NITROGEN FIXATION BY ASSOCIATIVE CYANOBACTERIA IN THE CANADIAN ARCTIC

by

Katherine Stewart

B.Sc., Lakehead University, 2001
H.B.O.R., Lakehead University, 2001
M.Sc., Lakehead University, 2004

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ABSTRACT

Atmospheric N_2-fixation by cyanobacteria is a key source of newly fixed N in nutrient-poor arctic ecosystems. To further determine the causes of N limitation and predict long-term responses to climate change the controls of biological N_2-fixation must be better understood. Using acetylene reduction assays we evaluated the spatial and temporal variation in N_2-fixation by associative cyanobacteria in various ecosystem types in both the low and high Canadian Arctic. The direct and indirect effects of soil moisture, plant community functional composition, and bryophyte and lichen abundance on rates of N_2-fixation were examined at sites varying in latitude and vegetation type. The linkages between N and C cycling processes in arctic systems were examined through paired measurements of N_2-fixation, inorganic soil N with surface greenhouse gas fluxes, including CO_2, N_2O and CH_4.

Total growing season N_2-fixation input across a low arctic landscape was estimated at 0.68 kg $ha^{-1}yr^{-1}$, which is slightly less than twice the estimated average N input 0.39 kg $ha^{-1}yr^{-1}$ via precipitation. N_2-fixation by bryophyte-cyanobacterial associations appear to be very important across the Canadian Arctic. Increasing soil moisture was strongly associated with an increasing presence of bryophytes and increasing bryophyte abundance was a major factor determining higher N_2-fixation rates at all sites. Shrubs had a negative effect on bryophyte abundance; competition from vascular plants, potentially through shading, may negatively influence N_2-fixation.

Soil N status was linked to rates of N_2-fixation in both the high and low Arctic indicating that these N_2-fixing associations act as important point sources of soil N. Higher rates of nitrification may be associated with warmer and drier vegetation types; however, increasing NO_3-N availability does not appear to increase rates of denitrification. Loss of N through

denitrification was not a significant factor in the N cycling at the high arctic sites examined. We found many factors control both the spatial and temporal variability of N_2-fixation, including topography, microtopography, vegetation characteristics, microclimatic conditions, *nif*H abundance and availability of other nutrients, such as phosphorus. Moisture, however, appears to be a key factor not only in determining N_2-fixation but also by influencing related nutrient cycling processes.

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CHAPTER 1: BACKGROUND

N_2-Fixing Organisms in the Arctic

Cyanobacteria are ubiquitous in the Arctic and Antarctic where they are the primary source of newly fixed N in these nutrient-poor ecosystems (Alexander & Schell, 1973; Alexander, 1974; Granhall & Lid-Torsvik, 1975; Davey, 1983 Henry & Svoboda, 1986; Chapin et al., 1991; Chapin & Bledsoe, 1992; Liengen, 1999a; Hobara et al., 2005; Solheim et al., 2006). The principal genera of cyanobacteria in the Arctic are *Nostoc*, *Anabaena*, *Scytonema*, *Stigonema*, *Hapalosiphon*, *Tolyphothrix* and *Fischerella* (Vincent, 2000). There is a high diversity of cyanobacterial species in the Arctic and in several ecosystems they can be the dominant microorganisms both in terms of biomass and productivity (Vincent, 2000; Zielke et al., 2005). Cyanobacteria can be found in both symbiotic relation and free-living as a component of biological soil crusts (BSCs), which are communities, composed of bacteria, cyanobacteria, algae, mosses, liverworts, fungi and lichens. Cyanobacterial species, such as *Nostoc* spp., that form both free-living colonies on the soil surface and grow epiphytically on bryophytes are perhaps the most important contributors to N_2-fixation in both arctic and antarctic environments (Fogg & Stewart, 1968; Alexander, 1974; Davey, 1983; Henry & Svoboda, 1986; Lennihan & Dickson, 1989; Chapin & Bledsoe, 1992; Solheim et al., 1996; Zielke et al., 2005). Cyanobacterial symbioses with lichens are also highly important as lichens are a major source of N_2-fixation (Schell & Alexander, 1973; Kallio & Kallio, 1975; Crittenden & Kershaw, 1978; Gunther, 1989). While cyanobacterial symbioses with lichens and bryophytes are common in the Arctic, symbiotic bacteria in associations with legumes or other higher plants are not as common (Gunther, 1989; Solheim et al., 2006). However, in

the low Arctic some symbioses with legumes (e.g. *Oxytropis* spp. and *Astragalus alpines*) with *Rhizobium*-type root nodules and with *Dryas* spp. with *Alnus*-type root nodules, as well as associations with *Carex* spp. have been observed (Alexander & Schell, 1973; Alexander et al., 1978; Karagatzides et al., 1985; Gunther, 1989; Henry & Svoboda, 1986). The importance of N_2-fixation in terrestrial ecosystems could vary widely depending upon the presence of species that harbour symbiotic bacteria (Boring et al., 1988; Schlesinger, 1997; Hobara et al., 2006).

Biological soil crusts

In cold deserts, semi-arid grasslands and arctic and alpine communities, N_2-fixation by soil crust microorganisms can be a dominant source of N (Evans & Ehleringer, 1993; Davidson et al., 2002). Cyanobacteria associated with BSCs are likely one of the major contributors to N inputs in arctic ecosystems due their prevalence across the landscape (Alexander & Schell, 1973, Alexander et al., 1978). As the primary source of N input in many arid and semi-arid ecosystems, BSCs make an important contribution to the ecosystem N budget. BSCs play an essential role in soil stability and nutrient cycling and contribute significantly to soil fertility (Eldridge, 1998; Issa et al., 2001; Issa et al., 2007; Hu et al., 2003; Veluci et al., 2006). Soil surface structure is altered by BSCs through the creation of a rough surface microtopography, which alters the movement and retention of nutrients by diminishing the impact of surface runoff and wind (Veluci et al., 2006; Housman et al., 2007). Several studies have found BSCs to have natural abundance ^{15}N values indicative of fixation of atmospheric sources, higher total and mineralizable N and higher dissolved nitrogenous compounds in porewater compared with adjacent soils (Evans & Ehleringer, 1993; Belnap, 1996; Evans & Belnap, 1999; Smith et al., 2002; Johnson et al., 2005; Marsh et al., 2006).

BSCs often retain N within the ecosystem that would otherwise be lost to leaching (Hawkes, 2003).

Lichens

Lichen species are often more successful under extreme conditions and are dominant in barren habitats where vascular plants maintain much of their biomass below the surface or are unable to establish (Tehunen et al., 1992; Kurina & Vitousek, 1999). The abundance and diversity of lichens in arctic ecosystems tend to be high and lichens can have a major influence on nutrient cycling by bringing carbon, nitrogen and other elements into nutrient-poor environments (Longton, 1998; Kurina & Vitousek, 1999 Bjerke et al., 2003; Solheim et al., 2006). Under extreme environmental conditions, organisms with wide ecological amplitudes may be selected for (Bolter, 1992). Lichens are highly sensitive to desiccation, but their ability to cease metabolic processes under unfavourable conditions and resume these processes under favourable conditions may allow them to occupy stressful environments. *Stereocaulon* spp., *Peltigera* spp. and *Nephroma arcticum* are common cyanolichens in arctic ecosystems and have N_2-fixation rates often exceeding that of other cyanobacterial symbioses. Many studies have found higher rates of N_2-fixation where lichens are abundant (Alexander & Schell, 1973; Hobara et al., 2005).

Bryophytes

Bryophytes influence N_2-fixation in many ecosystems by forming facultative associations with cyanobionts (DeLuca et al., 2002; Turetsky, 2003). Cyanobacteria have been found in association with many different moss, liverwort and hornwort species. *Pleurozium schreberi*, *Hylocomium splendens*, *Bryum* spp., *Sphagnum* spp., *Racomitrium lanuginsoum*,

Jamesoniella colorata, *Ditrichum strictum*, *Clasmatocolea humilis* and *Anthoceros punctatus* are just a few of the bryophyte species that are known to form symbiotic relations with cyanobacteria (Turetsky, 2003). Cyanobacteria in association with bryophytes can be epiphytic or endophytic and can reside in a number of different localities including gametophyte cavities and leaf crevices or margins (Granhall & Selander 1973; Rai et al., 2000; Turetsky, 2003). Cyanobacteria found in association with bryophytes may gain a supply of carbohydrates, protection against desiccation and UV-radiations, and bryophytes may in turn gain fixed N (Zielke et al., 2005). Moss-associated cyanobacteria can provide 2-58% of N in arctic ecosystems (Dodds et al., 1995; Solheim et al., 2006) and while variation is often high within and between bryophyte species, several studies have found the highest rates of N_2-fixation in arctic landscapes are associated with cyanobacteria moss symbioses (Alexander & Schell, 1973; Henry & Svoboda, 1986; Solheim et al., 1996).

