TUNDRA BRYOPHYTE REVEGETATION:

NOVEL METHODS FOR REVEGETATING NORTHERN ECOSYSTEMS

by

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ABSTRACT

Reclamation of northern disturbances is of increasing importance as industrial activities and associated infrastructure expands to accommodate growing human reliance on world ecosystems. Bryophytes are recognized as ecologically essential to northern ecosystems and effectively promoting their growth is critical for reclamation. They include pioneer species, facilitating soil and microhabitat development, providing biomass and ground cover and promoting germination and growth of higher trophic species. This pioneering role of bryophytes is critical in challenging northern ecosystems, where substrates are low or lacking in organic matter and where plant growth is restricted by environmental limitations such as the short growing season. Bryophyte revegetation is a new field of study that will fill an essential gap in northern reclamation.

The objective of this research was to assess bryophyte propagation and to determine most effective treatments for land reclamation. Bryophyte samples were collected near Lac de Gras in the Northwest Territories, Canada, and grown in the laboratory for twelve weeks. Treatments were small (< 1 mm), medium (< 2 mm) and large (< 40 mm) bryophyte fragment sizes, with beer, buttermilk and distilled water slurries. The fragment sizes were further assessed in a field experiment, with cheesecloth as an erosion control material. The field experiment was replicated on three substrates at Diavik Diamond Mine, in the Northwest Territories, Canada (crushed rock, lake sediment, processed kimberlite) and on two substrates at Heiðmörk, Iceland (plateau, road).

Relatively short term (12 weeks in laboratory, 2 growing seasons in field) results show that some fragmentation is beneficial to bryophyte propagation. Medium fragment size (leaf sized) led to highest bryophyte density and cover in the laboratory experiment. Medium fragment size produced highest density, species occurrence and species diversity when in direct contact with

soil in the field. Large fragments were less susceptible to the effects of wind and rain, resulting in greater live cover, likely due to higher total cover (retention).

Water and beer were significantly more effective at propagating bryophytes than buttermilk. Since water and beer did not differ significantly in their effects on bryophytes, the more affordable and accessible water is recommended for large scale reclamation use.

The effect of erosion control on cover and species occurrence was positive, varying with substrate. Intact cheesecloth had a positive effect on bryophyte retention and propagation. Most striking was the promotion of colonization under the cheesecloth in all but one substrate. Erosion control material had a tempering effect on soil volumetric water content and temperature, reducing their variability. Cloth decomposition occurred in three of five substrates.

Substrates with more heterogeneous surfaces had greater live bryophyte cover, volume retention, density and spontaneous colonization. Success of bryophyte propagation and colonization was highly dependent on species specific microhabitat requirements. Environment invariably impacts reclamation outcomes, with wind, precipitation and temperature having the most impact on experiment results.

The novel bryophyte propagation methods evaluated in these experiments were effective in promoting propagation and growth of tundra bryophytes on denuded and disturbed substrates. The positive outcomes in both the Northwest Territories and Iceland leads to the assumption that these methods would likely be effective in a number of different reclamation scenarios where bryophyte revegetation is a focus.

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I. BACKGROUND

1. INTRODUCTION

1.1. Northern Reclamation

Scientific, political and societal interests in reclamation and ecological restoration have intensified due to growing reliance on world ecosystems from the increasing human population. To meet these needs, relatively undisturbed northern ecosystems have become vulnerable due to increased interest and activities in natural resource extraction of fossil fuels and minerals such as gold, diamonds, nickel, copper and tungsten (Rey 1987). Industrial activities and associated infrastructure leads to denudation of vegetation and consequential impacts on fauna dependent on plants for survival.

The highly delicate and complex Arctic tundra biome is one of the world's last pristine wildlife sanctuaries (Rey 1987). The Arctic tundra biome (hereafter the north) lies north of the treeline and is characterized by short growing seasons, long, cold winters, low rainfall and slow nutrient release (Forbes 2015). These features make the north a difficult place to live for most species, result in slow vegetation establishment and growth, lead to slow recovery after anthropogenic disruption and make it extremely challenging to reestablish original ecosystems (Drozdowski et al. 2012, Nilsson and Aradóttir 2013). Natural recovery may take 100 to 1000 years or more (Forbes and Jeffries 1999), depending on the scale and degree of impact (Lawson et al. 1978, Harper and Kershaw 1996, Davis 1998, Forbes et al. 2001).

According to the Mining Association of Canada (2012), limiting environmental impacts of mining is a top priority for Canadian industry. One of industry's main targets for sustainable development is conservation and management of biodiversity. Reclamation research is thus critical to sustainable development of Canada's mining industry and is of specific interest in the north, where little research has been conducted.

The purpose of reclamation is to facilitate biodiversity, rehabilitate ecosystem functions and reconstruct original, healthy ecosystems after disturbance to promote an appropriate successional trajectory (van Diggelen et al. 2001). Reclamation is the umbrella term for returning a disturbed system to equivalent capability relative to its predisturbance state, which may differ from its original structure and purpose. Ecological restoration is defined as the "process of assisting the recovery of an ecosystem that has been degraded, damaged or

destroyed" (Society for Ecological Restoration International Science and Policy Working Group 2004), usually to its former structure, function and composition. The first step in ecological restoration is to increase biodiversity of disturbed sites (van Diggelen et al. 2001) and is the primary focus of the current research. Research on bryophytes, essential to the north, would facilitate the second and third steps, rehabilitation of ecosystem functions and reconstruction of the original ecosystem.

1.2. Bryophytes In Northern Reclamation

Difficult environmental conditions and a limited understanding of northern ecosystem processes and community dynamics impede restoration (Cargill and Chapin 1987, Forbes and McKendrick 2002). With every degree travelled north, fewer vascular and bryophyte species are capable of survival or revegetation (Forbes 2015). Revegetation attempts have focused on restoring vegetation cover, easily ensured by using agronomic, non native species (Forbes and McKendrick 2002). Introduced species naturally spread and replace native vegetation, and encroachment of local species is often slow. Recent research has focused on introduction of native shrub and grass species (Adams and Lamoureux 2005). Despite their major role in tundra ecosystem function and structure, there have been few attempts to establish bryophytes (Steere 1978, Forbes and Jeffries 1999, Adams and Lamoureux 2005, Jägerbrand et al. 2011). Greater consideration of community interactions, roles and growth mechanisms of bryophytes in northern ecosystems is essential (Rastorfer 1978), and elucidation of structure and function is key to successful restoration or reclamation (Cargill and Chapin 1987).

Bryophytes are the most successful plant group, after angiosperms, in geographical distribution, habitat diversification and species differentiation (Slack 2011). Presence of vascular plant species decreases towards higher latitudes (Rydin 2009) and that of bryophytes increases (Vitt and Pakarinen 1977). Bryophytes are recognized as ecologically essential to northern ecosystems (Rastorfer 1978, Steere 1978, Jägerbrand et al. 2011), which they easily dominate with their opportunistic and resilient nature.

Bryophytes play a critical role in tundra ecosystems, influencing function and structure (Schofield 1972, Jägerbrand et al. 2011). They act as pioneer species in ecosystem development, facilitating soil and microhabitat development (Schofield 1972, Kershaw and Kershaw 1987, Klokk and Rønning 1987, Longton 1988, Jandt et al. 2008, Rydgren et al. 2011). Tundra bryophytes provide biomass and ground cover, phytomass for nutrient cycling and food for consumers and decomposers (Longton 1988). Moss species and nitrogen fixing

microorganisms form a symbiotic relationship, exchanging a favourable habitat for nitrogen and growth regulators (Rodgers and Hendriksson 1976). Nitrogen fixing microorganisms are especially important in early stages of soil formation, when substrates are void of nitrogen.

Bryophytes in tundra ecosystems help sustain life for many other species of microorganisms and plants and animals. Early presence of bryophytes facilitates establishment and growth of other flora, such as lichens and vascular plants (Longton 1988, Forbes and Jeffries 1999, Hilty et al. 2004, Jägerbrand et al. 2011). Their role as nurse plants, providing protection and microsites for propagules germination, is important for plant assemblage establishment (Forbes and Jeffries 1999). Bryophytes and lichens are regarded as the most important pioneer species in northern ecosystems, colonizing disturbed areas before vascular plants (Kershaw and Kershaw 1987, Jandt et al. 2008, Rydgren et al. 2011). Whether lichens best colonize bare rock is contested (Longton 1988).

Tundra bryophytes are important for sustaining faunal life (Pakarinen and Vitt 1974). Mosses are a dietary component of many arctic rodents, such as *Spermophilus parrayi* (ground squirrels) (Batzli and Sobaski 1980), *Clethrionomys rutilis* (voles) (West 1982) and *Dicrostonyx groenlandicus* (collared lemmings) (Longton 1980, 1988). *Rangifer tarandus* (caribou) supplement their lichen based diet with moss (Thompson and McCourt 1981, Longton 1988). Winter rumen samples of Alaskan caribou had 13 to 58 % moss and 2 to 15 % lichen (Thomas and Edmonds 1983). Bryophytes have historically had a role in the daily lives of northern First Nations, and have been used for absorption in diapers, cleaning hands and tables, fueling fire and insulating shelters (Andre and Fehr 2002).

Bryophytes could fill an essential gap in northern reclamation (Forbes and McKendrick 2002), comprising an exciting new field of revegetation research in severely degraded northern sites (Adams and Lamoureux 2005). Although bryophytes have great potential as colonizing, indicator and biomonitoring species and restoration will likely not succeed without them (Davy 2002), their importance in establishment and maintenance of northern ecosystems is usually overlooked (La Farge et al. 2013).

Bryophytes can prevent erosion in denuded or low cover areas and promote nutrient retention by reducing leaching of dust deposited minerals (Klokk and Rønning 1987). They are important in triggering active nutrient cycling and soil genesis (Adams and Lamoureux 2005). Many moss species are natural pioneers adapted to colonize disturbed areas (Longton 1988) and their presence or absence could be a determining factor in the progress of ecological succession (Klokk and Rønning 1987, Forbes and Jeffries 1999, Hilty et al. 2004), and hence reclamation.

They may offer solutions for metal affected substrates, by absorbing and holding metals and providing habitat for metal tolerant plant survival (Adams and Lamoureux 2005), providing a contamination remediation role.

Information on bryophyte species and health could be useful in monitoring and classifying environmental change in the north (Forbes 1994, Tuba et al. 2011). They are capable of tolerating a wide range of environmental conditions and are accessible for study in almost any ecosystem (Slack 2011). Some moss species can indicate specific ecosystem qualities, such as hydrologic conditions or metals. Northern bryophytes have potential for biomonitoring heavy metal concentrations and regular bryophyte tissue assessment could be more efficient and require less time, money and labour than present methods (Wilkie and La Farge 2011). They are excellent indicators of ecosystem health (Forbes 1994, Naeth and Wilkinson 2008), due to long distance dispersal mechanisms, species specific fidelity to climatically sensitive habitats and an opportunistic and tolerant life strategy (Gignac 2011). Species presence or absence can indicate level of disturbance, health, hydrologic regime, acidity and nutrient concentrations (Gignac et al. 1991). A better understanding of the relationships between bryophyte species and ecosystem health are necessary for responsible reclamation of northern disturbance.

2. BRYOPHYTE REPRODUCTION

2.1. Natural Colonization

The colonization process is of variable temporal length, depending on size, shape and surrounding environment of the revegetation, and extent of the disturbance. A small area can be rapidly colonized in under a decade (Schenk 1997, Campbell and Bergeron 2012) but some ecosystems may take centuries or even millennia before bryophyte species composition is fully established (Forbes and Jeffries 1999).

2.2. Sexual Reproduction

In sexual reproduction, male gametes (sperms) access the female gametophore sex organ (archegonium), fertilize the egg, and if successful, produce a sporophyte (Schofield 1985). The sporophyte matures and produces a sporangium containing spores. Each spore is the first cell of the gametophyte generation; spore release completes the life cycle. Sexual reproduction may be limited by water and nutrient availability and distance of separation of egg and sperm.

Several experiments have attempted to propagate bryophytes from spores. The most common method is spore extraction from undehisced capsules and transfer to soil or agar (Longton and Greene 1979, Miles and Longton 1990, Schenk 1997). Large scale collection and sowing of spores would be challenging and would exclude species in which sporophyte production is infrequent or unobserved. Sexual reproduction is an important method of propagation; however asexual reproduction is generally more common for bryophytes (Miles and Longton 1990).

2.3. Asexual Reproduction

Regeneration is often fulfilled by asexual reproduction. Brood bodies, such as gemmae and tubers, are either miniature gametophores or cell clusters that can be produced and released by the parent gametophyte to develop into a gametophore (Malcolm and Malcolm 2000).

The simplest and most common means of propagation is fragmentation. Minute fragments of brittle gametophore leaves or stems can detach and regenerate into entire gametophores. Robinson and Miller (2013) found alpine diaspore fragments were 0.4 to 7.0 mm in the longest dimension, with 98 % of fragments less than 2.0 mm long (mean 1.3 mm). These totipotent cells are able to differentiate into a meristematic state and reprogram themselves for development of whole new organisms (La Farge et al. 2013). Fragments are either deposited near the plant (Caners et al. 2009) or transported by wind (Miller and Ambrose 1976, McDaniel and Miller 2000) or animals (Heinken et al. 2001) to a more distant location. In some species, gametophyte fragments, rather than spores, may be primarily responsible for establishment of new colonies (Mishler and Newton 1988, Miles and Longton 1990, Giordano et al. 1996, McDaniel and Miller 2000, Robinson and Miller 2013). Little is known about the capacity for regeneration regarding age, sex, type or original location of fragments. Young leaves may regenerate more frequently, particularly those arising close to the stem apex (Miller and Ambrose 1976, Longton and Greene 1979). The extreme habitat range and resilience of bryophytes is likely in large part due to totipotency and asexual reproduction.

Many methods are used to approximate the natural effect of fragmentation; including pulverization, sieving and hand crumbling or clipping. Fragment size will vary with treatment, as it does naturally with different species and environmental conditions. Propagules can be reduced to a small, dust like consistency, by pulverizing or blending (McDowell 1972, Shaw 1986, Schenk 1997, Svenson 2000, McDonough 2006, Moss and Stone Gardens 2011, Apartment Therapy 2012, WikiHow 2013). This size would approximate the effect of broken leaf tips or other minute fragments capable of wind dispersion.

Propagation from detached leaves has been observed (Gemmell 1953, Longton and Greene 1979, Wilmot-Dear 1980, Miles and Longton 1990, Giordano et al. 1996, Hugonnot and Celle 2012). Leaf wounding has also been shown to promote growth (Gemmell 1953). To approximate a medium leaf or stem piece fragmentation size, samples can be grated through a mesh sieve (Shaw 1986, Schenk 1997).

Larger fragmentation would imitate translocation of whole or partial plants by soil or water movement or by transportation on another living organism. For large fragments, dried material can be broken up by hand (Iwatsuki and Kodama 1961, Belnap 1993, Glime 2007, Magnúsdóttir and Aradóttir 2011, Aradóttir 2012). This method has traditionally been used in Japanese moss gardens for centuries (Flora of North America Editorial Committee 1993a). Bryophyte material can be clipped to a known size or length, usually 0.5 to 3.0 cm (Graf and Rochefort 2010), sometimes only planting the apex of the piece (Brown and Bates 1990, Miles and Longton 1990). With every increase in fragment size, fragments of all sizes up to a maximum are included. For example, when hand crumbling, some fragments of dust particle size will be included with others of full stem size.

Slurries can promote regeneration and or fastening of fragmented bryophyte material to substrate. A multitude of slurry preparations have been tested, including mixes of bryophyte material and soil (Iwatsuki and Kodama 1961, Buxton et al. 2005, McDonough 2006), distilled water (McDonough 2006), fertilizer (Buxton et al. 2005) or glue such as epoxy resin (Glime 2007). Regular household mixes have been successful (Flora of North America Editorial Committee 1993a), including beer (Gillis 1991), milk (McDowell 1972), buttermilk (Gillis 1991, Apartment Therapy 2012, WikiHow 2013), yogurt, compost (Schenk 1997, Buxton et al. 2005), manure (Schenk 1997) or eggs (Schenk 1997).

Fragments or slurries are sometimes planted directly onto desired surface substrate materials (Iwatsuki and Kodama 1961) or hardened before out planting (Flora of North America Editorial Committee 1993 a, McDonough 2006, Glime 2007). Some harden mosses on top of (Gillis 1991, Glime 2007) or between two layers of cheesecloth (Whitner 1992), which can easily be rolled and transferred to the field during reclamation activities (McDowell 1972). Overlaying cheesecloth on porous bricks in standing water will ensure the cheesecloth and bryophyte material remain damp but not saturated (Gillis 1991, Schenk 1997). If slurry is not directly applied in a consistent cover layer, hardened portions of bryophyte material can be laid in a grid, each square expanding and eventually filling in the entire surface, as the cheesecloth decomposes (Glime 2007).

2.4. Diaspore Bank Transplantation

The entire diaspore bank, including spores, sporophytes and gametophytes, can be mixed to enable sexual and asexual reproduction. Diaspore banks can contain bryophyte fragments, brood bodies, spores and myriad other materials such as decomposing organic particulates, insects, rhizomes and seeds (Cobbaert et al. 2004, Robinson and Miller 2013).

Topsoil or litter fermented humus (LFH) can be harvested and incubated in a laboratory or greenhouse to promote bryophyte growth (Bell et al. 1991, During 2001, Caners et al. 2009, Robinson and Miller 2013) for identification or transplanting. Type, age and provenance of diaspore material will affect longevity (During 2001). Short lived, acrocarpous species may be more common in diaspore banks than long lived, pleurocarpous species. Growth conditions will affect which species grow. Light, for example, will affect species composition with more pioneer species in high light and more light sensitive species in low light (Caners et al. 2009).

Many experiments focused on direct transfer of vegetative and top layers of substrate to denuded land (Longton and Greene 1979, Bell et al. 1991, Svenson 2000, Glime 2007, Aradóttir 2012, Aradóttir and Óskarsdóttir 2013). For a rapid establishment of species cover and composition, material can be directly transferred from donor site in entire blocks of 5.0 to 30.0 cm^2 (Svenson 2000, Aradóttir 2012, Aradóttir and Óskarsdóttir 2013). Substrate can be excluded from harvested blocks, however keeping some substrate on the bottom of a transplant may benefit propagation by protecting rhizoids, and mycorrhizal and microbial associations. Direct transplantation is challenging, as the turf block tends to shrink and pull away from the substrate as it dries out (Flora of North America Editorial Committee 1993a).

Harvested material can be broken and spread (Campeau and Rochefort 1996, Cobbaert et al. 2004, Graf and Rochefort 2008, Aradóttir 2012). A 1:16 ratio of diaspore collection to receiving areas may be enough for *Sphagnum* propagation on bare peat (Campeau and Rochefort 1996); species and site specific ratios should be based on time frame and desired outcome. Manually spreading diaspore material was most effective for species introduction and propagation.

2.5. Other Revegetation Considerations

Sample collection and preparation are important considerations before propagation use. Water content and temperature during storage vary with purpose of samples. Fiedl collected samples can be dried before storage, by leaving them out for days or weeks to completely desiccate. Complete drying is beneficial when samples will be stored for long periods. Fresh, wet samples

can be propagated. Storing in a cool and dark place, such as a refrigerator (approximately 4.0 °C) in aluminum foil packets (Jones and Rosentreter 2006) or sealable plastic bags (McDonough 2006) can extend freshness of field collected samples (Bell et al. 1991, McDonough 2006, Caners et al. 2009). These samples are best used within one to three days, and no more than three weeks after collection (McDonough 2006, Graf and Rochefort 2010).

Drr samples can be washed before propagating, to remove extraneous material and diaspores or contaminants (Miller and Ambrose 1976, Jones and Rosentreter 2006). However, washing dried materials may cause leakage of cell solutes during rehydration (Bates 2009). Hydrating with distilled water is preferred to tap water with variable mineral richness (Miller and Ambrose 1976, Giordano et al. 1996, Bates 2009, Robinson and Miller 2013). Samples can be cleaned with bleach (Fletcher 1991, Jones and Rosentreter 2006), ethanol (McDaniel and Miller 2000) or hypochlorite solution (Giordano et al. 1996) to sterilize plants, killing algae, fungus or spores.

Misting with distilled water helps bryophytes absorb water (Longton and Greene 1979, Shaw 1986, Brown and Bates 1990, Bell et al. 1991, Fletcher 1991, McDonough 2006, Glime 2007). Frequency and amount of misting depend on ambient temperature and humidity, and bryophyte growth stage and species (Giordano et al. 1996, Graf and Rochefort 2010). The protonemal growth stage may require more water, to maintain approximately 70 % relative humidity (Giordano et al. 1996), later life stages may require less (Shaw 1986, McDonough 2006).

Bryophyte propagation material can be kept damp by placing it in water. Capillary movement will draw water to the surface of the material and minimize disturbing sensitive fragments (Shaw 1986). Biodegradable absorbent polymer crystals can conserve water (Schenk 1997). Covers of clear plastic, micropore tape (Duckett et al. 2004, McDonough 2006, Robinson and Miller 2013) and cloth conserve humidity and provide shade (Buxton et al. 2005). A consistent water level should be maintained to promote optimal net assimilation and growth (Davey and Rothery 1997, Klimkowska et al. 2010). Frequent wetting and drying can be detrimental to bryophytes, as they will spend wet periods repairing damage from desiccation instead of growing (Glime 2007).

Temperature, light and their effects on evapotranspiration are challenging to control in the field. A protective cover is often used to maintain low temperatures, light and water loss (Gorham and Rochefort 2003, Cobbaert et al. 2004, Rochefort and Lode 2006, Mälson and Rydin 2007). Many covers have been used, including fabric netting (McDowell 1972, Flora of North America Editorial Committee 1993a, Gorham and Rochefort 2003, Mälson and Rydin, 2007, Graf and Rochefort 2010), straw (Gorham and Rochefort 2003, Rochefort and Lode 2006, Graf and Rochefort 2008) and paper (Longton and Greene 1979).

In the greenhouse and laboratory, many researchers recreate natural photoperiods and temperatures (Miller and Ambrose 1976, Miles and Longton 1990, Giordano et al. 1996, Graf and Rochefort 2010, Hugonnot and Celle 2012, Xiang et al. 2013) and others optimize growth by increasing temperature and photoperiod to ideals (Hoffman 1966b, Longton and Greene 1979, Robinson and Miller 2013). Longton and Greene (1979) found rate of growth increased with increase in temperature independently of photoperiod over 5.0 to 20.0 °C.

Substrate type and amendments are important for bryophyte growth in the field, laboratory, greenhouse or growth chamber. Fragments can grow on various substrates including, but not limited to, sand (Shaw 1986), perlite (Jones and Rosentreter 2006), vermiculite and peat (Bell et al. 1991, Hugonnot and Celle 2012). Substrate from the natural habitat is preferable (Shaw 1986, Jones and Rosentreter 2006), although bryophytes can grow in vitro in petri dishes with agar (Fletcher 1991, Robinson and Miller 2013) or test tubes with nutrient mix (Fletcher 1991).

Amendments can be added to improve substrates. Polyacrylamide (Bowker 2007), vascular plants (Bowker 2007, Graf and Rochefort 2010), buried (Bowker 2007) or overlaid (Gorham and Rochefort 2003, Rochefort and Lode 2006, Graf and Rochefort 2008) straw, light distilled water spray (Svenson 2000, Glime 2007) or polymer water absorbing crystals (Gillis 1991) can help stabilize soil, minimizing erosion and water loss. Bryophytes can store nutrients but, growing in vitro, can deplete reserves (Longton and Greene 1979, Brown and Bates 1990, Giordano et al. 1996); thus requiring fertilizer or a nutrient solution (Jones and Rosentreter 2006) (Quinty and Rochefort 2003). Chemical fertilizers should be avoided (Iwatsuki and Kodama 1961, Stubbs 1973) as they may alter osmotic relationships, causing membrane damage or water loss (Glime 2007). Other potential amendments include manure, egg whites, buttermilk, milk, beer, rice water, carrot water, potato water and water (Ellis 1992, Schenk 1997). Powdered sulfur (Schenk 1997), ammonium sulfate (Glime 2007), buttermilk (Flora of North America Editorial Committee 1993a) and skimmed or powdered milk, diluted with distilled water (McDowell 1972, Svenson 2000, Glime 2007) can be beneficial for limiting nutrient concentration (Klimkowska et al. 2010) and establishing soil acidity near pH 5.5, but varying by species (Schenk 1997, Glime 2007).

