Bryophyte and lichen communities in boreal forests are maintained through the dispersal and establishment of asexual propagules

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A Thesis

Submitted to the Faculty of Graduate Studies of

The University of Manitoba

in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

Bryophyte and lichen communities in boreal forests are partly shaped by the dispersal and establishment of propagules (meiotic spores and asexual propagules), yet it is not well known how asexual propagules shape these communities. This thesis examined some of the roles of asexual propagules in shaping the extant cryptogam community through propagule trapping and association of asexual propagules (number, size, and type) within different tree-dominated forest stands and environmental conditions (Chapter 2, 3), the characterization of wind dispersal-deposition patterns of select epiphytic and ground-dwelling mosses and lichens (Chapter 4), the assessment of abundance and richness of local bryophyte and lichen communities and that of the soil bank environmental DNA (eDNA) (Chapter 5), and the exposure of bryophytes to different temperature treatments to understand establishment outcomes (Chapter 6). Asexual propagules of small size were dispersed throughout the year, but lichen thallus fragments were dispersed in higher numbers than other propagules—particularly, during winter. Asexual propagules were deposited in higher numbers in coniferous forest stands and on trees, which are linked to their bryophyte and lichen communities having higher abundance and/or richness when compared to the communities on hardwood stands or the forest floor substrata. Bryophyte and lichen communities on trees having a higher dispersal capability than those on the forest floor may allow for their communities to be maintained and to be formed at farther distances. The dispersal capacity of bryophytes and lichens on the forest floor may be limited to maintaining the local community and its soil bank. Though the soil bank is partly maintained by the local dispersal of propagules, the eDNA in the soil bank may represent a past community structure and/or long-dispersal events. Once the soil bank is disturbed, the establishing propagules may change the extant community composition. Of the moss propagules that become established, the higher

propagule regeneration via protonemata and branch production at low-medium temperatures may facilitate ground-dwelling species to occupy their heterogenous substrata while spores may be required for epiphytic species to become established. Overall, bryophyte and lichen asexual propagules are fragmented and dispersed from the local community, with propagule establishment helping to maintain or change the local bryophyte and lichen communities in boreal forests.

ACKNOWLEDGMENTS

I would like to thank Dr. Michele D. Piercey-Normore for her commitment, guidance, and support that allowed me to learn about the wonderful world of lichens and the ecology of both bryophytes and lichens in Manitoba and Newfoundland. Dr. René J. Belland provided great advice and reinvigorated my love for bryophytes. Many thanks are given to the members of my committee (Dr. Anita Brûlé-Babel and Dr. Kevin Fraser) whom with their instruction facilitated my growth as a scientist. I am more than grateful to Dr. Tom Booth because he kindly provided his cabin which served as a base for field work. He also motivated discussions about lichen ecology, prepared great and new food during the field season, assisted in field work, and showed me the wonders that are the northern lights.

I am grateful to the laboratory members who provided friendship and assistance during field and laboratory work: Brittany Ropson, Katherine Flores-Hutten, Kamaldeep Chhoker, Chris Deduke, Jennifer Doering, Jennifer Otissi, Umi Aden, Kyle Fontaine, Mohanad Zraik, Anh Dang, Jasmine Pinksen, Duleeka Gunawardana, Vivian Brautigan, Sarangi Athukorala, and Mostafa Sami—in no particular order. I also would like to thank Tiago Booth and André Dufresne who assisted with field work and scientific photography.

I have made many friends, which have become family, that allowed me to enjoy the diverse culture found in Canada: Jordan Specker, Mike Y. Zhang, Stephanie McEwan, Liz McEwan and family, Cole Slater, Oiza Atta, Michael Frawley, Jordan Joseph and family, Benjamin Kissinger, and Shelby Thomas.

From a distance, I also received messages of support and friendship, and am indebted to them: Juan G. García-Cancel, my family, Benjamin Suarez, Carlos Aponte, Jaynise Pérez, Carlos León, and Gerardo Quintero.

Financial assistance was provided by the University of Manitoba Faculty of Graduate Studies Graduate Enhancement of Tri-Council Stipends (GETS) Program and Natural Sciences and Engineering Research Council of Canada – Discovery Grant (NSERC-DG) to Dr. Michele Piercey-Normore.

Thesis, the publication status of the chapters and the role of each coauthor in each chapter

Chapter	Author	Contribution of each author	Article status
1	Carlos J. Pasiche-Lisboa	I reviewed the literature, organized the chapter, and wrote the chapter. MPN guided the process and edited the chapter.	To be submitted for publication in Nova Hedwigia
2	Carlos J. Pasiche-Lisboa, Tom Booth, and Michele D. Piercey-Normore	I did field work, analyzed the data, and wrote the chapter. T. Booth helped with field work and discussed results. MPN and I developed the idea behind the project, MPN guided the process and edited the chapter.	To be submitted for publication in Evansia
3	Carlos J. Pasiche-Lisboa, Tom Booth, René J. Belland, and Michele D. Piercey-Normore	I did field work, collected the data, analyzed the data, and wrote the chapter. TB helped with field work and edited the chapter. RB helped form the original idea, verified the identity of bryophytes, and edited the chapter. MPN helped form the original idea, helped with field work, verified the identity of the lichens, guided the process and edited the chapter.	Published in Ecosphere
4	Carlos J. Pasiche-Lisboa and Michele D. Piercey-Normore	I developed and performed the experiment, analyzed the data, and wrote the chapter. MPN collected the moss and lichen material. MPN helped develop the idea, guided the process, and edited the chapter.	Under review in Herzogia

5	Carlos J. Pasiche-Lisboa, Tom Booth, and Michele D. Piercey-Normore	I developed the experiment, analyzed the data, and wrote the chapter. TB collected the soil samples The Jonah Ventures company extracted, sequenced, and identified taxa from the soil environmental DNA. MPN helped develop the experiment and write part of the chapter. MPN and TB guided the process and edited the chapter.	To be submitted for publication in Molecular Ecology
6	Carlos J. Pasiche-Lisboa, René J. Belland, and Michele D. Piercey-Normore	I developed the experiment, collected and analyzed the data, and wrote the chapter. RB guided the process, and edited the chapter. MPN guided the process and edited the chapter.	Published in Botany
7	Carlos J. Pasiche-Lisboa	I discussed the major points found in each chapter, contrasted the major points to the current literature, and wrote the chapter. MPN and RB guided the process and edited the chapter.	Not to be published

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Chapter 1: Dispersal of bryophytes and lichen asexual propagules, from detachment to establishment

Abstract

Bryophytes and lichens produce asexual propagules that are dispersed locally and over long distances. The dispersal and establishment of asexual propagules may shape the communities of bryophytes and lichens, such as those in boreal forests, which may help to further understand maintenance of the communities. This chapter highlights the goals of this thesis by reviewing the literature on Pre-Dispersal (if), Dispersal (how), and Post-Dispersal (where) of lichen and bryophyte asexual propagules.

Introduction

A diverse community of bryophytes and lichens occupy various kinds of substrata in boreal forests. Bryophytes and lichens (cryptogams) can be found growing on leaves, branches and the trunks of trees. Cryptogams may also be found on protruding boulders, rock faces, soil, plant debris, pebbles, and on artificial materials (plastic, metals, etc.) and may be present near/within bodies of water (streams, marshlands, lakes). For the cryptogam communities inhabiting these different substrata, dispersal of the populations contributes to increased genetic diversity. The increased genetic diversity results from the dispersal and establishment of meiotically produced spores or through the dispersal of mitotically produced asexual propagules (Frey and Kürschner 2011). Both sexually and asexually produced propagules can serve in the formation of new populations at distances far away from the parent colony. Studies have shown that asexual propagules are an important part of the airborne propagules and their numbers vary with vertical height above ground (Armstrong 1991, Marshall 1996, Marshall and Convey 1997). The dispersal and deposition of asexual propagules also depend on the environmental conditions

allowing for seasonal patterns that either facilitate or limit the establishment of asexual propagules (Armstrong 1991, Marshall 1996, Marshall and Convey 1997). Because the dispersal of asexual propagules and their roles in habitats may vary, I studied how asexual propagules are involved in the formation or maintenance of the present community and past soil bank community in boreal forests through a series of studies based in Manitoba, Canada.

Goals of the thesis

The main goal of the thesis is to link the dispersal of asexual propagules to the extant cryptogam community and soil bank community in a boreal forest of Manitoba. The dispersal of asexual propagules of boreal cryptogam communities was studied in five chapters, in which I address the following research goals.

2) A pilot study was developed to select the best type of trap to capture asexual propagules and secondarily to explore the dispersal of asexual propagules during the winter in **Chapter 2**. The best type of trap was selected for studies on asexual propagules (*i*). Traps were placed in three different forest stands, and on a lake, (*ii*) to determine differences in the number and size of asexual propagules detached and dispersed in a three-day period. Traps were also placed on trees and on the forest floor (*iii*) to explore how the number and size of deposited propagules were influenced by the species identified in the tree communities. Methods employed in this chapter are discussed to better study the dispersal of asexual propagules in boreal forests (*iv*).

3) The dispersal of asexual propagules was further examined in boreal forests in **Chapter 3** by (*i*) assessing the deposition of asexual propagule number and size at various times of the year (winter, summer, fall), (*ii*) by determining differences in asexual propagule number and size deposition among forest stands (balsam fir, poplar, white spruce), and (*iii*) by determining differences in asexual propagule number and size deposition between the tree and forest floor substrata, and the aspect of trees. The cryptogam diversity in the forest stands was characterized to compare with the type of asexual propagules and species captured in the traps (*iv*).
4) The dispersal of asexual propagules was studied in a wind tunnel experiment for a select number of lichens and mosses found in boreal forests for **Chapter 4**. The deposition of the number and size of asexual propagules was examined on a one-meter tape away from the fan. Differences in dispersal outcomes were compared for (*i*) the cryptogam located on tree and the forest floor, (*ii*) lichens and mosses, and for (*iii*) each of the nine lichen and moss species selected for the study.
5) The community of cryptogams present in the soil represented by environmental DNA (eDNA), was studied in **Chapter 5**. One objective was to study eDNA abundance and richness of cryptogams in the soil bank to determine (*i*) the association with forest stand properties in the boreal forests. The other objective was to show (ii) the association between eDNA cryptogam richness with the moss family richness and the lichen photobiont richness, and determine if a disturbance to the soil bank will likely change or maintain the extant cryptogam community.
6) The survival and regeneration of fragments from three boreal mosses (*Dicranum polysetum*, *Orthotrichum obtusifolium*, and *Pleurozium schreberi*) was assessed in

Chapter 6. The survival and regeneration of protonemata and branches from the gametophore fragments was examined to determine how they were influenced by (*i*) the nature of the temperature exposure (abrupt and gradual), (*ii*) temperature (43, 22, 6, –18, –40, –80 °C), (*iii*) duration of exposure (time, 1–6 months), and (*iv*) the size of the gametophore fragments.

7) The general significance of the experiments presented in Chapters 2–6, linking the dispersal of asexual propagules from and to the boreal cryptogam communities, was discussed in Chapter 7.

Literature Review

To better understand the influence of dispersal of asexual propagules in the maintenance of the cryptogam communities in a forest, the biological and non-biological aspects of nature that facilitate dispersal and influence dispersal of propagule establishment have been reviewed. The goal of this literature review is to highlight the factors that determine if, how, and where bryophyte and lichen asexual propagules are dispersed. The factors are highlighted by dividing the literature review into sections which include the Pre-Dispersal (if), Dispersal (how), and Post-Dispersal (where).

Pre-Dispersal (if)

Pre-dispersal involves any aspect, abiotic and biotic, tied to bryophytes and lichens that facilitates dispersal. Abiotic factors (environmental conditions) allow for the growth of the bryophyte gametophyte and the lichen thallus, which influence the genetic cues that affect the development of asexual propagules. During the growing season (rainy season, or summer) the

production of asexual propagules increases more than during any other season (dry season, or winter); however, asexual propagules can be dispersed in high quantities during periods of time that seem unfavorable for propagule establishment (Armstrong 1991, McDaniel and Miller 2000, Goward 2003). Biotic factors that facilitate dispersal include the growth of the bryophyte stem and lichen thallus with and without algae, and the production of the asexual propagules. The detachment via different processes also increases or decreases the chances of dispersal of the diverse types of asexual propagules. The asexual propagule is defined as a unit from the bryophyte or lichen formed by mitosis, which produces a genetic replicate of the parent colony (Frey and Kutchner 2011). The genetic replicate can become established on a substratum similar to where the parent colony is located (Frey and Kutchner 2011) or elsewhere. The ploidy level of these propagules is thought to be mostly haploid. However, recent research has indicated that the ploidy level may vary within the same gametophore (diploid to polyploid) for bryophytes or the mycelia of the lichen fungi (monokaryotic to dikaryotic; see Crosby 1980, Skaked et al. 2001, Tripp et al. 2017). The type of asexual propagule can be broadly defined as a unit from the bryophyte or lichen that is developed or specialized for dispersal. The type of asexual propagule can also be a unit that is not primarily made by the plant or fungus for dispersal, but once it is detached or fragmented it can serve as an asexual propagule.

For bryophytes, a defined asexual propagule may include specialized deciduous organs (leaves, leaf apices, shoots, branches, bulbils), specialized propagules (protonemata brood cells, gemmae, tubers), and mitospores (Frey and Kutchner 2011). Furthermore, an undefined asexual propagule includes the gametophyte stem with or without leaves, leaves, rhizoids, and even pieces of the sporophyte. The defined asexual propagule is produced in various parts of the plant such as on the stalk along the plant stem axis or directly from the stem axial meristem. Asexual

propagules can be produced on the tip and/or throughout a leaf, from protonemata, and/or from rhizoids. For lichens, the common specialized asexual propagules include soredia, isidia, phyllidia, schizidia, and conidia (Tripp and Lendemer 2018). The unspecialized asexual propagules of lichens include fragments of the thallus, which may or may not contain the photobiont. These unspecialized asexual propagules that fragment and detach from the lichen include pieces of the lichen lobes, branches, rhizines, cilia, and/or pieces of mycelia. Both bryophyte and lichen asexual propagules vary in size, with sizes ranging from 100 μm (soredia clusters) to the length (cm) of whole bryophyte gametophores or lichen thallus (Armstrong 1990). The shape of the asexual propagule may also vary, with some asexual propagules resembling spheres, columns, needles, and even stars.

Dispersal (how)

Many vectors help with the dispersal of asexual propagules from a few centimeters to as far as thousands of kilometers away from the parent colony (see Pohjamo et al. 2006, Frey and Kürschner 2011, Lewis et al. 2014, Glime 2017). The vectors include dispersal by animals, water, wind, and gravity. Dispersal by animal vectors occur by two means, through gut-passage or externally. During the dispersal by gut passage, the animal ingests asexual propagules, the asexual propagules pass through the gut, and then the asexual propagules are excreted. While an asexual propagule passes through the gut, which can take a few hours to days, the animal may move far away or stay close to the parent colony of the asexual propagule (Wilkinson et al. 2017, Pasiche-Lisboa and Sastre-De Jesús 2018). After the propagules pass through the gut, the propagules that remain viable regenerate into shoots or from protonemata differentiated from the gametophore. Dispersal by gut passage has been recorded to disperse the protonemata and

gametophore of mosses and the lichen thallus via flying foxes, snails, and water fowl (Parsons et al. 2007, Boch et al. 2013, Wilkinson et al. 2017, Pasiche-Lisboa and Sastre-De Jesús 2018). During external dispersal by animals, the animal brushes against the parent colony allowing the asexual propagule to fragment or detach and become attached to the fur, hooves, feathers, secretions (snail), or other exterior parts (Heinken et al. 2001, Pauliuk et al. 2011, Boch et al. 2013, Lewis et al. 2014). The movement of the animal from one location to another helps with the transfer of the propagule, enabling the propagule to settle on a substratum. Dispersal by water can occur by raindrops hitting and dislodging an asexual propagule or by water flowing through a surface and dislodging an asexual propagule (Equihua 1987, Pasiche-Lisboa and Sastre-De Jesús 2018). Rain drops have been recorded to move moss protonemata, liverwort gemmae, and lichen soredia close to the parent colony (Bailey 1966, Equihua 1987, Pasiche-Lisboa and Sastre-De Jesús 2018). Water flow has been inferred to move fragments of the moss *Rhynchostegium riparioides* and *Fontinalis antipyretica* (Hutsemekers et al. 2013, Ares et al. 2014). Wind is thought to be the vector of most importance for the dispersal of cryptogams and their asexual propagules. Gust of wind have been shown to move the liverwort *Anastrophyllum hellerianum* over 10 m and moss protonemata up to two meters (Pohjamo et al. 2006, Pasiche-Lisboa and Sastre-De Jesús 2018). Gravity, as a vector has been poorly studied for cryptogam asexual propagules; however, it is mostly linked to other vectors. Gravity has been observed to affect the rate of fall for meiotically produced spores when interacting with the air or affecting the force of the splash drop when falling from different vertical distances from the canopy (Nanko et al. 2006, Zanatta et al. 2016). Gravity, as a vector, needs to be further studied to better understand its influence on the dispersal of asexual propagules produced by cryptogams.

Several aspects of nature influence the dispersal vectors to affect the distance where the asexual propagules are deposited. These aspects include the likelihood of the asexual propagules being detached or fragmented from the parent colony and moved to various distances away from the source. For example, warmer temperature during the year may increase animal activity and thus increase the likelihood of the animal to interact with a cryptogam colony (Vickery and Bider 1981, Hagemoen and Reimers 2002), while the contrary is observed during the winter unless the animal is adapted to the cold. In contrast to mild environmental conditions, extreme environmental conditions (blizzard, hurricane, flood, heat waves) may prompt animals to seek shelter, such as by hibernating (Strumwasser 1958, Speakman and Rowland 1999, Sausmann et al. 2004). The likelihood of asexual propagules to be dispersed by animals will most likely be decreased during the winter also because of animals hibernating or becoming less mobile due to the snow accumulation on the landscape.

Dispersal by water requires the temperature to be above zero degrees Celsius for raindrops to form, drop, and hit the propagules (Glime 2017). Once the water has fallen and started to accumulate, then water flows among leaves and branches of a tree, the tree trunk, soil and rocks, and in a stream and enables dispersal of the asexual propagules. If the water is frozen, such as during winter at northern latitudes, then the asexual propagules may be blown across cold snow or ice surfaces. Water in the form of snow can also accumulate on a branch and thaw during warm periods of the winter, allowing for chunks of accumulated snow to fall and break branches at lower parts of a tree. The accumulated snow falling and breaking tree branches with bryophytes and lichens may facilitate asexual propagule dispersal to the lower branches and to the forest floor.

Dispersal by wind is enabled when wind is in high force, there is low moisture, and a variable temperature (Glime 2017). If the air has a high moisture content, the moisture may adhere and give weight to the asexual propagule. The air with high moisture makes the asexual propagule descend to the ground faster when dispersed by wind. Furthermore, if the air has high moisture content, the asexual propagule may be too heavy to be picked up by wind, disabling its dispersal (Armstrong 1991). A low moisture content and elevated temperatures allow for the propagule to stay in the air for a longer period of time. The low moisture content and elevated temperatures of air faciliate the asexual propagules to be dispersed to farther distances rather than to shorter distances (Armstrong 1991). If many aspects of the vectors and many vectors combine, then the asexual propagule may be dispersed many times and to distances well beyond the parent colony. Glime (2017) has suggested that secondary dispersal may occur for species that produce asexual propagules that remain dormant during stressful periods of the year (dispersal in time). For example, *Leptobryum pyriforme* (Hedw.) Wilson. can be found along the soil bank of streams and produces rhizoidal tubers that may disperse in time. During floods the asexual propagule of *L. pyriforme* may be submerged, dislodged, and moved to other areas (Glime 2017). Studies on vascular plants have suggested that secondary dispersal is not uncommon (Green and Johnson 1997, Griffith and Forseth 2002, Eichberg et al. 2005). Seeds have been observed to fall by gravity near the parent plant and then being carried by waterflow to other areas in wetlands (Griffith and Forseth 2002). Seeds have also been primarily dispersed by wind or frugivores and then dispersed by rodents or ants to different areas of the forests (Forget and Milleron 1991, Levey and Byrne 1993). Although secondary dispersal have been suggested for bryophytes (Glime 2017), there are no studies that have assessed these modes of dispersal for both bryophytes and lichens, and offer a new field of research for scientists.

Properties of the parent cryptogram and habitat may also facilitate or deter the dispersal of asexual propagules. The boundary layer, a layer of calm air surrounding a surface such as that of a cryptogam colony and its asexual propagules, can protect the asexual propagule from turbulent wind and limit their fragmentation or detachment and thus their dispersal. The location of the cryptogam colony may have an influence on the chance of its asexual propagules being dispersed. Cryptogams that occupy the edge of the forest, areas where there are more open canopies in the forests, and grasslands are more likely to interact with wind and their propagules to be dispersed by wind (Raynor et al. 1974, Amiro 1990, Heinken 1999). In contrast to these areas, a forest with a closed canopy, the interior of the forests, and areas with a higher number of terrain or other features (boulders, snags) can protect cryptogam colonies from incoming wind. The areas less exposed to wind may require other vectors (raindrops, water flow, or animals) for their dispersal of asexual propagules. Within a forest, cryptogams that occupy the trees will interact with wind in a higher likelihood than cryptogams on the forest floor (Parker 1995). However, the species in the higher canopy of the trees will interact more with wind than species at mid or lower parts of the tree because wind speeds decrease semi-logarithmically from the tree canopy to the forest floor (Parker 1995).

Post-Dispersal (where)

The distances where the asexual propagules fall once dispersed varies with the vector. The fragment being dispersed by animals depends on the animal encountering the parent colony, consuming the cryptogam because it is palatable or by accident, and/or brushing against the parent colony (Pasiche-Lisboa and Sastre-De Jesús 2018). For water dispersal, particularly by raindrops, the asexual propagule needs to be hit by a drop. Once the asexual propagule is hit by

the raindrop, it is more likely to move away from the parent colony rather than remain where the parent colony is located. Water dispersal by water flow requires the asexual propagule to be hit with sufficient force to detach, or make the propagule float, and be carried beyond the parent colony. Wind dispersal of asexual propagules requires the asexual propagule to be light and dry to be moved (Armstrong 1990, Pasiche-Lisboa and Sastre-De Jesús 2018). All of these conditions determine whether the asexual propagule will remain near the parent colony or dispersed far away from the source.

Most asexual propagules fall in the first few meters from the parent colony, independent of the vector responsible for dispersing the asexual propagules (Equihua 1987, Pohjamo et al. 2006, Frey and Kürschner 2011, Pasiche-Lisboa and Sastre-De Jesús 2018). Furthermore, a higher number of asexual propagules is less likely to be present at farther distances from the parent colony. This pattern of number of asexual propagules with distance from the parent colony determines the distribution of asexual propagules. Most dispersed asexual propagules have been found to have a leptokurtic distribution (Equihua 1987, Kimmerer and Young 1995, Pohjamo et al. 2006, Pasiche-Lisboa and Sastre-De Jesús 2018). A leptokurtic distribution shows a high number of asexual propagules that are dispersed close to the source (parent colony), and only a few asexual propagules disperse beyond the parent colony. However, the leptokurtic distribution may be shifted further away from the parent colony depending on the type of vector. For example, if the wind speed or the height of the drop is increased, the force of impact of wind on the propagule is increased causing it to travel further before falling to the ground. The shift in numbers of asexual propagules away from the parent colony helps with not only maintaining the population of the parent colony, but also to increase the likelihood of forming new populations

on substrata away from the parent colony (Laaka-Lindberg et al. 2003, Frey and Kürschner 2011, Pasiche-Lisboa and Sastre-De Jesús 2018).

Size of the asexual propagule can also determine the distance where propagules are dispersed and if it is likely to regenerate. For example, large propagules will mostly deposit near the parent colony, while smaller propagules will be carried more easily away from the parent colony in terrestrial habitats (Armstrong 1987, Pasiche-Lisboa and Sastre-De Jesús 2018). However, large propagules may also remain for longer periods of time on the fur of animals (sheep), due to the fur length and structure, which may allow their dispersal at longer distances from the parent colony (Pauliuk et al. 2011). The size of propagules of aquatic bryophytes helps their dispersal in a stream. Larger propagules of aquatic species may be more buoyant than those of non-aquatic species (Boedeltje et al. In Press). Aquatic bryophytes that have larger propagules are able to disperse for longer distances than small propagules (Boedeltje et al. In Press). The size of the propagule may have variable effects on regeneration. Propagules of larger sizes may have more resources which facilitate their survival and regeneration than those of smaller sizes, or the size may not have any influence on regeneration (Campeau and Rochefort 1996, Løe and Söderström 2001).

The relationship between dispersal time and viability may determine if the dispersed propagule regenerates and establishes on a substratum. For animal dispersal, the asexual propagule passing through the gut may increase or decrease the chances of the asexual propagule to survive and fall onto a suitable substratum. Because the asexual propagule does not have a thick wall layer to protect its cells, or a seed coat, it is subjected to the digestive enzymes and the time spent within the gut of an animal (Renison et al. 2010). For example, if the asexual propagule remains within the gut for an extended period of time, then the gastric juices, the

microbial flora, and the immune response of the animal may limit the chances of the propagule to be viable once it is excreted (Meyer and Witmer 1998). However, if the asexual propagule quickly passes through the gut and is excreted, then the moisture associated with the gut passage is suggested to increase the chances of the propagule surviving and thus becoming established (Pasiche-Lisboa and Sastre-De Jesús 2018). Water dispersal might not be detrimental to the viability of the asexual propagule if the water is low in salinity, a rather neutral pH, and the asexual propagule is not immersed in water for an extended period of time (Dalen and Söderström 1999). When these conditions are met, then the water from dispersal aids with the survival of the asexual propagule by facilitating the regeneration from asexual propagules (Rochefort et al. 2002, Pasiche-Lisboa and Sastre-De Jesús 2018). Once the conditions in the water (abrupt changes in pH, salinity, time) are changed, the regeneration of the asexual propagule may diminish, but this needs to be further studied (but see Pouliot et al. 2013). Wind dispersal may present a trade-off between dispersal and viability. Asexual propagules are dispersed by wind farther distances away from the parent colony, when compared to the distances achieved by the dispersal of other vectors (raindrops). However, 3/4 of the asexual propagules dispersed lose viability, possibly as a result of the desiccation caused by wind on the asexual propagule (Pasiche-Lisboa and Sastre-De Jesús 2018). The faster rate of desiccation of the propagule when dispersed by wind may explain the limited regeneration capability of the asexual propagule when establishing; however, it is noted that the degree of regeneration to the rate of desiccation is species and habitat dependent (Deltoro et al. 1998, Stark et al. 2013).

Conclusion

Bryophytes and lichens produce asexual propagules mostly during the growing season, but they can be dispersed throughout the year. These propagules are a result of mitotic cell

division that help form propagules of many types and sizes that can be specialized or unspecialized (fragments of the lichen thallus or bryophyte gametophores). When the propagules are exposed to the different vectors (animals, gravity, water, and wind), the propagules may fragment and detach from the parent colony. The detachment or fragmentation of asexual propagules may likely occur when animals brush on and/or eat the parent colony. The detachment or fragmentation of asexual propagules may also occur when water is in liquid form and flows through or drop on with force on asexual propagules. Wind may detach or fragment asexual propagules when in high intensity and accompanied by increased temperatures and low humidity. The dispersal of asexual propagules may occur more than once, but more evidence is needed to pinpoint how this happens. The dispersal of asexual propagules may be limited by the structure of the parent colony, the airboundary layer, and palatability of the bryophyte gametophore or lichen thallus. Size of asexual propagules and the location in a forest (closed forest, forest interior) or on the landscape (sheltered by topography) may also reduce the asexual propagule dispersal number and distance. Dispersal may be promoted by the increase in harshness or force of the dispersal vectors on the asexual propagules, location of parent colony in more open habitats, and size of the asexual propagules. Once the asexual propagule is dispersed, where it lands away from the parent colony will be affected by the dispersal vectors. An increase in the amount of time that the propagule spends within or on an animal, the interaction of water with the propagule buoyancy, size of the asexual propagules, and force of the vector helps the asexual propagule to move beyond the parent colony. Many avenues of research may come up to deepen our knowledge of the importance of asexual propagules, the vectors, and the location where dispersal occur that enables the maintenance of communities in forested and non-forested

systems. In this thesis, I expand our understanding of the roles of asexual propagules in the maintenance of communities in boreal forests in five chapters (Chapter 2—Chapter 6).

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Chapter 2: Dispersal during winter: a snapshot of the diversity of lichen and moss and asexual propagule dispersal in boreal forests of northern Manitoba

Abstract

Winter ecology (high wind speed, cold temperatures, snow) allows for moss and lichen asexual propagules to be dispersed within and among forest stands and to nearby areas. However, little is known about the moss and lichen asexual propagules dispersed from species in boreal forest stands, the dispersal within substrata of boreal forests, and the dispersal to a lake (Payuk Lake) during winter. The winter dispersal of the propagules asexually produced by moss and lichens was studied in mixed forest stands dominated by white spruce, balsam fir, and poplar. The primary goal was to test two types of traps for capturing asexual propagules and the secondary goal was to gain a understanding of the dispersal occurring in boreal forests and to a nearby lake. Environmental data were associated with the dispersal of asexual propagules. Moss and lichen species were collected from tree twigs and bark to assess the diversity associated with forest stands. Two types of traps (meshed-buckets and petri dishes) were set on trees and snow surface and a lake to capture the number and size propagules dispersed by wind. This study showed that petri dishes were the best of the traps tested since the petri dish traps captured asexual propagules produced by moss and lichens in higher quantities than bucket mesh traps. Lichen thallus fragments of small sizes were the most numerous propagules in traps. Propagules found in traps belonged to the more common epiphytic species in the forests, where most species were reproducing asexually (66%). Epiphytic species were found in higher quantities than the forest floor species in all forest stand types (balsam fir, poplar, and white spruce). Propagules from the forests were dispersed to the lake margin, but there were not as many as in the forest

stands. In addition, the study suggests that the dispersal of moss and lichen asexual propagules in boreal forests during winter may help to maintain the epiphytic communities more than the ground-dwelling species, leading to the hypotheses that were addressed in chapter 3.

Introduction

Winter conditions may have an effect on the fragmentation and dispersal of propagules (meiotic spores and asexual propagule) produced by cryptogams (mosses and lichens). Winter conditions include lower temperatures, variable humidity, presence of snow on the ground, and greater wind speed with fewer obstacles because of the bare trees and covered ground vegetation. The temperature may lower the photosynthetic activity and survival of the lichen and moss species (Rastorfer 1970, Kennedy 1991, Pannewitz et al. 2003, Bjerke 2011), and humidity can alter propagule development (Armstrong 1991). Snow can cover species and limit what is being dispersed in the season. In addition, wind may fragment, detach, and direct where propagules will be deposited. For example, propagules of mosses and liverworts can be transported from alpine forests to snowbeds (McDaniel and Miller 2000, Robinson and Miller 2013), hair lichens can be scattered by wind in subalpine forests to a meadow (Goward 2003), and moss spores can be dispersed kilometers from the source population (2 km, Marshall and Convey 1997; ca.128 km, Sunberg 2005). In addition, dispersal during winter may be influenced by environmental variables. For example, Armstrong (1991) found that during different periods of time in a year, including winter months, the deposition of soredia of *Hypogymnia physodes* (L.) Nyl was in higher numbers when the humidity was low, the wind speeds were high, and temperature was high. However, Marshall and Convey (1997) found that fewer moss spores and asexual propagules were dispersed during the winter than in summer, but the environmental variables

(overall) did not explain such depositions in their study. A few studies have looked at the dispersal of cryptogams during winter. The onset of winter has been shown to diminish the dispersal of asexual propagules, have no pronunced effect on the dispersal of asexual propagules when compared with other seasons, or seem to have increased the dispersal of asexual propagules than during summer (Armstrong 1991, Marshall and Convey 1997, McDaniel and Miller 2000). A study on the dispersal of moss and lichen asexual propagules during winter may help understand the maintenance and formation of the communities in boreal forests by providing details of the quantities of asexual propagules and the species dispersed.

Asexual propagules that can be dispersed during winter, as in other seasons, are diverse in type and sizes,. However, the majority of asexual propagules dispersed must come from epiphytic and epilithic species that grow above the level of the snow. These asexual propagules include soredia, isidia, and thallus fragments of lichens. Asexual propagules also include gemmae, branchlets and gametophyte fragments of mosses. The dispersal of these asexual propagules is important in forests because their dispersal close to the parent colony aids in the formation and maintenance of moss and lichen populations (Kimmerer 1991, Nash 1996, McDaniel and Miller 2000). A way to assess the asexual propagule dispersal in boreal forests is by trapping propagules and comparing the dispersed propagules with the diversity of moss and lichen species in the same forests. Traps used for capturing propagules can be complex to simple (Burkard Jet Traps, Tauber traps, plastic pools; Marshal and Convey 1997, Marshall and Chalmers 1997). The Burkard and Tauber traps are effective in trapping debris of small sizes, and at specific times. They have been employed in seasonal studies of spores, pathogens, and debris dispersal (Jenky 1974, Bonny 1980, Ebner and Haselwandter 1989, Calderon et al. 1995). However, these traps may not capture propagules that are of large sizes. For example, hair lichen

(*Bryoria* and *Usnea* spp.) have been known to have fragments with sizes up to 38 cm in length dispersed by wind (Goward 2003), which would not be captured by the Burkard or Tauber traps. In addition, these traps can be quite expensive (hundreds to thousands of dollars), and the cost can limit the number of traps placed at various locations within and outside of forest stands. Entrapment methods that account for different propagule sizes, are relatively inexpensive, easy to modify, and/or replicate, are the most feasible for studying the dispersal of asexual propagules. Petri dishes are an example of an entrapment method with these qualities. Petri dishes have been used to capture spores of *Lycopodium* (Gregory and Stedman 1953), to study the dispersal range of a pathogenic fungus in forests (Gothier et al. 2001), as well as the pollen of conifers (Giesecke et al. 2010). In addition, petri dishes can be modified to be attached onto different surfaces. Thus, petri dish traps can be used to study moss and lichen asexual propagules dispersal on substrata in boreal forests.

The goal of this study was to conduct a pilot experiment to select the best type of propagule and debris traps for understanding the dispersal of asexual propagules (number and size) in boreal forests (different stands and substrata) during winter. Another goal of this study was to associate the moss and lichen species present in boreal forests with the dispersed propagules captured in the traps.

Methods

Study Sites

The four study sites, which include three forest stands and the surface of the frozen lake, were selected to study where asexual propagules may land during winter. These sites were located on the Precambrian Shield on the eastern shore of Payuk Lake, Manitoba. Three boreal

forest stands were mainly on granite-gneiss rock, and the stands were dominated by white spruce (*Picea glauca* (Moench) Voss), balsam fir (*Abies balsamea* (L.) Mill.), and poplar trees (*Populus tremuloides* Michx.). The surrounding forests also included other trees and shrubs such as Jack pine (*Pinus banksiana* Lamb.), larch (*Larix laricina* (Du Roi) K. Koch), and alder (*Alnus* sp.)(Fontaine et al. 2014). Three types of stands (habitats) were selected for this preliminary study to understand the type and number of moss and lichen asexual propagules that might be expected in this area. Three habitat types were chosen and included the mixed forest (MF), shoreline forest (SF), and open poplar forest (PF). The properties of the forest stands were not determined in this pilot study. The mixed forest had a mix of balsam fir and white spruce trees, the shoreline forest was dominated by white spruce trees, and the open poplar forest by poplar trees. The shoreline forest was closest to Payuk Lake, and this forest stand type was located near the mixed forest. The poplar forest was located the farthest away from the other forests (Fig. 2.1 a–b).

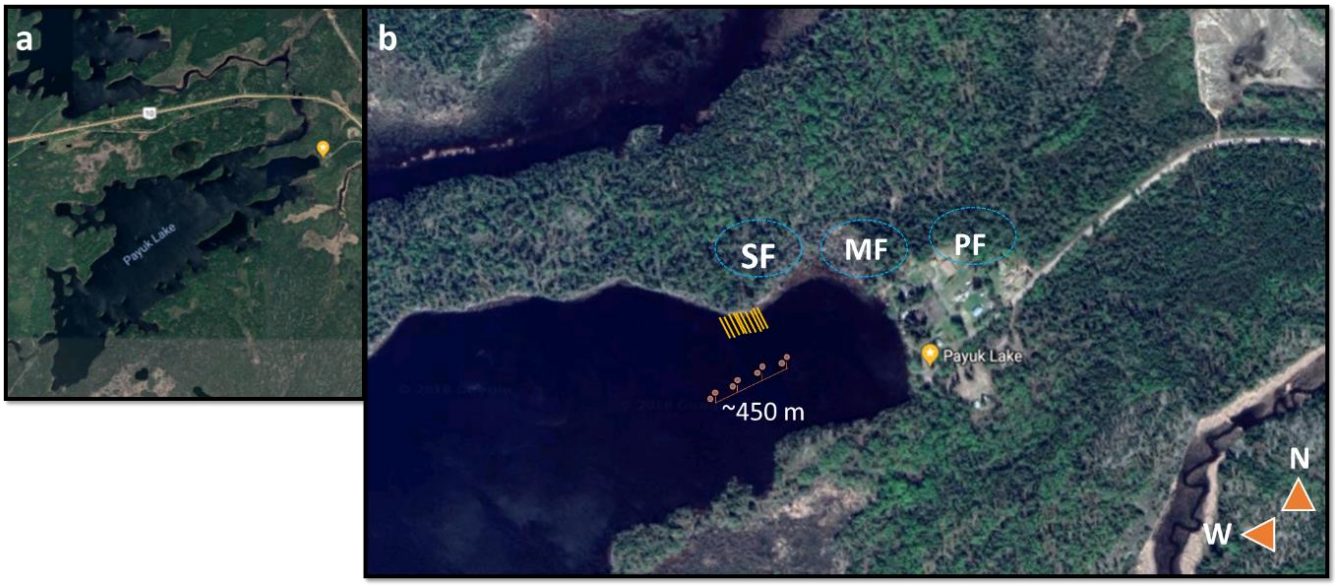

Figure 2.1. Aerial view of Payuk Lake, Manitoba (**a**). Aerial view and diagram of the relative location of the forest stand types (SF = shoreline forest, MP = mixed forest, PF = poplar forest), transect (orange line) with buckets (meshed-buckets facing north and west) on the lake, and transects near the shoreline (yellow lines) where the meshed-bucket and petri dish traps were placed to study dispersal of bryophyte and lichen asexual propagules during winter (**b**). ©2018 Google Maps.

Climate Data

Climate data were retrieved from the two nearest monitoring sites: Flin Flon Airport and The Pas (Table 2.1). Mean minimum, mean, mean maximum temperature (°C), mean speed at maximum gust ($km \cdot h^{-1}$), mean direction from the north pole of maximum gust (10s degree, 1° = 10°; 36° = North, 9° = East, 18° = South, 27° = West), and snow on the ground (cm) were recorded. The data were recorded and compared between the month of February (2015) and the three-day study period (February 17, 18, 19, 2015) to understand how weather might change during winter, and how weather affects asexual propagule dispersal. The GPS coordinates, the distance from the lake for all locations, and the elevation were obtained from Google Maps (2015).

Table 2.1. Habitat and monitoring site locations for the study of asexual propagule dispersal near Payuk Lake, Manitoba, Canada. Weather stations (Environment Canada) were assessed for climate data via climate.weather.gc.ca. *GPS Coordinates and elevations for sites at Payuk Lake are from Google Maps (2015), na = not applicable.

Location	GPS Coordinates of site (Latitude and Longitude)	Elevation of site (m)	Distance from Payuk Lake	Environmental data available (2015)
Mixed forest	54.651, -101.498	301	88.16 m	na
Poplar forest	54.651, -101.496	304	110.77 m	na
Shoreline forest	54.650, -101.500	300	30.33 m	na
Payuk Lake	54.648, -101.504	300	0 m	na
Flin Flon	54.680, -101.680	303.9	9.27 km	Hourly and Daily

Flin Flon A	54.770, -101.880	320	25.74 km	Daily
Flin Flon A	54.680, -101.680	304.2	9.27 km	Hourly
Flin Flon A	54.680, -101.680	3.04.2	9.27 km	Hourly
The Pas	53.970, -101.090	270.6	79.47 km	Hourly and Daily
The Pas A	53.970, -101.100	274	79.45 km	Hourly and Daly

Specimen collection and identification

Lichen and moss specimens were collected from the mixed, shoreline, and open poplar forests to record the presence and absence of species. Twig and bark samples were removed from trees, vouchers were placed in labeled bags for further identification (one bag per tree, and 10 trees per forest). No species were collected from the forest floor because it was covered with snow. After the three-day period of study, the bags were then transferred to the laboratory, where the species were identified using the keys in Ireland (1982), Goward et al. (1994), Goward (1999), Brodo et al. (2001), Thompson (2003), and Hinds and Hinds (2007). The collected specimens were air-dried and deposited as vouchers in the cryptogram division at the Sir Wilfred Grenfell Campus (SWGC) Herbarium at Memorial University of Newfoundland.

Experimental design

Two types of traps were used to select the most effective and inexpensive method for capturing asexual propagules dispersed during winter: plastic petri dishes (1.5 cm in height, 4.5 cm in radius, totalling 95.4 cm^3) and buckets lined with mesh (1 cm in height, 15 cm in radius, totaling 706.8 cm^3). Bucket traps were placed on the frozen lake on two aspects facing the incoming winds (north and west; $n = 4$ per aspect, $n = 8$ in total) about 150 meters apart in the snow on the ground. The bucket traps were located 300 to 400 meters from the shoreline. The bucket traps were used to capture propagules blown from the forest stands and across the snow.

Buckets (n = 3, n = 9 total buckets) and petri dishes (n = 5, n = 15 total petri dishes) on the snow surface were placed about 1 m away from any nearby trees in each stand type.

Petri dishes (n = 5, n = 15 total petri dishes) with a popsicle stick glued to the back of the dish were anchored to a hole drilled at breast-height of the trees selected for each stand type. Petri dish traps were also placed at different distances from the source of propagules (shoreline forest, closest forest to the lake) to see if wind influenced the deposition pattern of the propagules on the snow surface of the frozen lake. These traps were placed in a line southwest of the shoreline forest at different distances (0 m, 2 m, 4 m, 6 m, and 8 m) onto the lake. The distance between each line with petri dishes (10 total lines, n = 10 total petri dishes per distance) was c.a. two meters. Figure 2.1 shows the relative location of the experimental setting described above.

All the traps were covered before and while they were being placed on the various locations. Once the traps were stabilized, the lids were removed from traps to begin the capture of the debris (moss and lichen asexual propagules and other debris) for the three-day period. After the three-day period, petri dishes were covered with a lid, the mesh on the bucket folded, stored in boxes outside until transportation to the laboratory, and then refrigerated (4 °C) until further processed.

Debris count and size estimation

Once in the laboratory, the debris was examined under a dissection microscope (Leica MZ6) and a compound microscope (4× to 40×; Nikon YS100). All debris were categorized as animal parts, wood/bark fragments, soil, algae, leaves of vascular plants, pollen grains, and/or the asexual propagules of lichens and mosses.

The longest dimension of the lichen fragments (diameter), the most common asexual propagule, was measured with a micrometer (Nikon YS100). The dimensions of this asexual propagule were measured to understand the size distribution of propagule being dispersed during winter. A histogram of the frequency of lichen fragments (n = 100) per size classes (six classes: 0–500 µm, 501–1,000 µm, 1,001–1,500 µm, 1,501–2,000 µm, 2,001–2,500 µm) was constructed.

Statistical analyses

The presence and absence of the lichen and moss species were used to estimate the richness (S) and diversity (Chao and Shannon-Wiener) for each forest. Chao's number of class estimator was used to estimate the number of species that should be present in each forest (Chao 1984). The Shannon-Wiener index ($H = -\Sigma p_i \times \ln p_i$) was changed to Hill's number ($N_1 = e^H$) to have a better representation of the species diversity in the forests by transforming the entropy values to richness values, or the effective species richness: the closer N_1 is to the maximum S per site, the higher the diversity of the forest (Hill 1973). The relative evenness of the forest type (E) was obtained by dividing the effective species richness with the species richness of the forest ($E = [N_1/S] \times 100$). Similarly, the higher the E value, the more diverse and the fewer the dominant and rare species are present in the forest (Hill 1973). The relative dominance of the species within the forest stand types was obtained by subtracting E from 100. Both Chao and Shannon-Wiener values were obtained by bootstrapping (10,000 resamplings) the species presence-absence data at 95% confidence intervals (Efron and Efron 1982).

To support the diversity estimates, rank-abundance curves (RAC) were created and graphed for the boreal forests. The RAC shows the log-transformed species abundance plotted

for the rank species present in each forest stand type. The abundance refers to the presence of each species on the trees sampled, such as Species A being present on the twigs of eight out of the ten trees sampled. The steeper the curve, the higher the dominance of a few species per forest stand type; thus, the forest stand is less diverse. The lesser the curve steepness, the more diverse and even is the community assemblage for the respective forest (Whittaker 1965).

A binary matrix was developed for the presence-absence (1 and 0, respectively) data of the species in the different boreal forests. The Jaccard's coefficient of similarity (1908) converted the presence-absence data into distances by taking the one–complement of the data. Then a cluster analysis was performed using UPGMA (Unweighted pair group arithmetic average; Sokal and Michener 1958) from which a dendrogram was constructed to compare the forest stand communities according to the species present.

Data from the traps were explored with basic statistics and visualization tools (histograms, graphs, and boxplots). Before implementing any major statistical analyses, the counts from the traps were modified to similar unit/volume, as the petri dishes and the bucket mesh traps had different volumes. Volume of the traps was used to standardized counts, since volume influence the airboundary layer and how particules interact with the trap surface (Roger and Eaton 1990). The standarization was done by multiplying the asexual propagule counts in petri dishes by 7.42 (706.8 cm^3/95.4 cm^3). The histograms of the data showed a non-normal distribution. As a result, the data were log-transformed to improve data into a normal distribution. A Shapiro-Wilk's Test (a = 0.05) and a Levine's Test (a = 0.05), respectively, was used on the log-transformed data to check for normality and homoscedasticity to fulfill the assumption of the statistical tests (two-tailed T-Test and ANOVAs). For the ANOVAs, Tukey

Post Hoc Tests (a = 0.05) were used to see if there were differences between the habitat treatments.

A two-tailed T-Test was used for the climate data to see if there were differences between the month of February and the three-days when the winter dispersal study was done (2015). A Two-Way ANOVA was used to test if there were differences between the type of traps (petri dish and bucket with mesh) used to capture the types of debris (propagules and debris) overall, or those in the boreal forests. A One-Way ANOVA was used to test if aspect (N and W) towards the prevailing wind affected the debris deposition on the lake. Two-Way ANOVA was also used to test if there were interactions and differences between type of propagules and debris that dispersed in the habitats (boreal forests and lake), and to depict differences in propagule and debris dispersal interaction among boreal forests (poplar, shoreline, and mixed forests)—as well as substratum (snow surface and tree). For the Two-Way ANOVA to be run on these data, some of the values were removed (outliers: n = 5 of traps from the lake; n = 7, traps on trees from the shoreline forest). The removal of these data would not affect the interpretation of the tests on the factors being assessed and including these data would require the use of a non-parametric test even when it would be unnecessary. However, the non-parametric Kruskal-Wallis Test (Post Hoc; Pairwise Test, a = 0.05) with the outlier data was also run to compliment the results of the ANOVAs.

Some data analysis could not fulfill the assumptions (non-normal and heteroscedastic). For these data, a Kruskal-Wallis Test (Post Hoc; Pairwise Test, a = 0.05) was preferably run instead. The ANOVAs were run as well to complement the results from the non-parametric statistics. This test was used to see if there were differences in the dispersal of the different types of propagules (debris and propagules) on the substrata (snow surface and trees) in the boreal

forests (mixed, shoreline, and poplar forests). Elimination of data values, to ameliorate the data into a normal distribution and homoscedasticity, as it was done previously, was not possible as the data values were important in explaining part of the above process. Consequently, the interpretation of these results should be taken with caution. Lastly, a Two-Way ANOVA was used to test for differences among the distance (0 m, 2 m, 4 m, 6 m, 8 m) and types of propagules and debris that were dispersed from the shoreline forest to the lake.