Nitrogen Inputs via Biological N_2-Fixation

Plant productivity in cold arctic regions is limited both by low soil temperatures and low soil moisture, but during the growing season soil nitrogen (N) is believed to be the primary factor limiting plant growth (Crittenden & Kershaw, 1978; Shaver & Chapin, 1980; Nadelhoffer et al., 1992; Liengen & Olsen, 1997a; Dickson, 2000; Zielke et al., 2005). Biological N_2-fixation is a main source of N input in arctic ecosystems; however, there are relatively few estimates of annual N inputs via N_2-fixation (Bazely & Jefferies, 1989, Chapin & Bledsoe, 1992; Hobara et al., 2006). Using acetylene reduction assays (ARAs) to determine the rates of N_2-fixation across differing northern landscapes, several studies have found an average rate of approximately 8 μmol C_2H_4 $m^{-2}hr^{-1}$ (Alexander et al., 1978; Chapin et al., 1991; Dickson, 2000; Zielke et al., 2002; Hobara et al., 2006). Estimates of arctic N inputs via N_2-

fixation are extremely variable ranging from 0.06 to 9.4 kg N $ha^{-1}y^{-1}$(Cleveland et al., 1999; Hobara et al., 2006). For the majority of sites, however, estimates range from 0.10 to 1.20 kg N $ha^{-1}y^{-1}$ (Table 1).

Table 1. Estimated mean annual ecosystem N input via nitrogen fixation from various arctic and antarctic locations.

Study location	Ecosystem type	Mean annual N input via N_2-fixation (kg N $ha^{-1}y^{-1}$)	Source
Barrow, USA	Sedge meadow	0.7	Alexander et al., 1978
Imnavait Creek, USA	Tussock tundra	1.06	Hobara et al., 2006
Brooks Lake, USA	N. boreal forest/tundra	0.10	Gunther, 1989
Devon Island, Canada	Beach ridge	0.19	Chapin & Bledsoe, 1992
	Polar desert	3.03	Dickson, 2000
	Sedge/moss meadows	1.30	Cleveland et al., 1999
	Sedge/moss meadows	3.00	Chapin et al., 1991
Ellesmere Island, Canada	Sedge meadow	0.85	Henry & Svoboda, 1986
Stordalen, Sweden	Mire	1.20	Granhall & Lid-Torsvik, 1975
Hardangervidda, Norway	Lichen heath	0.56	Chapin & Bledsoe, 1992
	Wet meadow	0.94	Chapin & Bledsoe, 1992
	Dry meadow	2.55	Chapin & Bledsoe, 1992
Sweden, Norway, Finland	N. boreal forest	1.70	DeLuca et al., 2002
Signy Island, Antarctica	Mire/grassland	2.40	Cleveland et al., 1999
Marion Island, Antarctica	Mire/grassland	0.42	Cleveland et al., 1999

Compared with the rates of N_2-fixation in temperate and tropical ecosystems, N_2-fixation rates at higher latitudes tend to be low. However, compared with atmospheric deposition these inputs may be relatively high. Inputs from snow and rain tend to be very low ranging from 0.08 to 0.56 kg N $ha^{-1}yr^{-1}$ and are generally less than 0.30 kg N $ha^{-1}y^{-1}$ (Barsdate & Alexander, 1975; Van Cleve & Alexander, 1981; Solheim et al., 2006). N_2-fixation, therefore, can contribute four times the amount of N deposited via precipitation. While some studies put the contribution of N_2-fixation to ecosystem N inputs at approximately

50%-70% (Chapin & Bledsoe, 1992; Henry & Svoboda, 1986), other studies have found N_2-fixation contributed 80% or higher to total landscape N inputs (Hobara et al., 2006; Solheim et al., 2006).

Spatial and Temporal Variability

There is substantial spatial and temporal heterogeneity in N_2-fixation not only among various locations in the Arctic, but also across individual sites. Several studies have reported strong spatial and temporal variation across arctic landscapes (Alexander & Schell, 1973; Alexander et al., 1978; Chapin et al., 1991; Chapin & Bledsoe, 1992; Zielke et al., 2005). Chapin et al. (1991) found that although N_2-fixation by cyanobacteria was ubiquitous across the landscape there was considerable spatial and temporal variation. The highest rates were more often associated with brackish environments and the lowest rates occurred on beach ridges. Variation in N_2-fixation activity within a given landscape may be due to vegetation type, water status of vegetation and size and structure of the cyanobacterial community (Zielke et al., 2005).

Diurnal patterns of N_2-fixation have been observed in some studies (Crittenden & Kershaw, 1978; Davey, 1983; Coxson & Kershaw, 1983b; Chapin et al., 1991). The highest rates of N_2-fixation were generally found during the afternoon (approximately 13:00 hr) and the lowest rates generally in the early morning. Some studies have suggested that light may be the primary factor in driving diurnal patterns. However, the role of light may be coupled with temperature, and temperature may be a more important driving force in northerly latitudes.

Several studies have detected distinct seasonal patterns in N_2-fixation rates (Alexander & Schell, 1973**;** Crittenden & Kershaw, 1979; Henry & Svoboda, 1986; Chapin et al., 1991; Zielke et al., 2005)**.** The most common pattern of seasonal N_2-fixation shows an increase in rates soon after snowmelt (March-June) followed by the highest rates coinciding with the peak growing season and declining rates in late July to August depending on latitude. However, this seasonal pattern is often not uniform among all ecosystem types or communities. Chapin et al. (1991) found marked variation in seasonal patterns across different topography and vegetation types.

Variation in N_2-fixation across the season and/or between years is often due to interactions with temperature and moisture (Solheim et al., 2006). Immediately following snowmelt the vegetation is saturated with water and this may be one of the most important times for N_2-fixation in northern landscapes (Zielke et al., 2005), therefore, spatial and temporal differences in snow accumulation may play an important role in determining seasonal and interannual variation.

An understanding of the spatial and temporal variation in N_2-fixation, as well as the environmental controls of N_2-fixation has broad significance in both assessing nutrient cycling in arctic ecosystems and predicting the impact of future climatic changes (Chapin & Bledsoe, 1992). Since N is limiting in arctic environments, even minor changes to N inputs or availability could have significant consequences for arctic ecosystems (Solheim et al., 2006). Due to the high spatial and temporal variation in N_2-fixation, estimation of ecosystem N input via N_2-fixation is difficult. Attempts to quantify N inputs via N_2-fixation have been made in several studies (Alexander & Schell, 1973; Schell & Alexander, 1973; Gunther, 1989; Hobara et al., 2005). However, many of these studies have been limited by failing to

simultaneously consider N_2-fixation on multiple scales. Not only is it necessary to include all N_2-fixing associations, but the representation of these N_2-fixers within different ecosystems units must also be determined. Finally, in order to scale-up estimates of N_2-fixation to a landscape level the areal extent of different ecosystem types within a given landscape must also be known.

Factors Influencing Spatial and Temporal Variation in N_2-fixation

Wherever N is limiting N_2-fixers should have a competitive advantage over non-fixers, therefore, N_2-fixers should be selected for and their activity in turn should reverse N limitation (Vitousek & Howarth, 1991; Kurina & Vitousek, 2001; Zehr et al., 2003). Where N is abundant N_2 fixers may be competitively excluded by non-fixing species due to the high energetic costs of N_2-fixation. While this paradigm may adequately explain N dynamics in oceanic environments, it is inadequate for explaining the global distribution of N_2 fixation in terrestrial environments (Vitousek & Howarth, 1991; Houlton et al., 2008). There are several factors including energetic, ecological and physical constraints that may act individually or in concert to limit the abundance of N_2-fixers and/or control the rates of N_2-fixation. The activity of N_2-fixing organisms is not only dependent on the abundance and diversity of the N_2-fixing species present, but also on several environmental factors that control their activity. Cyanobacteria are adapted to extreme environmental conditions, such as prolonged desiccation and low temperatures. However, environmental conditions still play a major role in determining the rates of fixation and hence N input. Microclimatic variables are largely driven by topography but are strongly influenced by vegetation type. In addition, the availability of Carbon (C) and mineral nutrients including N, Phosphorus (P), Molybdenum (Mo), Cobalt (Co) and Calcium (Ca) can also affect N_2-fixation rates and the

distribution of free-living and associative cyanobacteria (Chapin & Bledsoe, 1992; Vitousek et al., 2002).