3. CHALLENGES TO ARCTIC BRYOPHYTE REVEGETATION

The ecology of northern organisms is dependent on their ability to tolerate extreme environmental conditions to which they are subjected (Davey and Rothery 1997). Bryophytes have evolved many adaptations that permit positive net assimilation under severe

environmental conditions and are key to their success (Longton 1988). Abiotic factors have a strong influence on bryophyte productivity and community structures.

3.1. Water

Most northern bryophyte growth coincides with water availability, which occurs during snow melt (Vitt and Pakarinen 1977, Longton 1988). Bryophytes are classified in order of decreasing availability of water as hydrophytes, hygrophytes, mesophytes, hemixerophytes and xerophytes (Steere 1978). Bryophyte gross and net photosynthesis and growth, increased from xeric to mesic to hydric habitats in a photosynthetic ranking of fourteen Antarctic species, in direct correlation with habitat water availability (Davey and Rothery 1997). Species specific habitat adaptation matched their water requirements. Some hydrophilic species require water content of up to 400 % dry weight for optimal net assimilation and functioning (Kallio and Heinonen 1973), whereas others can sustain themselves through years of desiccation.

Bryophytes are poikilohydric, with hydration entirely controlled by osmotic interaction with environmental hydrologic levels (Bowen 1933). Amount of water conducted over the external surface of bryophytes generally exceeds internal conduction. Water availability is considered the most important factor in regulating bryophyte community composition (Davey and Rothery 1997) and rapidity and luxuriance of growth (Steere 1978).

To survive unfavourable hydrologic conditions, bryophytes evolved an alternative dormancy life strategy, desiccation tolerance, whereby free intracellular water can be lost without impeding recovery of function upon rehydration (Proctor et al. 2007). This allows bryophytes to actively grow and photosynthesize when water is available and suspend metabolism when it is not. Most bryophytes can withstand drying to water content of 10 % their dry weight and restore normal function on wetting, although longevity and species specific tolerance is unknown (Dilks and Proctor 1974). Tolerable desiccation periods vary with species and factors such as propagule type. Many species have a desiccation survival period of months or years (Breuil-Sée 1993). Although contested, the record reviviscence of dry bryophytes is 70 years (Malta 1921, Bewley 1972, Mansour 1981). Most other research denotes some viability retention after 3 to 24 years of dry state (Malta 1921, Maheu 1922, Keever 1957, Breuil-Sée 1993).

Desiccation tolerance varies with species, growth form, size and habitat (Steere 1978, Proctor et al. 2007). Compact cushion and thick mat growth forms dry slower than isolated shoots (Proctor et al. 2007). Species habituated to moist, shady places are typically less tolerant of

desiccation than epiphytic and species of open, exposed, arid sites (Steere 1978, Davey 1997, Proctor et al. 2007). Many factors affect desiccation tolerance, with dry bryophyte survival declining with increasing desiccation temperature (Hearnshaw and Proctor 1982).

The process and rate of recovery varies among species. Recuperation commonly follows a sigmoid curve on a logarithmic time scale, with photosynthesis rising slowly at first, then progressively faster until asymptotically reaching a limiting value (Dilks and Proctor 1974, Proctor et al. 2007). Half recovery time ranges from 20 seconds to several hours, depending on species. Full recovery to positive net assimilation can be attained in minutes by some species, and may take hours, days, or never be reached for others.

Water is critical to bryophyte life cycles and necessary for reproduction. In low water times, plants cannot produce sporophytes and if they could, fertilization is impossible without free water as sperm cannot reach archegonia (Schofield 1972, Longton 1988). Asexual reproduction is most prominent in arctic mosses (Longton 1988). Some species produce leaf or rhizoidal axillary gemmae, with others more prone to propagation by fragmentation. In asexual reproduction, water content, size and type of propagule impact diaspore regeneration after dry periods (Mishler and Newton 1988, Proctor et al. 2007). Water is a main factor for peat decomposition, which mostly occurs near the water table during dry periods (Xiang et al. 2013).

Mean annual precipitation in arctic tundra is approximately 200.0 to 250.0 mm, varying regionally (Yukon Ecoregions Working Group 2004). Approximately 60 % of annual precipitation occurs as rain and 40 % occurs as snow, which is expected in every month of the year (Ecosystem Classification Group 2012). Terrain plays a large role in watershed processes of the north. In sloped terrain runoff is significant and flash floods common due to limited infiltration by underlying permafrost and low evapotranspiration rates (Yukon Ecoregions Working Group 2004). In flat terrain low drainage and moderate temperatures can impede runoff and lead to formation of wetlands and mesic habitats (Longton 1988). Many streams have no flow from November to April.

3.2. Temperature

Temperature is often regarded as one of the most important abiotic factors controlling bryophyte productivity and carbon flux (Davey and Rothery 1997). Photosynthesis may be temperature limited during daylight hours of the growing season although some suggest otherwise (Warren-Wilson 1957). If temperatures are too low, snow will not melt, and covered vegetation will not be

photoactive (Longton 1988). For some species, a specific number of degree days above zero may be necessary after snow melt before growth initiation (Genet et al. 2013).

Bryophytes can photosynthesize at a broad range of temperatures, the exact range varying inter and intra specifically (Kallio and Heinonen 1973). Polar bryophytes are adapted to large temperature fluctuations to which they are regularly subjected (Longton 1988). Davey and Rothery (1997) found optimal polar bryophyte temperature of 0.0 to 20.0 °C for gross photosynthesis and 10.0 to 20.0 °C for net photosynthesis. High temperatures may lead to increased growth when no other factors are limiting, with maximum net assimilation rates for cool arctic bryophyte species around 14.0 °C (Longton 1988). Net assimilation rates vary intra specifically; many species show lower maximums in arctic than temperate populations.

Bryophytes resort to cryptobiosis in extreme cold temperatures, by entering the same ametabolic state triggered by desiccation (Roads et al. 2014). La Farge et al. (2013) recently observed regeneration of subglacial bryophytes following the retreat of a 400 year old glacier. The longest period of cryptobiosis ever recorded is over 1530 years, in bryophytes regrown from previously frozen permafrost (Roads et al. 2014).

Temperature plays a role in determining ecological community structure. The treeline is largely delineated by temperature limits inhibiting tree growth (Pienitz et al. 2004). Tree absence in the tundra has a profound impact on microclimate affecting cryptogamic vegetation (Longton 1988, Yukon Ecoregions Working Group 2004, Ecosystem Classification Group 2012). Shrubs and tussocks provide shade and water and catch windborne bryophyte fragments (Schofield 1972).

The Canadian north is characterized by long, cold winters and short, cool summers (Yukon Ecoregions Working Group 2004, Ecosystem Classification Group 2012). Mean annual temperatures in the arctic tundra are -11.0 to -7.0 °C. The growing season is much cooler than elsewhere, with a mean daily temperature of 0.0 °C in the higher latitudes to 12.0 °C in lower latitudes (Longton 1988). The warmest month in the tundra is July, with average temperatures from 10.0 to 12.0 °C (Ecosystem Classification Group 2012). January is coldest, with average temperature -30.0 °C.

3.3. Irradiance

Irradiance is important in carbon flux regulation (Davey and Rothery 1997), photosynthesis, species assemblage development and individual species response (Caners et al. 2009). Richness and cover of acrocarpous, common pioneer species, may be reduced under low light

but leave pleurocarpous mosses unaffected, and vice versa. Leaf and stem size (Hoffman 1966b), Shannon diversity (Caners et al. 2009) and sexual and asexual reproduction may be significantly higher with high light, until species specific tolerance is surpassed. Amount of light bryophytes receive at ground level in polar habitats is generally equivalent to the requirements of shade plants (Davey and Rothery 1997). Irradiance is therefore likely the abiotic factor least limiting in polar ecosystems, with the exception of dark winter months. Antarctic bryophyte photosynthesis is saturated at 30.0 to 270.0 μmol m^2 s^{-1}.

Photoinhibition, or the halting of the process of photosynthesis triggered by strong light, may be a factor limiting growth of some polar bryophytes (Oechel and Sveinbjornsson 1978, Adamson et al. 1988). To avoid damage to the photosynthetic apparatus, bryophytes will shut them down (Adamson et al. 1988). Low levels of light or other limiting factors, such as temperature, may increase sensitivity to photoinhibition (Adamson et al. 1988, Davey and Rothery 1997, Genet et al. 2013). However, high levels of irradiance to which arctic bryophytes are subjected are likely not high enough to be limiting overall (Adamson et al. 1988, Longton 1988, Davey and Rothery 1997, Genet et al. 2013). Steere (1978) postulates that tundra bryophyte species, with few exceptions, are tolerant of insolation. Instead of strong light halting photosynthesis, a red pigmentation occurs in the plant biomass in full sun.

Average annual daily solar input in the tundra low arctic ecoregion is 9.0 to 10.0 mJ m^{-2} day^{-1}, varying with slope and aspect (Ecosystem Classification Group 2012). Lowest average inputs occur in December at 0.7 mJ m^{-2} day^{-1} and are highest in June at 22 mJ m^{-2} day^{-1}. Mean annual solar radiation is directly correlated to temperature decrease, which declines with increasing latitude (Longton 1988). Seasonal variation in day length increases with increased latitude. Beyond 66° 33' north and south the sun stays in the sky 24 hours a day in midsummer, with a corresponding period of continuous 24 hour darkness in midwinter. The short growing season of up to 750 growing degree days is enhanced by long photoperiods (Longton 1988). The 24 hour daylight does not necessarily impact daily rhythms of polar bryophyte species (Steere 1954).

3.4. Nutrients

Bryophyte community composition is affected by nutrient concentration and availability (Steere 1978, Zoltai and Vitt 1995). Variations of pH can significantly alter cation exchange capacity, total nutrient availability and nutrient uptake (Glime 2007), indirectly affecting growth and distribution of bryophytes (Steere 1978, Glime 2007). Most mosses and hepatics either prefer acidic or calcareous habitats (Glime 2007). Although many bryophytes are considered

calcicoles or calcifuges, it is unclear to what extent tolerance is associated with calcium concentrations or merely indirectly by pH or associated factors (Longton 1988).

Bryophytes require many of the same mineral macronutrients and trace elements as tracheophytes, although at smaller concentrations (Glime 2007, Bates 2009). Most bryophytes require, in order of importance, nitrogen, phosphorus and sulfur and to a lesser extent potassium, calcium and magnesium (Voth and Hamner 1940, Tamm 1953, Hoffman 1966a, Glime 2007). Nutrient concentrations exceeding bryophyte requirements can be damaging, as there is very little resistance to excessive uptake (Voth 1943, Bates 2009).

Uptake systems are limiting (Bowen 1933). Passive sorption is the primary pathway for nutrient uptake (Pickering and Puia 1969), with active uptake possible by transporter proteins and proton pumps (Bates 2009). Most bryophytes are capable of storing nutrients (Brown and Bates 1990, Bates 2009). Bryophytes lack roots and absorb minerals over the entire gametophyte surface (Glime 2007, Bates 2009) therefore the atmosphere is the main source of all mineral elements (Tamm 1953, Rieley et al. 1979, Brown 1982, Brown and Bates 1990, Glime 2007). Atmospheric sources of nutrients can be wet as precipitation and leachates, or dry as dust and gas deposition (Tamm 1953, Bates 2009). In symbiotic relationships in biological soil crusts, nitrogen fixation by microorganisms (Belnap 2001) is provided in exchange for water held in bryophyte community structures (Bates 2009).

The dominant soils in the arctic tundra are relatively nutrient poor Cryosols, both turbic and static, and permafrost is continuous (Yukon Ecoregions Working Group 2004, Ecosystem Classification Group 2012). Brunisols and Regosols are present, as is exposed bedrock (Ecosystem Classification Group 2012). Mineral nutrient availability can be very limiting to bryophyte growth, especially in some inland northern regions (Longton 1988). In many cases, nitrogen and phosphorus are the most limiting elements.

3.5. Other Physical Challenges

Remote locations of northern reclamation sites present challenges for a variety of reasons. There are few named communities in the north (Ecosystem Classification Group 2012), making environmental information and reference documents scarce or obscure or difficult to find. This lack of various types of information extends to information on cryptogamic floras. Assessment of size, geographical affinity and history of bryophyte communities is made more difficult by taxonomic uncertainty and lack of distribution data (Longton 1988). There is virtually no

information on northern bryophytes prior to the 1970s, when sample collection and observation was mostly incidental to other research (Schofield 1972).

North of the treeline, there is little protection from wind. Mean wind speed in the arctic tundra is 18.0 km h^{-1} with only 3 % calms (The Government of Canada 1999). Wind plays a significant role in snow redistribution (Longton 1988, Ecosystem Classification Group 2012). Cooling and desiccating consequences of wind have a negative impact on bryophyte growth (Longton 1988). Northern bryophytes are thus more likely in sheltered depressions, where fine soil and humidity might accumulate. Wind likely plays an important role in regulating bryophyte growth.

Bryophytes are inherently slow growing prganisms, mainly due to their opportunistic life strategy (Longton 1988). Research on bryophytes in tundra environments has shown that natural recovery after disturbance may be possible, but only likely in the long term (Bliss and Wein 1972, Davis 1998).

4. PAST NORTHERN BRYOPHYTE REVEGETATION

Land reclamation, in general, is a relatively new field of research. Only in the last 40 years has concern been expressed over the impacts of large scale resource development in the northern environments (Forbes et al. 2001). Reclamation research in the arctic tundra has mostly focused on revegetation either without reintroduction of vegetation or by seeding exotic or native grass and herbaceous species (Bliss and Wein 1972, Cargill and Chapin 1987, Densmore and Holmes 1987, Elliott et al. 1987, Elmarsdóttir et al. 2003, Reid and Naeth 2005, Rausch and Kershaw 2007, Deshaies et al. 2009). Little research has focused on reintroduction of bryophyte species (Forbes and Jeffries 1999).

4.1. Natural Revegetation Of Bryophytes

Tundra plant community succession is a relatively long process. The general consensus is that complete natural revegetation does not occur in large disturbance areas within 50 years of disturbance (Harper and Kershaw 1996, Davis 1998). Forbes et al. (2001) found only the smallest and wettest patches of disturbed, level ground recovered after disturbance without assistance, approaching their surrounding environment in less than 75 years. It may even take up to centuries for northern ecosystems to develop to successional stages past primary succession and millennia before plant species composition resembles the pre disturbance community state (Forbes and Jeffries 1999).

Bryophytes can revegetate small patches of disturbed area, however, their susceptibility to disturbance and slow growth make it difficult for them to colonize large bare areas (Bliss and Wein 1972, Harper and Kershaw 1996). Slow revegetation is likely linked to limited pedogenic processes in the north (Harper and Kershaw 1997). On the CANOL pipeline project, denuded areas such as borrow pits were warmer, drier, less acidic, had lower organic matter content, and were thus suggestive of slow soil development since the disturbance 50 years prior (Harper and Kershaw 1997). It is difficult to say whether the poor substrate conditions are the result of restricted plant cover or vice versa.

Several studies have been conducted on natural revegetation on the CANOL pipeline corridor through Northwest Territories and its associated disturbances. Fifty years after disturbance, bryophytes were among the richest taxonomic groups in small disturbance areas, such as vehicle tracks (Kershaw and Kershaw 1987, Harper and Kershaw 1996, Davis 1998). The pipeline, recovered with original topsoil material, was better revegetated than borrow pits, which still remained in early stages of revegetation (Davis 1998). Vegetation in borrow pits was sparse, consisting mostly of lichens and bryophytes (Harper and Kershaw 1996).

Grettarsdóttir et al. (2004) found Icelandic sites revegetated by seeding with exotic grass species almost 25 years earlier had 0 to 2 % cover of those species, plant cover consisting primarily of native vascular and non vascular species. Three Icelandic barren lands aerially seeded with *Festuca rubra* L. (creeping red fescue) were assessed after 2, 10 and 25 years (Greipsson and El-Mayas 1999). The high cover of seeded grass after two years was reduced by ten years, and almost completely eliminated after 25 years. Native vegetation had recolonized the area, and cover of native species after 25 years was 25 %.

The bank of knowledge on ecosystem specific observations is too small at present for any meaningful comparison. Present day estimations of colonization time and success would be speculative at best without more data. Thus this is an important area for further research to be conducted in the north.

4.2. Anthropogenically Assisted Revegetation Of Bryophytes

Most of the research conducted on reintroduction of bryophytes to disturbed sites focused on harvested peatland restoration (Pfadenhauer and Klötzi 1996, Pfadenhauer and Grootjans 1999, Lamers et al. 2002, Gorham and Rochefort 2003, Vasander et al. 2003, Cobbaert et al. 2004, Rochefort and Lode 2006, Mälson and Rydin 2007, Sottocornola et al. 2007, Similä et al.

2011). Interest in peatlands is due in part to the economic benefits provided by sustainable harvesting of peat (Gorham and Rochefort 2003) and their importance as carbon sinks (Pfadenhauer and Grootjans 1999, Gorham and Rochefort 2003). The process relies strongly on restoration of original hydrologic regime (Pfadenhauer and Grootjans 1999, Lamers et al. 2002, Sottocornola et al. 2007, Similä et al. 2011) and thus differs greatly from tundra restoration, where water availability is much lower. Peatland bryophyte revegetation is generally accomplished through manual spreading of *Sphagnum* fragments (Rochefort and Lode 2006), donor diaspore material (Cobbaert et al. 2004) or transplantation of entire blocks of vegetation (Rochefort and Lode 2006).

Tundra restoration methods are mainly adapted from successful peatland methods. Aradóttir (2012) transplanted turf blocks for reclamation at a geothermal power plant in southwestern Iceland. Different size turfs containing a mix of grasses, sedges, forbs, dwarf shrubs, mosses and lichens were directly transplanted. After two years, moss cover increased with all sizes of transplants; spread of turf was low with loss of rare species and species with low cover (< 6 %). Live turf transplanting may be effective in quickly establishing species composition of a reclaimed area, but relative abundance of some native species may be different from the donor site (Aradóttir and Óskarsdóttir 2013). Turf transplanting can quickly reintroduce native cover, and there is a potential for salvaging industrial sites where development is planned by translocating the entire turf material to decommissioned areas. There is potential damage to donor sites, slow spread from turf and loss of rare or sensitive species.

Magnúsdóttir and Aradóttir (2011) assessed the potential of *Racomitrium lanuginosum* for regeneration from fragments in a greenhouse. Results were promising, and they postulated fragmentation would accelerate colonization on disturbed areas with little disturbance to a donor site. This may not be effective to regenerate rare or sensitive species, and little is known of vegetative regeneration capacities of many bryophyte species. Manually shredded and distributed turf materials positively affected moss cover with time (Magnúsdóttir and Aradóttir 2011, Aradóttir 2012). This method can reduce effects on donor sites if collection is strategic and minimal, and can increase cover by spreading material and including all diaspore types. Klokk and Rønning (1987) in Svalbard, Norway found establishment of bryophytes and other species was stimulated by application of fertilizers. These findings could be applicable to other nutrient poor northern applications.

The science of reintroduction of bryophyte species to disturbed lands is relatively novel. A deeper understanding of life history traits of individual species, community interactions and

facilitation of growth is necessary for restoration of disturbed tundra ecosystems (Cargill and Chapin 1987). Almost any treatment will likely hasten reintroduction relative to natural revegetation in northern ecosystems (Grettarsdóttir et al. 2004).

5. MEASURING BRYOPHYTE GROWTH

5.1. Percent Cover

Ocular estimation of cover is the most common method of measuring bryophyte growth (Belnap 1993, Belland and Vitt 1995, Buxton et al. 2005, Newmaster et al. 2005, Caners et al. 2009, Graf and Rochefort 2010, Aradóttir 2012). It is important to understand and distinguish types of estimations (Fehmi 2010). Aerial cover can be assessed as the uppermost vegetation layer expressed by a percentage area occupied per species, totaling 100 for all species. Cover for each species can be independently estimated, wherein cover of individual species must not exceed 100, but the sum of species might. Leaf cover includes all layers of vegetation from uppermost to soil surface.

The sum of individual species may exceed 100 %, as might the sum of species. When cover is difficult to assess, brackets or ranges may be used (Usher 1983, Aradóttir 2012). For example: 1 = < 1 %; 2 = 1 to 5 %, 3 = 6 to 10 %; 4 = 11 to 15 %; 5 = 16 to 25 %; 6 = 26 to 50 %; 7 = 51 to 75 % and 8 = 76 to 100 % (Aradóttir 2012). When bracketing, mean values are assumed to be at the mid point of the ranges (Usher 1983). Cover can be estimated in fixed plots (Belnap 1993, Aradóttir 2012) or linear transects (Usher 1983).

Visual cover estimations are non destructive, relatively easy to do, require little equipment and provide a calculable measure of growth. Cover values are limited by subjective estimations of the observer and results varying with different assessors and the species being assessed. Thjis can be overcome in part by using visual guides of percent cover as a reference. Percent cover estimations give a snapshot of revegetation success, and do not measure other important qualities of a living plant (Belnap 1993). Thus other methods or a combination of methods and analyses may be necessary.

5.2. Photographic Technologies

It is possible to photograph and trace colonies over time to visually assess and compare their expansion (Longton 1988). Vitt (1989) used a hoop at a marked position over a colony for

consistent comparison of size. This would be useful for non destructive, visual qualification of growth and expansion, but limiting as it does not give concrete data for analysis.

New technologies are currently being developed to combine photographic technologies with cover or point sampling measurements, employing digital algorithms (Song et al. 2015), remote sensing data (Chen et al. 2010, Trimble Geospatial 2015), shape and colour spectrum image processing (ImageJ 2015), manual point intercept (Booth et al. 2006) and colour spectrum classification software (Trimble Geospatial 2015, VegMeasure 2015). The benefits of digitizing cover estimates include reduced subjectivity and reduced field time for an increased amount of data. Knowledge of computer programming, GIS or other advanced technologies may limit accessibility of some of the tools presently available.

5.3. Radial Expansion

Cryptogamic growth can be assessed through quantification of radial expansion, by measuring colony average diameter (Longton 1988, Vitt 1989). This is most useful when colonies grow in a uniform outward fashion like rock lichens, although it has been used for bryophytes (Vitt 1989). Diameter measurement is non destructive but would not capture species expansion through spread of fragments or spores, overall health of colonies or changes in mixed species patches.

5.4. Density

Counting shoots might be effective to assess new growth. Bell et al. (1991) categorized and counted *Polytrichum formosum* shoot growth as old, old with new growth, small new shoots (< 1 cm) and large new shoots (> 1 cm). Others counted new branched and lateral shoots at intervals (Longton and Greene 1979). Shoot counting could be an effective, non destructive way to monitor early growth. It would not be practical in a large, established, community patch.

5.5. Species Richness

Frequency or abundance percentages are commonly employed to estimate species richness. The scale of species richness diversity is important whether calculations are based on entire landscape, site, plot or microhabitat (Vitt et al. 1995, Newmaster et al. 2005). Frequency is determined by counting species occurrence in an area, by analyzing the entire plot (Rastorfer 1978) or using a point frame (May and Hollister 2012, Belland 2014). Plots can be located randomly in an area or microhabitats used as sampling units or strata (Newmaster et al. 2005).

Frequency can be combined with cover for a robust estimate of abundance (Vitt et al. 1995, Newmaster et al. 2005). With species richness, evolution of community composition may be monitored over an extended period. Species richness does not indicate health, but can provide an estimate of what exists in a delimited area.

5.6. Length And Height

Height measurements have been used to assess size of cryptogamic species and may be useful to monitor long term growth (Pitkin 1975, Bell et al. 1991, Belnap 1993). A vertical wire or stick can be pinned to the ground for a consistent measure of growth (Clymo 1970, Longton 1988, Bell et al. 1991). Markers can be tied to shoots at a known distance from the apex and measured extension based on growing distance (Clymo 1970, Longton and Greene 1979, Bell et al. 1991). Height can be measured by planting mosses cut to a known length, then measuring them after a period of growth (Clymo 1970, Longton 1988).

Measurement of height may be useful for non destructively determining growth of a single species and for repeated measurements on large, prostrate, branched mosses. Pinning would be difficult in a natural environment, where fauna or effects of ground frost action might disturb pin placement. Tying would not be useful in measuring colony growth, and string markers may affect capillary growth (Longton 1988). Cutting bryophytes fragments to a determined length would be useful for a single species, however, time consuming and destructive for large scale work. Selecting an initial size would be difficult and the disturbance of cutting off the bottom part of the plant may disrupt growth (Longton 1988). Measurement of applied markers would be limiting for mixed species.

Innate markers can be used to measure seasonal variation in leaf length (Clymo 1970, Longton 1988). Individual plants can be sampled and dissected. This type of assessment would provide accurate and precise data, but would require destructive sampling. Such measurements would be time consuming and would likely not be applicable to all taxa or even comparable among taxa (Longton 1988).