Simple calculations were done in excel (sum, mean, standard deviation, transformations, and the Two-Tailed T-Test), while the more advanced statistics (Multivariate analyses, ANOVAs, Kruskal-Wallis Tests) were run on Infostats (2015).

Results

Environmental Data

The average temperature during the month of February was -24.5 °C, with a mean relative humidity of 68%, and wind had a mean maximum gust of 21 km/h from the northeast (Table 2.2). The minimum and mean temperatures of the three-day period (Feb. 17, 18, 19) was significantly colder than the mean and minimum temperatures of the month ($P = 0.01$ and $P = 0.001$, respectively; $n = 168$ and $n = 25$, respectively).

Table 2.2. Environmental data for the month of February and the three-day period (February 17, 18, and 19, 2015). The data were obtained from weather stations in Flin Flon Airport and The Pas. *Environmental data were obtained via the Environment Canada website. Data analyzed with the daily values are not marked, while the data analyzed with hourly values are marked with †. Different letters indicate significant differences between the data from the time environmental

log-transformed variables ($P < 0.05$), the differences were estimated with two-tailed T-Tests ($a = 0.05$).

Time	Min. Temp. (°C)	Mean Temp (°C)	Max. Temp (°C)	Relative Humidity (%)†
Feb., 2015	-27.1 ± 5.2a	-21.7 ± 4.7a	-16.4 ± 5.2a	68.0 ± 8.8a
Feb. 17, 18, 19	-31.1 ± 3.7b	-24.5 ± 1.2b	-18.1 ± 2.2a	68.35 ± 8.0a

Time	Speed of Max. Gust (km·h^{-1})	Direction of Max. Gust (10s degree)	Snow on Ground (cm)
Feb., 2015	20.8 ± 11.8a	27.5 ± 9.2a	32.6 ± 9.4
Feb. 17, 18, 19	18.2 ± 13.8a	29.4 ± 6.0a	30.7 ± 10.1

For the three-day period, the environmental conditions were mostly clear, with a few instances where the day was cloudy and there was snow or ice crystals blowing. The mean wind speed was the highest on the first day of the traps being exposed in the different areas (Payuk Lake and forests), but the mean wind speed diminished between the 17th and 18th of February (2015). Fig. 2.2 shows that the wind speeds increased for the third day of sampling (ca. 12 km/h), but it was not as high as the wind speed for the first sampling date (ca. 27 km/h).

Figure 2.2. Mean wind speed (km/h) of maximum gust for the dates of Feb. 17th, 18th, and 19th (2015). Data are from the Flin Flon Airport and The Pas weather stations, Manitoba, Canada.

Lichen and moss diversity in three boreal forest stands

A total of 27 species of lichens and mosses were collected from twig and bark of trees (see Appendix, Table A2.1). Twenty-five of these species were lichens, while the remaining two species were mosses that were present only in the poplar forest. The mixed forest had the highest richness, followed by the shoreline forest, and lastly the poplar forest: 19, 15, and 10 species, respectively. The mixed forest has the highest diversity, followed by the shoreline forest and then the poplar forest. The estimated effective species richness indicates that there are differences in diversity for each forest, and lower effective species richness value in comparison to the richness. These differences result from a few species with greater dominance and abundance than others in each forest. Sixteen percent (16.4%), 19%, and 20 % of the species in the mixed, poplar, and shoreline forests were dominant. This diversity and dominance are verified with the rank-abundance curves for each forest (Fig. 2.3.). The steepness of the RACs line for each forest indicate that the diversity among the forest stands was not even, but was dominated by four to five moss and/or lichen species. Since the steepness of the RAC for the mixed forest is less than the shoreline and poplar forests, the diversity and evenness are higher in this forest. Although the mixed forest has higher richness and diversity than other forests, the Chao's estimate of the expected species richness for this forest suggests that there are other species of lichen and moss that have not been sampled, as well as for the other forests near Payuk Lake (Table 2.3).

A cluster analysis of the species present in the different boreal forests shows that the assemblage of lichens from the mixed forest is similar to that of the shoreline forest (Fig. 2.4.). These two forests shared 14 lichen species. However, these forests were dissimilar in assemblages with that of the poplar forest. Only three and two species were shared between the poplar forest and the mixed and shoreline forests, respectively.

Table 2.3. Diversity indices estimation based on the lichen and moss species collected from three boreal forests along Payuk Lake: mixed, shoreline, and poplar forests. The Shannon-Weiner index was transformed to Hill's number to give the effective species richness of each boreal forest stand. The value of the Shannon-Weiner index and species richness estimator (Chao1) were estimated by 1000 bootstrappings at 95% confidence interval.

Indices	Mixed forest	Shoreline forest	Poplar forest
Species Richness (S)	19.0	15.0	10.0
Chao1	29.0	29.0	28.5
Effective species richness ($N_1 = e^H$)	15.9	12.0	8.1
Evenness (E = H/S) ×100; (Dominance)	83.6% (16.4%)	80.0% (20%)	81.4% (19.6%)

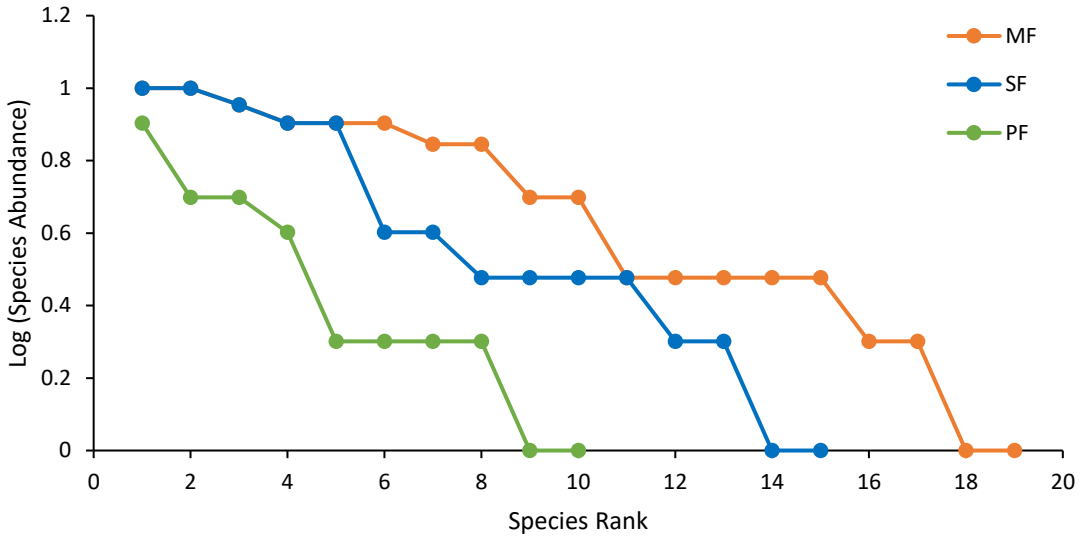

Figure 2.3. Moss and lichen species rank-log (abundance) curves of three boreal forests near Payuk Lake: mixed, shoreline, and poplar. MF = mixed forest, SF = shoreline forest, and PF = poplar forest.

The dissimilarity of communities between the forest types resulted from the presence of seven rare species out of the 10 species located in the poplar forest (Appendix, Table A2.1). These rare species include the lichens *Caloplaca holocarpa* (Hoffm. ex Ach.) M. Wade) and *Lecanora barkmaniana* Aptroot and van Herk, and the mosses *Orthotrichum obtusifolium* Brid. and *Platydictya subtilis* (Hedwig) H. A. Crum, Mich. Both the mixed and shoreline forests had fewer species that were rare: *Lecanora circumborealis* H.T. Lumbsch., *Usnea filipendula* Stirton, and *Vulpicida pinastri* (Scop.) J.-E. Mattsson and M. J. Lai.; and, *Lecanora. hybocarpa* (Tuck.) Brodo and *Usnea cavernosa* Tuck., respectively.

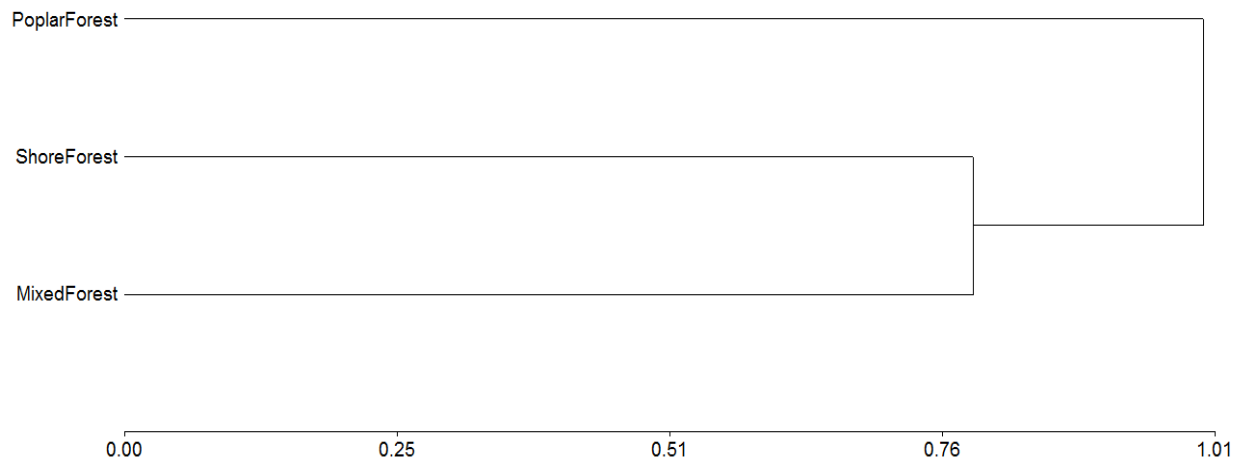

Figure 2.4. Dendrogram (UPGMA) of the cluster analysis of boreal forests (mixed, shoreline, and poplar forests) near Payuk Lake. The cluster analysis was performed with Jaccard's coefficient of similarity distances on the presence and absence of mosses and lichens in each forest.

Dominant species (species proportion ≥ 10%) of the mixed and shoreline forests include *Parmelia sulcata* Taylor, *Evernia mesomorpha* Nyl., and *Usnea hirta* (L.) Weber ex F.H. Wigg (Appendix, Table A2.1). The first two species are the dominant species in the mixed forest, while the shoreline forest had five dominant species. The dominant species in the poplar forest were the mosses *Orthotrichum obtusifolium* and *Platydictya subtilis*, as well as the lichen *Physcia adscendens* (Fr.) H. Olivier. The abundance of these dominant species ranged between 25% to 16% in the poplar forest. The rare species were *Bryoria fuscescens* (Gyelnik) Brodo and D. Hawksw. and *Cyphelium tigillare* ((Ach.) Ach.) for the mixed forest, *Bryoria furcellata* (Fr.)

Brodo and D. Hawksw and *Lecanora hybocarpa* for the shoreline forest, and *Melanelia septentrionalis* (Lynge) Essl. and *P. sulcata* for poplar forest.

Differences among Petri dish and bucket traps in capturing asexual propagules

Both petri dishes and bucket traps captured debris, which included moss and lichen asexual propagules. However, there were no significant differences between the mean number of debris that were dispersed to traps placed on the frozen lake or boreal forests (n = 7 to 45, df = 1, F = 0.95, P = 0.33). Furthermore, there were no differences in the mean number of propagules and other debris in traps (n = 17 to 85, df = 1, F = 0.19, P = 0.66). However, the petri dishes trapped significantly higher number of debris (asexual propagules and other types of debris) than the bucket traps (n = 34 to 170, df = 1, F = 64.71, P < 0.0001).

The type of trap, bucket and petri dish traps, did not seem to influence the type of debris that was captured (n =10 and 18 per treatment, respectively; df =1, F = 3.48, P = 0.0677). However, the petri dishes captured, overall, more debris than the bucket traps (n = 36 and 20, respectively; df = 1, F = 51.94, P < 0.0001). Overall, there were more debris than moss and lichen propagules in the traps (n = 28, df = 1; F = 26.28, P < 0.0001).

Dispersal of asexual propagules during winter

Lichen and moss asexual propagules, as well as plant and animal debris were dispersed onto the traps in the boreal forests and to the lake. A total of 1241 lichen and moss propagules, and 2456 pieces of debris were recovered from the petri dishes and bucket traps. Debris dispersed onto the lake in varied proportions to the two aspects, north and west (Table 2.4), but

was not affected by the aspect of the bucket on the lake. However, it was observed that a higher deposition of debris than moss and/or lichen propagules occurred on the lake.

When comparing the dispersal of debris between the lake and the boreal forests, it was found that the average number of propagules and debris that were dispersed differed between locations. A higher number of propagules and debris were dispersed in the forest than onto Payuk Lake (Table 2.4, $P = 0.006$). However, by only looking at the data from petri dishes, there was a high level of variation in the debris (other debris and propagules) in both locations (df = 1, $F = 0.02$, $P = 0.8941$) and between forest stands (df = 1, $F = 0.07$, $P = 0.7962$). Lichen and moss propagules were fewer in number than the debris on all the traps in both locations (Table 2.4, df = 1, $F = 54.36$, $P < 0.0001$).

Table 2.4. Differences in propagule and/or debris dispersal among the three sites. A Two-Way ANOVA and Kruskal-Wallis Tests ($a = 0.05$) were used to test the difference between or among treatments. The semi colon divides the *n* [total number of replicates (replicates for treatment 1, replicates for treatment 2)] and *P* values according to the statistic used: ANOVA and Kruskal-Wallis Test, respectively. *n* = replicates, df = degrees of freedom, *F* = *F* statistics associated with the ANOVA, *H* = *H* statistics associated with the Kruskal-Wallis Test.

Treatment	*n*	df	*F*	*H*	*P*
Comparison between lake aspect and debris					
Lake: aspect N × W, debris vs asexual propagules	4 per treatments	1	1.29	10.43	0.2783; 0.01416
Lake: aspect, N × W	4 per aspect	1	2.74	9.93	0.1237; 0.6277
Lake: debris × asexual propagules	8 per debris type	1	19.67	9.93	0.0008; 0.0006
Comparison between lake and the boreal forests					
Lake × Boreal forests	(13, 104); (18, 111)	1	7.95	29.53	0.006; < 0.0001
Debris × asexual propagules	(59); (66)	1	30.48	8.27	0.0003; 0.0040
Comparison between the boreal forests, the substratum, and debris					
Boreal forest: poplar, shoreline, × mixed forests; substratum (tree vs snow surface); debris × asexual propagules	6-9, 8-9 ,9-14, respectively	2	1.71	62.27	0.1856; < 0.0001

Boreal forest: poplar, shoreline, × mixed forests; substratum (tree × snow surface)	12-18, 16-18, 19-28, respectively	2	6.82	20.31	0.0017; 0.0011
Boreal forest: poplar, shoreline, × mixed forests	30, 27, 39; 30, 34, 39	2	0.08	0.48	0.922; 0.7855
Boreal forest: substratum (trees ×snow surface)	28 and 56, respectively	1	15.88	13.42	0.0001; 0.0002
Boreal forest: debris ×asexual propagules	56	1	60.08	35.77	< 0.0001

Dispersal of debris and asexual propagules to substrata of the boreal forests

Among the boreal forests, the dispersed debris (asexual propagules and other debris) on the traps varied between the trees and the snow surface (substrata). However, all boreal forest stands had more debris dispersed to traps on trees than to traps on the snow surface (Table 2.4 and Table 2.5). Yet, the boreal forest stands were not different in the number of debris that was dispersed among stands. Also, fewer moss and lichen propagules dispersed to the snow surface traps in comparison with the debris dispersed to the snow surface (Table 2.5). In addition, Table 2.5 shows that the debris found on the snow surface and the propagules on the trees were dispersed in similar numbers, but there was more debris on the tree traps.

Table 2.5. Tuckey Post Hoc Test (a = 0.05) on the boreal forest treatments (habitat, substratum, and debris and asexual propagules). Different letters indicate differences between and/or among treatments. n = number of replicates, st. error = standard error.

Habitat	Substratum	log (Means)	*n*	st. error	Tuckey Post Hoc Test		
Mixed forest	Snow surface	1.41	28	0.07	a		
Poplar forest	Snow surface	1.74	12	0.11	a	b	
Shoreline forest	Snow surface	1.75	16	0.10	a	b	
Shoreline forest	Tree	1.84	18	0.09		b	
Poplar forest	Tree	1.92	18	0.09		b	
Mixed forest	Tree	2.11	19	0.09		b	
Substratum	Debris	log (Means)	*n*	st. error	Tuckey Post Hoc Test		
Snow surface	moss and lichen fragments	1.34	28	0.08	a		
Trees	moss and lichen fragments	1.65	27	0.08		b	
Snow surface	debris	1.92	28	0.08		b	
Trees	debris	2.27	28	0.07			c

Dispersal on the frozen surface of Payuk Lake

Lichen and moss propagules, as well as other debris, were dispersed onto the petri dish traps placed at different distances (0 m, 2 m, 4 m, 6 m, and 8 m) on the surface of the frozen lake near the lake shore. There were no significant differences in the mean number of debris, overall, away from the source (n = 18, df = 4, F = 0.31, P = 0.87), nor between the mean number of propagules and debris dispersed per distance (n = 9, df = 4, F = 0.29, P = 0.88). However, more debris was dispersed onto the petri dishes on the lake than lichen and moss propagules (n = 40, df = 1, F = 42.68, P < 0.0001).

Size of the lichen propagules

Lichen propagules (soredia and thallus fragments) were trapped and varied in size, ranging from 25 to 3,000 µm in diameter. Most (71%) of the propagules were of smaller sizes (Fig. 2.5.). The frequency of lichen propagules in a size class decreased with an increase in propagule size. However, there was a slight increase in the relative frequency of propagules of the size range, 1,501 to 2,000 µm.

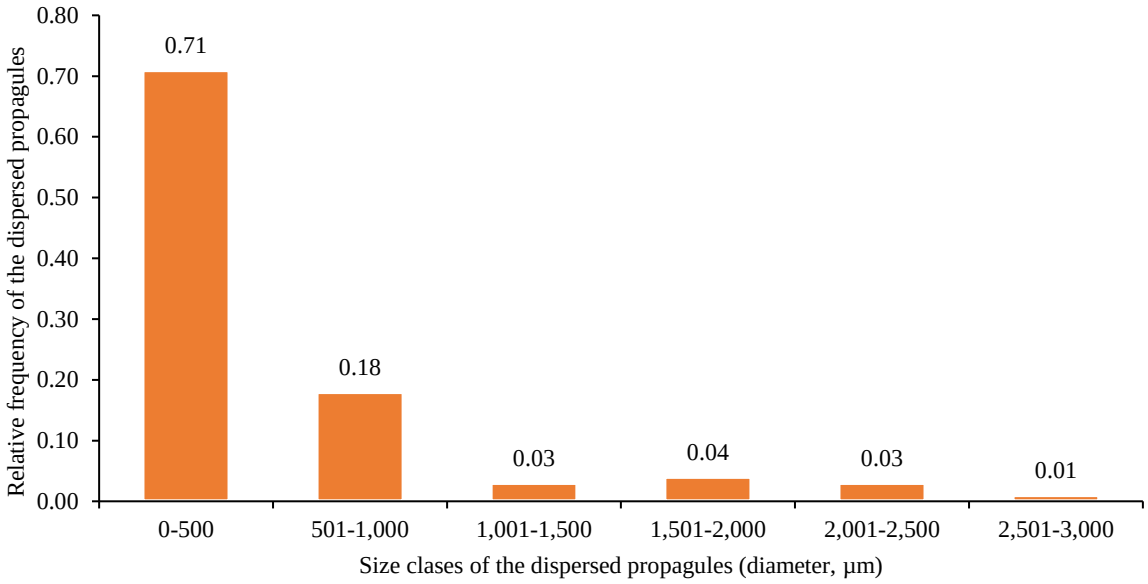

Figure 2.5. The relative frequency of 100 dispersed lichen propagules in different size (diameter) classes. Lichen propagules were from traps placed on Payuk Lake and surrounding forests, Manitoba.

Discussion

Environmental data

During the three-day period of this pilot study, the average wind speeds were recorded to be 18.2 ± 13.0 km·h^{-1}. These speeds are in the range where dispersal of bryophyte and lichen propagules have been recorded to occur. For example, the liverwort *Anastrophyllum hellerianum* (Nees ex Lindenb) R.M. Schust. had both sexual and asexual propagules dispersed by wind speeds ranging from 3.24 ± 2.16 km·h^{-1} to 5.04 ± 2.88 km·h^{-1} (Pohjamo et al. 2006). At such speeds, the propagules were scattered up to 10 m from the source, and Pohjamo et al. (2006) suggested that the dispersal of these propagules could go beyond those distances. It should be noted that the study by Pohjamo et al. (2006) was performed on a log-growing species (*A.*

hellerianum) in an open area, and they suggested that the dispersal of this liverwort may have changed in a more complex habitat. For *H. physodes*, a species present in this study, soredia were dispersed by wind speeds of 37.08 $km \cdot h^{-1}$ and these propagules fell 10–25 m from the source (Armstrong 1987). Furthermore, granules (aereoles) from the lichen *Cladonia pyxidata* (L.) Hoffm. can disperse by wind turbulence from wind speeds ranging from 3.6 $km \cdot h^{-1}$ to 29.16 $km \cdot h^{-1}$, even though the granules produce on the podetia and inside the cups seem to have been selected for dispersal by raindrops (Brodie and Gregory 1953). Propagules produced by bryophyte and lichens in these boreal forests would benefit not only from the dispersal by wind speeds reported during the study period, but also from lower wind speeds.

Lichen and moss diversity in three boreal forest stands during winter

During the winter, mosses and lichens growing on bark and twigs on trees are often unobstructed by snow cover. Cryptogam species on those substrata are affected primarily by wind dispersal by wind fragmenting and dispersing the propagules of these species to other areas. Lichens and mosses growing on the forest floor have limited or no dispersal due to the forest floor being covered with snow. However, of the species on trees, those that have a higher dominance in the forests would most likely be represented in the traps during winter rather than rarer species.

In this study, foliose species comprise about 36% of the lichen species, while the fruticose and crustose lichen comprise 32% of the species. Only one foliose species collected was reproducing sexually as suggested by the presence of apothecia on the lichen thallus (*Tuckermannopsis americana* (Spreng.) Hale), while none of the fruticose species were reproducing sexually. However, six of the eight crustose species had apothecia, indicating that

the crustose species may be benefiting from the dispersal of both meiotically produced spores and asexual propagules during winter. Piercey-Normore (2005) suggested that the dispersal of meiotic spores is advantageous for crustose and foliose lichen species, particularly in disturbed or changing environments.

Most of the species living on tree bark, twigs, and/or branches were dispersing by asexual means (66%), thus dispersal in the boreal forests during winter months happens by asexual means. The asexual propagules collected in the traps may represent species assemblages present in the forest, but establishment of thalli in suitable habitats or substrata may alter the species composition observed in the forest. The local species assemblages represented by the dispersed propagules may be linked to those in the soil bank, but not always (Rydgren and Hestmark 1997, Ross-Davis and Frego 2004, Rydgren et al. 2004, Caners et al. 2009). However, the number of terrestrial species growing on, or above, the soil may differ from the epiphytic species present in the forest (Ross-Davis and Frego 2004). This study suggests that winter dispersal should facilitate the dispersal of species growing on twigs or bark of trees. As a result of such dispersal, the composition of species on the substratum after dispersal and establishment should remain similar over time. However, long distance dispersal events may introduce propagules of species that change can the local flora.

Maintenance of moss and lichen communities as a result of dispersed propagules

The lichen and moss species in the poplar forest have a different species assemblage from that of the conifer forests, and the maintenance of community within the poplar forest may little benefit from the propagules dispersed from conifer stands. In contrast to the communities in the poplar forest, the communities in mixed and shoreline forests can supply one another with

propagules and maintain the diversity in and between the forest stands. However, when considering the similarity between these forest stands, rare moss and lichen species for each forest would need similar forest stands in order to supply other stands with the same propagule species. This is particularly holding true in case of a disturbance altering the species composition of the forest where only the dominant species are present.

Trap type to capture asexual propagules

Two types of traps were used in the forest stands and/or on the lake to gain an understanding of the dispersal of moss and lichen propagules during winter. Of those traps, the plastic petri dishes captured more propagules than the meshed-buckets. This indicates that petri dishes are better at collecting propagules and debris than the meshed-buckets. A reason that the bucket traps were not as effective as the petri dishes may have been the movement of the mesh by wind during the study period. The movement of the mesh by wind could have scattered already captured propagules and debris during the study period. The movement of mesh may have resulted in the low retention of propagules and debris. For winter dispersal, a solid container seems to be the most effective trap type to hold the propagules and other debris being dispersed.

Because of the exploratory nature of this work, there was a low number of traps used for the different treatments. The low number of traps resulted in a high number of outliers in the data. Using a higher number of petri dish traps per studied area may limit the effects of outliers on the data. In addition, a higher number of petri dishes on the trees and on the floor of the different forest types may clarify whether the properties associated with the forest stand affects the number and sizes of propagules dispersed in the boreal ecosystem. The study of dispersal

during seasons other than winter might also prove useful in the entrapment of the propagules and debris as it would disentangle how dispersal may be affected by the environmental conditions related to different times of the year.

Dispersal in the forest stands and on the lake

Moss and lichen propagules were dispersed toward the lake, as well as among the mixed, shoreline, and poplar forests. The dispersal of the debris onto the lake varied as seen by the variable number of propagules captured in buckets facing two aspects of the lake (N and W). This suggests that features of the lake, such as the openness that allow for wind circulation, could cause the dispersal of debris and propagules to be more variable than the dispersal that occurs in the boreal forest stands. In one study, it was shown that propagules and spores have been variably deposited and reworked on the surface of lake sediment due to circulation of snow (Wilmshurst and McGlone 2005). Interestingly, the buckets on the lake trapped fewer asexual propagules than in the forest stands, which either indicate that the propagules quickly settle in the forest stands or are moved across the lake where the structural features of the forest canopy trap asexual propagules (Raynor et al. 1974).

Comparison of dispersed asexual propagules and species on trees

A similar number of propagules produced per forest stand was shown in this study. Although there was no difference in the propagule production per forest stand, propagules seem to be produced in higher quantities by the common bryophyte and lichen species in the forest stands. The more common lichen and moss species in the forest stands may have produced more asexual propagules and have a higher proportion of dispersed propagules than the rare species (Ross-Davis and Frego 2004). The moss and lichen propagules in this study fell near, or on the

trees, rather than on the snow surface since the traps on the snow surface had fewer propagules than those on the trees. The propagules dispersed near trees may stay in the air column near individual trees, or they may have been carried away to other areas (Marshall 1996). This supports the function of asexual propagules, in general, where the asexual propagules are thought to maintain populations of the parent colony.

Propagule and debris dispersal with distance

Dispersal of the propagules and debris by wind should reflect a density-dependence with distance from the source (forest) to an open area, such as the frozen lake if individual propagules were traced. A density-dependent pattern would be expected since the dispersal deposition pattern of moss and lichen propagules have a leptokurtic distribution: most of the dispersed structures fall near the source, with the density of the dispersed propagules decreasing as the distance from the source increases (Wyatt 1977, McQueen 1985, Kimmerer 1991, Miles and Longton 1992, Pohjamo et al. 2005). However, no differences in propagule and debris numbers were found in this study. Since the petri dishes placed on the lake were near the source (0 m to 10 m from the shoreline forest), these distances were expected to have fallen within the peak of the leptokurtic distribution where most of the propagules in a density-distance distribution fell. Similar observations were made by Marshall (1996) on the data set present in Armstrong's study (1992, 1994); where, Armstrong (1992, 1994) did not find a density distribution pattern for soredia dispersal of *H. physodes*. The lack of a certain distribution with distance may be also explained by the variables winds and number of propagules observed for the two aspects (N and W) in the lake, indicating the dispersal of propagules from opposite sides of the lake. This suggestion is also supported by Robertson and Piercey-Normore (2007) who studied gene flow

on *Cladonia arbuscula* (Wallr.) Rabenh. and found that there were haplotypes that were shared among populations located on islands in Payuk Lake with populations located on the margins of the lake, indicating that propagules were dispersed across the lake. The variable number of propagules in traps in the distances studied suggests that traps should be placed at farther distances on the lake to test whether there is a density-dependence of dispersed propagules. However, there is the plausibility that the dispersal of propagules is not density-dependent with distance, but random due to the dispersal of propagules between lake margins and beyond.

Size of the lichen propagules

Propagules that dispersed during winter were mostly of small sizes (< 1,000 µm). In addition, there were more small-sized propagules in comparison with large-sized propagules—particularly those of lichens. Dispersal of small-sized propagules seem to be favored because these propagules are more easily carried by wind than larger propagules.

Interestingly, what was mostly being dispersed was lichen thallus fragments in comparison to other propagules that were also present (moss: gametophyte fragments, leaves, and gemmae; lichen: soredia, soredial clumps, and rhizines). Considering that some of the dominant species (*Evernia mesomorpha, Parmelia sulcata, Hypogymnia physodes, Usnea* spp.) in these boreal forest stands produce small-sized propagules (soredia and isidia), more of these propagules in the traps were expected. For example, soredia of *H. physodes* size ranges from ~25 to 100 µm in diameter (Armstrong 1992), and measurements of soredia sizes from specimens collected show that soredia of *E. mesomorpha* are 21.0 ± 5.6 µm in diameter (n = 10), while soredia of an *Usnea* spp. can be 28.5 ± 7.5 µm (n = 10) in diameter. These propagules should have been abundant in the traps located on the trees due to the dispersal of these propagules

occuring near the colony source (up to 5 cm for *H. physodes*; Armstrong 1994), but the presence of these propagules on the traps was seldom observed. Since the propagules were not observed in the traps, these could have been dispersed to further distances (up to 80 cm away from the source, Armstrong 1994), or, little dispersal may occur for these propagules during winter in these forests. Similarly, Armstrong (1991) found that *H. physodes* soredia does not disperse well during winter and this may account for the low dispersal of this lichen structure in this study.

A few of the lichen and the moss larger-sized propagules (>1,000 µm) were identifiable to genera and even species. For example, traps in the mixed and shoreline forests were observed to have fragments of *Bryoria* spp., *Lepraria* sp., *P. sulcata*, and *Tuckermannopsis americana*; while, *Physcia adscendens* and *Orthotrichum obtusifolium* were observed in the poplar forest. These species had high to medium abundance on the tree branches and/or bark in proximity to the forest in which the study was conducted. Thus, propagule dispersal in the forests may likely reflect the dominance of a few lichen and moss species in the forest. Some of these dominant species fragments were present in the lake traps: gemmae and leaves of *O. obtusifolium* and thallus fragments of *Usnea* spp. were observed in the traps placed on the lake. However, the number of fragments that were identified were fewer on the lake traps, than those trapped in the boreal forests.

Some large-size propagules were dispersed in higher numbers than others, as seen by the slight bimodal distribution of the relative abundance of propagules per size classes (diameter µm, Fig. 2.5). These propagule sizes range from 1,500 to 2,000 µm in diameter. It is unknown why these size ranges were dispersed in higher numbers, but it can be speculated that the area to weight ratio of the propagule may be influencing their dispersal effectiveness more than the propagules larger than 2,000 µm in diameter. The fragmentation and dispersal of large

propagules are not limited to these boreal forests. Goward (2003), showed that in a sub-alpine forest in British Columbia (CA), fragments of large and variable sizes (6 cm, 8, cm, and 38 cm) from *Bryoria* spp. were dispersed onto a meadow up to 2 km away from the propagule source. Furthermore, on the Bathurst Island in Nunavut (CA), high winds have been suggested to move fragments (1 to 10 mm) of mosses onto snowbeds (Miller and Ambrose 1976). The frequency of dispersal of these large fragments seems to be low, but the dispersal of these fragments is pivotal for maintaining the population in these forests.

Considerations for future studies

Some considerations for a better understanding of the dispersal of moss and lichen asexual propagules in this study include the study of dispersal during various time periods and its association with environmental variables. Furthermore, the addition of two more stands for each forest type and an increase in the number of trees sampled for species collection may provide a more precise estimate of the lichen and moss diversity as well as dispersal in these forests. The measurement of forest stand properties (tree aspect, slope of the terrain, tree density, and canopy openness) may clarify their influence on dispersal within or outside of the forest stands. Adding more petri dish traps to the habitats within these sites may also show a better representation of the propagule quantities and sizes dispersed. Because most of the asexual propagules were dispersed within the forest stands, further studies should focus on the dispersal in these forest stands and reveal the factors that facilitate or limit the maintenance of community of bryophyte and lichens in boreal forests.

Conclusion

This study showed that petri dish traps were more effective than the bucket traps at capturing a larger number of asexual propagules produced by bryophytes and lichens, most likely resulting from the asexual propagules not adhering to the mesh and/or by the mesh being lost from the bucket by wind. This study also showed that during winter, wind and low temperatures aided in the dispersal of moss and lichen small-sized propagules in northern Manitoba, Canada. In addition, there were varied numbers of debris items dispersed. The study indicates that the propagules dispersed are representative of the species diversity in these boreal forests. Species with higher abundance (dominance) have larger numbers of asexual propagules dispersed than the species with lower abundance. Therefore, the propagules were likely to have originated from the studied forests rather than other forest stands further away with different assemblages of species. Finally, modifications needed to improve the dispersal study in these locations for the winter season, as well as other seasons, are proposed.

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Chapter 3: Moss and lichen asexual propagule dispersal may help to maintain the extant community in boreal forests

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Abstract

Asexual propagules produced by mosses and lichens may help to maintain their community composition in boreal forests. Understanding the factors affecting the deposition of asexual propagules and their link with the community composition may reveal how the community is maintained. The goal of this study was to understand how weather, the community of lichens and mosses, the dominant tree species in a stand, substrata, and tree aspect influenced and were linked to the deposition of asexual propagules (quantity, size, and type) in boreal forests. Species richness and cover were assessed for the substrata within the tree stands. Traps attached to trees and the ground (substrata) in balsam fir, white spruce, and poplar dominated stands were used to capture asexual propagules. Species and quantity of trapped asexual propagules were linked to the species richness and cover of the extant community. Propagules captured were dominated by lichen thallus fragments and were smaller and in higher quantities during colder times of the

year, and in higher quantities and smaller sizes mostly on trees of conifer stands. Moss propagules were captured in low quantities compared to lichen propagules, on the forest floor, but mostly during warmer times of the year. The dispersal of mosses and lichen asexual propagules helps to maintain and are linked to their community in boreal forests. The linkage between asexual propagule deposition and the community (richness and abundance) was observed among lichen and moss communities on poplar trees, conifer trees, or the forest floor of boreal forest stands.

Key words: Fungi dispersal; Cryptogams; Plant dispersal; Propagule dispersal

Introduction

Moss and lichen communities produce a variety of sexual and asexual propagules. Sexually produced propagules include moss spores and lichen ascospores. Some of these spores can be produced in high quantities, and dispersed spores aid with gene flow among populations (van Zanten 1976 and 1978, Stoneburner et al. 1992, Clayden 1997, Sundberg 2005, Lättman et al. 2009). Asexually produced propagules by mosses and lichens are diverse in types. Moss asexual propagules include bulbils, gemmae, gametophore fragments (leaves, rhizoids, stems), and protonemata (Frey and Kürschner 2011). Lichen asexual propagules include conidiospores, isidia, soredia, and fragments of the thallus (pieces of cilia, rhizines, and/or lobes/branches) (Brodo et al. 2001). The primary biological role of asexual propagules is thought to be colonization and dispersal of species, especially if spores are rare. However, other roles include an increased surface area for photosynthesis while attached to the thallus or gametophore (Tretiach et al. 2005), maintenance of the combination of symbiont partners after dispersal, and/or the facilitation of algal switching among lichens (Robinson 1975, Lange et al. 1989,

Piercey-Normore and DePriest 2001). Dispersal studies on a few types of asexual propagules such as bryophyte stem fragments (Pauliuk et al. 2011), gemmae (Pohjamo et al. 2006), brood branches (Kimmerer and Young 1995), lichen soredia (Stubbs 1995, Marshall 1996), and lichen thallus fragments (Heinken 1999) have suggested that most asexual propagules disperse short distances and the primary role is in the maintenance of populations of nearby sites. The role of asexual propagules on the maintenance of moss and lichen communities in boreal forests may depend on the quantity, size, and type of asexual propagules that are dispersed (Heinken 1999, Goward 2003, Pohjamo et al. 2006); in addition to the frequency of sexually produced spores of species (Pyatt 1969, Sundberg and Rydin 1998). Understanding the variation in quantity, size, and type of asexual propagules dispersed in relation to the moss and lichen communities in boreal forests may reveal how the communities are maintained.

Dispersal vectors and weather (wind speed, temperature, precipitation) are known to affect and link to the dispersal of asexual propagules. For example, higher quantities of variable-sized soredia from *Hypogymnia physodes* (L.) Nyl. were dispersed by wind when wind speeds were high and moisture levels were low under a laboratory setting (Armstrong 1994). Marshall (1996) showed how the quantities of asexual propagules deposited resulted from an increased rainfall, temperature, and wind speeds during early summer and autumn, or no correlations were found during winter. Since an increase in the quantity of dispersed propagules may be correlated with low moisture and an increase in wind speed, it would be expected that patterns in asexual propagule deposition are also linked to the weather in boreal forests of Manitoba (https://goo.gl/GPkoqm, weather.gc.ca).

The deposition of moss and lichen asexual propagules may depend on habitat, which influences the dispersal distance, wind speeds, and effects of water and animals on the movement

of propagules (Heinken 1999, Goward 2003, Pohjamo et al. 2006). Habitat may include open grasslands and two pine forests of different ages (Heinken 1999), open lawn and closed boreal pine forests (Pohjamo et al. 2006), and dominant tree species (forest stand types: deciduous, coniferous, mixed) (Hoyt and Hannon 2002). Forest stand types are habitats for which the canopy structure varies per tree species (Amiro 1990, Parker 1995) and may affect how the moss and lichen communities and propagule dispersal interact with weather. For example, forest stands with a closed canopy have been shown to have less wind turbulence and trap propagules in higher quantities within and below the canopy than open stands (Raynor et al. 1975, Amiro 1990, Parker 1995). Thus, habitat (forest stand type) and vegetation layer (canopy vs. below canopy) can influence the dispersal of asexual propagules. In contrast to the studies above, for the liverwort *Anastrophyllum hellerianum* (Nees ex Lindenb.) R.M. Schust., the distance that asexual propagules travelled was not dependent on the habitat but rather the size of the propagule (Pohjamo et al. 2006). Since most of these studies show that bryophyte and lichen asexual propagule dispersal is affected by habitat (forest stand type), and the size of propagules (Heinken 1999, Marshall and Convey 1997), then an interaction between propagule quantities and size with habitat structure and substratum location might be expected in different forest types: for instance, a forest stand with a higher canopy cover may allow for a higher deposition of asexual propagules.

Dispersal patterns have been shown for a few species by directly studying dispersal in a laboratory or field setting (Bailey 1976, Armstrong 1991, Armstrong 1994, Marshall 1996, McDaniel and Miller 2000), but a larger number of studies have inferred dispersal and gene flow from observed haplotype patterns based on the distribution of species (van der Velde and Bijlsma 2000, O'Brien et al. 2009, Wirtz et al. 2012, Shaw et al. 2014). Understanding the influence of

weather at various times in a year, the species abundance and richness (composition) within communities, and habitat (forest stand types and location of substratum) will shed light on the ability of the existing species to colonize substratum within their own environment (Young and Klay 1971, Marshall and Convey 1997, McDaniel and Miller 2000). Thus, to elucidate how the dispersal of moss and lichen asexual propagules might be maintaining the community composition in boreal forests, the goal of this research was to link the deposition of asexual propagules during three times of the year with the cover and richness of the community of mosses and lichens growing on the forest floor and trees in three types of forest stands (balsam fir, poplar, white spruce).

Methods

Study area

Nine boreal forest stands on the Precambrian Shield in northern Manitoba (Canada), near the eastern shore of Payuk Lake (Appendix, Table B3.1), were selected for this study. Three replicate stands were chosen for each of three types of boreal forest stands (balsam fir, *Abies balsamea* (L.) Mill.; white spruce, *Picea glauca* (Moench) Voss; poplar., *Populus tremuloides* Michx.), which are here defined as three types of habitats. The stands grow in humic to sandy soils or on thin soil over granite-gneiss rock. Detailed information about the forest stands and their properties are found in the Appendix, Table B3.1. Average temperatures recorded during various times of the year ranged from -29 °C to 21 °C (Flin Flon weather station data; see https://goo.gl/GPkoqm).

Four readings (N, S, E, W) at each of three locations within each forest stand (12 readings all together) using a spherical densiometer (Model A, Forest Densiometers, Bartlesville,

OK, USA) were recorded and averaged to obtain canopy cover. Slope (± %) was recorded depending on ease of access from the middle of the stand using a PM-5/360 PC handheld clinometer (Suunto, Vantaa, Finland). Aspect was measured in degrees facing downslope using a MC-2 G Mirror Compass (Sunnto, Vantaa, Finland). Tree Density was determined using BAF 5 and 15 (Basal Area Factor) prisms (Cruise Master Prism, Sublimity, Oregon). Vascular flora species were identified in the field.

Assessing moss and lichen cover and richness

Lichen and moss richness was assessed with two methods in the forest stands in 2015–2016. In the first method, lichens and mosses from all substrata were collected in summer of 2015. The second method employed a 20-m transect placed in the middle of the stand, in which five quadrats (0.5 m^2) were selected from randomly generated numbers between 1–20, to assess the species richness on the forest floor during the summer of 2016. Five dead and/or alive standing trees were also selected at random in each forest stand, and for each tree five alive and dead branches from the lower canopy (~ 1.82 m) were removed to assess moss and lichen richness. Abundance (relative % cover) of both forest floor or bark components (moss, lichen, ground, and/or bark) was assessed during the summer of 2016. The abundance was estimated from the 0.5 m^2 quadrats placed on the forest floor, or malleable tape-made quadrats (0.25 m^2, one per tree) placed on the tree trunk at breast height (1.4 m) of five dead and/or alive trees that were also selected at random. Ground cover included areas occupied by decaying wood, litter, rock, and vascular plant vegetation. Cover values presented here are from the average of three cover estimates for each quadrat. Samples were air dried and placed in packets, brought to the laboratory, and stored prior to identification.

Identification of asexual propagules and species

Identification of mosses and morphology of asexual propagules used the taxonomy of Ireland (1982); while, the identification of lichens and morphology of asexual propagules used Goward et al. (1994), Goward (1999), and Hinds and Hinds (2007). Nomenclature is based on indexfungorum.org for lichens and tropicos.org for mosses. Thin-layer chromatography using solvent A was performed on lichen podetia or squamules of *Cladonia* spp. (Culberson 1972) to confirm their identity. Asexual propagules that displayed defined morphological traits were identified to the lowest taxonomic rank possible, which was limited by propagule size. All specimen vouchers were deposited in the cryptogram herbarium at the Memorial University of Newfoundland, Grenfell Campus (MUN).

Trapping of asexual propagules

Polystyrene Petri dishes (100 mm x 15 mm; Fisher Scientific, Pittsburgh, PA, USA) were used as traps to capture debris that included moss and lichen asexual propagules. The Petri dish was glued at the base to a wooden popsicle stick. Filter paper (Whatman™, 15 mm; Sigma-Alrich, Darmstadt, Germany) that was cut to the dimensions of the petri dish was placed in it. In each forest stand, traps were attached to two substrata, standing trees as well as logs and stumps on the forest floor. Traps were placed at each aspect (N, S, E, W; 10 trees/stand), perpendicular to the trunk of the tree, and facing upward with the wooden stick inserted into small drilled holes at breast height (~1.4 m). Traps were also placed on two sides of logs/stumps in any direction (10 logs/stumps per stand). A total of 60 traps were installed in each stand for a total of 540 traps in nine stands.

Traps were exposed for three-day time periods for each of three times of the year (June 2015, August 2015, February 2016: Table 3.1), air-dried, covered, labeled and boxed, then transported to the laboratory, and stored at room temperature (21.9 ± 0.4 °C, n = 25 days). In some cases, the papers were blown out of the traps, even when the filter papers were glued with petroleum jelly to the petri dishes, and data could not be retrieved from these traps (58 traps, June 2015; 99 traps, August 2015; 71 traps, February 2016). During February 2016, traps were only placed on trees, as snowfall would have covered the traps on the ground.

Recording the quantity, size, and type of asexual propagules

In the laboratory, papers from all traps were examined for debris. Debris containing asexual propagules was placed in water on a microscope slide, the quantities were counted, the size was measured, and the types were identified using dissection (Leica MZ6 mag. 6.3x; Concord, ON, Canada) and compound (Nikon YS100; Melville, NY, USA) microscopes. All of the moss and lichen asexual propagules were classified based on their morphology, and only two moss leaf fragments were classified with uncertainty.

Due to the large quantity of debris placed on the microscope slide, only a subset was used to assess asexual propagule deposition in boreal forests (50 debris). The subset of debris was counted throughout the whole area covered by the glass slip: starting from the top of the cover slip, going left to right, in an S-shaped pattern, until the 50 debris were counted. Of the debris counted, the occurrence of different debris (invertebrates and appendages, leaf pieces, soil, wood pieces) was recorded but only the frequently captured moss and lichen asexual propagules were statistically assessed. The longest dimension (size, µm) of captured moss and lichen asexual

propagules was measured using an eyepiece micrometer under a compound light microscope (Nikon YS100; Melville, NY, USA).

Statistics

Moss and lichen asexual propagule quantity and size data, cover data, and weather data were explored with descriptive statistics. A One-Way Analyses of Variance (ANOVA), followed by Tuckey's HDS pairwise comparison tests ($\alpha = 0.05$), was performed on the stand property data (aspect (˚), cover, % slope, tree diameter, tree density) as well as the weather data (relative humidity, %; temperature, °C; wind direction, 10s degrees: direction of the maximum gust from which the wind blows from the geographic north pole; and wind speed, km/h).

To assess how the community of lichens and mosses within a stand might impact asexual propagule dispersal, and vice versa, the lichens and mosses association of the boreal forest stands were analyzed with hierarchical clustering analyses. The hierarchical clustering analyses (HCA) was performed with 100,000 bootstraps and the unweighted pair-group method algorithm, using the arithmetic average and analyzed with the Jaccard's similarity coefficient on the species presence/absence data for each stand. The presence (1) and absence (0) of the 127 lichens (81) and mosses (46) collected during the summer of 2015 and 2016 from the nine stands and the within stand substrata (trees and forest floor) were analyzed with HCA on the software Past v. 3.16 (Hammer et al. 2001). For the list of species collected see the Appendix, Table B3.2. The relative frequency (%) of each asexual propagule type captured was assessed visually via a stacked column graph for each substratum, forest stand type, and time of the year.

Kruskal-Wallis and/or Conover-Inman non-parametric tests were used on the data (quantity data was added X +1, since zeroes were present), since it is less stringent about the data distribution (Kruskal and Wallis 1952, Conover 1999). Kruskal-Wallis and Conover-Inman tests

were performed on the average cover data of each stand, and evaluated if there were difference in moss, lichen, and ground or bark cover, as well as their nested interaction with the forest types. Kruskal-Wallis tests, followed by Conover-Inman pairwise comparison tests, ($\alpha = 0.05$) also tested if the asexual propagule quantity or size significantly differed among the time of the year, forest stand types, substrata or tree aspects, and their nested interactions. Since February 2015 traps were placed only on trees, two types of data emerged that accounted for the variance observed within the substrata data (log/stump and tree) or just the tree aspect data within the highest nested data (times of year and forest stand types). Since February 2015 only had the tree aspect data, the tree aspect data were analyses using the highest nested data. All ANOVAs and Kruskal-Wallis/Conover-Inmam tests were carried out with Infostat (Di Rienzo et al., 2015).