Topography and microtopography

Topography and microtopography create abiotic and biotic variation in northern landscapes, which in turn affect both N_2-fixation and N biogeochemical cycling. Many studies have suggested that temperature (Oberbauer et al., 1991; Hobbie, 1996; Hartley et al., 1999;) and soil moisture (Johnson et al., 1996; Mueller et al., 1999) are major determinants of nutrient cycling rates in arctic soils, however there is increasing evidence that topographic patterns also play a role in controlling nutrient turnover (Walker et al., 2004; Mueller et al., 1999; Biasi et al., 2005). Topography is the primary determinant of soil moisture patterns across tundra landscapes, and therefore plays a major role in determining the distribution of vegetation types (Walker, 2000) and their cyanobacterial associations. Topographic gradients can control C availability (i.e. higher C availability downslope), which could have important implications for rates of N_2-fixation. Lichens tend to be found on higher soil positions (crests and beach ridges) and can dominate under harsh and exposed conditions. Bryophytes are usually found in lower slope positions in less severe tundra habitats, where there is greater moisture availability (Schell & Alexander, 1973; Tehunen et al., 1992; Hobara et al., 2006).

In hummock-hollow tundra ecosystems mosses tend to dominate the hollows, which are expected to exhibit low growth rates, slow decomposition rates, relatively high C/N ratios and long nutrient turnover times (Hobbie, 1995; Biasi et al., 2005). In contrast hummocks can be dominated by graminoids and are expected to have higher growth rates, lower C/N

ratios and more rapid nutrient turnover (Chapin et al., 1995; Hobbie, 1995; Biasi et al., 2005). However, some studies have found higher rates of N_2-fixation in lower lying trough and interhummock areas (Schell & Alexander, 1973; Henry & Svoboda, 1986). Higher N pools, N mineralization rates, microbial activity and decomposition have also been noted in interhummock areas (Mueller et al., 1999; Baisi et al., 2005). Both the proximity of permafrost to the soil surface and moisture retention by bryophytes help to create a moist environment in these low lying areas, which is likely crucial in maintaining higher rates of N_2-fixation. In addition, leaching from high mounded areas may increase dissolved organic and inorganic N in depressions. Henry & Svoboda (1986) suggest that increased rates of fixation in interhummocks are not due to moisture differences, but due to shading of the ground surface by vegetation on the hummocks that prevents the growth of cyanobacteria. Microaspect can also affect N_2-fixation by altering the distribution of N_2-fixing organisms. Differences in colonization frequencies, abundance, and distribution of microorganisms comprising BSCs have been demonstrated as a function of mound aspect (George et al., 2000; Davidson et al., 2002). The association of different organisms on a particular aspect are likely due to distinctive and favourable microhabitats on these exposures.

Vegetation

The interactions between plant communities and environmental factors can be important in determining both the ability of N_2-fixers to survive and the rates at which they can fix N_2. Vegetation type plays a major role in determining the moisture, light and temperature regimes under which N_2-fixers operate. Differences in the capacity for various vegetation types to retain moisture and make it accessible to cyanobacteria have been correlated with rates of N_2-fixation (Zielke et al., 2002; 2005). Line (1992) found that plants from

waterlogged mire habitats or ponds were associated with epiphytic cyanobacteria but plants in drier habitats were not. Vegetation types can also alter the underlying soil moisture regime affecting cyanobacteria fixing on the surface soils (Bolter, 1992). The relationships between water availability and vegetation type are further complicated by the fact that different types of vegetation harbour different cyanobacterial communities, which have varying adaptations to water availability. Vegetation also alters surface temperatures. Soil surface and near-surface midday temperatures are 5-8^{o}C cooler under moss dominated BSCs and 10-11^{o}C cooler under fruitcose lichen dominated BSCs than non-crusted soils (Gold et al., 2001). Shading by different vegetation types can reduce the light intensities received by various N_2-fixers. Epiphytic cyanobacteria found in association with moss species are expected to be exposed to lower light intensities due to the shadowing effects of moss leaves (Basilier & Granhall, 1978; Zielke et al., 2002).

Microclimatic controls on N_2-fixation

Several studies have investigated the effect of microclimatic variation on the process of N_2-fixation in different symbioses. Microclimatic conditions including moisture, temperature and light are the most important factors controlling N_2-fixation and variation in these conditions can lead to spatial and temporal variability in N_2-fixation rates (Basilier & Granhall, 1978; Chapin et al., 1991; Dickson, 2000; Hobara et al., 2005; Solheim et al., 2006). An understanding of how environmental factors affect N_2-fixation not only helps to explain variation within the landscape, but can also provide insight into the ecophysiological functioning of various N_2-fixing organisms. Water availability is often cited as the primary controlling factor of N_2-fixation, but alteration of light and temperature regime can also act as limiting factors. Other factors, which will not be discussed here, but require consideration

include oxygen and carbon dioxide levels (Norby & Sigal, 1989; Chapin & Bledsoe, 1992; Billings et al., 2003; Zak et al., 2003) and UV-B levels (Solheim et al., 2002; Bjerke et al., 2003; Convey & Smith, 2006; Solheim et al., 2006). Although concurrent measurements of microclimatic parameters provide important information about the operational environment of N_2-fixing organisms, prior conditions must also be considered. Despite rapid recovery of nitrogenase activity (NA) following desiccation (Davey, 1983; Coxson & Kershaw, 1983a; Kurina & Vitousek, 2001), there is often a lag time between when an organism reaches optimal conditions for N_2-fixation and the onset of N_2-fixation (Crittenden & Kershaw, 1978; Gunther, 1989). The lag time can be variable depending on the prior conditions experienced by the N_2-fixing organism.

Moisture appears to be the most important environmental factor controlling N_2-fixation across various arctic environments (Alexander, 1974; Alexander et al., 1978; Davey, 1983; Chapin & Beldsoe, 1992; Line, 1992; Zielke et al., 2002, 2005; Convey & Smith, 2006). Correlation of N_2-fixation rates with soil moisture or water content of lichen thalli or moss tissues provide evidence of the important role of moisture for free-living and symbiotic cyanobacteria. Moisture enhances the metabolic activity of N_2-fixers directly by increasing C and energy supplies for N_2-fixation. In addition, higher input of water can also affect N_2-fixers indirectly by stimulating net primary production, thereby increasing soil organic matter inputs and by transporting dissolved organic carbon and nutrients downslope (Wierenga et al., 1987; Hartley & Schlesinger, 2002). Moisture can affect seasonal changes within individual communities that are at least partly reversible and spatial differences that reflect cyanobacterial biomass and long-term characteristics of the community moisture regime (Chapin et al., 1991).

Protection from desiccation is likely one of the major reasons that cyanobacteria are so often associated with bryophytes. A common feature of plants supporting N_2-fixation in dry habitats is dense packing of stems and leaves enabling water translocation to the cyanobacterial zone (Line, 1992). The ability of moss-mats to retain moisture and obtain moisture from the soil via capillary action makes them an ideal habitat for N_2-fixing cyanobacteria (Chapin & Bledsoe, 1992). In lichens, such as *Stereocaulon* spp., NA is critically dependent on water content at values less than 120% and N_2-fixation is negligible at water contents lower than 50% (Crittenden & Kershaw, 1978). Lichens are often established on drier exposed habitats and due to NA limitation by moisture, N_2-fixation by lichens during the summer may be reduced to a few comparatively short episodes when moisture conditions are suitable (Crittenden & Kershaw, 1979). Soil moisture alone can account for 56% of the variation in N_2-fixation of BSCs. However, NA in BSCs may be more desiccation-tolerant than lichens as N_2-fixation rates may decrease only after water content is less than 50% (Zielke et al., 2005). Although lichens require relatively high levels of moisture for NA, they may also rely on wetting and drying cycles to maintain the metabolic requirements of both the phycobiont and fungal partner. Tysiaczny & Kershaw (1979) suggest that the retention of photosynthate at low levels of thallus saturation is essential to supply the phycobiont with basic metabolic requirements, whereas the requirements of the fungal partner are satisfied at higher levels of thallus saturation.