5.7. Net Primary Production

Positive net photosynthesis will increase plant dry weight due to accumulation of assimilates (Longton 1988). Dry weight is a common measure of bryophyte growth (Clymo 1970). Bryophytes can be oven dried at 70 °C for 24 hours then cooled over an absorbent product such

as calcium chloride ($CaCl_2$), in a desiccator prior to dry weighing (Rastorfer 1978). Dry weight is sometimes calculated at intervals to compare and track growth over time (Dilks and Proctor 1974, Davey and Rothery 1996, Davey 1997, Davey and Rothery 1997, Benscoter and Vitt 2007, Caners et al. 2009), or compared between samples of equal size (Rastorfer 1978). Benscoter and Vitt (2007) present a conceptual model for *Pleurozium schreberi* L. (red stemmed feather moss) whereby length of branches can be used to infer a corresponding weight increase. Models for every environmental expression of every species would reduce destructive sampling.

Dry weight provides the most direct measure of plant growth (Davey and Rothery 1997). It does not account for buildup of non photosynthetic organic material included in the sample or for plant species that naturally have a low phytomass per unit area. The assumption that increased dry weight is an indicator of health is not necessarily correct for plants with opportunistic growth responses (Longton 1988). For example, to survive, bryophytes may be alive but not growing and hence the absence of growth may be misinterpreted.

Net primary production can be calculated through carbon dioxide flux measurements. Normally, the flux measurements are repeated on replicate patches several times throughout the growing season to measure seasonal variations, using infrared gas analyzers (Adamson et al. 1988, Davey and Rothery 1996, Davey 1997, Davey and Rothery 1997, Street et al. 2012). Carbon dioxide flux measurements allow for indirect measures of respiration and photosynthesis (Davey and Rothery 1997). However, many variables may affect the results obtained, such as decomposition of plant material and activity of soil microorganisms (Landhausser 2014). Irradiance will impact results, as it directly impacts rates of photosynthesis and respiration (Davey and Rothery 1997).

5.8. Chlorophyll Analysis

Spectrophotometry provides chlorophyll contents of plant materials (Talling et al. 1978, Belnap 1993, Davey and Rothery 1997). Chlorophyll is chemically extracted from plant material, centrifuged and then analyzed in a spectrophotometer. Variation in proportions of non photosynthetic material will not affect final chlorophyll results (Davey and Rothery 1997), however, samples must be collected and treated in a short time period to reduce seasonal variability (Belnap 1993). This can provide accurate and precise data, although collection is time consuming and results are often more oriented to plant physiology than to plant ecology (Landhausser 2014).

6. SUMMARY

This literature review provided a brief summary of the current scope of scientific knowledge of bryophytes and their propagation, to be used to address best methods for reclamation in this research program. Strict restoration of original bryophyte communities is unlikely, however many effective methods for revegetation of some or most of desired species do exist.

Anthropogenic involvement in the north would ideally be kept to a minimum, but disturbance is unavoidable. To speed ecosystem restoration, all strata of northern biota must be considered. Starting with a foundation of resilient colonizers that have adapted quick regeneration strategies could be key to reducing erosion and creating microsites supporting higher trophic species.

Natural colonization is a lengthy process and is not ideal in all northern ecosystem revegetation scenarios. Land reclamation practitioners should attempt to use both sexual and asexual reproduction when propagating bryophyte material. Bryophyte communities from similar ecosystems in proximity to the disturbed area would be best adapted and accessibly located, thus reducing time, effort and cost of translocation. Whenever possible, diaspore material should be translocated with bryophyte vegetative material, as it might contain sexual elements, asexual fragments and propagules, soil and a microbial community.

A number of effective methods exist for promoting bryophyte propagation, including transplantation and fragmentation. Where industrial development is advancing as reclamation is occurring, it would be appropriate to harvest as much turf material as possible, and replant it on the reclamation site. Fragmentation of small volumes of bryophyte material is more appropriate when attempting to limit disturbance to donor area. The most effective size of material is a subject for further research. Wind erosion of planted material could be reduced with the use of a light fabric cover. Material should be biodegradable within the time frame of reclamation, taking into account the slower decomposition rates in the north, and fine enough to allow bryophytes to grow through it and have access to sunlight and precipitation. Revegetated areas should be monitored for successful establishment of bryophytes; methods for doing so should be selected on a case by case basis.

7. THESIS APPROACH

This thesis research focused on determining the most effective methods for promoting bryophyte growth for northern reclamation. Chapter 2 covers a laboratory experiment that

assessed three slurry mixtures; beer, buttermilk and water, and three bryophyte fragment sizes; small (< 1 mm), medium (< 2 mm) and large (< 40 mm). Chapter 3 covers a field experiment using the same three fragment sizes of bryophytes in a water slurry, with cheesecloth as an erosion control material. Northern field sites in Canada and Iceland were compared to explore the impacts of different substrates and climates on effectiveness of treatments. Chapter 4 summarizes the research, addresses research limitations and provides some ideas for future research. Chapter 5 contains references for all of the chapters.

II. BEER, BUTTERMILK, WATER AND PLANT FRAGMENTS FOR LABORATORY PROPAGATION OF TUNDRA BRYOPHYTES

1. INTRODUCTION

Mosses are capable of both sexual and asexual reproduction. Spore production can be impeded by availability of water, nutrients and light, and distance of gamete separation; it has never been observed in a number of species (Miles and Longton 1990). Regeneration is therefore often by asexual reproduction, either through release of miniature clones (brood bodies, gemmae) (Malcolm and Malcolm 2000) or regeneration of gametophore fragments, totipotent cells able to differentiate into a meristematic state and reprogram themselves for development of whole new organisms (La Farge et al. 2013).

Several methods are used to replicate natural fragmentation; including pulverization, sieving, hand crumbling and clipping. Fragment size varies with treatment, as it does naturally with species and environmental conditions. Propagules can be reduced to small, dust sized particles, by pulverizing or blending (McDowell 1972, Shaw 1986, Schenk 1997, Svenson 2000, McDonough 2006, Gignac 2010, Moss and Stone Gardens 2011, Apartment Therapy 2012, WikiHow 2013). Grating material through a mesh sieve can produce medium, leaf sized fragments (Shaw 1986, Schenk 1997, Jones and Rosentrerer 2006). This size approximates multicellular fragments, easily transported by wind and known to propagate, such as detached (Longton and Greene 1979, Wilmot-Dear 1980, Miles and Longton 1990, Giordano et al. 1996, Hugonnot and Celle 2012) or wounded leaves (Gemmell 1953). Larger fragments approximate whole or partial plants translocated by soil or water movement or transportation on another living organism. Large fragments can be produced by manually breaking dried material (Iwatsuki and Kodama 1961, Belnap 1993, Glime 2007, Magnúsdóttir and Aradóttir 2011, Aradóttir 2012) or clipping to a standard length (Graf and Rochefort 2010). All broken material can be used or sometimes only the apex (Brown and Bates 1990, Miles and Longton 1990). Little research comparing effectiveness of fragment size has been conducted.

Many home renovation and craft websites, magazines and blogs feature moss propagation. These generally imply that moss material, collected from almost anywhere, can be blended and sprayed or painted on walls or sidewalks to make art or a low maintenance yard. To support and promote moss regeneration, numerous slurry preparations have been suggested, including mixes of bryophyte material and soil (Iwatsuki and Kodama 1961, McDonough 2006, Buxton et

al. 2005), distilled water (McDonough 2006), fertilizer (Buxton et al. 2005) or glue such as epoxy resin (Glime 2007). Regular household mixes including beer (Gillis 1991), milk (McDowell 1972), buttermilk (Gillis 1991, Apartment Therapy 2012, WikiHow 2013), yogurt, corn syrup, sugar (WikiHow 2013), compost (Schenk 1997, Buxton et al. 2005), manure (Schenk 1997), eggs (Schenk 1997) and water retention gel (Goodier 2010) have been recommended. Most common slurries include a combination of water, beer and buttermilk or yogurt. No rigorous testing of slurries has been conducted.

2. RESEARCH OBJECTIVES AND HYPOTHESES

2.1. Research Objectives

This research helps to address the goal of starting early successional tundra ecosystems through bryophyte introduction. Methods to facilitate species introduction were explored, including collection, slurrying and propagation, to determine most effective practices for reclamation. Specific research objectives are as follow.

- To determine effectiveness of three fragment sizes for promotion of diaspore regeneration.
- To determine effectiveness of three slurry mixtures for promotion of diaspore regeneration.
- To determine capacity of different species for effective propagation.
- To select treatments for use in a reclamation field experiment, based on effectiveness of sizes, slurries and species.

2.2. Research Hypotheses

The following hypotheses are postulated regarding the revegetation of bryophytes.

- If fragmentation is an effective means of bryophyte revegetation, evidence of growth will be detectable after several weeks under laboratory conditions.
- If slurry mixtures aid propagation, bryophytes under these treatments will grow faster and or more abundantly than with water alone.
- If bryophyte communities evolve along a successional gradient, some species will be more prone to colonizing and others more suited to establishment in stable conditions.
- If bryophyte species have specific physical fragmentation and environmental adaptations, those that are adapted to the fragment sizes and slurry treatments will do so faster or more abundantly than those that are not.

3. MATERIALS AND METHODS

3.1. Collection And Identification

Bryophyte biomass samples were collected in fall 2013 from natural areas located near Diavik Diamond Mine, on Lac de Gras, in the Northwest Territories, Canada. Collection microsites were randomly selected and represented a variety of hydrologic regimes, soils, disturbance levels and plant communities. Sample homogeneity and species composition varied with microhabitat properties. Fist sized bunches of biomass were separated and pulled from substrate or surrounding vegetation by hand. Biomass bunches were deposited into paper bags labeled with microsite type and description. Samples were transported to the University of Alberta and air dried in the laboratory by opening paper bags for approximately one month at room temperature.

To facilitate identification and sorting, samples were rehydrated with a distilled water dilution of the surfactant Aerosol OT (sodium bis(2-ethylhexyl) sulfosuccinate) (Belland 2014). Bryophytes were identified according to Crum (2004) and Atherton et al. (2010), with the assistance of Dr. René Belland (Belland 2014). Species were sorted and air dried in open trays on a laboratory bench for three days, until a constant weight was achieved, prior to experimental applications. The final dry weight of each species was determined for a general estimate of initial abundance.

3.2. Treatments

Three fragment sizes were assessed for growth potential. Large fragments (< 40 mm) were entire, hand separated individual plants. Medium fragments (< 2 mm) were created by sifting plant material through a 1.0 mm soil sieve. Small fragments (< 1 mm) were produced by grinding dried samples in a standard hand held electric coffee grinder.

The three fragment sizes were evaluated in three slurries. Slurries were made by hand mixing 2.0 g of bryophytes with 50.0 mL distilled water and 50.0 mL of either beer, buttermilk or more distilled water, using glass beakers and stir sticks. The beer, donated by Alley Kat Brewery, had a pH of 4.0 and an alcohol content of 5.0 %. The beer was unpasteurized, contained no preservatives and was made from organic malts in Edmonton, Alberta. Dairyland old fashioned buttermilk was sourced from a local grocery store; it contained 3.3 % fat and had a pH of 4.3. Distilled water, pH 6.3, was sourced from the University of Alberta laboratory. The slurries stood for a minimum of 5 minutes to rehydrate the desiccated bryophytes.

The species in the bryophyte mass used to make the slurries were *Aulacomium turgidum* *Cephalozia* sp, *Ceratodon purpureus*, *Cynodontium alpestre*, *Funaria hygrometrica*, *Pohlia* sp, *Polytrichum juniperinum*, *Polytrichum strictum*, *Ptilidium ciliare*, *Racomitrium lanuginosum* and *Tetralophozia setiformis* (Table 2.1). Lichens were included, as well as species too few or small to identify, labeled as unknown.

3.3. Experimental Design And Laboratory Procedures

Slurries were applied to plastic sponges overlaid with a double layer of natural, white, 100 % cotton, 1.0 mm^2 mesh cheesecloth (20 threads per inch), cut to fit the 11 x 9 cm top of the sponges. The cheesecloth was fastened to the sponge at each of its four corners using plastic toothpicks. The damp sponges and cheesecloth stood in open trays of distilled water, at an ambient temperature of approximately 23.0 °C. Bryophyte slurries were poured on to the cheesecloth in a circular 7.5 cm diameter metal frame to concentrate their location. Each of the sponges received a single replicate of slurry.

There were 45 experimental units consisting of 3 slurry materials x 3 fragment sizes x 5 replicates. Replicates and fragment sizes were randomized in trays of designated slurry composition to avoid contamination, for a total of 9 trays, 3 per slurry treatment.

To minimize potential limiting factors, the sponges were misted with distilled water twice weekly and were injected with distilled water, using a 500.0 mL squirt bottle, when dry; however some visible desiccation occurred between waterings. Trays were moved weekly by shifting them on the laboratory bench, moving the furthest left tray to the right side of the bench to reduce impact of irregular drafts or light. To approximate northern summer conditions, fluorescent lights were on 24 hours every day.

Trays were covered with clear plastic lids, then removed after 2 days when mold grew on 7 of the 9 trays. Only 1 distilled water tray had mold. Replicates with mold were treated with a distilled water dilution (50 %) fungicide of 0.3 % potassium salts and 0.2 % sulphur on day 4 of the experiment. Fuzzy white growth immediately receded in some replicates but persisted in all buttermilk and some beer replicates for the duration of the experiment, mostly in a white hyphae form (up to 98 % cover at times), although a few small mushrooms did grow and persist in four different replicates (3 beer, 1 buttermilk). Fungicide application was halted after one month when most of the mold growth had receded, as it had a desiccating effect on sponges and to limit its potential harmful effects on bryophyte growth. Duration of the experiment was 12 weeks.

3.4. Vegetation Assessments

Vegetation growth was assessed weekly. Percent cover of live bryophytes (fragments green and or regenerating) was visually estimated separately within the circular frame and outside the frame on the rest of the sponge. Cover estimates were calibrated to a visual guide created for this purpose. Thickness of vegetation was measured using a standard ruler, with point of measurement at the edge of the circular planted area at the point of highest visually estimated thickness. Diameter was measured with a standard ruler to determine spread outside the planted area. Diameter and thickness measurements were halted after three weeks, as there were never any perceptible variations in these parameters.

Weekly density of individuals per sponge was determined separately inside and outside the circular frame using a magnifying lamp and click counter. After 12 weeks, species that regenerated inside and outside the circular frame were identified (Crum 2004, Atherton et al. 2010, Belland 2014) and counted. Species counts were approximate, as plants were often small and growing in compact mats. Although weight of individual species would have been more desirable, it was not taken at the end of the experiment due to difficulty removing plants from the degrading cheesecloth.

Photographs were taken to document interesting occurrences such as fungal and protonemal development and growth throughout the experiment. Entire trays were photographed at weeks 0, 1, 5 and 12 for a visual record of bryophyte growth.

3.5. Statistical Analyses

Data were checked for errors using the identify tool in R (R Core Team 2015); outliers were marked and analyzed. Outliers did not correspond to any possible batch error and thus were not altered or removed.

Scatter plots were used to assess overall data trends. Boxplots and Shapiro-Wilks tests were used to assess normality. Data transformations, including square root, log and inversion, were performed, but failed to achieve normality; therefore permutational analyses were conducted. Permutational non parametric testing is considered a powerful approach (Good 2013). It does not rely on assumptions of normality or equal variance, and thus is often used for a number of ecological applications.

A permutational two way analysis of variance (permANOVA) was performed in R to examine the influence of independent variables (size and slurry) on dependent variables (cover, density). To

raise confidence in the significance of permutational output, tests that were not strongly significant were run 10 times, and the most common output was selected. Significance was accepted at $p < 0.05$. The lowest value output from R is $p < 2.2\ e^{-16}$. Permutational ANOVA tests were conducted for all weeks combined and repeated every week for comparison. This repeated testing was to determine if statistical significance increased or lessened with time. Results from week 12, the end of the experiment, were different from all weeks combined, therefore both will be discussed. A Tukey multiple comparison of means was used to evaluate significance of differences between sizes and slurries at week 12.

Relative abundance of identified bryophytes was calculated from weights at the beginning of the experiment and by individual plant densities at the end of the experiment (Table 2.1). Week 0 relative abundance was categorized as high (> 23.2 g), medium (11.6 to 23.2 g) and low (< 11.6 g). Week 12 relative abundance was categorized as high (> 1233 individuals), medium (117 to 1233 individuals) and low (< 117 individuals). Treatment specific densities were categorized as high (> 212 individuals), medium (106 to 212 individuals) and low (< 106 individuals).

4. RESULTS AND DISCUSSION

4.1. Treatment Effects On Cover And Density

Fragment size and slurry type significantly affected plant live cover when averaged over the duration of the experiment ($p < 2.2\ e^{-16}$). However, at the end of the experiment in week 12, only size (p 6.6 e^{-3}) was significant (p 0.9 for slurry) (Table 2.2). Medium fragments generally had a higher live cover than small or large fragments; buttermilk generally had a lower cover than beer or water (Figures 2.1, 2.2). All sizes and slurries were statistically distinct and no interaction effects occurred. The statistical significance of this effect was decreased by week 12. Small fragments did not differ significantly from medium or large fragments but medium and large were statistically different (p 5.6 e^{-3}) (Figure 2.3). No slurries were significantly different at that time. Figure 2.1 also shows the rate at which each fragment size lost its green colour. Large and medium fragments remained alive for one month, whereas small fragments lost their colour earlier. Its possible that the green colour of live plants was more difficult to distinguish due to the fine texture of the small fragments or that these small fragments died faster.

Plant density responded to treatments similarly to cover. Fragment size and slurry type had a significant effect on density overall ($p < 2.2\ e^{-16}$). After 12 weeks, size (p 6.0 e^{-4}) and slurry were significant (p 4.4 e^{-3}) (Figure 2.4; Table 2.3). The slight loss of treatment significance over time

is evident through considerable overlapping of error bars especially approaching week 12 (Figure 2.5). No interaction effects occurred. After 12 weeks, beer had the highest plant density, followed by water and buttermilk (Figure 2.6). Overall, beer and water were statistically distinct from buttermilk (p 0.0), but did not differ from each other. Buttermilk and water were the only two slurries that differed significantly (p 1.1 e^{-2}) at the end of the experiment. After 12 weeks, slurries may have been too dilute to have an effect. Slurries may be more important for initial propagation even though their effect is neutralized over time.

The pH of the liquids used to make the slurries was expected to play a role in promoting propagation. This did not occur. Beer was most acidic, followed by buttermilk then distilled water. Slower propagation of bryophytes from buttermilk slurries may be related to the fungal growth that affected buttermilk, the fungicide used to treat it, or specific chemical composition of buttermilk. The increase in density with buttermilk around week 10 could be due to dilution and leaching of the buttermilk and fungicide or a delayed growth after mold was reduced.

The weakening statistical significance of fragment size after 12 weeks shows effectiveness of some treatments for hastening initial propagation and establishment but not for long term growth of bryophytes. This may be important in northern ecosystems where the short growing season makes early growth imperative for sustainable community development.

The highest density was found in medium size fragments, with less in small and least in large size fragments (Table 2.2). After 12 weeks, medium size fragments had almost double the density of that in large or small fragments (Figures 2.4, 2.5, 2.6). Overall and at week 12, medium fragments were statistically distinct from small and large fragments, although the statistical difference between small and medium was weakening by week 12 (Figure 2.6).

Higher density and live cover with medium size fragments relative to small or large is likely due to it most closely resembling the size of plant parts that naturally occur with bryophytes, many of which evolved capacity to propagate from leaf or stem pieces. The biological potential for growth of detached leaves and stem apices is well known; with many species relying primarily on this method for regeneration (Longton and Greene 1979, Robinson and Miller 2013). Small pieces may not have had enough stored energy for propagation, with stress of desiccation and fragmentation intolerable. The large fragments had low and slow rates of regeneration, possibly due to elevated desiccation stress or energy requirement in supporting the entire plant.

The hydrologic and atmospheric conditions in the laboratory were different from what occurs in tundra. Higher temperatures, no water deficits and lack of wind likely had a beneficial effect on

bryophyte regeneration. Thus results may be more optimistic than might occur in the field.

4.2. Treatment Effects On Species

After 12 weeks, *Bryum pseudotriquetrum*, small unidentifiable plants and an abundance of protonemal growth (latter two were classified as unknown) dominated the vegetation regardless of treatment (Table 2.4). Relative abundance of most species was low by week 12.

Approximately half of the species planted propagated by week 12 (Table 2.4). Total densities in all fragment size and slurry treatments were highest for *Bryum pseudotriquetrum* and *Aulacomium turgidum*, followed by those of *Ceratodon purpureus*, *Polytrichum strictum*, *Ptilidium ciliare*, *Tetralophozia setformis* and *Funaria hygrometrica*, in decreasing order. Approximately 1345 individuals were too small to identify (unknown). No lichens propagated during the 12 weeks.

Protonemal growth dominated the bare cloth outside the circular frame, on the edges on the sponges, and was found in all treatments. Edge protonemal growth was highest in buttermilk treatments with medium (41.0 % mean live cover) and large fragments (17.8 %), and with beer and medium fragments (6.4 %). Protonemal growth in the planted area was also highest on buttermilk (medium 67.4 %, small 20.0 %, large 8.0 %).

Bryum pseudotriquetrum and protonemal growth extended to the edges of the sponges, likely due to the effect of water runoff from the main sponge area, which pooled around the plastic toothpicks in the corners. Growth on outer edges of sponges around toothpicks often surpassed growth on the planted area. This occurred mainly on buttermilk treated sponges, where 749 individuals were counted. No individuals were counted outside the main planted area of sponges treated with only distilled water and only 35 were found on beer treated sponges. Unidentifiable protonema was found on almost all outer sponge edges and inside planted areas, under all treatments.

Species that propagated were likely adapted to the hydrologic, light and temperature conditions in the laboratory and were able to propagate from fragments or regenerate from entire stems. *Aulacomium turgidum* likely propagates preferentially through fragmentation or branching, as it is rarely seen with capsules (Atherton et al. 2010). *Ceratodon purpureus* is a common colonizer, known to be tolerant of sterile or disturbed substrates (Crum 2004). *Bryum pseudotriquetrum* was not found in the initial assessment of vegetation prior to slurry mixing, It may have been present in a limited number of stems or a form that was too small to be detected as very small

plants, fragments or spores. It is a common marsh species (Crum 2004); therefore it was likely well adapted to the hydrologic condition of the sponges.

4.3. Reclamation Applications

Results of this experiment were used to develop field experiment protocols. The future application of the fragmentation methods assessed are supported by their simplicity and effectiveness for short term bryophyte establishment in a field reclamation scenario. Rapid establishment of vegetative cover is of primary importance in many erosion prone reclamation scenarios. Our results support those of others in determining that rough hand pulverization may be useful for stimulating bryophyte propagation (Glime 2007, Hugonnot and Celle 2012).

Although direct planting of bryophyte material in the field would be quicker and more cost effective, preparation of bryophyte propagation materials for transplantation in the field may be required. For example, if an insufficient amount of bryophyte propagation material is available for field planting or if specific species are targeted for revegetation as important in the developing plant community, then growing plants in the greenhouse to increase the planting material would be necessary.

Our results support those of others in that laboratory growth is plausible method of revegetating bryophytes. Laboratory or greenhouse growth and subsequent transplantation has been effective (Flora of North America Editorial Committee 1993a, McDonough 2006, Glime 2007). Material could be planted and hardened on top (Gillis 1991, Glime 2007) or between two layers of cheesecloth (Whitner 1992) then transferred to the field during reclamation (McDowell 1972). Grown bryophytes, transplanted to the field, could assist in rapidly revegetating and providing a diaspore bank in difficult environments with minimal impact to donor ecosystems. A different material may be necessary, as the cheesecloth was very degraded after 12 weeks.

The use of food products in a field setting is impractical. Cost of purchasing and transplanting materials to remote sites would be high, and their application would likely attract wildlife, potentially disturbing the fragile site and endangering workers. There are countless varieties of beer and buttermilk, and testing each one would be time consuming. Since water and beer did not differ significantly in this experiment, further investigation of other varieties is unwarranted and water is recommended for large scale field application. Although distilled water was used in this experiment and would be considered expensive for field application, it is similar to rain water or other water sources that would be available on northern sites.

The spread of protonemal material to depressions is of significant importance in field reclamation. Fragments that are carried by runoff have potential to be more productive than directly deposited material, which may die or be blown away, particularly in northern environments with high and frequent wind. Promoting and accounting for mobility of planted bryophyte material could be an important consideration for field application. Since material will likely be transported by precipitation or wind to cervices or micro depressions, creating a substrate surface that is heterogeneous would likely be beneficial for formation of small bryophyte islands.