Results

Stand properties and weather

Balsam fir, poplar, or white spruce forest stands were not different with respect to aspect (°; $F_{2, 6} = 1$, $R^2 = 0.3$, $P = 0.331$), % slope ($F_{2, 6} = 0$, $R^2 = 0.1$, $P = 0.765$), and tree density (#trees/hectare; $F_{2, 6} = 2$, $R^2 = 0.4$, $P = 0.189$). The average canopy cover was marginally different among forest stands ($F_{2, 6} = 5$, $R^2 = 0.6$, $P = 0.052$): poplar stands had the lowest canopy cover, balsam fir had the highest cover, and the cover of white spruce stands was similar to the cover in the poplar and balsam fir stands (Appendix, Table B3.1).

Low relative humidity was observed in February 2016 and June 2015, but it was higher in August 2015 (%; $F_{2, 212} = 4$, $R^2 = 0$, $P = 0.024$). Wind speed was low in August 2015 and June 2015, and high in February 2016 (°C; $F_{2, 212} = 9$, $R^2 = 0.1$, P 0.0001). Temperature was lowest in

February 2016, increased for August 2015, and was the highest in June 2015 ($F_{2, 212} = 576$, $R^2 = 0.8$, $P < 0.0001$; Table 1). Wind direction varied during the study ($F_{2, 212} = 2$, $R^2 = 0$, $P = 0.1347$).

Table 3.1. Weather data (average ± standard error) for three times of the year during the three-day periods when the asexual propagules of mosses and lichens were captured in boreal forests around Payuk Lake in Manitoba, Canada. Different superscript letters indicate significant differences between times of the year according with the untransformed weather variable tested (Tukey's Test, $p < 0.05$).

Time of year	Relative humidity (%)	Temperature (°C)	Wind direction (10s deg)	Wind speed (km/h)
25–28 June 2015	62.5 ± 4.5^{ab}	21.8 ± 2.2^{a}	23.2 ± 3.1^{a}	10.3 ± 2.5^{a}
23–26 August 2015	68.3 ± 4.3^{a}	17 ± 2^{b}	20.3 ± 2.8^{a}	10.1 ± 2.1^{a}
25–28 February 2016	60.8 ± 3.4^{b}	-12 ± 3^{c}	20.1 ± 3.6^{a}	14.2 ± 2.9^{b}

Note: 10s deg = direction of the maximum gust from which the wind blows: e.g., 9 means 90 degrees true or an east wind, and 36 means 360 degrees true or a wind blowing from the geographic north pole (https://goo.gl/pq0rpR).

Moss and lichen richness and abundance in boreal forest stands

A hundred and twenty-seven lichen and moss species, 81 lichens and 46 mosses, were collected in the boreal forest stands from the 2015–2016 collections. Forty-nine lichens and 43 mosses occurred on the forest floor, while 37 lichens and three mosses occurred on trees. Mosses well represented in these habitats included *Bryum* (2–4 spp.), *Brachythecium* (5 spp.), and *Dicranum* (8 spp.). Lichens well represented in these habitats included *Cladonia* (24 spp.), *Peltigera* (9 spp.), and *Usnea* (4 spp.). Fifty-six species were found on average per stand (range 32–83).

The community of mosses and lichens shared little to no species between the forest floor and tree substrata (bootstrap support, 100; Fig. 3.1). Within the tree community, poplar stands shared some species with the conifer stands, but the poplar community was also distinct from that in conifer stands. The community on the forest floor shared many species between the poplar stands and conifer stands, but the community in the poplar stands were slightly clustered away from the conifer stands. The community on the forest floor of the conifer stands shared many species as suggested by the indistinct clustering of balsam fir and white spruce stands.

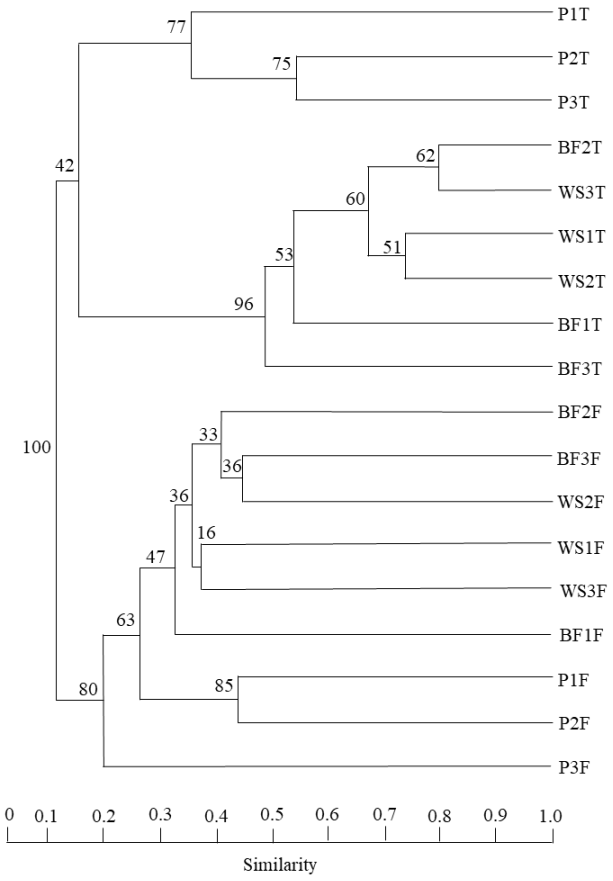

Figure 3.1. Cluster analyses of the presence and absence of lichen and moss species collected in the summer 2015 and 2016 from tree (T) and forest floor (F) substrata in nine tree dominant stands in boreal forests, which include three balsam fir (BF), three poplar (P), and three white spruce stands (WS) in Manitoba, Canada.

Moss and lichen cover varied among taxa (Appendix, Table B3.3), with lichen and moss cover on the forest floor reaching 1% for some species (*Campylium hispidulum* (Brid.) Mitt., *Platygyrium repens* (Brid.) Schimp.); while, other species formed extensive mats in quadrats such as *Cladonia amaurocraea* (Flörke) Schaerer (up to 50%) and *Hylocomium splendens* (Hedw.) Schimp. (up to 62%). Poplar stands had higher ground cover than the conifer stands, and moss and lichen had the lowest cover on the forest floor in all stands (Table 3.2, $P < 0.0001$). Bark cover was higher than moss and lichen cover on trees ($P < 0.0001$). However, lichen cover was higher on bark of the three types of stands than moss cover, with moss cover delimited to poplar stands ($P < 0.0001$).

The species and types of asexual propagule captured

Only a few (large) asexual propagules could be identified to species level (Table 3.3). Two epiphytic mosses, seven ground-dwelling mosses, 11 epiphytic lichens, and two ground-dwelling lichens were identified. Asexual propagules of epiphytes were captured in higher quantities (375) than ground-dwelling species (34). *Bryoria* spp. (57), *Evernia mesomorpha* (99), and *Parmelia sulcata* (103) were species with numerous propagules captured on trees, while other species had 1–35 asexual propagules and were absent from most forest stand types and substrata (Table 3.3). Of the ground-dwelling species, *Cladonia* spp. (cup-bearing lichen), *Sanionia uncinata*, and *Pleurozium schreberi* had the highest quantities of propagules in traps and were mostly found in conifer stands.

Table 3.2. A nested Kruskal-Wallis (*H*) test on moss, lichen, and ground or bark components of cover (percent) assessed during the summer of 2016 in boreal forests around Payuk Lake in Manitoba, Canada. Compared the cover of forest type (balsam fir, poplar, white spruce), floor component, bark components, and their interactions. Different letters indicate significant differences among ranks (*P* = 0.05; Conover-Inman pairwise tests).

Location	n	Avg. cover ± st. error	Ranks	df	*H*	*P* value
Forest type: Floor components						
poplar × moss	10	7.6 ± 2.1	18.1a	8	48	<0.0001
poplar × lichen	7	9.8 ± 3.4	21.5ab			
balsam fir × lichen	6	13.7 ± 7.8	23.5ab			
white spruce × lichen	10	41.58 ± 12.4	47.1bc			
white spruce × ground	11	42.9 ± 10.4	47.6bc			
balsam fir × moss	15	44.4 ± 6.7	53c			
white spruce × moss	12	51.1 ± 11.6	56.8c			
balsam fir × ground	14	53.8 ± 7.0	59.5c			
poplar × ground	15	90.4 ± 2.9	84.93d			
Floor components						
Lichen	23	24.6 ± 6.5	33.1a	2	21	<0.0001
Moss	37	36.6 ± 5.4	44.8a			
Ground	40	64.5 ± 5.0	65.8b			
Forest types: Bark components						
poplar × moss	3	1	5a	6	69	<0.0001
white spruce × lichen	11	4.2 ± 1.3	13.2a			
poplar × lichen	9	6.6 ± 2.	15.4a			
balsam fir × lichen	15	36.4 ± 6.6	33.6ab			
balsam fir × bark	15	63.5 ± 6.9	43.6b			
poplar × bark	15	95.8 ± 1.5	67.4c			
white spruce × bark	15	97 ± 1.1	67.8c			
Bark components						
Moss	3	1	5a	2	54	<0.0001
Lichen	35	18.6 ± 3.95	22.5a			
Bark	45	85.5 ± 3.3	59.6b			

Note: n = number or replicates, Avg. = average, st. error = standard error, df = degrees of freedom, *H* = Kruskal-Wallis test

Table 3.3. Quantity of asexual propagules frequently captured (2015–2016), which could be identified to the genus or species, from traps in the different forest stand types and substrata in boreal forests around Payuk Lake in Manitoba, Canada.

Habit	Taxa	Species	Balsam Fir		White Spruce		Poplar		Total
			Log/Stump	Tree	Log/Stump	Tree	Log/Stump	Tree	
Epiphytic									375
	Lichen	*Bryoria* spp.	3	23	1	27	0	0	54
		Bryoria furcellata	0	1	0	0	0	0	1
		Bryoria fuscescens	0	2	0	0	0	0	2
		Evernia mesomorpha	1	41	0	53	0	4	99
		Hypogymnia physodes	2	14	0	12	0	0	28
		Lecanora allophana	0	1	0	1	0	0	2
		Lecanora circumborealis	0	0	0	1	0	0	1
		Melanelia spp.	0	0	0	1	0	0	1
		Melanelia subaurifera	0	1	0	0	0	0	1
		Parmelia sulcata	1	63	0	38	0	1	103
		Physcia spp.	4	4	0	7	1	1	17
		Physcia adscendens	0	1	0	4	0	0	5
		Physcia aipolia	0	0	0	1	0	0	1
		Tuckermanopsis americana	0	7	0	4	0	0	11
		Usnea spp.	0	17	0	18	0	0	35
	Moss	*Orthotrichum obtusifolium*	0	0	0	0	0	7	7
		Platydictya subtilis	0	0	0	5	0	2	7
Ground-Dwelling									34
	Lichen	*Biatora vernalis*	0	0	0	1	0	0	1
		Cladonia spp.	4	0	2	1	0	0	7
	Moss	*Brachythecium* spp.	1	0	0	0	0	0	1
		Dicranum polysetum	0	0	0	0	0	1	1
		Eurhynchium pulchellum	1	0	1	0	0	0	2
		Mnium spinulosum	1	0	0	0	0	0	1
		Mnium spp.	1	0	0	1	0	0	2
		Pleurozium schreberi	1	0	4	0	0	0	5
		Pohlia nutans	0	0	0	1	1	0	2
		Sanionia uncinata	3	0	1	1	0	0	5
		Sanionia uncinata/Pylasiella polyantha?	4	0	0	0	0	0	4
		Sphagnum spp.	0	0	1	0	0	0	1

The types of asexual propagules captured included lichen thallus fragments, isidia, rhizines, soredia, and moss gametophore fragments, gemmae, and leaves or pieces of leaves (Fig. 3.2). Lichen thallus fragments were captured in all forest stand types and substrata, and had the highest frequency of the types of propagules captured (74%–98%) throughout the sampling periods. Lichen rhizines were captured in all forest types (up to 7%), but not on all substrata during the different times of the year. Lichen isidia and soredia were captured in low frequency mostly during the colder times of the year. Moss gametophore fragments were deposited in higher frequency on logs/stumps than on trees (1%–16% and 2%–10%, respectively), while moss leaves were captured in most substrata of forest stands (0%–10%). Moss gemmae were only captured in traps on poplar trees (1%).

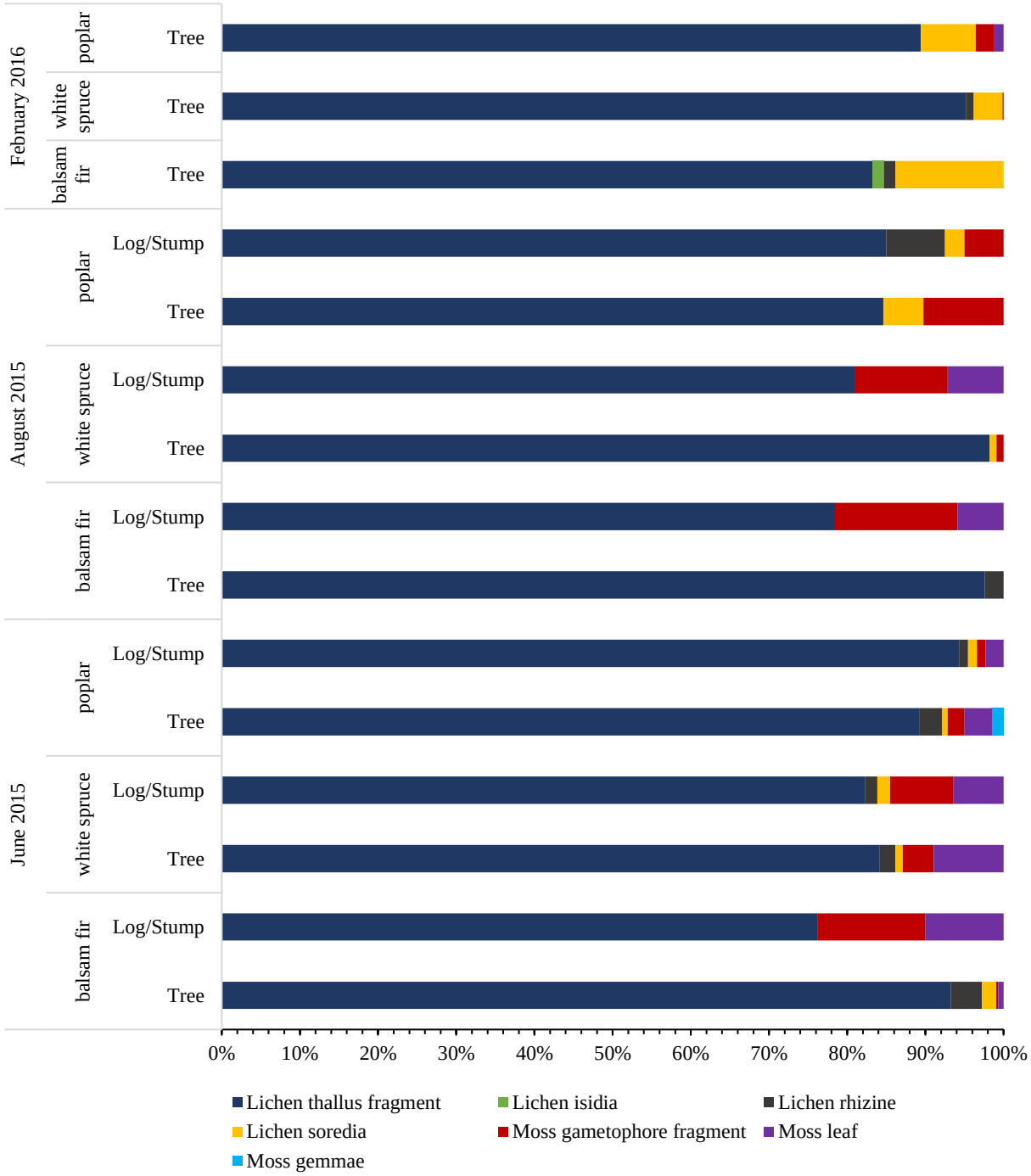

Figure 3.2. The relative frequency of identifiable bryophyte and lichen asexual propagule morphological types captured during different times of the year (2015–2016) on the substrata of the different forest stand types in boreal forests around Payuk Lake in Manitoba, Canada.

The quantity and size of asexual propagules captured

Asexual propagules were on an average of 3.0 ± 0.1 (standard error; range, 0–48) out of 50 debris counted for each trap; however, asexual propagules outnumbered other debris with 48 asexual propagules in one trap from August. Traps placed during February and June captured similar quantities of asexual propagule, but these times of the year had higher quantities of asexual propagules captured than in August (Appendix, Table B3.4; $P < 0.0001$). Conifer stands had similar quantities of captured asexual propagules, and both stand had larger quantities of propagules captured than in poplar stands ($P < 0.0001$). A larger quantity of asexual propagules were captured in traps on trees than those on the logs/stumps on the forest floor (Appendix, Table B3.4; $P < 0.0001$). The quantities of asexual propagules did not differ among the tree aspects. All of the nestedness (interactions) variables were important in affecting the quantities of asexual propagules captured (Appendix, Table B3.4).

The size of the captured asexual propagules in boreal forests ranged from 0.01 to 55 mm, but most asexual propagules captured were of relatively small sizes (average and standard error: 0.9 ± 0.1 mm). The sizes of asexual propagules captured did not differ among the forest stand types, nor tree aspects. (Appendix, Table B3.5). However, asexual propagules of smaller sizes were captured during February than during June and August, and smaller propagules were found on tree traps than on log/stump traps ($P< 0.0001$). All of the nestedness (interactions) variables were important in affecting the size of asexual propagules captured (Appendix, Table B3.4).

Discussion

Propagule captured may be linked to their communities in boreal forests

Distinct moss and lichen communities were associated with poplar trees, conifer trees, or the forest floor of boreal forests, in part influencing the community composition within the stands via the dispersal, establishment, and colonization from propagules (species and their genotypes) (Ross-Davis and Frego 2004, Barbé et al. 2016 a,b, Kövendi-Jakó et al. 2016). The influence of the community composition along with their cover explains the low abundance of species and asexual propagules found within the debris captured, but also how similar the captured propagules are to the communities detected within the boreal forest stands.

The identified asexual propagules were mostly found on tree traps and pertained to epiphytic lichens (Table 3.3). *Bryoria* spp., *Evernia mesomorpha* Nyl., *Parmelia sulcata* Taylor, and *Usnea* spp. are epiphytic species that reproduce primarily by asexual means (fragments of the thallus, isidia, soredia) and that had higher quantities of asexual propagules in traps than other epiphytic lichens identified, suggesting that these species produce and dominate asexual propagules dispersed to maintain their populations when compared to species that reproduce sexually (*Physcia* spp., *Lecanora* spp., and *Tuckermanopsis americana* (Spreng.) Hale) (see Piercey-Normore 2005). The ground-dwelling species, which were mostly mosses, were occasionally found in the traps on the forest floor (logs/stumps) and were rarely observed in tree traps. Though the ground-dwelling species can form extensive mats that cover the floor of boreal forests (Clayden and Bouchard 1983), the occasional presence of propagules found in the traps indicate that these species (*Pleurozium schreberi* (Brid.) Mitt., *Dicranum polysetum* Sw., *Sanionia unicanata* (Hedw.) Loeske, and *Cladonia* lichens) might not rely as much on asexual propagule dispersal and establishment for population maintenance, but rather on horizontal expansion and/or mixed reproductive strategies (Lloret 1994, Frego 1996, Halvorsen 2000, Sundberg 2002, Maciel-Silva 2016).

The frequency of asexual propagules types captured during the times of the year (Fig. 3.2) is explained by the growth and senescence of mosses and lichens. Lichens are adapted to photosynthesize and grow both during warm and cold times of the year (e.g., -20–5 °C during periods of high humidity; McCarthy 1989, Pannewitz et al. 2003, Barták et al. 2007), which explain their higher frequency in traps throughout the year—particularly during winter. Bryophyte gametophytes have high growth rates during the warmer times of the year (Skre and Oechel 1981, Mironov et al. 2018), which explains the production and dispersal of propagules in higher frequency than bryophytes that are frozen or covered with ice during colder times of the year (Barbé et al. 2017).

More and smaller propagules were captured in February and June than in August

Moss and lichen asexual propagules of mostly small sizes were dispersed in different quantities at different times of the year, indicating the linkage between weather and asexual propagule production and dispersal (Barbé et al. 2017). A decreased humidity and temperature decreased the water weight of the asexual propagules via drying, while an increased wind speed facilitated propagule fragmentation and dislodgment (Armstrong 1994), which explains the increased deposition of propagules in February and June than in August. Alternatively, biological processes such as the senescence of a lichen thallus and moss gametophyte (McCarthy 1989), developmental times for asexual propagules (Marshall 1996), and animal interactions that may loosen or consume/disperse some of the propagules (Heinken et al. 2001, Barbé et al. 2016a,b) played a role in the quantity and size of propagules which were available for dispersal.

Boreal forest stand types affect the quantity of propagules captured

Stand type (balsam fir, white spruce, and poplar) affected the quantity but not the size of asexual propagules captured, suggesting that weather may not create a strong enough selective force to filter asexual propagule sizes in different habitats (see Heinken 1999). While aspect (°), average canopy cover (%), % slope, and tree density (#trees/hectare; Appendix, Table B3.1) could not explain differences in propagule quantities in these forest stands, the higher canopy cover of conifer stands than that of poplar stands does explains the higher asexual propagule quantities captured. Raynor et al. (1975) also showed that most propagules (spores) when dispersed from high among the canopy and along a vertical gradient are intercepted by a higher tree foliage density. This study and Raynor et al. (1975) reveal the influence of tree canopy density within the forest stand types in the deposition of propagules.

More propagules were captured on tree traps than ground traps

The deposition of the propagules in higher quantities on trees rather than on the forest floor traps is supported by this study (asexual propagules) and Raynor et al. (spores; 1975). The high quantities of asexual propagules in the tree traps than in forest floor traps is explained by the higher wind speeds in the canopy facilitating the fragmentation and dispersal of epiphytic species, but these asexual propagules may remain in the air and then get attached to the trees, leaving only a few propagules fall to the forest floor (Parker 1995). Raynor et al. (1975), in contrast to our study, showed that propagules of large sizes were intercepted by trees, while small-sized spores vertically travelled further along the air and deposited on the forest floor. The opposite might be true for bryophyte and lichens asexual propagules in boreal forests. However, further research is needed to unveil the quantity and sizes of asexual propagules that are

intercepted in the different areas (stem, leaves, branches) within the forest canopy to understand the relationship with the location of moss and lichen colonies and their dispersal of propagules.

The low quantities of asexual propagules captured on the forest floor may be explained by the airboundary layer surrounding and protecting the bryophyte gametophore and the lichen thallus from incoming wind (Marshall 1996, Bohuslavová et al. 2018), the reduced force of the wind on the forest floor (Parker 1995), and the dispersal of asexual propagules in proximity to the parental colony (Frego 1996) that limited the dispersal of asexual propagules.

Conclusions

This study shows that the communities of mosses and lichens (diversity and cover) were more similar between the trees of coniferous stands rather than those in the poplar stands, suggesting that the dispersal of asexual propagules may in part help with the maintenance of the communities of mosses and lichens only if dispersed within the coniferous or the poplar stands but rarely among them. The moss and lichen communities on the forest floor might be maintained if the stand receives propagules from forest stands with similar community composition. Asexual propagules were dispersed and captured in higher quantities and smaller sizes during the colder times of the year, and mostly in higher quantities and smaller sizes on the trees of coniferous stands, indicating that weather, the properties of the forest stand, and the communities associated with the forest stands influence which asexual propagules are dispersed and where they are deposited in boreal forests.

Acknowledgments

Special thanks to U. Aden, K. Fontaine, K. Chhoker, S. Arekar, C. Deduke, J. Otisi, M. Zraik, Tiago Booth, A. Dang, and A. Dufresne who assisted with field or laboratory work. We are indebted to J. Doering who assisted in collecting the forest property data used in this study. Funding for this study was obtained from the Department of Biological Sciences and the Faculty

of Graduate Studies (University of Manitoba) and from the Natural Sciences and Engineering Research Council of Canada (NSERC-DG to MPN).

CPL, RB, and MPN conceived the study project. CPL, TB, RB, and MPN designed the experiments. CPL, TB, and MPN set up the experiments in the forests. MPN gathered the forest stand type data (tree density/area, slope, canopy cover, vascular plant). CPL and MPN collected the cryptogam species and/or cover. CPL identified lichens and mosses, collected and analyzed the data from the cover, community composition, traps, and weather data. RB and MPN verified the identified species. All authors read, wrote, and edited the manuscript.

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Chapter 4: Wind tunnel dispersal of boreal lichen and moss asexual propagules shows a strong letptokurtic distribution

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Abstract

Bryophytes and lichens produce asexual propagules in different numbers and sizes that are dispersed mainly by wind. Habitat and geographic location of the lichen and bryophyte species, the propagule size, dispersal vector (wind), and propagule establishment influence their population composition by allowing propagules to be dispersed near, and maintain, the parent colony, or be moved to new areas. However, little is known about the dispersal of asexual propagules produced and how they may influence the composition (abundance and richness) of boreal moss and lichen in a habitat. The goal of this study was to test whether the size of asexual propagules impacts dispersal distance by wind. Moss gametophore and lichen thallus material of nine species from tree and forest floor (ground) were collected from boreal forest stands in Manitoba, Canada. In the laboratory, the material was crushed to form varied-sized propagules, which were exposed to horizontal winds (9.05 ± 0.06 km/hr) in a wind tunnel experiment. Propagules were deposited on a one-meter tape extending away from the fan. Dispersal of these

species was studied by assessing if there were significant associations of number and size of asexual propagules with distance traveled (cm) away from the fan. Asexual propagule number and size were compared between epiphytic and ground-dwelling species, and between mosses and lichens. Results showed that small fragments (204.1 ± 5.8 μm) of the moss gametophore and thallus fragments of lichens were mostly dispersed within 10 cm from the fan. The size and number of propagules decreased with an increase in dispersal distance. However, the ground rather than epiphytic species, and mosses rather than lichens had a stronger negative association between numbers or sizes of propagules and distance, suggesting their limited dispersal in boreal forests. This study suggests that dispersal limitations may influence the population composition of these boreal species, with the dispersed propagules allowing the maintainence of the population of the propagule source.

Key words: bryophyte ecology; diaspore; plant dispersal; soil bank; wind tunnel

Introduction

Wind-dispersed propagules vary in number and size, and in addition to other abiotic and biotic interactions (competition, herbivory, climate), influence the lichen and moss composition in a landscape (Ross-Davis and Frego 2004, Hedenås et al. 2003). Once the propagules have been deposited, the ability for the propagules to become established may influence the species presence away from the colony (Sillett et al. 2000, Werth et al. 2006). Understanding the ecological roles of wind-dispersed propagules with respect to the population source (parent colony) has been pivotal in addressing how lichen and moss populations are maintained, changed, or go extinct. For example, Heinken (1999) studied lichen fragment dispersal from

Cetraria muricata (Ach.) Eckfeldt and five *Cladonia* species, and found that these fragments were dispersed by wind and animals to distances which varied according to habitats with different vegetation types. The fragments were dispersed to a maximum distance in a dry sand grassland (970 cm), and shorter distances in an open pioneer pine forest or old-growth pine forest (7cm) (Heinken 1999). Heinken's (1999) study suggests that features of the propagules (number, size, and morphology) and landscape properties affected the effective dispersal distance of propagules on the landscape. Because effective dispersal results in the deposition of the propagules at different distances from the parent colony, dispersal limitations select against establishment of some species allowing for other species to colonize suitable habitats. Dispersal limitations create dissimilarity in species abundance and richness (composition) with distance (Dettki et al. 2000, Lenoir et al. 2012), and a patchy occupancy based on the availability of suitable habitats (Belinchón et al. 2017). A better understanding of the linkage between dispersal and deposition away from the parent colony may elucidate some of the factors that drive their presence in a habitat.

Habitat structural differences may influence the effective dispersal of the propagules associated with lichen and moss species by intercepting wind and interfering with its velocity and direction. Low wind velocities on the ground of the forest floor, the air boundary layer, and the presence of litter may alter the dispersal capacity of asexual propagules of ground-dwelling species (Gregory 1973, Marshall 1996, Eldridge and Leys 1999). In contrast to ground-dwelling species, epiphytic species of mosses and lichens may also be affected by the air boundary; however, they would not be expected to be as constrained in their dispersal as ground-dwelling species because they are higher above the ground and intercept higher wind velocities. High wind velocities against tree trunks or the canopy may cause propagules to become easily

detached, fragmented, and dispersed. The higher wind velocities in the canopy may allow for propagules to be dispersed in higher numbers due to increased frequency of detachment and fragmenting, and to further distances, until the propagules settle on trees and/or the forest floor (Reynor et al. 1975, Parker 1995, Marshall 1996, Dettki 1998). The habitat differences between ground and tree species may allow for propagules associated with each habitat to have different dispersal capacities. The effectiveness of wind dispersal may depend on the number, size, and morphology of propagules of bryophyte and lichens from different substrata and habitat, but it needs further study.

Studies suggest that sexually produced propagules (meiotic spores) correspond with long distance dispersal, population formation, and increased genetic variation. On the other hand, the dispersal of asexually-produced propagules (lichens: isidia, soredia, and thallus fragments; mosses: brood cells, bulbils, gemmae, leaf and stem fragments) correspond with the maintenance of a population or colonization of substrata adjacent to the parent colony (van Zanten 1976, van Zanten 1978, Armstrong 1991, Marshall 1996, Heinken et al. 2001, Goward 2003, Frey and Kürschner 2011). The larger size of asexual propagules compared with that of spores may limit their dispersal distances by wind. However, an increasing body of literature is suggesting that wind dispersal distances of asexual propagules is highly variable, which may affect their role in subsequent population composition, more than previously thought (Young and Clay 1971, Hedenås et al. 2003, Pasiche-Lisboa and Sastre-De Jesús 2018). For example, even for the same type of asexual propagule (lichen thallus fragments) there may be differences in wind-dispersed outcomes due to *Bryoria* having a smaller size than *Alectoria sarmentosa* Ach. (Goward 2003). Larger numbers of fragments of *Bryoria* were observed to disperse farther than those of *A. sarmentosa* (Goward 2003). As a result, Goward (2003) suggested that *A. sarmentosa* had a less

effective dispersal of the two taxa, even when *A. sarmentosa* is a dominant species in the forests. Furthermore, Armstrong (1987) showed that dispersal outcomes may vary for different asexual propagule types produced in the same lichen, with soredia dispersing further distances than thallus fragments of *Hypogymnia physodes* L. (Nyl.). Because more variation is apparent in the types and structure of asexual propagules than sexually produced propagules (spores), there might be differences in dispersal outcomes that allow for differences in the way populations maintenance occur. If the population composition depend on propagule dispersal, we need to better understand how the structural variability of propagules associated with a species affects their dispersal.

The main goal of this study was to evaluate the horizontal dispersal distance of moss and lichen asexual propagules using nine species from the forest floor and trees, by simulating wind dispersal in a laboratory setting (wind tunnel experiment), to gain insights into how wind-dispersed propagules may influence their presence away from the parent colony in boreal forests. The more specific objectives were 1) to describe the number and size of propagules dispersed and their distribution one-meter away from the fan because of the low number of propagules after this distance, 2) to determine the association between the number and size data with the distance from the fan, and 3) to investigate how the average propagule number and size change, with respect to specific distances away from the fan (3, 33, 66, 99 cm).

Methods

Plant material

Lichen and moss mats of nine species were collected from three boreal forest stands (balsam fir, poplar, and white spruce) in Manitoba (Pasiche-Lisboa et al. 2018) to be used for their

propagules. The mats were left to dry in ambient temperature before use. The species included five lichens: *Cladonia amaurocraea* (Flörke) Schaer., *C. arbuscula* (Opiz) Brodo, *Evernia mesomorpha* Nyl., *Tuckermannopsis americana* (Spreng.) Hale, *Usnea* spp. (*U. filipendula* Stirt., *U. hirta* (L.) F.H.Wigg., *U. subfloridana* Stirt.). The species also included four mosses: *Dicranum polysetum* Sw., *Hylocomium splendens* (Hedw.) Schimp., *Orthotrichum obtusifolium* Brid., and *Pleurozium schreberi* (Brid.) Mitt. (see Appendix Table C4.1). *C. amaurocraea*, *C. arbuscular*, *D. polysetum*, *H. splendens*, and *P. schreberi* are species found on the forest floor substrata while the other species are epiphytic. The species identification and nomenclature for lichens followed Hinds and Hinds (2007), and the mosses followed that of Ireland (1982).

Experimental settings

A fan (Home-Line 16", Oscillation fan; Canarm, Brockville, Ontario, C.A.) was used to simulate wind dispersal events in a laboratory setting (21.6 °C, Appendix Fig. C4.1). The fan speed setting was measured 26 cm high and 13 cm in front and center of the fan with an anemometer (Kestrel 100; Kestrel Meters, Minneapolis, Minnesota, U.S.A.) prior to the experiment. The measured winds speeds were 9.05 ± 0.06 km/hr. Lichen or moss dry material of the respective species, was manually ground in a ceramic mortar and pestle for up to five minutes to form different sized propagules such as pieces of the lichen thallus, whole soralia, detached soredia, pieces of moss gametophore and leaves, whole leaves, and detached moss gemmae, which were placed in a plastic bag for each species. Due to the structural properties of the lichen thallus or the moss gametophore, some species may crush more easily than other species. However, a time period of five minutes and visual comparison of propagule sizes, were used to ensure consistency between species of the material used for the dispersal experiment.

Dry material in each bag was mixed to ensure randomized sizes of the propagules were represented in each replicate. Dry material, 1.25 ml (measuring spoon), was placed 26 cm high and 13 cm in front and center of the fan on a microscope slide, in a square area (2 x 2.2 cm) that was designated as the propagule source. The fan was turned on and let adjust for 30 seconds before exposure. Dry material was then exposed to the fan mid-wind velocities (9.05 ± 0.06 km/hr, n =15) over a period of three minutes to ensure propagules were lifted and dispersed during that time. Some propagules dispersed onto the sticky side of a one-meter transparent tape (1.8 cm width) that was placed horizontally in the front and center of the fan (Appendix, Fig. C4.1). Some propagules dispersed beyond and to the sides of the tape, which were not included in the analyses. Only those propagules stuck on the tape were measured. The one-meter distance was used because most propagules fell within that distance, and were rarely seen beyond that distance. When the propagules were collected, the tape was sealed with an additional layer of tape to limit the movement of captured asexual propagules. Then the area was cleaned, and the experiment was repeated until all replicates were completed for each species.

Each one-meter tape with propagules was assessed under a compound microscope. For every centimeter of tape, starting from the tape closest to the fan, the number and size of asexual propagules were counted and measured. Since the high density and the overlap of propagules in the first two centimeters limited the accuracy of the number of deposited propagules, the propagules were counted from 3 to 100 centimeters. A total of 2,646 data points were gathered for the number data (nine species × 98 centimeters × three replicates per species).

The size (longest dimension, micrometer = μm) of captured bryophyte and lichen asexual propagules was measured using an eyepiece micrometer under a compound light microscope (at 4×, Nikon Eclipse E200; Melville, New York, U.S.A). Propagules with less than 25 μm in

diameter were not assessed in this study because of difficulty to accurately estimate the propagule dimensions at higher magnifications. The size of the asexual propagule was only measured for the first 48 cm, excluding centimeter one and two, because the propagules were rarely in enough numbers to be measured beyond those distances. For each centimeter, the size of five randomly selected propagules were measured giving a total of 240 data points for each replicate of a species. A total of 6480 data points were gathered for the size data (nine species × 48 centimeters × five sizes × three replicates per species).

Statistics

Descriptive statistics were used to detail the traits associated with the propagule number and size (Tables 4.1 and 4.2). Data were also visually assessed with scatter plots (x-axis, distance cm; y-axis, number or size data) to understand the distribution of number (discrete data) or size (continuous data) of propagules wind-dispersed by the fan from the propagule source (Fig. 4.1 and 4.2). The data were organized to observe the deposition patterns from all the boreal species, ground and epiphytic species, lichens and mosses, and each of the respective nine species studied.

A constant (C) was added to the number and size data (1 or 25 µm, respectively) to account for the zeroes in the data structure (Osborne 2002). The number data were square-rooted and size data were log-transformed prior to the analyses to fulfill the assumptions (normalization) needed to perform One-Way ANOVA and Pearson's correlation coefficient analyses (Osborne 2002). The linear correlation between categories (all the boreal species, ground and epiphytic species, lichens and mosses, and the respective nine species) was assessed with the Pearson's correlation coefficient (r). Values were given between +1 and -1, values between 0.1 and 1 indicated a positive association, values between -0.1 and -1 indicated a

negative association, and a value of 0 indicated there was no association (Lee Rodgers and Nicewander 1988). The R^2 values were accompanied to Pearson's correlation coefficient in the graphs to denote the variance explained between the number (sqrt (x + c)) or size (log (x + c)) data and the dispersal distance. Models for each category are found in the Appendix, Table C4.2.

A One-Way ANOVA assessed whether the number and size of the dispersed propagules were significantly different among each category (ground and epiphytic species, lichens and mosses, and nine species). The ANOVA was also used to test how the average number and size of propagules changed with specific distances (3, 33, 66, and 99 cm) for each category. Tukey's Test ($\alpha = 0.05$) was used to compare the pairs of means of the square-rooted or log-transformed data, different letters indicate significant differences between pairs (Tukey 1949).

Results

Number of dispersed propagules on the one-meter tape

A large number of propagules were dispersed at short distances (< 10 cm) and fewer propagules were dispersed long distances beyond 10 cm (Fig. 4.1 a–d). Furthermore, for all factors tested, there was a negative log-linear association (leptokurtic distribution) between the number of dispersed propagules and the horizontal distance. A maximum of 20,127 propagules belonging to epiphytic and ground mosses and lichens from the boreal forests were wind-dispersed at horizontal distances of 3 cm to 100 cm of the 1 m tape from the fan, depending on the number available for counting at each replicate (Table 4.1). Propagule numbers per centimeter ranged from 0 to 638. An average of 7.6 ± 0.6 propagules was found per centimeter, making the propagules found per centimeter represent less than 0.5 to 2% of the propagules dispersed for all categories. The number of propagules captured differed between species of

different substrata, where the epiphytic species had a significantly higher number (10.1 ± 1.1) of propagules dispersed than the ground species (5.6 ± 0.5; $P < 0.0001$). The cryptogams also differed where moss propagules were dispersed in higher quantities than lichen propagules (8.4 ± 0.7 and 7.0 ± 0.9, respectively; $P < 0.0001$). The number of dispersed propagules per centimeter varied for each lichen and moss species: *Orthotrichum obtusifolium* had significantly the highest number of propagules dispersed (13.4 ± 1.7; $P = 0.0001$), and *Cladonia arbuscula* had significantly the lowest number of propagules dispersed (3.6 ± 0.9; $P = 0.0001$, Table 4.1).

The Pearson's correlation coefficient (r) showed a negative relationship between the number of propagules deposited and distance away from the fan for these boreal bryophytes and lichens (r = -0.50, $P < 0.0001$; Fig. 4.1a), ground and epiphytic species (r = -0.50, $P < 0.0001$; r = -0.43, $P < 0.0001$; Fig. 4.1b), lichens and mosses (r = -0.46, $P < 0.0001$; r = -0.54, $P < 0.0001$; Fig. 4.1c), and the respective nine species studied (r = -0.60 to -0.40, $P < 0.0001$; Fig. 4.1d).

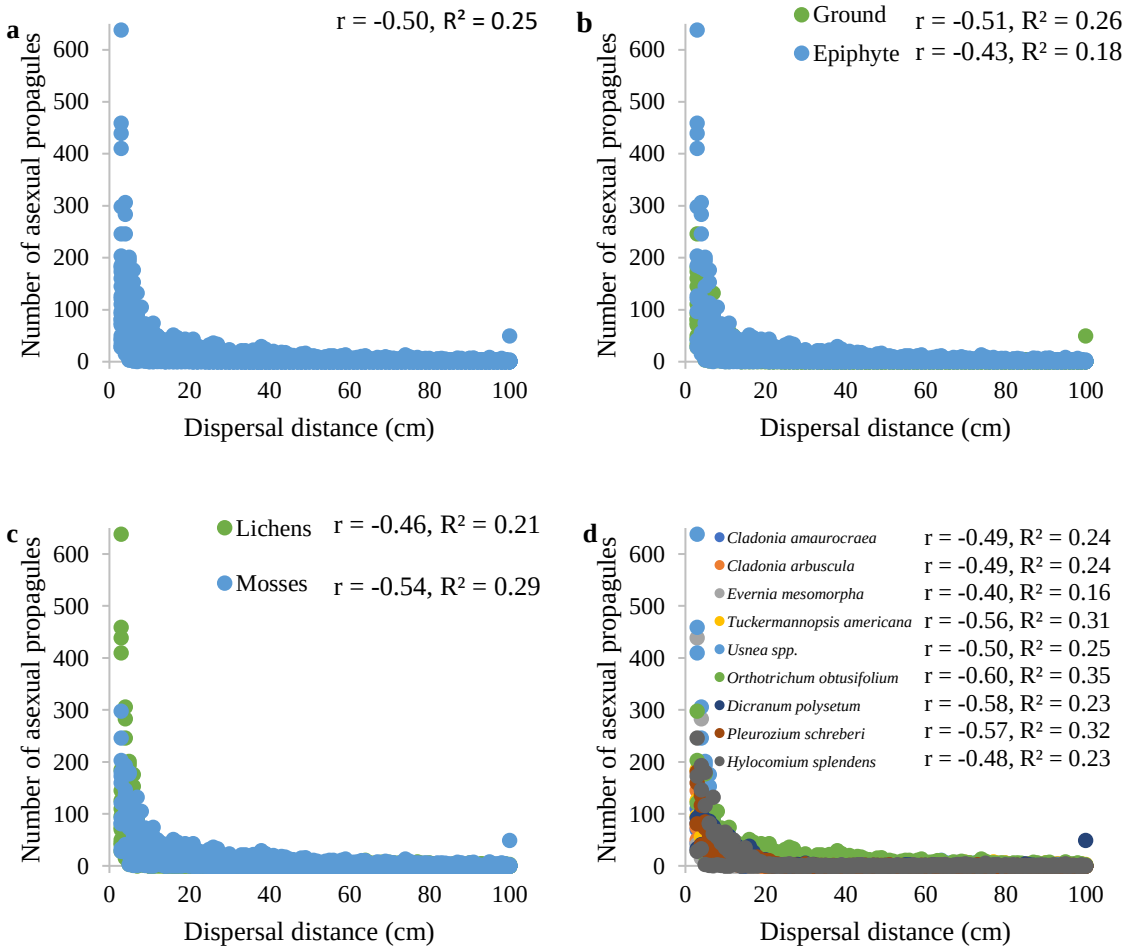

Figure 4.1. Scatter plots of the number of propagules dispersed horizontally on a one-meter tape (3 cm to 100 cm) showing: (**a**) all propagules (boreal species), (**b**) propagules by substrata (epiphyte and ground), (**c**) propagules by cryptogams (mosses and lichens), and (**d**) propagules by species. Pearson's correlation coefficient (r) and R^2 indicate whether an association/correlation are present between the sqrt (number +1) of propagules and dispersed distances. All associations were significant ($P < 0.0001$).

Table 4.1. Assessment of the dispersal of the number of boreal lichen and moss propagules from ground and epiphytic species dispersed on a one-meter tape in the laboratory. The One-Way ANOVA (*F*) compared the sizes of dispersed propagules among treatments (substrata, cryptogam, species). Different letters indicate significant differences within treatments (α = 0.05; Tukey Tests). *n* = replicates, avg. = average, # = number of propagules, st. error = standard error, df = degrees of freedom.

Treatments		*n*	total #	avg. #	st. error #	df	*F*	*P*
Substrata						1	48.98	< 0.0001
Epiphyte		1176	11927	10.14b	1.14			
Ground		1470	8200	5.58a	0.53			
Cryptogams						1	32.94	< 0.0001
Lichens		1470	10244	6.97a	0.87			
Mosses		1176	9883	8.4b	0.74			
	Species					6	4.72	0.0001
Lichen	*Cladonia amaurocraea*	294	1188	4.01ab	0.83			
Lichen	*Cladonia arbuscula*	294	1068	3.63a	0.89			
Lichen	*Evernia mesomorpha*	294	1844	6.27ab	1.93			
Lichen	*Tuckermannopsis americana*	294	2001	6.81bc	1.11			
Lichen	*Usnea* spp.	294	4143	14.09c	3.53			
Moss	*Dicranum polysetum*	294	1923	6.54abc	1.05			
Moss	*Hylocomium splendens*	294	2268	7.71abc	1.68			
Moss	*Orthotrichum obtusifolium*	294	3939	13.4d	1.79			
Moss	*Pleurozium schreberi*	294	1753	5.96ab	1.22			

Fewer propagules were dispersed farther distances (66, 99 cm) and a larger number of propagules were found at the smallest distances from the fan (3, 33 cm) for both the ground and epiphytic species, but the epiphytic species dispersed farther distances than the ground species (df = 3, $F = 7.04$, $P = 0.0003$; Appendix, Table C4.2). The propagule number varied per distance for both the mosses and the lichens. No significant differences were found between the number of propagules of the cryptogams and distance. Appendix Table C4.2 also shows that fewer propagules were dispersed longer distances (66, 99 cm) and most propagules were found at short distances from the fan (3, 33 cm), with *Usnea* spp. having the highest number of propagules

dispersed near the fan and *D. polysetum* having fewer propagules dispersed near the fan (df = 21, $F = 2.30$, $P = 0.0049$).

Size of dispersed propagules on the one-meter tape

The sizes of propagules had a negative association with distance (Fig. 4.2a–d), with most propagules with larger sizes being close to the fan. Dispersed propagules belonging to epiphytic and ground mosses and lichens from the boreal forests varied in sizes, ranging from 25 µm to 6,050 µm and on average per centimeter they were 204.10 ± 5.83 µm in size (Table 4.2). The average size of the dispersed propagules per centimeter for the ground species (263.65 ± 9.05 µm) were significantly larger than the epiphytic species (129.66 ± 6.36 µm; $P = 0.0040$). Mosses had asexual propagules that, on average per centimeter, were significantly larger the lichen asexual propagules (251.99 ± 8.95 µm and 165.79 ± 7.61 µm, respetively; $P < 0.0001$). The sizes of dispersed propagules varied for each lichen and moss species: *Orthotrichum obtusifolium* had the largest propagules dispersed per centimeter, and *Evernia mesomorpha* had significantly smallest of propagules dispersed ($P < 0.0001$, Table 4.2).

The Pearson's correlation coefficient showed for the boreal forest species (r = -0.55, $P < 0.0001$), ground and epiphytic species (r = -0.66, $P < 0.0001$; r =-0.38, $P < 0.0001$), lichens and mosses (r = -0.51, $P < 0.0001$; r = -0.60, $P < 0.0001$), and the respective nine species studied (r = -0.67 to -0.22, $P < 0.0001$) that as distance increased from the fan, the deposited asexual propagules decreased in size. No significant differences were found among the sizes at the specific distances (3, 33, 66, 99 cm) for the categories.

