but not their survival (**Table 3.6**). Correlation analyses showed that the mean F_v/F_m values decreased as moss cover increased, with a correlation coefficient of -0.30 (**Fig. 3.9**). Similarly, the mean F_v/F_m values also declined as forb cover increased, showing a correlation coefficient of -0.17 (**Fig. 3.10**).

Percent cover

Analysis of the percent cover data collected in 2024 showed no significant differences between the coverages on different substrates, but a significant difference between the fragment sizes (**Table 3.7**). The highest mean covers were present with the medium fragments (21.9%), followed by the large fragments (14.5%), and the small fragments had the lowest covers (7.8%).

Discussion

This study was designed to determine the effect of fragment size and substrate on the survival and health of reindeer lichen fragments. Several studies have shown that reindeer lichens can successfully be transplanted through fragments (Roturier and Bergsten 2009; Duncan 2015; Rapai et al. 2017; Roturier et al. 2024), and these transplants can have long-term success (Rapai et al.

2023). Developing knowledge about the performance of different sizes of lichen fragments on different substrates is an important next step in applying this knowledge at larger scales for caribou habitat restoration.

When interpreting the chlorophyll fluorescence data, it is important to note that almost all of the F_v/F_m values of the alive lichen sampled were within a healthy range. The F_v/F_m of most healthy lichens ranges from 0.6 to 0.76, with healthy crustose and cyanolichens having lower values ranging from 0.5–0.6 (Jensen and Kricke 2002; Fernandez-Salegui et al. 2006). Values ranging from 0.2–0.3 indicate irreversible damage to photosynthetic pathways (Angelini et al. 2001; Dzubaj et al. 2008). Within our data, there was a bimodal distribution of F_v/F_m values, with lichen fragments either scoring 0, or else over 0.6, with very few in between. This indicates that within the two-year duration of the study, fragments were either dead or were healthy, with very few in an intermediate stressed condition.

Overall, this study demonstrated that both fragment size and substrate affect lichen transplant success. The survival rates of large-sized fragments and F_v/F_m of *S. tomentosum* were comparable to those of medium-sized fragments, but significantly higher than small-sized fragments (Fig 3.3b and Fig. 3.8). Small-sized fragments also had the lowest F_v/F_m values for C. stellaris compared to both large- and medium-sized fragments, although the effect size was not significant (Table 3.3).

Similarly, Roturier et al. (2007) found that larger (3 cm diameter) transplanted fragments of *C. mitis* resulted in higher percent coverages than smaller (1 cm diameter) fragments in forested environments in Sweden; however, their study showed no significant differences in clear-cut environments, which would more closely correlate with this study.

The effect of substrate on lichen transplants was less consistent, as might be expected given the different ecologies of the species present. Overall, there were no differences in lichen survival between the different substrates (**Table 3.3**), although surviving lichens on the moss substrate had significantly lower F_v/F_m values than those on soil or pine needles (**Fig. 3.5**). It would be expected that the moss substrate would retain the highest amount of moisture versus the other substrates, so these lower health values may be due to the moss substrate not providing as much of the desiccation that these lichens are adapted to as poikilohydric organisms. This may especially be the case as our sites were mostly imperfectly to poorly drained, with subhygic to subhydric moisture regimes (**Table 3.2**). In contrast, terrestrial lichen communities in Alberta's boreal forest are typically dominant on either rapid to well-drained sites with xeric to submesic moisture regimes, or else in *Sphagnum*-rich peatlands that experience frequent surface desiccation (Beckingham and Archibald 1996; Beckingham et al. 1996). In this study, we also observed that lichen fragments in plots or portions of plots that were lower in micro-elevation and subject to pooling appeared to have poor lichen survival, which is consistent with the idea that excessive moisture may have led to poorer lichen health.

Living moss cover was also significantly negatively correlated with living lichen F_v/F_m values (**Fig. 3.9**). In the field, the transferred moss substrate was observed to have remained partially to

fully alive throughout the study, especially in lower, moister areas. Thus, the lower F_v/F_m values both on the moss substrate and with living moss may also be due to competition or other interspecific interactions with the moss. Several studies have shown that reindeer lichen cover is negatively associated with that of feather mosses (Coxson and Marsh 2001; Nelson et al. 2015; Norbert et al. 2020; Cichowski et al. 2022), which is consistent with this pattern. The forb cover was also negatively correlated with lower lichen F_v/F_m values, which could also be due to competition, or that forb cover was higher in moister microsites (or those with other parameters) (**Fig. 3.10**), which also resulted in poorer lichen health.

Other studies investigating the impact of substrate on reindeer lichen transplants have found that lichen cover and fragment retention was significantly higher on moss and bare mineral soil than on twig and bark substrates in a clear-cut site in northern Sweden (Roturier et al. (2007), and that fragments had significantly higher fragment retention on moss and litter substrates than on bare soil at a 12-year-old reforested site in the Boreal Mixedwood Natural Subregion of Alberta (Duncan 2011). Tolpysheva and Timofeeva (2008) showed a significantly higher growth rate of *C. mitis* on soil than rocks or wood in a lichen-pine forest and a bilberry-cowberry-green-moss pine forest. The study design, measured parameters, and ecological systems in these studies are different from the current study, so comparisons are difficult to make. It may be that moss provides a good substrate for lichen fragment retention but may not provide as ideal a substrate for lichen health, depending on site characteristics.

When analyzed by species, *C. uncialis* had significantly higher F_v/F_m values on wood versus on moss (**Fig. 3.6**). In our collection data, *C. uncialis* was only abundant in subxeric to submesic sites under pine, though very small amounts were collected in a subhydric bog site with black spruce (data not shown). This is consistent with *C. uncialis* growing better with the higher, dryer microsites up on the wood pieces, as opposed to on the moister moss. Lechowicz and Adams (1973) found *C. uncialis* to be tolerant of a wide range of environments, from hot, dry, and sunny to cool, damp, and shaded. This is consistent with the fact that, despite the difference, the mean F_v/F_m of surviving *C. uncialis* fragments on all the substrates was still within a healthy range (**Fig. 3.6**), and *C. uncialis* had an overall high survival rate of 97.9% (**Table 3.4**).

Cladonia stellaris followed the overall trend of having the lowest F_v/F_m values on the moss substrate and with small fragments and furthermore demonstrated a strong interaction response (**Table 3.5**), with the small fragments on moss performing the most poorly (**Fig. 3.7**). *C. stellaris* is a slow-dispersing, long-lived, competitive species, with individual thalli estimated to survive over 100 years (Scotter 1963b; Yarranton 1975) and thus may be more sensitive to less ideal dispersal situations. This is also consistent with its moderate overall survival of 80.4% (**Table 3.4**).

Cladonia stygia similarly showed a significant interaction effect of substrate and fragment size (**Table 3.5**) and also had its lowest F_v/F_m results on mosses. This is somewhat unexpected, as *C. stygia* is known to prefer humid, peat bog ecosystems (Ahti and Hyvönen 1985; Oset et al. 2008), and it would seem that feather mosses would be the closest analogue to this of the substrates used. *C. stygia* also had much lower overall survival compared to the other species, with only 40.7% of

collected fragments being alive in 2023, and 65.0% alive in 2024 (**Table 3.4**). It may be that none of the substrates used were very conducive to the growth of this species. It may also be that there was bias in the collection and identification of *C. stygia*. Fragments of this species were most easily recognized in the field by being relatively sparsely branched, and having a black stereome on the interior of lower portions of the thallus. It may be that fragments recognizable as *C. stygia* were more likely to be from lower, less healthy portions of the fragmented thalli that were less likely to be alive. Regardless, given that *C. stygia* is an uncommon and tracked species in Alberta (ACIMS 2022), it is likely not a good candidate for transplantation projects.

It is notable that neither *C. mitis* nor *C. rangiferina* showed any significant effects of fragment size or substrate (**Table 3.5**). These two species were the most abundant in our collections, with *C. mitis* representing 52% of the identified fragments in 2024, and *C. rangiferina* 13%. This bodes well for potential larger-scale lichen transplantation projects, indicating that the most abundant species are also the least sensitive to variations in fragment size and substrate. Microhabitat preferences for *C. mitis* are for warm, moderately open, protected sites, while *C. rangiferina* prefers cool, relatively shaded, mesic sites among vascular vegetation (Lechowicz and Adams 1974). This is consistent with the overall survival of the two species, with the open habitat of the harvested blocks allowing for a higher survival rate of 92.0% for *C. mitis* but a lower rate of 73.0% for *C. rangiferina* (**Table 3.4**).

Stereocaulon tomentosum had the highest survival of all the lichen species, with 98.3% of fragments surviving overall (**Table 3.4**). Limited ecological information about this species could be found, but it is known from mineral soil, rock, and moss substrates (Goward 1999; Brodo et al. 2001), and in our collections was only found on submesic sites under pine and appeared to be associated with historic disturbances. This is the only lichen in this study that contains a nitrogen-fixing cyanobacterial photobiont. *Stereocaulon* species can be an important source of nitrogen in lichen-dominated ecosystems (Crittenden and Kershaw 1979; Larsen 1980), which are typically nutrient-poor. Thus, the good transplantation success of this species may help establish higher-nitrogen soils on restored sites.