On a long term scale, applying any of the three fragmentation methods would likely be beneficial for bryophyte propagation purposes. When considering field application, rapid establishment and growth are critical factors in preventing water runoff and wind erosion of bare substrates. Hydrologic and atmospheric conditions in the laboratory were ideal relative to a field setting. Thus 12 weeks in the laboratory may be equivalent to many months or years in the field, and the short term benefits provided by fragmentation in the laboratory could have important consequences on first few growing seasons of revegetation.

5. CONCLUSIONS

Bryophytes were propagated in the laboratory using small (< 1 mm), medium (< 2 mm) and large (< 40 mm) fragment sizes and beer, buttermilk and distilled water slurries. Medium size fragments were more effective than large or small fragments for propagating bryophytes over a 12 week period. Water and beer were both effective at short term propagation of bryophytes, although water, being more readily available and less variable, would be more efficient for field application. After 12 weeks, the effects of slurries were lessening. *Bryum pseudotriquetrum* was the most abundant species propagated, followed by *Aulacomium turgidum* and *Ceratodon purpureus*. Species specific growth did not respond to fragment or slurry treatment and is likely more dependent on individual species capacity for regeneration in the laboratory conditions.

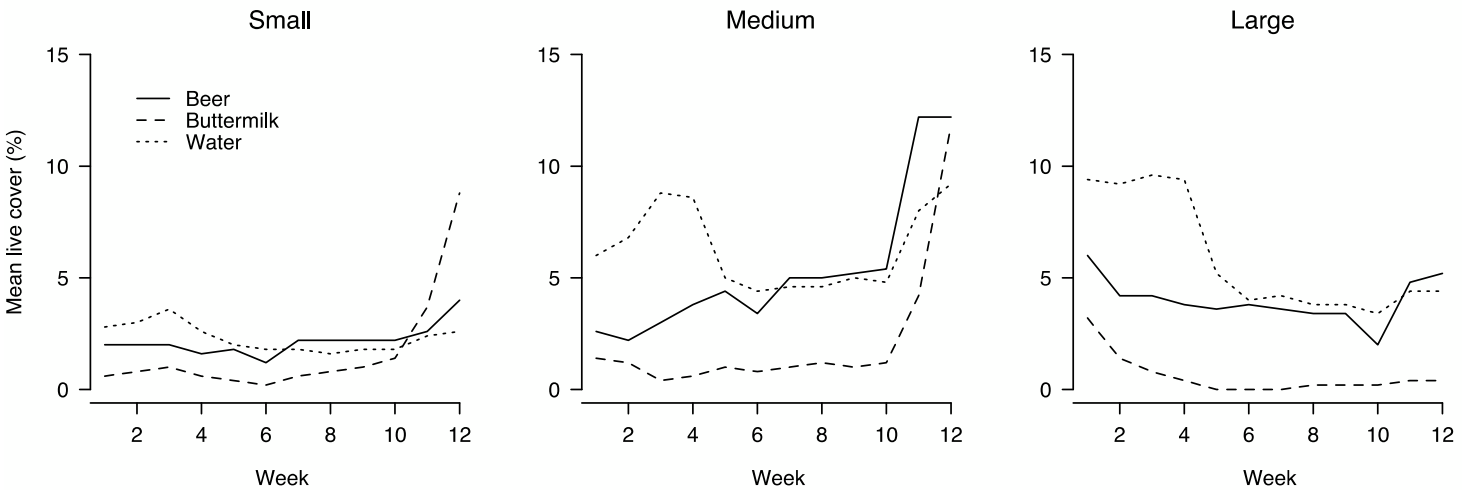

Figure 2.1. Weekly mean live cover of bryophytes in fragment size and slurry treatments (without error bars).

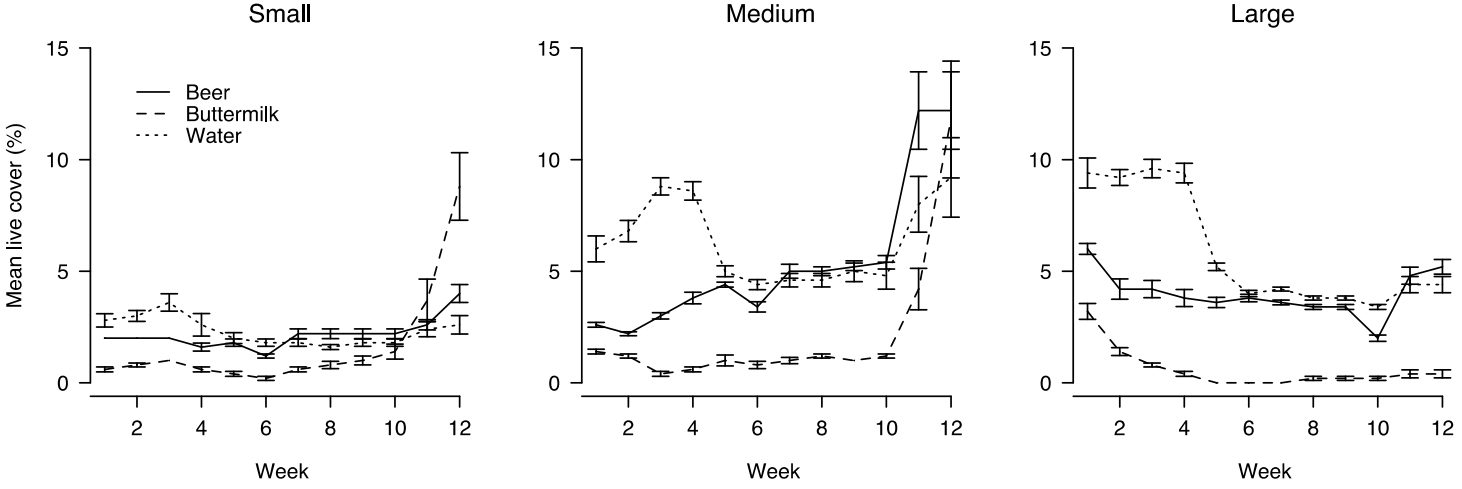

Figure 2.2. Weekly mean cover of bryophytes in fragment size and slurry treatments (error bars are standard error of the mean).

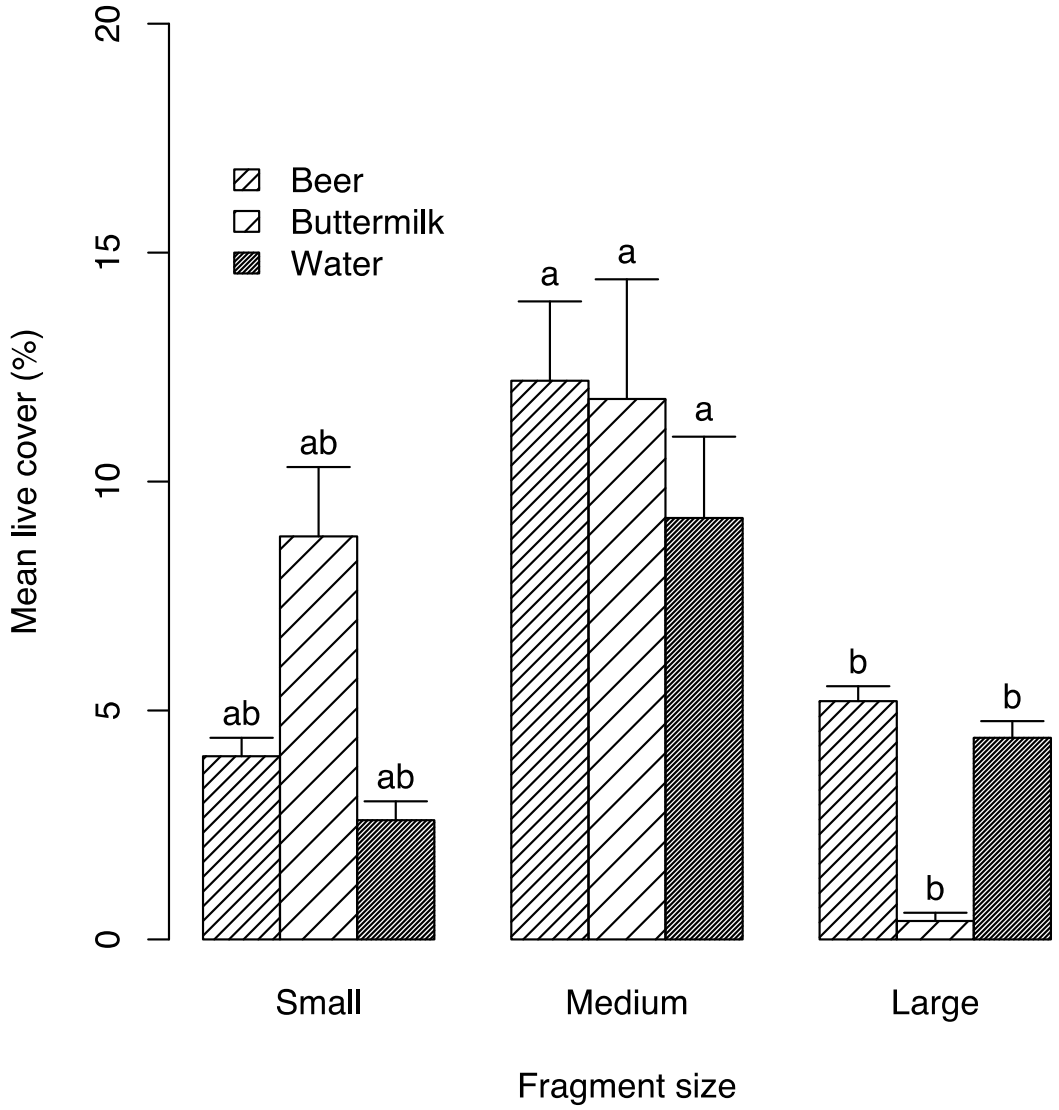

Figure 2.3. Week 12 mean live cover of bryophytes in fragment size and slurry treatments. Different letters denote significant fragment size treatment differences at p 0.05, error bars are standard error of the mean.

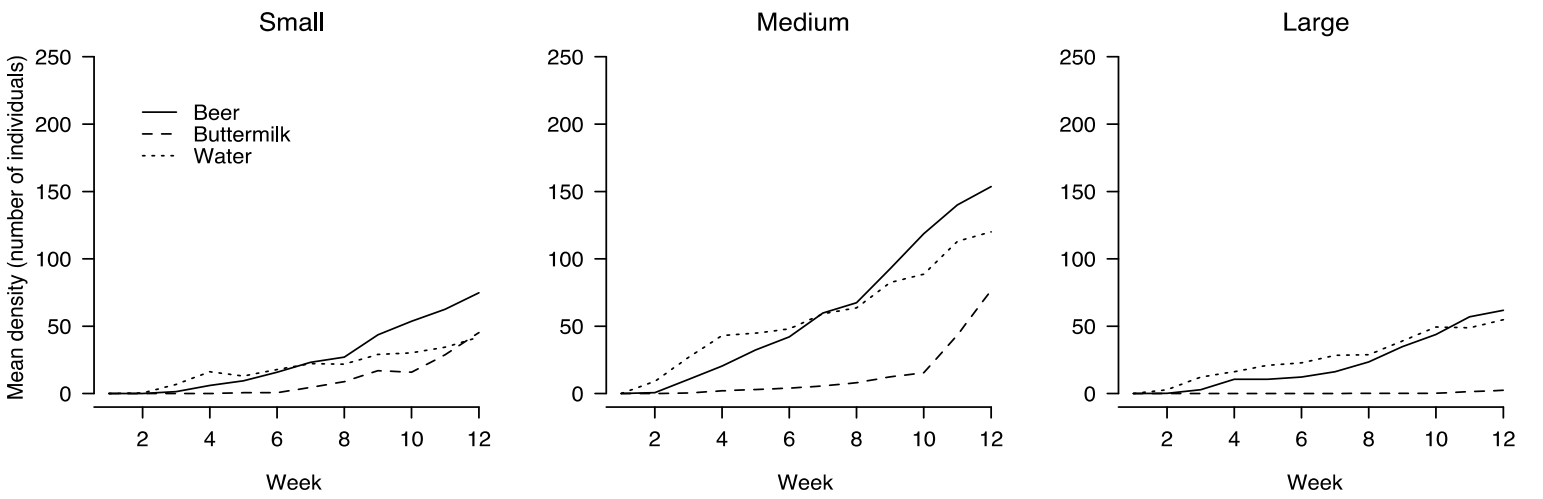

Figure 2.4. Weekly mean density (per sponge) of bryophytes in fragment size and slurry treatments (without error bars).

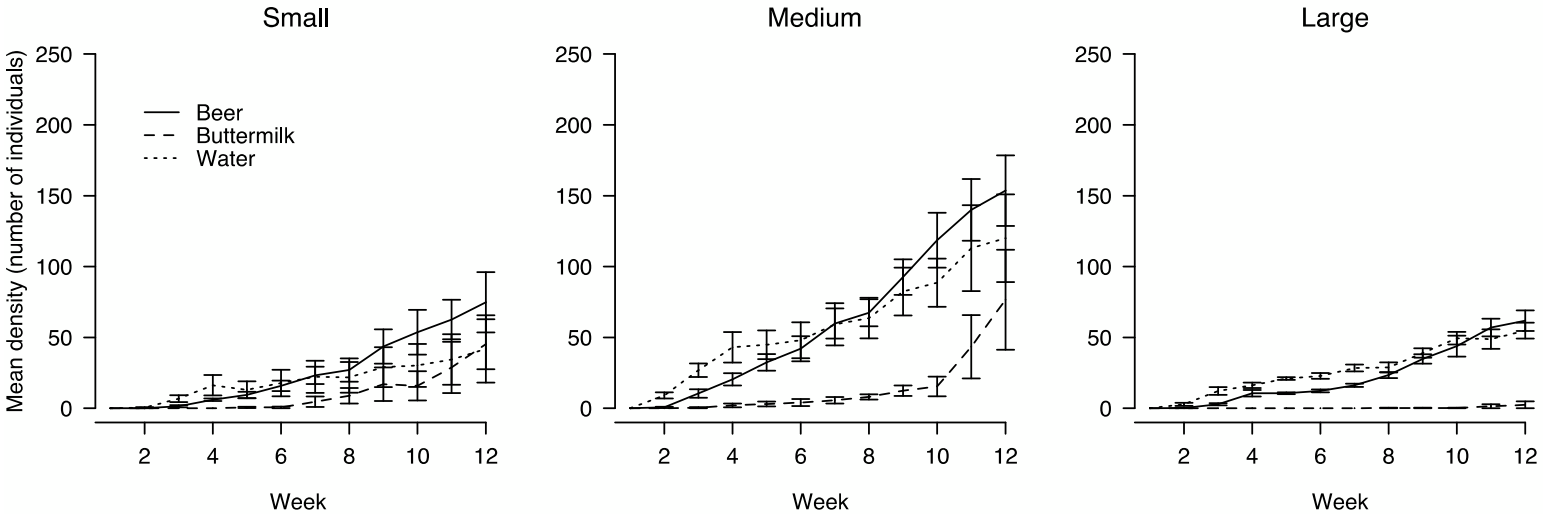

Figure 2.5. Weekly mean density (per sponge) of bryophytes in fragment size and slurry treatments (error bars are standard error of the mean).

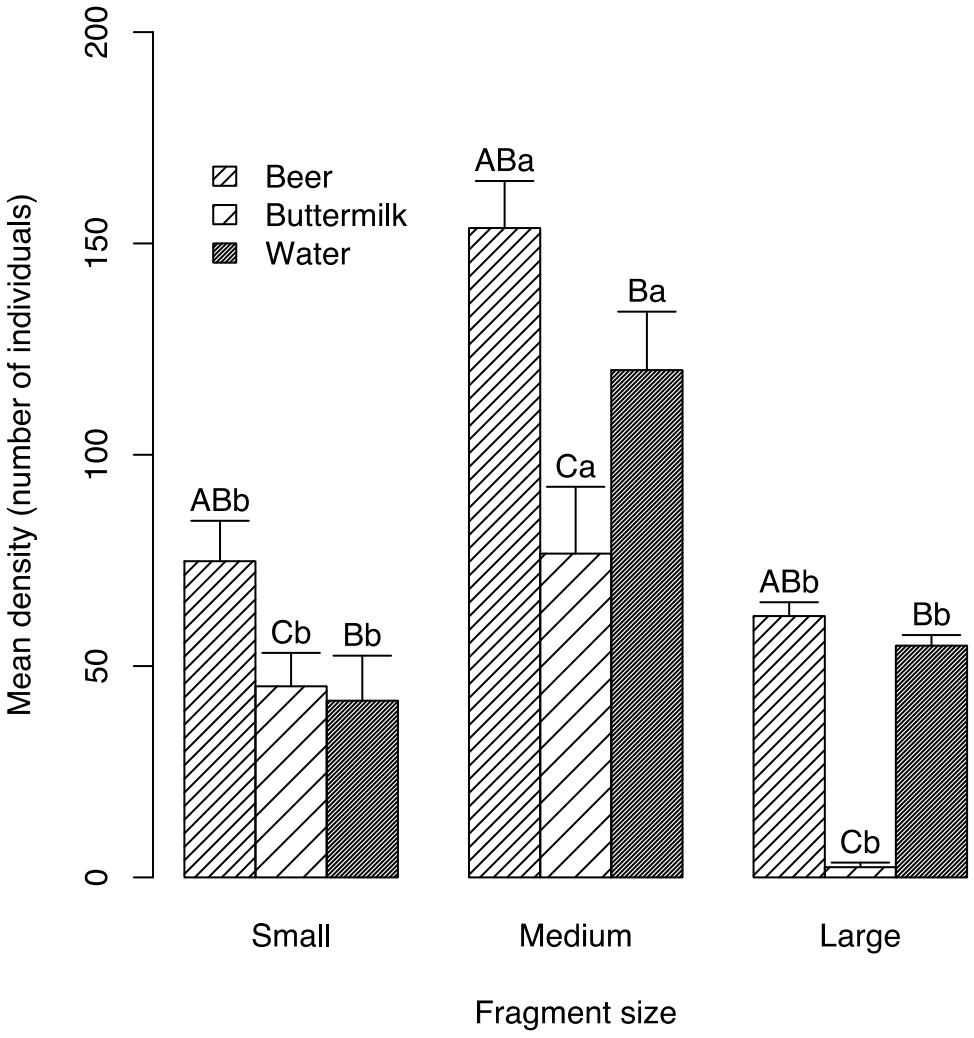

Figure 2.6. Week 12 mean density (per sponge) of bryophytes in fragment size and treatments. Different letters denote significant slurry (upper case) and fragment size (lower case) differences at p 0.05, error bars are standard error of the mean.

Table 2.1. Relative and total abundance of bryophyte species in fragment size (small, medium, large) and slurry (beer, buttermilk, water) treatments combined at beginning (week 0) and end (week 12) of the experiment.

	Relative abundance		Total abundance	
Species	Week 0	Week 12	Week 0 (g)	Week 12 (individuals)
Aulacomnium turgidum	Low	Medium	5.0	245
Bryum pseudotriquetrum		High	0.0	1328
Cephalozia sp	Low		1.4	0
Ceratodon purpureus	High	Medium	27.2	199
Cynodontium alpestre	Low		0.4	0
Funaria hygrometrica	Low	Low	1.9	1
Pohlia sp	Low		< 1.0 e^{-3}	0
Polytrichum juniperinum	Low		8.1	0
Polytrichum strictum	Medium	Low	13.8	18
Ptilidium ciliare	Low	Low	1.6	19
Racomitrium lanuginosum	High		34.8	0
Tetralophozia setiformis	Low	Low	1.4	4
Unknown	Low	High	5.3	1345
Lichens	Low		3.3	0

Blanks = not found.

Table 2.2. Weekly mean live cover of bryophytes in fragment size and slurry treatments.

		Week												
Size	Slurry	1	2	3	4	5	6	7	8	9	10	11	12	All
Small	Beer	2.0	2.0	2.0	1.6	1.8	1.2	2.2	2.2	2.2	2.2	2.6	4.0	2.2
Small	Buttermilk	0.6	0.8	1.0	0.6	0.4	0.2	0.6	0.8	1.0	1.4	3.7	8.8	1.7
Small	Water	2.8	3.0	3.6	2.6	2.0	1.8	1.8	1.6	1.8	1.8	2.4	2.6	2.3
Medium	Beer	2.6	2.2	3.0	3.8	4.4	3.4	5.0	5.0	5.2	5.4	12.2	12.2	5.4
Medium	Buttermilk	1.4	1.2	0.4	0.6	1.0	0.8	1.0	1.2	1.0	1.2	4.2	11.8	2.2
Medium	Water	6.0	6.8	8.8	8.6	5.0	4.4	4.6	4.6	5.0	4.8	8.0	9.2	6.3
Large	Beer	6.0	4.2	4.2	3.8	3.6	3.8	3.6	3.4	3.4	2.0	4.8	5.2	4.0
Large	Buttermilk	3.2	1.4	0.8	0.4	0.0	0.0	0.0	0.2	0.2	0.2	0.4	0.4	0.6
Large	Water	9.4	9.2	9.6	9.4	5.2	4.0	4.2	3.8	3.8	3.4	4.4	4.4	5.9

Table 2.3. Weekly mean density of bryophytes in fragment size and slurry treatments.

		Week												
Size	Slurry	1	2	3	4	5	6	7	8	9	10	11	12	All
Small	Beer	0.0	0.0	1.4	6.0	9.4	15.8	23.2	27.0	43.6	53.6	62.6	74.8	26.5
Small	Buttermilk	0.0	0.0	0.0	0.0	0.6	0.6	4.6	8.8	17.0	15.8	28.8	45.2	10.1
Small	Water	0.0	0.4	6.8	16.2	13.0	17.8	22.2	21.8	29.0	30.2	34.4	41.8	19.5
Medium	Beer	0.0	0.6	10.4	20.4	32.4	42.0	59.8	67.4	92.6	118.6	140.0	153.6	61.5
Medium	Buttermilk	0.0	0.0	0.4	2.0	3.0	4.0	5.6	8.0	12.4	15.4	43.4	76.6	14.2
Medium	Water	0.0	9.0	26.8	43.0	44.8	48.0	59.2	63.6	82.4	88.6	113.0	120.0	58.2
Large	Beer	0.0	0.2	2.8	10.6	10.6	12.2	16.2	23.4	34.8	43.8	57.0	61.8	22.8
Large	Buttermilk	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.2	1.4	2.4	0.4
Large	Water	0.0	2.8	12.2	16.2	21.0	22.8	28.4	28.8	39.0	49.4	48.8	54.8	27.0

Density = individuals per sponge

Table 2.4. Relative abundance of bryophyte species in fragment size and slurry treatments after 12 weeks of the experiment.

Species	Beer			Buttermilk			Water		
	Small	Medium	Large	Small	Medium	Large	Small	Medium	Large
Aulacomnium turgidum	Low	Low	Low		Low	Low	Low	Low	Low
Bryum pseudotriquetrum	Medium	High	Medium	Low	High	Medium	Low	Medium	Low
Cephalozia sp									
Ceratodon purpureus	Low	Low	Low			Low	Low	Low	Low
Cynodontium alpestre									
Funaria hygrometrica							Low		
Lichens									
Pohlia sp									
Polytrichum juniperinum									
Polytrichum strictum	Low	Low	Low				Low	Low	Low
Ptilidium ciliare	Low						Low	Low	Low
Racomitrium lanuginosum									
Tetralophozia setiformis		Low							Low
Unknown	Low	High	High	Low	Low	Low	Medium	High	Low

Blanks = not found

III. REVEGETATION OF NATIVE TUNDRA BRYOPHYTES IN CANADA AND ICELAND

1. INTRODUCTION

Development in the relatively undisturbed northern ecosystems is occurring at an unprecedented rate. Reducing the impact of this expansion, through timely reclamation of decommissioned sites, is critical for sustainable development of the north. Natural recovery is limited by short growing seasons, long, cold winters, low rainfall and slow nutrient release (Drozdowski et al. 2012, Nilsson and Aradóttir 2013, Forbes 2015) and could take 100 to 1000 years or more (Forbes and Jeffries 1999), depending on the scale and degree of impact (Lawson et al. 1978, Harper and Kershaw 1996, Davis 1998, Forbes et al. 2001).