Regardless of the N_2-fixing organism, rates of N_2-fixation are strongly affected by temperature. Several studies have found N_2-fixation is significantly correlated with temperature in both the Arctic and Antarctic (Alexander et al., 1974; Davey, 1983; Smith, 1984; Chapin et al., 1991; Lennihan et al., 1994; Liengen & Olsen 1997b; Zielke et al.,

2002). For example, Zielke et al., (2005) found that where water content was higher than 80% throughout the season N_2-fixation in high arctic vegetation was correlated with temperature. Seasonal temperature fluctuations have a very important role in determining annual ecosystem rates of N_2-fixation.

Temperature optimum estimates for N_2-fixation in the Arctic vary from 15-30^{o}C (Alexander, 1974; Kallio & Kallio, 1975; Chapin & Bledsoe, 1992; Hobara et al., 2005). Most N_2-fixers appear to reach optimal rates of N_2-fixation at approximately 21^{o}C and show a rapid increase in rates at temperatures above 10^{o}C, while N_2-fixation rates at or below 0^{o}C are low but detectable (1-3 μmol C_2H_4 $m^{-2}h^{-1}$) (Davey & Marchant, 1983; Chapin et al., 1991; Chapin & Bledsoe, 1992; Zielke et al., 2002; Hobara et al., 2005).

Detectable NA under low temperature conditions may reflect an evolutionary cold adaptation in polar strains of cyanobacteria (Liengen, 1999a). Cyanobacteria have been able to survive long-term freezing at -20^{o}C (Davey, 1983). Photosynthesis by *Nostoc commune* can continue at very low temperatures (-4^{o}C), which enables NA to proceed indefinitely until it is inhibited by complete cellular freezing (Davey, 1983). Coxson & Kershaw (1983c) found no winter inactivation of the nitrogenase enzyme and suggest that the elimination of NA in lichens under snowpack is likely due to depletion of carbohydrate pools supplying energy to the reaction in the dark, rather than direct inactivation of NA by freezing temperatures. Cell-free extracts of the nitrogenase enzyme have a cold labile nature, however the nitrogenase enzyme within an intact thallus may be unaffected by temperatures as low as -41^{o}C (Kershaw & MacFarlane, 1982). Average arctic surface temperatures in the high Arctic at Truelove lowland are approximately 7^{o}C and optimal temperatures for most N_2-fixing organisms are

above 20°C; therefore, despite an adaptation to cold temperatures, temperature is still an important limiting factor for N_2-fixing organisms in the Arctic (Chapin et al., 1991).

Of the microclimatic factors discussed here, light is likely the least limiting factor in polar environments. The ability of cyanobacteria to use stored energy for fixation combined with continuous or near continuous daylight over the growing season, as well as, a reduced plant canopy, limit the potential for light to act as a controlling factor on N_2-fixation rates in the Arctic (Chapin & Bledsoe, 1992). Some studies have found N_2-fixation to be light-dependent (Granhall & Lid-Torsvik, 1975; Alexander et al., 1978) while others have found little light dependence as photosynthetic rates tend to saturate at low light levels (<500 $\mu molm^{-2}s^{-1}$) (Coxson & Kershaw, 1983c; Chapin & Bledsoe, 1992; Zielke et al., 2002).

Light limitation is often cited as the reason that some lichens do not persist into later successional stages (Kershaw, 1976; Foster, 1985; Kurina & Vitousek, 1999). Remote sensing, repeat photography, and warming experiments in combination with nutrient addition studies all suggest that current warming trends in the low Arctic may be promoting shrub growth and expansion within various topographic positions (Chapin et al., 1995; Sturm et al., 2001; Goetz et al., 2005). Declining macrolichen abundance in warming sub- and mid-arctic environments may be a function of increased growth and abundance of shrubs, which may inhibit lichen performance through shading (Cornelissen et al., 2001). N_2-fixation rates and persistence of other N_2-fixing associations in these environments may also be similarly influenced by reduced light availability. In addition, light intensity may still play an important role through its coupling with surface temperature.

Influence of mineral and nutrient availability on N_2-fixation

Most soil nutrients do not have a homogeneous spatial distribution across an ecosystem and soil chemistry varies among plant type and between microsites (Biasi et al., 2005; Housman et al., 2007). Ponzetti & McCune (2001) found soil chemistry gradients were the strongest explanatory variable in the abundance and composition of BSC in shrub-steppe communities of eastern Oregon. Higher rates of fixation by asymbiotic N_2-fixers have been demonstrated in litter with low lignin, low N and high P (Thompson & Vitousek, 1997). Differences among *nif*H gene pools have been correlated with physiochemical parameters including texture, total C and total N contents (Poly et al., 2001). There is evidence that moss and lichen cover are correlated with complex water-nutrient availability gradients, with higher cover occurring where water and nutrients are more available (Bowker et al., 2005).

While some N_2-fixing organisms (i.e. BSCs) may be well correlated with underlying soil properties others may not. For example, cyanolichens that lack a rooting system are limited to nutrient acquisition from atmospheric sources and nutrients concentrated at the surface of the substrate (Hyvarinen & Critteneden 1998; Weiss et al., 2005). Mat-forming lichens also tend to form large quantities of basal necromass that can isolate the living thalli from chemical influences of the soil beneath (Crittenden, 1991; Hyvarinen & Crittenden, 1998).

Although N limitation is often cited as the main factor limiting ecosystem productivity, several studies have suggested that in many terrestrial ecosystems, P is central to the regulation of N budgets and may ultimately be more responsible than N for controlling plant biomass production (Cole & Heil, 1981; Eisele et al., 1989; Smith, 1992; Crews, 1993). Any ecological advantage given to N_2-fixing organisms may not be evident if the limit set by

another nutrient, such as P availability, is also low (Crittenden et al., 1994). The rates of dissolution of inorganic mineral P, as well as processes of mineralization and immobilization of soil organic P may play a controlling role in many critical N dynamics (Cole & Heil, 1981; Crews, 1993). The biologically active pool of P may also influence N mineralization from organic matter, the ability of plants to recover mineral N from the soil and affect the rates of free-living and symbiotic N_2-fixation. High demand for P by N_2-fixing organisms may link the global cycles of N and P, with P availability being the ultimate limit on both N availability and net primary production (Smith, 1992).

Several studies have found evidence to suggest that N_2-fixing organisms increase in both abundance and fixation rate when P supply is high, especially in ecosystems with a relatively low supply of N (Eisele et al., 1989; Chapin et al., 1991; Vitousek & Howarth, 1991; Smith 1992, Crews, 1993, Kurina & Vitousek, 1999; Davidson et al., 2002; Vitousek et al., 2002; Weiss et al. 2005; Benner & Vitousek, 2007; Benner et al., 2007). Due to the high P requirements of N_2-fixing organisms, free-living N_2-fixation rates in soil have been shown to correlate with availability of P in some ecosystems (Eisele et al., 1989; Chapin et al., 1991; Smith, 1992; Reed et al., 2007). N_2-fixing lichens, in particular, may be sensitive to changes in the availability of P and the limit set for growth by N availability is likely close to that set by P income (Crittenden et al. 1994; Kurina &Vitousek, 1999). Both epiphytic and terricolous cyanolichens have shown higher rates of N_2-fixation under P fertilization treatments and in some cases higher thallus N concentrations (Benner & Vitousek, 2007; Benner et al., 2007; Weiss et al., 2005). Enhanced nutrient supply even at modest doses can significantly alter productivity and nutrient recycling behaviours of bryophytes (Crews, 1993; Gordon et al. 2001; Phuyal et al., 2008). However, increases in NA with the addition

of P may also be due to an increase in host plant biomass rather than a direct effect of P on N_2-fixation rates (Gordon et al. 2001).