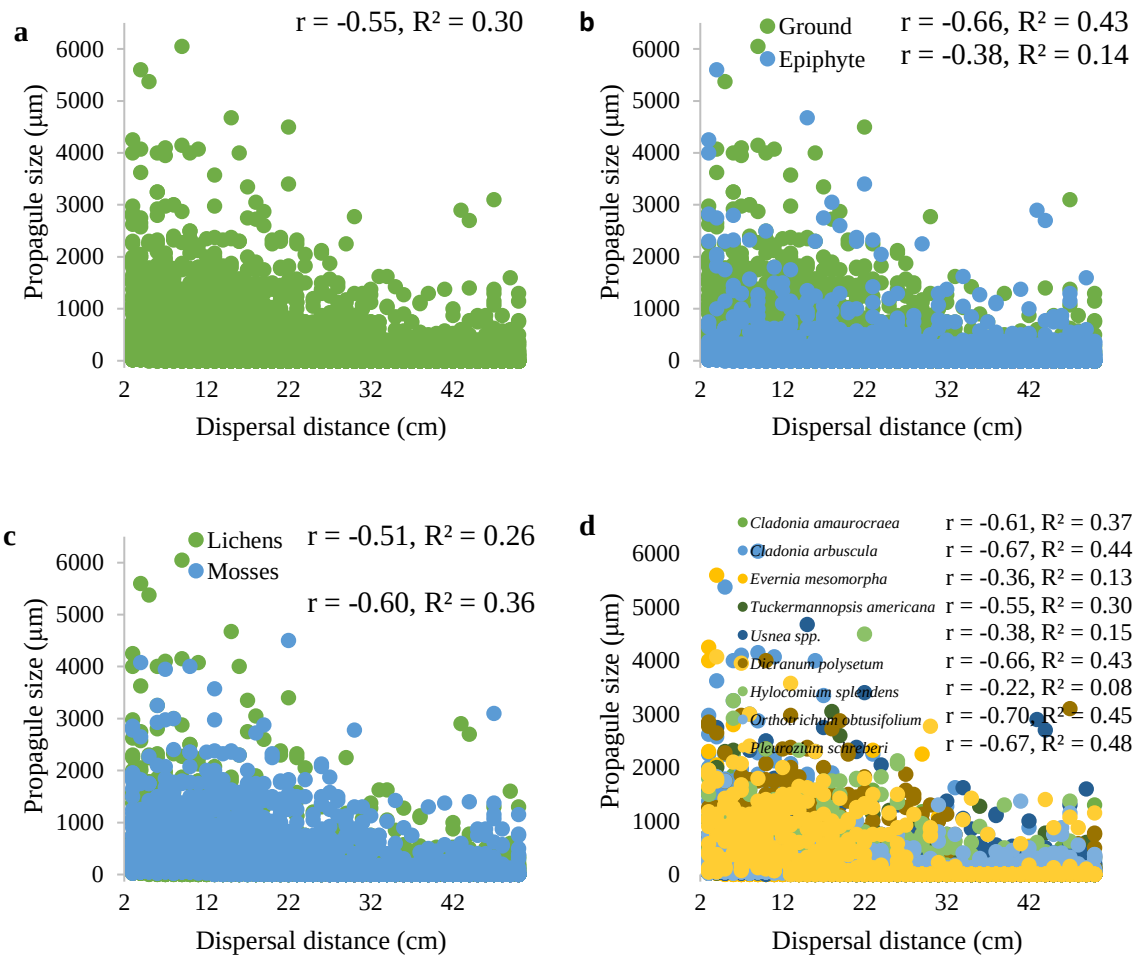

Figure 4.2. Scatter plots of the sizes of propagules dispersed horizontally on a one-meter tape (3 cm to 100 cm) showing: (**a**) all propagules (boreal species), (**b**) propagules by substrata (epiphyte and ground), (**c**) propagules by cryptogams (mosses and lichens), and (**d**) propagules by species. Pearson's correlation coefficient (r) and R^2 indicate whether an association/correlation are present between the log (size + 25 µm) of the propagules and dispersed distances, all associations were significant ($P < 0.0001$).

Table 4.2. Assessment of the sizes of boreal lichen and moss propagules from ground and epiphytic species dispersed on a one-meter tape in the laboratory. The One-Way ANOVA (*F*) compared the sizes of dispersed propagules separated by treatment (substrata, cryptogam, species). Different letters indicate significant differences within treatments (α = 0.05; Tukey Tests). *n* = replicates, avg. = average, st. error = standard error, df = degrees of freedom.

Treatments		n	avg. size (μm)	st. error	df	*F*	*P*
Substrata					1	8.28	0.0040
Epiphyte		2880	129.66a	6.36			
Ground		3600	263.65b	9.05			
Cryptogams					1	101.49	< 0.0001
Lichens		3600	165.78a	7.61			
Mosses		2880	251.99b	8.95			
	Species				8	19.71	< 0.0001
Lichen	*Cladonia amaurocraea*	720	154.32b	7.01			
Lichen	*Cladonia arbuscula*	720	278.02b	26.30			
Lichen	*Evernia mesomorpha*	720	62.53a	13.61			
Lichen	*Tuckermannopsis americana*	720	144.79bc	11.64			
Lichen	*Usnea* spp.	720	189.26c	16.31			
Moss	*Dicranum polysetum*	720	317.19c	22.11			
Moss	*Hylocomium splendens*	720	255.52c	17.06			
Moss	*Orthotrichum obtusifolium*	720	122.05c	7.01			
Moss	*Pleurozium schreberi*	720	313.19c	20.45			

Discussion

Dispersal ability varies between species of different substrata

Th epiphytic species had propagules (number and size) that were negatively associated with distance from the source, but in higher number and in smaller size than the ground-dwelling species . These differences are suggested to reflect the dispersal adaptations needed to facilitate the occupancy on trees species. A high number of asexual propagules being dispersed can increase the chances of the following scenarios to occur: (1) propagules may be deposited and

retained on bark of branches, trunk, and twigs of the same tree; (2) propagules may be deposited, then recirculated in the air by wind, and finally deposited and retained on the same tree; and/or (3) variations of scenarios 1 and 2 but on neighboring trees. A higher number of dispersed propagules may also be required to ensure establishment on trees, particularly because trees having specific microsite traits (pH and humidity) and being exposed to high irradiation and wind, may limit the establishment of most deposited propagules (Armstrong 1990, Wiklund and Rydin 2004, Löbel and Rydin 2010). There are a small number of studies that have looked at the association between lichen and moss asexual propagule size and distance dispersed (Löbel et al. 2009, Pohjamo et al. 2006, Pasiche-Lisboa and Sastre-De Jesús 2018). Most of these studies observed no association between the two variables. The studies that have reported an association, surmised that larger propagules (protonemata) dispersed farther distances than smaller propagules with the caveat that further replication may clarify if this dispersal pattern is true (Pasiche-Lisboa and Sastre-De Jesús 2018). The discrepancy between the associations found in Pasiche-Lisboa and Sastre-De Jesús (2018) and this study may result from the type of asexual propagules used, protonemata and other propagules produced from the moss gametophore/lichen thallus such as gemmae, leaves, stem pieces, soredia, isidia, or thallus fragments. Based on Pasiche-Lisboa and Sastre-De Jesús (2018), the observation that the protonemata formed a thin flake when air dried by wind may allow the prediction that a larger flake allowed the propagule to remain in the air current longer and disperse farther distances than smaller protonemata flakes. However, this study found that most asexual propagules dispersed from the epiphytic species are of small size and do not have a strong correlation with distance as they do in the ground species. These findings are suggested to allow for propagules from the tree species to disperse farther

distances than those of the ground species, and it may suggest that the small size of some asexual propagules may facilitate being carried by wind.

In contrast to the epiphytic lichens and mosses, the stronger negative relationship between the number and size of deposited propagules with distance travelled by the ground species showed their limited dispersal capabilities when dispersed by wind. The limited dispersal ability illustrated in this study may result from the forest floor species having larger-sized asexual propagules, which may have resulted from less grinding or from tougher gametophores/thallus than the epiphytic species. Studies on some vascular plants have shown that larger propagules deposit more rapidly and closer to the parent colony than smaller propagules (Morse and Schmitt 1985, Greene and Johnson 1993). If wind limits the propagule dispersal capabilities, then ground lichens and mosses may require other vectors such as animals (carried or ingested) and/or water (rain drops or water flow) to disperse longer distances (Rudolphi 2009, Boch et al. 2013, Pasiche-Lisboa and Sastre-De Jesús 2018). If the dispersal distance is the limiting factor for establishment on the forest floor, then dispersal with an ability to remain dormant (dispersal in time) may be of benefit for these species. Dispersal in time requires the production of propagules that fall into the soil, form a propagule bank that resists decay and remains dormant until a suitable disturbance occurs to enable establishment in a suitable habitat (Jonsson 1993, Rydgren and Hestmark 1997, Caners et al. 2009). The lower number, but larger size of the dispersed asexual propagules at short distances may help localized dispersal and propagule bank formation for the lichens and mosses on the forest floor.

Dispersal variation among cryptogams

Among mosses and lichens in the boreal forests, this study shows that mosses have a more limited dispersal due to the stronger association between asexual propagule number and distance from the fan. A limited dispersal capability allows for moss populations to be maintained and occasionally being formed at short distances from the parent colony. However, the formation of new populations at longer distances from the parent colony is more likely to occur for lichen populations. The dispersal capability shown for lichens is suggested to be important to increase the chance of becoming established, since lichens require the presence of the mycobiont and the photobiont to interact, develop into a thallus, and in a suitable habitat. If the propagule deposited has both symbionts (large thallus fragments, isidia, soredia), then both population and thallus formation is facilitated. However, the dispersal of only one of the symbionts as an asexual propagule (conidia, rhizines, small thallus fragments with a single symbiont) will require the resynthesis of the lichen fungus with a free-living photobiont, with a photobiont that was deposited with an asexual propagule, or the mycelia stealing a photobiont from a nearby lichen (Honegger and Nash 2008). In contrast to lichens, mosses are single organisms and would only require the deposited propagule to become established on a suitable substratum. The study of a larger number of moss or lichen species would reveal patterns between species with regards to their deposition, establishment, and occupancy on substrata in boreal forests.

Dispersal variation among species

The negative associations between distance with size and number of asexual propagules found for the species in this study may represent the combination of wind strength and ease of detachment of the propagule from the gametophore or thallus. The effect of this combination may influence the size and number of asexual propagules resulting from the force of wind,

degree of desiccation, or other environmental factors that may affect fragmentation. The ability to form asexual propagules may also result from the moss gametophore or lichen thallus being brittle, easy to fragment, and form asexual propagules; or, the moss gametophore or lichen thallus not being brittle, or easy to fragment, and form asexual propagules. Fragmentation and detachment of asexual propagules in combination with the dispersal vector are pivotal to propagule deposition in the forest. Fragmentation, detachment, and dispersal of asexual propagules requires further study to better understand how this affect their presence in a habitat.

Conclusions

The core finding of this study indicates that boreal lichens and mosses when exposed to wind are dispersed close the the propagule source, with propagule number and size decreased with increased distance. The dispersal partterns observed are suggested to result from differences in the dispersal ability of the lichen and moss studied here, resulting from the the ease of detachment of asexual propagules from the parent colony. However, it can be noted that these results may also be confounded by the ability of the moss or the lichen to withstand the grinding by mortal and pestel when preparing the material for dispersal, which may skew the number and sizes of propagules produced and dispersed on the tape as well as the different values observed in the analyses. Further studies would need to address the role and structural properties of the lichen thallus and the moss gametophore when exposed to a force that helps form the propagule number and size observed in this study. Understanding the integrity of the gametophore or lichen thallus when exposed to a force may disentangle if the dispersal ability of lichens and mosses studied here are in part concordant with the dispersal ability and the presence of these species in boreal habitats.

Acknowledgments

Special thanks to J. Pinksen who helped with the wind dispersal experiment, and to T. Booth for help with the collection of the lichen and moss material. Funding for this study was obtained from the Department of Biological Sciences, the Faculty of Graduate Studies (University of Manitoba), and from the Natural Sciences and Engineering Research Council of Canada (NSERC-DG to MPN).

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Chapter 5: The eDNA community composition in soil banks does not mirror that of the extant cryptogam community but is partly associated with the local forest stand properties

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Abstract

Soil banks contain bryophyte and lichen propagules that facilitate the formation, change, and/or maintenance of the extant community in boreal forest stands when the soil is disturbed, and viable propagules become established. A traditional method, the emergence method, has been used to culture and assess the community composition (abundance and richness) that has become established from soil samples to understand how the community is affected by forest stand properties, and if it mirrors the extant community and their dispersal patterns. Because the emergence method relies on viable propagules in the soil to establish colonies under a set of specific laboratory conditions, some of the viable propagules may not germinate, thus leaving a knowledge gap on how soil banks influence the extant community. High throughput sequencing technologies may supplement the information obtained with the emergence method by depicting the abundance and richness of species in the environmental DNA (eDNA) using barcoding genes. Thus, in this study, high throughput sequencing technologies are used to describe the soil

community of bryophytes and lichens in boreal soils, to understand their association with forest stand properties, and to reveal if they correlate with the extant community of bryophytes and lichens. The eDNA from soil within nine boreal forest stands (three stands dominated each by balsam fir, poplar, or white spruce) was examined using barcoding genes and assigned a taxonomic rank to each operational taxonomy unit (OTU, species). The barcoding genes included the following taxa: 23S rDNA for embryophytes, eukaryotic algae, and cyanobacteria; trnL intron c-h for embryophytes; rbcL for embryophytes; and, ITS rDNA for fungi. The OTU abundance (number of amplicons per OTU) and richness (number of OTUs) were compared with that of the extant bryophyte and lichen community, and with forest stand properties. The OTU abundance and richness was partly associated with forest canopy cover, tree density, slope, and/or the extant cryptogam cover and richness. This study suggests that the lichen or bryophyte OTUs increase in the soil bank when the growth of the aboveground lichens and bryophytes are favoured due to the properties of the forest stand. The results of this study also show that eDNA richness of moss families or the lichen photobiont of the soil community does not mirror that of the extant community composition, suggesting that the soil community represents a past community composition and/or dispersal events external to the forest stands.

Key words: asexual propagules; diaspore banks; liverworts; mosses

Introduction

Soil banks, diaspore banks, or propagule banks are repositories of propagules produced, dispersed, and deposited in the soil from organisms (such as bryophytes and lichens) present in the extant community (Jonsson 1993, During 2001). Viable propagules remain dormant for

months to even millennia in the soil (Furness and Hall 1981, Sundberg and Rydin 2000, Roads et al. 2014). When the soil is disturbed, and under ideal conditions (light, moisture, temperature), some of the viable propagules may germinate and become established (Caners et al. 2009, Kövendi-Jakó et al. 2016). Depending on the species that become established from the soil bank, a new community may be formed, or the extant community may be maintained. The composition of the extant community, here defined as the abundance and richness of species within the community, may change if the emerging species from the soil bank are different from the extant community. The composition of the community is maintained if the emerging species are the same as that of the extant community (Jonsson 1993, Caners et al. 2009, Maciel-Silva et al. 2012, Kövendi-Jakó et al. 2016). Furthermore, a new community may be formed during small scale disturbances such as a denuded substratum during succession (Jonsson 1993), or large-scale events (fire, drought) that facilitate change of the properties of forest stands (Jean et al. 2017, Boudreault et al. 2018). However, the degree of difference between the soil bank and the extant community composition, and the potential for the soil bank propagule community to supply species for the extant community, is not well known.

Three non-mutually exclusive explanations can be attributed to the formation of the community in soil banks. When propagules of the species in the soil bank emerge, changes to the extant community may be attributed to 1) the propagule soil bank representing dispersal-deposition events from a past or present community, 2) the propagule soil bank representing dispersal events from communities at long-distances (Iglesias et al. 2015, Barbé et al. 2017), and/or 3) the past community may have been a different community than that of the extant community. Dispersal of asexual propagules have been linked to forest stand properties (aspect, canopy cover, tree density, slope), which in turn may link to the formation and/or maintaince of

the composition of the soil bank community. For example, an open canopy can limit the number of propagules deposited in the forests from within (Chapter 3, Heinken 1991) because of higher wind velocities carrying the propagules outside of the forest than in closed canopy forests. Furthermore, a closed canopy forest may form a barrier to the propagules dispersed from outside sources. However, a higher tree density can increase the availability of substrata for some bryophytes and lichens (Hazell et al. 1998), and in turn allow for more colonization events and thus a higher production of propagules. Aspect and slope of the landscape underlying a forest stand may influence the direction and speed of wind acting on the bryophytes and lichens, or the amount of light and humidity (Måren et al. 2015), and it may augment or diminish the likelihood of propagules to fragment, detach, and disperse within and among forest stands (Hylander 2005, Åström et al. 2007). Since stand properties have been shown to affect the extant community in terms of dispersal of propagules, it would be expected that stand properties may have also influenced past communities (abundance and richness) and hence the soil bank composition.

Methods that have linked the soil bank community with that of the extant community include comparisons of the emergence of propagules from the soil bank by culturing, entrapment of dispersed propagules from extant communities, and surveys on the cover and richness of bryophytes and lichens in the extant community (Ross-Davis and Frego 2004, Caners et al. 2009, Ingerpuu and Vellak 2018, Ingerpuu et al. 2019). The emergence method employs culturing of propagules from the soil bank to determine the species present and infer the community composition in the soil. The emergent method has shown that the soil bank may facilitate the rapid colonization of species after a disturbance (Jonsson 1993), abundance and richness of emerging species may change with time (Jonsson 1993, Pasiche-Lisboa and Sastre 2014), and establishment from the soil bank is strongly dependent on edaphic and light conditions of the

forest (Caners et al. 2009, Kövendi-Jakó et al. 2016). However, the emergence method may not provide the necessary conditions for all viable propagules to establish, leaving gaps of information on the composition of the soil bank community. High-throughput sequencing technologies can be used on environmental DNA (eDNA) from propagules, and the use of barcoding genes allow the identification of taxa in the form of an operational taxonomic unit (OTU ≈ species) present in the soil (Taberletet al. 2012, Thomsen and Willerslev 2015). The use of high-throughput sequencing technologies to identify species in the soil bank can fill the knowledge gap left by the culturing method.

The species composition of the extant lichen and bryophyte community in three boreal forest types was explored in Chapter 3, which reported that species overlapped between balsam fir and white spruce forests, but the species composition of poplar forests was different from the conifer forests. The diversity patterns observed in these forest stand types reflect the dispersal capability of species from the extant community rather than past dispersal patterns. Because of the similarity in diversity patterns in conifer forest stands or in poplar stands, the bryophytes and lichens eDNA taxonomic diversity in the soil bank was expected to mirror that of the extant community for these forest types (Chapter 3).

The main goal of this study was to examine the dispersal potential of propagules by comparing the bryophyte and lichen diversity in the soil bank (eDNA) of three boreal forest types with that of the above-ground diversity (extant community). The more specific objectives were 1) to identify taxa present in the soil to the lowest rank possible using standard barcoding genes, 2) to compare the OTU richness and abundance of reads from the barcoding genes with forest stand properties and stand types, and 3) to compare the similarity of the bryophyte and lichen species composition in the propagule bank inferred from the eDNA richness with that of

the extant species composition among three stand types. Because the selected primers were universal and amplified other groups of organisms (fungi and vascular plants) in addition to bryophytes and lichens, the findings from these groups were assessed for the three objectives.

Methods

Boreal forest sites and their properties

Nine boreal forest stands on the Precambrian Shield in northern Manitoba (Canada), near the eastern shore of Payuk Lake (Appendix, Table B3.1), were selected for this study. Three replicate stands were chosen for each of three types of boreal forest stands (balsam fir, *Abies balsamea* (L.) Mill.; white spruce, *Picea glauca* (Moench) Voss; poplar., *Populus tremuloides* Michx.), which are here defined as three types of habitats. The stands grow in humic to sandy soils or on thin soil over granite-gneiss rock. Detailed information about the forest stands and their properties are found in the Appendix, Table B3.1. Average temperatures recorded during various times of the year ranged from -29 °C to 21 °C (Flin Flon weather station data; see https://goo.gl/GPkoqm).

Four readings (N, S, E, W) at each of three locations within each forest stand (12 readings all together) using a spherical densiometer (Model A, Forest Densiometers, Bartlesville, OK, USA) were recorded and averaged to obtain canopy cover. Slope (± %) was recorded depending on ease of access from the middle of the stand using a PM-5/360 PC handheld clinometer (Suunto, Vantaa, Finland). Aspect was measured in degrees facing downslope using a MC-2 G Mirror Compass (Sunnto, Vantaa, Finland). Tree Density was determined using BAF 5

and 15 (Basal Area Factor) prisms (Cruise Master Prism, Sublimity, Oregon). Vascular flora species were identified in the field.

Assessing moss and lichen cover and richness

Lichen and moss richness was assessed with two methods in the forest stands in 2015–2016. In the first method, lichens and mosses from all substrata were collected in summer of 2015. The second method employed a 20-m transect placed in the middle of the stand, in which five quadrats (0.5 m^2) were selected from randomly generated numbers between 1–20, to assess the species richness on the forest floor during the summer of 2016. Five dead and/or living standing trees were also selected at random in each forest stand, and for each tree five living and dead branches from the lower canopy (~ 1.82 m) were removed to assess moss and lichen richness. Abundance (relative % cover) of both forest floor or bark components (moss, lichen, ground, and/or bark) was assessed during the summer of 2016. The abundance was estimated from the 0.5 m^2 quadrats placed on the forest floor, or malleable tape-made quadrats (0.25 m^2, one per tree) placed on the tree trunk at breast height (1.4 m) of five dead and/or alive trees that were also selected at random. Ground cover included areas occupied by decaying wood, litter, rock, and vascular plant vegetation. Cover values presented here are from the average of three cover estimates for each quadrat. Samples were air dried and placed in packets, brought to the laboratory, and stored prior to identification.

Identification of asexual propagules and species

Identification of mosses and morphology of asexual propagules followed the taxonomy of Ireland (1982); while, the identification of lichens and morphology of asexual propagules

followed that of Goward et al. (1994), Goward (1999), and Hinds and Hinds (2007). Nomenclature is based on indexfungorum.org for lichens and tropicos.org for mosses. Thin-layer chromatography using solvent A was performed on lichen podetia or squamules of *Cladonia* spp. (Culberson 1972) to confirm their identity. Asexual propagules that displayed defined morphological traits were identified to the lowest taxonomic rank possible, which was limited by propagule size. All specimen vouchers were deposited in the cryptogram herbarium at the Memorial University of Newfoundland, Grenfell Campus (MUN).

Soil sampling for eDNA

Three soil samples were collected from the upper soil layer (first five centimeters), one meter apart from each other at the center of each stand, resulting in a total of 27 soil samples (August 25, 2017). One sample was lost; thus, it was not included in further analyses (balsam fir stand 3, replicate 1). A clean plastic spoon was used for each soil sample to avoid potential contamination. The soil sample was stored in Corning tubes (50 ml; Milipore Sigma, Darmstadt, Germany) and labeled. Because samples had moisture in them, covers of tubes were loosened and placed in a precision drying oven (Chicago Surgical and Electrical Co, Cat. no. 200; Serial no. 0767; Illinois, USA) in the glass drying mode with the fan on and heat off (75 hrs., 28˚C) to dry. Samples were stored at room temperature until they were processed. In the laboratory, samples were independently mixed (homogenized) with a clean metal rod, then a homogenized subsample was transferred to a clean vial (5–7 mL) and labeled under sterile conditions. The sub-samples were sent to Jonah Ventures in Boulder, Colorado (USA, jonahventures.com) for eDNA extraction, sequencing, and bioinformatics.

eDNA extraction, sequencing, and bioinformatics

The end of a sterile cotton swab (Fisher, cat# 23-400-100) was coated each sample and then genomic DNA was extracted by the MoBio PowerSoil htp-96 well Isolation Kit (Carlsbad, CA, USA) according to the manufacturer's protocol. Four genes were amplified from the eDNA. The 23S rDNA gene from the nuclear ribosomal RNA gene region was selected to amplify the DNA of cyanobacteria, eukaryotic algae, and the embryophytes (Sherwood and Presting 2007); both the $trnL_{UAA}$ intron (trnL) and ribulose-bisphosphate carboxylase (rbcL) genes located in the chloroplast were selected to amplify the DNA of embryophytes (Taberlet et al. 2007, Murphy et al. 2011); and, the internal transcribed spacer (ITS) rDNA from the nuclear ribosomal RNA gene region was selected to amplify the DNA of fungi (lichenized and non-lichenized) (Gardes and Bruns 1993; Bellemain et al. 2010). Embryophytes amplified for the respective genes contain both bryophyte and vascular plant OTUs. The selected genes (23S rDNA, trnL intron, rbcL, and ITS rDNA; see Table 5.1) were amplified from each genomic environmental DNA sample. Each 25 µL PCR reaction was mixed according to the Promega PCR Master Mix specifications (Promega catalog # M5133, Madison, WI, USA) which included 12.5 µl master mix, 0.2–0.5 µM of each primer, 1.0–1.2 µl of gDNA, and 10.3–10.5 µl DNase/RNase-free H_2O. DNA was amplified by PCR according to conditions presented in Table 5.1. Amplicons were then cleaned by incubating with Exo1/SAP for 30 minutes at 37 °C followed by enzyme inactivation at 95 °C for 5 minutes. A second round of PCR was performed to give each sample a unique 12-nucleotide index sequence. The indexing PCR included Promega Master mix, 0.5 µM of each primer and 2 µl of template DNA (cleaned product from the first PCR reaction) and consisted of an initial denaturation of 95 °C for 3 minutes followed by 8 cycles of 95 °C for 30 seconds, 55 °C for 30 seconds and 72 °C for 30 seconds. The final indexed amplicons (25 µl)

from each sample were cleaned and normalized using SequalPrep Normalization Plates (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's protocol. Samples were then pooled together in equimolar concentrations. Pooled amplicon libraries were sequenced on an Illumina MiSeq using the v2 500-cycle kit (San Diego, CA, USA) at the University of Colorado Boulder BioFrontiers Sequencing Center.

Sequences were demultiplexed by taking advantage of Golay barcodes via QIIME v1.9.1 (Caporaso et al. 2012). The following options were used to output raw unfiltered fastq files for both forward and reverse reads: split_libraries_fastq.py -q 0 --max_bad_run_length 250 --min_per_read_length_fraction 0.0001 --sequence_max_n 250 --store_demultiplexed_fastq. Forward and reverse read sequences were trimmed to 150-235 nucleotides, according to the amplicons of interests (see Table 5.1), via the usearch8 option -fastx_truncate.

Table 5.1. Primer and PCR conditions used to amplify different gene regions specific to taxonomic groups from environmental DNA (cyanobacteria, eukaryotic algae, embryophytes [bryophytes and vascular plants], and fungi) extracted from soils of forest stands surrounding Payuk Lake, Manitoba, Canada. PCR steps: Initial denaturation occurred for five minutes at 95˚C for the 23s rDNA and ITS rDNA genes, while for the trnL and rbcL genes the reactions occurred at 94˚C (three and five minutes, respectively). Den. = Denaturating temperatures; # Cycles = Number of cyles; Elongation occurred for 10 minutes (75˚C) for all genes PCR amplified.

Gene (primers)	Taxa		Primer sequence (5'– 3')	Amplicon size (nucleotides)	Den. (Time)/# Cycles	Source of primers
23S rDNA (p23SrV_f1, Diam23Sr1)	Cyanobacteria, Eukaryotic algae, Embryophytes	F	GGACAGAAAGACCCTATGAA	235	95˚C (45s), 50˚C (60s), 72˚C (90s), 35 cycles	Sherwood and Presting 2007
		R	TGAGTGACGGCCTTTCCACT	235		

trnL (intron c-h)	Embryophytes	F	CGAAATCGGTAGACGCTACG	235	94˚C (30s), 55˚C (30s), 72˚C (60s), 40 cycles	Taberlet et al. 2007
		R	CCATTGAGTCTCTGCACCTATC	235		
rbcL (rbcLF2, rbcLR3a)	Embryophytes	F	TGTTTACTTCCATTGTGGGTAATG	200	94˚C (30s), 52˚C (30s), 72˚C (60s), 40 cycles	Murphy et al. 2011
		R	TTCGGTTTAATAGTACAGCCCAAT	200		
ITS rDNA (ITS1-F, ITS2R)	Fungi	F	CTTGGTCATTTAGAGGAAGTAA	200	95˚C (45s), 50˚C (60s), 72˚C (90s), 35 cycles	Gardes and Bruns 1993; Bellemain et al. 2010
		R	GCTGCGTTCTTCATCGATGC	150		

Sequences were then merged using the -fastq_mergepairs option in usearch8 (Edgar 2010). The forward primer and reverse primer were removed using cutadapt (Martin 2011). Then, the different gene amplicons were processed via the UPARSE pipeline (Edgar 2013) and taxonomy was assigned via the SINTAX protocol (http://www.drive5.com/usearch/manual/utax_user_train.html) available in usearch (v8.1.1861) for amplicons of gene 23S rDNA (Edgar 2010), NCBI GenBank records using the UTAX protocol for amplicons of the genes, trnL intron c-h and rbcL (Taberlet et al. 2007), or the uclust option with assign_taxonomy.py in QIIME 1.9 (Caporaso et al. 2010) and the UNITE database for amplicons of the gene ITS rDNA (Kõljalg et al. 2013) released on 2/3/2015. Specifically, sequences were quality trimmed to have a maximum expected number of errors per read of less than 0.5 and operational taxonomic units (OTU) were clustered at 85%–99% similarity with de novo chimera checking enabled (23S rDNA, 97%; trnL intron c-h 99%, rbcL, 85%–90%; ITS rDNA, 99%). For amplicons of gene 23S rDNA and trnL intron c-h, the taxonomy to each OTU was assigned with an `in-house` SINTAX 23S rDNA/trnL intron c-h reference database, which was constructed by downloading any annotated GenBank entry (Benson et al. 2005). Any OTUs with taxonomy assignments not meeting these criteria were removed from the OTU table. All resulting amplicon regions were dereplicated to 99%–100% sequence identity and any identical

sequence across lineages are collapsed to the lowest-common-ancestor. To ensure increased specificity of OTU assignment against the reference database the -maxaccepts and –maxrejects usearch options were set to 0. These parameters help to ensure that individual reads are correctly mapped to their respective OTUs.

The taxonomic identification of OTUs depended on the availability of sequences in the reference databases. If specific taxa were absent from the database, OTUs were assigned to species that were closely related to that of the query sequence. The OTUs from the genes that amplified Embryophytes were separated into two data sets for further analyses, those containing bryophyte or vascular plant OTUs. For the amplicons of the 23S rDNA gene, bryophytes included OTUs in the Amblystegiaceae, Funariaceae, Pottiaceae, Ptilidiaceae, Sphagnaceae, and Tetraphisdaceae. For the trnL intron c-h gene, bryophytes included OTUs in the Amblystegiaceae, Anastrophyllaceae, Aulacomniaceae, Bartramiaceae, Bauxbaumiaceae, Bryaceae, Calliergonaceae, Dicranaceae, Ditrichaceae, Funariaceae, Grimmiaceae, Hedwigaceae, Hylocomiaceae, Hypnaceae, Orthotrichaceae, Mniaceae, Plagiotheciaceae, Polytrichaceae, Pottiaceae, Ptilidiaceae, Rhabdoweisiaceae, Scapaniaceae, Sphagnaceae, and the Splachnaceae families. For the rbcL gene, bryophytes included OTUs in the Dicranaceae, Polytrichaceae, and Sphagnaceae families. For the amplicons of the 23S rDNA gene, the lichen photobiont was labeled as any cyanobacterial OTU present in the Nostocaceae, Trebouxiaceae -*Trebouxia*, Trebouxiaceae -*Coccomyxa*; eventhough, there are species within these families that do not form any partnership with the fungi of the lichen symbiosis (Nash 2008). However, since many lichen photobionts have not been described, and those species that have been are within these three groups, the usage of these groups can provide a good representation of species within the soil bank and allow for comparisons between the the richness found among the soil bank and

extant communities. Vascular plant OTUs within different families were also selected for further analyses from the 23S rDNA, trnL intron c-h, and the rbcL genes. For the fungal ITS rDNA gene, all OTUs present in the Lecanoromycetes were defined as lichen forming fungi, since most lichen-fungi belong to the Lecanoromycetes (Nash 2008): the Lecanoromycetes were represented by seven OTUs, and one lichen fungi family (Parmeliaceae). Fungal families, excluding the lichen fungi, were also included in the analyses, but only those OTUs that had a clear taxonomic assignment to compliment the finding from the lichenized fungi. A summary of all the OTUs and their identifications from the different genes (28S rDNA, trnL intron c-h, rbcL, and ITS rDNA) is given in the Appendix Table D5.1.

Statistical analyses

Descriptive statistics (number, average, standard error) were used to understand the variation in forest stand properties (Appendix, B3.1) and OTU abundance and richness present in the boreal soil were performed for each gene (28S rDNA, trnL intron c-h, rbcL, and ITS rDNA). The OTU abundance refers to the number of sequences or reads for each OTU sequence present in a soil sample, while the OTU richness refers to the number of different OTU sequences present in the soil sample. The average and standard error for the OTU abundance and richness were calculated from the two to three samples gathered from within each stand, which was then used to calculate the average and standard error for the OTU abundance and richness of the forest stand types (balsam fir, poplar, white spruce).

A One-Way Analyses of Variance (ANOVA), followed by Tuckey's HDS pairwise comparison tests ($\alpha = 0.05$), was performed on the stand property data (aspect (˚), cover, %

slope, tree diameter, tree density). The Kruskal-Wallis Test, Conover-Inman non-parametric Test, and Spearman Correlation Coefficient (Spearman's Rho) were carried out with Infostat (Di Rienzo et al. 2012). Kruskal-Wallis and Conover-Inman non-parametric tests were used on the data, since it is less stringent about the data distributions (Kruskal and Wallis 1952, Conover 1999). Nested Kruskal-Wallis Tests, followed by Conover-Inman pair-wise comparison tests, were performed to determine if the OTU abundance and richness differed between the forest stand types, and the stands within each type, and independently for the genes of interest (28S rDNA, trnL intron c-h, rbcL, and ITS rDNA). No pilot experiment, or prior analyses, were done to estimate the number of replicates needed to limit Type 1 errors in the data set for this study. Because the data were based on an already established experimental setting (Chapter 3), we opted a posteriori for the use of an α of 0.10 to better understand the biological differences in OTU abundance and richness among treatments (McDonald 2014). When appropriate, various levels of significant differences for the outcomes of the respective tests were indicated (*P < 0.10, **P < 0.05, ***P < 0.01).

Spearman Correlation Coefficient or Spearman's Rho (r_s, $\alpha = 0.10$) was used to test if there was an association between the OTU abundance or richness with that of the stand properties (aspect, average percent canopy cover, slope, and tree density) and/or the richness (number of species) and abundance (percent cover of species) in the extant community. The OTUs from the fungi and vascular plants were associated solely with the forest stand properties since that was the only data available for analysis. Spearman's Rho values range between 1 and -1, with positive values indicating a positive association and negative values indicating a negative association.

Two approaches were used to assess how similar the moss and lichen communities in the boreal soil bank were to the extant bryophyte and lichen communities. In the first approach, the relative proportion of the number of eDNA OTUs and the number (richness) of species from the extant community for each moss family were compared among the forest stand types. Similarly, this procedure was done, but on the OTU richness for each lichen photobiont type (23s rDNA: Nostocaceae, Trebouxiaceae -*Trebouxia* and allies, Trebouxiaceae -*Coccomyxa*). The comparison of the photobiont type between the OTU and the extant communities was based on the literature description of the photobiont for each lichen species collected from the extant community in Chapter 3, see Appendix Table D5.2. When the algae were described as Trebouxioid, *Trebouxia*-like, or chlorococcoids, they were presumed to be related to *Trebouxia*. Species that contained *Asterochloris* as its photobiont were treated as having *Trebouxia* in the data set, since no OTU was assigned as *Asterochloris* in the OTU data set. For the lichen photobionts of the extant communities, if there were no information in the literature indicating the photobiont type, then information of a related species was used to fill in the gap of information. The vascular plant families and fungal families were also included as comparison with the moss families and the lichen photobionts. In the second approach, a hierarchical clustering analyses was performed using 100,000 bootstrap replicates, unweighted pair-group method algorithm (UPGMA), arithmetic average, and analyzed with the Bray-Curtis similarity coefficient. A total of nine stands (three balsam fir, three poplar, and three white spruce stands), the stands within these forest types, moss and lichen photobiont type OTU richness, and the extant moss and lichen photobiont type richness (127 species; 81 lichens and 46 mosses) were included in the analyses. The trnL intron c-h gene, out of the three genes that had moss amplicons, had the most OTU information for the moss families and was the gene used to

compare OTU richness with that of the extant richness in these two approaches. Similarly, the vascular plant families were better represented in the trnL intron c-h gene. The non-lichenized fungal OTUs in the ITS rDNA genes were also used in this analysis. The clustering analyses were done on the software Past v. 3.16 (Hammer et al. 2001).

Results

Stand properties and weather

Balsam fir, poplar, or white spruce forest stands were not different with respect to aspect (°; $F_{2, 6} = 1$, $R^2 = 0.3$, $P = 0.331$), % slope ($F_{2, 6} = 0$, $R^2 = 0.1$, $P = 0.765$), and tree density (#trees/hectare; $F_{2, 6} = 2$, $R^2 = 0.4$, $P = 0.189$). The average canopy cover was marginally different among forest stands ($F_{2, 6} = 5$, $R^2 = 0.6$, $P = 0.052$): poplar stands had the lowest canopy cover, balsam fir had the highest cover, and the cover of white spruce stands was similar to the cover in the poplar and balsam fir stands (Appendix, Table B3.1).

Abundance of DNA sequences in boreal soils

A total of 1,278,934 reads were retrieved from the 26 boreal soil samples. The number of reads retrieved for each of the following genes include 209,876 reads from the 23S rDNA, 603,249 reads from trnL intron c-h, 133,819 reads from rbcL, and 331,990 from ITS rDNA. The number of bryophyte reads varied for each gene with 9,178 reads were from 23S rDNA, 26,844 reads from trnL intron c-h, and 17,330 reads from the rbcL. Lichen photobionts were identified from 21,915 reads from the 23S rDNA gene. The vascular plant reads were identified from 146,828 reads from the 23S rDNA, 116,480 reads from trnL intron c-h, and 576,405 from the

rbcL genes. Fungi were identified by 331,990 reads from the ITS rDNA region (487 lichenized and 331,503 non-lichenized). The average number and standard error of reads for the stands of the forest stand types are presented in the Appendix Table D5.3.

The number of reads of Bryophyte-rbcL were higher in conifer forest stands rather than in the poplar stands (df = 2, H =5.6, P = 0.05; Table 5.2). Fewer reads of the lichen photobiont-23S rDNA were found in poplar stands (1–2) and white spruce stand (2), intermediate abundance in the balsam fir stands, and the read numbers were the highest in white spruce 2 stand (df = 8, H =15.11, P = 0.0569; Appendix Table D5.4). Most of the other taxonomic groups showed insignificant differences in read numbers among the forest stand types.

The association between OTU reads with forest stand properties are found in Table 5.3. The canopy cover and slope of the forest stands were positively associated with the number of bryophyte-rbcL reads; however, the slope was negatively associated with the vascular plant-rbcL reads. Extant lichen cover was positively associated with the number of bryophyte-23S rDNA, lichen photobiont-23S rDNA, and bryophyte-trnL intron c-h reads. Extant moss cover and the total cryptogam cover was positively associated with the number of bryophyte-rbcL reads

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Table 5.2. Difference in bryophyte, fungi, lichen photobiont, lichen fungi (Lecanoromycetes) reads, or vascular plant number of sequences (abundance) from sequences of different genes (23S rDNA, trnL intron c-h, rbcL, ITS rDNA) obtained from environmental DNA in boreal soil samples. Boreal soil samples pertained to different stands that were dominated by a tree species (balsam fir, poplar, white spruce) and surrounding Payuk Lake in Manitoba, Canada. Different letters indicate significant differences among the treatments from the Conover-Iman Tests (α = 0.10) after performing a Kruskal-Wallis Test on the data. avg. = average, st. error = standard error, df = degrees of freedom, *H* = Kruskal-Wallis Test.

Gene	Taxa	Forest stand type	avg. read (abundance)	st. error.	df	*H*	*P*
23S	Bryophyte	balsam fir	464.1	130.9	2	4.36	0.1268
		poplar	53.3	5			
		white spruce	508.2	125.3			
	Lichen photobiont	balsam fir	325.38	73.3	2	3.29	0.2173
		poplar	336.4	73.2			
		white spruce	1777.2	236			
	Vascular plants	balsam fir	5575	1707	2	2.16	0.3395
		poplar	7334.3	606			
		white spruce	4368.1	1865			
trnL	Bryophyte	balsam fir	705.1	251	2	3.2	0.2536
		poplar	107.7	9.3			
		white spruce	2175.7	515.8			
	Vascular plants	balsam fir	4229	1396.3	2	0.66	0.7196
		poplar	5807.1	637.3			
		white spruce	3197.6	917.9			
rbcL	Bryophyte	balsam fir	1150.2 b	424.2	2	5.6	0.05
		poplar	19.1 a	3.7			
		white spruce	927.9 b	108.7			
	Vascular plants	balsam fir	21358	1185.1	2	3.71	0.1663
		poplar	21840.8	1519.7			
		white spruce	23459.1	1411.5			
ITS	Lichen fungi	balsam fir	1.8	0.6	2	1.07	0.6643
		poplar	3.7	0.6			
		white spruce	48.8	46.1			
	Fungi	balsam fir	12403.2	1648.5	2	2.98	0.2249
		poplar	11242.4	955.3			
		white spruce	14300.8	1632			

Table 5.3. Pairwise correlation (Spearman's correlation coefficient (r_s)) between each of the stand properties (aspect, canopy cover, tree density, slope, cryptogam cover, cryptogam richness) and the OTU abundance for each of four gene regions (23S rDNA, trnL intron c-h, rbcL, ITS rDNA) sequenced from environmental DNA of boreal soil samples. Values represent the Spearman's correlation coefficient (r_s) between the variables presented, $*P < 0.10$, $**P < 0.05$, $***P < 0.01$.

	Abundance (# of reads)								
	23S			trnL		rbcL		ITS	
Variables	Bryophyte	Lichen photobiont	Vascular plants	Bryophyte	Vascular plants	Bryophyte	Vascular plants	Lichen fungi	Fungi
Aspect (°)	-0.12	0.25	0.37	-0.07	0.60*	-0.03	-0.133	0.02	-0.08
Average Canopy Cover (%)	0.42	-0.07	-0.12	0.18	0.1	0.63*	-0.32	-0.57	-0.1833
Tree Density (#trees/hectare)	-0.03	0.43	0.03	0.13	0.05	-0.42	0.18	0.53	0.17
% Slope	0.38	0.25	-0.33	0.35	-0.13	0.72**	-0.82**	0.22	0.25
Extant lichen cover	0.58*	0.77**	-	0.68*	-	0.25	-	0.07	-
Extant moss cover	0.38	-0.08	-	0.17	-	0.67*	-	-0.17	-
Extant lichen and moss cover	0.38	0.13	-	0.27	-	0.62*	-	-0.3	-

OTU richness of DNA sequences in boreal soils

A total of 15,476 OTUs were retrieved from the 26 boreal soil samples. The number of OTUs from each of the following genes include 5,492 OTUs from 23S rRNA, 1,530 OTUs from trnL intron c-h, 2,655 OTUs from rbcL, and 5,799 OTUs from ITS rDNA. The number of bryophyte OTUs varied for each gene with 60 OTUs obtained from 23S rRNA, 73 OTUs from trnL intron c-h, and 56 OTUs from rbcL. Vascular plant OTUs obtained were 2460 OTUs from 23S rRNA, 1237 OTUs from trnL intron c-h, and 2426 OTUs from rbcL. The lichen photobiont OTUs obtained were 216 OTUs from the 23S rRNA gene. Fungi were identified with 5,784 OTUs from the ITS rDNA gene (7 lichenized and 5,777 non-lichenized). The average number and standard error of OTUs for the stands of the forest stand types are presented in the Appendix Table D5.5.

The number of bryophyte-rbcL OTUs, overall, were higher in conifer forest stands rather than in the poplar stands (Table 5.4.). However, balsam fir stands had similar OTU numbers as the poplar and white spruce stands (df = 2, H =7.2, P = 0.0036). The OTU richness of lichen photobiont-23S rDNA was significantly higher in white spruce stands 1–2, than in the other forest stand types (df = 8, H =13.92, P = 0.0832; Appendix Table D5.6). Most of the other taxonomic groups showed insignificant differences in OTU richness among the forest stand types.

The association between the number of OTUs with forest stand properties are found in Table 5.5. Aspect of the forest was positively and negatively associated with the vascular plant OTU richness. Canopy cover of the forest was negatively associated with the number of lichenized and non-lichenized fungi OTUs. Tree density was positively associated with the number of lichenized and non-lichenized fungi. Slope of the forest was negatively associate with OTU richness of vascular plants. Extant lichen richness was positively associated with the

number of bryophyte-rbcL OTUs, and total cryptogam richness was positively associated with bryophyte-rbcL OTUs.

Table 5.4. Difference in bryophyte, fungi, lichen photobiont, lichen fungi, or vascular plants OTU richness from sequences of four genes (23S rDNA, trnL intron c-h, rbcL, ITS rDNA) obtained from environmental DNA of boreal soil samples. Boreal soil samples were collected from stands dominated by tree species (balsam fir, poplar, white spruce), near Payuk Lake in Manitoba, Canada. Different letters indicate significant differences among the treatments from the Conover-Iman Tests ($\alpha = 0.10$) after performing a Kruskal-Wallis Test on the data. avg. = average, st. error = standard error, df = degrees of freedom, *H* = Kruskal-Wallis Test.

Gene	Taxa	Forest stand type	avg. OTUs richness	st. error.	df	*H*	*P*
23S	Bryophyte	balsam fir	9	2.8	2	0.8	0.7143
		poplar	6.3	1.5			
		white spruce	10.7	3.7			
	Lichen photobiont	balsam fir	17.7	5.3	2	2.4	0.3607
		poplar	18.2	8.9			
		white spruce	39.7	11.1			
	Vascular plants	balsam fir	68.4	14.2	2	2.83	0.2424
		poplar	116.2	48.4			
		white spruce	97.4	35.5			
trnL	Bryophyte	balsam fir	8.8	2	2	3.2	0.2536
		poplar	7.7	2.3			
		white spruce	16.7	5.2			
	Vascular plants	balsam fir	42.8	9.3	2	1.38	0.5016
		poplar	54.1	7			
		white spruce	43.3	9.2			
rbcL	Bryophyte	balsam fir	8.3 ab	1.5	2	7.2	0.0036
		poplar	4 a	0.2			
		white spruce	13.7 b	0.8			
	Vascular plants	balsam fir	68	21.1	2	1.96	0.3742
		poplar	87.3	18.9			
		white spruce	122.3	40.5			
ITS	Lichen fungi	balsam fir	0.6	0.2	2	0.96	0.6214
		poplar	1.1	0.4			
		white spruce	0.8	0.2			
	Fungi	balsam fir	195.2a	7	2	4.85	0.0881
		poplar	242b	10.4			
		white spruce	227.7ab	15.9			

Table 5.5. Pairwise correlation (Spearman's correlation coefficient (r_s)) between each of the stand properties (aspect, canopy cover, tree density, slope, cryptogam cover, cryptogam richness) and the OTU richness for each of four gene regions (23S rDNA, trnL intron c-h, rbcL, ITS rDNA) sequenced from environmental DNA of boreal soil samples. Values represent the Spearman's correlation coefficient (r_s) between the variables presented, $*P < 0.10$, $**P < 0.05$, $***P < 0.01$.

Variables	Richness (# OTUs)								
	23S			trnL		rbcL		ITS	
	Bryophyte	Lichen photobiont	Vascular plants	Bryophyte	Vascular plants	Bryophyte	Vascular plants	Lichen fungi	Fungi
Aspect (°)	-0.13	0.22	0.67*	-0.07	-0.65*	0.27	0.58*	0.2	0.45
Average Canopy Cover (%)	-0.03	-0.03	-0.13	0.18	-0.09	0.48	-0.08	-0.63*	-0.83**
Tree Density (#trees/hectare)	0.32	0.47	0.37	0.13	-0.14	-0.17	0.22	0.60*	0.62*
% Slope	0.26	0.13	-0.63*	0.35	-0.11	0.52	-0.55	0.26	-0.40
Extant lichen species richness	0.27	0.5	-	0.5	-	0.92***	-	0.06	-
Extant moss species richness	0.07	-0.16	-	0.19	-	0.51	-	-0.03	-
Extant lichen and moss richness	0.23	0.35	-	0.37	-	0.8*	-	0.11	-

The relative proportion of soil OTU richness (trnL intron c-h) from the eDNA analyses and the species identified for the extant community richness at the family level of mosses is reported in Fig. 5.1. The moss families showed differences between soil OTUs and extant

species within the boreal forest stand types. For example, the extant species richness of the Brachytheciaceae are represented in the balsam fir, poplar, and white spruce stands, but no OTUs from the Brachytheciaceae were found as eDNA in the soil bank within these forests. In contrast to the Brachytheciaceae, a few families (Buxbamiaceae, Calliergonaceae, Grimmiaceae) are represented only as OTUs from eDNA (not as extant species) within these forests.