Challenging environmental conditions and limited knowledge of ecosystem processes impede northern restoration (Cargill and Chapin 1987, Forbes and McKendrick 2002). Revegetation attempts have focused on using non native species (Forbes and McKendrick 2002), which have potential to spread and replace native vegetation. Recent research focused on native shrub and grass species introduction (Adams and Lamoureux 2005), with few attempts to establish bryophytes (Rastorfer 1978, Steere 1978, Forbes and Jeffries 1999, Adams and Lamoureux 2005, Jägerbrand et al. 2011) despite their major role in tundra ecosystems, and a belief they fill a fundamental gap (Davy 2002, Forbes and McKendrick 2002, Adams and Lamoureux 2005).

Bryophytes are critical to the function and structure of northern ecosystems (Schofield 1972, Jägerbrand et al. 2011). A number of bryophytes are pioneer species, facilitating soil and microhabitat development (Schofield 1972, Kershaw and Kershaw 1987, Klokk and Rønning 1987, Longton 1988, Adams and Lamoureux 2005, Jandt et al. 2008, Rydgren et al. 2011). They provide biomass and ground cover, reducing erosion, increasing nutrient cycling and retention and providing food for consumers and decomposers (Klokk and Rønning 1987, Longton 1988). They can form symbiotic relationships with nitrogen fixing microorganisms, trading a favourable habitat for nitrogen and growth regulators (Rodgers and Hendriksson 1976); this could be of critical importance in early stages of soil formation when substrates are void of nitrogen. Early presence of bryophytes facilitates establishment and growth of other flora, such as lichens and vascular plants (Longton 1988, Forbes and Jeffries 1999, Hilty et al. 2004, Jägerbrand et al. 2011). Bryophytes provide protection and microsites for propagule germination, important for plant assemblage establishment (Forbes and Jeffries 1999). Tundra bryophytes are important for sustaining faunal life (Pakarinen and Vitt 1974), including arctic

rodents (Batzli and Sobaski 1980, West 1982, Longton 1980, 1988) and *Rangifer tarandus* (caribou) (Thompson and McCourt 1981, Thomas and Edmonds 1983, Longton 1988). Bryophytes may provide remediation solutions for metal affected substrates, absorbing and holding metals and providing habitat for metal tolerant plants (Adams and Lamoureux 2005).

Research on bryophyte propagation for northern reclamation is lacking, with only a few studies to date. Entire blocks of diaspore material have been transplanted (Longton and Greene 1979, Bell et al. 1991, Svenson 2000, Glime 2007, Aradóttir 2012, Aradóttir and Óskarsdóttir 2013), which heavily impacts donor sites and should be avoided in sensitive ecosystems. Manual spreading of bryophyte turf material has less impact on donor sites and provides an equivalent or better outcome over time (Aradóttir 2012). Fragmentation of bryophyte turf material may be an effective alternative effective but has never been tested for use in northern reclamation.

Fragmentation encourages totipotent cells to differentiate into a meristematic state and reprogram for development of whole new organisms (La Farge et al. 2013). To emulate natural fragmentation, material can be reduced to small, dust like fragments by pulverizing or blending (McDowell 1972, Shaw 1986, Schenk 1997, Svenson 2000, McDonough 2006, Moss and Stone Gardens 2011, Apartment Therapy 2012, WikiHow 2013). Leaf detachment and wounding can promote bryophyte growth (Gemmell 1953, Longton and Greene 1979, Wilmot-Dear 1980, Miles and Longton 1990, Giordano et al. 1996, Hugonnot and Celle 2012). To produce leaf and stem fragments, bryophyte material can be grated through a mesh sieve (Shaw 1986, Schenk 1997).

Erosion control is likely to be essential for retaining bryophyte fragments in the wind swept tundra. Commonly employed straw and coconut mats are too thick for bryophytes to grow through, therefore another material will be necessary. Cheesecloth has been used in a number of bryophyte revegetation applications (McDowell 1972, Gillis 1991, Whitner 1992, Schenk 1997, Glime 2007). Cheesecloth has never been employed as an industrial erosion control material however, if effective, could provide a faster decomposing, weed free and cost effective alternative for northern reclamation.

2. RESEARCH OBJECTIVES AND HYPOTHESES

2.1. Research Objectives

The research objective is to establish early successional tundra ecosystems through bryophyte introduction. Specific research objectives are as follow.

- To determine effectiveness of three fragment sizes for bryophyte propagation and growth.
- To determine effectiveness of erosion control material for bryophyte propagation and growth.
- To assess the influence of substrates and climate on bryophyte propagation and growth.
- To develop bryophyte revegetation recommendations for use in northern reclamation.

2.2. Research Hypotheses

The following hypotheses are postulated regarding revegetation of bryophytes.

- If bryophyte fragmentation and propagation are effective means of revegetation, evidence of bryophyte growth will be detectable after one growing season.
- If appropriately selected, the right type of erosion control material will promote propagation and retention of planted bryophyte material.
- If procured from local populations, a number of bryophytes planted on a reclamation site will be adapted to conditions and will successfully colonize.
- If bryophyte communities evolve along a successional gradient, some species will be more prone to colonizing and others more suited to stable conditions.

3. MATERIALS AND METHODS

3.1. Research Site Descriptions

The Diavik Diamond Mine (hereafter referred to as Diavik) research site is located at 64°49' N 110°27' W, approximately 320 km northeast of Yellowknife in the Northwest Territories, Canada (Figure 3.1). It consists of a denuded and decommissioned area where three waste substrates, crushed rock, lake sediment and processed kimberlite, were deposited and levelled. Crushed rock is overburden from mining; lake sediment is overburden from mining under Lac de Gras; processed kimberlite is residual material from diamond removal (Table 3.1). Soil is 0.5 to 1.0 m higher than surrounding dry heath tundra to the north and wet tussock tundra to the south. Upland, dry heath is dominated by dwarf shrubs, such as willow (*Salix* sp), bog bilberry (*Vaccinium uliginosum* L.) and crowberry (*Empetrum nigrum* L.). Low lying areas are marshy with sedges and bryophytes, such as *Aulacomium turgidum* and *Dicranum groenlandicum*. Climate at Diavik is characteristic of continental polar environments (The Government of Canada 1999). The growing season occurs mainly in the months of July to August (Ecosystem Classification Group 2012).

Heiðmörk research sites are located at 64°06' N 21°75' W in southeast Iceland, approximately 10 km from Reykjavík, in a municipal conservation area. One site consists of a plateau, approximately 3 m high, which is naturally eroded by frost heaving (hereafter plateau). The second site is a relatively unused road consisting primarily of crushed lava rock, placed atop the original 5 to 15 cm thick volcanic silt loam (Russi-Colmenares 2014) (hereafter road). Both sites are located atop postglacial basaltic lava. The surrounding ecosystem consists of a dwarf shrub, moss heath dominated by large *Racomitrium lanuginosum* hummocks interspersed with resin birch (*Betula glandulosa* Michx.) shrub islands. The climate of Iceland is maritime, with mild fluctuations in mean monthly temperature (Einarsson 1984). The months of June to August are generally frost free, and winter thaws are common.

Heiðmörk and Diavik are located at the same latitude, 64° N. The sun remains below the horizon for 24 hours per day in the winter, and daylight is continuous for much of the summer (Einarsson 1984, Longton 1988). Both are polar islands, one oceanic and the other lacustrine. Their mean humidity levels over 2014 and 2015 differed by less than 3.0 % (Table 3.2). Wind speeds were also very similar, slightly higher at Heiðmörk.

The main climate differences between Diavik and Heiðmörk are precipitation and temperature. Diavik received less than half the precipitation of Heiðmörk in 2014 and 2015 (Table 3.2). The form of precipitation also differs; Diavik gets many more snow events than Heiðmörk. Snow events occurred in all months with the exception of July and August at Diavik in 2014 and 2015 (The Weather Channel 2015a), whereas May to September 2014 and June to September 2015 were without snow at Heiðmörk (The Weather Channel 2015b).

Temperature fluctuations at Diavik are more extreme than those at Heiðmörk (Table 3.2). Heiðmörk has a higher mean annual temperature, positively impacting number of growing degree days and frequency of snow melt, both important for opportunistic cryoptogam growth.

3.2. Donor Sites And Bryophyte Material Collection

Donor sites for bryophytes at Diavik and Heiðmörk were low, medium and high disturbance areas within several kilometers of the research sites. Bryophyte species collected from the donor sites are listed in Table 3.3. Collection site habitat characteristics and surrounding vegetation are provided in Tables 3.4 and 3.5.

In June 2014, bryophyte samples were separated from substrate or surrounding vegetation by hand. Microhabitat information was documented (Tables 3.4 and 3.5) and a photograph was

taken of the sample before removal. The material was placed in a marked paper bag for transport to the laboratory for identification and preparation for field application. Equal portions of donor material were taken from areas of low, medium and high disturbance to ensure diverse species composition. Sample homogeneity and species composition varied, but were representative of different microsites and levels of disturbance at those sites. At Diavik 7 L of bryophyte material was collected at Diavik and at Heiðmörk 5 L was collected.

3.3. Experimental Design

Natural microtopography of the research site was retained, except when extremely large boulders were present, or when vegetation needed to be uprooted. All vegetation in the plots was removed prior to application of propagation materials. Diavik sites were fenced by 2.5 cm^2 mesh size plastic wire attached to 0.5 m tall wooden or metal stakes. Heiðmörk sites were not fenced as the risk of animal or human disturbance was low.

The experimental design consisted of three substrates at Diavik and one at Heiðmörk. There were three fragment sizes of bryophyte material and two erosion control treatments. At the crushed rock, lake sediment and processed kimberlite sites at Diavik and the plateau site at Heiðmörk, there were ten replicates of each fragment size treatment with and without erosion control on each of the substrates, totalling 60 quadrats per substrate. The road site at Heiðmörk is half the size of the other sites, and therefore only five replicates of each fragment size treatment were assessed with and without erosion control, totalling 30 quadrats.

On each of the substrates, 4 m x 2 m plots were established, each with four rows of 50 cm^2 quadrats, separated by 20 cm. Plots were halved; one was randomly selected for erosion control material and one for no erosion control. In the middle of each 50 cm^2 quadrat, 10 cm x 10 cm areas were delineated and randomly assigned replicates and fragment size treatments.

Three fragment sizes were assessed at Diavik and Heiðmörk, each made with one third of the collected material (by volume). Large fragments (< 40 mm) were prepared by hand crumbling, approximating entire individual plants. Medium, (< 2 mm) leaf and stem sized fragments were produced by pushing material through a 1 mm sieve. Small (< 1 mm), dust or particle sized fragments were produced by grinding dried material in a standard hand held coffee grinder.

Erosion control material was natural, white, 100 % cotton, 20 threads per inch, 1 mm^2 mesh cheesecloth overlaying soil. Single layers of cloth were laid directly on the substrate. Bryophyte slurries were applied on top of this layer, and then another layer of cloth was laid, sandwiching

the bryophyte slurry. Cloth was secured with metal staples or small boulders when staples were impractical. The cloth was expected to degrade slightly and fuse to soil with time.

Bryophyte turf plugs were transplanted into the lake sediment substrate at Diavik, for observational purposes. Turf samples were collected from the vegetation surrounding the research site at Diavik using a shovel and were immediately planted into the soil by digging a small hole. Plugs were selected for their homogenous species composition. There were 8 replicates of 4 species, *Aulacomium turgidum, Ceratodon purpureus, Polytrichum* sp (included *piliferum* and *strictum*) and *Dicranum groenlandicum*, for a total of 32 plugs. Plugs were approximately 5 cm^3 and were planted 50 cm apart in a grid.

3.4. Treatment Application

After bryophyte material was separated and fragmented, it was soaked in plastic buckets with clean water for at least 20 minutes for full hydration to form a slurry. At Diavik, water was sourced from the lake (pH 5.82) to approximate practical reclamation methods. The remote diamond mine does not have a large supply of distilled water and sources all water from the lake. At Heiðmörk, distilled water (pH 5.47) from the University of Iceland laboratory was used.

The half plot for the erosion control treatment was overlaid with a single layer of cheesecloth. Quadrats were saturated with water before slurry application, to slow material desiccation and promote adherence to soil. Slurries were applied to plots, directly on cheesecloth or directly on substrate, using 10 x 10 cm square wooden frames and tablespoons to ensure homogeneity among replicates. A total of 15.0 mL (1.0 tablespoon) of slurried small and medium fragments and 22.5 mL (1.5 tablespoons) of slurried large bryophyte fragments were added to each quadrat. Volumes were determined by wet slurry material weight. As large fragments contained more airspace and compressing was not easily standardized, more of the uncompressed material was required. Slurries were spread out as evenly as possible over rocks, depressions and soil within the 10 x 10 cm quadrat. Target cover of propagation material was 80 to 90 %.

After application, all quadrats were hydrated then photographed. Plots without erosion control material were left bare, and those with the treatment were covered with a second layer of cloth.

3.5. Substrate Characterization

Soil volumetric water content, electrical conductivity and temperature sensors were installed to document surface soil conditions in the second growing season of the experiment. 5TE soil

water sensors and Em50 digital analog data loggers (Decagon Devices, United States) were installed horizontally at approximately 5 cm depth adjacent to the research plots on each substrate. Two data loggers with 5 sensors each were installed in each substrate, 1 with erosion control material covering and one bare. Logger and sensor locations were recorded and mapped. Data were logged at 30 minute intervals for the duration of the study. Data loggers were downloaded at the end of the growing season. Data spanned 31 May to 3 August 2015 (65 days) at Diavik sites and 19 June to 29 August 2015 (72 days) at Heiðmörk.

Substrates were sampled in June 2015. The upper 5 cm of soil was collected from five locations around plot peripheries and mixed in a plastic bag, for a minimum of 200 g per bag. This was repeated six times per substrate for 6 composite samples per substrate. Soil pH and electrical conductivity of substrates were assessed in the laboratory using an Oakton Portable Waterproof pH/CON 300 Meter (Oakton, United States) (Hendershot et al. 2007). The substrate composites were air dried for two days, then 10 g of soil from each was mixed with 20 mL of distilled water in a small glass beaker, stirred with a glass rod intermittently for 30 minutes and let to stand for 1 hour. The electrodes were then fully immersed into the clear supernatant and recordings were taken once readings were constant for 3 seconds. Sensors were rinsed with distilled water and calibrated before and after each measurement. Standards of pH 4.0 and 7.0 and electrical conductivity of 15000.0 µS were measured between each substrate. Data were mathematically corrected to account for calibration liquid differences. Data were averaged for each substrate.

3.6. Vegetation Assessments

Density was assessed by counting individuals in the 10 x 10 cm quadrat with a click counter. Cover of living (regenerating, green) and total (dead, alive) bryophyte material was ocularly estimated, using a bryophyte cover guide designed specifically for calibration of observations.

In June and August 2014 and 2015, a photograph was taken of each individual field plot, from a distance of approximately 20 cm. To ensure accuracy of photos, a plot measurement tool marked with exact plot locations and dimensions was used and included in the photograph. The scale included on the tool facilitated cropping of all photos to 10 cm x 10 cm during processing. All photos were labeled, cropped and edited to minimize shadows and to boost colours. Photos were analyzed using SamplePoint (Booth et al. 2006). The maximum number of points were analyzed in a 15 x 15 point grid (total 225 points). Each point was manually identified as living (green) bryophyte, dead bryophyte, unknown (living or dead) bryophyte, lichen, substrate, forb, grass, other (included leaf litter, scat) or unknown (impossible to identify).

At the final site assessment, species thought to be propagating were counted and collected with forceps and brought to the University of Alberta laboratory for identification and confirmation of regeneration. All plots with erosion control material were cut open, to assess and photograph each layer of the material. Care was taken to reduce disturbance to the plots when each layer was removed. Assessments were conducted directly on the substrate, under the two layers of erosion control cloth (hereafter under layer), and in the middle of the two layers where material was planted (hereafter middle layer). Ten 10 cm x 10 cm plots without slurry applied were randomly selected between the planted grid, on each substrate, half with erosion control and half without. Unplanted plots were assessed in the same way as planted plots.

Bryophyte volume retention was measured by comparing original and final volume of the large fragment size. The three highest visual cover quadrats were selected for analysis in all substrates, with and without erosion control. As much material as possible was collected using fine forceps, including from between and under cloth layers in erosion control plots. Samples were stored in paper envelopes and taken to the University of Alberta. They were rehydrated with distilled water and left to stand for 20 minutes, to mimic their field application. Volume was assessed in the same plastic measuring spoons that had been used for field application, with minimum compression of material.

Transplanted plugs were visually assessed upon planting in June 2014 to estimate cover of green (living) material. In August 2015, total live cover and total cover of exposed material were assessed, relative to what was planted.

3.7. Statistical Analyses

Summary statistics of all data were calculated to assess data trends. Each treatment, including fragment size, erosion control, substrate and site, was summarized individually. For live and total bryophyte cover, means were calculated, and for density totals per 10 x 10 cm quadrats were calculated. Individual species abundance was analyzed by adding the number of plots in which each individual species was found; species richness was calculated from the total species present in the unit. All of the soils data were assessed as daily means and all of the climate data were assessed as monthly means (collected daily for weather network data, hourly for Diavik meteorological station). Loss of live cover in the turf transplants was calculated by subtracting August 2015 live cover from June 2014 live cover of the transplants. Biomass volume sample data were used to calculate volume of biomass lost during the experiment, calculated as ((original volume - final volume) / original volume) x 100.

Statistical analyses were conducted in R (R Core Team 2015) to assess bryophyte fragment sizes and erosion control treatments in August 2015. Data and residuals were assessed for normality using histograms and Shapiro-Wilks tests, and transformations such as square root and logarithm were performed in an attempt to normalize non normal data. Both were tested for homogeneity of variance, using boxplots and Bartlett's test.

Since most data and residuals were non normal and of non equal variance, they were analyzed using permutational analysis of variance (permANOVA) in R. To be sure of significance of permutational output, each test was run 10 times, and most common output selected. Pairwise comparisons, Tukey's test for honest significant difference, were run in R to compare treatments within variables and build interaction plots when ANOVA showed significant interaction. Tukey's tests were conducted on digitally assessed cover data (live, total) and visually assessed density. All interaction levels were considered, site, substrate, erosion control and fragment size.

Means for visually estimated and digitally assessed cover differed by less than 5 %. Since the two did not differ significantly, and both data sets would be interpreted the same way, the less subjective digital assessments were reported.

4. RESULTS AND DISCUSSION

4.1. Substrate Characterization

Icelandic soils were more acidic than Canadian soils, as expected with their volcanic origins (Table 3.1). Processed kimberlite was most basic, followed by crushed rock and lake sediment. Sand and silt content varied among substrates, with all having sand to loamy textures. Mean volumetric water content was similar among substrates and treatments (Table 3.6). Mean soil temperatures were slightly higher at Diavik than Heiðmörk (Table 3.7). Maximum temperatures and the spread of values were greater and minimum temperatures were lower at Diavik than Heiðmörk. Erosion control material elevated mean and minimum temperatures by less than 0.5 °C in most substrates. Mean electrical conductivity was extremely low and similar among substrates and sites (Table 3.8).

4.2. Erosion Control And Substrate Effects

Small divots were observed under erosion control materials. These microtopographical variations could have significant impact on water infiltration and plant propagule retention and

germination. Erosion control material decomposition occurred only at Diavik, in 9 plots in crushed rock, 5 in lake sediment and 1 in processed kimberlite. Decomposition occurred only in the bottom layer of cloth when in direct contact with soil and had progressed so entire 5 to 7 cm^2 patches of material were entirely dissolved. No decomposition occurred at Heiðmörk.

Substrate had a significant effect on overall live and total cover (both $p < 2.2\ e^{-16}$) (Figures 3.2 to 3.4; Table 3.9). Crushed rock and road had highest live cover; total cover was highest in processed kimberlite and crushed rock, followed closely by lake sediment. Erosion control had a significant effect on overall live (p 2.2 e^{-3}) and total ($p < 2.2\ e^{-16}$) cover at Diavik, where the material remained intact (Figure 3.5; Table 3.9). At Heiðmörk the material was no longer intact; likely due to the effect of strong winds forcing the cloth to rub against the rocky substrate; the effect of the material was thus neutralized (live p 0.9, total p 0.1) (Figure 3.5).

Substrate impacted effectiveness of erosion control on total cover of plants in the middle and under layers of cloth ($p < 2.2\ e^{-16}$) and volume retained. The lowest bryophyte volume loss occurred in road without erosion control and highest occurred in processed kimberlite without erosion control (Table 3.9). The more topographically homogenous substrates (lake sediment, processed kimberlite) held a high total cover with erosion control, but did not show as much regeneration as more heterogeneous substrates (plateau, road), perhaps because these could provide more microtopographic protection from wind through heterogeneity of their surfaces (Figure 3.4). These substrates had lowest retention of bryophyte material when no erosion control was present. Crushed rock may have presented the best balance of texture; it had high live and total cover. In addition to providing protective microsites, the presence of microbiota in crushed rock, road and plateau likely had a positive impact on regeneration of bryophytes. Lake sediment and processed kimberlite are both nearly sterile, due to their origins deep underwater (lake sediment) or very far below the surface (processed kimberlite).

Erosion control had a positive effect on density in all treatments (Table 3.9); in many cases density was doubled with erosion control material. Density under the cloth, directly in contact with the soil, and density in the plots without erosion control, varied with substrate. Crushed rock had highest density of all substrates overall and under erosion control material (Figure 3.6); this was observed in the field with tight tufts of *Bryum argenteum* and *Ceratodon purpureus*. Densities in processed kimberlite and lake sediment were lowest in all erosion control treatments. Overall, substrate had a significant impact ($p < 2.2\ e^{-16}$) on density in the mid, lower and combined layers. However, when assessing sites individually, only Diavik substrates appear to have had an impact (mid, lower and combined $p < 2.2\ e^{-16}$). There was no significance

of substrate at Heiðmörk (mid and under p 1.0, combined p 0.5). The two substrates at Heiðmörk may have been too similar to provide major differences. Densities of plants in and under the erosion control material varied greatly.

The effect of erosion control on density of regenerative shoots in the middle layer of cloth was not significant overall at Diavik (p 0.5) or at Heiðmörk (p 0.1). Under the cloth, however, it was significant at both sites (Diavik p 1.1 e^{-2}, Heiðmörk p < 2.2 e^{-16}). Total densities between and under the cloth were significant at both sites (Diavik p 4.0 e^{-4}, Heiðmörk p 3.2 e^{-3}). Fragment size had a significant effect on mid layer regeneration density of plants in between the two layers of cloth (p 2.9 e^{-2}), interactions between these two treatments were highly complex. Mean density was highest with large fragments, with erosion control (Table 3.9). Lowest mean density occurred with small fragments and no erosion control.

Overall, erosion control had a positive impact on species abundance. Species abundance was highest under erosion control material, followed by no material, then the middle layer (Table 3.10), indicating the importance of direct bryophyte substrate contact. *Racomitrium lanuginosum* occurred most frequently in the middle layer, under the material and in combined layers. *Ceratodon purpureus* occurred most frequently without erosion control.

The greatest benefit of erosion control material may be for growth of colonizers and planted individuals under the material, possibly due to protection from wind erosion and dessication and higher soil water content in the substrate. Highest live cover was in an unplanted plot with erosion control material, in crushed rock substrate. Despite some growth in unplanted plots, in all substrates except processed kimberlite, that is the only instance where an unplanted plot surpassed planted plot cover. Species that occurred in plots under erosion control material, that were not planted, included the following, with their abundance in parentheses after their names; *Bryum argenteum* (7), *Bryum pseudotriquetrum* (6), *Ceratodon purpureus* (6), *Diplophyllum obtusifolium* (5), *Funaria hygrometrica* (1), *Polytrichum strictum* (4), *Racomitrium lanuginosum* (6). In unplanted plots without cloth, there were fewer colonizers, including *Bryum argenteum* (4), *Bryum pseudotriquetrum* (2), *Cephalozia* sp (2), *Ceratodon purpureus* (2), *Diplophyllum obtusifolium* (1), *Polytrichum strictum* (1), *Racomitrium fasciculare* (2) and *Racomitrium lanuginosum* (4).

Substrate significantly impacted species occurrence and diversity at both sites. Results do not appear to be related to soil temperature, pH or volumetric water content. Microtopography, nutrient concentrations and presence of microbial populations could be more important to consider. Substrate with highest number of species occurrences and highest spontaneous

colonization was crushed rock; highest species diversity was plateau (Table 3.11). The lowest number of occurrences was in lake sediment, and lowest diversity was in processed kimberlite, both substrates considered to be nearly sterile. There were no colonizers in either lake sediment or processed kimberlite. Three species successfully propagated in all five substrates: *Bryum pseudotriquetrum*, *Ceratoron purpureus* and *Racomitrium lanuginosum*. *Bryum pseudotriquetrum* (Olech and Massalski 2001) and *Ceratoron purpureus* (Kershaw and Kershaw 1987, Olech and Massalski 2001) are known to colonize disturbed tundra.