The percent coverage data should be interpreted with caution, because the initial percent covers of lichens within the plots were not determined. However, similar quantities of lichens were initially dispersed within each plot. The fact that there were no significant differences between substrates supports the survival data, which also showed no significant differences between substrates. The significant difference between fragment sizes may be due to how effectively the fragments spread out across the plot, how well they survived, how much they grew, or a combination of these factors. The lowest percent cover being produced by the small fragments (**Table 3.6**) does correspond with their lowest overall survival (**Fig 3.3**). This also corresponds with field observations noting that smaller fragments often became partially buried, and many became too brittle to collect and/or recognize. However, the medium fragments having a higher percent cover is the opposite of the larger fragments having the highest survival, although the survival difference was not significant.

It may be that the higher covers seen with the medium fragments are the result of a higher survival than the small fragments, but a wider coverage than the large.

Multiple parameters have been used as indicators of establishment for transplanted lichens, including vigor, photographic areal cover, microscopic growth, and potential photosynthetic activity (Roturier et al. 2007; Duncan 2011; Rapai et al. 2023). In this study, lichen fragment dispersal was not determined; thus, it may be possible that lichen fragments within our plots may have become dispersed outside of the plots.

Overall, this study indicated that small lichen fragments had the lowest survival and produced the lowest coverages. Large lichen fragments had higher survival rates, but medium fragments resulted in the highest covers. The moss substrate, higher living moss cover, and higher forb cover resulted in lower health of the surviving fragments, as indicated by F_v/F_m values (**Fig. 3.5**). However, the mean health values of all surviving lichens were still within a healthy range.

Differences in responses to transplant type and site condition were also seen between species. In particular, the uncommon *C. stygia* generally had a low survival rate under any method and on any substrate and may not be a suitable candidate for transplantation projects.

C. stellaris had moderate survival under any method and on any substrate, and its health was affected by fragment size and substrate, with the small fragments on moss having the poorest health. *C. uncialis* and *S. tomentosum* both had high survival rates, although the health of *C. uncialis* was lower on the moss substrate, and that of *S. tomentosum* was better with larger fragments. The two most common lichen species in the trials, *C. mitis* and *C. rangiferina,* did not demonstrate significant differences in survival or health based on fragment size or substrate, although *C. rangiferina* did have a lower overall survival rate. The overall high survival and health of transplants supports several other studies (Duncan 2015; Rapai et al. 2023; Routier et al. 2024), demonstrating that lichen transplantation may be an effective tool in caribou habitat restoration, and that larger-scale projects have the potential to transplant diverse terrestrial lichen species without being overly complex regarding lichen fragment size and substrate. Additional studies investigating the impact of other microsite characteristics on lichen transplants may also help to increase transplant success.

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Table 3.1 Site information for lichen collection locations. Dates are provided as year and month. Ecosites follow Beckingham and Archibald (1996) and are within the Boreal Mixedwood (BM) Ecological Area. Species genus abbreviations are *Cladonia* (C), *Peltigera* (*P*), and *Stereocaulon* (*S*), and *Cladonia arbuscula* ssp. *mitis* is abbreviated as *C. mitis*.

Plot	Location	Date	Latitude	Longitude	Ecosite	Major species
01-B	Peace River	2021.08	56.16724	-116.808	a.1.1	*C. mitis, P. malacea*
01-C	Peace River	2021.08	56.16724	-116.808	a.1.1	*C. mitis, P. malacea*
01-D	Peace River	2021.08	56.16724	-116.808	a.1.1	*C. mitis, P. malacea, C. rangiferina*
01-E	Peace River	2021.08	56.16724	-116.808	a.1.1	*C. mitis, P. aphthosa, P. malacea*
02-C	Peace River	2021.08	56.16828	-116.811	a.1.2	*C. mitis, C. stellaris*
03-B	Peace River	2021.08	56.6681	-118.062	h.1.1	*C. mitis, C. stellaris*
03-C	Peace River	2021.08	56.6681	-118.062	h.1.1	*P. malacea, C. 82rispate, C. stygia, C. mitis, C. stellaris*
03-D	Peace River	2021.08	56.6681	-118.062	h.1.1	*C. stellaris, C. stygia, C. crispata*
03-E	Peace River	2021.08	56.6681	-118.062	h.1.1	*C. stygia, C. stellaris, C. mitis, P. aphthosa*
04-A	Peace River	2021.08	56.66726	-118.259	k.2.1	*C. mitis, C. stygia*
04-B	Peace River	2021.08	56.66726	-118.259	k.2.1	*C. stellaris, C. stygia, C. mitis*
04-C	Peace River	2021.08	56.66726	-118.259	k.2.1	*C. stygia, C. mitis*
BR1-01	Fox Creek	2022.05	54.33603	-116.253		*C. mitis*
BR1-02	Fox Creek	2022.05	54.336	-116.253		*C. mitis, C. uncialis*
BR1-03	Fox Creek	2022.05	54.33561	-116.253		*C. mitis, C. stygia*
BR1-04	Fox Creek	2022.05	54.3359	-116.253		*C. mitis, C. stellaris*
BR2-01	Fox Creek	2022.05	54.43363	-116.345		*C. mitis, C. rangiferina*
BR2-02	Fox Creek	2022.05	54.43663	-116.345		*P. aphthosa, C. mitis, C. uncialis*
ANPI-01	Fox Creek	2022.05	54.22231	-116.046		*C. mitis, Cetraria islandica*
ANPI-02	Fox Creek	2022.05	54.22234	-116.046		*C. mitis, S. tomentosum*
ANPI-03	Fox Creek	2022.05	54.22252	-116.045		*S. tomentosum, P. aphthosa, C. mitis*
ANP2-01	Fox Creek	2022.05	54.18552	-116.189		*C. mitis, C. uncialis*
ANP2-02	Fox Creek	2022.05	54.18555	-116.189		*C. mitis, C. stellaris, C. uncialis*
ANP2-04	Fox Creek	2022.05	54.18461	-116.188		*C. mitis, C. stygia*
SHI-01	Fox Creek	2022.05	54.60935	-115.378		*C. mitis, P. aphthosa*
SHI-02	Fox Creek	2022.05	54.60906	-115.378		*C. mitis, C. rangiferina*

Table 3.2 Ecological site information summary for transects assessed in 2023. Terminology and procedures follow Beckingham and Archibald (1996) and Soil Classification Working Group (1998).

	ANP (B)	Block 270-Unit A (D)	Block 690-Unit 347 (EM & ES)	Block 690-Unit 2031 (F)	Block 690-Unit 338 (G)
Percent Shrub Cover	60	20	20	30	20
Percent Cover of Mainly Graminoids (HG) or Forbs (HF)	HG 40	HF 80	HF 80	HF 70	HG 80
Slope (%)	0	0	5	2	4
Aspect (degrees)	n/a	n/a	355	130	360
Surface Expression	Undulating	Rolling	Rolling	Undulating	Rolling
Surface Shape	Concave	Concave	Straight	Convex	Straight
Slope Position	Level	Mid-slope	Upper Slope	n/a	Mid-slope
Drainage	Poor	Imperfect	Imperfect	Mod. Well	Very Poor
Moisture Regime	Subhydric	Hygric	Hygric	Subhygric	Subhydric
Nutrient Regime	Medium	Rich	Rich	Poor	Medium
Total Organic Thickness (cm)	15	7	0	12	42
Soil Surface Texture	Sandy Clay Loam	Silty Clay Loam	Silt Loam	Silt Loam	Organic-Mesic
Soil Effective Texture	Sandy Clay Loam	Silty Clay	Silt	Sandy Clay	Organic-Humic
Water Table Depth (cm)	23	>60	>60	>60	>60
Humus Form	Mull	Mull	Mull	Mor	Peatymor
Parent Material	Till	Glaciolacustrine	Glaciolacustrine	Till	Swamp
Soil Type	SWm	SWm	SWm	SM4	SR
Percent Surface Substrate[a]	30 DW, 5 MS, 63 OM[b], 2 W	20 DW, 4 MS, 76 OM	20 DW, 10 MS, 70 OM	40 DW, 2 MS, 58 OM[b]	30 DW, 70 OM

[a]Percent Surface Substrate codes: DW=decaying wood, MS=mineral soil, OM=organic matter, W=water

[b]Surface organic material primarily mulched wood

Table 3.3 Analysis of deviance for the F_v/F_m values and survival of lichen collected from plots with small, medium, and large fragments on soil, moss, pine needles, and woody debris substrates in 2022, 2023, and 2024.

	df	Survival		F_v/F_m	
		Chisq	Pr(>Chisq)	Chisq	Pr(>Chisq)
Substrate	3	2.28	0.517	12.08	0.007**
Size	2	10.41	0.005**	4.02	0.134
Year	2	7.22	0.027*	133.87	< 0.001 ***
Substrate*size	6	1.70	0.945	8.61	0.197
Substrate*year	6	2.56	0.861	5.27	0.510
Size*year	4	6.42	0.170	9.91	0.042*
Substrate*size*year	12	11.94	0.451	17.64	0.127

* Significant at P<0.05, ** significant at P<0.01, and *** significant at P<0.001.