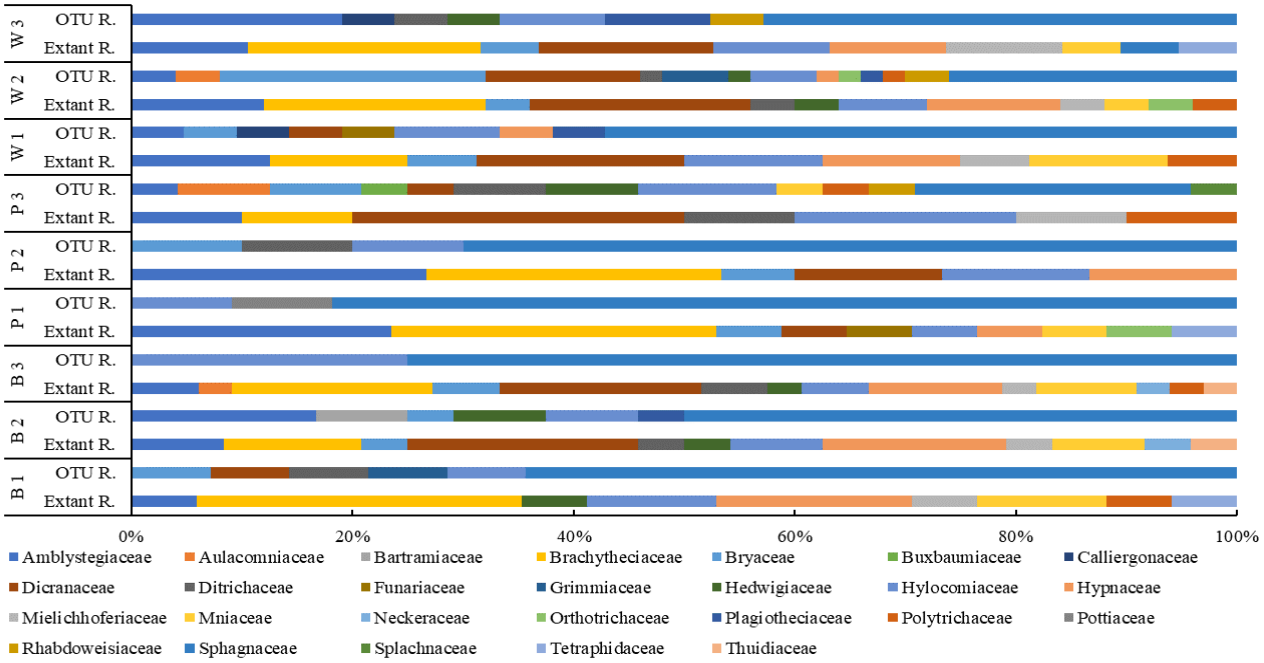

Figure 5.1. Relative proportion of the richness, number of different operational taxonomic units (OTUs) in soils and extant species in the community, of moss families from mosses and lichens collected in summer 2015/2016, or environmental DNA sequences (moss, trnL intron c-h) from soil samples collected in summer 2017 in nine tree dominant stands in boreal forests (three balsam fir, three poplar, and three white spruce stands) in Manitoba, Canada.

The relative proportion of the lichen photobiont type (*Trebouxia*, *Coccomyxa*, or *Nostoc*) richness also varied between the OTU richness and the extant richness among the forests stand,

but with the *Trebouxia* photobiont represented in higher proportions than the other two photobionts (Fig. 5.2). Furthermore, the photobiont may not be represented in the lichens present in the extant community, but it can be present in the photobiont OTU richness within the soils of forests such as it was observed for the relative proportion of photobionts *Coccomya* and *Nostoc* in poplar forests.

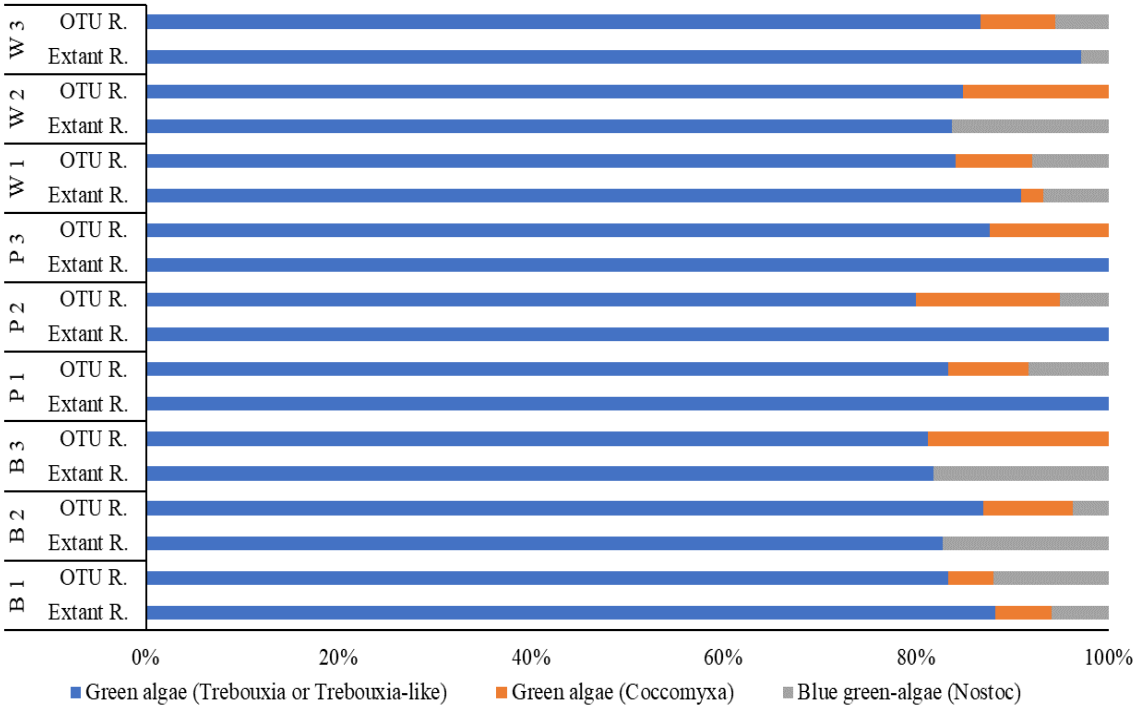

Figure 5.2. Relative proportion of richness, number of different operational taxonomic units (OTUs) in soils and extant species in the community of the lichen photobiont types (*Trebouxia*, *Coccomyxa*, or *Nostoc*), from lichens collected in summer 2015/2016, or environmental DNA sequences (lichen photobiont, 23S rDNA) from soil samples collected in summer 2017 in nine tree dominant stands in boreal forests (three balsam fir, three poplar, and three white spruce stands) in Manitoba, Canada.

The proportion of vascular plant OTU families (trnL intron c-h) show similarities between the balsam fir and white spruce stands, and less with the poplar stands (Fig. 5.3a) . The proportion of the fungal OTU families represented in the soil as eDNA show similarities between the poplar and white spruce stands, and less with the balsam fir stands (Fig. 5.3b).

Separation of soil OTU richness (trnL intron c-h) of the moss families from the extant moss family richness in the boreal forest stand types (B, P, W) is strongly supported (Fig. 5.4a). The separation of stand types between the OTU and the extant richness of the lichen photobiont type is marginally supported (Figure 5.4b). The separation of the forest stand types by the family OTU richness of vascular plants (trnL intron c-h) is not well supported (Fig. 5.4c). Similarly, the separation of the forest stand types by the family OTU richness of fungal families (ITS rDNA) is not well supported (Fig. 5.4c).

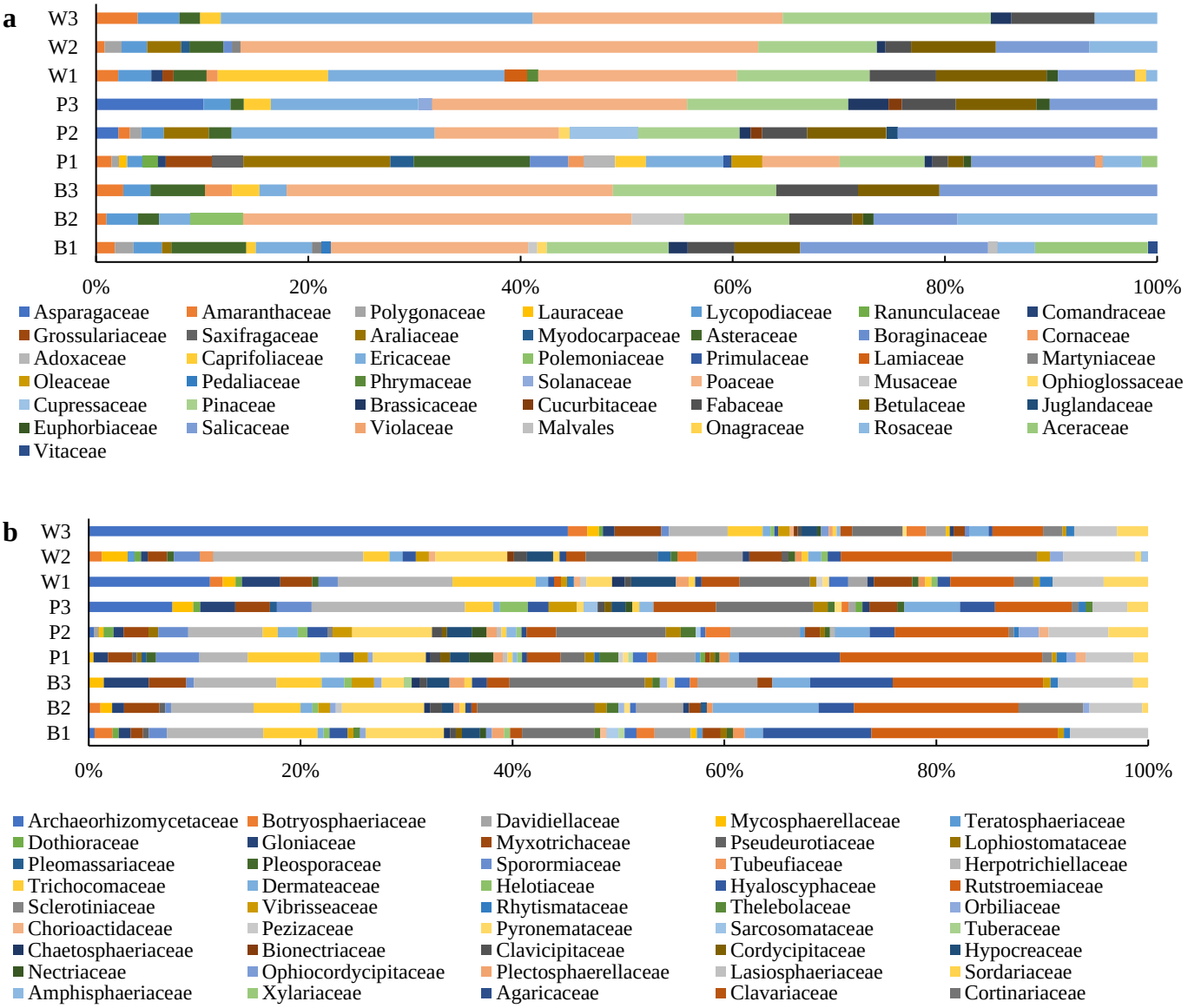

Figure 5.3. Relative proportion of the richness, number of different operational taxonomic units (OTUs) in soils, of vascular plant (**a**) and fungal families (**b**) from environmental DNA sequences (trnL intron c-h and ITS rDNA, respectively) from soil samples collected in summer 2017 in nine tree dominant stands in boreal forests (three Balsam Fir, three Poplar, and three White Spruce stands) in Manitoba, Canada.

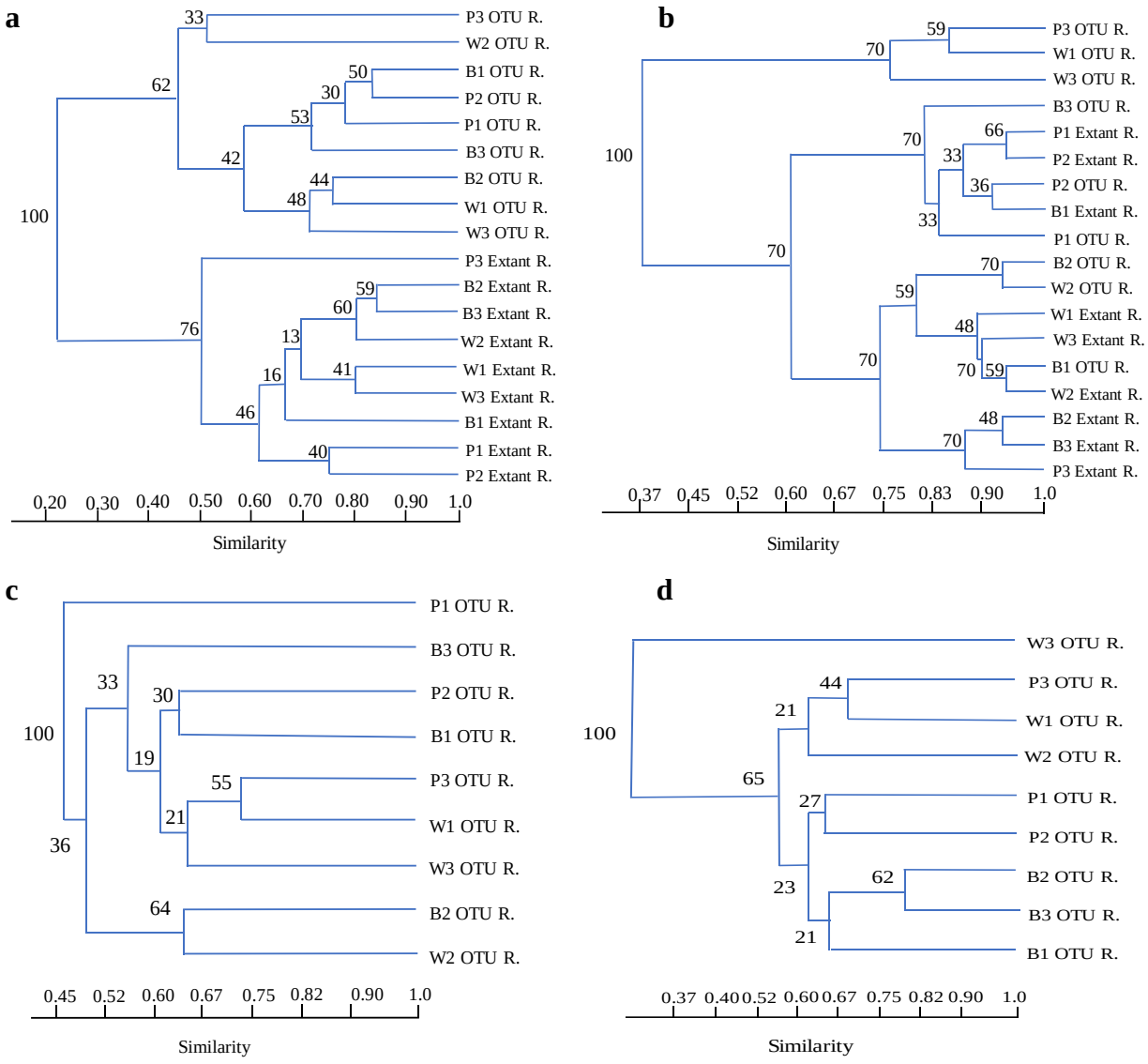

Figure 5.4. Dendrograms from the cluster analyses (UPMGA algorithm, Bray-Curtis similarity index) performed on the richness (R), number of different operational taxonomic units (OTUs) in soils and/or extant species in the community of moss families (trnL intron c-h; **a**), lichen photobiont types (*Trebouxia*, *Coccomyxa*, or *Nostoc*; 23S rDNA; **b**), vascular plant families (trnL intron c-h; **c**) and fungal families (ITS rDNA; **d**) from mosses and lichens collected in summer 2015/2016 or environmental DNA sequences (moss, trnL intron c-h gene, lichen photobiont, 23S rDNA) from soil samples collected in summer 2017 in nine tree dominant stands

in boreal forests. The tree-dominant forests include three balsam fir (B1–3), three poplar (P1–3), and three white spruce stands (W1–3) in Manitoba, Canada. Dendrograms (**a**) and (**c**) show the richness associated with each forest stand type, while (**b**) and (**d**) show the richness associated with the stands within each forest stand type.

Discussion

eDNA abundance and richness among boreal forest stands

Bryophyte and lichen eDNA abundance and richness in the soil bank were marginally higher in conifer stands than in poplar stands in this study, while the vascular plant and fungal eDNA diversity did not vary among these forest stand types. The higher eDNA abundance and richness suggest that conifer stands provide a suitable environment for a higher number of cryptogams than poplar stands, allowing for the production of propagules in higher abundance that are eventually deposited in the soil. A higher abundance and richness of cryptogams in conifer forests than in poplar forests is consistent with the findings of richness and/or abundance in conifer vs. deciduous stands in other studies (Chapter 3, Holt et al. 2015, Kumar et al. 2018, and Bartels et al. 2018). The eDNA abundance and richness of bryophytes and lichens may have been influenced by the forest stand properties such as aspect, canopy cover, tree density, and slope. However, vascular plant eDNA abundance and richness had mixed results by being both positive and negatively associated with aspect of the forest stands for different genes, most likely resulting from the variation in their diversity with the forest stands. Yet, an increase in canopy cover may account for the eDNA abundance of bryophytes, but showed lower richness of fungi (lichen and non-lichen). An increase in ground cover and competition (dominance) of feather mosses over fungi (lichenized and non-lichenized) on the forest floor of a closed canopy forest may explain this discrepancy (Pharo and Vitt 2000, Sulyma and Coxson 2001).

High tree density was correlated with a higher richness of lichenized fungi most likely resulting from the larger number of substrata available for epiphytic lichens, which increases the surface area for the colonization and expansion of lichen populations (Rolstad et al. 2001, Friedel et al. 2006), and may also increase the course woody debris on the forest floor that serve as substrata for both lichenized and non-lichenized fungi (Critesand Dale 1998, Norden et al. 2004). An increase in the slope of the forest stand, mostly facing the south, was correlated with an increase in the bryophyte abundance, but a decrease in vascular plant abundance and richness. The sloped forest stands in this study were located near rock faces which provided protection from the prevailing wind, and most likely increased humidity levels and lower light levels that allowed the bryophyte cover to increase resulting in more propagules being deposited in the soil bank. The association between cryptogam cover and eDNA abundance was consistent with more propagule formation of bryophytes and the lichen photobiont which could be eventually stored in the soil as eDNA. The photobiont in this study may potentially include many non-lichenized algae and cyanobacteria because of the universal primers used for the study.

The discordance between the extant cryptogam cover and the eDNA abundance of bryophyte suggests that within the forest stands there might be microhabitats for bryophytes to occupy such as decayed and intact logs (Pharo and Vitt 2000) that influence eDNA diversity even when the forest stands are dominated by other cryptogams. For example, the more open stands were dominated by the lichen cover (Chapter 3), but the logs and tree bases within the stands that were not occupied by lichens may serve as a substratum on which bryophytes grow and for bryophyte propagules to be produced and stored in the soil bank. On the other hand, the discordance between the extant cryptogam cover and the eDNA abundance of bryophyte may reflect past forest stand properties that facilitated bryophyte growth, production of propagules,

and their dispersal to the soil bank. Once the forest stands properties changed, the cryptogam community presumably changed to the current composition, which is different from that of the composition present in the soil bank. A similar explanation can be used for the eDNA richness of bryophytes, which was associated with the lichen and total cryptogam richness.

The community composition of moss families and lichen photobiont type richness is distinct between the soil and the extant community

Both the richness of the eDNA moss families and the photobiont type indicate that the community composition within the soil is different from that of the community composition in the extant community, which is contrary to our expectations. Other studies have found comparable results, where the extant community does not necessarily mirror the soil community and mostly represent a fraction of the species in the extant community (Ross-Davis and Frego 2002, Pasiche-Lisboa and Sastre-De Jesús 2014). The difference between the community in the soil bank and extant community may be explained by the soil bank community containing propagules from past dispersal-deposition events (Hock et al. 2008) over a long period of time. The dispersal-deposition events may also have been the result of dispersal from a different community at long distances from the soil bank (Iglesias et al. 2015, Barbé et al. 2016). Additionally, through ecological succession the extant community and its propagule rain may have changed because of the changes in the forest stand properties (Chapter 3, Jonsson 1993, Ross-Davis and Frego 2002, Barbé et al. 2017). The type of disturbance that may have exposed the propagules in the soil bank and the edaphic and light conditions would be expected to aid in the propagule establishment could change or maintain the local community (Jonsson 1993, Caners et al. 2008). Interestingly, in the case of the richness of the lichen photobiont, it was

noted that the photobiont was present in the soil bank even when the photobiont was not present in extant community. This may suggest the presence of the lichen symbionts (soredia, isidia) as dormant propagules or as free-living entities in the soil (Ahmadjian 1988), long distance dispersal events, or the OTUs representing non-lichenized green algae and cyanobacteria. The less separation of the forest types by photobiont types richness (OTU and extant) in the dendrograms (Fig. 5.2c and d) may be the result of lack of photobiont identification at lower taxonomic ranks, or it may be the result of greater dispersal ability of the photobiont between forest types. The explanation of less separation of the forest types by photobiont types richness (OTU and extant) may be also used to explain why the vascular plant and fungal family richness (OTU) showed similar results for the forest stand types.

On the OTU taxa within the soil bank

Many species were identified from the OTUs in the soil bank of boreal forest stand types. Among bryophyte OTUs that were expected in the boreal forest stands (see Chapter 3), forest floor species common on boulders, humic soil, decaying wood, and tree bases included *Aulacomnium palustre* (Hedw.) Schwagr., *Ceratodon purpureus* (Hedw.) Brid., *Dicranum polysetum* Swartz and *D. scoparium* Hedw, *Funaria* sp., *Hedwigia ciliata* (Hedw.) P.Beauv., *Hylocomium splendens* (Hedw.) B.S.G., *Pleurozium schreberi* (Michx.) Trevis, *Sanionia uncinata* (Hedw.) Loeske, and *Tetraphis pellucida* Hedwig; however, their occurrence was sporadic in the soil bank and is likely explained by their patchy distribution on substrata within the forest stand types. One OTU was detected as *Orthotrichum obtusifolium* Hedwig, which grew as an epiphyte on poplar trees, but the species was even found in the soil bank of tree stands dominated by conifers probably due to these stands having a few poplar trees in them.

Bryophytes that were likely in the area, were detected as OTUs, but were not detected during the surveys in Chapter 3 include *Barbilophozia barbata* (Schmid. ex Schreb.) Loeske, *Bryum psuedotriquetrum* (Hedw.) G. Gaertn., *Buxbaumia aphylla* Hedwig, *Polytrichum commune* Hedwig and *P. strictum* Bridel, J. Bot. (Schrader)., *Ptilidium pulcherrimum* (Weber) Vain., *Sphagnum* (*S. fuscum* (Schimp.) H.Klinggr., *S. magellanicum* Bridel, *S. riparium* Ångström, *S. squarrosum* Crome, *S. teres* Ångström), and *Tetraplodon* sp., which may indicate their broad dispersal capility or the occurrence as part of past boreal communities. *Sphagnum* was the genus with the highest number of OTUs and reads within the forest stand types for all genes amplified (13 OTUs, trnL intron c-h; ~17 OTUs, 23S rDNA; 52 OTUs, rbcL). However, only one extant species (*S. capillifolium* (Ehrh.) Hedw.) was surveyed in a boggy habitat within a white spruce stand (WS3). The occurrence of *Sphagnum* OTUs within these forest stands may be explained by their broad dispersal capability in the landscape, but also by the location where the forest stand types are present having boggy habitats that allowed for *Sphagnum* communities to proliferate, which further supports the hypothesis that the OTUs in the soil bank represent past communities.

A few bryophyte OTUs where present in the soil banks that seemed to be unlikely representatives of the local or regional flora: *Ambuchanania leucobryoides* (T. Yamag., Seppelt. & Z. Iwat.) Seppelt & H. A. Crum, is a sphagnioid moss endemic to Tasmania (Jonhson et al. 2008); *Sphagnum microporum* Warnst. ex Card., is distributed through China and Korea (efloras.org); and, *Phyllodon truncatulus* (Müll. Hal.) W. R. Buck, is a sub-tropical to tropical species (Buck 1998). The presence of these bryophytes within the soil bank may indicate rare long-distance dispersal events or the misidentification of the OTU due to the inadequacies of using primers that amplify short nucleotide sequences (200–300) and that are unable to detect a

higher level of diversity within the stands (Taberletet al. 2012, Bohmann et al 2014., Thomsen and Willerslev 2015).

The gene (23s rDNA) that was used to characterize the lichen photobiont richness and abundance in the soil banks had numerous OTUs amplified and some of which were classified into eight species, pertaining to free-living species (*Coccomyxa dispar* Schmidle) as well as to species that form symbiosis with lichenized fungi (*Coccomyxa subellipsoidea* E.Acton, *Nostoc* sp. of *Peltigera malacea* (Ach.) Funck, *Trebouxia asymmetrica* Friedl & Gärtner). *C. subellipsoidea* have been known to associate with basidiomycetes of the genus *Omphalina* (Zoller and Lutzoni 2003), which was present in forest stands where the basidiomycete of *Lichenomphalia umbellifera* (L.: Fr.) Redhead, Lutzoni, Moncalvo & Vilgalys was present or absent. *Nostoc* OTUs of the lichen *P. malacea* were also found in the same stands where *P. malacea* was collected and in stands where the species was absent. *T. asymmetrica* and related species are commonly associated with species from the genus that were abundant within forest stands (Appendix, Table D5.2.: *Bryoria*, *Caloplaca*, *Cladonia*, *Evernia*, *Usnea*; Ahmadjian 1993), and many OTUs of this species were found within all the forest stand types. The presence of these different photobiont OTUs, and different OTUs for the same species (genotypes), within the same area where the lichenized fungi were collected or absent, may indicate the dispersal strategies employed by asexually reproducing lichens to facilitated lichenization and/or retain the morphology of the lichen in mild to stressful environments (Doering and Piercey-Normore 2009).

Considering the abundance of lichenized fungi in the forest stands (Chapter 3), there should have been a higher representation of their major groups within the OTUs that were amplifed (eg., *Bryoria*, *Cladonia*, *Physcia*, *Usnea* spp.). However, only one OTU was identified

to species (*Alectoria sarmentosa* (Ach.) Ach.) that was not found within the studied area, and only seven OTUs were classified with the lichenized fungi defined in this study. The lack of representation of lichenized fungi within the soil may represent their limited dispersal into the soil bank, the rapid degradation of eDNA from the lichenized fungal thallus in the soil, and/or the misindentification of OTUs.

Discussion of the identities of vascular plant and fungi are located in the Appendix **Discussion D5.7**.

Disentangling the soil bank diversity studies using eDNA

One of the challenges encountered in this study was the difficulty to accurately match the soil eDNA richness and abundance with that of the dispersal patterns from the extant community composition. The sequencing and databasing of the genes representing the extant species within the landscape where the sites were located would provide a valuable baseline with which to compare the eDNA results. The sequencing and databasing of species will provide the same type of data with which to compare the richness and abundance of the species, rather than the families, which are present in the soil eDNA. The use of the same type of data (DNA sequences) would increase the likelihood of a correct taxonomic assignments for the OTUs in the soil. Furthermore, the sequencing and databasing of species from the landscape and its comparison to the species in the soil may clarify the extent to which the eDNA diversity in soil results from past dispersal events or by the dispersal of propagules from communities located at long distances.

Conclusions

The eDNA studies have detected cryptogams and vascular plants in soil cores. However, no study has made a link between the OTU abundance and richness in soil bank with dispersal events resulting from the extant community and the abundance and richness of the extant community—particularly for bryophytes and lichenized fungi (see Thomsen and Willerslev 2015 and citations within). However, here it is shown that the collection of few grams of top layer soil allowed for the detection of OTUs for select barcoding genes (23S rDNA, trnL intron c-h, rbcL, ITS rDNA) that enabled the characterization and association of bryophytes, vascular plants, the lichen photobiont, lichenized fungi, and non-lichenized fungi abundance and/or richness with that of the stand properties of boreal forest stand dominated by balsam fir, poplar, and white spruce. The characterization of cryptogams and vascular plants using eDNA helped gain novel insight into the species or groups that were influencing the soil bank. These novel findings show that eDNA OTU richness and abundance for the taxa studied here were marginallly different between forest stand types. Since the stands were relatively close to one another they were likely within the range of the dispersal capacity of species that influence the soil bank. The presence of bryophyte and lichen eDNA in the soil is partly correlated with the properties of the stand (aspect, canopy cover, tree density, slope) as well as with the extant community richness and abundance. The correlation with the stand properties suggests that the bryophyte and lichen growth in these stands that may either hinder or facilitate the dispersal and deposition of propagules and influence the community within the soil bank. Although the bryophyte and lichen richness and abundance of soil eDNA is partly influenced by the extant community richness and abundance, the community represented in the soil is different from that of the extant community suggesting that the soil eDNA represents a past community composition and/or long-distance dispersal events. This study provides an innovative approach to better understand the

composition of the soil bank communities of bryophytes and lichens by using high-throughput sequencing technologies, moving forward our understanding on how dispersal might be influencing the soil bank.

Acknowledgments

Funding for this study was obtained from the Department of Biological Sciences and the Faculty of Graduate Studies (University of Manitoba) and from the Natural Sciences and Engineering Research Council of Canada (NSERC-DG to MPN).

CPL and MPN conceived the study project. TB collected the soil samples. CPL designed the experiments and analyzed the data from the experiments. All authors wrote and edited the manuscript.

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Chapter 6: Responses differ among three boreal mosses after exposure to extreme temperatures

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Abstract

Many factors may affect the survival and establishment of a moss's vegetative propagules after dispersal, but little is known about the species-specific nature of the response. This study examined the survival and regeneration of gametophore fragments after exposure to laboratory temperature changes for three boreal forest mosses from different habitats: *Dicranum polysetum*, *Orthotrichum obtusifolium*, and *Pleurozium schreberi*. Fragments were cultured on water agar and the survival and regeneration responses were recorded. Logistic regression analyses and AIC modeling evaluated the association between the response with the size of the gametophore fragments exposed to five abrupt or gradual temperatures for up to six exposure durations. The increased survival and regeneration were best explained when species were exposed to gradual

rather than abrupt temperatures, lower rather than higher temperatures, and when the fragments had larger, rather than smaller sizes. The mosses had different survival and regeneration responses that may be species-specific, including clonal growth via the production of gametophore branches and protonemata, or mostly protonemata, even when exposed to elevated temperatures.

Key words: asexual propagules; bryophyte; desiccation tolerance

Introduction

Moss propagules dispersed to different habitats must be able to withstand varying environmental conditions to survive and become established. Competition for resources such as nutrients, light, moisture, and temperature influence the successful establishment of these fragments (Glime 2007). For example, moisture and light levels influence the ability of mosses to establish in an environment (Cleavitt 2002, Fenton and Bergeron 2006). Low and high levels of light during culturing affect gametophyte fragments and propagules from a diaspore bank by contributing to the increase in richness and cover on soil in petri dishes placed at high light levels (Caners et al. 2009). Species of *Polytrichum* (*P. commune* Hedw., *P. formosum* Hedw., *P. juniperinum* Hedw., *P. piliferum* Hedw.) and *Pogonatum* (*Po. aloides* (Hedw.) P. Beauv., *Po. urnigerum* (Hedw.) P. Beauv.) growing at 20 °C were shown to have varied regeneration responses when detached leaves were cultured, with *Po. aloides* not regenerating and other species regenerating by protonemata or buds directly from leaves (Wilmot-Dear 1980). The liverwort *Blasia pusilla* L. produces two types of propagules that showed different responses to the environment, allowing for colonization during the growing season, or survival over the

winter months and germination in the next growing season (stellate and ellipsoid gemmae, respectively; Duckett and Renzaglia 1993). It is evident from these studies that bryophytes show species-dependent responses to changes in the environment, but few species have been examined under controlled conditions.

The gametophyte, which produced the fragment, or the fragment itself before regeneration and establishment, are exposed to varying temperatures for a period of time. Ambient temperatures (5–25 °C) have been shown to favor growth for some boreal mosses (Furness and Grime 1982, Proctor 2000), but exposure to extreme temperatures (55–65 °C for dry bryophytes and 35 °C to 40 °C for moist bryophytes) decreased survival or had no effect on the plant (Proctor 2000), such as during heatwaves and fires. Gradual changes in seasonal temperatures may also cause changes in moss cover and species composition, depending on the moss's life history strategies and habitat requirements (Bates et al. 2005). Changes in temperature may also be abrupt, such as the short outburst of sunflecks on forest mosses that have been shown to warm up the gametophyte to 39°C within a short period of time even when the air temperature was 20 °C (Proctor 2000, Glime 2017*a*). Because mosses are exposed to both abrupt and gradual temperature changes, this may lead to regeneration responses that are specific to each species.

The response of mosses to environmental conditions (time and temperature) may also depend on gametophyte fragment size, where the size of the fragment dispersed may influence the ability of the fragment to regenerate. An increase in *Hylocomium splendens* (Hedw.) W.P. Schimp gametophyte size, has been shown to improve their regeneration and resilience on the forest floor (Økland 1995). However, it is unknown how these responses, because of the size of the gametophore, differ among species. Regeneration from the gametophore has also been shown

to vary among species. For example, some species, such as *Pleurozium schreberi* (Michx.) Trevis have been shown to establish poorly from gametophore fragments in garden experiments when compared with *Dicranum, Aulacomnium,* and *Sphagnum* species (Bayfield 1976). Successful establishment depends on the regeneration response from fragments before gametophyte growth occurs. For example, the rapid production of gametophore and protonemata mats from fragments of *P. commune*, *A. palustre*, *D. scoparium* Hedw., *Hypnum cupressiforme* Hedw., *P. schreberi*, *Sphagnum papillosum* Lindb., and *Leptobryum pyriforme* (Hedw.) Wilson facilitated the development of growth in pots within a few weeks (Bayfield 1976). Additionally, the extent of development of protonematal shoots of these seven species varied, but on average within five months they occupied 25% of the soil surface in a pot (Bayfield 1976). These results may suggest that the type of regeneration response may determine the extent of successful establishment in nature. Species that regenerate by both branch and protonemata production from fragments may be more successful at establishment than species regenerating by only one of the two types of regeneration structures.

Studies have shown that moss establishment and regeneration responses may be linked to temperature, nature of temperature change (abrupt vs gradual), exposure time, and fragment size. These establishment and regeneration responses may reflect conditions of the habitat or life history strategy of the moss. However, habitat conditions and their microclimate have also been shown to influence sporulation in *Sphagnum* species—depending on water availability (Sundberg 2002), to influence a higher regeneration from moss gametophyte fragments (and rarely spores) on tree mounds (Kimmerer 2005), and cause a varied regeneration and establishment responses from fragments of fen mosses exposed to changes in nutrients (Li and Vitt 1994). Mosses that are growing in terrestrial habitats and are competitive species would be

expected to show different responses than those in epiphytic habitats or that are pioneer species. Additionally, within each habitat (terrestrial), these responses may vary and be related to the growth forms of mosses (pleurocarpy and acrocarpy). For example, pleurocarpous mosses produce stems and branches with the ability to grow both indeterminately and determinately (main stem and short branches, respectively), whereas acrocarpous mosses produce a main stem, usually with determinate growth and few or no short branches (Frey and Kürschner 2011), which possibly affects the regeneration response of dispersed fragments. However, few studies have linked the survival and regeneration of mosses with their growth forms within a habitat.

The objective of this study was to examine how the survival and regeneration of gametophore fragments from mosses were affected by different temperature regimes and gametophore fragment sizes, in an effort to understand the ecology of three species of boreal mosses (*Dicranum polysetum* Sw., *Orthotrichum obtusifolium* Brid., and *Pleurozium schreberi*). Furthermore, the influence of habitat and growth forms on moss survival and regeneration was explored in this study.

Methods

Moss gametophores were collected during the month of June (2015) from boreal forests on the Precambrian shield in northern Manitoba, near the eastern shore of Payuk Lake (54°39’03.6”N, 101°29’52.8”W). Mean winter and summer temperatures in this area vary from -29°C to 21°C, with temperatures ranging between -35 °C and 29 °C, and extreme temperatures rarely reaching -46.1°C (December, 1930) in winter months and 40 °C (July, 1929) in summer months (Flin Flon Airport, the nearest weather station to the boreal forests, https://goo.gl/GPkoqm). These boreal forest stands are composed of balsam fir *Abies balsamea*

(L.) Mill.), white spruce (*Picea glauca* (Moench) Voss), poplar (*Populus tremuloides* Michx.), Jack pine (*Pinus banksiana* Lamb.), larch (*Larix laricina* (Du Roi) K. Koch), and alder (*Alnus* spp., Fontaine et al. 2014). The forest floor is dominated by reindeer lichens (*Cladonia* spp.) in drier areas, feather mosses in dry to wet areas, and *Sphagnum* spp. in the wettest areas.

Three moss species were studied: *Dicranum polysetum*, *Orthotrichum obtusifolium*, and *Pleurozium schreberi*. *Dicranum polysetum* is an acrocarpous moss species that grows 3–10 cm in height, in loose to dense tufts on soil or humus in boreal woodlands. *Orthotrichum obtusifolium* is also an acrocarpous moss which grows ca. 6–16 mm long on the trunks of deciduous trees in boreal regions, but rarely on coniferous trees (*Juniperus virginiana* L.; Churchill 1985). *Pleurozium schreberi* is a large pleurocarpous moss that grows 6–15 cm long in loose mats on humus and soil on the boreal forest floor, and sometimes on stumps. The identification, nomenclature, and biological/habitat information for the moss species followed that of Ireland (1982). Specimen vouchers from the plant material we gathered were air-dried and deposited in the cryptogram division of the Sir Wilfred Grenfell Campus (SWGC) Herbarium at Memorial University of Newfoundland. The plant material was air dried and stored at room temperature until used (six months). The unavoidable collection at different times of the season from site to site, and without dehardening may have affected the outcomes of this study.

The effect of gametophore size on regeneration was studied by cutting the shoot apex of a branchless gametophore (here called fragments) stem for *D. polysetum* and *P. schreberi* into two sizes, 0.5 cm or 1.0 cm in length. Whole gametophores of *O. obtusifolium* that were growing on poplar bark were used for the study (0.16 ± 0.06 cm). Air dried fragments were placed in aluminum foil packets (3 mm x 5 mm; Alcan ®) and exposed to temperature treatments consisting of abrupt (coded as 0) and gradual changes (coded as 1). The fragments were not

hydrated prior to the exposure treatment, as prior hydration was shown to be detrimental to moss physiology and subsequent growth (Proctor 2000). The fragments for the abrupt treatments were exposed to 22 °C (room temperature) for six months beginning from the month of collection, then moved immediately to the respective abrupt temperature treatment (43, 22, 6, -18, -40, or -80°C). Fragments exposed to gradual temperature treatments were exposed to 22 °C for six months beginning from the month of collection, moved to the next temperature treatment, exposed for a month, and then the process was repeated until they were exposed to the last temperature treatment (one to four temperature changes). For example, if the last temperature treatment was -80 °C, then the treatment would start at 22 °C, then after a month moved to 6 °C, exposed for one month in 6 °C, then moved to -18 °C, exposed for one month in -18 °C, and subsequently repeated the procedure until it eventually was exposed to -80 °C (Appendix Fig. E6.1). Fragments of both the abrupt and gradual treatments were kept in the final temperature treatment for one to six months to test the influence of exposure time on survival and regeneration.

A pilot experiment was performed on fragments of the three species to limit contamination (data not shown), where fragments were sterilized in 5% of commercial bleach for 150 s. The bleach killed the stems; thus, a sterilization procedure was not incorporated in the study. However, to limit the influence of contaminants before and after culture on the survival and regeneration outcomes, the fragments were handled with clean tweezers and inoculated under aseptic conditions in a biosafety cabinet (Purifier+ Class II, Type A2, Labconco). Once the target temperature/time treatments were reached, the fragments were placed onto the medium (1% water agar) in plastic petri dishes (16 mm diam.) with stems placed postrate and then the dishes were sealed with parafilm. In addition, no more than two fragments from the same

gametophyte, out of the 10 fragments placed on the water agar, were placed in each plate for the respective species. The plates were stored in a growth chamber (18°C) at 14/10 hr day/night cycles (11.9 μmol $m^{-2}s^{-1}$), where they were maintained for six weeks.

Survival and regeneration of the moss fragments

After six weeks of culture, fragments were assessed for color (as an indication of survival; living = green or dead = brown) and the data were recorded as binary values (0 and 1, respectively). No color standards were used in this study. Regeneration was assessed by determining whether each fragment produced branches and/or protonemata, by forming new branches from the main stem or apices, or protonemata from within the leaves and/or stem of the gametophore (coded as 1). The absence of branches or protonemata indicated that there was no regeneration (coded as 0). The identification of branches and protonemata was based on these following definitions. Protonemata were defined as the presence of filamentous caulonemata and chloronemata cells: chloronema cells have large chloroplasts and have by a slow tip growth mechanism; caulonema cells are more elongated, have few smaller chloroplasts, and have by rapid tip growth (Menand et al. 2007). The moss's gametophore branches are developments from the vegetative body (stem) with and without leaves or rhizoids (Sharp et al. 1994). All of the regenerating moss fragments were examined under a dissecting microscope.

Statistical Analyses

Logistic regression analyses (Peng et al. 2002) were used on the categorical response (binary data, 0 and 1) for each of three boreal mosses, and tested whether the nature of temperature changes (abrupt/gradual), temperature, time of exposure, and the size of the

fragment influence survival and regeneration responses of mosses in different temperature treatments. Specifically, the logistic regression predicted three response variables: one survival response [color, 0 = brown (dead), and 1 = green (alive)] and two regeneration responses to each of four predictor variables, which included protonemata and/or branch production (0 = no protonemata or branch production; 1 = protonemata or branch production) for *D. polysetum*, *O. obtusifolium*, and *P. schreberi*. A total of 10 replicates (fragments) were used per treatment for each of three species, giving a total of 3600 data points (10 replicates × 2 abrupt/gradual temperature changes × 6 temperatures × 6 time treatments (durations), and × 2 fragment sizes) for each of the three species (*D. polysetum*, *O. obtusifolium*, and *P. schreberi*) resulting in 1440, 720, 1440 data points, respectively. During processing, some gametophore fragments were lost or damaged reducing the total to 3147 gametophore fragments [*D. polysetum* (1264), *O. obtusifolium* (614), and *P. schreberi* (1269)]. The probabilities of survival (green or brown) and regeneration (presence or absence of protonemata/branches) from the logistic regressions were fitted onto a graph to better visualize the response outcome (*y*-axis, probability values between 0 and 1) per treatment (*x*-axis, categorical or binary data) for each of the species.

The variance inflation factors (VIF) assessed whether collinearity or lack of independence was present between predicting variables (abrupt/gradual temperature changes, temperature, time of exposure, and fragment size) in the logistic regression. The VIF measures how much the variance of the estimated regression coefficients (*b*) for the positive or negative association between the response and predicting variables was inflated compared with when the predicting variables are not linearly related: VIF =1 suggests no correlation; 1< VIF <5 suggests moderate correlation; VIF >5 to 10 suggests high correlation (Mansfield and Helms 1982; Minitab 2000).

Odds ratios helped us to interpret the logistic regression models by looking at the degree of likelihood of each response to the predictors (Minitab 2000, Peng et al. 2002). The odds ratio is a measure of association, as it approximates how much more likely or unlikely (in terms of odds) it is for the survival and regeneration to increase with the predictor variables. An odds ratio <1 indicates that the response is less likely to be affected by the higher values of the predictor, an odds ratio = 1 indicates no association between the response and the predictor, and values >1 indicate a higher likelihood of the higher values of the predictor affecting the response (Minitab 2000).

Deviance R^2 evaluated the overall model fit for each species based on the survival and regeneration responses to the four predicting variables. The Deviance R^2, which is similar to McFadden's pseudo R^2 (McFadden 1973, Menard 2000), is the proportion of deviance in the data that the model explains: the larger the deviance R^2 (0–100%) the better the model fits the data for logistic and Poisson regression models (Cameron and Windmeijer 1996, Minitab 2000). We reported the adjusted deviance R^2 or (adj. dev. R^2) because it adjusts to the number of predictors in the model. Akaike's Information Criterion (AIC) was used to evaluate and compare all of the possible models. For the AIC, the smaller the AIC, the better the model fits the data using the fewest parameters, properly balancing the errors from over- and under-fitting (Minitab 2000, Burnham and Anderson 2001). Because there was a small sample size per treatment, the adjusted AIC (AIC_c) was used because of its lower bias in evaluating the most parsimonious model (lowest AICc; Cavanaugh 1997). Akaike weights (*W)* were calculated to evaluate the relative support for each model (Burnham and Anderson 2001). Models included in the results were those that had substantial support (Δ AIC_c = 0–2), and all of the AIC_c models can be found in the

Appendix (Table E6.1). All of the analyses were run and the fitted probabilities were built in Minitab (2000, v. 17).

Results

The survival and regeneration (protonemata and branch production) responses significantly increased for *D. polysetum* when the fragments were exposed to gradual as opposed to abrupt temperature changes (b = 0.16 to 1.04, P = 0.0001) (Fig. 6.1; Table 6.1). Except for survival and protonemata production, the regeneration response increased with greater duration of exposure (b = 1.32, P = 0.001). The three responses significantly decreased when the fragments were exposed to warmer temperatures (b = -0.03 to -0.74, P = 0.0001), and increased in fragment size (b = 1.17 to 1.96, P = 0.0001; Fig. 6.1).

The survival and regeneration of protonemata by *O. obtusifolium* significantly increased when the fragments were exposed to gradual as opposed to abrupt temperature changes (b = 1.17 to 1.96, P = 0.0001; Fig. 6.2; Table 6.1), but the abrupt and gradual temperature changes had no influence on branch regeneration. Branch regeneration increased after longer exposure times at the final temperature treatments (b = 0.13 to 0.63, P = 0.0001), and it significantly decreased with warmer temperatures (b = -0.03 to -0.02, P = 0.0001).

Fragment survival and protonemata production significantly increased for *P. schreberi* when the fragments were exposed to gradual as opposed to abrupt temperature changes (b = 0.50 to 0.043, P = 0.002 to 0.0001) (Fig. 6.3; Table 6.1). Branch production significantly increased with abrupt changes in temperature (b = -0.90, P = 0.0001), and survival increased with exposure duration (b = 0.41, P = 0.001). However, survival and protonemata regeneration

decreased when exposed to warmer temperatures (b = -0.03 to -0.02, P = 0.0001), and also increased with increased fragment size (b = 0.76 to 1.70, P = 0.001 to 0.0001; Fig. 6.3).