Success of each species on a given substrate is dependant on its related preference to microhabitat (Table 3.12). *Bartramia ithyphylla*, *Dicranum scoparium*, *Diplophyllum albicans*, *Fissidens* sp, *Ptilidium ciliare*, *Racomitrium canescens*, *Rhytidiadelphus loreus*, *Tortella tortuosa* prefer low disturbance microhabitats. They were collected from medium and low disturbance sites at Heiðmörk (Table 3.3). *Sphagnum capillifolium* and *warnstorfii* are peatland species, and their likelihood of survival was considered low on these substrates. They were collected from a low disturbance wetland at Diavik and added in expectation they would help hold soil water for other bryophytes.

Polytrichum juniperinum is a pioneer species adapted to exposed, acidic soils (Table 3.12). It was collected at high, medium and low disturbance sites at Heiðmörk (Table 3.3). It is interesting that it did not flourish on the research site, and it may propagate more successfully from sexual reproduction than fragmentation. *Tetralophozia setiformis* was collected at Diavik medium and low disturbance sites mixed with *Ptilidium ciliare* and other moss species. It is common on exposed rocks (Atherton et al. 2010), but likely did not fare well without the protective presence of other mosses and liverworts.

Five bryophytes species without previous detection in initially planted samples successfully colonized the research sites. *Bryum argenteum* is widespread in disturbed habitats that may become very dry and are rich in nutrients (Atherton et al. 2010). It was found at Diavik, in crushed rock and lake sediment substrates (Table. 3.11). Its spread and abundance indicates it was probably dispersed by wind blown spores. *Diplophyllum obtusifolum* is a liverwort known to be a pioneer of open, crumbling acidic soil (Atherton et al. 2010). It was found on both substrates at Heiðmörk. Number of individuals of this plant may have been overestimated, as it propagates in a rosette of plants emanating from a single spore (Atherton et al. 2010). *Polytrichum piliferum* is a known colonizer, especially in exposed, loose, dry and acidic substrates; *Polytrichum strictum* is usually found in open, damp, peaty areas (Atherton et al. 2010); both occurred infrequently at Heiðmörk.

4.3. Fragment Size Effects

Fragment size had a significant effect on live ($p < 2.2\ e^{-16}$) and total cover ($p < 2.2\ e^{-16}$) at both Diavik and Heiðmörk. Large fragments had significantly highest mean live and total cover after two growing seasons, followed by medium, then small (Figures 3.2, 3.3; Table 3.13). Large fragment size had highest density with erosion control; however, without erosion control medium fragments had highest density. Medium fragments were sifted through the erosion control material and may have been unable to access enough sunlight or water to propagate.

Fragment size had an impact on species abundance, which was highest in medium fragments, followed by large and small fragments (Table 3.14). The species occurring most frequently in small and large fragments was *Ceratodon purpureus*, *Racomitrium lanuginosum* was slightly higher in medium fragments. Protonema was produced in a few plots in small and medium fragments, but not large fragments.

Large fragments had higher total retention on substrates, contributing to their higher cover. Large fragments were likely more susceptible to wind displacement, whereas medium fragments could find protection in micro depressions in the substrate. They may have had quickest regeneration potential, or better capacity to remain hydrated and retain nutrients. Large fragments were more likely to be detected with digital or visual analysis. Small and medium fragments were more likely to have been transported into crevices or under rocks where they would not be counted.

Both sites and their substrates had very complex interaction effects on live and total cover ($p < 2.2\ e^{-16}$). Substrate texture impacted retention and regeneration of different sizes of fragments. Therefore climate, parent material and species present at a site all represent important factors in the success of bryophyte propagation in the field, measured in regeneration and retention of fragment sizes. The interplay between substrate and fragment size is likely affected by species specific substrate and diaspore colonization adaptations.

4.4. Site And Climate Effects

Mean live cover of bryophytes was significantly higher at Heiðmörk (2.2 %) than at Diavik (1.9 %) ($p < 2.2\ e^{-16}$). Higher total precipitation and mean temperature at Heiðmörk (Table 3.1) likely helped accelerate regeneration and or increase survival of planted bryophytes.

Mean total cover of bryophytes was significantly higher at Diavik (25.9 %) than at Heiðmörk (9.5 %) ($p < 2.2\ e^{-16}$). One climate difference that may have impacted total retention is wind.

Maximum wind speeds at Heiðmörk were much higher than at Diavik (Table 3.1). Wind speed varies depending on landscape location and microsite, and from an observational perspective, it was always much windier at plateau than at road or any other site. Wind velocity likely had an impact on volume redistribution, translocating materials from flat surfaces deeper into crevices, although differences in volume do not occur between the sites.

Total density of plants at Diavik (8073 individuals) were more than double that at Heiðmörk (2932 individuals). Most of these individuals were found under erosion control material. Site had a weakly significant impact on density in the mid layer (p 2.0 e^{-2}) but not the under layer (p 1.0). Total density was likely highly dependent on total material retention, therefore, higher total densities in the middle layer at Diavik may be related to higher total retention there. The higher density under erosion control material and without it may be related to the effect of substrate. The under layer was generally dominated by site specific pioneer species, whose capacity for colonization is adapted to climate conditions.

The species that occurred most frequently at Diavik was *Ceratodon purpureus*; *Racomitrium lanuginosum* was most common at Heiðmörk (Table 3.11). *Ceratodon purpureus* is a known colonizer (Kershaw and Kershaw 1987, Olech and Massalski 2001, Atherton et al. 2010); *Racomitrium lanuginosum* propagates by fragmentation (Magnúsdóttir and Aradóttir 2011).

Of the 18 species originally planted at Diavik, 9 regenerated in addition to one colonizer not found in the original samples, *Bryum argenteum* (Table 3.15). At Heiðmörk, 11 of 21 species planted were growing with the addition of 5 species that were not planted (*Diplophyllum obtusifolium*, *Polytrichum commune*, *piliferum* and *strictum* and *Rhytidium rugosum*).

Five species were planted and grew at both sites: *Bryum pseudotriquetrum*, *Ceratodon purpureus*, *Hylocomium splendens*, *Racomitrium lanuginosum* and *Sanionia uncinata* (Table 3.15). These species could be targeted as successful propagators. Since the species were collected and planted in their original climate, species presence or absence at Diavik and Heiðmörk is likely more related to the absence of their specific microhabitat, substrate, or propagation methods than to any climate variations between the two.

4.5. Turf Plug Transplant Effects

None of the bryophytes in the transplanted plugs spread to the adjacent substrate. Live cover loss was least for *Ceratodon purpureus* (64.1 %), followed by *Polytrichum* sp (74.9 %) and *Dicranum groenlandicum* (82.3 %). The greatest live cover loss occurred in *Aulacomium*

turgidum plugs (86.9 %). Species with highest survival were more adapted to dry conditions, whereas the two with lowest survival were from wetter habitats. New growth was difficult to ascertain but suspected in three plots, one in each of *Ceratodon purpureus*, *Polytrichum* sp and *Dicranum groenlandicum*.

Growth of fragmented materials was more successful than propagation of transplanted plugs. In the two growing seasons, turf transplants did not spread and produced very little new growth. Poor growth of the plugs may have been due to unfavourable substrate conditions and could have been more productive in crushed rock, road or plateau.

4.6. Reclamation Applications

Bryophyte propagation occurred with all fragment size, erosion control and substrate treatments at research sites in both Canada and Iceland. Our research supports the statement that any treatment will likely improve bryophyte reintroduction relative to natural revegetation in northern ecosystems (Grettarsdóttir et al. 2004).

When collecting bryophyte material for propagation, it is important to focus on habitats that are similar to the one being revegetated. Collecting some soil along with bryophyte material would likely be beneficial. A small amount of collected bryophyte material can go far when fragmented. Collecting in handfuls and avoiding sensitive areas and species can minimize the impact to donor sites. The exception to this would be in cases where large, bryophyte vegetated areas are being cleared for development, where as much material as possible should be harvested. Turf plug transplantation was not successful in the lake sediment substrate at Diavik, but may have been on a more fertile substrate.

The large and medium fragments have the most regrowth potential. Large fragments produced greater live and total cover; medium fragments produced higher density, species occurrence and species diversity when in direct contact with substrate. Some amount of fragmentation could be beneficial for propagating bryophytes. A rough chop, emulating a mixture of medium (< 2 mm) and large (< 40 mm) fragments, could provide a variety of sizes of propagules, some larger and smaller. Placing this material directly on soil and covering with a light erosion control material could yield a better outcome than using the double layer method that was tested.

Erosion control material had a moderating effect on soil volumetric water content and temperature. Erosion control material promoted bryophyte colonization in all but the least favourable substrate. The effect of erosion control on cover and species occurrence was

positive but varied according to a number of factors, including fragment size and substrate. The most positive impacts of erosion control material occur when it is intact and in direct contact with substrate and bryophytes. Material decomposition did begin within the two growing seasons, but only at Diavik and only in the lower layer, which was directly in contact with soil. Cheesecloth erosion control was beneficial in this experiment, and is recommended for areas where erosion is a concern. The benefits of cheesecloth include affordability, ease of transportation (light, compact), light colour (important in northern ecosystems for conservation of permafrost) and relatively rapid decomposition (important in northern ecosystems where decomposition processes are slow).

Substrate and environment will vary with reclamation project, and methods should address site specific challenges. Topographically flat substrates had high total cover, but low regeneration. These substrates are both likely nearly sterile, due to their origins deep underwater (lake sediment) or very far below the surface (processed kimberlite). Promoting soil fertility and development of a microbial community could be beneficial. Substrates with more heterogeneous surfaces (rocky, not sandy) had higher live cover, volume retention, density and spontaneous colonization. These substrates provide more micro topographic protection from wind due to the natural heterogeneity of their surfaces. Building soils that are texturally heterogeneous (1 to 5 cm surface variability) would be ideal for bryophyte revegetation.

Methods for bryophyte propagation that were used in this research could be used in reclamation on targeted sensitive areas to produce patches of vegetated islands from which more plants can spread, when resources are limited or disturbed area is small. They could be used to blanket entire areas when resources permit or a short timeline for reclamation is expected. Either of these bryophyte application approaches would likely facilitate at least short term propagation of bryophytes with great potential for longer term stability and continued growth and development, not only of the bryophytes, but also of later successional communities.

5. CONCLUSIONS

The novel methods of bryophyte propagation for revegetation assessed in this study promoted propagation and growth of bryophytes at Diavik Canada and Heiðmörk Iceland. Large (< 40 mm) and medium (< 2 mm) size fragments had the highest regrowth potential. Retention and live cover were higher after two growing seasons with large fragments, whereas medium fragments yielded higher density, species abundance and species diversity when in direct

contact with substrate. A combination of the two would likely be ideal for reclamation. *Ceratodon purpureus* was the most frequent species at Diavik and *Racomitrium lanuginosum* was most frequent at Heiðmörk.

Cheesecloth has great potential for future use as an erosion control material in northern ecosystems. The material minimized fluctuations in soil water content and temperature and promoted the most bryophyte colonization when it remained intact and in direct contact with substrate and bryophytes.

The more topographically homogeneous substrates (lake sediment, processed kimberlite) had high total bryophyte cover, but did not show much regeneration. Substrates with more heterogeneous surfaces (crushed rock, plateau, road) yielded higher live cover, volume retention, density and spontaneous colonization by providing protection from wind.

Effectiveness of fragment size and erosion control differed slightly due to site related factors such as climate (wind, temperature, precipitation), bryophyte species collected and substrate parent material.

Figure 3.1. Map of Diavik Canada and Heiðmörk Iceland research site locations (adapted from Google Maps).

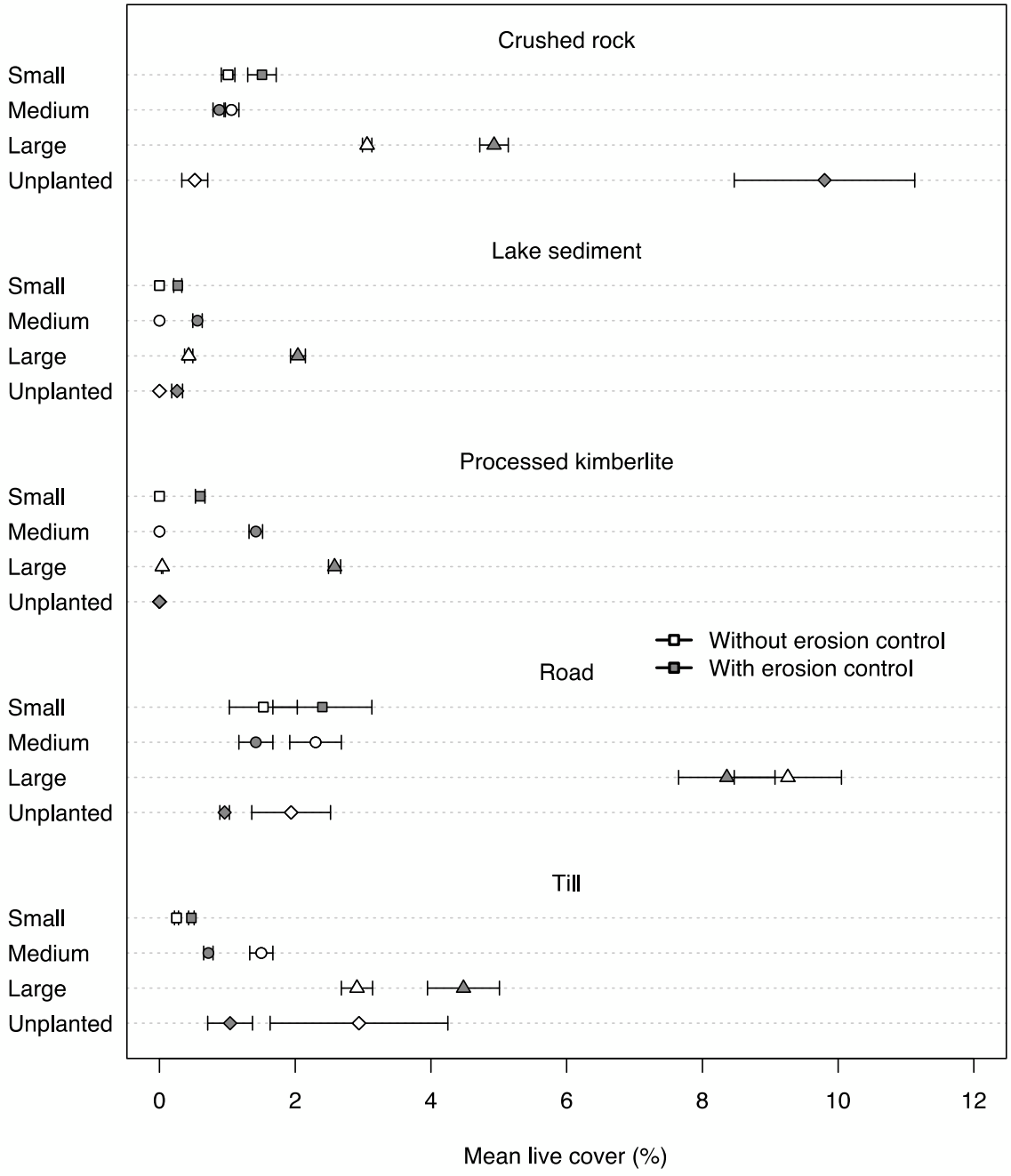

Figure 3.2. Mean live cover for fragment size, erosion control and substrate treatments at Diavik Canada (crushed rock, lake sediment, processed kimberlite) and Heiðmörk Iceland (road, plateau) research sites in August 2015. Error bars are standard error of the mean.

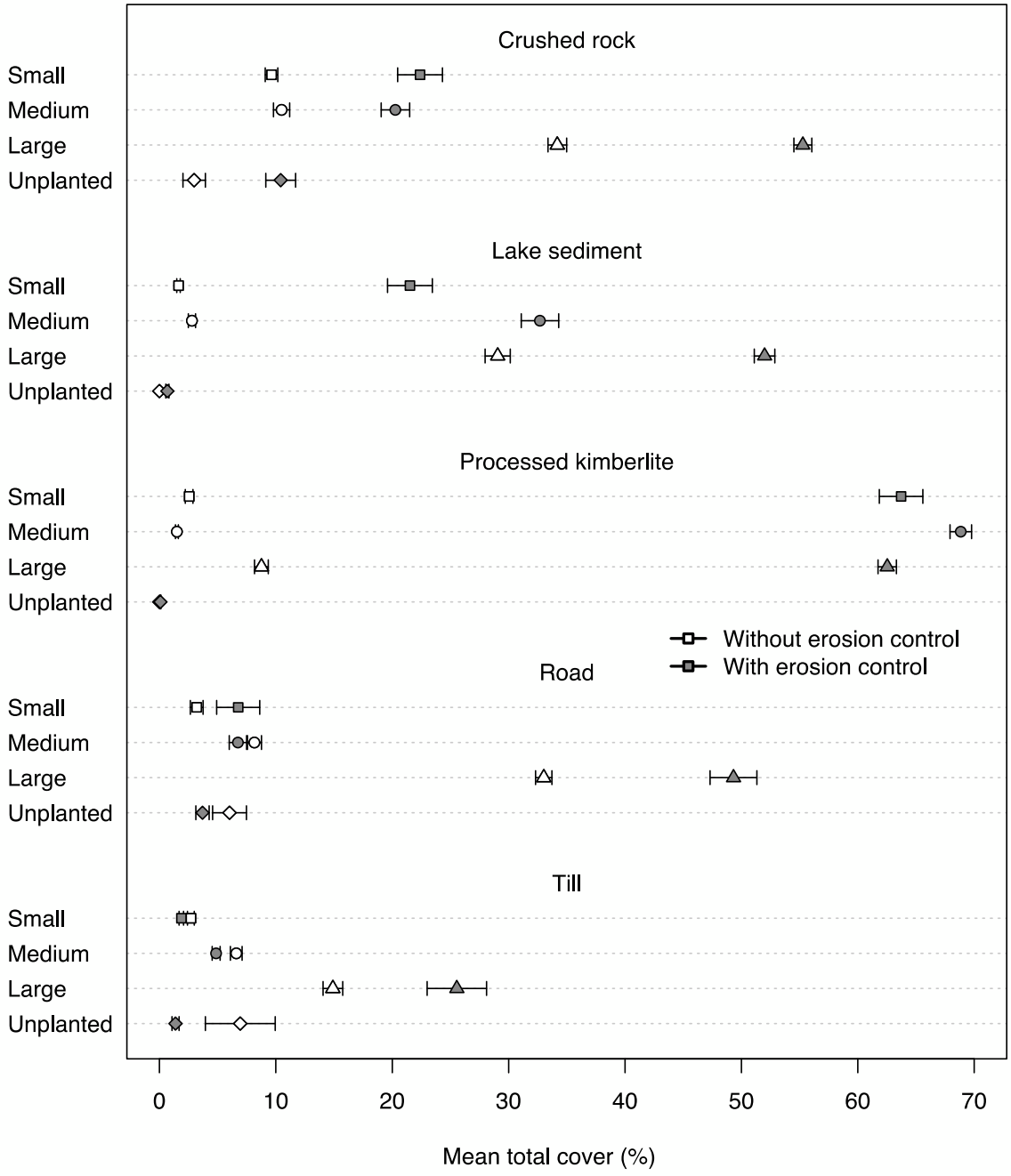

Figure 3.3. Mean total cover for fragment size, erosion control and substrate treatments at Diavik Canada (crushed rock, lake sediment, processed kimberlite) and Heiðmörk Iceland (road, plateau) research sites in August 2015. Error bars are standard error of the mean.

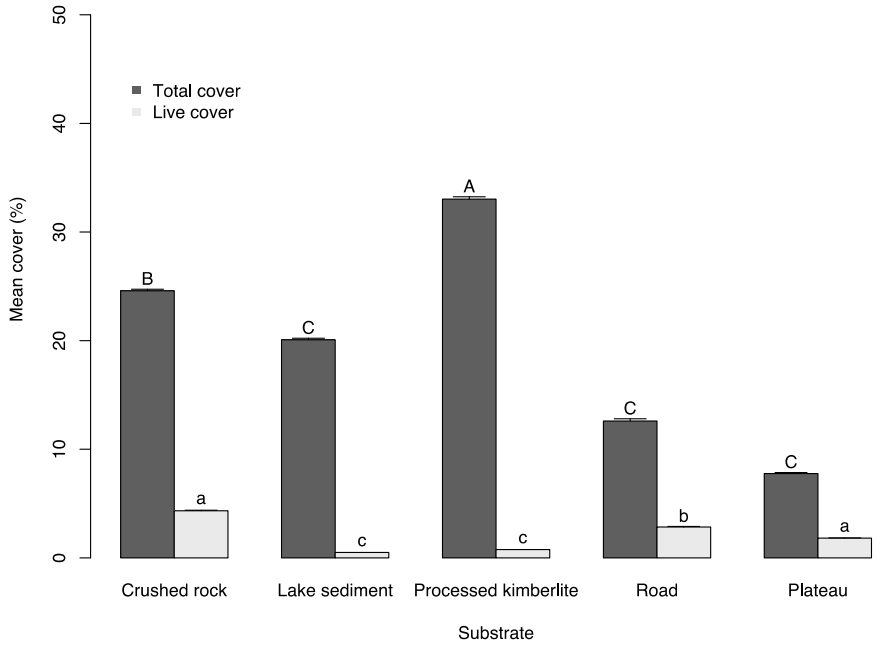

Figure 3.4. Mean total and live cover on substrates at Diavik Canada (crushed rock, lake sediment, processed kimberlite) and Heiðmörk, Iceland (road, plateau) research sites in August 2015. Error bars are standard error of the mean, letters denote significant differences in total (upper case) and live (lower case) cover at p 0.05.

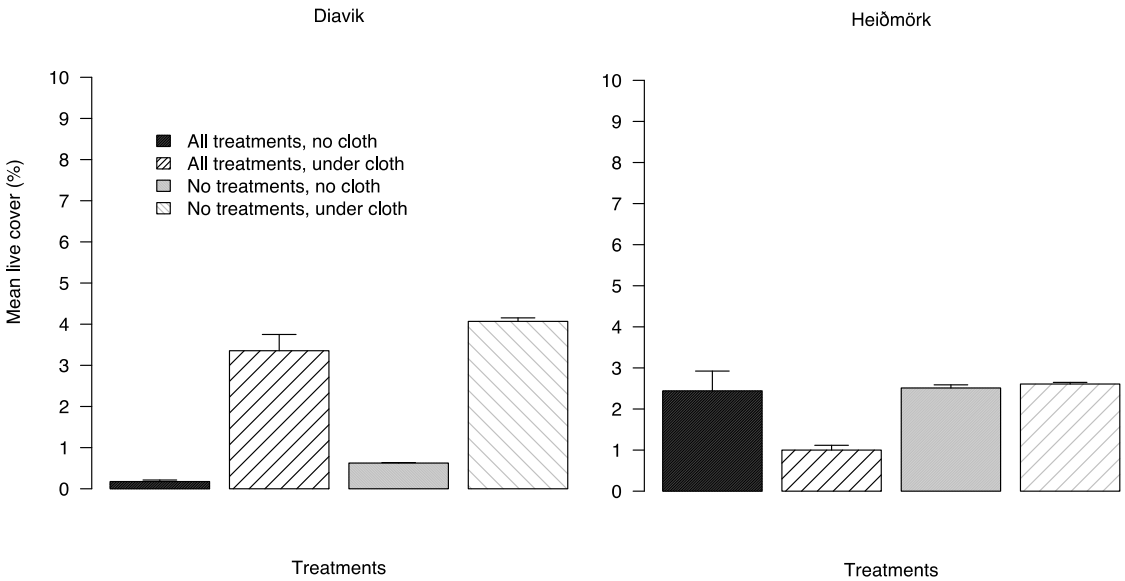

Figure 3.5. Mean live cover under erosion control material, without erosion control treatment and in unplanted areas at Diavik Canada and Heiðmörk Iceland research sites. Error bars are standard error of the mean.