Table 3.4 Percent survival of lichen fragments by species in 2023 and 2024, and with both years combined.

	C. mitis	*C. rangiferina*	*C. stellaris*	*C. stygia*	*C. uncialis*	*S. tomentosum*	Mean
2023	89.1	77.2	78.8	65.0	96.3	100.0	85.1
(n)	(119)	(57)	(52)	(40)	(54)	(34)	(356)
2024	93.5	69.0	82.0	40.7	100.0	96.2	86.1
(n)	(217)	(58)	(50)	(27)	(40)	(26)	(418)
Combined	92.0	73.0	80.4	55.2	97.9	98.3	85.7
(n)	(336)	(115)	(102)	(67)	(94)	(60)	(774)

Table 3.5 Analysis of deviance for the F_v/F_m values of alive lichen fragments of six lichen species collected from plots with small, medium, and large fragments on soil, moss, pine needle, and woody debris substrates in 2023 and 2024.

		2023			2024		
		df	Chisq	Pr(>Chisq)	df	Chisq	Pr(>Chisq)
C. mitis	Substrate	3	1.33	0.722	3	1.4	0.706
	Size	2	2.64	0.267	2	0.92	0.63
	Substrate*size	6	6.09	0.413	6	4.99	0.545
C. rangiferina	Substrate	3	0.89	0.827	3	3.29	0.348
	Size	2	3.04	0.219	2	0.08	0.961
	Substrate*size	6	11.69	0.069	N/A	N/A	N/A
C. uncialis	Substrate	3	0.61	0.895	3	17.33	<0.001***
	Size	2	2.4	0.301	2	3.33	0.189
	Substrate*size	6	5.6	0.47	6	6.9	0.33
C. stellaris	Substrate	3	1.27	0.737	3	35.34	<0.001***
	Size	2	3.66	0.16	2	9.28	0.009**
	Substrate*size	6	21.7	0.001**	6	33.28	<0.001***
C. stygia	Substrate	3	9.24	0.026*	2	2.49	0.288
	Size	2	4.54	0.104	N/A	N/A	N/A
	Substrate*size	6	16.52	0.011*	N/A	N/A	N/A
S. tomentosum	Substrate	3	1.39	0.707	3	0.3	0.961
	Size	2	8.52	0.014*	2	7.12	0.028*

N/A=not available, * Significant at P<0.05, ** significant at P<0.01, and *** significant at P<0.001.

Table 3.6 Analysis of deviance for the F_v/F_m values and survival of lichens collected from plots with small, medium, and large fragments on soil, moss, pine needles, and woody debris substrates in 2024 using the cover of woody, forbs, graminoids, moss, and lichen as covariates.

	df	Survival		F_v/F_m	
		Chisq	Pr(>Chisq)	Chisq	Pr(>Chisq)
Substrate	3	6.10	0.107	5.98	0.113
Size	2	4.24	0.120	1.82	0.402
Woody plants	1	0.98	0.322	0.16	0.690
Forbs	1	0.53	0.467	4.27	0.039*
Graminoids	1	3.70	0.055	3.03	0.082
Mosses	1	1.39	0.239	12.65	<0.001***
Lichens	1	0.00	0.990	0.86	0.354
Substrate*size	6	1.90	0.929	10.39	0.109

* * Significant at P<0.05 and *** significant at P<0.001.

Table 3.7 Mean percent covers of lichens from plots with small, medium, and large fragments on soil, moss, pine needles, and woody debris substrates in 2024. Standard deviations are presented in brackets.

	Soil	Moss	Pine	Wood	Total***
Large	14.0 (9.8)	12.0 (7.5)	16.5 (7.3)	15.4 (8.1)	14.5 (8.1)
Medium	19.2 (14.9)	26.3 (13.9)	20.1 (15.9)	22.0 (18.3)	21.9 (15.5)
Small	6.2 (6.3)	11.5 (6.4)	5.1 (3.7)	8.5 (3.5)	7.8 (5.6)
Total	13.1 (11.9)	16.6 (11.8)	13.9 (11.9)	15.3 (12.6)	

*** significant at P<0.001.

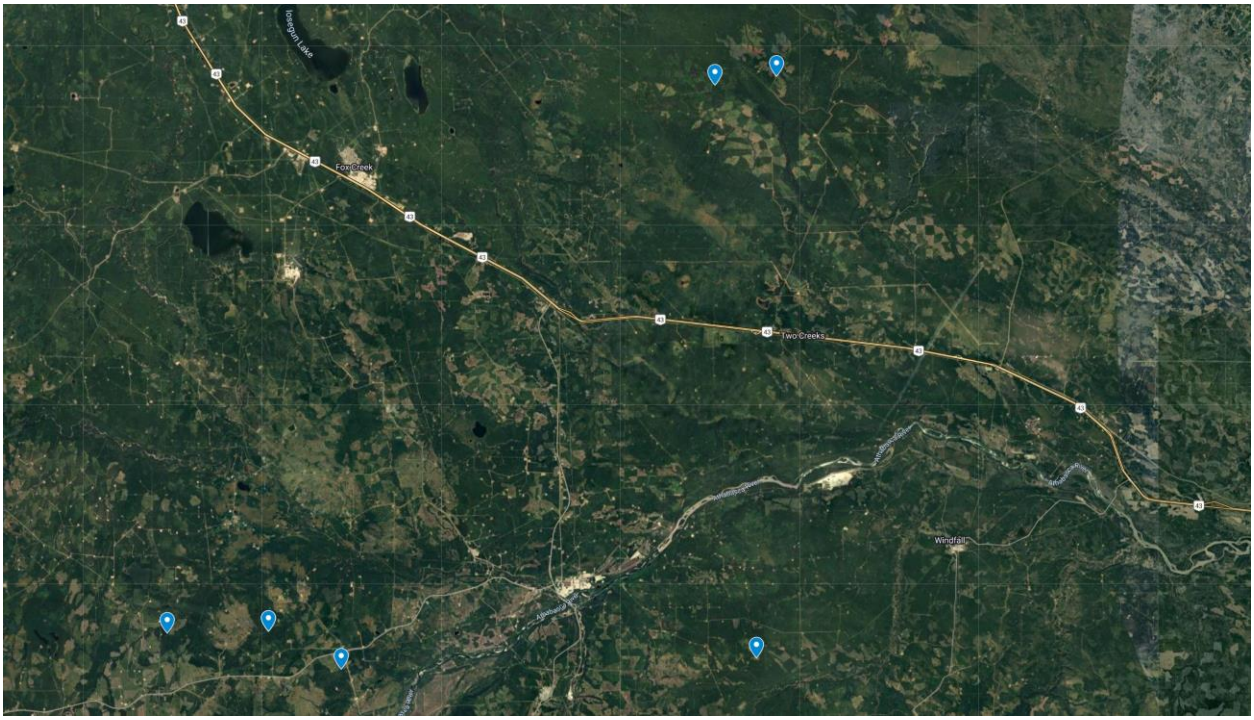

Fig. 3.1 Lichen transplant trial sites near Fox Creek, AB

Fig. 3.2 Study plots showing substrates of **(a)** soil, **(b)** moss, **(c)** pine needles, and **(d)** woody debris.