The mechanism by which P limitation may exert its effect on N_2-fixation is unclear. Cole & Heil (1981) suggest that close linkages between P and N cycling processes are related to the large energy requirements of N transformations. N_2-fixation is an energy intensive process and requires an abundant source of P. For the reduction of one molecule of N_2 16 molecules of adenosine triphosphate (ATP) are converted to adenosine diphosphate (ADP). Low phosphorus availability may reduce rates of photosynthesis, which in turn may inhibit nitrogenase by reducing photosynthate supplies and in particular the supply of ATP (Layzell, 1990; Crews, 1993; Hartley & Schlesinger, 2002). Phosphorus stimulation of N_2-fixation may also reflect the enhancement of cyanobacterial biomass and/or heterocyst number in addition to the direct effects of P on heterocyst activity (Chapin et al., 1991; Smith, 1992). N_2-fixation may diminish short of the limit set by P availability. N_2-fixing organisms have a greater demand for P than non-fixers, reducing their ability to compete effectively for low levels of P (Vitousek & Howarth, 1991). N_2-fixation often decreases at P levels where most primary producers can still obtain P, therefore, at equilibrium N_2-fixers may be P limited while other primary producers remain N-limited. N and P can thus be co-limiting. Increases in N deposition or N mineralization due to soil warming may lead to changes in C cycling, but the magnitude of response may also depend on the ratios in which N and P availability increase (Gordon et al., 2001; Arens et al., 2008).

The global deposition rates of P tend to range between <0.1 and 1 kg P $ha^{-1}yr^{-1}$ (Newman, 1995; Gordon et al., 2001). Malmer & Nihlgard (1980) found that deposition over the growing season in the low Arctic was at the low end of this range, near 0.01 kg P $ha^{-1}yr^{-1}$. In

tundra soils a large proportion of the total N and P is found in dead vegetation and organic mats in the upper 10 cm of the soil profile, resulting in tight nutrient retention, but lower nutrient availability and productivity (Cole & Heil, 1981). Low soil temperatures and shallow organic horizons lead to slow net mineralization of P in arctic soils, which can even be negative due to microbial immobilisation (Schmidt et al., 1999; Arens et al., 2008). Strong responses to N addition have been noted in arctic ecosystems, but the strongest responses to N addition have often occurred with combined addition of N and P (Shaver & Chapin, 1980; Henry et al., 1986; Shaver et al., 1998; Arens et al., 2008).

Phosphate has been shown to be a limiting factor for N_2-fixation by cyanobacteria in arctic habitats (Basilier & Granhall, 1978; Chapin et al., 1991; Liengen, 1999a). High Arctic free-living cyanobacteria (i.e. *Anabaena* sp.) have shown increasing rates of N_2-fixation in response to P fertilization (Liengen, 1999a). The highest rates of N_2-fixation were obtained with the addition of approximately 300 μM of phosphate, which greatly exceeds the expected natural P inputs. Not all studies have found higher rates of NA with addition of P (Alexander et al., 1978; Hartley & Schlesinger, 1992). The response of arctic free-living and associative cyanobacteria to short-term ecologically relevant P addition requires further study.

The energetic costs of fixing dinitrogen are often higher than that of assimilating ammonium or nitrate. Symbiotic N_2-fixers must expend 8-12 g of glucose to acquire 1g of N via fixation, not including the construction or maintenance costs of specialized structures, such as heterocysts (Gutschick, 1981: Vitousek & Howarth, 1991). For free-living diazotrophic bacteria acquiring N may be more energetically expensive, requiring the utilization of 100g of C to fix 1 to 5 g of N (Marschner, 1995; Kurina & Vitousek, 2001). Significant increases

in soil N_2-fixation have been demonstrated with the addition of carbon (glucose) and water (Hartley & Schlesinger, 2002).

In addition to P there are several other elements that could limit N_2-fixation and hence impact overall primary production. Molybdenum (Mo) and iron are two micronutrients that may limit N_2-fixation as both are essential components of the nitrogenase enzyme (Smith, 1992; Hartley & Schlesinger, 2002). In addition, Cobalt (Co) may also act to limit fixation due to its involvement in coenzyme and nucleotide reductase activity. Higher availability of Mo and Co has been found to increase N_2-fixation rates (Alexander et al., 1978). Bowker et al. (2005) found that the micronutrients Mn and Zn had a prominent and consistent positive correlation with BSC development, suggesting that they may act as limiting factors in the establishment of crust species, such as *Collema* sp. A positive correlation between the amount of extractable Mg and Ca and N_2-fixation has been found in the high Arctic (Liengen & Olsen, 1997a, 1997b). Organisms relying on combined N uptake from the soil as their sole N source likely do not require the same concentrations of these nutrients (Vitousek & Howarth, 1991).

Release of N from N_2-Fixing Organisms

Ecosystem structure and function result from a dynamic exchange of energy and materials between organisms and their environment. The distribution of abiotic resources, such as available soil N, affects both the structure and function of a given ecosystem; however biotic factors can play an equally important role in the distribution of abiotic factors (Lovett et al., 2005; Housman et al., 2007). Most arctic studies that have investigated N_2-fixation have examined the cycling of N only at the level of fixation. The role that various N_2-fixing associations play in altering nutrient availability and the extent and importance of other N

transformations remains understudied or controversial (Belnap, 2001; Johnson et al., 2005; Knowles et al., 2006; Lagerstrom et al., 2007). Organic soil N inputs originate from fixation of atmospheric N_2 by diazotrophic organisms, but may also accumulate via assimilation of inorganic nitrogen from wet and dry deposition (Figure 1). Organic forms of N are converted to inorganic forms through ammonification and nitrification. Inorganic N is lost from the soil through volatilization or denitrification, or can be returned to the organic N pool through assimilation by microorganisms or plants (Evans & Ehleringer, 1993). To gain a more comprehensive understanding of N_2-fixation within the larger context of N cycling within arctic ecosystems, the release and transfer of N from N_2-fixing organisms must be considered.

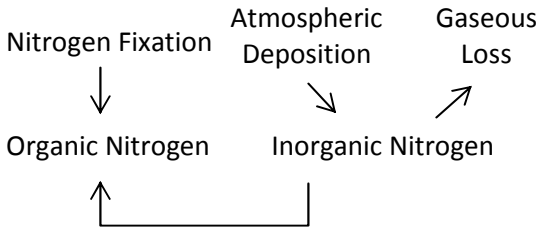

Figure 1. Sources of input and loss for nitrogen within the soil (Evans & Ehleringer, 1993, p. 314).

Atmospheric N_2 that is fixed by associative cyanobacteria not only serves to meet the nutritional needs for cyanobacterial growth, but cyanobacteria also excrete significant amounts of N compounds into their surroundings. N_2-fixing cyanolichens and soil cyanobacteria may release up to 70% of the fixed N_2 into the surrounding soil environment where it is available to associated organisms including vascular plants, mosses, fungi and other microbes (Mayland & MacIntosh, 1966; Stewart, 1967; Alexander & Schell, 1973; Harper & Belnap, 2001). Organic N (e.g. peptides, amino acids and amides) often comprises a large portion of this total dissolved N released from N_2-fixing organisms providing

evidence for the cellular source of the nitrogenous compounds (Alexander & Schell, 1973; Johnson et al., 2005). In arctic environments N fixed by cyanobacteria can be a source of readily available N, because it is released relatively rapidly through decomposition and is rapidly taken up by plants (Alexander et al., 1978; Chapin & Bledsoe, 1992). However, soil N availability within the tundra may be patchy due to the high spatial variability in N_2-fixation (Gold et al., 2001).

Associative N_2-fixing organisms likely have several functions in the nitrogen dynamics of an ecosystem and may play a dual role both supplying and competing for N. While BSCs are often considered a source of N input, when a given element is extremely scarce within the environment, microorganisms in BSCs may compete with vascular plant roots for these elements. The relative abundance of N_2-fixing (e.g. cyanobacteria) and nonfixing microbes (e.g. heterotrophs) will determine whether a BSC acts as a net N source or sink (Hawkes, 2003). A lack of N accumulation may suggest similar rates of N input and output. The detection of steady-state concentrations have been explained by the reassimilation of leaked inorganic and organic N compounds by microbes within BSCs (Johnson et al., 2005).

Since N not only enters the ecosystem via N_2-fixation but also through atmospheric deposition, lichens, bryophytes and BSCs also act as filters through which exogenous N must pass. Mat-forming lichens in polar ecosystems can retain >80% of NH_4^+ and NO_3^- deposited in summer rainfall (Crittenden, 1983; 1998). Similarly, atmospheric deposition must pass through BSCs before reaching the underlying soil; therefore crusts may regulate the capture and release of atmospheric N (Hawkes, 2003; Veluci et al., 2006). Release of chemical compounds from lichens, bryophytes and BSCs is not limited to N. Efflux of solutes from cellular pools in lichens and mosses may also be a source of exogenous sugars (e.g. fructose,

mannitol, glucose, erythritol and sucrose) that can influence the processes of microbial decomposition and asymbiotic N_2-fixation (Tearle, 1987; Coxson et al., 1992; Wilson & Coxson, 1999). Extracellular polymers secreted from organisms within BSCs can represent a major source of C inputs. Cyanobacteria, green algae, fungi and bacteria also secrete metal chelators (Lange, 1974; McLean & Beveridge, 1990) that maintain metals in biologically-available forms (Harper & Belnap, 2001). Cyanobacteria secrete glycollate which stimulates the uptake of P (Fogg, 1966), as well as, vitamins (e.g. B12), auxin-like compounds and other substances that promote growth and cell division in plant tissue (Harper & Belnap, 2001).