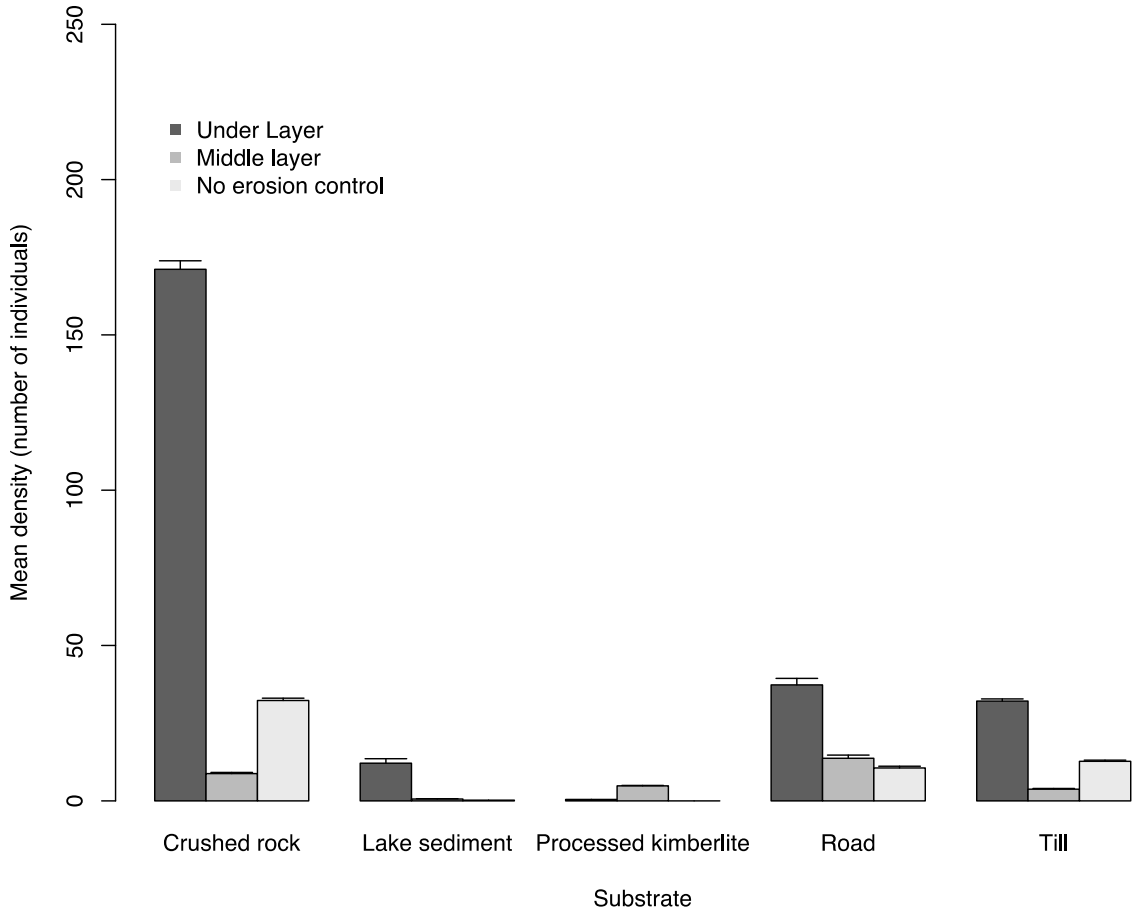

Figure 3.6. Mean density (per 10 x 10 cm quadrat) under and in the middle of erosion control material layers and without erosion control for substrates at Diavik Canada (crushed rock, lake sediment, processed kimberlite) and Heiðmörk Iceland (road, plateau) research sites in August 2015. Error bars are standard error of the mean.

Table 3.1. Properties of substrates at Diavik Canada and Heiðmörk Iceland research sites.

Site	Substrate	PH	% Sand	% Silt	% Clay	Texture
Diavik	Crushed rock	7.3	87.1	10.0	2.9	Sand, loamy sand
Diavik	Lake sediment	6.4	66.9	27.7	5.4	Sandy loam
Diavik	Processed kimberlite	8.5	88.2	8.2	3.6	Sand
Heiðmörk	Road	5.3	81.0	16.0	3.0	Loamy sand
Heiðmörk	Plateau	5.6	48.0	48.0	4.0	Sandy loam

Table 3.2. Climate characterization of Diavik Canada and Heiðmörk Iceland in 2014 and 2015.

Climatic parameter	Year	Diavik	Heiðmörk
Average temperature	2014	-9.4 °C	5.8 °C
	2015	-6.5 °C	5.1 °C
Maximum temperature	2014	27.5 °C	19.0 °C
	2015	25.2 °C	21.0 °C
Minimum temperature	2014	-38.3 °C	-10.0 °C
	2015	-39.6 °C	-10.0 °C
Total precipitation	2014	117.5 mm	396.5 mm
	2015	156.9 mm	295.9 mm
Average monthly precipitation	2014	0.1 mm	1.1 mm
	2015	2.0 e^{-2} mm	1.0 mm
Maximum total monthly precipitation	2014	33.4 mm (July)	79.5 mm (December)
	2015	81.1 mm (August)	58.7 (March)
Mean relative humidity	2014	70.0 %	72.4 %
	2015	70.1 %	72.2 %
Mean annual wind speed	2014	19.6 km h^{-1}	17.4 km h^{-1}
	2015	19.7 km h^{-1}	18.2 km h^{-1}
Maximum wind speed	2014	84.0 km h^{-1}	87.0 km h^{-1}
	2015	84.0 km h^{-1}	108.0 km h^{-1}

Adapted from DDMI 2015 (Diavik) and The Weather Channel 2015b (Heiðmörk).

Table 3.3. Bryophyte species collected at high, medium and low disturbance areas near Diavik Canada and Heiðmörk Iceland.

Species	Diavik			Heiðmörk		
	High	Medium	Low	High	Medium	Low
Aulacomium turgidum	x	x	x			
Bartramia ithyphylla					x	x
Brachythecium albicans				x		
Bryum pseudotriquetrum	x	x	x	x	x	
Calliergon richardsonii			x			
Cephalozia sp		x	x			x
Ceratodon purpureus	x		x	x	x	
Dicranum fulvum				x		
Dicranum groenlandicum	x	x	x			
Dicranum scoparium	x				x	
Diplophyllum albicans						x
Fissidens sp						x
Hylocomium splendens			x	x	x	x
Pleurozium schreberi			x		x	x
Polytrichum juniperinum				x	x	x
Polytrichum piliferum	x		x			
Polytrichum strictum	x	x	x			
Ptilidium ciliare	x	x	x			x
Racomitrium canescens					x	x
Racomitrium fasciculare				x		x
Racomitrium lanuginosum		x	x	x	x	x
Rhytidiadelphus loreus						x
Rhytidiadelphus squarrosus				x	x	
Rhytidiadelphus triquetrus						x
Rhytidium rugosum		x	x			
Sanionia uncinata	x			x	x	x
Sphagnum capilifolium			x			
Sphagnum warnstorfii			x			
Tetralophozia setiformis		x	x			
Tortella tortuosa						x

Table 3.4. Habitat characteristics and plant species at high, medium and low disturbance bryophyte collection sites near Diavik Canada.

Low Disturbance	Uphill, pristine dwarf shrub heath tundra several km from anthropogenic impact on an island near diamond mining operations. Some low lying areas were collected near a lake.		
	Arctostaphylos rubra	(Rehder & Wilson) Fernald	Bearberry
	Betula glandulosa	Michx.	Resin birch
	Carex sp	L.	Sedge
	Empetrum nigrum	L.	Crowberry
	Koeleria macrantha	(Ledeb.) J.A. Schultes	June grass
	Ledum groenlandicum	Oeder	Labrador tea
	Salix sp	L.	Willow
	Vaccinium vitis-idaea	L.	Lingonberry
Medium Disturbance	Rocky dwarf shrub heath in proximity to diamond mining operations, characterized by rock lichen communities on exposed till boulders and bedrock, with seepage zones supporting localized wetlands of sedge, moss and lowland dwarf shrubs.		
	Arctostaphylos rubra	(Rehder & Wilson) Fernald	Bearberry
	Betula glandulosa	Michx.	Resin birch
	Carex sp	L.	Sedge
	Empetrum nigrum	L.	Crowberry
	Ledum groenlandicum	Oeder	Labrador tea
	Lichen		Lichen
	Poa sp	L.	Bluegrass
	Vaccinium uliginosum	L.	Blueberry
	Vaccinium vitis-idaea	L.	Lingonberry
High Disturbance	At edges of an access road, with sparsely vegetated sand and gravel.		
	Betula glandulosa	Michx.	Resin birch
	Carex sp	L.	Sedge
	Empetrum nigrum	L.	Crowberry
	Epilobium angustifolium	L.	Fireweed
	Ledum groenlandicum	Oeder	Labrador tea
	Poa sp	L.	Bluegrass

Table 3.5. Habitat characteristics and plant species at high, medium and low disturbance bryophyte collection sites near Heiðmörk Iceland.

Low	Bouldery lava field, at least 20 m from anthropogenic impact.		
Disturbance	*Carex* sp	L.	Sedge
	Empetrum nigrum	L.	Crowberry
	Lichen		Lichen
	Picea glauca	(Moench) Voss	White spruce
	Racomitrium	(Hedw.) Brid.	Racomitrium moss
	Salix sp	L.	Willow
	Vaccinium uliginosum	L.	Blueberry
Medium	Near a secondary road, in small protected ditch.		
Disturbance	*Equisetum* sp	L.	Horsetail
	Lupinus nootkatensis	Donn ex Sims	Nootka lupine
	Picea glauca	(Moench) Voss	White spruce
	Poa sp	L.	Bluegrass
	Salix sp	L.	Willow
	Taraxacum officinale	F.H. Wigg	Dandelion
	Equisetum sp	L.	Horsetail
High	High traffic gravel parking lot and walking path areas.		
Disturbance	*Carex* sp	L.	Sedge
	Equisetum sp	L.	Horsetail
	Galium sp	L.	Bedstraw
	Lupinus nootkatensis	Donn ex Sims	Nootka lupine
	Phleum pratense	L.	Timothy
	Poa sp	L.	Bluegrass
	Potentilla sp	L.	Cinquefoil
	Taraxacum officinale	F.H. Wigg	Dandelion
	Trifolium sp	L.	Clover

Table 3.6. Soil volumetric water content with and without erosion control treatment on substrates at Diavik Canada (crushed rock, lake sediment, processed kimberlite) and Heiðmörk Iceland (road, plateau) research sites from June to August 2015.

Substrate	Erosion control	Mean ($m^3\ m^{-3}$)	Standard deviation ($m^3\ m^{-3}$)	Maximum ($m^3\ m^{-3}$)	Minimum ($m^3\ m^{-3}$)	Spread ($m^3\ m^{-3}$)
Crushed rock	No	0.1	$1.8\ e^{-02}$	0.2	0.1	0.2
Crushed rock	Yes	0.1	$1.8\ e^{-02}$	0.3	0.0	0.2
Lake sediment	No	0.1	$1.7\ e^{-02}$	0.4	0.0	0.3
Lake sediment	Yes	0.1	$1.6\ e^{-02}$	0.3	0.1	0.3
Processed kimberlite	No	0.2	$5.3\ e^{-03}$	0.2	0.1	0.1
Processed kimberlite	Yes	0.2	$8.7\ e^{-03}$	0.2	0.1	0.1
Road	No	0.1	$8.7\ e^{-03}$	0.2	0.1	0.1
Road	Yes	0.1	$7.8\ e^{-03}$	0.2	0.1	0.1
Plateau	No	0.2	$1.2\ e^{-02}$	0.3	0.1	0.1
Plateau	Yes	0.2	$1.0\ e^{-02}$	0.2	0.1	0.1

Table 3.7. Mean soil temperature with and without erosion control treatment on substrates at Diavik Canada (crushed rock, lake sediment, processed kimberlite) and Heiðmörk Iceland (road, plateau) research sites from June to August 2015.

Substrate	Erosion control	Mean (°C)	Standard deviation (°C)	Maximum (°C)	Minimum (°C)	Spread (°C)
Crushed rock	No	13.7	4.3	30.6	0.3	30.3
Crushed rock	Yes	13.4	4.2	29.6	0.2	29.4
Lake sediment	No	13.0	4.3	28.3	0.1	28.2
Lake sediment	Yes	13.1	4.1	27.2	0.3	26.9
Processed kimberlite	No	13.5	4.2	26.7	0.4	26.3
Processed kimberlite	Yes	14.1	4.0	29.5	0.5	29.0
Road	No	12.2	1.5	20.1	5.8	14.3
Road	Yes	12.5	1.5	23.0	6.5	16.5
Plateau	No	11.1	1.0	15.6	6.0	9.6
Plateau	Yes	11.4	1.0	15.8	6.4	9.4

Table 3.8. Mean soil electrical conductivity with and without erosion control treatment on substrates at Diavik Canada (crushed rock, lake sediment, processed kimberlite) and Heiðmörk Iceland (road, plateau) research sites from June to August 2015.

Substrate	Erosion control	Mean (dS cm^{-1})	Standard deviation (dS cm^{-1})	Maximum (dS cm^{-1})	Minimum (dS cm^{-1})	Spread (dS cm^{-1})
Crushed rock	No	6.4 e^{-03}	3.1 e^{-03}	0.1	0.0	0.1
Crushed rock	Yes	7.7 e^{-03}	6.2 e^{-03}	0.2	0.0	0.2
Lake sediment	No	0.1	0.1	2.0	0.0	2.0
Lake sediment	Yes	0.1	0.1	1.3	0.0	1.3
Processed kimberlite	No	0.1	1.6 e^{-02}	0.4	1.0 e^{-02}	0.4
Processed kimberlite	Yes	0.0	1.1 e^{-02}	0.3	0.0	0.3
Road	No	0.0	0.0	0.0	0.0	0.0
Road	Yes	4.2 e^{-04}	1.1 e^{-04}	1.0 e^{-02}	0.0	1.0 e^{-02}
Plateau	No	4.2 e^{-03}	1.7 e^{-03}	2.0 e^{-02}	0.0	2.0 e^{-02}
Plateau	Yes	3.5 e^{-03}	1.3 e^{-03}	1.0 e^{-02}	0.0	1.0 e^{-02}

Table 3.9. Mean live and total cover, volume loss and density of substrates at Diavik Canada (crushed rock, lake sediment, processed kimberlite) Heiðmörk Iceland (road, plateau) research sites in August 2015.

Substrate	Erosion control	Live cover (%)		Total cover (%)		Volume lost (%)	Density (number of individuals)	
		Mean	Standard error	Mean	Standard error		Mean	Standard error
Crushed rock	No	1.7	4.0 e^{-02}	18.1	0.4	31.9	33.8	0.9
Crushed rock	Yes	5.6	8.0 e^{-02}	31.5	0.2	33.3	56.8	1.0
Lake sediment	No	0.1	1.0 e^{-02}	11.2	0.5	51.9	0.2	0.0
Lake sediment	Yes	0.7	1.0 e^{-02}	27.5	0.2	33.3	5.0	0.4
Processed kimberlite	No	1.0 e^{-2}	0.0	4.3	0.2	93.0	0.0	0.0
Processed kimberlite	Yes	1.2	2.0 e^{-02}	50.0	0.3	25.2	2.1	0.1
Road	No	4.6	0.3	15.6	1.0	7.4	9.3	0.5
Road	Yes	3.3	8.0 e^{-02}	16.2	0.4	22.2	21.6	0.8
Plateau	No	1.6	6.0 e^{-02}	8.1	0.3	35.6	12.6	0.4
Plateau	Yes	2.1	3.0 e^{-02}	9.0	0.2	20.7	11.1	0.2

Density = individuals per 10 x 10 cm quadrats

Table 3.10. Species abundance with and without erosion control treatment at Diavik Canada and Heiðmörk Iceland research sites in August 2015.

Species	With erosion control			Without erosion control
	Middle layer	Under layer	All layers	
Aulacomium turgidum	9	2	11	8
Bartramia ithyphylla				
Brachythecium albicans		2	2	2
Bryum argenteum	4	35	39	25
Bryum pseudotriquetrum	5	17	22	16
Calliergon richardsonii				
Cephalozia sp				
Ceratodon purpureus	37	50	87	40
Dicranum fulvum	1	2	3	1
Dicranum groenlandicum	2		2	1
Dicranum scoparium				
Diplophyllum albicans				
Diplophyllum obtusifolium	2	24	26	7
Fissidens sp				
Funaria hygrometrica				
Hylocomium splendens	3	1	4	
Pleurozium schreberi	2	14	16	10
Polytrichum commune	2		2	
Polytrichum juniperinum				
Polytrichum piliferum		1	1	1
Polytrichum strictum		8	8	2
Ptilidium ciliare	1		1	
Racomitrium canescens				
Racomitrium fasciculare	8	14	22	23
Racomitrium lanuginosum	44	32	76	33
Rhytidiadelphus loreus				
Rhytidiadelphus squarrosus	5	21	26	24
Rhytidiadelphus triquetrus				4
Rhytidium rugosum	34	5	41	6
Sanionia uncinata	5	23	28	25
Sphagnum capilifolium				
Sphagnum warnstorfii				
Tetralophozia setiformis				
Tortella tortuosa				
Unknown	11	25	36	26
Protonema		3	3	1
Total	164	251	417	228

Total does not include unknown or protonema. Protonemata are counted in groups.

Table 3.11. Species abundance on substrates at Diavik Canada (crushed rock, lake sediment, processed kimberlite) and Heiðmörk Iceland (plateau, road) research sites in August 2015.

Species	Substrates				
	Crushed rock	Lake sediment	Processed kimberlite	Plateau	Road
Aulacomium turgidum	15	2	2		
Bartramia ithyphylla					
Brachythecium albicans				2	2
Bryum argenteum	58	6			
Bryum pseudotriquetrum	31	1	1	2	3
Calliergon richardsonii					
Cephalozia sp					
Ceratodon purpureus	59	8	24	19	17
Dicranum fulvum					4
Dicranum groenlandicum	2	1			
Dicranum scoparium					
Diplophyllum albicans					
Diplophyllum obtusifolium				1	32
Fissidens sp					
Funaria hygrometrica					
Hylocomium splendens			1	3	
Pleurozium schreberi				11	15
Polytrichum commune				2	
Polytrichum juniperinum					
Polytrichum piliferum					2
Polytrichum strictum				8	2
Ptilidium ciliare	1				
Racomitrium canescens					
Racomitrium fasciculare				16	29
Racomitrium lanuginosum	16	1	19	33	40
Rhytidiadelphus loreus					
Rhytidiadelphus squarrosus				20	30
Rhytidiadelphus triquetrus					4
Rhytidium rugosum	25	5	16		
Sanionia uncinata	2			20	31
Sphagnum capilifolium					
Sphagnum warnstorfii					
Tetralophozia setiformis					
Tortella tortuosa					
Unknown	14	6	6	7	29
Protonema		1		2	1
Total	209	24	63	137	211

Total does not include unknown or protonema. Protonemata are counted in groups.

Table 3.12. Bryophyte species microhabitat preferences.

Type	Latin name	Hydrologic regime	Substrate	Disturbance level
Moss	*Aulacomium turgidum*	Mesic	Soil, rock	Low
Moss	*Bartramia ithyphylla*	Mesic	Soil, rock	Low
Moss	*Brachythecium albicans*	Mesic	Soil, rock	Low
Moss	*Bryum argenteum*	Mesic	Soil	High
Moss	*Bryum pseudotriquetrum*	Hygric	Soil, rock	Moderate
Moss	*Calliergon richardsonii*	Hydric	Peatland	Low
Liverwort	*Cephalozia* sp	Hygric to mesic	Peatland	Low
Moss	*Ceratodon purpureus*	Mesic	Soil, rock	High
Moss	*Cynodontium alpestre*	Hygric to mesic	Rock	Low
Moss	*Dicranum fulvum*	Mesic	Soil, rock	Low
Moss	*Dicranum groenlandicum*	Mesic	Soil, humus	Low
Moss	*Dicranum scoparium*	Mesic	Soil, humus	Low
Liverwort	*Diplophyllum albicans*	Hygric	Soil, peat, rock	Moderate
Liverwort	*Diplophyllum obtusifolium*	Mesic	Soil	High
Moss	*Fissidens* sp	Hygric	Soil, rock	Low
Moss	*Funaria hygrometrica*	Mesic	Soil, disturbed	High
Moss	*Hylocomium splendens*	Mesic	Soil, humus, rock	Low
Moss	*Pleurozium schreberi*	Mesic	Soil, humus, rock	Low
Moss	*Pohlia* sp	Mesic	Various	Low to High
Moss	*Polytrichum commune*	Hydric	Peatland	Moderate
Moss	*Polytrichum juniperinum*	Mesic	Soil	High
Moss	*Polytrichum piliferum*	Mesic to xeric	Soil	High
Moss	*Polytrichum strictum*	Hydric	Peatland	Low
Liverwort	*Ptilidium ciliare*	Mesic	Soil, peat, rock	Low
Moss	*Racomitrium canescens*	Xeric	Soil, humus	Low
Moss	*Racomitrium fasciculare*	Hygric	Rock	Low
Moss	*Racomitrium lanuginosum*	Xeric	Soil, rock	Low to moderate
Moss	*Rhytidiadelphus loreus*	Mesic	Soil, humus, rock, logs	Low
Moss	*Rhytidiadelphus squarrosus*	Mesic	Soil, rock, sand	Moderate
Moss	*Rhytidiadelphus triquetrus*	Mesic	Soil, humus	Low
Moss	*Rhytidium rugosum*	Mesic	Rock	Moderate
Moss	*Sanionia uncinata*	Mesic	Soil, rock, logs	Low
Moss	*Sphagnum capilifolium*	Hydric	Peatland	Low
Moss	*Sphagnum warnstorfii*	Hydric	Peatland	Low
Liverwort	*Tetralophozia setiformis*	Mesic	Rock	Low
Moss	*Tortella tortuosa*	Hygric	Rock	Low

Adapted from Atherton et al. 2010, Flora of North America Editorial Committee 1993a and b.

Table 3.13. Mean cover and density for size and erosion control treatments at Diavik Canada and Heiðmörk Iceland research sites in August 2015.

Size	Erosion control	Mean live cover (%)	Standard error (%)	Mean total cover (%)	Standard error (%)	Mean density (number of individuals)
Unplanted	No	1.1	0.1	3.2	0.3	10.3
Unplanted	Yes	0.8	$4.0\ e^{-02}$	1.1	0.0	19.8
Small	No	0.4	$2.0\ e^{-02}$	4.1	0.1	9.4
Small	Yes	1.5	$2.0\ e^{-02}$	22.3	0.2	17.2
Medium	No	0.8	$3.0\ e^{-02}$	5.7	0.1	13.6
Medium	Yes	1.5	$2.0\ e^{-02}$	25.9	0.2	15.3
Large	No	2.5	0.1	23.0	0.3	11.2
Large	Yes	4.5	0.1	36.2	0.2	24.7

Density = individuals per 10 x 10 cm quadra

Table 3.14. Species abundance for size treatments at Diavik Canada and Heiðmörk Iceland research sites in August 2015.

Species	Fragment sizes		
	Small	Medium	Large
Aulacomium turgidum	2	3	14
Bartramia ithyphylla			
Brachythecium albicans		4	
Bryum argenteum	21	21	22
Bryum pseudotriquetrum	14	10	14
Calliergon richardsonii			
Cephalozia sp			
Ceratodon purpureus	32	43	52
Dicranum fulvum		2	2
Dicranum groenlandicum			3
Dicranum scoparium			
Diplophyllum albicans			
Diplophyllum obtusifolium	12	11	10
Fissidens sp			
Funaria hygrometrica			
Hylocomium splendens			4
Pleurozium schreberi	5	16	5
Polytrichum commune			2
Polytrichum juniperinum			
Polytrichum piliferum		1	1
Polytrichum strictum	2	5	3
Ptilidium ciliare		1	
Racomitrium canescens			
Racomitrium fasciculare	10	16	19
Racomitrium lanuginosum	21	45	43
Rhytidiadelphus loreus			
Rhytidiadelphus squarrosus	17	24	9
Rhytidiadelphus triquetrus	2	1	1
Rhytidium rugosum	15	17	14
Sanionia uncinata	12	27	14
Sphagnum capilifolium			
Sphagnum warnstorfii			
Tetralophozia setiformis			
Tortella tortuosa			
Unknown	23	25	14
Protonema	1	3	
Total	165	247	232

Total does not include unknown or protonema. Protonemata are counted in groups.

Table 3.15. Species occurrence in planted samples (June 2014) and final collection (August 2015) at Diavik Canada and Heiðmörk Iceland research sites.