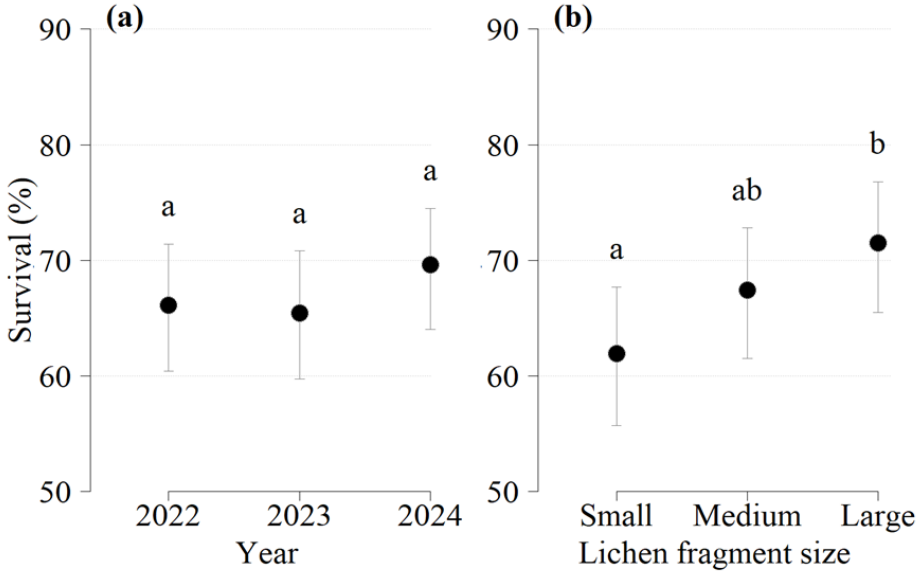

Fig. 3.3 (a) Survival (%) of lichen collected in 2022, 2023, and 2024 across soil, moss, pine needles, and woody debris substrates with small, medium, and large fragments. **(b)** Survival (%) of lichen collected from plots with small, medium, and large fragments across soil, moss, pine needles, and woody debris substrates in 2022, 2023, and 2024. The dots indicate the means, the horizontal bars indicate the horizontal bars indicate asymptotic lower and higher confidence limit. Treatments with different letters are significantly different at $P<0.05$

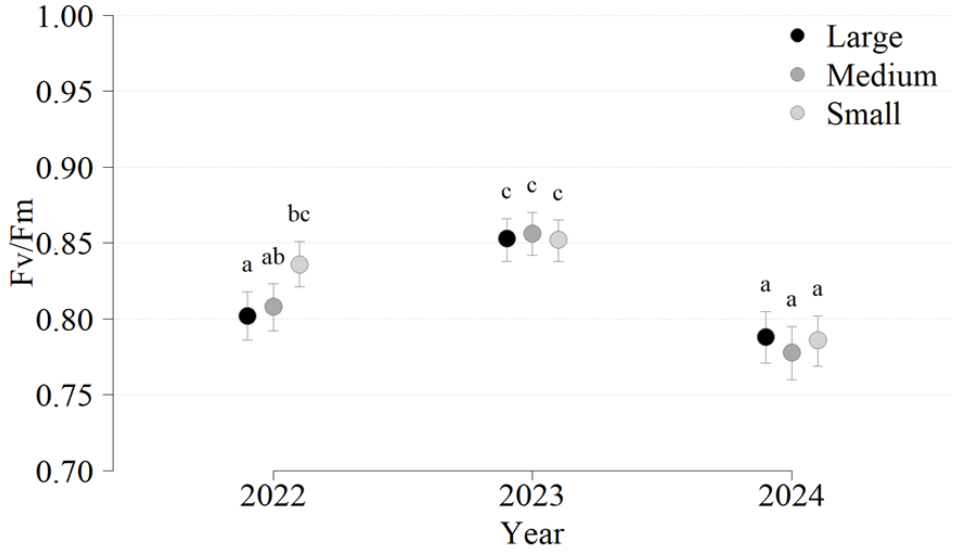

Fig. 3.4 Mean F_v/F_m values of lichen collected from plots with small, medium, and large fragments in 2022, 2023, and 2024 across soil, moss, pine needles, and woody debris substrates. The dots indicate the means, the horizontal bars indicate the horizontal bars indicate asymptotic lower and higher confidence limit. Treatments with different letters are significantly different at $P<0.05$.

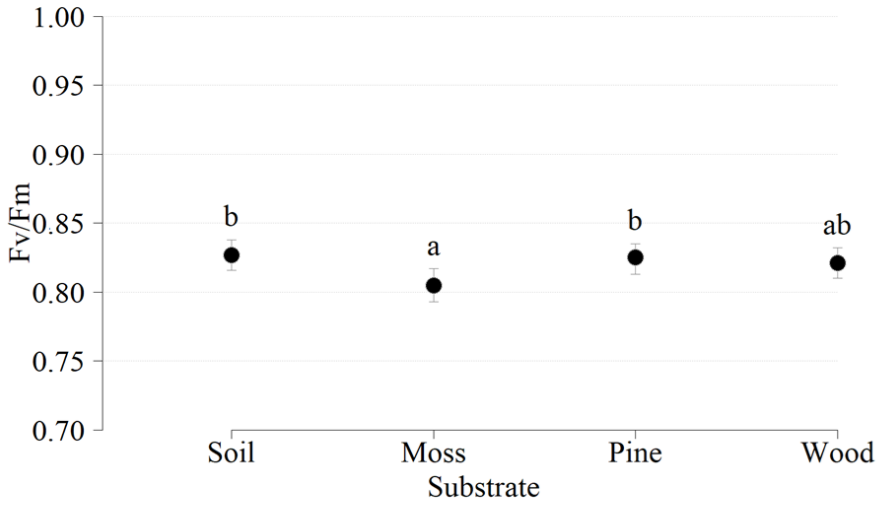

Fig. 3.5 Mean F_v/F_m values of lichen collected from plots on soil, moss, pine needles, and woody debris substrates across small, medium, and large fragments in 2022, 2023, and 2024. The dots indicate the means, the horizontal bars indicate the horizontal bars indicate asymptotic lower and higher confidence limit. Treatments with different letters are significantly different at $P<0.05$.

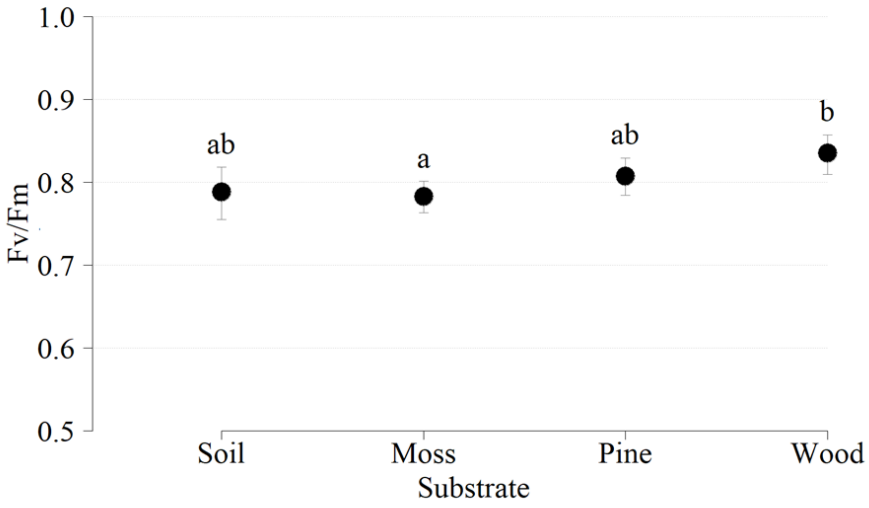

Fig. 3.6 Mean F_v/F_m values of *C. uncialis* collected from plots on soil, moss, pine needles, and woody debris substrates across small, medium, and large fragments in 2024. The dots indicate the means, the horizontal bars indicate the horizontal bars indicate asymptotic lower and higher confidence limit. Treatments with different letters are significantly different at P<0.05.

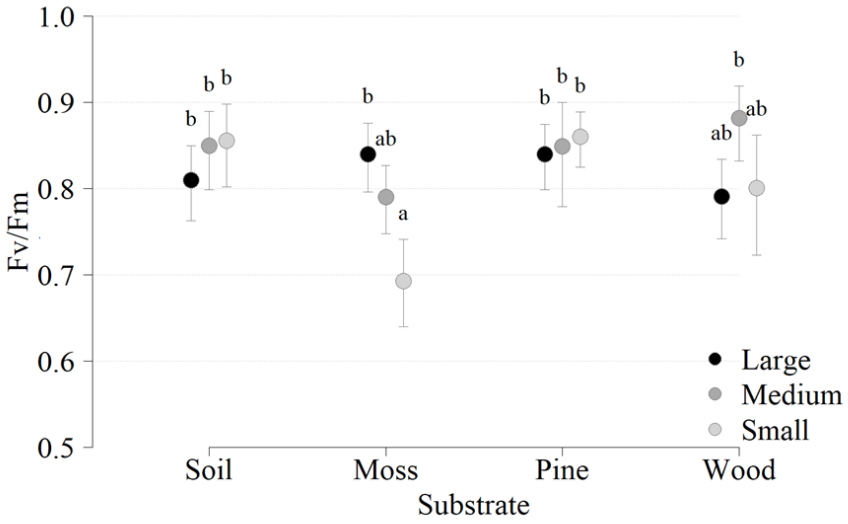

Fig. 3.7 Mean F_v/F_m values of *C. stellaris* collected from plots with small, medium, and large fragments on soil, moss, pine needles, and woody debris substrates in 2024. The dots indicate the means, the horizontal bars indicate the horizontal bars indicate asymptotic lower and higher confidence limit. Treatments with different letters are significantly different at P<0.05.