The concentrations and/or amount of nutrients released during a given precipitation event can vary widely and may not be solely affected by the nutrient concentrations within the N_2-fixing organism. Higher intensity rainfall events (i.e. high volume of precipitation over short time) may result in greater amounts of N or C being released as organisms are not able to reassimilate any losses (Crittenden, 1983; Wilson & Coxson, 1999). In addition, the rate of drying and time spent desiccated prior to a rewetting event can alter cellular integrity and hence the magnitude of the initial pulse of leachates. In general the greatest release of leachates occurs upon initial rewetting after a prolonged period of desiccation.

Biological Soil Crusts

BSCs have an important influence on both the chemical and physical characteristics of surface soils. Several studies have demonstrated a strong correlation between major alterations in the inorganic chemistry of surface soils and the presence of BSCs (Shields & Durrell, 1964; MacGregor & Johnson, 1971; Harper & Pendleton, 1993; Belnap, 1995;

Pendleton & Warren, 1995; Harper & Belnap, 2001). The presence of BSCs is often correlated with an increase in OM content, alteration of pH and contributions of C and N to soils altering the distribution of these resources within the landscape (Kleiner & Harper, 1977; Housman et al, 2007). The distribution of soil fauna can also be altered by BSCs, as cyanobacteria may attract larger ciliates, amoebae and tardigrades, as well as, those organisms which feed on these predators (e.g. nematodes) (Housman et al., 2007).

Few studies have focussed on the functional role of BSCs; however, there is accumulating evidence that BSCs play an important role in both the development and maintenance of vascular plant communities in arctic environments (Breen & Lévesque, 2006; Gold et al., 2001). BSCs tend to increase the N content, as well as, the uptake of Cu, K, Mg and Zn of associated seed plants (Belnap & Harper, 1995; Harper & Belnap, 2001). Some studies have shown negative associations between BSCs and plant tissue concentrations of Fe, P and N, which may reflect competition for these nutrients between BSCs and plants (Gold et al., 2001). The rooting morphology of plant species may influence the extent to which BSCs act as a source of nutrients for vascular plants. Short-lived herbs that are rooted primarily within surface soils are more influenced by the co-occurrence of BSCs than deeply rooted plant species (Harper & Belnap, 2001). In addition, the presence of mycorrhizal fungi also plays a role in the interaction between BSCs and plant N through the competition between mycorrhizae and soil microbes. Mycorrhizal plant species may have greater access to organic N and N released from BSCs (Cole & Heil, 1981; Harper & Pendleton, 1993; Belnap & Harper, 2001; Hawkes, 2003). BSCs may also affect vascular plants by preventing soil surface drying, altering surface thermal environments and reducing physical stresses of soil

movement caused by freeze-thaw action (Gold & Bliss, 1995a, 1995b, Bliss & Gold, 1999, Dickson, 2000; Gold et al., 2001).

Lichens

Leaching of metabolites from, and decomposition of, the lichen thallus are two potential pathways by which N can be released subsequent to fixation (Crittenden & Kershaw, 1978). Several studies have suggested that N-enriched leachate from terricolous cyanolichens contribute labile N to the soil on both a localized scale and can act as a significant N source on a landscape scale (Gunther, 1989; Knowles et al., 2006; Veluci et al., 2006). In environments with N limitation the inputs from N_2-fixing lichens can create temporal and spatial variability in the supply of labile N and in turn variation in soil microbial communities (Crittenden, 1983; Millbank & Olsen, 1986; Knowles et al., 2006). Compared with moss crusts and bare soil, Veluci et al. (2006) found lichen crusts had higher losses of NH_4^+ through leaching. In a boreal forest environment, Knowles et al. (2006) found a zone of influence extending 1.5m from *Peltigera* spp. with significant increases in soil N availability, potentially mineralizable N and soil % N. Losses of nitrogenous material from lichen thalli by elution may vary by season, but can average 4.5-9% of N_2-fixed (Millbank & Olsen, 1986). In addition, there is further evidence that mosses found in association with lichens derive direct nutritional benefit from N compounds leached from lichen thalli. Fixed N may also be maintained within the lichen due to tight N recycling within the thalli of mat forming lichens. There is evidence of translocation of N from the degenerating lower thallus to the apices where there is an N sink for new growth (Crittenden, 1991; Hyvarinen & Crittenden, 1998; Ellis et al., 2004).

Bryophytes

During drying and rewetting events protein N leaked from both green and senescent segments of *Pleurozium schreberi* has been detected (Carleton & Read, 1991; Turetsky, 2003). Ayres et al. (2006) demonstrated direct uptake of N from the soil by mosses, which suggests mosses may have access to multiple N pools and are likely highly competitive at acquiring N. Bryophytes have adapted to nutrient poor environments and are extremely efficient both in their use of N and their ability to retain N and may exert control over the N retention efficiency of the ecosystem (Bowden, 1991; Aldous, 2002; Phuyal et al., 2008). Weber & Van Cleve (1984) found that isotopically enriched feather moss mats released very little available N to underlying vascular plant roots, and suggest that bryophyte mats function as a major ecosystem sink for available N. However, under some circumstances, stored N pools can be released from moss mats. Wilson & Coxson (1999) demonstrated pulse release of N and C from moss mats during rewetting episodes, and concluded that mosses can act as system capacitors, absorbing nutrients from atmospheric sources over long time periods at low concentrations, which can later be released at much higher concentrations during episodic events. Similar to lichens, mosses also appear to have the ability to recycle N from older to newer segments allowing for long-term sequestration (Eckstein 2000; Lagerstrom et al., 2007). Sedia & Ehrenfeld (2006) found N cycling rates and available N were notably lower beneath lichen mats than mosses or vascular plants. However, N released from bryophytes may be in less biologically available forms and bryophytes may reduce nutrient turnover rates through the production of acidic nutrient-poor organic matter, retention of N in recalcitrant compounds and by reducing soil temperatures and hence lowering decomposition rates (Eckstein, 2000; Turetsky, 2003; Lagerstrom et al., 2007).

N_2-Fixation and Related Nutrient Cycling Processes

Knowledge of the relative importance of different N cycling processes is needed for predicting the long-term stability of ecosystems and their susceptibility to change (Rosswall, 1982; Boring et al., 1988; Evans & Ehleringer, 1993). While climate warming undoubtedly has direct influences on arctic systems, several multifactor experiments have shown that tundra ecosystems are more responsive to additions of N and P than to changes in temperature, light or carbon dioxide (CO_2) (Chapin et al., 1995; Hobbie & Chapin, 1998; Shaver et al., 1998; Van Wijk et al., 2002; Hill & Henry, 2011). The indirect influences of climate change, therefore, may be of greater importance in determining plant productivity. Many N_2-fixing surfaces are able to provide enough N to meet the needs of these low biomass systems and may even be able to sustain additional plant growth (Dickson, 2000; Sorenson et al., 2006). Nutrient inputs, especially via biological means, and nutrient cycling vary greatly between different vegetation communities in the Arctic. Nutrient cycling processes in soil also vary with plant community composition (Ehrenfeld et al., 2005; Sedia & Ehrenfeld, 2006). Vegetation communities in the high Arctic, therefore, will not necessarily respond in a similar manner to climatic changes. To fully understand the implications of climate change on N availability, the interactions among the processes that drive the nitrogen cycle in these arctic environments need to be more clearly understood.

Differences in the biogeochemical cycle of nitrogen compared with other elements could initiate or accentuate N limitation in many ecosystems (Vitousek & Howarth, 1991). Nitrogen can be extremely mobile and relatively labile forms of organic N are depleted disproportionately in comparison to other elements. This disproportionate depletion is due to the many avenues (i.e. leaching, volatilization and denitrification) through which nitrogen

moves across ecosystem boundaries (Crews, 1993). In addition, residual N tends to occur in organic forms that are highly recalcitrant to decomposition, which can lead to N limitation where decomposition is slow.