Species	Diavik		Heiðmörk	
	June 2014	August 2015	June 2014	August 2015
Aulacomium turgidum	x	x		
Bartramia ithyphylla			x	
Brachythecium albicans			x	x
Bryum argenteum		x		
Bryum pseudotriquetrum	x	x	x	x
Calliergon richardsonii	x			
Cephalozia sp	x		x	
Ceratodon purpureus	x	x	x	x
Dicranum fulvum			x	x
Dicranum groenlandicum	x	x		
Dicranum scoparium	x		x	
Diplophyllum albicans			x	
Diplophyllum obtusifolium				x
Fissidens sp			x	
Funaria hygrometrica				
Hylocomium splendens	x	x	x	x
Pleurozium schreberi	x		x	x
Polytrichum commune				x
Polytrichum juniperinum			x	
Polytrichum piliferum	x			x
Polytrichum strictum	x			x
Ptilidium ciliare	x	x	x	
Racomitrium canescens			x	
Racomitrium fasciculare			x	x
Racomitrium lanuginosum	x	x	x	x
Rhytidiadelphus loreus			x	
Rhytidiadelphus squarrosus			x	x
Rhytidiadelphus triquetrus			x	x
Rhytidium rugosum	x	x		
Sanionia uncinata	x	x	x	x
Sphagnum capilifolium	x			
Sphagnum warnstorfii	x			
Tetralophozia setiformis	x			
Tortella tortuosa			x	
Unknown		x		x
Protonema		x		x
Total	18	10	21	15

Total does not include protonema or unknown.

IV. SYNTHESIS AND FUTURE RESEARCH

1. RESEARCH SUMMARY

Research was conducted in the laboratory and in the field at Diavik Diamond Mine in Northwest Territories, Canada and Heiðmörk, Iceland to address the need for use of bryophytes in reclamation of northern ecosystems. Research objectives were to determine effectiveness of bryophyte fragment size (small < 1 mm, medium < 2 mm and large < 40 mm), slurry mixtures (beer, buttermilk, water) and cheesecloth as an erosion control material in promoting bryophyte regeneration and revegetation. Research was conducted on substrates of crushed rock, lake sediment and processed kimberlite at Diavik and road material and plateau at Heiðmörk. The capacity of different bryophyte species for effective propagation and the influence of different environments on reclamation success were assessed.

Results of the two experiments showed that in the relative short term (12 weeks in laboratory, 2 growing seasons in field), fragmentation promoted bryophyte growth. Medium bryophyte fragments produced higher density and cover than small or large fragments in the laboratory experiment and produced the highest density, species abundance and species diversity when in direct contact with soil in the field experiment. The large fragments were less susceptible to the effects of wind and rain and yielded higher total and live cover. Greater live cover was likely due to higher total retention of material on the substrates.

The effect of erosion control material on bryophyte cover and species abundance was positive, varying with substrate and climate. At Heiðmörk, the erosion control cloth became frayed after one growing season, likely due to the combination of wind and jagged substrates. At Diavik, the erosion control cloth remained intact and had a positive effect on bryophyte retention and propagation. The most striking effect was the promotion of colonization under the cloth in all but one substrate. This was likely due to the minimization of wind erosion and the reduction in variation of soil water content and temperature. Erosion control material had a tempering effect on soil volumetric water content and temperature, narrowing ranges of recorded values. Early stage cloth decomposition was observed after two growing seasons in 3 of 5 substrates.

Slurry had a significant impact on bryophyte propagation. Beer and water had higher bryophyte cover and density than buttermilk, although buttermilk did generate a wealth of protonemal growth by week 12. Beer and water did not differ significantly from each other; thus beer is not recommended for large scale bryophyte propagation in reclamation. The effect of slurry was

stronger early in the experiment, and likely more important for short term bryophyte propagation than long term reclamation success.

Substrates with more heterogeneous surfaces (crushed rock at Diavik; plateau and road at Heiðmörk) had higher live cover, volume retention, density and spontaneous colonization of bryophytes. More material was retained in the erosion control material on the relatively homogeneous substrates (processed kimberlite, lake sediment at Diavik), likely due to better contact between material and soil particles. However, retained material did not yield much regeneration. Results of an observational turf transplantation experiment were inconclusive, likely as it was only replicated an unfavourable substrate.

Environment invariably impacts reclamation outcomes. The factors that had the most impact on experiment results were climate related. Regeneration (live cover and density of new individuals) was higher at Heiðmörk, where there is more precipitation and less variation in temperature. Retention of planted material was higher at Diavik, where wind speeds were lower. These factors considerably impacted the outcome of fragment sizes, likely due to their impact on material displacement and bryophyte species specific regeneration requirements.

2. APPLICATIONS FOR RECLAMATION

2.1. Fragment Size

Some fragmentation and separation of biomass can promote propagation of bryophytes by activating the plants evolved capacity to produce clonal offspring. Fragments created using a 1 mm soil sieve (medium fragment size) and hand crumbling (large fragment size) had the most short term potential. Thus a rough chop, emulating a mixture of medium (< 2 mm) and large (< 40 mm) fragments, would provide a variety of sizes of propagules to utilize the effective aspects of each of the sizes. Heterogeneous fragment mixtures would likely be more beneficial than no fragmentation. The use of other available tools, such as mixers or grinders to roughly chop the material or sprayers to distribute it, could increase efficiency for large scale applications.

2.2. Slurries

The testing of food ingredients, such as beer and buttermilk, for promotion of bryophyte growth yielded interesting results but are not recommended for large scale use. Beer had a positive effect on bryophyte growth but not enough to outweigh the problems it would bring. Food

ingredients are likely to attract wildlife, potentially disturbing the fragile site and endangering workers. Cost of purchasing and transporting materials to remote sites would be high. Water was equally as effective as beer and does not present any of these practical challenges. For these reasons, the reclamation recommendation is to use whatever clean water source is most easily accessible.

2.3. Erosion Control Materials

2.3.1. Reclamation Suitability

Northern climates add a number of challenges to reclamation. High winds are common, leading to an increased likelihood of fine particulate soil loss. Average temperatures and soil water content are low, leading to slow decomposition. Erosion control is necessary in many northern sites, however conventional tools may not be best for the job. The large mesh size of straw and coconut matting would likely not be very effective at retaining fine materials, and may be an obstacle to growth of small stature, important tundra plants such as bryophytes and lichens. Straw is thought to degrade within 12 months in a temperate climate, and coconut will persist up to 36 months (Coldstream Concrete 2015, Nilex Inc. 2015). In northern climates, the thick, fibrous materials may persist for decades after site closure or, if non biodegradable materials are used as binding, persist indefinitely. Economically, these materials are expensive to transport due to their bulk and weight. There is also a risk of introducing non native seed to the tundra when using straw matting. It may be economically and ecologically beneficial to consider other, lighter and more easily degradable materials.

Cheesecloth is most commonly used in food preparation, polishing, staining and filtering (Cheesecloth.ca 2015, Vantex Innovations 2015a). It is produced from cotton bleached with peroxide, non-chlorine bleach that does not contain chemical binders; unbleached cotton is also available (Vantex Innovations 2015b). Each individual thread in cheesecloth measures less than 0.5 mm, and number of threads per inch determines the grade of cloth, from open to extra fine weaves. Grade 10 is the most open weave commonly available, with 20 x 12 threads per square inch (TPI) (Vantex Innovations 2015a). The weave tightens with grade 40 (24 x 20 TPI), 50 (28 x 24 TPI), 60 (32 x 28 TPI) and 80 (40 x 32 TPI) up to the finest weave, grade 90 (44 x 36 TPI).

Cheesecloth promoted retention of fine particulate soil and fragmented bryophyte materials in the field experiment, and could be practical for northern reclamation. Of primary importance is its capacity to reduce erosion. Structural integrity was lost in very high winds but the material

stayed intact in moderate to high winds. The presence of the cloth helped regulate fluctuations of soil volumetric water content and temperature. Overall, the material was effective at retaining planted bryophyte material and promoting growth of planted material and outside colonizers.

Estimated decomposition time of cheesecloth is 10 to 20 years in northern climates based on the amount of decomposition observed after two growing seasons at research site in Canada and Iceland. Faster decomposition is desirable in northern environments, where the process is especially slow, and could shorten reclamation timelines. Light colour of the material will reduce soil warming by deflecting sunlight, critical for conserving or building permafrost soils. The fine weight of material is necessary if mosses or biological soil crusts are being considered, as it permits penetration of sunlight and precipitation in addition to protection from elements. It reduces risk to wildlife that could get snagged or caught in some of the more bulky materials. The risk of introducing non native seed to the tundra is nonexistent with cheesecloth.

Cheesecloth material may degrade too quickly to be effective as a long term erosion solution in warmer climates. Bleached materials should be avoided, especially if the bleaching poses a risk to sensitive flora and fauna species.

2.3.2. Cost Analysis

Various comparable products are depicted in figure 4.1. These values were calculated based on a number of sources (Bean's Farm Landscape Supply 2015, Cascade Geotechnical Inc. 2015. Cheesecloth.ca 2015, Enviro-Pro Geosynthetics 2015, Home Depot 2015, Layfield Canada Ltd. 2015, Nilex Inc. 2015. Nusso Textiles Ltd. 2015, Vantex Innovations 2015b). Straw, coconut and a combination of the two, are most commonly employed industrial scale erosion control blankets (Enviro-Pro Geosynthetics 2015). Straw matting is slightly less costly, averaging \$0.64 m^{-2}, compared to coconut at \$1.23 m^{-2} and a combination of both materials at \$0.89 m^{-2}. Prices for both types increase when biodegradable netting is used as a binder. Aspen and jute fibers are relatively new materials being used for erosion control, and cost on average \$1.13 m^{-2} and \$1.47 m^{-2}, respectively.

Economically, cheesecloth is comparable to other commonly employed erosion control materials. The lowest grades of cheesecloth (10, 20) averages \$0.68 m^{-2} and \$0.81 m^{-2}, comparable to straw and straw coconut combination matting. Costs increase for mid grade cheesecloth (40, 50) \$1.02 m^{-2} and \$1.12 m^{-2}, but are still more affordable than coconut, jute or aspen matting. The highest grades (80, 90) cost on average \$1.88 m^{-2} and \$2.09 m^{-2}.

Preliminary testing shows that this very old product can provide solutions to modern problems. Benefits include promotion of bryophyte growth, reduced persistence time in northern climates, increased stability of soil water content and temperature, sunlight deflection and minimized risk of injury to wildlife and of non native species introduction. Economically, cheesecloth offers reduced transportation costs, due to lighter weight and lower bulk, and material costs less than or comparable standard reclamation materials.

3. STUDY LIMITATIONS

3.1. General Study Limitations

These experiments provided short term information on bryophyte propagation and revegetation success. Conservative long term projections based on these results can be made but it is difficult to interpret the early stages of the bryophyte revegetation process. Long term study of bryophyte revegetation methods in disturbed northern ecosystems will be required to better assess the effect of early bryophyte establishment on community development.

Digital cover assessments were conducted to reduce analysis subjectivity and increase data collection precision. Since this technique is relatively novel, no standard method exists. Quantification of cover was limited by a learning curve and technology. Initial photo quality was poor, but improved with practice in balancing light and colour for optimal digital representation. It was challenging to match photos to exact original position. A plot measurement tool, comparison to original photos, when taking and when cropping photos helped to improve accuracy. Small flags or a dot of spray paint could potentially be used in the future, as long as these do not impact bryophyte growth. A better quality camera, to reduce pixilation, and an updated program, to improve efficiency and statistical output, could further refine the method.

Bias and subjectivity are challenges faced in almost any experiment. Results of the field experiment may have been influenced by a success bias of large fragments. Small and medium fragments may have been propagating after transport into a crevice or under a rock and were therefore not observed or evaluated. Minute fragments were very difficult to differentiate from soil particles, and were likely more overlooked in the digital assessments due to pixilation and difficulty in differentiating colour. Attempts to reduce subjectivity included the use of a visual cover calibration tool, a single observer and the use of digital assessment.

3.2. Plant Cover Assessment Issues

The majority of plant studies rely on visual percent cover as a quantitative indicator of ecosystem characteristics. Variations include timed assessments, different quadrat sizes and consideration of different vegetation strata and layering. These methods are a standard practice that efficiently and economically provides simple and straightforward data. More objective methods, such as point sampling grids or biomass collection, are time consuming, bulky, destructive, impractical for remote work and not precise enough for larger or smaller scale work. These tools are therefore less commonly employed.

The subjectivity of visual estimation is taken for granted in most vegetation cover studies. The observer bias, regardless of attempts at statistical correction, likely impacts research results to some degree. The extent to which this potential margin of error is accepted is apparent in that very few digital tools of assessment have been developed. With technology available for scientific analysis of countless environmental parameters, it is reasonable to expect more researchers to employ digital assessment tools to reduce error and bias in vegetation cover assessments. A number of different technologies are slowly being introduced into the toolkit of plant scientists, including digital algorithms (Song et al. 2015), remote sensing data (Chen et al. 2010, Trimble Geospatial 2015), shape and colour spectrum image processing (ImageJ 2015) and colour spectrum classification software (VegMeasure 2015, Trimble Geospatial 2015).

Digital percent cover assessment tools could fill the gap in vegetation cover assessment technology. One such tool, SamplePoint (Booth et al. 2006), was employed to provide a more quantitative assessment of plot percent cover. SamplePoint was chosen for its low learning curve; use of the software does not require advanced GIS, Java or spectral classification experience. The grid overlay allowed for objective analysis of 225 points per 10 x 10 cm plot, precision that would not be feasible with a point count grid in the field. Programs such as this could allow for reduced field time for cover assessments and are capable of dealing with very small (bryophytes) or very large (forest canopy) scale assessments.

The challenges faced with this program could easily be solved with more advanced technology. Detection of cover below 5 % is unreliable, especially when assessing the very small fragments of bryophytes. If the points do not fall on the few fragments present in the plot, they are not reflected in the data set. A larger number of points would reduce omissions. The quality of photos took some time to perfect, and would have been enhanced by a higher resolution camera, to reduce pixilation and improve colour, and the earlier knowledge of the importance of

suitable lighting, to reduce shading and overexposure. The data output function of the program is awkward. The program can create summary statistics files, but it is not an automatic function and if it is forgotten one is left with the very complex and impractical metadata output. Improved software design could improve effectiveness of output and efficiency. The process was time consuming, although less so than if undertaken in the field. More automation in selecting certain parameters for assessment, for example all things of a certain colour and shape could be instantly computed.

The extent to which visual estimates differed from digital photography analysis in field assessments of bryophyte cover was evaluated by comparing visual estimates of cover to digital quantifications of cover for treatments at field research sites in Canada and Iceland. Visual and digital assessments of cover were made for bryophyte revegetation experiments and were compared (Table 4.1). In almost every case, visual assessments were higher than digital. Interestingly, the average difference decreased by order of size, for both live and total cover (Table 4.2). Mean cover assessments differed most for large fragments, less for medium fragments and least for small fragments.

Overall, when considering all data collected from May 2014 to August 2015, visual cover was greater than digital cover by 3.0 %. The maximum difference of live cover assessments (visual – digital cover) in large points was 15.9 %, medium was 11.4 % and small was 3.1 % (Table 4.2). The differences were significantly higher for total cover, at 59.1 %, 53.4 % and 33.8 %, respectively, meaning that for larger measures of cover, the visual method had a positive skew.

In conclusion, visual estimates of cover were positively skewed relative to digital quantification methods. The positive skew was greater for larger estimates of cover and for large bryophyte fragments and was minimized when replicated a number of times.

4. FUTURE RESEARCH

Bryophytes are notoriously slow growing organisms. The short span of this experiment did allow for some trends to become apparent, however with more time, it is likely that treatment effects would become more differentiated. Long term study of bryophyte revegetation success would be of great value for estimating and predicting bryophyte revegetation outcomes.

The treatments assessed in this experiment succeeded to some extent in two different but equally severe tundra ecosystems. Future research should focus on applying similar methods to a wider range of ecosystem types.

Study of species specific relationships to soil properties, including but not limited to fertility, texture, and microbiotic community, warrants in depth study and would assist in developing a more targeted approach to species selection. Selecting species that are colonizers or that are specifically adapted to the disturbed area would likely increase their propagation relative to those that had different microhabitat requirements.

If slurry assessments are considered in the future, they should focus on the chemical composition of slurries, to determine what exactly is benefitting the bryophytes. Regular slurry addition would reduce the chance of diminishing effects as regular watering dilutes initial slurry composition with time.

A plethora of bryophyte fragment sizes could be evaluated, however the outcomes are likely more species dependent and less important than the fragmentation method itself. Different methods of fragmenting bryophyte material could be explored, to find the most effective and efficient options for reclamation purposes. Use of industrially available tools such as grinder and mixers would be ideal. Higher application rates would likely yield faster revegetation and could be considered.

Bryophyte fragments in the erosion control treatment were not planted directly on to the surface of the soil and covered with material due to space limitations and the uncertainty of material displacement under the cloth. However, the profusion of spontaneous colonization under the erosion control material, in addition to the benefits provided to plants by the presence of erosion control, points to a high potential for positive outcome. Future experiments could explore planting the bryophyte material on soil before covering with a fine material such as the one used in this experiment, or applying a tackifier to adhere the material to the substrate. Methods of directly imbedding bryophyte material into erosion control material would also be of interest.

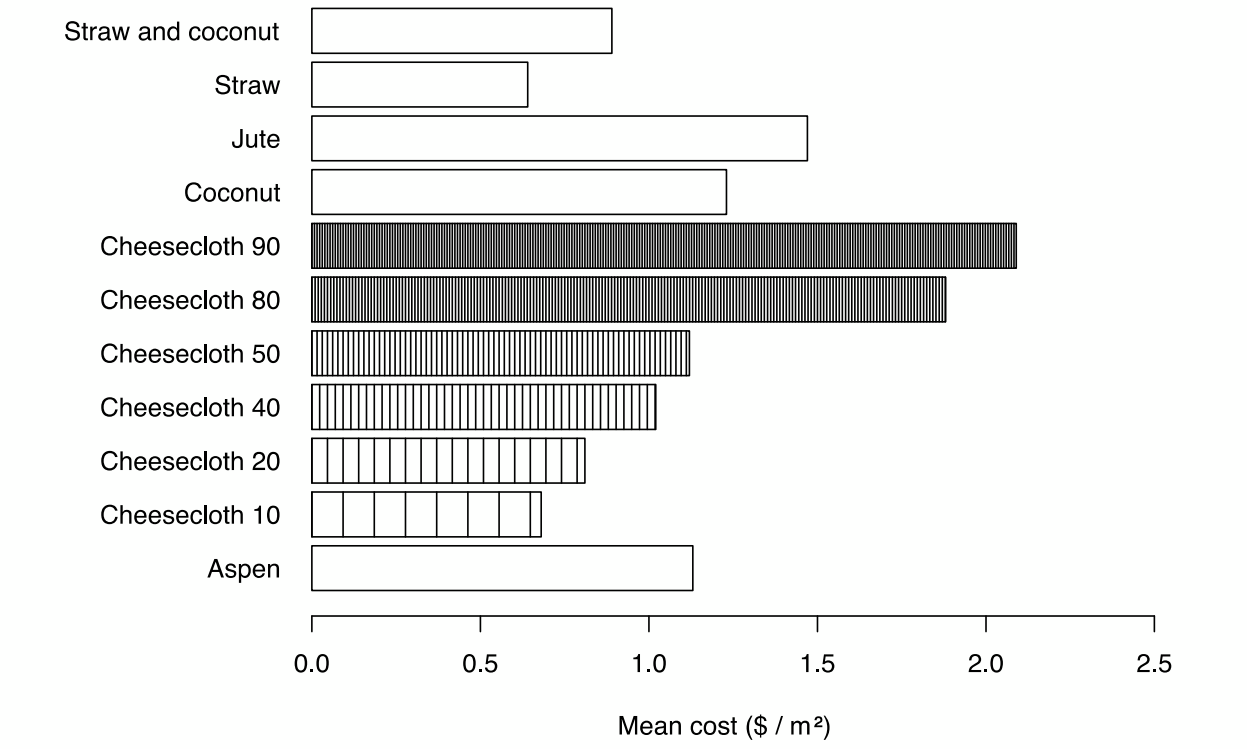

Figure 4.1. Mean cost comparison of common erosion control blankets. Numbers after cheesecloth refer to grade based on threads per inch.

Table 4.1. Visual and digital mean cover of bryophytes for fragment size and erosion control treatments and unplanted plots at Diavik Canada and Heiðmörk Iceland research sites in August 2015.

Treatment	Erosion control	Mean live cover (%)			Mean total cover (%)		
		Visual	Digital	Visual minus digital	Visual	Digital	Visual minus digital
Unplanted	No	2.1	1.1	1.0	4.5	3.2	1.3
Unplanted	Yes	1.8	0.8	1.0	2.0	1.1	1.0
Large	No	3.9	2.5	1.5	35.9	23.0	12.9
Large	Yes	5.6	4.5	1.1	46.4	36.2	10.2
Medium	No	2.0	0.8	1.2	8.3	5.7	2.7
Medium	Yes	2.3	1.5	0.8	25.2	25.9	-0.6
Small	No	0.7	0.4	0.3	4.6	4.1	0.6
Small	Yes	2.1	1.5	0.6	23.1	22.3	0.8

Table 4.2. Maximum, minimum and mean difference (visual – digital cover) for fragment size cover assessments at Diavik Canada and Heiðmörk Iceland research sites from May 2014 to August 2015.

Size	Maximum difference		Average difference	
	Live	Total	Live	Total
Large	15.6	59.1	1.1	13.5
Medium	11.4	53.4	0.6	2.9
Small	3.1	33.8	0.2	-0.3
All	15.6	59.1	0.6	5.4

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APPENDIX

Table 6.1. Bryophyte species scientific and common names.

Type	Scientific name	Common name
Moss	*Aulacomium turgidum* (Wahlenb.) Schwägr.	Swollen thread moss
Moss	*Bartramia ithyphylla* Brid.	Stiff apple moss
Moss	*Brachythecium albicans* (Hedw.) Schimp.	Whitish feather moss
Moss	*Bryum argenteum* Hedw.	Silver moss
Moss	*Bryum pseudotriquetrum* (Hedw.) P. Gaertn., B. Mey. & Scherb.	Marsh bryum
Moss	*Calliergon richardsonii* (Mitt.) Kindb. ex Warnst.	Richardson's calliergon moss
Liverwort	*Cephalozia* sp (Dumort. emend. Schiffn.) Dumort.	Pincerwort
Moss	*Ceratodon purpureus* (Hedw.) Brid.	Redshank
Moss	*Cynodontium alpestre* (Wahlenb.) Milde	Cynodontium moss
Moss	*Dicranum fulvum* Hook.	Dicranum moss
Moss	*Dicranum groenlandicum* Brid.	Greenland dicranum moss
Moss	*Dicranum scoparium* Hedw.	Broom fork moss
Liverwort	*Diplophyllum albicans* (L.) Dumort.	White earwort
Liverwort	*Diplophyllum obtusifolium* (Hook.) Dumort.	Blunt leaved eartwort
Moss	*Fissidens* sp Hedw.	Fissidens moss
Moss	*Funaria hygrometrica* Hedw.	Bonfire moss
Moss	*Hylocomium splendens* (Hedw.) Schimp.	Glittering wood moss
Moss	*Pleurozium schreberi* (Brid.) Mitt.	Red stemmed feather moss
Moss	*Pohlia* sp Hedw.	Pohlia moss
Moss	*Polytrichum commune* Hedw.	Common haircap
Moss	*Polytrichum juniperinum* Hedw.	Juniper haircap
Moss	*Polytrichum piliferum* Hedw.	Bristly haircap
Moss	*Polytrichum strictum* Brid.	Strict haircap
Liverwort	*Ptilidium ciliare* (L.) Hampe	Ciliated frigewort
Moss	*Racomitrium canescens* (Hedw.) Brid.	Hoary fringe moss
Moss	*Racomitrium fasciculare* (Hedw.) Brid.	Green mountain fringe moss
Moss	*Racomitrium lanuginosum* (Hedw.) Brid.	Wooly fringe moss
Moss	*Rhytidiadelphus loreus* (Hedw.) Warnst.	Little shaggy moss
Moss	*Rhytidiadelphus squarrosus* (Hedw.) Warnst.	Springy turf moss
Moss	*Rhytidiadelphus triquetrus* (Hedw.) Warnst.	Big shaggy moss
Moss	*Rhytidium rugosum* (Sull.) Kindb.	Wrinkle leaved feather-moss
Moss	*Sanionia uncinata* (Hedw.) Loeske	Sickle leaved hook moss
Moss	*Sphagnum capilifolium* (Ehrh.) Hedw.	Red bog moss
Moss	*Sphagnum warnstorfii* Russow	Warnstorf's big moss
Liverwort	*Tetralophozia setiformis* (Ehrh.) Schljakov	Monster pawwort
Moss	*Tortella tortuosa* (Hedw.) Limpr.	Frizzled crisp moss

Sources: Crum 2004, Atherton et al. 2015, USDA 2015.