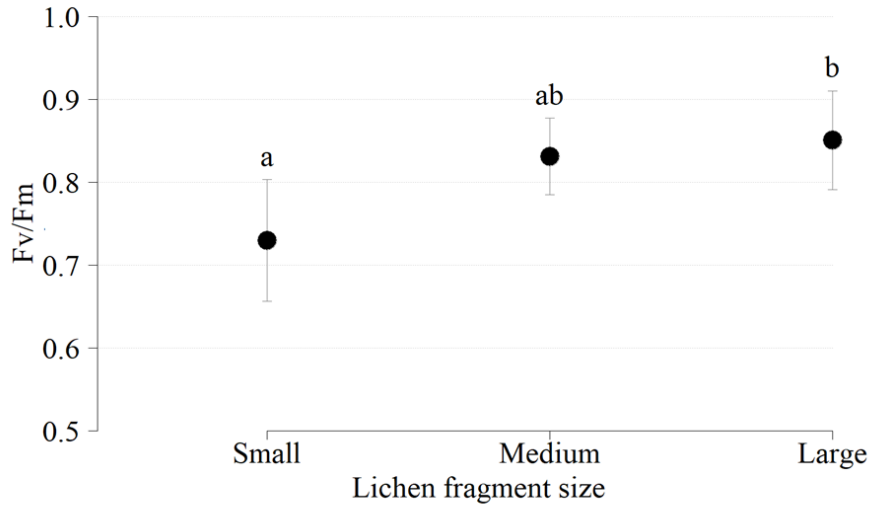

Fig. 3.8 Mean F_v/F_m values of *S. tomentosum* collected from plots with small, medium, and large fragments across soil, moss, pine needles, and woody debris substrates in 2023. The dots indicate the means, the horizontal bars indicate the horizontal bars indicate asymptotic lower and higher confidence limit. Treatments with different letters are significantly different at $P<0.05$.

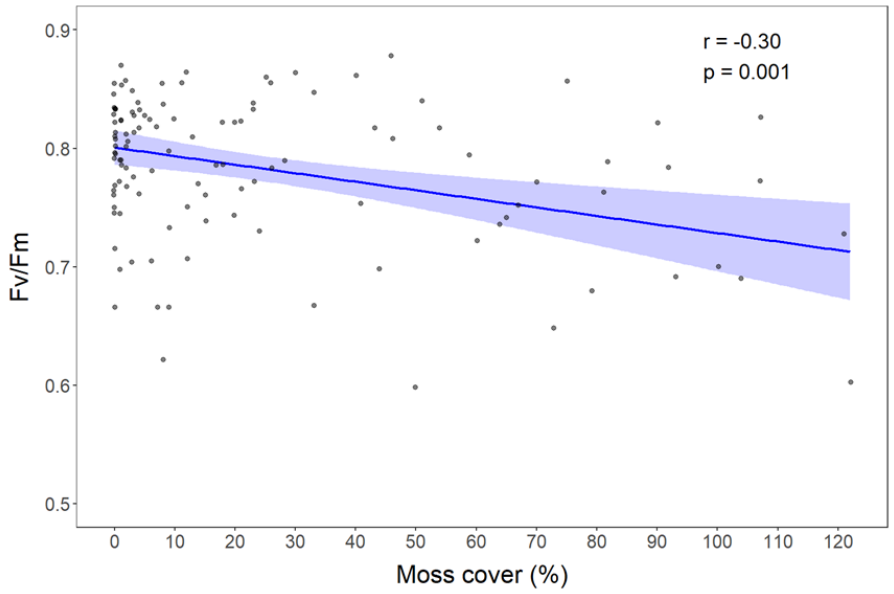

Fig. 3.9 Scatter plot between moss cover (%) and the F_v/F_m values of lichen collected from plots with small, medium, and large fragments on soil, moss, pine needles, and woody debris substrates in 2024. r=correlation coefficient, p=probability.

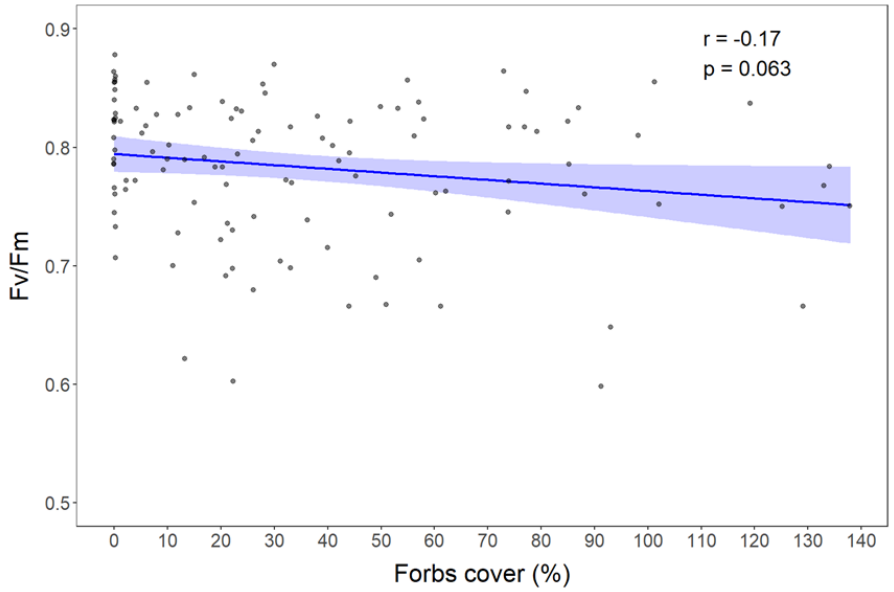

Fig. 3.10 Scatter plot between forbs cover (%) and the F_v/F_m values of lichen collected from plots with small, medium, and large fragments on soil, moss, pine needles, and woody debris substrates in 2024. r=correlation coefficient, p=probability.

Application of Hydroseeding to Deploy Terrestrial Lichens

Abstract

Lichens are essential components of ecosystems, particularly for woodland caribou, which rely on them as a food source during harsh winter months when other vegetation is buried under deep snow or otherwise unavailable. According to Heggbertget et al. (2002), lichens can make up more than 80% of a caribou's winter diet. Restoration of these lichen communities, especially reindeer lichens, is significant for caribou conservation, but challenges exist in terms of propagation and dispersal. The study explored the feasibility of hydroseeding as a potential method to disperse and adhere lichen to exposed substrates in field settings. Specifically, the study assessed the density, survival, and health of lichen fragments applied with and without tackifiers in four harvested blocks with various site preparations. The study found that hydroseeding effectively distributed lichen material, but survival rates were generally low. The plots treated with tackifiers retained a significantly higher number of lichen fragments than those sprayed without tackifiers in 2023 but not in 2024. However, the use of tackifiers did not improve lichen survival or growth compared to treatments without tackifiers in the following two years after the application.

Introduction

Lichens cover approximately 8% of the Earth's land surface and play an important role in terrestrial ecosystems (Asplund and Wardle 2017). However, little is known about the effective restoration of these communities after disturbance. Terrestrial lichens make up 60-83% of the winter diet of the threatened woodland caribou (Thomas et al. 1996, Heggberget et al., 2002), which is considered threatened under Canada's federal Species at Risk Act (SARA, 2002). These are primarily composed of species of *Cladonia*, particularly *C. arbuscula*, *C. rangiferina*, *C. stellaris*, *C. stygia* and *C. uncialis*, herein referred to as "reindeer lichens". The restoration of reindeer lichen communities has the potential to become a significant component of management practices for caribou conservation (Thomas et al. 1996; Duncan 2015) but has yet to achieve practical implementation. This is partly due to a lack of information regarding how to propagate and disperse reindeer lichens cost-effectively.

Lichens have several modes of reproduction that may be sexual or asexual (Brodo et al. 2001). Reindeer lichens mainly reproduce asexually via fragmentation of the thallus, which contains both mycobiont and photobiont cells (Kiss 1985; Honegger 1996; De Santis 1999; Duncan 2011; Roturier et al. 2017). Wind, rain, and animals serve as significant media for the dispersal of thallus fragments (Heinken 1999; Duncan 2011; Ronalds and Grant 2018). In open habitats, thallus fragments contribute to effective dispersal over short distances but not over long distances (Heinken 1999). By imitating the natural dispersal of thallus fragments, the artificial dispersal of reindeer lichens may accelerate and promote their re-establishment (Heinken,1999; Liden et al. 2004; Ballesteros et al. 2017).

In practice, lichens can be transplanted as fragments or as entire mats (Duncan 2015; Rapai et al. 2017; Ronalds and Grant 2018). Compared to the transplantation of mats, transplanting fragments requires fewer materials, likely causes less impact to source populations (Roturier et al. 2017), and is less time-consuming to perform. A variety of methods have been used to distribute lichen fragments over larger areas, including spreading them by hand, leaf blower, helicopter, or hydro-mulcher (Enns 1998; Krekula 2007 in Duncan 2015; Rapai et al. 2017; Roturier et al. 2017; Ronalds and Grant 2018). Roturier et al. (2017) estimated the cost of hand application at $650-$3,250 USD/ha depending on the lichen density used, but this process is very labour-intensive and time-consuming. Ronalds and Grant (2018) estimated a cost of $125,000 CAD/ha for aerial application by plane, which was much faster but very expensive.