A negative feedback mechanism between N_2-fixers and available N may also contribute to the maintenance of N limitation. The activity of symbiotic and free-living N_2-fixers is repressed by high levels of available N (Alexander et al., 1978; Chapin et al., 1991; Liengen, 1999a; Vitousek & Field, 1999; Weiss et al., 2005). Ammonium inhibits heterocyst differentiation and expression of *nif* genes through regulation of specific mRNA levels (Meeks et al., 1983; Ramos et al., 1985; Wolk et al., 1994; Liegnin, 1999a). Although high levels of mineral N often result in low levels of N_2-fixation, Chapin & Bledsoe (1992) suggest that the lack of a strong correlation between NH_4^+ and N_2-fixation rates indicate that levels of mineral N in arctic soils do not tend to reach a point where N_2-fixation is appreciably reduced.

Rates of N_2-fixation are strongly influenced not only by other N cycling processes, but also by C cycling processes. Photosynthesis and respiration are two major processes that must be considered in conjunction with N_2-fixation. Rates of photosynthesis are important to consider not only due to a positive relationship with rates of fixation, but also due to the potential for direct coupling with other nitrogen transformations. Carbon availability plays a crucial role in N cycling. Soil microbial activity and consequently N immobilization not only depend upon the supply of N, but also depend on the availability of C in soil (Hawkes, 2003). Garcia-Pichel & Belnap (1996) suggest that increased oxygenation levels within BSCs due to photosynthetic activity may result in higher levels of ammonium oxidation. Ammonium oxidation (the first step in the nitrification process) is an important process in N cycling

turning high amounts of biologically fixed N into oxidized forms (Johnson et al., 2005). The fate of oxidized products include export to bulk soils with percolating water, uptake by plant roots and *in situ* use in denitrification. Respiration indirectly affects N_2-fixation by removing oxygen, an element that can inactivate the nitrogenase enzyme, therefore an understanding of substrate controls on respiration are important in estimating N_2-fixation rates (Hicks et al., 2003). Soils with BSCs tend to have higher microbial biomass and often higher rates of respiration.

The addition of N via N_2-fixation has been demonstrated to stimulate mineralization rates enhancing the flow of nutrients to associated organisms, but this effect may only be transient (Sundstrom & Huss, 1975; Ingham et al., 1985; Harper & Belnap, 2001; Smith et al., 2002). Gold et al. (2001) did not find BSCs significantly influenced net N mineralization.

Slower rates of nitrification have been associated with N_2-fixing soils, which could reduce N_2O production and corresponding losses (Smith et al., 2002). However, N limitations may not be the result of slow cycling within soils. Low rates of N accumulation can also result where rates of N transformations and loss are relatively fast (Peterjohn & Schlesinger, 1991; Evans & Belnap, 1999). Rapid cycling of N and higher turnover, however, may offset reduced N_2O. While rates of N transformations are generally thought to be quite slow in arctic soils, nitrogen cycling through litter and tundra soils may increase due to warming climatic conditions (Hobbie, 1996).

Veluci et al. (2006) detected N_2-fixation by BSCs throughout the season in a dry sand savannah; however they did not detect a net accumulation of N in soil. They suggest that in regions where rainfall dominates during warm periods, the highest rates of N_2-fixation and

release likely occur when N leaching rates are also greatest leading to greater losses of N relative to N inputs. In addition, denitrification processes could simultaneously compete with plants and microbes for newly released N (Belnap, 2001; Veluci et al., 2006). Higher summer temperatures could lead to increased denitrification rates, but N_2O fluxes may be minimal if most of the N_2 fixed is consumed by plants and microbes during these times (Veluci et al., 2006). Paired measurements of N_2-fixation rates and inorganic soil N with surface greenhouse gas fluxes, including CO_2, N_2O and CH_4, can provide insight into the linkages between N and Carbon (C) cycling processes in arctic systems.

Measurements of N_2-Fixation

Almost all N_2-fixation studies in northern environments have used Acetylene Reduction Assays (ARAs). Most, however, use only theoretical conversion ratios to estimate the difference between the quantity of acetylene reduced and the rate at which atmospheric N_2 would be fixed under a given set of conditions. The conversion factor between ARA rates and N_2-fixation rates has been a long-standing challenge when using ARA rates to estimate N input via N_2-fixation. The basis of the problem lies in the fact that eight electrons are required to reduce one mole of dinitrogen gas, including the obligatory reduction of two protons to hydrogen gas, and six electrons would reduce three moles of acetylene to ethylene. Therefore, the theoretical ratio of acetylene reduced:nitrogen reduced is 3:1, however in practice the ratio differs significantly (Gunther, 1989). Differences between the theoretical and actual conversion ratio are due to the higher solubility of acetylene and differences in the electron-transfer efficiencies between dinitrogen and acetylene (Smith, 1982). In addition, conversion ratios vary between different N_2-fixing associations and are likely not consistent over different environmental conditions. Conversion ratios from various studies range as

widely as <0.01 to 25 (Bergersen, 1970; Nohrstedt, 1983; Millbank & Olsen, 1986; Zechmeister-Boltenstern & Kinzel, 1990; Liengen, 1999b; Hobara et al., 2005). Liengen (1999b) found conversion ratios of 0.11-0.48 for *Nostoc commune* and 0.022-0.073 for BSCs under optimal conditions. Conversion ratios for cyanolichen species may vary widely depending on the moisture conditions. Under moist conditions *Peltigera* spp. had a ratio of 8-10, while under drier conditions the ratio increased to over 20 (Millbank, 1981). In an attempt to correct for the effect of moisture differences on the conversion ratio, Gunther (1989) used a variety of conversion ratios to reflect the various moisture conditions experienced by a single fixing species within the landscape. In contrast, Hobara et al., (2005) used a single conversion ratio (4.9) to represent all N_2-fixing associations under all environmental conditions within the landscape. The accuracy of estimating N input via N_2-fixation using this type of simplified approach is likely compromised.

ARA rates or N_2-fixation rates have been expressed in a number of different ways, including by area, dry weight, chlorophyll *a* as a measure of cyanobacterial biomass, heterocyst abundance and as a function of *nif*H abundance and/or diversity. Most studies have expressed N_2-fixation rates either by dry weight (Crittenden & Kershaw, 1978; Coxson & Kershaw, 1983a; Solheim et al., 2002) or area (Alexander & Schell, 1973; Henry & Svoboda, 1986; Zielke et al., 2002, 2005). The use of weight or area is primarily dependent on the type of N_2-fixing association present, with N_2-fixation by lichens being commonly expressed on a per unit weight basis, while N_2-fixation rates by BSCs are more commonly expressed on a per unit area basis.

Although not employed in this study, chlorophyll *a* and/or bacteriochlorophyll *a* are often used as a surrogate measure of soil cyanobacterial biomass (Stal et al., 1984). For C inputs,

chlorophyll *a* may be the best assay as it directly relates to potential C fixation (Bowker et al., 2002). Chlorophyll fluorescence provides a useful tool for assessing the physiological state of the photosynthetic apparatus, which may provide insight into N_2-fixation potential which depends on C fixation rates (Davidson et al., 2002). Quantification of the abundance of heterocystous forms can also be useful in predicting the potential for N_2-fixation. The relative proportion of heterocystous forms may increase in early summer and remain high throughout the arctic growing season (Alexander & Schell, 1973). Expression of N_2-fixation on a per cyanobacterial biomass basis can lead to higher N_2-fixation per unit of biomass toward the end of the growing season, which can be explained by higher proportions of heterocysts.

Molecular methods are often used to determine the diazotrophic community composition and may also provide a means of expressing N_2-fixation. It is generally assumed that genes are ultimately not retained by microorganisms unless they are functional and thus, are selected for in the environment (Zehr et al., 2003). Deslippe et al. (2005) found a poor relationship between *nif*H community structure and NA in a high arctic polar oasis and suggested that the factors that control the distribution of *nif*H genotypes in soil may not be directly related to expression of *nif*H genes. However, Steppe & Pearl (2005) were able to relate diel patterns of NA to alterations in the phototrophic community. The composition of N_2-fixing species in BSCs may not differ between poorly developed and mature crusts; however, the abundance of *nif*H sequences can be 7.5 times greater in mature BSCs (Yeager et al., 2004). The relative abundance of *nif*H gene copy numbers in roots has been demonstrated to have a positive correlation with the N uptake of adjoining plants. PCR-based *nif*H gene quantification in combination with ARAs and N-content analysis may provide a way to

evaluate the direct contribution of N_2-fixing organisms (Juraeva et al., 2006). The presence of a gene in a habitat, however, must be interpreted cautiously as presence may not be directly linked to the process catalysed by the expressed protein (Zehr et al., 2003). Neither measurement of genomic *nif*H gene abundance, nor detection of specific *nif*H transcripts, unequivocally indicates that the organisms were actively fixing (Steppe & Pearl, 2005). The use of ethidium monoazide bromide for the differentiation of genes extracted from viable and non-viable microorganisms may help to ensure that the genes detected were actively fixing N_2 (Nogva et al., 2003; Pisz et al., 2007).