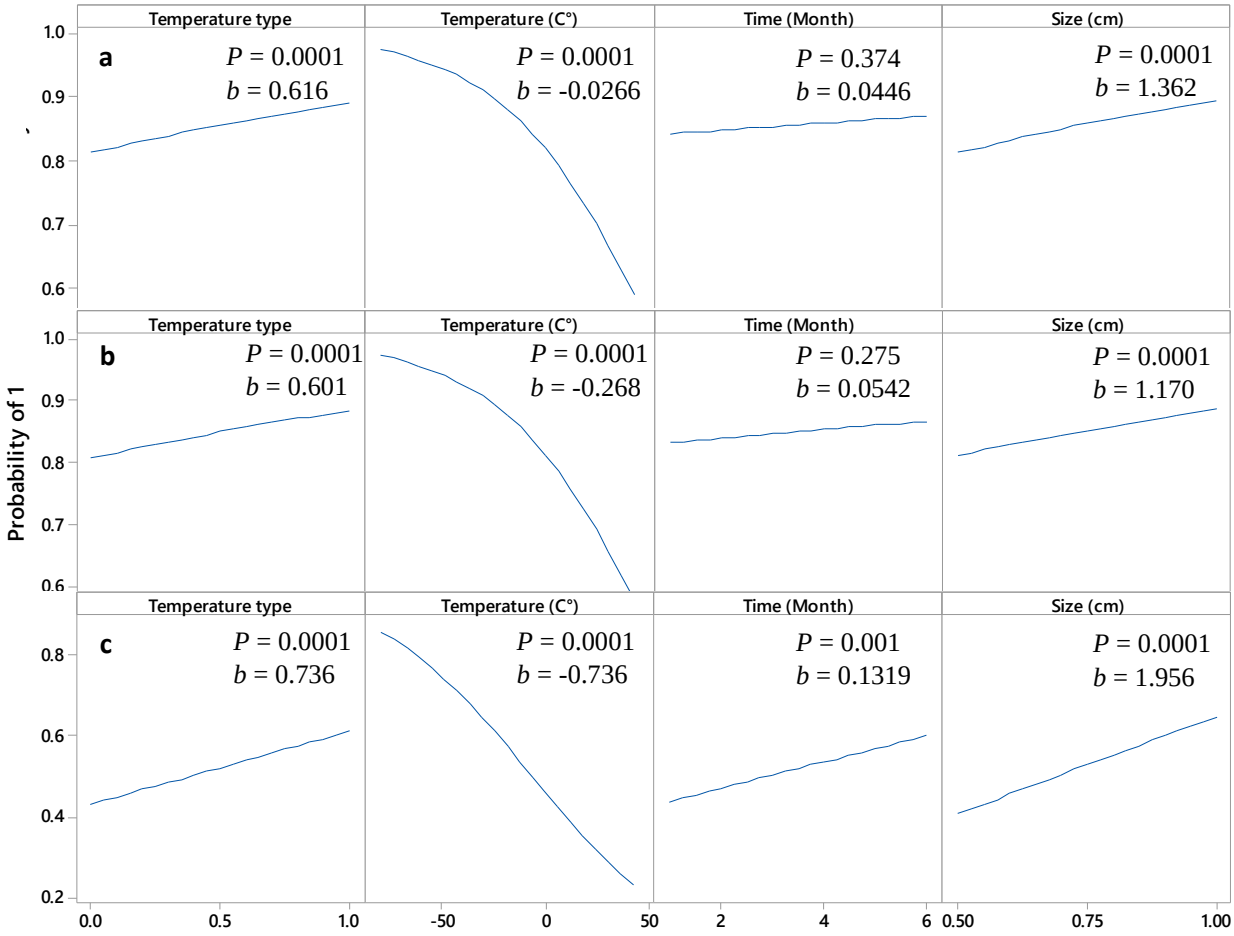

Fig. 6.1. Fitted probabilities of the response (survival, protonemata and branch production) to abrupt/gradual temperature changes (0 and 1 respectively), exposure temperature (43, 22, 6, -18, -40, -80 °C), exposure duration (1–6 months), and fragment size (0.5 and 1.0 cm) treatments (x-axis) for the boreal moss *Dicranum polysetum* (n = 1264). (**a**) Survival. (**b**) Protonemata production. (**c**) Gametophyte branch production. See Table 6.1 for details of the statistics.

Table 6.1. Summary of multiple logistic regression models predicting the survival and growth response (protonemata and gametophyte branch development) to abrupt/gradual temperature changes (0 and 1 respectively), exposure temperature (43, 22, 6, -18, -40, and -80 °C), exposure duration (1–6, months), and size (0.5 and 1.0 cm) for three boreal forest mosses: *Dicranum polysetum* (*n* = 1264), *Orthotrichum obtusifolium* (*n* = 614), and *Pleurozium schreberi* (*n* = 1269).

Species, Response	Variable	coefficient (b)	std. error	VIF	adj dev. R^2	*P*
D. polysetum						
Survival	Intercept	-0.006	0.318		14.49	0.0001
	Abrupt/Gradual Temperature Changes	0.616	0.156	1.02		0.0001
	Temperature (°C)	-0.02669	0.0025	1.01		0.0001
	Time	0.0446	0.0502	1.02		0.374
	Sizes	1.362	0.313	1.02		0.0001
Protonemata	Intercept	0.062	0.315		14.32	0.0001
	Abrupt/Gradual Temperature Changes	0.601	0.154	1.02		0.0001
	Temperature (°C)	-0.02680	0.00247	1.01		0.0001
	Time	0.0542	0.0497	1.02		0.275
	Sizes	1.170	0.308	1.01		0.0001
Branches	Intercept	-2.511	0.285		17.89	0.0001
	Abrupt/Gradual Temperature Changes	0.736	0.131	1.04		0.0001
	Temperature (°C)	-0.736	0.131	1.06		0.0001
	Time	0.1319	0.0413	1.03		0.001
	Sizes	1.956	0.261	1.04		0.0001

O. obtusifolium						
Survival	Intercept	-1.906	0.319		22.48	0.0001
	Abrupt/Gradual Temperature Changes	1.014	0.219	1.13		0.0001
	Temperature (°C)	-0.02511	0.00302	1.06		0.0001
	Time	0.6160	0.0756	1.19		0.0001
	Sizes	na				
Protonemata	Intercept	-2.015	0.321		23.06	0.0001
	Abrupt/Gradual Temperature Changes	1.064	0.22	1.14		0.0001
	Temperature (°C)	-0.02531	0.00301	1.04		0.0001
	Time	0.6286	0.07758	1.2		0.0001
	Sizes	na				
Branches	Intercept	-2.419	0.303		10.93	0.0001
	Abrupt/Gradual Temperature Changes	-0.040	0.19	1.02		0.8833
	Temperature (°C)	-0.01669	0.00237	1.02		0.0001
	Time	0.3438	0.0624	1.03		0.0001
	Sizes	na				
P. schreberi						
Survival	Intercept	0.014	0.289		16.85	0.0001
	Abrupt/Gradual Temperature Changes	0.495	0.14	1		0.0001
	Temperature (°C)	-0.02893	0.00220	1.01		0.0001
	Time	0.0138	0.0455	1.02		0.762
	Sizes	0.759	0.278	1.02		0.006
Protonemata	Intercept	-0.283	0.286		16.22	0.0001

	Abrupt/Gradual Temperature Changes	0.425	0.139	1.02		0.002
	Temperature (°C)	-0.02798	0.00214	1.01		0.0001
	Time	0.0599	0.045	1.02		0.182
	Sizes	0.906	0.275	1.01		0.001
Branches	Intercept	-5.054	0.47		16	0.0001
	Abrupt/Gradual Temperature Changes	-0.903	0.195	1.01		0.0001
	Temperature (°C)	-0.01975	0.00234	1.03		0.0001
	Time	0.4098	0.669	1.02		0.0001
	Sizes	1.690	0.391	1.01		0.0001

Note: VIF, variance inflation factors; adj. dev., adjusted deviance R^2; A/G, abrupt/gradual; na, not applicable.

The odds ratio for the survival response for the three species are shown in Fig. 6.4. The odds ratio values and 95% CI values are presented in the Appendix (Table E6.2). For *D. polysetum*, the likelihood of survival and regeneration from the gametophore fragments increased 1.82× to 2.09× when they were gradually exposed to different temperatures, decreased 0.96× to 0.97× after exposure to the in warmer temperatures, increased 1.05× to 1.14× with increased duration of exposure, and increased 3.22× to 7.07× when the fragment size was 1.0 cm. For *O. obtusifolium*, the likelihood of survival and regeneration from gametophore fragments increased 2.76× to 2.90× when they were gradually exposed to different temperatures, but not branch regeneration, which decreased (0.96×), branch regeneration decreased 0.98× to 0.99× after exposure to the warmer temperatures, and increased 1.41× to 1.88× with increased duration of exposure.

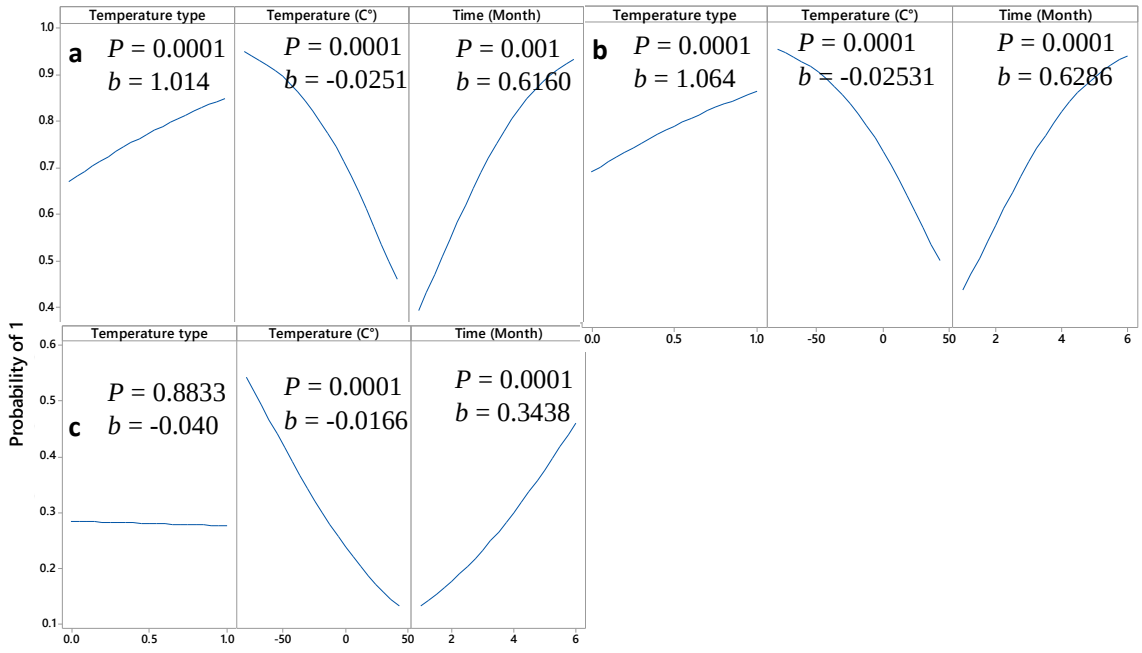

Fig. 6.2. Fitted probabilities of the response (survival, protonemata and branch production) to abrupt/gradual temperature changes (0 and 1 respectively), exposure temperature (43, 22, 6, -18, -40, -80 °C), exposure duration (1–6 months), and fragment size (0.5 and 1.0 cm) treatments (*x*-axis) for the boreal moss *Orthotrichum obtusifolium* (*n* = 614). The higher the probability (between 0 and 1), reflects the higher the likelihood of the moss surviving, producing protonemata, or producing branches. (**a**) Survival. (**b**) Protonemata production. (**c**) Gametophyte branch production. See Table 6.1 for details of the statistics.

For *P. schreberi*, the likelihood of survival and regeneration for the gametophore fragments increased 1.52× to 1.64× when they were gradually exposed to different temperatures, but not branch regeneration, which decreased (0.41×); branch regeneration decreased 0.97× to 0.98× for fragments exposed to the warmer temperatures; branch regeneration increased 1.01× to 1.06× with increased duration of exposure; branch regeneration increased 2.13× to 5.42× when size was 1.0 cm.

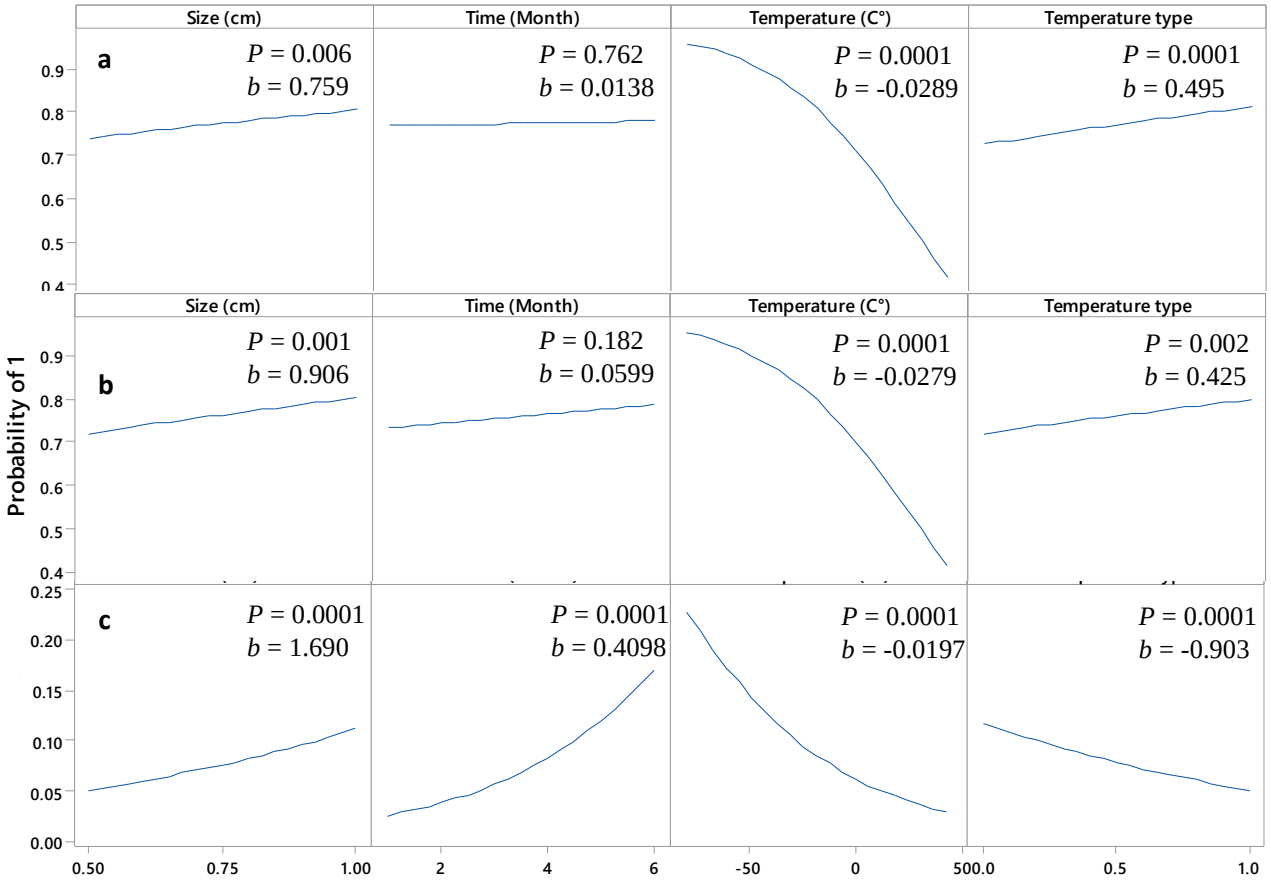

Fig. 6.3. Fitted probabilities of the response (survival, protonemata and branch production) to abrupt/gradual temperature changes (0 and 1 respectively), exposure temperature (43, 22, 6, -18, -40, -80°C), exposure duration (1–6 months), and fragment size (0.5 and 1.0 cm) treatments (*x*-axis) for the boreal moss *Pleurozium schreberi* (n = 1269). (**a**) Survival. (**b**) Protonemata production. (**c**) Gametophyte branch production. See Table 6.1 for details of the statistics.

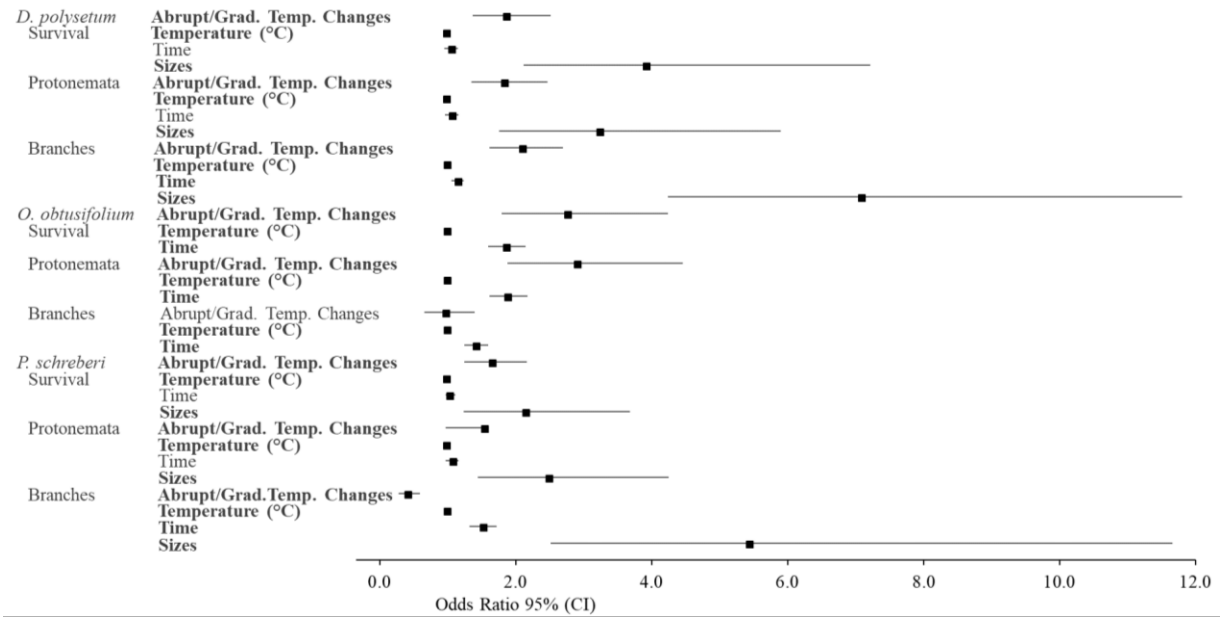

Fig. 6.4. Forest plot showing the odds ratio (95% confidence interval) of the survival and growth (protonemata and branch development) response (*x*-axis) of three boreal moss species to abrupt/gradual temperature (Grad. Temp.) changes (0 and 1, respectively), exposure temperature (43, 22, 6, -18, -40, -80 °C), exposure duration (1–6, months), and size (0.5 and 1.0 cm). The forest plot graph shows how strong the association is a black square <1 (low association with higher response values); =1 (none); or >1 (higher association with higher response values). Models with treatments in bold font were considered statistically significant ($P < 0.05$).

The VIF values ranged from 1 to 1.20, indicating that the predicting variables had low to no correlation, or showed no lack of independence (Stine 1995, O'Brien 2007), and thus none of the predictors (treatment variables) were removed when assessing models with multiple predicting variables. The variance in the response variables that could be explained by the predicting variables was derived from Table 6.1. For the three species, the regression models explained between 14.49% to 22.48% (adj. dev. R^2, $P = 0.0001$) of the variance in the survival response, 14.32% to 23.06% (adj. dev. R^2, $P = 0.0001$) of the variance in the protonemata

regeneration response, and between 10.93% to 17.89% (adj. dev. R^2, P = 0.0001) of the variance in the branch regeneration response.

The best models (ΔAICc=0) for explaining each of the three responses for all three species were those that incorporated three or four predicting variables (abrupt/gradual temperature changes, temperature, and/or duration of exposure, and fragment size) (Table 6.2). The Akaike weight (*W*) for the survival response ranged from 0.25 to 0.67; for the protonemata regeneration response ranged from 0.20 to 0.50, and for the branch regeneration response ranged from 0.11 to 0.99.

Table 6.2. Corrected AIC (AICc)-based model selection (Δ AICc = 0–2) for the logistic regression of survival and growth (protonemata and branches development) responses of three boreal mosses (*Dicranum polysetum*, *Orthotrichum obtusifolium*, and *Pleurozium schreberi*) to abrupt/gradual temperature changes (0 and 1, respectively), exposure temperature (43, 22, 6, –18, –40, –80°C), exposure time (1–6, months), and size (0.5 and 1.0 cm).

Species	Response	Model variables	-LogLikelihood	*n*	*K*	AIC_c	Δ AIC_c	AIC
D. polysetum	Survival	Abrupt/Gradual Temperature Changes, Temperature (°C), Sizes	530.07	1264	4	1068.17	0.00	0.6477
		Abrupt/Gradual Temperature Changes, Temperature (°C), Time, Sizes	529.68	1264	5	1069.40	1.23	0.3509

	Protonemata	Abrupt/Gradual Temperature Changes, Temperature (°C), Sizes	539.80	1264	4	1087.62	0.00	0.5998
		Abrupt/Gradual Temperature Changes, Temperature (°C), Time, Sizes	539.20	1264	5	1088.45	0.83	0.3969
	Branches	Abrupt/Gradual Temperature Changes, Temperature (°C), Time, Sizes	717.38	1264	5	1444.80	0.00	0.2592
O. obtusifolium	Survival	Abrupt/Gradual Temperature Changes, Temperature (°C), Time	287.12	614	4	582.3	0.0	0.2592
	Protonemata	Abrupt/Gradual Temperature Changes, Temperature (°C), Time	287.61	614	4	583.29	0.00	0.2058
	Branches	Temperature (°C), Time	336.15	614	3	678.33	0.00	0.2966
		Abrupt/Gradual Temperature Changes, Temperature (°C), Time	336.12	614	4	680.31	1.98	0.1103
P. schreberi	Survival	Abrupt/Gradual Temperature Changes, Temperature (°C), Sizes	626.61	1269	4	1261.25	0.00	0.6772
		Abrupt/Gradual Temperature Changes, Temperature (°C), Time, Sizes	626.57	1269	5	1263.18	1.93	0.2586
	Protonemata	Abrupt/Gradual Temperature Changes, Temperature (°C), Sizes	639.06	1269	4	1286.15	0.00	0.5075

	Abrupt/Gradual Temperature Changes, Temperature (°C), Time, Sizes	638.17	1269	5	1286.39	0.24	0.4510
Branches	Abrupt/Gradual Temperature Changes, Temperature (°C), Time, Sizes	385.30	1269	5	780.65	0.00	0.9998

Note: The model variables in all cases were abrupt/gradual temperature changes, temperature (°C), time, and size; n, number of replicates; K, number of parameters in the model; AIC=, Akaike information criterion; ΔAICc, measure of the model relative to the best model; AIC W, weight or strength of the model.

Discussion

Survival and regeneration responses varied with abrupt/gradual temperature changes, temperature, and duration of exposure

Gradual temperature changes facilitated fragment regeneration in *Dicranum polysetum* and/or*thotrichum obtusifolium*, whereas abrupt temperature changes (5% to 15%) only facilitated fragment regeneration for *Pleurozium schreberi*. Gradual changes (slow freezing) in temperature temporarily negatively affected the metabolism of gametophytes of *Tortula ruralis* ([Hedw.] Gaertn., whereas abrupt changes (rapid freezing) deteriorated the gametophytes through decreased production of ribosomes, proteins, and ATP (Malek and Bewley 1978). Gradual changes in temperature permit the removal of water through gradual cooling of plant cells (10 °C per hour) and limit the rupture of cells through ice formation (Marchand 1991, Glime 2017*b*). Gradual changes in warm temperatures may also allow for the moss to prepare for heat stress by producing heat shock proteins that limit the denaturation of proteins (Saidi et al. 2005).

We expected the habitat to influence the adaptation of the moss whereby the epiphyte, *O. obtusifolium*, exposed to more extreme temperatures, would be better adapted to abrupt temperature changes. However, the more competitive nature of the acrocarp and pleurocarp species from the forest floor, *D. polysetum* and *P. schreberi* (respectively), may endow these species with a greater tolerance to abrupt temperature changes than that found in the epiphytic species.

The increased likelihood of survival and regeneration at low temperatures (22 °C to -80 °C) and the decreased survival and regeneration at higher temperatures (> 22 °C to 43 ˚C) are consistent with the findings that prolonged periods of high temperatures (>30 °C) rapidly kill *Brachythecium rutabulum* (Hedw.) Schimp and *Funaria hygrometrica* Hedw. (Furness and Grime 1982). However, the results reported by Furness and Grime (1982) were observed for plants that were grown at varying temperatures in a hydrated state, which contrasts with our study where the plants were air dried, exposed to the temperature treatment, and then grown in an ideal temperature (~22°C). Because the moss fragments were air-dried prior to the temperature exposure, the drying may have had some effect on the survival and regeneration response. This hypothesis is backed by other studies showing that the gradual removal of water by air-drying allows moss fragments to ameliorate the effects of stress (heat or freezing; Marchand 1991, Saidi et al. 2005, Glime 2017*a*, 2017*b*). The ability to regenerate after exposure to the temperature treatments suggests the tolerance of these three species to fluctuations in temperature within their habitat.

Duration of exposure in the final temperature treatment had varied effects on the survival and regeneration of the three species, with time of exposure increasing the probability of branch regeneration only in *D. polysetum* and *P. schreberi*, but both survival and regeneration of *O.*

obtusifolium. In the desiccation experiments, moss gametophyte fragments usually deteriorate as time passes (Proctor et al. 2007, Segreto et al. 2010), but *Tortula ruralis* remained unaffected for up to 10 months (Bewley 1973), which is longer than the six-month time-period of this study. If *D. polysetum* and *P. schreberi,* forming tufts or mats on the forest floor, are subject to snow cover and warming periods during the year, they may be equipped with mechanisms that harden the gametophytes (Minami et al. 2005), and allow them to regenerate and survive for a prolonged period of time. On the other hand, gametophore fragments of *O. obtusifolium* remain attached to the plant colonies on the tree trunk when exposed to the air, and are adapted to withstand desiccation and extreme temperatures throughout the year. *O. obtusifolium* may have mechanisms that allow to better withstand the extreme temperatures used in this study for a longer prolonged period of time in this study than *D. polysetum* or *P. schreberi,* which in nature are protected by snow cover for several months of the year.

Survival, protonemata, and gametophore regeneration increase with gametophore size

An increase in gametophore fragment size facilitated the survival of plants and allowed for more protonemata and branches to be produced for *D. polysetum* and *P. schreberi.* This response suggests that the availability of more resources in larger sized gametophore fragments help them to prepare via metabolism (hardening) to withstand the varying temperature and regenerate, which helps establishment in their natural habitat. The findings of Segreto et al. (2010) as well as Løe and Söderström (2001) are consistent with those of this study, by showing that larger fragments tend to regenerate more easily as these have more resources (metabolites) and a higher number of specialized initial cells for regeneration. However, Campeau and Rochefort (1996) found that *Sphagnum* regeneration in bare peat surfaces was not affected by

gametophore size, suggesting that the influence of gametophore size on survival and regeneration may be species dependent.

The increased gametophore fragment size of *D. polysetum* allowed the fragment to survive and regenerate with a greater likelihood (3.2× to 7.07×) than the fragments of *P. schreberi* (2.1× to 5.4×), suggesting that the species difference in survival and regeneration response to fragment sizes dispersed in a similar habitat (Fig. 6.2). These differences between the two species might be explained by the fragments of *D. polysetum* having wider stems with densely tomentose rhizoids. The tomentose rhizoids might act as insulation for the passage of hot or cold airflow towards the plant. The effect of temperature changes could be further ameliorated by the dense tuft *D. polysetum* forms, which would provide the interior of the tuft, and the stem fragments, with a more regulated environment that allows fragments within the tuft to harden and better tolerate changes in temperature than stems (external individuals) on the outer part of the tuft.

The fragments of *P. schreberi*, which has narrower stems, lack tomentose rhizoids, and grow in an open mat, are more exposed and therefore less likely to tolerate changes in temperature. However, *P. schreberi* forms mats on the forest floor, which could limit the effects of freezing through snow cover in winter, or be protected from full sun exposure by shading during the summer time. Although not statistically assessed in this study, it was observed that gametophores of *P. schreberi* were produced more often from bud development in the protonemata than from stem regeneration. This observation suggests different adaptations for establishment and horizontal expansion between the two species related to their growth form (acrocarp vs. pleurocarp). For example, the acrocarp *D. polysetum* relies on both protonemata and branch regeneration, whereas the pleurocarp *P. schreberi* relies mostly on protonemata for

the production of gametophores and for the growth and expansion of tufts or mats (respectively) on boreal substrata. In addition, this strategy might explain why *P. schreberi* dominates the forest floor more readily than *D. polysetum*; however, the establishment response of *D. polysetum* helps the species to compete for space in areas dominated by *P. schreberi* (Frego 1996).

Model with all predictor variables best explains the responses

The survival and regeneration responses were best explained when all or almost all the predicting variables studied were included in the models. Although most of the models for all the three species were significant, models for *O. obtusifolium* had higher adj. dev. R^2 than the two other boreal mosses (Table 6.1). We hypothesize that the lower model performance for *D. polysetum* and *Pleurozium schreberi* may reflect the widespread distribution of the species in the boreal biome, allowing them to tolerate a wider range of conditions. *O. obtusifolium*, being mostly found on hardwoods (Caners et al. 2010), appears to experience low levels of competition with other lichen and moss species, and may not need a wide range of responses to maintain populations. Because the regeneration of *O. obtusifolium* was better explained by the modeled variables, it may also indicate that this species may be at a greater risk of being negatively affected by environmental changes than the other two species.

Asexual propagule response to stress, a hypothesis

A shoot fragment from the gametophore is one of the asexual propagules that help with establishment via colonization and maintenance of moss populations (*sensu lato*; Frey and Kürschner 2011). Shoot fragments form via cell and cell wall disintegration, and mechanical

force; whereas, the formation of specialized asexual propagules such as gemmae, bulbils, and/or tubers occurs by biologically-determinate processes. The regeneration ability and response to stress might differ between fragments and the specialized asexual propagules, with the shoot fragment regeneration most likely sharing similar regeneration and stress responses as the unfragmented gametophore. However, Stark et al. (2016), studying how the response to stress (desiccation) differs between the shoot and the asexual propagule, found that adult shoots and asexual propagules of *Syntrichia pagorum* (Milde) J. J. Amann can withstand and be revived from rapidly desiccating conditions (humidity, duration of exposure, and temperature), enabling them to rapidly establish in habitats that are exposed to drying. Furthermore, Stark et al. (2016) note that this ability is likely present in mosses that inhabit xeric habitats, and mosses in more mesic habitats may require slow drying to survive and become established. Considering that within the boreal biome both xeric (denuded terrain, open canopy forests, trees) and mesic habitats (closed canopy forests, forest floor) are present, both responses may also be present for the gametophore and specialized asexual propagules to facilitate establishment, but this needs further study.

Conclusions

The regeneration of gametophore fragments of three boreal moss species mostly increased in gradual rather than abrupt temperature changes, lower temperatures (43, 22, 6, -18, -40, or -80°C), had mixed effects with the duration of exposure to the final temperature, and increased with gametophore size. The type of regeneration response, protonema or branch, may have biological significance for the survival of the plant that allow for the colonization of the species on the forest floor or on trees. The more variable regeneration in *P. schreberi* and *D.*

polysetum may explain the occurrence on the different substrata on the forest floor, while the limited regeneration observed for *O. obtusifolium* explained the occurrence solely on poplar trees. Eucidatationing the tolerance of moss species in the boreal forest will help understand which species will persist or perish in the community, and whether this is attributable to the type of habitat in the landscape. Performing this study on a larger number of species will test how habitat facilitates or diminishes survival and regeneration from dispersed fragments.

Acknowledgments

Author Contribution: C.P.L., R.B., and M.P.N. designed the research study, C.P.L. collected and analyzed the data with guidance from R.B. and M.P.N., and all authors wrote the manuscript.

The authors thank K. Fontaine and J. Otisi for their help with moss culture, in vitro, and T. Booth for help with field collection. Funding for this study was obtained from the Department of Biological Sciences and the Faculty of Graduate Studies (University of Manitoba) and from the Natural Sciences and Engineering Research Council of Canada (NSERC-DG to MPN).

Author Contribution: C.P.L., R.B., and M.P.N. designed the research study, C.P.L. collected and analyzed the data with guidance from R.B. and M.P.N., and all authors wrote the manuscript.

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Chapter 7: Discussion and Conclusions

The dispersal and establishment of asexual propagules have shaped the extant and soil bank communities of bryophytes and lichens in boreal forests. Knowledge of the influence of environmental conditions on asexual propagule dispersal in boreal forests (Chapter 2 and 3), the dispersal of asexual propagules within boreal forests and nearby areas (Chapter 2, 3, 4), the comparison between the diversity of propagules with the diversity of bryophytes and lichens in the extant communities in boreal forests (Chapter 2, 3), the dispersal ability of boreal mosses and lichen when exposed to wind (Chapter 4), the propagule diversity in the soil bank and its maintenance in boreal forests (Chapter 5), and the regeneration of asexual propagules (Chapter 6) contribute to a novel understanding of the community composition of bryophytes and lichens in the boreal forests. This chapter concludes with remarks that detail the overall findings of this thesis, significance of the findings in a broader context, as well as future studies and directions exploring the impact of asexual propagule dispersal and establishment on the composition of bryophyte and lichen communities in boreal forests.

The influence of local environmental conditions on asexual propagule dispersal

Asexual propagules were dispersed by wind during the winter, summer, and fall, but the largest number of propagules was dispersed during the winter months (Chapter 2 and 3). Asexual propagules represented around 10% of particulates (incl. detritus, fibers, pieces of bark, invertebrate, leaves, wood) dispersed and deposited in boreal forests and surrounding bodies of water during portions of the year. The low representation of asexual propagules as part of the particulate matter is not uncommon, as other propagules such as meiotic spores outnumbered

asexual propagules in the air column throughout the year in open habitats of Antarctica (Marshall and Convey 1997). The low representation of asexual propagules among the dispersed particulate matter may indicate the difficulty (or tolerance) of the lichen thallus and the moss gametophyte to be fragmented and dispersed by the local environmental conditions during the study period. However, during the winter study period a combination of low humidity (60.8 ± 3.42%), low temperatures (-12 ± 3.05 °C), desiccation, and variable wind speeds (14.2 ± 2.90 km/h) may have facilitated the fragmentation of fragile parts of the lichen thallus and moss gametophyte. In this study, the fragmentation and dispersal of asexual propagules of larger sizes and higher quantities during winter rather than during the warmer environmental conditons suggested the facilitation of dispersal by the low humidity, low temperature, and high wind velocities. The dispersal of numbers of asexual propagules have also been observed to vary with habitat and the seasonal conditions in other studies, with propagules of Antarctic mosses and the lichen *Hypogymnia physodes* (L.) Nyl. being dispersed in higher quantities during warmer times of the year (Marshall and Convey 1997, Armstrong 1991), or varying throughout the year (Barbé et al. 2017). However, this study reveals that during winter rather than in other periods of the year, a greater number of asexual propagules are dispersed, most likely due to the environmental harshness associated with boreal winters, such as those in Manitoba.

During the winter the forest floor and surrounding water bodies were covered with snow, which explained why most of the trapped asexual propagules were parts of epiphytic bryophytes and lichens rather than species on the forest floor, and possibly to some species that occupy nearby rockfaces devoid of snow. Lichens and bryophytes covered in snow may become exposed during times of increased temperature and snow melt, which may increase the chances of species on rockfaces and the forest floor to become fragmented and dispersed. Although most propagules

were dispersed during the winter, these propagules will not likely become established on a substratum because of the cold temperatures, and instead be dispersed through time as dormant propagules in the snow (McDaniel and Miller 2000). Once the environmental conditions favor the propagule to become metabolically active, such as temperatures above −15°C for lichens and −5°C for bryophytes (Rastorfer and Higinbotham 1968, Lange and Kappen 1972, Goulden and Crill 1997, Barták et al. 2007), then the asexual propagules can become established during the warmer periods of the year.

Dispersal within the boreal forest stands

Bryophyte and lichen asexual propagules and fragments were captured by traps placed on trees and on the forest floor (Chapter 2 and 3). Some propagules may have remained airborne, which may have been deposited into nearby forest stands and bodies of water. However, many asexual propagules appeared to have remained within the forest stands suggesting their dispersal to be local (Chapter 2), which may have helped to maintain the local communities of bryophytes and lichens. Alternatively, propagules from the same species may have entered the stands from elsewhere, contributing to the propagule load within the studied stands. Within these forest stands, more propagules were captured by traps in trees, particularly those in coniferous stands, than in forest floor traps. The trees may have acted like a net where the canopy cover trapped large asexual propagules and allowed for the rapid deposition of propagules (Raynor et al. 1975). Asexual propagules that escaped the entrapment by the canopy were most likely of smaller sizes and lower numbers, and were deposited on the forest floor (Chapter 3). Because less asexual propagules from the forest floor species were trapped in the forest floor traps, the horizontal

expansion of the forest floor species is likely to be the main source for the maintenance of the forest floor community.

Diversity of propagules and communities in boreal forests

Asexual propagules produced by bryophytes and lichens appeared to have been wind dispersed to the trees and the forest floor, were diverse in morphology, and those that were identified belonged to species reported within the habitat (Chapter 2 and 3). Most of the asexual propagules captured by traps were lichen thallus fragments; however, other lichen and bryophyte asexual propagules were also found in the traps (lichen soredia and isidia, moss gametophyte fragments, moss gemmae, liverwort gametophyte). The presence of different asexual propagule types may suggest that specialized and unspecialized propagules (sensu lato Frey and Kürschner 2011) may contribute to community composition. However, if unspecialized propagules (fragments) are as effective as specialized propagules, it is not clear why specialized propagules have evolved. The role of asexual propagules in aiding in the establishment of bryophyte and lichens needs to be further studied. The further study of difference between specialized and unspecialized asexual propagules may clarify how these propagules respond to environmental factors (desiccation, humidity, herbivory).

Most asexual propagules trapped belonged species in the extant community. For examples, asexual propagules trapped on the trees mainly belonged to species that grew on trees. Similar findings were observed on the forest floor. Furthermore, the species present in traps and in the forest stands reflected a community composition (abundance and richness) relatively specific to the forest stand types where these species were present (balsam fir, poplar, and white

spruce forest stands). Other studies support these findings (Frey and Kürschner 2011, Barbé et al 2017, Pasiche-Lisboa and Sastre-De Jesús 2018) and they have observed that asexual propagules help with the maintenance of the parent colony on a substratum. However, the findings in this thesis may suggest that asexual propagule abundance and richness helps to maintain, and occasionally form new bryophyte and lichen communities in boreal forests.

Soil bank formation and maintenance in boreal forests

Some bryophyte and lichen asexual propagules may not become established during the warmer times of the year resulting in loss or viability to increase in temperature, or may be lost to herbivory or parasitism. Other propagules remain viable for prolonged periods of time and be deposited within the soil bank (Jonsson 1993, During 2001, Hock et al. 2008). Asexual propagules aid in the formation of the soil bank by their deposition in varying numbers, sizes, and types that represent species from past communities, the extant community, and from dispersal events of communities located at long distances from the soil bank (Rydgren and Hestmark 1997, Kimmerer 2005, Maciel-Silva et al. 2012). The representation of asexual propagules by the amplification of environmental DNA, and thus the species, in the propagule bank was related to the forest stand properties and to the abundance as well as the richness of these species in the forests. The influence of the forest stand properties to the propagule bank indicates that part of the local community is affecting the community composition in the soil bank. For example, an increase in forest cover (canopy cover) and tree densities were correlated with a higher abundance and richness of bryophytes and lichen species, since these conditions provided a suitable habitat for their growth (Chapter 3 and Chapter 5). Because there was a higher richness and abundance of species in these forests with these stand properties, and

because the lichen thallus or the moss gametophytes interacted with the dispersal vectors, the interaction between forest properties with the species as well as wind led to a higher abundance of species represented in airborne propagules (Chapter 4). Thus, the higher representation of lichen and bryophytes in the airborne propagules, once deposited, was also represented in the soil bank (Chapter 5).

The family richness of bryophytes and the richness of the lichen photobiont represented in the soil bank were not similar to that of the extant community, which was not unexpected as other studies have made similar observations (see citations in Chapter 5). However, since the species captured as propagules in the forest stands were similar to the species in the extant community (Chapter 3), and the propagules in the soil bank were influenced by the dispersed propagules from the extant community, some similarity was expected. The difference between bryophyte and lichen photobiont richness in the soil bank and extant community, and within the soil bank of the forest types can be understood by three possible explanations (Chapter 5): the soil bank represents dispersal and deposition events from a past community, and/or from communities located at long distances from the soil bank, or a combination of past communities at the same site and at a distance over long periods of time. Furthermore, because of the distinctness of the richness (moss families and lichen photobiont types) between the extant and soil bank communities and within the soil bank communities, when the soil bank is disturbed, the emerging species may change the abundance and species of the extant communities. However, the species present in the extant community will depend on the conditions of the habitat when the soil bank is disturbed (Caners et al. 2009). If the conditions (forest stand properties) in the forest stands remain the same after the soil bank is disturbed, then the emerging species may be the same as the ones in the extant community, but the changes in the community results from

changes in abundances rather than changes in the species richness. If the conditions (forest stand properties) in the forest stands change after the soil bank is disturbed, then the emerging species may be different from the ones in the extant community, resulting in changes in abundances and richness of species and thus a change of the extant community.

Regeneration of asexual propagules from species in boreal forests

Viable asexual propagules dispersed and deposited on a substratum have the possibility of becoming established (survive and regenerate) when substratum conditions are suitable for growth (Chapter 5 and 6). The asexual propagules that overcome the abiotic and biotic conditions on tree and forest floor substrata will then regenerate protonemata, branches, or both. However, larger asexual propagules rather than smaller propagules, may more likely become established on a substratum because they have more resources available for growth in the larger fragment or propagule. Yet, for the different propagule sizes, regeneration will likely occur if the propagules were not exposed to elevated temperatures or temperatures that fluctuate abruptly for at least six months of dormancy prior to establishment (Chapter 6).

Growth requirements of different species will affect the regeneration responses once the propagules become established on a substratum. Chapter 6 shows that species on the forest floor were more likely to regenerate from stem fragments than species on trees. The increased likelihood of regenerating from asexual propagules of species on the forest floor was explained by the tolerance that these species needed to grow on the forest floor (humic to sandy soil, decaying wood, rock). The forest floor species may rely on the horizontal expansion of the parent colony through short distance propagule dispersal, to maintain their parent colony and

become more competitive with other species on similar substrata. The asexual propagules that regenerate on trees may likely maintain the parent colony. However, the low regeneration output of the tree species studied suggests that the species may require both asexual and sexual reproduction to maintain the parent colony and form new colonies. The more competitive nature of the acrocarp and pleurocarp species from the forest floor, *D. polysetum* and *P. schreberi* (respectively), may allow these species to have a greater regeneration to temperature changes in comparisons to the acrocarpic epiphytic species.

Concluding remarks and future directions

This study shows that asexual propagules produced by bryophytes and lichens were dispersed in low quantities among the trees and the forest floor of different boreal forest types (balsam fir, poplar, whites spruce) year-round. Most of the propagules captured belonged to lichen fragments, indicating that lichen benefit from the dispersal of asexual propagules in boreal forests due to the dispersal of both symbionts in the asexual propagule, and since there might be limited maintenance of the communities via sexually produced spores as a result of the stress associated with lichenization. Propagules were captured in relatively higher quantities during winter, particularly on conifer trees and from epiphytic species rather than on the forest floor and from ground-dwelling species, but allowing for both tree and ground-dwelling communities to be partly maintained if the propagules become established. Yet, the ground-dwelling communities might rely on horizontal expansion more than epiphytic communities for community maintenance. The development of bryophyte and lichen colonies during the growing season on suitable substrata, the production of asexual propagules, the dispersal of asexual propagules, and the forest stand properties may shape the forests communities by the numbers and sizes of

asexual propagules interacting with wind and facilitating the dispersal throughout the forest, with some of asexual propagules dispersed helping form the communities present and dormant in the soil bank. Although the dispersal of asexual propagules may influence the richness and abundance of propagules in the soil bank, the propagules in the soil bank may represent dispersal events from a mix of communities such as present communities, communities at long distances, or from past communities due to the richness of species in the soil bank not mirroring the richness of species in the extant community. However, if the soil bank is disturbed and the propagules start regenerating, the ground-dwelling moss species were more likely to rely on the regeneration of both stems and protonemata to colonize, horizontally expand, and compete on substrata on the forest floor due to their resilience and ability to grow on many substrata. In contrast the ground-dwelling species, the epiphytic species may have a more limit regeneration output on trees and may require spore dispersal for the community maintenance.

The thesis provides novel insights into the factors that influence the community composition of bryophytes and lichens in boreal forests due to the dispersal and regeneration of asexual propagules. These novel insights come from a multidiciplinary approach within ecology where it was shown how the dispersal of asexual propagules captured with a relatively inexpensive type of trap (Petri dishes) changed with environmental conditions and stand properties specific to boreal forests (field ecology), how the diversity of dispersed asexual propagules was linked to the diversity of the extant community (field and community ecology), or how a select number of lichens and mosses were dispersed away from a propagule source when exposed to a fan to mimic wind dispersal in a laboratory setting (experimental ecology). It was also shown how the diversity of bryophyte and lichen propagules from environmental DNA

from a few grams of the upper layer of soil obtained from the soil bank of forest stands was linked to the diversity of bryophytes and lichens in the extant community (molecular ecology). In addition, it was shown what were the regeneration responses of small and large moss stems when exposed to variables (the nature of temperature, temperature, time of exposure) associated with the environmental conditions of boreal forests (plant physiology and experimental ecology). The usage of this multidisciplinary approach to understand the link between bryophyte and lichen asexual propagule dispersal and regeneration with the bryophyte and lichen community composition in boreal forests, moves bryology and lichenology forward into elucidating the selective forces shaping communities in a landscape and provide the queries to undertake further studies needed to advance these fields of science.

Further studies are needed to understand the role of asexual propagules in boreal forests. One venue of research is to determine how the fragility (tensile and yield strength) of the lichen thallus and moss gametophyte when exposed to different forces and environmental conditions is likely to form and detach from the individual as an asexual propagule. Determining how bryophytes and lichens are detached and fragmented will help understand propagule dispersal throughout the year. Another venue of research is to understand the likelihood of species on rock faces and the forest floor to be dispersed during winter: either by direct fragmentation of the lichen and bryophyte or by the substratum (rocks and tree bark/branches) breaking off from the main substrata and dispersing multiple species to nearby areas. These forms of dispersal may help understand population formation and maintainence in different habitats. There is also a need to characterize the extant species within a habitat, using high-throughput molecular sequencing technologies, along with the emergence methods, to more accurately compare the abundance and richness of species represented as airborne propagules and how these are influenced by, or are of

influence on, the local and nearby communities. Additional regeneration experiments with a higher number of species from various substrata in the laboratory and in the field may explain how the regeneration outcomes of asexual propagules facilitate the propagule establishment in boreal forests.

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Appendix A: Dispersal during winter: a snapshot of the diversity of lichen and moss and asexual propagule dispersal in boreal forests of northern Manitoba

Table A2.1. Presence and absence of the lichen and moss species from the mixed, shoreline, and poplar boreal forests near Payuk Lake. The asterisk (*) indicates the rare species for each forest.