Hydroseeding, also known as hydraulic seeding, is an effective and efficient method for establishing vegetation and preventing soil erosion, particularly on challenging terrain (Schiechtl 1980; Simcock and Ross 1995; Gudyniene et al. 2021). Developed in the United States and Europe during the 1950s, this method involves spraying a special slurry that typically contains seeds, soil-adhesive tackifiers, and sometimes additional elements, such as fertilizers, to promote rapid plant growth and immediate soil stabilization (Schiechtl 1980; Simcock and Ross 1995; Gudyniene et al. 2021). Tackifiers, a vital component, are commonly used in hydroseeding and hydromulching for various purposes, including landscaping, restoring areas after wildfires, and stabilizing unstable slopes (Robichaud et al. 2010; Blankenship et al. 2020). While previous studies have examined the influence of tackifiers on the development and recovery of communities comprising lichens, mosses, fungi, and algae (Park et al. 2017; Chandler et al. 2019; Blankenship et al. 2020), and have demonstrated their effectiveness in restoring dryland mosses, a significant gap remains in understanding their impact on the survival and growth of reindeer lichen (Blankenship et al. 2020). Although the estimated industrial cost ranges from $8,500 to $10,000 per hectare (A. Bertchi, personal communication, February 17, 2024), hydroseeding offers a swift application process that can be customized depending on the available equipment and site access. Ultimately, hydroseeding is a powerful tool for environmental restoration and erosion control; however, more research is needed into its specific ecological impacts on sensitive organisms.

In this study, we examined hydroseeding, with and without tackifiers, as an alternative method for dispersing and adhering lichen to exposed substrates in a field setting. The project had two objectives: 1) to examine the feasibility of hydroseeding lichens to restore lichen in harvested forest areas, which could be an important component of habitat recovery for woodland caribou, and 2) to assess the viability and growth of hydroseeded lichens across different forestry treatments, with or without tackifier.

Materials and Methods

Lichen and substrate collection

Lichen samples were collected from bog, swamp, and pine forests around Peace River and Lac La Biche in 2021 and Fox Creek in 2022. Collection occurred by laying out 1 × 1 m quadrats in high-lichen density sites, selected to represent the diversity of lichen species present in each area. At the time of collection, the percent cover of each species (including vascular plants, bryophytes, and terrestrial lichens) in each plot was assessed, and ecological site information such as soil types, moisture and nutrient regimes, and ecological site classifications were determined. All lichen material within each plot was collected by hand, placed in paper bags, and allowed to air dry completely. They were then stored in paper bags at room temperature in the dark at the Centre for Boreal Research (CBR) in Peace River.

Experimental field trials

The study was set up on June 7, 2023, at eight sites within three conifer forest harvested areas that had been subjected to differing forestry treatments. This included a site that had been mounded (Block 690-Unit 347; Site EM), treated with a RipPlow (Block 690-Unit 338; Site GRP), burned (Block 690-Unit 338; Sites GB), and untreated (Block 690-Unit 2031; Site F). Ecological site information was collected from each site in August and September 2023 and is summarized in **Table 4.1**. At each site, two 10 m by 10 m plots were created and subdivided into four 5 m by 5 m subplots. In each plot, two subplots were sprayed with lichen and water mix only (LW), and two were sprayed with a mix that also included tackifier (LWT). Subplots with the same mixtures were always placed diagonally across from one another.

Clean water was brought on-site using a water truck and was used for all operations. The equipment used was a Turfmaker 800 hydroseeder with a 3 m^3 tank, and a 23 m long, 5 cm diameter hose with a fan nozzle (**Fig. 4.1 a**). The hydroseeder was filled with 2 m^3 of water, to which was added approximately 0.025 m^3 of dried lichen material. After allowing the material to mix, the mixture was evenly sprayed across the LW subplots, using approximately 0.1 m^3 of LW per subplot. An assistant moved a 2.4 m wide piece of corrugated aluminum sheeting around the perimeter of the plot as the hydroseeding progressed, to prevent material from being sprayed outside the subplots.

For the applications including tackifier, approximately two-thirds of a 22.7 kg bag of Flexterra® HP-FGM (High Performance-Flexible Medium) were added per 1 m^3 of LW solution. The resulting LWT solution was applied in the same manner as the LW solution. A second batch with approximately 1.2 m^3 water was required to complete all eight plots, and this was performed using the same ratios of lichens, water, and tackifier as required. After using the tackifier, the hydroseeder and hose were thoroughly rinsed before re-adding water for the LW plots. All of the plots were completed on the same day.

Field data collection

Baseline data was collected on the day of application, but only from the LW plots because the tackifier made it impossible to see the lichen fragments, and we did not want to disturb the drying tackifier by walking on it. To estimate lichen density, a 10 x 10 cm quadrat was thrown at random around each subplot, and all fruticose lichen fragments 5 mm or more in widest dimension that were at least half within the quadrat were counted (**Fig. 4.1 b**). This was repeated ten times per subplot.

At this time, we also collected one fragment from every other quadrat (i.e. five per subplot) to analyze using chlorophyll fluorescence. The fruticose lichen fragment that was at least 1 cm in widest dimension that was closest to the center of the quadrat was collected, to ensure that the fragments were large enough to perform the analysis with. If there were no sufficiently large fragments within the quadrat, the fragment of sufficient size that was closest to the quadrat was collected. Collected lichens were individually placed in labelled coin envelopes and allowed to air-dry completely before being sent for analysis.

In August/September 2023 (hereafter referred to as "September") and July/August 2024 (hereafter referred to as "August"), this procedure was repeated, and this time performed in the subplots with tackifier as well. By this time, the tackifier was sufficiently degraded that it was noticeably present only in occasional small patches, mostly in grassy areas and in deeper crevices in woody material.

Chlorophyll fluorescence analysis

All collected fragments were measured for chlorophyll fluorescence. Measurements were conducted by the University of Northern British Columbia, Coxon Research Group. All thalli were preconditioned by spraying them with de-ionized water until they fully hydrated. The samples were kept in a container under Saran wrap sitting on a damp paper towel in the light at moderate illumination of 200 µmol m^{-2} s^{-1} and temperature of 15 °C for 24 h. Immediately after the preconditioning, the F_v/F_m was recorded with a pulse-modulated chlorophyll fluorescence unit (Hansatech, Norfolk, United Kingdom) with a 6 mm measurement disc after a 5-min period of dark adaptation following the methods of Gauslaa et al. (2012).

Statistical analysis

For the data collected from LW plots in June and September 2023, a two-sample t-test was used to compare the mean number of lichen fragments counted in a 10 x 10 cm quadrat for a subplot in each site. To determine survival of lichen fragments collected from each subplot, the number of samples with F_v/F_m values over 0 was divided by the total number of samples with available F_v/F_m values. A two-sample t-test was used to compare the survival of lichen fragments collected from each subplot in each site. Due to the limited number of lichen samples with F_v/F_m values over 0, the mean of all F_v/F_m values over 0 within each site was used to conduct the two-sample t-test.

For the data collected from LW and LWT plots in September 2023 and August 2024, the mean number of lichen fragments counted in a 10 x 10 cm quadrat for a subplot was used, the mean

F_v/F_m of alive lichen fragments, and the survival of lichen fragments collected from each subplot was calculated as described above. Differences in the mean number, mean F_v/F_m, and survival among treatments and years were analyzed using the "glmmTMB" package in R. Treatment and year serve as fixed factors. Subplot nesting in plot and plot nesting in site serve as random factors. Model assumptions were checked with a histogram of residuals and diagnostic plots of fitted and residual values. Pairwise comparisons between treatments were conducted using Tukey's HSD method with the R package "emmeans" (Lenth et al. 2021). Letter codes indicating significant differences in groupings for the pairwise tests were assigned using the "cld" function from the R package "multicomp" (Bretz et al. 2011).

Results

Hydroseeding procedure overview

The hydroseeding procedure worked remarkably well to evenly distribute the lichen material, both with and without added tackifier. Even though the lichens had not been significantly fragmented before being placed in the hydroseeder, they did not clog the machine or nozzle, although the nozzle did become clogged twice during the treatments by black spruce (*Picea mariana*) cones. To get a rough estimate of the resulting fragment sizes, after applying the LW to the first plot, a 10 x 10 cm quadrat was placed in a representative area, and all of the lichen fragments were counted and measured to the nearest millimeter in the widest dimension. The 80 fragments measured were 4.8 ± 3.1 mm (SD), ranging from 2-33 mm. Although subsequent measurements were not performed, it was observed that the lichen fragments appeared smaller as the lichen mixture was in the hydroseeder for longer, likely due to the additional saturation and agitation they received.

Lichen performance after the first season and by forestry treatment

After the first three-month growing season, the fragment density in the LW plots was reduced from a mean of 9.02 fragments/10 cm^2 to a mean of 4.70 fragments/10 cm^2 for the burned treatment, 8.47 to 2.95 for the mounded treatment, 9.10 to 4.15 for the RipPlow treatment, and 6.80 to 4.45 for the untreated site (**Table 4.2**). The mean number of lichen fragments in September was significantly lower than in June for the mounded and RipPlow treatments but not for burned and untreated sites. The mean survival of collected lichen fragments in September was significantly lower than in June for all forestry treatments (**Table 4.3**); an average decline of 72%. Of the living lichen fragments, there was no significant difference in the mean F_v/F_m between June and September 2023 (**Fig. 4.2**). The mean F_v/F_m of alive lichens collected in both June and September was over 0.6.

Lichen performance after the second season and with or without tackifiers

The lichen fragment densities were significantly different by forestry treatment, year, and their interaction when the September 2023 and August 2024 data were compared (**Table 4.4**). The mean LWT density declined significantly between September 2023 and August 2024, from 8.13

fragments/10 cm^2 to 3.77 (**Fig 4.3**). The LW density in September (4.06 fragments/10 cm^2) was significantly lower than that of the LWT but had not significantly changed by August 2024 (2.88 fragments/10 cm^2), at which time there was no significant difference between the LWT and LW plots. However, the survival of lichen fragments was not significantly different between the two treatments over both years (**Table 4.4** and **Fig. 4.4**): all treaments had survival lower than 25%. There was also no significant difference in the mean F_v/F_m of alive lichens between the treatments LW and LWT over both years (**Table 4.4** and **Fig. 4.5**). The mean F_v/F_m of alive lichens collected in LW and LWT was over 0.65. Further statistical analyses could not be performed among forestry treatments due to insufficient replication.

Discussion

This field trial demonstrated that lichen fragments can be successfully dispersed using hydroseeding treatments. The application ran smoothly except for the two occurrences of clogging due to black spruce cones, but this could be easily remedied by a more careful sorting of collected lichen material. The hydroseeding process is well-understood and already applied at commercial and industrial scales and thus has the potential for broader use in caribou habitat restoration. Its application and cost-effectiveness would depend on the availability of suitable water supplies. While most hydroseeding equipment, such as that used in this trial, requires that roads or other flat grounds be in somewhat proximity, other hydroseeding equipment exists that is capable of traversing rougher ground as well.

The survival and growth of the hydroseed fragments of the lichens, however, was not as ideal. There was a significant decline in lichen density in the LW subplots after the first three-month assessment period, indicating that in this time, approximately half of the lichen fragments either died and were no longer recognizable, or were dispersed elsewhere (**Table 4.2**). The 72% decline in the percentage of live lichen fragments within the LW subplots during this period supports the idea that mortality may have been a significant contributing factor. Combined, this means that only about 14% of the lichen fragments initially sprayed in the LW subplots were still present and alive. However, the remaining alive lichens appeared to be healthy and relatively unstressed, with a mean F_v/F_m of 0.72, which was not significantly different than the initial F_v/F_m values of the living lichen fragments assessed in June 2023 (0.63; **Fig. 4.2**). This is supported by the results of the August 2024 assessment, where the was no significant change in the density, survival, or health of lichen fragments as compared to the September 2023 treatments.

The effect of tackifier on the lichens appeared to be significant but temporary. The subplots with tackifier had significantly more lichen fragments than those without in September 2023, but this had declined so that there was no significant density difference by August 2024 (**Fig. 4.3**). Because we were not able to assess the lichens within the tackifier plots initially in June 2023, it is also possible that they had more fragments to begin with, despite our attempts to maintain consistency. However, the equalization seen by August 2024 between the LW and LWT treatments makes this seem unlikely. There was also no significant difference between the survival or health of the

lichens with or without tackifier between the years (**Figs. 4.4** and **4.5**). Overall, this indicates that the tackifier initially helped the lichens to maintain a higher density of healthy fragments, but as the tackifier dissipated, this benefit was lost. However, it is also important to note that the tackifier had no significant negative effect on the fragments either. The initial density decline we observed could have been due to mortality or dispersal out of the plot.

Mortality of lichen fragments may have been caused by the length of time lichens were submerged within the hydroseeder, physical damage caused by the agitators and the fragments travelling through the hose and nozzle and impacting the ground, or other factors. The lichens were submerged for a longer period than would be typical for hydroseeding due to the time it took to set up and record information at each plot, move the machine between plot locations, and engage in discussions between the experimenters, hydroseeding operator, and water truck operator. Because it was also observed that the lichen fragments seemed to become smaller as the mixture sat longer in the tank, it is possible that minimizing the time the lichens spend in the tank would result in higher survival. Physical damage to the lichens could also potentially be reduced by reducing the pressure within the machine, and/or by spraying at a less direct angle. To keep the mixtures within the marked subplots, a fairly direct angle spray was used in our treatments.

Dispersal of lichen fragments is another potential contributor to the decline in the number of fragments within the first three-month period. This may have occurred due to wind, water, animals, or other mechanisms (Heinken 1999; Duncan 2011; Ronalds and Grant 2018).

The potential effect of tackifier was unknown in this study, as tackifiers have not yet been utilized with fruticose lichens to our knowledge. Blankenship et al. (2020) examined the effect of three common tackifiers (guar, psyllium, and polyacrylamide (PAM)) on the growth of two dryland mosses. They found that psyllium increased the growth of mosses grown in growth chambers compared to distilled water, guar, or PAM.

The effect of forestry treatments could not be statistically analyzed in this study, but our data indicate that lichen survival within the forestry treatments was highly variable (**Table 4.3**). The lowest mean survival as of September 2023 was in the RipPlow treatment, followed by the untreated site, the mounded treatment, and the burned treatment. However, the high variability of this data suggests that other site variables may play a more significant role.

Overall, this preliminary field trial indicates that hydroseeding has the potential to be used as a fast, cost-effective method for reindeer lichen dispersal. However, due to the relatively high proportion of lichen fragments that died and/or were dispersed in our trial, it is recommended that further study be done to optimize lichen survival during the process. Our observations lead us to suspect that survival could improve if the time lichens spend within the hydroseeding tank is minimized, lower pressures are used, and a less direct angle of spray is utilized. The tackifier used in this study appeared to have an initially positive effect on lichen retention, but this effect was not significant after the next growing season. The potential effect of other tackifier materials, fertilizers, or potentially other additives warrants investigation as well.

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Table 4.1 Ecological site information summary. Terminology and procedures follow Beckingham et al. (1996) and Soil Classification Working Group (1998).

	Block 690-Unit 347 (EM)	Block 690-Unit 2031 (F)	Block 690-Unit 338 (GB1 & GRP)	Block 690-Unit 338 (GB2)
Percent Shrub Cover	20	30	30	20
Percent Cover of Mainly Graminoids (HG) or Forbs (HF)	HF 80	HF 70	HG 70	HG 80
Slope (%)	5	2	2	4
Aspect (degrees)	355	130	320	360
Surface Expression	Rolling	Undulating	Rolling	Rolling
Surface Shape	Straight	Convex	Straight	Straight
Slope Position	Upper Slope	N/A	Mid-slope	Mid-slope
Drainage	Imperfect	Mod. Well	Poor	Very Poor
Moisture Regime	Hygric	Subhygric	Subhydric	Subhydric
Nutrient Regime	Rich	Poor	Medium	Medium
Total Organic Thickness (cm)	0	12	14	42
Soil Surface Texture	Silt Loam	Silt Loam	Sandy Clay Loam	Organic-Mesic
Soil Effective Texture	Silt	Sandy Clay	Sandy Clay Loam	Organic-Humic
Water Table Depth (cm)	>60	>60	18	>60
Humus Form	Mull	Mor	Raw Moder	Peatymor
Parent Material	Glaciolacustrine	Till	Till	Swamp
Soil Type	SWm	SM4	SWm	SR
Percent Surface Substrate[a]	20 DW, 10 MS, 70 OM	40 DW, 2 MS, 58 OM[b]	15 DW, 5 MS, 80 OM[b]	30 DW, 70 OM

[a]Percent Surface Substrate Codes: DW=Decaying Wood, MS=Mineral Soil, OM=Organic Matter, W=Water

[b]Surface organic material primarily mulched wood

Table 4.2 Mean number of lichen fragments counted in 10 cm^2 quadrats within lichen and water only (LW) plots in June and September 2023.

	23-Jun	23-Sep	*P* value[a]
Burned	9.02 ± 2.90	4.70 ± 1.80	0.052
Untreated	6.80 ± 1.04	4.45 ± 3.45	0.271
Mounded	8.47 ± 1.18	2.95 ± 1.03	0.000
RipPlow	9.10 ± 1.80	4.15 ± 0.61	0.008

Values: mean ± SD.