Species per forest	Boreal forests		
Species	Mixed forest	Shoreline forest	Poplar forest
Lichens			
Unknown crust	1	1	0
Bryoria fuscescens (Gyel.) Brodo and D. Hawksw	1*	0	0
Bryoria furcellata (Fr.) Brodo and D. Hawksw	1	1	0
Bryoria lanestris (Ach.) Brodo and D. Hawksw	1	1	0
Bryoria simplicior (Vainio) Brodo and D. Hawksw	1	0	0
Caloplaca holocarpa (Hoffm. Ex Ach) M. Wade.	0	0	1*
Cyphelium tigillare (Ach.) Turner and Borrer	1	1	0
Evernia mesomorpha Nyl.	1	1	0
Hypogymnia physodes (L.) Nyl	1	1	0
Lecanora allophana Nyl.	1	1	1
Lecanora barkmaniana Aptroot and van Herk	0	0	1*
Lecanora circumborealis Brodo and Vitik	1*	0	0
Lecanora hybocarpa (Tuck.) Brodo	0	1*	0
Lecidella euphoria (Florke) Hertel	0	0	1*
Lepraria sp.	1	1	0
Melanelia septentrionalis (Lynge) Essl	1	0	1
Melanelia subaurifera Nyl.	1	1	0
Parmelia sulcata Taylor	1	1	1

Physica aipolia (Ehrh. Ex Humb.) Fürnr.	0	0	1*
Physcia adscendens (Fr.) H. Oliver	0	0	1*
Tuckermannopsis americana (Sprengel) Hale	1	1	0
Usnea cavernosa Tuck.	0	1*	0
Usnea filipendula Stirton	1*	0	0
Usnea hirta (L.) F.H. Wigg	1	1	0
Usnea lapponica Vain	1	1	0
Vulpicida pinastri (Scop.) J.E. Mattsson and M.J.	1*	0	0
Mosses			
Orthotrichum obtusifolium Shrad. Ex. Brid	0	0	1*
Platydictya subtile (Hedw.) H.A. Crum	0	0	1*
Total	19	15	10

Appendix B: Moss and lichen asexual propagule dispersal may help to maintain the extant community in boreal forests

Table B3.1. Description of boreal forest stand types (balsam fir, white spruce, and poplar) and their vascular flora. Boreal forest stands are located around Payuk Lake, Manitoba, Canada. The data and description of the study sites were gathered during the summer, between 2014–2016. Vascular species are not listed in any particular order.

Forest Types and stand	Latitude and Longitude (NAD83)	% Slope	Aspect (°)	Average Canopy Cover (%)	Tree Density (#trees/ha)	Site Description	Vascular Plants
balsam fir 1	N54°39'05" W101°29'47.5"	-15	142	94.0	672.5	Balsam fir stand near cabin, forest floor mostly covered with litter, but with a few patches with moss and tree stumps. Tree stumps covered with mosses and fruticose lichens (*Cladonia* spp.).	*Abies balsamea*, *Picea glauca*, *Aralia nudicaulis*, *Cornus canadensis*, *Linnaea borealis*, *Ribes* sp., and *Clintonia borealis*.
balsam fir 2	N54°39'7.5" W101°29'50"	28	166	90.4	751.2	Balsam fir stand near granite-gneiss rock outcrop, moss-covered floor and with a few trunks and wood on the floor.	*Aralia nudicaulis*, *Picea glauca*, *Abies balsamea*, *Clintonia borealis*, *Ribes* sp., *Arctostaphylos uva-ursi*, and *Cornus canadensis*.
balsam fir 3	N54°39'6.5" W101°29'55.6"	10	194	94.4	542.6	Balsam fir stand with a closed canopy, near granite-gneiss rock outcrop. The forest floor was covered with feather mosses.	*Lycopodium lagopus*, *Abies balsamea*, and *Betula papyrifera*, *Picea glauca*, *Coptis* sp., *Aralia nudicaulis*, *Arctostaphylos uva-ursi*, and *Linnaea borealis*.
white spruce 1	N54°39'6.6" W101°29'50.1"	1	278	92.4	810.1	Forest floor covered with moss, reindeer lichen, and tree stumps—covered with moss, lichen, and fungi. Forest stand with an open canopy near the trail and closed canopy away from the trail.	*Picea glauca*, *Abies balsamea*, *Betula papyrifera*, *Linnaea borealis*, *Cornus canadensis*, *Rosa acicularis*, *Arctostaphylos uva-ursi*, and *Maianthemum racemosum*.
white spruce 2	N54°39'7.1" W101°29'50.8"	16	186	90.1	950.1	White spruce stand near granite-gneiss rock outcrop. Trees seem to be mature in age, deadwood abundant on the forest floor. Areas not covered in dead wood is covered in moss.	*Linnaea borealis*, *Picea glauca*, *Betula papyrifera*, *Abies balsamea*, *Maianthemum racemosum*, *Aralia nudicaulis*, *Chimaphila umbellata*, and *Ribes* sp.
white spruce 3	N54°39'6.8" W101°29'52.5"	-2	356	83.9	1525.9	White spruce stand in boggy area, the forest floor is covered with moss. Stand is close to the lake, one of the dominant moss on the forest floor is *Sphagnum* sp. There are feather mosses near the trail.	*Rhododendron groenlandicum*, *Picea glauca*, *Betula papyrifera*, *Equisetum pratense*, grasses, *Salix* sp., *Linnaea borealis*, and *Alnus viridis*.

Table B3.2. (Continued). Description of boreal forest stand types (balsam fir, white spruce, and poplar) and their vascular flora. Boreal forest stands are located around Payuk Lake, Manitoba, Canada. The data and description of the study sites were gathered between 2014–2016. Vascular species are not listed in any particular order.

Forest Types and stand	Latitude and Longitude (NAD83)	% Slope	Aspect (°)	Average Canopy Cover (%)	Tree Density (#trees/ha)	Site Description	Vascular Plants
poplar 1	N54°39'5.5" W101°29'45"	5	60	87.9	569.4	Poplar stand with an open canopy, mature and young trees. Lots of dead wood, some covered with moss and lichen. The bases of the trees were covered with moss.	*Rosa acicularis*, *Alnus viridis*, *Populus tremuloides*, *Picea glauca*, *Ribes* sp., *Aralia nudicaulis*, fern sp., *Epilobium angustifolia*, and *Maianthemum racemosum.*
poplar 2	N54°39'13.5" W101°29'20.8"	-4	250	73.1	2176.4	Poplar stand on sandy soil with young and mature poplar trees, a mix of closed and open canopy in a windy area.	*Populus tremuloides*, *Picea glauca*, *Aralia nudicaulis*, *Arctostaphylos uva-ursi*, *Clintonia borealis*, *Maianthemum racemosum*, *Juniperus communis*, and *Ribes* sp.
poplar 3	N54°39'12.8" W101°29'29.9"	-3	263	78.4	2567.0	Poplar stand on sandy soil dominated by young trees, canopy open. The forest floor is covered with litter, and there are a few stumps with moss and reindeer lichen.	*Populus tremuloides*, *Picea glauca*, *Maianthemum racemosum*, *Prunus pensylvanica*, *Fragaria* sp., *Alnus viridis*, and *Arctostaphylos uva-ursi.*

Table B3.2. Presence and absence of moss and lichen species collected (summer 2015–2016) from the forest floor (F) and trees (T) of three balsam fir (BF), poplar (P), and white spruce (WS) dominated stands in boreal forests next to Payuk Lake in northern Manitoba, Canada.

Taxa	Species	BF1T	BF1F	BF2T	BF2F	BF3T	BF3F	P1T	P1F	P2T	P2F	P3T	P3F	WS1T	WS1F	WS2T	WS2F	WS3T	WS3F
Lichens	*Arthonia patellulata*	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0
	Biatora subduplex	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1
	Biatora vernalis	0	1	0	0	0	1	1	1	0	0	0	0	0	0	1	1	0	1
	Bryoria furcellata	0	0	1	1	1	1	0	0	0	0	0	0	1	1	1	0	1	1
	Bryoria fuscescens	0	0	1	0	1	1	0	0	0	0	0	0	1	1	1	1	1	1
	Bryoria lanestris	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0
	Caloplaca cerina	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0
	Caloplaca holocarpa	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
	Caloplaca pyracea	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	Candelariella lutella	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	Chaenotheca chrysocephala	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	Cladonia amaurocraea	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0
	Cladonia arbuscula	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	1	0	1
	Cladonia cenotea	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1
	Cladonia chlorophaea	0	0	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1
	Cladonia coniocraea	0	1	0	0	0	1	0	1	0	0	0	1	1	0	1	1	0	1
	Cladonia cornuta	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
	Cladonia crispata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
	Cladonia deformis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	Cladonia fimbriata	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1
	Cladonia gracilis sbspp gracilis	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0
	Cladonia gracilis sbspp turbinata	0	0	0	1	0	1	0	0	0	0	0	1	0	1	0	1	0	1

Cladonia macilenta	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	1	0	0
Cladonia merochlorophaea	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Cladonia multiformis	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	1	0	0
Cladonia ochrochlora	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Cladonia phyllophora	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	1	0	0
Cladonia pleurota	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Cladonia pyxidata	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Cladonia rangiferina	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
Cladonia scabriuscula	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Cladonia stellaris	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Cladonia stygia	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
Cladonia sulphurina	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
Cladonia uncialis	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Diploschistes scruposus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Evernia mesomorpha	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0
Hypogymnia physodes	1	1	1	1	1	1	1	0	0	1	0	0	1	1	1	0	1	1
Lecanora allophana	0	0	0	0	0	1	1	0	0	1	0	0	1	0	1	0	0	0
Lecanora circumborealis	0	0	1	0	0	0	0	1	0	0	0	0	1	0	1	1	1	0
Lecanora pulicaris	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Lecidea berengeriana	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Lecidella euphorea	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Leptogium dactylinum	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptogium tenuissimum	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Loxospora elatina	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Melanelia septentrionalis	1	1	1	0	1	1	1	0	0	1	1	0	1	1	1	1	1	1
Melanelia subaurifera	0	0	1	0	1	1	0	0	0	0	0	0	0	0	1	0	1	1
Nephroma parile	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Omphalina umbellifera	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Parmelia sulcata	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	1

Parmeliella triptophylla	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0
Parmeliopsis ambigua	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Parmeliopsis capitata	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1
Parmeliopsis hyperopta	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	1
Peltigera aphthosa	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Peltigera canina	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0
Peltigera didactyla	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0
Peltigera elisabethae	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Peltigera horizontalis	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Peltigera lepidophora	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Peltigera malacea	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0
Peltigera polydactylon	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Peltigera rufescens	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Physcia adscendens	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	1	0	0
Physcia aipolia	1	0	1	0	1	1	1	0	0	1	1	0	1	0	1	1	1	1
Physcia millegrana	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Physconia detersa	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Ramalina dilacerata	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
Rhizocarpon grande	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Rinodina spp.	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
Stereocaulon tomentosum	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Tuckermannopsis americana	1	1	1	0	1	1	0	0	0	0	0	0	1	1	1	1	1	1
Tuckermannopsis sepincola	0	0	1	0	0	1	0	0	0	0	0	1	0	1	0	0	0	1
Usnea filipendula	1	1	1	1	0	1	0	0	0	0	0	0	1	0	1	1	1	0
Usnea hirta	1	1	1	0	0	1	1	0	0	0	0	0	1	0	1	1	1	1
Usnea lapponica	1	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0
Usnea subfloridana	1	1	1	0	0	0	0	0	0	1	0	0	1	0	1	1	1	1
Vulpicida pinastri	0	0	1	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1
Xanthoparmelia cumberlandia	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0

	Xanthoparmelia viriduloumbrina	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	Xanthoria hasseana	0	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0
Moss	*Abietinella abietina*	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	Amblystegium serpens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	Amblystegium varium	0	0	0	0	0	1	0	1	0	1	0	0	0	1	0	0	0	0
	Aulacomnium palustre	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	Brachythecium erythrorrhizon	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	1	0	0
	Brachythecium plumosum	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	Brachythecium salebrosum	0	1	0	1	0	1	0	1	0	1	0	0	0	1	0	1	0	1
	Brachythecium starkei	0	1	0	0	0	1	0	1	0	1	0	0	0	0	0	1	0	1
	Brachythecium velutinum	0	1	0	1	0	1	0	1	0	1	0	0	0	1	0	1	0	1
	Bryum capillare	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	Bryum spp.	0	0	0	1	0	1	0	1	0	1	0	0	0	1	0	1	0	1
	Callicladium haldanianum	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Campylium hispidulum	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	1	0	1
	Ceratodon purpureus	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	1	0	0
	Dicranella spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	Dicranum bonjeanii	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	Dicranum flagellare	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0
	Dicranum montanum	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	Dicranum polysetum	0	0	0	1	0	1	0	0	0	0	0	1	0	1	0	1	0	0
	Dicranum scoparium	0	0	0	1	0	1	0	1	0	1	0	1	0	0	0	1	0	0
	Dicranum undulatum	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	Dicranum viride	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
	Distichium capillaceum	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
	Eurhynchium pulchellum	0	1	0	0	0	1	0	1	0	1	0	1	0	0	0	1	0	1
	Funaria hygrometrica	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
	Hedwigia ciliata	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0
	Herzogiella turfacea	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0

Hylocomium splendens	0	1	0	1	0	1	0	0	0	1	0	1	0	1	0	1	0	1
Mnium marginatum	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Mnium spinulosum	0	1	0	1	0	1	0	0	0	0	0	0	0	1	0	1	0	1
Neckera pennata	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Onchophorus wahlenbergii	0	0	0	1	0	1	0	0	0	1	0	0	0	1	0	1	0	1
Orthotrichum obtusifolium	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0
Plagiomnium cuspidatum	0	1	0	1	0	1	0	1	0	0	0	0	0	1	0	0	0	0
Platydictya subtilis	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0
Platygyrium repens	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0
Pleurozium schreberi	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1
Pohlia nutans	0	1	0	1	0	1	0	0	0	0	0	1	0	1	0	1	0	1
Pohlia spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Polytrichum juniperinum	0	1	0	0	0	1	0	0	0	0	0	1	0	1	0	1	0	0
Ptilium crista-castrensis	0	1	0	1	0	1	0	0	0	1	0	0	0	1	0	1	0	1
Pylaisia polyantha	0	0	0	1	0	1	0	1	0	1	0	0	0	0	0	1	0	1
Sanionia uncinata	0	1	0	1	0	1	0	1	0	1	0	1	0	1	1	1	0	1
Sphagnum capillifolium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Tetraphis pellucida	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1

Table B3.3. Total percent cover or the percent cover range (min., max.) for the bryophyte and lichen species assessed in quadrats (0.5 m^2) on the forest floor of balsam fir, poplar, and white spruce dominated stands in boreal forests around Payuk Lake in Manitoba, Canada (summer 2016).

Taxa	Species/Forest Stand	Balsam Fir			Poplar			White Spruce		
		1	2	3	1	2	3	1	2	3
Lichen	*Biatora vernalis*	8	0	0	0	0	0	0	0	0
	Cladonia amaurocraea	0	0	0	0	0	0	14, 50	0	0
	Cladonia arbuscula	0	0	0	0	0	4, 10	8, 49	1	0
	Cladonia chlorophaea	0	0	0	0	0	0	0	5	0
	Cladonia cornuta	0	0	0	0	0	1, 5	0	0	0
	Cladonia crispata	0	0	0	0	0	0	10	0	0
	Cladonia deformis	0	0	3	0	0	0	0	0	0
	Cladonia gracilis	0	0	0	0	0	1, 2	1, 18	1	0
	Cladonia multiformis	0	6	0	0	0	1, 4	1	0	0
	Cladonia phyllophora	0	0	0	0	0	0	2	0	0
	Cladonia pleurota	0	0	0	0	0	0	6	0	0
	Cladonia spp.	1	1	1	1	1	0	0	1	0
	Cladonia uncialis	0	0	0	0	0	0	25	0	0
	Cladonia verticillata	0	0	0	0	0	0	0	1.3	0
	Crustose lichen	0	0	0	0	1	0	0	3	0
	Evernia mesomorpha	0	0	0	0	0	0	0	1	0
	Lichen spp.	10	0	0	24	0	0	0	39	0
	Nephroma parile	0	33	0	0	0	0	0	0	0
	Parmeliella triptophylla	0	0	0	0	0	0	0	3, 5	0
	Parmelia sulcata	0	12	0	0	0	0	0	1	0
	Peltigera malacea	0	0	0	0	0	0	2	0	0
	Peltigera spp.	0	0	0	0	0	0	0	1, 7	0
	Vulpicida pinastri	0	0	0	0	0	0	0	1	0
	Xanthoparmelia spp.	0	7	0	0	0	0	0	1	0
Total		0	1, 52	4	25	1	4, 17	29, 99	2, 53	0
Liverwort		9, 10	0	0	0	0	0	0	1, 7	1
Moss	*Brachythecium* spp.	6, 14	2, 14	0	1	1	0	3	1, 4	6.7
	Bryum sp./*Pohlia* sp.	0	0	0	0	0	4	0	3, 8	0
	Campylium hispidulum	0	0	0	1	0	0	0	0	0
	Dicranum spp.	0	1, 6	1, 4	0	0	1	8	1, 3	1
	Eurhynchium pulchellum	21.17	0	0	0	0	0	0	0	0
	Hedwigia ciliata	0	5	0	0	0	0	0	0	0

Table B3.3. (Continued). Total percent cover or the percent cover range (min., max.) for the bryophyte and lichen species assessed in quadrats (0.5 m^2) on the forest floor of balsam fir, poplar, and white spruce dominated stands in boreal forests around Payuk Lake in Manitoba, Canada (summer 2016).

Taxa	Species/Forest Stand	Balsam Fir			Poplar			White Spruce		
		1	2	3	1	2	3	1	2	3
Moss	*Hylocomium splendens*	2, 62	10, 20	8, 35	0	0	0	0	2.67	1, 5
	Mnium spp.	0	0	5, 7	1	0	0	0	1	1
	Moss	0	0	0	1	0	0	0	0	0
	Orthotrichum obtusifolium	0	0	0	0	0	0	0	2	0
	Plagiomnium cuspidatum	0.5	0	0	0	0	0	0	0	0
	Platygyrium repens	1	0	0	0	0	0	0	0	0
	Pleurozium schreberi	1, 4	12, 40	17, 82	1	0	0	40, 56	6, 11	19, 97
	Polytrichum spp.	0	6	0	0	0	15	7	0	0
	Pylasiella polyantha	0	0	0	1, 4	0	0	0	0	0
	Ptilium crista-castrensis	1	10	4	0	0	0	0	0	1, 80
	Sanionia uncinata	1, 12	15	0	1, 9	1	0	0	2	0
Total		15, 72	2, 72	27, 63	6, 20	1, 2	4, 15	43, 71	2, 20	46, 100

Table B3.4. A nested Kruskal-Wallis (*H*) test on the quantity of bryophyte and lichen propagules frequently deposited in traps in boreal forests around Payuk Lake in Manitoba, Canada. Compared the time of year (February, June, August), forest stand type, substrata, aspect (north, south, east, west), and their interactions. $X+1$ was added to all data values, since zeroes were present. Different letters indicate significant differences among ranks ($P = 0.05$; Conover-Inman pairwise tests).

Data type	n	df	Avg. quantities ± st. error	Ranks	*H*	*P* value
Times of the Year		2			115	<0.0001
February 2016	288		5.5 ± 0.3	523a		
June 2015	311		3.0 ± 0.2	484.7a		
August 2015	282		3.0 ± 0.3	309b		
Forest Stand Types		2			148	<0.0001
balsam fir	303		4.7 ± 0.2	500.4a		
white spruce	309		2.1 ± 0.1	284a		
poplar	269		5.4 ± 0.3	519.4b		
Substrata		1			60	<0.0001
Trees	881		4.1 ± 0.2	654.1a		
Logs/Stumps	330		2.2 ± 0.1	479.2b		
Aspects						
(N, S, E, W)	216, 230, 219, 216	3			0	0.9460
Interactions						
Times of the Year × Forest Stand Types × Aspects		35			367	<0.0001
Times of the Year × Forest Stand Types × Substrata		14			442	<0.0001
Times of the Year × Forest Stand Types		8			355	<0.0001
Times of the Year × Substrata		4			193	<0.0001
Times of the Year × Aspects		11			120	<0.0001
Forest Stand Types × Substrata		5			217	<0.0001
Forest Stand Types × Aspects		11			150	<0.0001

Note: n = number or replicates, Avg. =average, st. error = standard error, df = degrees of freedom, *H* = Kruskal-Wallis test

Table B3.5. A nested Kruskal-Wallis (*H*) test on the size (mm) of bryophyte and lichen propagules frequently deposited in traps in boreal forests around Payuk Lake in Manitoba, Canada. Compared the time of year (February, June, August), forest stand type, substrata, aspect (North, South, East, West), and their interactions. Different letters indicate significant differences among ranks (*P* = 0.05; Conover-Inman pairwise tests).

Data type	n	df	Avg. sizes ± st. error	Ranks	*H*	*P* value
Times of the Year		2			13	0.0014
August 2015	284		1.2 ± 0.2	1277.3a		
June 2015	842		0.7 ± 0.8	1222.5a		
February 2016	1246		0.9 ± 0.8	1141.5b		
Forest Stand Types		2			5	0.0827
balsam fir	1036		0.8 ± 0.7	1200.6		
white spruce	1062		0.6 ± 0.1	1101.4		
poplar	274		1.0 ± 1.0	1194.7		
Substrata		1			24	<0.0001
Logs/Stumps	372		0.1 ± 1.0	1561.0b		
Trees	2372		1.2 ± 1.9	1342.9a		
Aspects						
(N, S, E, W)	615, 594, 613, 550	3			1	0.6981
Interactions						
Times of the Year × Forest Stand Types × Aspects		35			90.4	<0.0001
Times of the Year × Forest Stand Types × Substrata		14			-163	<0.0001
Times of the Year × Forest Stand Types		4			33	<0.0001
Time of the Year × Substrata		4			-194	<0.0001
Times of the Year × Aspects		11			29	0.0018
Forest Stand Types × Substrata		5			-191	<0.0001
Forest Stand Types × Aspects		11			20	0.034

Note: n = number or replicates, Avg. = average, st. error = standard error, df = degrees of freedom, *H* = Kruskal-Wallis test

Appendix C: Wind tunnel dispersal of boreal lichen and moss asexual propagules shows a strong letptokurtic distribution

Table C4.1. Traits of lichen and moss species showing habitat and substratum, growth form, size of the intact lichen thallus or moss gametophore (size), and propagule type observed after crushing the gametophore/thallus. Size was measured for ten lichen thalli or moss gametophores, while 30 gametophores were measured for *O. obtusifolium*. Size values represent the average ± standard error.

Cryptogam	Species	Substrata	Form	Size (cm)	Propagule type
Lichen	*Cladonia amaurocraea* (Flörke) Schaer.	Ground-dwelling, on humus, in white spruce or balsam fir forest stands	Fruticose	6.73 ± 0.40	thallus fragment
	Cladonia stellaris (Opiz) Brodo	Ground-dwelling, on humus, in white spruce or balsam fir forest stands	Fruticose	5.48 ± 0.33	thallus fragment
	Evernia mesomorpha Nyl.	Epiphytic on white spruce, balsam fir, or poplar	Fruticose	1.52 ± 0.15	thallus fragment, soredia
	Tuckermannopsis americana (Spreng.) Hale	Epiphytic on white spruce or balsam fir	Foliose	2.08 ± 0.26	thallus fragment
	Usnea spp. *(filipendula* Stirt., *hirta* (L.) F.H.Wigg., *subfloridana* Stirt.)	Epiphytic on white spruce, balsam fir, or poplar	Fruticose	2.43 ± 0.21	thallus fragment, soredia
Moss	*Dicranum polysetum* Sw.	Ground-dwelling, on humus, in white spruce or balsam fir forest stands	Acrocarp	7.32 ± 0.35	gametophore fragment
	Hylocomium splendens (Hedw.) Schimp.	Ground-dwelling, on humus, in white spruce or balsam fir forest stands	Pleurocarp	6.35 ± 0.27	gametophore fragment

Orthotrichum obtusifolium Brid.	Epiphytic on poplar	Acrocarp	0.16 ± 0.01	gametophore fragment, gemmae
Pleurozium schreberi (Brid.) Mitt.	Ground-dwelling, on humus, in white spruce or balsam fir forest stands	Pleurocarp	7.03 ± 0.59	gametophore fragment

Table C4.2. Models summarizing the relationship between the quantities or sizes with distance for the moss and lichen propagules dispersed by a fan on a one-meter tape.

	Treatments	quantities eq.	quant. R^2	size eq.	size R^2
Boreal forests	All species	y = -0.0374 × sqrt (x + C) + 3.9391	0.25	y = -0.0234 × log (x + C) + 2.4864	0.30
Habitat	Ground	y = -0.0337 × sqrt (x + C) + 3.508	0.26	y = -0.0317 × log (x + C) + 2.7245	0.43
	Epiphyte	y = -0.0336 × sqrt (x + C) + 3.7824	0.18	y = -0.0131 × log (x + C) + 2.1888	0.14
Taxa	Mosses	y = -0.0415 × sqrt (x + C) + 4.2919	0.29	y = -0.0273 × log (x + C) + 2.6736	0.36
	Lichen	y = -0.0341 × sqrt (x + C) + 3.6569	0.21	y = -0.0203 × log (x + C) + 2.3367	0.26
Species	*Cladonia amaurocraea* (Flörke) Schaer.	y = -0.0254 × sqrt (x + C) + 3.0179	0.24	y = -0.0253 × log (x + C) + 2.4682	0.37
	Cladonia arbuscula (Opiz) Brodo	y = -0.026 × sqrt (x + C) + 2.8744	0.24	y = -0.0321 × log (x + C) + 2.6381	0.44
	Evernia mesomorpha Nyl.	y = -0.0291 × sqrt (x + C) + 3.2568	0.16	y = -0.0091 × log (x + C) + 1.8372	0.13
	Tuckermannopsis americana (Spreng.) Hale	y = -0.0358 × sqrt (x + C) + 3.9657	0.31	y = -0.0202 × log (x + C) + 2.4182	0.3
	Usnea spp. (*filipendula* Stirt., *hirta* (L.) F.H.Wigg., *subfloridana* Stirt.)	y = -0.0341 × sqrt (x + C) + 3.6569	0.25	y = -0.015 × log (x + C) + 2.3216	0.15
	Dicranum polysetum Sw.	y = -0.0402 × sqrt (x + C) + 3.9778	0.33	y = -0.0336 × log (x + C) + 2.8142	0.43
	Hylocomnium splendens (Hedw.) Schimp.	y = -0.0383 × sqrt (x + C) + 3.8776	0.23	y = -0.008 × log (x + C) + 2.1783	0.08
	Orthotrichum obtusifolium Brid.	y = -0.049 × sqrt (x + C) + 5.5198	0.35	y = -0.0318 × log (x + C) + 2.8059	0.45
	Pleurozium schreberi (Brid.) Mitt.	y = -0.0386 × sqrt (x + C) + 3.7923	0.32	y = -0.0358 × log (x + C) + 2.8961	0.49

Note: eq. =equation.

Table C4.3. Assessment of the number of boreal lichen and moss propagules from ground and epiphytic species dispersed on a one-meter tape in the laboratory. The One-Way ANOVA (*F*) compared the numbers of dispersed propagules separated by treatment (Substrata, cryptogams). Different letters indicate significant differences within treatments ($\alpha = 0.05$; Tukey Tests). *n* = replicates, sqrt = square root, avg. = average, C = constant (25 μm), st. error = standard error, df = degrees of freedom.

Treatments	Distance	n	sqrt (avg. size + C)	st. error	df	*F*	*P*
Substrata					3	7.04	0.0003
Ground	66a	15	1.06	0.54			
Ground	99a	15	1.1	0.54			
Ground	33a	15	1.19	0.54			
Epiphyte	99a	12	1.47	0.6			
Epiphyte	33a	12	1.72	0.6			
Epiphyte	66a	12	1.73	0.6			
Ground	3b	15	9.86	0.56			
Epiphyte	3c	12	14.77	0.6			
Cryptogams					3	44.91	0.6848
Moss	66a	12	1.24	0.6			
Moss	99a	12	1.25	0.6			
Lichen	99a	15	1.27	0.54			
Lichen	33a	15	1.33	0.54			

Lichen	66a	15	1.44	0.54			
Moss	33a	12	1.55	0.6			
Moss	3b	12	11.39	0.6			
Lichen	3b	15	12.56	0.56			
Species					21	2.30	0.0049
Cladonia arbuscula	99a	3	1	1.2			
Dicranum polysetum	99a	3	1	1.2			
Pleurozium schreberi	99a	3	1	1.2			
Pleurozium schreberi	66a	3	1	1.2			
Hylocomium splendens	66a	3	1	1.2			
Dicranum polysetum	66a	3	1	1.2			
Cladonia amaurocraea	66a	3	1.14	1.2			
Cladonia arbuscula	66a	3	1.14	1.2			
Evernia mesomorpha	33a	3	1.14	1.2			
Hylocomium splendens	33a	3	1.14	1.2			
Dicranum polysetum	33a	3	1.14	1.2			
Pleurozium schreberi	33a	3	1.14	1.2			
Cladonia amaurocraea	99a	3	1.24	1.2			
Hylocomium splendens	99a	3	1.24	1.2			
Cladonia amaurocraea	33a	3	1.28	1.2			

Cladonia arbuscula	33a	3	1.28	1.2
Evernia mesomorpha	99a	3	1.33	1.2
Evernia mesomorpha	66a	3	1.33	1.2
Usnea spp.	99ab	3	1.38	1.2
Tuckermannopsis americana	99ab	3	1.41	1.2
Usnea spp.	33ab	3	1.48	1.2
Tuckermannopsis americana	33ab	3	1.49	1.2
Orthotrichum obtusifolium	99ab	3	1.75	1.2
Tuckermannopsis americana	66abc	3	1.79	1.2
Usnea spp.	66abc	3	1.82	1.2
Orthotrichum obtusifolium	66abc	3	1.96	1.2
Orthotrichum obtusifolium	33abcd	3	2.78	1.2
Dicranum polysetum	3bcde	3	8.2	1.2
Cladonia amaurocraea	3cde	3	8.62	1.47
Cladonia arbuscula	3de	3	9.29	1.2
Evernia mesomorpha	3e	3	10.95	1.2
Hylocomium splendens	3e	3	11.43	1.2
Tuckermannopsis americana	3e	3	11.59	1.2
Pleurozium schreberi	3e	3	11.74	1.2
Orthotrichum obtusifolium	3e	3	14.21	1.2

Usnea spp.	3e	3	22.33	1.2

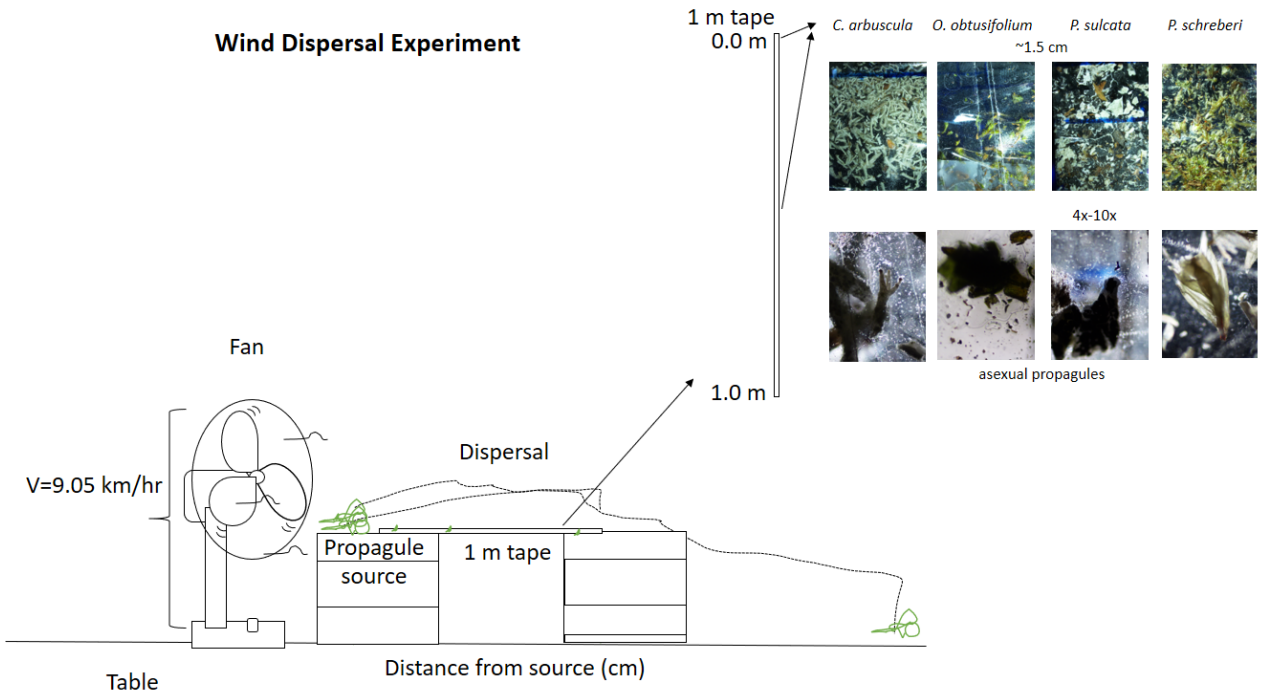

Figure C4.1 Diagram showing the experimental setting of the wind dispersal experiment on the asexual propagules of boreal mosses and lichens at wind velocity of 9.05 km/hr (V).

Appendix D: Then eDNA community structure in soil banks does not mirror that of the extant cryptogam community but is partly associated with forest stand properties in boreal forests

Table D5.1. Descriptive statistics (number, average, and standard error) of the number of sequences (reads) sequenced from the 23S rDNA, trnL intron c-h, rbcL, and ITS rDNA genes for the taxa (bryophytes, fungi, lichen photobiont, lichen fungi, and vascular plants) present in the environmental DNA in boreal soils of different tree dominated stand (three balsam fir, B1–3; three poplar, P1–3; and, three white spruce stands, W1–3) in Manitoba, Canada.

Note: An excel file attached to the thesis.

Table D5.2. Lichen and its photobiont richness, presence and absence of photobiont type (*Trebouxia* or *Trebouxia*-like, *Coccomyxa*, *Nostoc*), associated with the forest stand types and their stands (three balsam fir, B1–3; three poplar, P1–3; and, three white spruce stands, W1–3) of boreal forests surrounding Payuk Lake, Manitoba, Canada.

Species	Photobiont	Reference	B	P	W	B1	B2	B3	P1	P2	P3	W1	W2	W3
Arthonia patellulata	Trebouxioid	Wetmore 2005	0	1	0	0	0	0	1	1	1	0	0	0
Bryobilimbia berengeriana	Trebouxioid	Printzen and Ekman 2014	0	0	1	0	0	0	0	0	0	1	0	0
Biatora subduplex	Trebouxioid	Printzen and Tønsberg	1	0	1	1	0	1	0	0	0	1	0	1
Biatora vernalis	Trebouxioid	1999	1	1	1	1	0	1	1	0	0	0	1	1
Bryoria furcellata	Trebouxiaceae: *Trebouxia*		1	0	1	0	1	1	0	0	0	1	1	1
Bryoria fuscescens	Trebouxiaceae: *Trebouxia*	Lindgren et al. 2014	1	0	1	0	1	1	0	0	0	1	1	1
Bryoria lanestris	Trebouxiaceae: *Trebouxia*		0	0	1	0	0	0	0	0	0	1	1	1
Caloplaca cerina	Trebouxiaceae: *Trebouxia*		0	1	0	0	0	0	1	1	1	0	0	0
Caloplaca holocarpa	Trebouxiaceae: *Trebouxia*	Nyati et al. 2014	0	1	0	0	0	0	0	1	1	0	0	0
Caloplaca pyracea	Trebouxiaceae: *Trebouxia*		0	1	0	0	0	0	0	0	1	0	0	0
Candelariella lutea	Trebouxiaceae: *Trebouxia*	Beck et al. 1998	0	1	0	0	0	0	1	0	0	0	0	0
Chaenotheca chrysocephala	Trebouxiaceae: *Trebouxia*	Tibell 2001	0	0	1	0	0	0	0	0	0	0	0	1
Cladonia amaurocraea	Trebouxiaceae*: Trebouxia/Asterchloris*		0	1	1	0	0	0	0	0	1	1	1	0
Cladonia arbuscula	Trebouxiaceae*: Trebouxia/Asterchloris*		1	1	1	0	0	1	0	0	1	1	1	1
Cladonia cenotea	Trebouxiaceae*: Trebouxia/Asterchloris*		0	0	1	0	0	0	0	0	0	1	1	1
Cladonia chlorophaea	Trebouxiaceae*: Trebouxia/Asterchloris*	Rambold et al. 1998, Bačkor et al. 2010	1	1	1	0	1	1	1	1	1	1	1	1
Cladonia coniocraea	Trebouxiaceae*: Trebouxia/Asterchloris*		1	1	1	1	0	1	1	0	1	1	1	1
Cladonia cornuta	Trebouxiaceae*: Trebouxia/Asterchloris*		0	1	1	0	0	0	0	0	1	1	0	0
Cladonia crispata	Trebouxiaceae*: Trebouxia/Asterchloris*		0	0	1	0	0	0	0	0	0	0	1	1
Cladonia deformis	Trebouxiaceae*: Trebouxia/Asterchloris*		0	0	1	0	0	0	0	0	0	0	1	0

Cladonia fimbriata	Trebouxiaceae*: Trebouxia/Asterchloris*		0	1	1	0	0	0	0	0	1	0	0	1
Cladonia gracilis sbspp gracilis	Trebouxiaceae*: Trebouxia/Asterchloris*		0	1	1	0	0	0	0	0	1	1	1	0
Cladonia gracilis sbspp turbinata	Trebouxiaceae*: Trebouxia/Asterchloris*		1	1	1	0	1	1	0	0	1	1	1	1
Cladonia macilenta	Trebouxiaceae*: Trebouxia/Asterchloris*		0	0	1	0	1	0	0	1	0	1	1	0
Cladonia merochlorophaea	Trebouxiaceae*: Trebouxia/Asterchloris*		0	0	1	0	0	0	0	0	0	1	0	0
Cladonia multiformis	Trebouxiaceae*: Trebouxia/Asterchloris*		1	1	1	0	1	1	0	0	1	0	1	0
Cladonia ochrochlora	Trebouxiaceae*: Trebouxia/Asterchloris*		1	0	1	1	1	0	0	0	0	1	0	1
Cladonia phyllophora	Trebouxiaceae*: Trebouxia/Asterchloris*		1	1	1	0	0	1	0	0	1	1	1	0
Cladonia pleurota	Trebouxiaceae*: Trebouxia/Asterchloris*		0	0	1	0	0	0	0	0	0	0	1	0
Cladonia pyxidata	Trebouxiaceae*: Trebouxia/Asterchloris*		1	0	0	0	0	1	0	0	0	0	0	0
Cladonia rangiferina	Trebouxiaceae*: Trebouxia/Asterchloris*		0	1	1	0	0	0	0	0	1	1	0	0
Cladonia scabriuscula	Trebouxiaceae*: Trebouxia/Asterchloris*		0	1	0	0	0	0	0	1	0	0	0	0
Cladonia stellaris	Trebouxiaceae*: Trebouxia/Asterchloris*		0	0	1	0	0	0	0	0	0	1	0	1
Cladonia stygia	Trebouxiaceae*: Trebouxia/Asterchloris*		0	1	1	0	0	0	0	0	1	1	0	0
Cladonia sulphurina	Trebouxiaceae*: Trebouxia/Asterchloris*		0	1	1	0	0	0	0	0	1	1	0	0
Cladonia uncialis	Trebouxiaceae*: Trebouxia/Asterchloris*		0	0	1	0	0	0	0	0	0	1	0	0
Diploschistes scruposus	Trebouxiaceae*: Trebouxia/Asterchloris*	Bačkor et al. 2010	0	0	1	0	0	0	0	0	0	0	1	1
Evernia mesomorpha	Trebouxiaceae: *Trebouxia*	Piercey-Normore 2006	1	1	1	1	1	1	1	1	1	1	1	1
Hypogymnia physodes	Trebouxiaceae: *Trebouxia*	Bačkor et al. 2010	1	1	1	1	1	1	1	1	0	1	1	1
Lecanora allophana	Trebouxiaceae: *Trebouxia*		1	1	1	0	0	1	1	1	0	1	1	0
Lecanora circumborealis	Trebouxiaceae: *Trebouxia*	Beck et al. 1998	1	1	1	0	1	0	1	0	0	1	1	1
Lecanora pulicaris	Trebouxiaceae: *Trebouxia*		1	0	1	0	1	0	0	0	0	0	0	1
Lecidella euphorea	Trebouxiaceae: *Trebouxia*	Beck et al. 1998	0	1	0	0	0	0	1	0	0	0	0	0

Leptogium dactylinum	Nostocaceae: *Nostoc*	Otálora et al. 2010	1	0	0	0	1	0	0	0	0	0	0
Leptogium tenuissimum	Nostocaceae: *Nostoc*		1	0	0	0	0	1	0	0	0	0	0
Loxospora elatina	Chlorococcoid	Lendemer 2013	0	1	0	0	0	0	1	0	0	0	0
Lichenomphalia umbellifera	Trebouxiaceae : Coccomyxa	Zoller and Lutzoni 2003	1	0	0	1	0	0	0	0	0	0	0
Melanelia septentrionalis	Trebouxiaceae: *Trebouxia*	Dahlkild et al. 2001	1	1	1	1	1	1	1	1	1	1	1
Melanelia subaurifera	Trebouxiaceae: *Trebouxia*		1	0	1	0	1	1	0	0	0	1	1
Nephroma parile	Nostocaceae: *Nostoc*	Otálora et al. 2010	1	0	1	0	1	0	0	0	0	1	0
Parmelia sulcata	Trebouxiaceae: *Trebouxia*	Dahlkild et al. 2001	1	1	1	1	1	1	1	1	1	1	1
Parmeliella triptophylla	Nostocaceae: *Nostoc*	Magain and Sérusiaux 2014	1	0	1	0	0	1	0	0	0	1	0
Parmeliopsis ambigua	Trebouxiaceae: *Trebouxia*		0	0	1	0	0	0	0	0	1	0	0
Parmeliopsis capitata	Trebouxiaceae: *Trebouxia*	Ahmadjian 1993	1	0	1	0	0	1	0	0	1	0	1
Parmeliopsis hyperopta	Trebouxiaceae: *Trebouxia*		1	0	1	0	0	1	0	0	1	0	1
Peltigera aphthosa	Nostocaceae: *Nostoc*; Trebouxiaceae: *Trebouxia*	Otálora et al. 2010, Paulsrud et al. 2001	0	0	1	0	0	0	0	0	1	0	0
Peltigera canina	Nostocaceae: *Nostoc*		1	0	1	1	0	1	0	0	1	0	0
Peltigera didactyla	Nostocaceae: *Nostoc*		1	0	1	0	0	1	0	0	1	1	0
Peltigera elisabethae	Nostocaceae: *Nostoc*		1	0	0	0	1	1	0	0	0	0	0
Peltigera horizontalis	Nostocaceae: *Nostoc*	Otálora et al. 2010	1	0	1	0	1	0	0	0	0	1	1
Peltigera lepidophora	Nostocaceae: *Nostoc*		0	0	1	0	0	0	0	0	0	1	0
Peltigera malacea	Nostocaceae: *Nostoc*		1	0	1	0	0	1	0	0	1	1	0
Peltigera polydactylon	Nostocaceae: *Nostoc*		0	0	1	0	0	0	0	0	0	1	0
Peltigera rufescens	Nostocaceae: *Nostoc*		1	0	0	0	1	0	0	0	0	0	0
Physcia adscendens	Trebouxiaceae: *Trebouxia*		0	1	1	0	0	0	1	1	1	0	1	0
Physcia aipolia	Trebouxiaceae: *Trebouxia*	Dahlkild et al. 2001	1	1	1	1	1	1	1	1	1	1	1	1
Physcia millegrana	Trebouxiaceae: *Trebouxia*		1	0	0	0	0	1	0	0	0	0	0	0
Physconia detersa	Trebouxiaceae: *Trebouxia*		1	0	0	0	0	1	0	0	0	0	0	0
Ramalina dilacerata	Trebouxiaceae: *Trebouxia*	Ahmadjian 1993	1	0	1	1	1	0	0	0	0	0	1	0
Rhizocarpon grande	Trebouxiaceae: *Trebouxia*		0	0	1	0	0	0	0	0	0	0	0	1
Rinodina sp.	Trebouxiaceae: *Trebouxia*	Helms et al. 2001	0	1	0	0	0	0	0	1	1	0	0	0
Stereocaulon tomentosum	Trebouxiaceae: *Trebouxia*	Ahmadjian 1993	0	0	1	0	0	0	0	0	0	1	0	0
Tuckermannopsis americana	Trebouxiaceae: *Trebouxia*	Doering and Piercey-Normore 2009	1	0	1	1	1	1	0	0	0	1	1	1
Tuckermannopsis sepincola	Trebouxiaceae: *Trebouxia*		1	1	1	0	1	1	0	0	1	1	0	1
Usnea filipendula	Trebouxiaceae: *Trebouxia*		1	0	1	1	1	1	0	0	0	1	1	1
Usnea hirta	Trebouxiaceae: *Trebouxia*	Ahmadjian 1993	1	1	1	1	1	1	1	0	0	1	1	1
Usnea lapponica	Trebouxiaceae: *Trebouxia*		1	0	1	1	1	0	0	0	0	1	1	1
Usnea subfloridana	Trebouxiaceae: *Trebouxia*		1	1	1	1	1	0	0	1	0	1	1	1
Vulpicida pinastri	Trebouxiaceae: *Trebouxia*	Lindgren et al. 2014	1	1	1	0	1	1	0	0	1	1	1	1
Xanthoparmelia cumberlandia	Trebouxiaceae: *Trebouxia*	Ahmadjian 1993	1	0	1	0	1	0	0	0	0	0	1	0
Xanthoparmelia viriduloumbrina	Trebouxiaceae: *Trebouxia*		0	0	1	0	0	0	0	0	0	0	1	0

Xanthoria hasseana	Trebouxiaceae: *Trebouxia*	Dahlkild et al. 2001	0	1	0	0	0	0	1	1	1	0	0	0
Boreal forest stands			B	P	W	B1	B2	B3	P1	P2	P3	W1	W2	W3
Total			45	36	64	17	29	33	18	16	26	44	43	35
Green algae (Trebouxia or Trebouxia-like)			34	36	55	15	24	27	18	16	26	40	36	34
Green algae (Coccomyxa)			1	0	1	1	0	0	0	0	0	1	0	0
Blue green-algae (Nostoc)			10	0	8	1	5	6	0	0	0	3	7	1

Table D5.3. Descriptive statistics (number, average, and standard error) of the number of sequences (reads, abundance) for each operational taxonomic units (OTU) sequenced from the 23S rDNA, trnL, rbcL, and ITS rDNA genes for the taxa (bryophytes, fungi, lichen photobiont, lichen fungi, and vascular plants) present in the environmental DNA in boreal soils of different tree dominated stand (three balsam fir, B1–3; three poplar, P1–3; and, three white spruce stands, W1–3) in Manitoba, Canada.