[a]*P* value of the two-sample t-test between groups of 23-Jun and 23-Sep.

Table 4.3 Survival (%) of lichen fragments collected from plots with lichen and water only (LW) in June and September 2023.

	23-Jun	23-Sep	*P* value[a]
Burned	100.00 ± 0.00	41.25 ± 26.58	0.021
Untreated	90.00 ± 20.00	20.00 ± 23.09	0.004
Mounded	70.00 ± 25.82	25.00 ± 19.15	0.034
RipPlow	85.00 ± 10.00	10.00 ± 20.00	0.002

Values: mean ± SD.

[a]*P* value of the two-sample t-test between groups of 23-Jun and 23-Sep.

Table 4.4 Analysis of deviance for mean number of lichen fragments counted in 10 cm^2 quadrats, mean F_v/F_m values, and percentage of alive lichen fragments collected from plots with lichen and water only (LW) and those with a mix that included tackifier (LWT) with water in September 2023 and August 2024.

	df	Mean number		Mean F_v/F_m		Survival	
		Chisq	Pr(>Chisq)	Chisq	Pr(>Chisq)	Chisq	Pr(>Chisq)
Treatment	1	27.99	<.001***	2.88	0.090	0.61	0.433
Year	1	34.86	<.001***	0.22	0.640	1.81	0.178
Treatment*year	1	11.46	<.001***	0.02	0.900	0.24	0.624

***significant at P<0.001

Fig. 4.1 (a) Applying a mix of lichen, water, and tackifier using a hydroseeder. **(b)** Data collection using a 10 x 10 cm quadrat in hydroseeding trials.

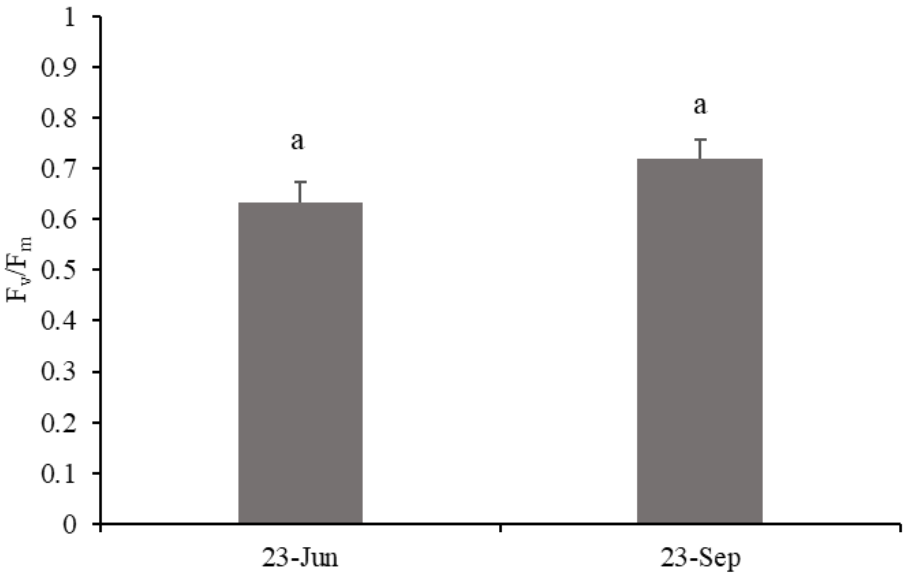


Fig. 4.2 Mean F_v/F_m of alive lichen fragments collected from plots with lichen and water only (LW) in June and September 2023. Treatments with the same letters are not significantly different at $P<0.05$. The horizontal bars indicate ± SD.

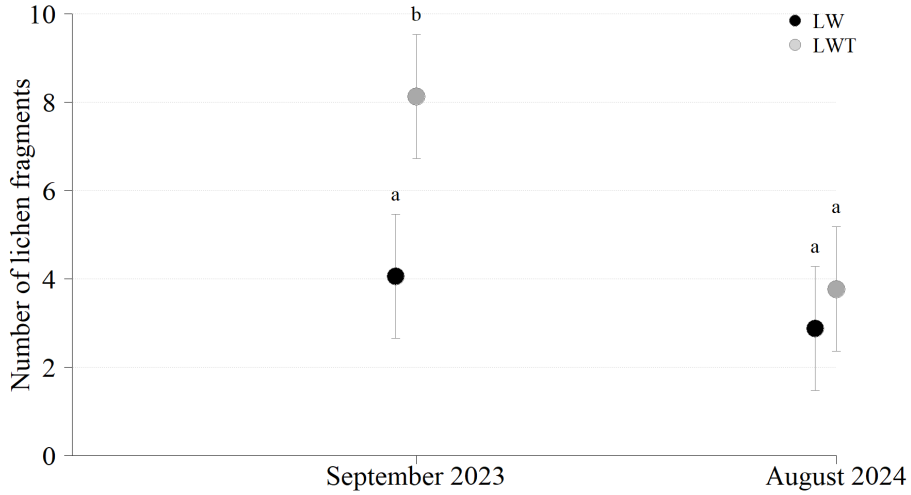
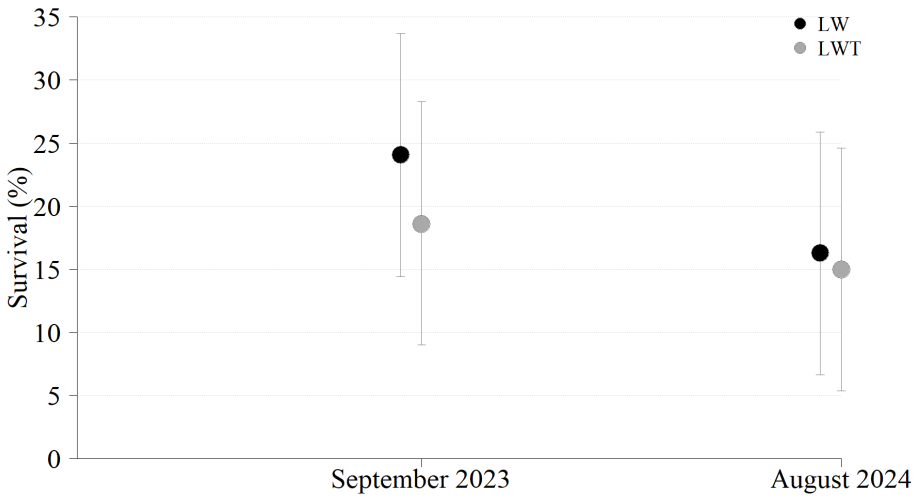


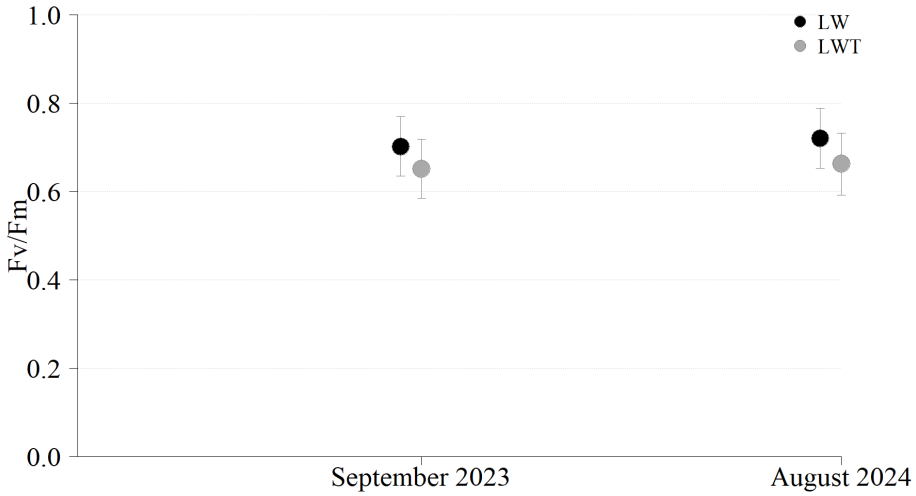
Fig. 4.3 Mean number of lichen fragments counted per 10 cm^2 quadrat within plots treated with lichen and water only (LW) and those with a mix that included tackifier (LWT) in September 2023 and August 2024. Treatments with different letters are significantly different at $P<0.05$. The horizontal bars indicate ± SD.



436

437 **Fig. 4.4** Survival (%) of lichen fragments collected within plots treated with lichen and water only
438 (LW) and those with a mix that included tackifier (LWT) in September 2023 and August 2024.
439 Treatments with the same letters are not significantly different at P<0.05. The horizontal bars
440 indicate ± SD.

441



442

443 **Fig. 4.5** Mean F_v/F_m of alive lichen fragments collected within plots treated with lichen and water
444 only (LW) and those with a mix that included tackifier (LWT) in September 2023 and August
445 2024. Treatments with the same letters are not significantly different at P<0.05. The horizontal
446 bars indicate ± SD.