Gene	Taxa	Sequence abundance (reads)	forest stands										
			B total	P total	W total	B1	B3	P1	P2	P3	W1	W2	W3
23S	Overall	# of reads	57847	81009	71020	21637	18528	23408	28575	29026	27692	21731	21597
		avg. # of reads per stand	7456.8	9001	7891.1	7212.3	9264	7802.7	9525	9675.3	9230.7	7243.7	7199
		st. error # of reads per stand	182.8	205.5	139.9	1214.5	1114	1889	1790.3	2450.3	1881.6	2033.6	2356.1
	Bryophyte	# of reads	4124	480	4574	1406	106	139	130	211	691	3287	596
		avg. # of reads per stand	464.1	53.3	508.2	468.7	53	46.3	43.3	70.3	230.3	1095.7	198.7
		st. error # of reads per stand	130.9	5	125.3	384.3	49	27.2	17.7	35	198.4	560.9	173.2
	Lichen photobiont (green and blue-green algae)	# of reads	2893	3028	15995	1041	69	463	221	2344	5873	8758	1364
		avg. # of reads per stand	325.3	336.4	1777.2	347	34.5	154.3	73.7	781.3	1957.7	2919.3	454.7
		st. error # of reads per stand	73.3	73.2	236	232.7	25.5	142.5	55.1	304.9	1248.1	1010.2	451.7
	Vascular plants	# of reads	41506	66009	39313	15834	17336	18378	24062	23569	19348	1943	18022
		avg. # of reads per stand	5574.9	7334.3	4368.1	5278	8668	6126	8020.7	7856.3	6449.3	647.7	6007.3
		st. error # of reads per stand	1706.6	606	1864.6	1712.3	1424	1298.2	704	2123.1	3131.2	305.7	2191

trnL	Overall	# of reads	175000	197536	230713	64426	47135	66998	57382	73156	77141	77748	75824
		avg. # of reads per stand	22063.1	21948.4	25634.8	21475.3	23567.5	22332.7	19127.3	24385.3	25713.7	25916	25274.7
		st. error # of reads per stand	758.2	1530	189.3	2206.7	1507.5	821.8	2871.6	3812	4132.7	3307.7	3607.6
	Bryophyte	# of reads	6294	969	19581	1042	103	326	268	375	3012	15822	747
		avg. # of reads per stand	705.1	107.7	2175.7	347.3	51.5	108.7	89.3	125	1004	5274	249
		st. error # of reads per stand	512.8	10.3	1564.4	235.7	15.5	55.7	30	59.8	845.5	1933.8	162.2
	Vascular plants	# of reads	35438	67181	28778	21029	5237	17811	13932	20521	10547	4418	13813
		avg. # of reads per stand	4228.5	5807.1	3197.6	7009.7	2618.5	5937	4644	6840.3	3515.7	1472.7	4604.3
		st. error # of reads per stand	1396.3	637.3	917.9	1712.4	2598.5	83.1	672.9	1514.1	1978.9	675.2	857.5
rbcL	Overall	# of reads	44245	52443	37131	21290	8327	17867	14010	20566	13931	8421	14779
		avg. # of reads per stand	5378.7	5827	4125.7	7096.7	4163.5	5955.7	4670	6855.3	4643.7	2807	4926.3
		st. error # of reads per stand	521.1	414.4	369.6	2844.2	1059.5	79.7	680.2	1509	1500.1	219.7	872.8
	Bryophyte	# of reads	8807	172	8351	261	3090	50	78	44	3382	4003	966
		avg. # of reads per stand	1150.2	19.1	927.9	87	1545	16.7	26	14.7	1127.3	1334.3	322
		st. error # of reads per stand	424.2	3.7	108.7	81	1539	8.3	19	7.6	664.1	479.8	287.6
	Vascular plants	# of reads	168706	196567	211132	63384	47032	66672	57114	72781	74129	61926	75077
		avg. # of reads per stand	21358	21840.8	23459.1	21128	23516	22224	19038	24260.3	24709.7	20642	25025.7
		st. error # of reads per stand	1185.1	1519.7	1411.5	2420.1	1523	872.3	2851.7	3811.2	4973.9	1448.6	3619.4
ITS	Overall	# of reads	101629	101215	129146	34892	20030	34863	38034	28318	33132	48745	47269
		avg. # of reads per stand	12404.9	11246.1	14349.6	11630.7	10015	11621	12678	9439.3	11044	16248.3	15756.3
		st. error # of reads per stand	873.6	65.8	723.3	2138.3	60	1213.8	1015.7	1212.7	1542.6	337.9	2842.7
	Lichen fungi	# of reads	15	33	439	4	1	6	5	22	2	421	16
		avg. # of reads per stand	1.8	3.7	48.8	1.3	0.7	2	1.7	7.3	0.7	140.3	5.3
		st. error # of reads per stand	0.6	0.6	46.1	1.3	0.5	1.5	0.9	2.9	0.7	140.3	3.2
	Fungi	# of reads	101614	101182	128707	34888	20029	34857	38029	28296	33130	48324	47253
		avg. # of reads per stand	12403.2	11242.4	14300.8	11629.3	10014.5	11619	12676.3	9432	11043.3	16108	15751
		st. error # of reads per stand	1648.5	955.3	1632	2137.5	59.5	1213.2	1014.9	1215.5	1543	476.4	2843

Table D5.4. Difference in bryophytes, fungi, lichen photobiont, lichen fungi, and vascular plant number of sequences (reads, abundance) from sequences of different genes (23S rDNA, trnL intron c-h, rbcL, ITS rDNA) obtained from environmental DNA in boreal soil samples. Boreal soil samples pertained to different stands (1–3) that were dominated by a tree species (B = balsam fir, P = poplar, W = white spruce) and surrounding Payuk Lake in Manitoba, Canada. Different letters indicate significant differences among the treatments from the Conover-Iman Tests ($\alpha = 0.10$) after performing a Kruskal-Wallis Test on the data. avg. = average, st. error = standard error, df = degrees of freedom, *H* = Kruskal-Wallis Test.

Gene	Taxa	Forest stand	avg. read abundance	st. error.	df	*H*	*P*
23s	Bryophyte	B1	468.67	384.25	8	9.1	0.3337
		B2	870.67	480.97			
		B3	53	49			
		P1	46.33	27.19			
		P2	43.33	17.7			
		P3	70.33	34.95			
		W1	230.33	198.37			
		W2	1095.67	560.92			
		W3	198.67	173.2			
	Lichen photobiont	B1	347.00 ab	232.66	8	15.11	0.0569
		B2	594.33 abc	256.29			
		B3	34.50 a	25.5			
		P1	154.33 a	142.5			
		P2	73.67 a	55.1			
		P3	781.33 abc	304.9			
		W1	1957.67 bc	1248.11			
		W2	2919.33 c	1010.16			
		W3	454.67 a	451.67			
	Vascular plants	B1	5278	1712.3	8	9.77	0.2812
		B2	2778.7	2315			
		B3	8668	1424			
		P1	6126	1298.2			

		P2	8020.7	704			
		P3	7856.3	2123.1			
		W1	6449.3	3131.2			
		W2	647.7	305.7			
		W3	6007.3	2191			
		B1	347.33	235.68	8	12.47	0.1315
		B2	1716.33	853.97			
		B3	51.5	15.5			
		P1	108.67	55.71			
	Bryophyte	P2	89.33	30			
trnL		P3	125	59.81			
		W1	1004	845.54			
		W2	5274	1933.75			
		W3	249	162.22			
		B1	7009.7	1712.4	8	5.9	0.7475
	Vascular plants	B2	3057.3	2568.3			
		B3	2618.5	2598.5			
		P1	5937	83.1			
		P2	4644	672.9			
		P3	6840.3	1514.1			
		W1	3515.7	1978.9			
		W2	1472.7	675.2			
		W3	4604.3	857.5			

rbcL	Bryophyte	B1	87	81.02	8	10.59	0.2221
		B2	1818.67	969.78			
		B3	1545	1539			
		P1	16.67	8.33			
		P2	26	19.01			
		P3	14.67	7.62			
		W1	1127.33	664.11			
		W2	1334.33	479.84			
		W3	322	287.64			
	Vascular plants	B1	21128	2420.1	8	10.12	0.2565
		B2	19430	990.1			
		B3	23516	1523			
		P1	22224	872.3			
		P2	19038	2851.7			
		P3	24260.3	3811.2			
		W1	24709.7	4973.9			
		W2	20642	1448.6			
		W3	25025.7	3619.4			
ITS	Lichen fungi	B1	1.33	1.33	8	6.28	0.5563
		B2	3.33	2.4			
		B3	0.71	0.5			
		P1	2	1.53			
		P2	1.67	0.88			
		P3	7.33	2.85			
		W1	0.67	0.67			
		W2	140.33	140.33			
		W3	5.33	3.18			
	Fungi	B1	11629.3	2137.5	8	12.32	0.1375
		B2	15565.7	3005.1			
		B3	10014.5	59.5			
		P1	11619	1213.2			
		P2	12676.3	1014.9			
		P3	9432	1215.5			
		W1	11043.3	1543			
		W2	16108	476.4			
		W3	15751	2843			

Table D5.5. Descriptive statistics (number, average, and standard error) of the number of different operational taxonomic units (OTU) sequenced from the 23S rDNA, trnL intron c-h, rbcL, and ITS rDNA genes for the taxa (bryophytes, fungi, lichen photobiont, lichen fungi, and vascular plants) present in the environmental DNA in boreal soils of different tree dominated stand (three balsam fir, B1–3; three poplar, P1–3; and, three white spruce stands, W1–3) in Manitoba, Canada.

Gene	Taxa	Sequence richness (OTU)	Forest stands											
			B total	P total	W total	B1	B2	B3	P1	P2	P3	W1	W2	W3
23S	Overall	# of OTUs per stand	1259	2281	1952	567	412	280	535	565	1181	795	478	679
		avg. # of OTUs per stand	155.4	253.4	216.9	189	137.3	140	178.3	188.3	393.7	265	159.3	226.3
		st. error # of OTUs per stand	16.8	70.2	30.9	64.3	23.4	64	24.6	38.1	42.2	71.8	47.1	52.8
	Bryophyte	# of OTUs per stand	76	57	96	27	41	8	16	13	28	21	54	21
		avg. # of OTUs per stand	8.9	6.3	10.7	9	13.7	4	5.3	4.3	9.3	7	18	7
		st. error # of OTUs per stand	2.8	1.5	3.7	4.2	5	2	2.2	0.3	2	2.5	3.8	4
	Lichen photobiont (green and blue-green algae)	# of OTUs per stand	151	164	357	56	79	16	27	29	108	138	165	54
		avg. # of OTUs per stand	17.7	18.2	39.7	18.7	26.3	8	9	9.7	36	46	55	18
		st. error # of OTUs per stand	5.3	8.9	11.1	3.8	4.8	2	7.6	4.3	9	9.6	20.2	17
	Vascular plants	# of OTUs per stand	537	1046	877	259	121	157	192	215	639	376	81	420
		avg. # of OTUs per stand	68.4	116.2	97.4	86.3	40.3	78.5	64	71.7	213	125.3	27	140
		st. error # of OTUs per stand	14.2	48.4	35.5	51.3	19.9	50.5	11.9	4.9	18.4	54.3	9.3	32.6

		# of OTUs per stand	434	556	540	198	174	62	217	173	166	174	253	113
	Overall	avg. # of OTUs per stand	51.7	61.8	60	66	58	31	72.3	57.7	55.3	58	84.3	37.7
		st. error # of OTUs per stand	10.6	5.3	13.5	5.5	23	7	15.6	6.6	8.5	11	8.7	6.7
		# of OTUs per stand	74	69	150	26	37	11	16	16	37	34	81	35
trnL	Bryophyte	avg. # of OTUs per stand	8.8	7.7	16.7	8.7	12.3	5.5	5.3	5.3	12.3	11.3	27	11.7
		st. error # of OTUs per stand	2	2.3	5.2	1.2	4.3	0.5	1.7	0.7	4.7	3.4	4.6	5.7
		# of OTUs per stand	360	718	390	172	137	51	201	157	129	140	172	78
	Vascular plants	avg. # of OTUs per stand	42.8	54.1	43.3	57.3	45.7	25.5	67	52.3	43	46.7	57.3	26
		st. error # of OTUs per stand	9.3	7	9.2	5.4	9	7.5	14.6	6.8	4	7.7	12.6	1.2
		# of OTUs per stand	605	825	1225	324	121	160	237	201	387	476	175	574
	Overall	avg. # of OTUs per stand	76.1	91.7	136.1	108	40.3	80	79	67	129	158.7	58.3	191.3
		st. error # of OTUs per stand	19.6	19	40	6.6	16.6	62	10.7	12.6	17	90.2	28.6	16.2
		# of OTUs per stand	66	36	123	17	32	17	13	11	12	44	43	36
rbcL	Bryophyte	avg. # of OTUs per stand	8.3	4	13.7	5.7	10.7	8.5	4.3	3.7	4	14.7	14.3	12
		st. error # of OTUs per stand	1.5	0.2	0.8	2.7	5.2	7.5	2.2	0.3	1.5	1.9	0.7	6.6
		# of OTUs per stand	539	786	1101	307	89	143	222	190	374	431	132	538
	Vascular plants	avg. # of OTUs per stand	67.8	87.3	122.3	102.3	29.7	71.5	74	63.3	124.7	143.7	44	179.3
		st. error # of OTUs per stand	21.1	18.9	40.5	8.8	20.3	69.5	8.7	12.3	17.3	88.3	28.2	16.1
		# of OTUs per stand	1555	2188	2056	589	551	415	693	790	705	613	666	777
	Overall	avg. # of OTUs per stand	195.8	243.1	228.4	196.3	183.7	207.5	231	263.3	235	204.3	222	259
		st. error # of OTUs per stand	6.9	10.2	16.1	33.1	13.3	17.5	38.8	18.9	17.7	6.4	28.5	30.1
		# of OTUs per stand	5	10	7	1	3	1	2	2	6	1	3	3
ITS	Lichen fungi	avg. # of OTUs per stand	0.6	1.1	0.8	0.3	1	0.5	0.7	0.7	2	0.3	1	1
		st. error # of OTUs per stand	0.2	0.4	0.2	0.3	0.6	0.5	0.3	0.3	0.6	0.3	1	0.6
		# of OTUs per stand	1550	2178	2049	588	548	414	691	788	699	612	663	774
	Fungi	avg. # of OTUs per stand	195.2	242	227.7	196	182.7	207	230.3	262.7	233	204	221	258
		st. error # of OTUs per stand	7	10.4	15.9	33	13.3	18	38.7	18.8	17.4	6.7	27.5	30.3

Table D5.6. Difference in bryophytes, fungi, lichen photobiont, lichen fungi, and vascular plant OTU richness from sequences of four genes (23S rDNA, trnL intron c-h, rbcL, ITS rDNA) obtained from environmental DNA of boreal soil samples. Boreal soil samples were collected from stands that were dominated by tree species (B = balsam fir, P = poplar, W = white spruce), near Payuk Lake in Manitoba, Canada. Different letters indicate significant differences among the treatments from the Conover-Iman Tests ($\alpha = 0.10$) after performing a Kruskal-Wallis Test on the data. avg. = average, st. error = standard error, df = degrees of freedom, *H* = Kruskal-Wallis Test.

Gene	Taxa	Forest stand	avg. OTU richness	st. error.	df	*H*	*P*
23S	Bryophyte	B1	9	4.16	8	10.65	0.2218
		B2	13.67	4.98			
		B3	4	2			
		P1	5.33	2.19			
		P2	4.33	0.33			
		P3	9.33	2.03			
		W1	7	2.52			
		W2	18	3.79			
		W3	7	4.04			
	Lichen photobiont	B1	18.67ab	3.76	8	13.92	0.0832
		B2	26.33ab	4.81			
		B3	8.00a	2			
		P1	9.00a	7.55			
		P2	9.67a	4.33			
		P3	36.00ab	9			
		W1	46.00b	9.64			
		W2	55.00b	20.22			
		W3	18.00a	17.01			
	Vascular plants	B1	86.3ab	51.3	8	14.1	0.079
		B2	40.3a	19.9			
		B3	78.5abc	50.5			
		P1	64abc	11.9			
		P2	71.7 abc	4.9			
		P3	213 c	18.4			
		W1	125.3 abc	54.3			
		W2	27 a	9.3			
		W3	140 bc	32.6			
trnL	Bryophyte	B1	8.67	1.2	8	12.83	0.1121
		B2	12.33	4.33			
		B3	5.5	0.5			
		P1	5.33	1.67			
		P2	5.33	0.67			
		P3	12.33	4.67			

		W1	11.33	3.4			
		W2	27	4.58			
		W3	11.67	5.67			
		B1	57.3 c	5.4	8	13.83	0.0855
		B2	45.7 abc	9			
		B3	25.5 ab	7.5			
		P1	67 c	14.6			
	Vascular plants	P2	52.3 bc	6.8			
		P3	43 abc	4			
		W1	46.7 abc	7.7			
		W2	57.3 c	12.6			
		W3	26a	1.2			
		B1	5.67	2.73	8	10.3	0.2403
		B2	10.67	5.21			
		B3	8.5	7.5			
		P1	4.33	2.19			
	Bryophyte	P2	3.67	0.33			
		P3	4	1.53			
		W1	14.67	1.86			
		W2	14.33	0.67			
		W3	12	6.56			
rbcL		B1	29.7 abc	20.3	8	13.59	0.0928
		B2	71.5 a	69.5			
		B3	67.8 abc	21.1			
		P1	74 abc	8.7			
	Vascular plants	P2	63.3 ab	12.3			
		P3	124.7 abc	17.3			
		W1	143.7 abc	88.3			
		W2	44 ab	28.2			
		W3	179.3 b	16.1			
		B1	0.33	0.33	8	5.6	0.5962
		B2	1	0.58			
	Lichen fungi	B3	0.5	0.5			
		P1	0.67	0.33			
ITS		P2	0.67	0.33			
		P3	2	0.58			
		W1	0.33	0.33			
		W2	1	1			

	W3	1	0.58			
	B1	196	33	8	9.07	0.3356
	B2	182.7	13.3			
	B3	207	18			
	P1	230.3	38.7			
Fungi	P2	262.7	18.8			
	P3	233	17.4			
	W1	204	6.7			
	W2	221	27.5			
	W3	258	30.3			

Discussion D5.7

Vascular plant representatives in the soil bank

The vascular plants present as OTUs in the soil banks included the genus or the species of trees that dominated the forest stand types (balsam fir, poplar, white spruce), and tree species that occasionally were present in the stands and in nearby areas (*Juniperus* sp, *Populus balsamifera* L. *Salix* sp; Table B3.1). Of the trees in the stands, there was a high diversity of genotypes of *Populus tremuloides* (469 OTUs) and *Picea glauca* (505 OTUs) amplified for the rbcL gene, and two OTUs for *Abies balsamea*. However, for *P. glauca* and *A. balsamea* the OTUs were presumily misidentified, since these OTUs were represented by *Picea sitchensis* (Bong.) Carr. and *Abies sibirica* Ledeb., which are not part of the local or regional flora (western North Americas and Siberia, respectively). Shrubs and herbs common on the forest floor of the surveyed sites and amplified as genus or species OTUs included *Aralia* sp., *Arctostaphylos uva-ursi* (L.) Spreng., *Cornus canadensis* L., *Linnaea borealis* L., *Maianthemum* sp.,and *Ribes* sp. *Musa acuminata* Colla (banana) and *Zea mays L.* (corn) were also found within the soil bank, which may reflect human activity on trails that pass by the forest stands and gardening activities in the lawns of houses near the forest stands.

Fungal representative in the soil bank

In contrast to the lichenized fungi, non-lichenized fungi were both abundant and richly represented within the soil bank in six phyla Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycota, Rozellomycota, and Zygomycota. Ascomycota was the phylum with the highest number of OTUs (851 OTUs) in nine classes, which included genus and species with various ecological

roles: *Venturia* spp., such as *V. inaequalis* Cooke (Wint.), forms scabs on apples (Parisi et al. 1993); *Paecilomyces marquandiimycosis* forms renal mycosis in fish and humans (Lightner et al. 1988, Naldi et al. 2000); and, *Wilcoxina rehmii* Chin S. Yang & Korf is an important ectomycorrhiza in forested ecosystems (Nedelin 2014). Basidiomycota followed Ascomycota with the highest number of OTUs (661 OTUs) in four classes: species in this phylum included *Cortinarius alpinus* Boud., an ectomycorrhiza with a boreal to subarctic distribution (Peintner 2008); *Cryptococcus pinus* Golubev et Pfeiffer, a yeast associated with pine needles (Bolubev et al. 2008); and *Rhodotorula lamellibrachii* Nagah., Hamam., Nakase & Horikoshi, an unlikely representative in the local flora due to its association with tubeworms in the deep sea (Nagahama et al. 2001). OTUs were less numerous in Chytridiomycota (3 OTUs), Glomeromycota (9 OTUs), Rozellomycota (9 OTUs), and Zygomycota (42 OTUs). The abundance of OTUs in the fungal phyla reflect their diversity and roles with the forest site, and their influence via competition, mutualism, and commensalism on the bryophyte and lichen propagules in the soil bank.

Appendix E: Regeneration responses differ among three boreal mosses after exposure to extreme temperatures

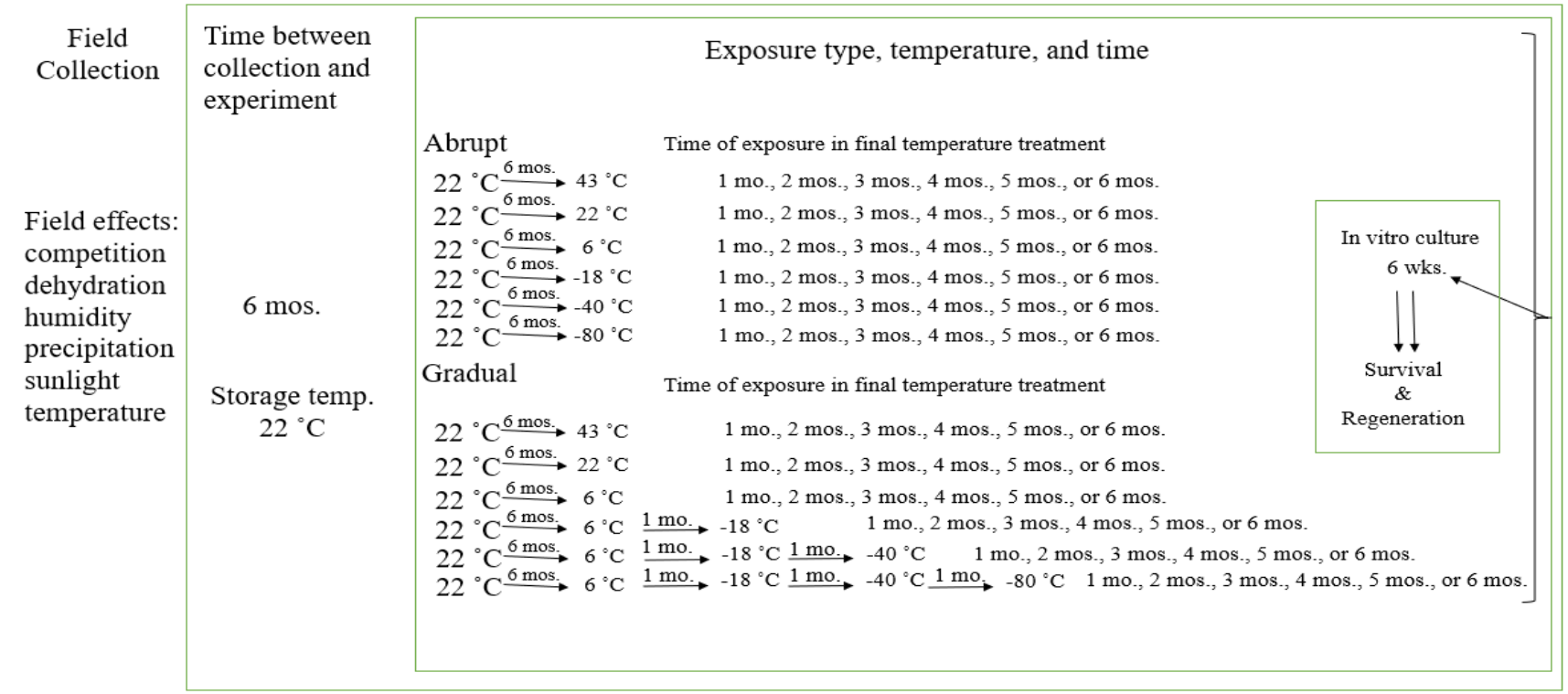

Figure E6.1. Experimental design of temperature experiments exposing gametophyte fragments of the three boreal mosses *Dicranum polysetum*, *Orthotrichum obtusifolium*, and *Pleurozium schreberi* to six different temperatures under an abrupt model and a gradual model over one to six months (mo./mos.) of time.

Table E6.1. AIC model selection for the logistic regression of three boreal moss species survival and growth (protonemata and branches development) responses to abrupt/gradual temperature changes (0 and 1 respectively), temperature (43, 22, 6, -18, -40, -80°C), time (1–6, months), and size (0.5 to 1 cm).

***D. polysetum*-survival**													
Model variables	-LogLikelihood	*n*	K	AIC	Delta (D)	exp(-0.5 × D)	weight	n/k	AIC_c	Delta (D)	exp(-0.5 × D)	weight	*P*
Abrupt/Gradual Temperature Changes	750.540	1269	2	1505.1	243.9	1.11E-53	0.0000	635	1505.1	243.8	1.13E-53	0.0000	0.001
Temperature (°C)	636.500	1269	2	1277.0	15.8	0.000374	0.0003	635	1277.0	15.8	0.000379	0.0003	0.0001
Time	755.770	1269	2	1515.5	254.3	5.96E-56	0.0000	635	1515.5	254.3	6.02E-56	0.0000	0.6
Sizes	752.730	1269	2	1509.5	248.2	1.25E-54	0.0000	635	1509.5	248.2	1.26E-54	0.0000	0.012
Abrupt/Gradual Temperature Changes, Temperature (°C)	630.385	1269	3	1266.8	5.5	0.062349	0.0422	423	1266.8	5.5	0.062746	0.0425	0.0001

Abrupt/Gradual Temperature Changes, Time	750.535	1269	3	1507.1	245.9	4.11E-54	0.0000	423	1507.1	245.8	4.14E-54	0.0000	0.005
Abrupt/Gradual Temperature Changes, Sizes	747.33	1269	3	1500.7	239.4	1.01E-52	0.0000	423	1500.7	239.4	1.02E-52	0.0000	0.0001
Temperature (°C), Sizes	632.88	1269	3	1271.8	10.5	0.005144	0.0035	423	1271.8	10.5	0.005176	0.0035	0.0001
Temperature (°C), Time	636.615	1269	3	1279.2	18.0	0.000123	0.0001	423	1279.2	18.0	0.000124	0.0001	0.0001
Time, Sizes	752.555	1269	3	1511.1	249.9	5.46E-55	0.0000	423	1511.1	249.9	5.49E-55	0.0000	0.035
Abrupt/Gradual Temperature Changes, Time, Sizes	747	1269	4	1502.0	240.8	5.19E-53	0.0000	317	1502.0	240.8	5.19E-53	0.0000	0.001
Temperature (°C), Time, Sizes	632.87	1269	4	1273.7	12.5	0.001911	0.0013	317	1273.8	12.5	0.001911	0.0013	0.0001
Abrupt/Gradual Temperature Changes, Temperature (°C), Time	630.32	1269	4	1268.6	7.4	0.024478	0.0165	317	1268.7	7.4	0.024478	0.0166	0.0001

Abrupt/Gradual Temperature Changes, Temperature (°C), Sizes	626.61	1269	4	1261.2	0.0	1	0.6760	317	1261.3	0.0	1	0.6772	0.0001
Abrupt/Gradual Temperature Changes, Temperature (°C), Time, Sizes	626.565	1269	5	1263.1	1.9	0.384812	0.2602	254	1263.2	1.9	0.381773	0.2586	0.0001
***P. schreberi*-survival**													
Model variables	-LogLikelihood	*n*	K	AIC	Delta (D)	exp(-0.5 × D)	weight	n/k	AIC_c	Delta (D)	exp(-0.5 × D)	weight	*P*
Abrupt/Gradual Temperature Changes	750.54	1269	2	1505.08	243.86	1.11E-53	7.52E-54	634.5	1505.089	243.8378	1.13E-53	7.62E-54	0.001
Temperature (°C)	636.5	1269	2	1277	15.78	0.000374	0.000253	634.5	1277.009	15.75783	0.000379	0.000256	0.0001
Time	755.77	1269	2	1515.54	254.32	5.96E-56	4.03E-56	634.5	1515.549	254.2978	6.02E-56	4.08E-56	0.6
Sizes	752.73	1269	2	1509.46	248.24	1.25E-54	8.42E-55	634.5	1509.469	248.2178	1.26E-54	8.53E-55	0.012

Abrupt/Gradual Temperature Changes, Temperature (°C)	630.385	1269	3	1266.77	5.55	0.062349	0.042151	423	1266.789	5.537327	0.062746	0.042494	0.0001
Abrupt/Gradual Temperature Changes, Time	750.535	1269	3	1507.07	245.85	4.11E-54	2.78E-54	423	1507.089	245.8373	4.14E-54	2.8E-54	0.005
Abrupt/Gradual Temperature Changes, Sizes	747.33	1269	3	1500.66	239.44	1.01E-52	6.86E-53	423	1500.679	239.4273	1.02E-52	6.91E-53	0.0001
Temperature (°C), Sizes	632.88	1269	3	1271.76	10.54	0.005144	0.003477	423	1271.779	10.52733	0.005176	0.003506	0.0001
Temperature (°C), Time	636.615	1269	3	1279.23	18.01	0.000123	8.3E-05	423	1279.249	17.99733	0.000124	8.37E-05	0.0001
Time, Sizes	752.555	1269	3	1511.11	249.89	5.46E-55	3.69E-55	423	1511.129	249.8773	5.49E-55	3.72E-55	0.035
Abrupt/Gradual Temperature Changes, Time, Sizes	747	1269	4	1502	240.78	5.19E-53	3.51E-53	317.25	1502.032	240.78	5.19E-53	3.52E-53	0.001
Temperature (°C), Time, Sizes	632.87	1269	4	1273.74	12.52	0.001911	0.001292	317.25	1273.772	12.52	0.001911	0.001294	0.0001

Abrupt/Gradual Temperature Changes, Temperature (°C), Time	630.32	1269	4	1268.64	7.42	0.024478	0.016548	317.25	1268.672	7.42	0.024478	0.016577	0.0001
Abrupt/Gradual Temperature Changes, Temperature (°C), Sizes	626.61	1269	4	1261.22	0	1	0.676045	317.25	1261.252	0	1	0.677238	0.0001
Abrupt/Gradual Temperature Changes, Temperature (°C), Time, Sizes	626.565	1269	5	1263.13	1.91	0.384812	0.26015	253.8	1263.178	1.92586	0.381773	0.258551	0.0001
***O. obtusifolium*-survival**													
Model variables	-LogLikelihood	*n*	K	AIC	Delta (D)	exp(-0.5 × D)	weight	n/k	AIC_c	Delta (D)	exp(-0.5 × D)	weight	*P*
Abrupt/Gradual Temperature Changes	367.818	614	2	739.6	157.4	6.64E-35	0.0000	307	739.7	157.3	6.79E-35	0.0000	0.0003
Temperature (°C)	331.230	614	2	666.5	84.2	5.15E-19	0.0000	307	666.5	84.2	5.27E-19	0.0000	0.0001

Time	342.860	614	2	689.7	107.5	4.58E-24	0.0000	307	689.7	107.4	4.69E-24	0.0000	0.0001
Abrupt/Gradual Temperature Changes, Temperature (°C)	328.285	614	3	662.6	80.3	3.6E-18	0.0000	205	662.6	80.3	3.65E-18	0.0000	0.0001
Abrupt/Gradual Temperature Changes, Time	331.575	614	3	669.2	86.9	1.34E-19	0.0000	205	669.2	86.9	1.36E-19	0.0000	0.0001
Temperature (°C), Time	298.425	614	3	602.9	20.6	3.35E-05	0.0000	205	602.9	20.6	3.39E-05	0.0000	0.0001
Abrupt/Gradual Temperature Changes, Temperature (°C), Time	287.12	614	4	582.2	0.0	1	0.2588	154	582.3	0.0	1	0.2592	0.0001
***D. polysetum*-protonemata**													
Model variables	-LogLikelihood	*n*	K	AIC	Delta (D)	exp(-0.5 × D)	weight	n/k	AIC_c	Delta (D)	exp(-0.5 × D)	weight	*P*
Abrupt/Gradual Temperature Changes	625.375	1264	2	1254.8	167.2	5.03E-37	0.0000	632	1254.8	167.1	5.09E-37	0.0000	0.0001
Temperature (°C)	554.350	1264	2	1112.7	25.1	3.53E-06	0.0000	632	1112.7	25.1	3.57E-06	0.0000	0.0001
Time	631.555	1264	2	1267.1	179.5	1.04E-39	0.0000	632	1267.1	179.5	1.05E-39	0.0000	0.713

Sizes	625.435	1264	2	1254.9	167.3	4.74E-37	0.0000	632	1254.9	167.3	4.79E-37	0.0000	0.0001
Abrupt/Gradual Temperature Changes, Temperature (°C)	547.11	1264	3	1100.2	12.6	0.001809	0.0011	421	1100.2	12.6	0.001821	0.0011	0.0001
Abrupt/Gradual Temperature Changes, Time	625.045	1264	3	1256.1	168.5	2.57E-37	0.0000	421	1256.1	168.5	2.59E-37	0.0000	0.001
Abrupt/Gradual Temperature Changes, Sizes	619.205	1264	3	1244.4	156.8	8.85E-35	0.0000	421	1244.4	156.8	8.91E-35	0.0000	0.0001
Temperature (°C), Sizes	547.11	1264	3	1100.2	12.6	0.001809	0.0011	421	1100.2	12.6	0.001821	0.0011	0.0001
Temperature (°C), Time	625.045	1264	3	1256.1	168.5	2.57E-37	0.0000	421	1256.1	168.5	2.59E-37	0.0000	0.001
Time, Sizes	625.355	1264	3	1256.7	169.1	1.89E-37	0.0000	421	1256.7	169.1	1.9E-37	0.0000	0.002
Abrupt/Gradual Temperature Changes, Time, Sizes	618.84	1264	4	1245.7	158.1	4.69E-35	0.0000	316	1245.7	158.1	4.69E-35	0.0000	0.0001
Temperature (°C), Time, Sizes	546.915	1264	4	1101.8	14.2	0.000809	0.0005	316	1101.9	14.2	0.000809	0.0005	0.0001

Abrupt/Gradual Temperature Changes, Temperature (°C), Time	546.55	1264	4	1101.1	13.5	0.001165	0.0007	316	1101.1	13.5	0.001165	0.0007	0.0001
Abrupt/Gradual Temperature Changes, Temperature (°C), Sizes	539.795	1264	4	1087.6	0.0	1	0.5979	316	1087.6	0.0	1	0.5998	0.0001
Abrupt/Gradual Temperature Changes, Temperature (°C), Time, Sizes	539.2	1264	5	1088.4	0.8	0.666977	0.3988	253	1088.4	0.8	0.661688	0.3969	0.0001
***P. schreberi*-protonemata**													
Model variables	-LogLikelihood	*n*	K	AIC	Delta (D)	exp(-0.5 × D)	weight	n/k	AIC_c	Delta (D)	exp(-0.5 × D)	weight	*P*
Abrupt/Gradual Temperature Changes	760.395	1269	2	1524.8	238.7	1.49E-52	0.0000	635	1524.8	238.6	1.51E-52	0.0000	0.006
Temperature (°C)	648.850	1269	2	1301.7	15.6	0.000414	0.0002	635	1301.7	15.6	0.000418	0.0002	0.0001
Time	760.010	1269	2	1524.0	237.9	2.19E-52	0.0000	635	1524.0	237.9	2.22E-52	0.0000	0.635
Sizes	759.430	1269	2	1522.9	236.7	3.91E-52	0.0000	635	1522.9	236.7	3.96E-52	0.0000	0.002

Abrupt/Gradual Temperature Changes, Temperature (°C)	644.5	1269	3	1295.0	8.9	0.011796	0.0060	423	1295.0	8.9	0.011871	0.0060	0.0001
Abrupt/Gradual Temperature Changes, Time	760.03	1269	3	1526.1	239.9	7.9E-53	0.0000	423	1526.1	239.9	7.95E-53	0.0000	0.017
Abrupt/Gradual Temperature Changes, Sizes	755.67	1269	3	1517.3	231.2	6.18E-51	0.0000	423	1517.4	231.2	6.22E-51	0.0000	0.0001
Temperature (°C), Sizes	643.345	1269	3	1292.7	6.6	0.037441	0.0189	423	1292.7	6.6	0.037679	0.0191	0.0001
Temperature (°C), Time	648.345	1269	3	1302.7	16.6	0.000252	0.0001	423	1302.7	16.6	0.000254	0.0001	0.0001
Time, Sizes	759.355	1269	3	1524.7	238.6	1.55E-52	0.0000	423	1524.7	238.6	1.56E-52	0.0000	0.008
Abrupt/Gradual Temperature Changes, Time, Sizes	755.365	1269	4	1518.7	232.6	3.09E-51	0.0000	317	1518.8	232.6	3.09E-51	0.0000	0.001
Temperature (°C), Time, Sizes	642.905	1269	4	1293.8	7.7	0.021386	0.0108	317	1293.8	7.7	0.021386	0.0109	0.0001
Abrupt/Gradual Temperature Changes, Temperature (°C), Time	643.64	1269	4	1295.3	9.2	0.010255	0.0052	317	1295.3	9.2	0.010255	0.0052	0.0001

Abrupt/Gradual Temperature Changes, Temperature (°C), Sizes	639.06	1269	4	1286.1	0.0	1	0.5057	317	1286.2	0.0	1	0.5075	0.0001
Abrupt/Gradual Temperature Changes, Temperature (°C), Time, Sizes	638.17	1269	5	1286.3	0.2	0.895834	0.4530	254	1286.4	0.2	0.888758	0.4510	0.0001
***O. obtusifolium*-protonemata**													
Model variables	-LogLikelihood	*n*	K	AIC	Delta (D)	exp(-0.5 × D)	weight	n/k	AIC_c	Delta (D)	exp(-0.5 × D)	weight	*P*
Abrupt/Gradual Temperature Changes	370.705	614	2	745.4	162.2	6.04E-36	0.0000	307	745.4	162.1	6.18E-36	0.0000	0.001
Temperature (°C)	333.995	614	2	672.0	88.8	5.29E-20	0.0000	307	672.0	88.7	5.42E-20	0.0000	0.0001
Time	345.370	614	2	694.7	111.5	6.08E-25	0.0000	307	694.8	111.5	6.22E-25	0.0000	0.0001
Abrupt/Gradual Temperature Changes, Temperature (°C)	329.5555	614	3	665.1	81.9	1.65E-18	0.0000	205	665.2	81.9	1.67E-18	0.0000	0.0001

Abrupt/Gradual Temperature Changes, Time	333.04	614	3	672.1	88.9	5.06E-20	0.0000	205	672.1	88.8	5.13E-20	0.0000	0.0001
Temperature (°C), Time	300.085	614	3	606.2	22.9	1.04E-05	0.0000	205	606.2	22.9	1.05E-05	0.0000	0.0001
Abrupt/Gradual Temperature Changes, Temperature (°C), Time	287.61	614	4	583.2	0.0	1	0.2052	154	583.3	0.0	1	0.2058	0.0001
***D. polysetum*-branches**													
Model variables	-LogLikelihood	*n*	K	AIC	Delta (D)	exp(-0.5 × D)	weight	n/k	AIC_c	Delta (D)	exp(-0.5 × D)	weight	*P*
Abrupt/Gradual Temperature Changes	865.125	1264	2	1734.3	289.5	1.37E-63	0.0000	632	1734.3	289.5	1.39E-63	0.0000	0.0001
Temperature (°C)	765.050	1264	2	1534.1	89.3	3.96E-20	0.0000	632	1534.1	89.3	4.04E-20	0.0000	0.0001
Time	874.200	1264	2	1752.4	307.7	1.57E-67	0.0000	632	1752.4	307.6	1.6E-67	0.0000	0.051
Sizes	852.510	1264	2	1709.0	264.3	4.12E-58	0.0000	632	1709.0	264.2	4.2E-58	0.0000	0.0001

Abrupt/Gradual Temperature Changes, Temperature (°C)	751.33	1264	3	1508.7	63.9	1.32E-14	0.0000	421	1508.7	63.9	1.34E-14	0.0000	0.0001
Abrupt/Gradual Temperature Changes, Time	861.875	1264	3	1729.8	285.0	1.3E-62	0.0000	421	1729.8	285.0	1.32E-62	0.0000	0.0001
Abrupt/Gradual Temperature Changes, Sizes	841.315	1264	3	1688.6	243.9	1.1E-53	0.0000	421	1688.6	243.9	1.12E-53	0.0000	0.0001
Temperature (°C), Sizes	736.7	1264	3	1479.4	34.7	2.99E-08	0.0000	421	1479.4	34.6	3.03E-08	0.0000	0.0001
Temperature (°C), Time	762.2	1264	3	1530.4	85.7	2.52E-19	0.0000	421	1530.4	85.6	2.56E-19	0.0000	0.0001
Time, Sizes	850.4	1264	3	1706.8	262.1	1.25E-57	0.0000	421	1706.8	262.0	1.27E-57	0.0000	0.0001
Abrupt/Gradual Temperature Changes, Time, Sizes	837.765	1264	4	1683.5	238.8	1.41E-52	0.0000	316	1683.6	238.8	1.42E-52	0.0000	0.0001
Temperature (°C), Time, Sizes	733.535	1264	4	1475.1	30.3	2.61E-07	0.0000	316	1475.1	30.3	2.63E-07	0.0000	0.0001
Abrupt/Gradual Temperature Changes, Temperature (°C), Time	746.67	1264	4	1501.3	56.6	5.15E-13	0.0000	316	1501.4	56.6	5.19E-13	0.0000	0.0001

Abrupt/Gradual Temperature Changes, Temperature (°C), Sizes	722.53	1264	4	1453.1	8.3	0.015686	0.0154	316	1453.1	8.3	0.015811	0.0156	0.0001
Abrupt/Gradual Temperature Changes, Temperature (°C), Time, Sizes	717.375	1264	5	1444.8	0.0	1	0.9846	253	1444.8	0.0	1	0.9844	0.0001

***P. schreberi*-branches**

Model variables	-LogLikelihood	*n*	K	AIC	Delta (D)	exp(-0.5 × D)	weight	n/k	AIC_c	Delta (D)	exp(-0.5 × D)	weight	*P*
Abrupt/Gradual Temperature Changes	448.280	1269	2	900.6	120.0	8.93E-27	0.0000	635	900.6	119.9	9.1E-27	0.0000	0.0001
Temperature (°C)	430.125	1269	2	864.3	83.7	6.85E-19	0.0000	635	864.3	83.6	6.98E-19	0.0000	0.0001
Time	439.695	1269	2	883.4	102.8	4.78E-23	0.0000	635	883.4	102.8	4.87E-23	0.0000	0.0001
Sizes	451.675	1269	2	907.4	126.8	3E-28	0.0000	635	907.4	126.7	3.05E-28	0.0000	0.0001
Abrupt/Gradual Temperature Changes, Temperature (°C)	416	1269	3	838.0	57.4	3.43E-13	0.0000	423	838.0	57.4	3.48E-13	0.0000	0.0001

Abrupt/Gradual Temperature Changes, Time	429.725	1269	3	865.5	84.9	3.76E-19	0.0000	423	865.5	84.8	3.81E-19	0.0000	0.0001
Abrupt/Gradual Temperature Changes, Sizes	438.72	1269	3	883.4	102.8	4.66E-23	0.0000	423	883.5	102.8	4.73E-23	0.0000	0.0001
Temperature (°C), Sizes	420.28	1269	3	846.6	66.0	4.75E-15	0.0000	423	846.6	65.9	4.82E-15	0.0000	0.0001
Temperature (°C), Time	406.09	1269	3	818.2	37.6	6.91E-09	0.0000	423	818.2	37.6	7.01E-09	0.0000	0.0001
Time, Sizes	430.705	1269	3	867.4	86.8	1.41E-19	0.0000	423	867.4	86.8	1.43E-19	0.0000	0.0001
Abrupt/Gradual Temperature Changes, Time, Sizes	420.41	1269	4	848.8	68.2	1.54E-15	0.0000	317	848.9	68.2	1.55E-15	0.0000	0.0001
Temperature (°C), Time, Sizes	396.625	1269	4	801.3	20.7	3.28E-05	0.0000	317	801.3	20.6	3.31E-05	0.0000	0.0001
Abrupt/Gradual Temperature Changes, Temperature (°C), Time	395.145	1269	4	798.3	17.7	0.000144	0.0001	317	798.3	17.7	0.000145	0.0001	0.0001
Abrupt/Gradual Temperature Changes, Temperature (°C), Sizes	405.945	1269	4	819.9	39.3	2.94E-09	0.0000	317	819.9	39.3	2.96E-09	0.0000	0.0001

Abrupt/Gradual Temperature Changes, Temperature (°C), Time, Sizes	385.3	1269	5	780.6	0.0	1	0.9998	254	780.6	0.0	1	0.9998	0.0001
***O. obtusifolium*-branches**													
Model variables	-LogLikelihood	*n*	K	AIC	Delta (D)	exp(-0.5 × D)	weight	n/k	AIC_c	Delta (D)	exp(-0.5 × D)	weight	*P*
Abrupt/Gradual Temperature Changes	378.789	614	2	761.6	83.3	8.21E-19	0.0000	307	761.6	83.3	8.29E-19	0.0000	0.471
Temperature (°C)	352.815	614	2	709.6	31.3	1.57E-07	0.0000	307	709.6	31.3	1.58E-07	0.0000	0.0001
Time	363.155	614	2	730.3	52.0	5.06E-12	0.0000	307	730.3	52.0	5.11E-12	0.0000	0.0001
Abrupt/Gradual Temperature Changes, Temperature (°C)	352.2605	614	3	710.5	32.2	1E-07	0.0000	205	710.6	32.2	1E-07	0.0000	0.0001
Abrupt/Gradual Temperature Changes, Time	363.146	614	3	732.3	54.0	1.88E-12	0.0000	205	732.3	54.0	1.88E-12	0.0000	0.0001
Temperature (°C), Time	336.145	614	3	678.3	0.0	1	0.2961	205	678.3	0.0	1	0.2966	0.0001

Abrupt/Gradual Temperature Changes, Temperature (°C), Time	336.121	614	4	680.2	2.0	0.376815	0.1116	154	680.3	2.0	0.371886	0.1103	0.0001

Table E6.2. Odds ratio of the survival and growth (protonemata and branch development) response of three boreal mosses (*Dicranum polysetum*, *Orthotrichum obtusifolium*, and *Pleurozium schreberi*) to abrupt/gradual temperature changes (0 and 1, respectively), temperature (43, 22, 6, -18, -40, -80 °C), exposure duration (1–6 months), and size (0.5 to 1.0 cm) treatments. The odds ratio with 95% confidence interval (CI) represents the relative measure of effect of the variable, the odds that an outcome will occur.

Species, Response	Variable	Odds Ratio	lower 95% CI	upper 95% CI
D. polysetum	Abrupt/Gradual Temperature Changes	1.8507	1.3638	2.5114
Survival	Temperature (°C)	0.9737	0.9689	0.9785
	Time	1.0456	0.9476	1.1538
	Sizes	3.9036	2.1129	7.2118
Protonemata	Temperature type	1.8236	1.3484	2.4662
	Temperature (°C)	0.9736	0.9688	0.9783
	Time	1.0556	0.9577	1.1636
	Sizes	3.2219	1.7609	5.8953
Branches	Abrupt/Gradual Temperature Changes	2.0867	1.6139	2.6982
	Temperature (°C)	0.9754	0.9720	0.9789
	Time	1.1410	1.0523	1.2372
	Sizes	7.0743	4.2395	11.8047

O. obtusifolium	Abrupt/Gradual Temperature Changes	2.7555	1.7925	4.2359
Survival	Temperature (°C)	0.9752	0.9695	0.9810
	Time	1.8515	1.5963	2.1474
Protonemata	Abrupt/Gradual Temperature Changes	2.8981	1.8800	4.4594
	Temperature (°C)	0.9750	0.9693	0.9808
	Time	1.8751	1.6161	2.1756
Branches	Abrupt/Gradual Temperature Changes	0.9606	0.6618	1.3944
	Temperature (°C)	0.9835	0.9789	0.9880
	Time	1.4102	1.2448	1.5938
P. schreberi	Abrupt/Gradual Temperature Changes	1.6413	1.2470	2.1602
Survival	Temperature (°C)	0.9712	0.9673	0.9757
	Time	1.0139	0.9715	1.1084
	Sizes	2.1365	1.2392	3.6835

Protonemata	Abrupt/Gradual Temperature Changes	1.5299	0.9683	0.9765
	Temperature (°C)	0.9724	0.9683	0.9765
	Time	1.0618	0.9721	1.1596
	Sizes	2.4749	1.4426	4.2457
Branches	Abrupt/Gradual Temperature Changes	0.4054	0.2768	0.5936
	Temperature (°C)	0.9814	0.9769	0.9859
	Time	1.5065	1.3213	1.7177
	Sizes	5.4201	2.5194	11.6609