RESEARCH OBJECTIVES

To develop a more comprehensive understanding of the spatial and temporal patterns of and controls on N_2-fixation by associative cyanobacteria in the Canadian Arctic I will examine N_2-fixation at varying scales and in several arctic locations. In Chapter 1, I will investigate the spatial and temporal variation at a landscape-scale within a typical low Arctic tundra landscape at Daring Lake, NWT (see objective 1). Estimates of N input in the low arctic landscape will be based on a microclimatically driven model. In Chapter 2, I will examine spatial and temporal patterns of N_2-fixation, *nif*H abundance and release of N at a smaller scale in a hummock-hollow environment within the Daring Lake landscape (see objective 2). The linkage between BSC N_2-fixation rates and soil N status, the effect of BSCs on soil fertility, and the influence of P supply on N_2-fixation rates will also be explored in the hummock-hollow environment. In Chapter 3, I will investigate the controls on N_2-fixation that may operate at a larger scale across the Canadian Arctic (see objective 3). The direct and indirect effects of soil moisture, plant community functional composition, and bryophyte and lichen abundance on rates of nitrogen fixation will be examined across four sites varying in latitude and vegetation type (i.e. Daring Lake, NWT, Truelove Lowland, Devon Island, Alexandra Fiord Polar Oasis and Polar Desert, Ellesmere Island). Finally, in Chapter 4 linkages between N and C cycling processes in arctic systems through paired measurements of N_2-fixation, inorganic soil N with surface greenhouse gas fluxes will be examined in two high arctic vegetation communities at Alexandra Fiord, Ellesmere Island (see objective 4).

Objective 1:

a) Evaluate spatial and temporal variation in N_2-fixation by associative cyanobacteria in various ecosystem types within a typical low Arctic tundra landscape.

b) Model N_2-fixation rates on the basis of incubation studies of the ecophysiological responses of individual N_2-fixing associations to moisture, temperature and light conditions over the growing season.

c) Estimate N input via N_2-fixation over the growing season using models based upon microclimatic conditions.

Objective 2:

a) Examine spatial and temporal patterns in N_2-fixation, *nif*H abundance and release of N in a hummock-hollow low arctic environment.

b) Examine the linkage between BSC N_2-fixation rates and soil N status and the effect of BSC on soil fertility between BSC type and location.

c) Determine if hummock-hollow BSC N_2- fixation activity is limited by P supply.

Objective 3:

a) Examine the direct and indirect effects of soil moisture, plant community functional composition, and bryophyte and lichen abundance on rates of N_2-fixation at four sites varying in latitude and vegetation type.

b) Compare the effects of these factors and the networks of interactions among them across sites to determine the influence of different N_2-fixing associations on fixation and key interactions driving N_2-fixation across the Arctic.

Objective 4:

a) Examine the linkages between N and C cycling processes in arctic systems through paired measurements of N_2-fixation, inorganic soil N with surface greenhouse gas fluxes, including CO_2, N_2O and CH_4, in two high arctic vegetation communities.

CHAPTER 2: NITROGEN INPUTS BY ASSOCIATIVE CYANOBACTERIA ACROSS A LOW ARCTIC TUNDRA LANDSCAPE

ABSTRACT

Atmospheric N_2-fixation by cyanobacteria is often the primary source of newly fixed N in nutrient-poor arctic environments. We examined temporal and spatial variation in N_2-fixation by the principal cyanobacterial associations (biological soil crusts, *Sphagnum* spp. associations, and *Stereocaulon paschale*) in a wide range of ecosystems within a Canadian low Arctic tundra landscape, and estimated N input via N_2-fixation over the growing season using a microclimatically driven model. Moisture and temperature were the main environmental factors influencing N_2-fixation. In general, N_2-fixation rates were largest at the height of the growing season, although each N_2-fixing association had distinct seasonal patterns due to ecosystem differences in microclimatic conditions. Ecosystem types differed strongly in N_2-fixation rates with the highest N input (10.89 kg $ha^{-1}yr^{-1}$) occurring in low-lying Wet Sedge Meadow and the lowest N input (0.73 kg $ha^{-1}yr^{-1}$) in Xerophytic Herb Tundra on upper esker slopes. Total growing season (June 3rd-September 13th) N_2-fixation input from measured components across a carefully mapped landscape study area (26.7 km^2) was estimated at 0.68 kg $ha^{-1}yr^{-1}$, which is approximately twice the estimated average N input via wet deposition. Although biological N_2-fixation input rates were small compared to internal soil N cycling rates, our data suggest that cyanobacterial associations may play an important role in determining patterns of plant productivity across low arctic tundra landscapes.

INTRODUCTION

Plant productivity in many arctic regions is constrained both by low soil temperature and low soil moisture content, but during the growing season soil nitrogen (N) is believed to be the primary factor limiting plant growth (Shaver & Chapin, 1980; Nadelhoffer et al., 1992; Liengen & Olsen, 1997a; Zielke et al., 2005). Atmospheric N_2-fixation is considered the primary source of new N input to arctic terrestrial ecosystems; however, there are relatively few estimates of annual N inputs via N_2-fixation (Alexander & Schell, 1973; Schell & Alexander, 1973; Bazely & Jefferies, 1989; Gunther, 1989; Chapin & Bledsoe, 1992; Hobara et al., 2006). For example, most estimates have failed to simultaneously consider all N_2-fixing associations present, the representation of different N_2-fixers within vegetation types or ecosystem types and the extent of ecosystem types within a given landscape.

New N inputs in nutrient-poor arctic ecosystems are primarily due to atmospheric N_2-fixation by cyanobacteria (Alexander, 1974; Granhall & Lid-Torsvik, 1975; Chapin & Bledsoe, 1992; Liengen, 1999a; Solheim et al., 2006). Cyanobacteria occur in symbiotic associations with a wide variety of lichens, and as a free-living component of biological soil crusts (BSCs), which are communities composed of bacteria, cyanobacteria, algae, mosses, liverworts, fungi and lichens. Facultative symbioses between cyanobacteria and mosses, liverworts and hornworts are also common (Smith, 1984; Granhall & Selander 1973; Rai et al., 2000; Turetsky, 2003).

Biological N_2-fixation inputs are determined by the abundance and diversity of these N_2-fixing associations, as well as several environmental factors that control their activity. For example, seasonal variation in moisture, temperature and light lead to large temporal

variability in N_2-fixation rates (Basilier & Granhall, 1978; Chapin et al., 1991; Dickson, 2000; Solheim et al., 2006). Furthermore, biological N_2-fixation inputs may be expected to vary greatly among and within vegetation-types due to spatial heterogeneities in environmental and microclimatic conditions. Accordingly, landscape-level estimates of biological N_2-fixation inputs must account for topographical variation since it is the primary determinant of soil moisture patterns and therefore of the distribution of vegetation types (Walker, 2000) and their cyanobacterial associations.

A landscape-level understanding of the temporal and spatial variation in N_2-fixation inputs, as well as the environmental controls on N_2-fixation has broad significance not just in understanding N cycling in low arctic ecosystems, but also in predicting the potential impacts of future climatic changes (Chapin & Bledsoe, 1992). Warmer temperatures and changes in moisture availability may directly affect N_2-fixation rates, but may also indirectly affect N inputs by altering the distribution of vegetation types and their particular cyanobacterial associations across the landscape. For example, enhanced shrub growth associated with climate warming trends in the low Arctic (Sturm et al., 2001; Goetz et al., 2005) may shade out lichens and possibly other N_2-fixing associations in mesic tundra. Evaluation of the relative importance of these potential effects requires a spatially explicit understanding of individual ecosystem N_2-fixation rates across the landscape.

The objectives of this study were to: a) evaluate temporal and spatial variation in N_2-fixation by associative cyanobacteria in various ecosystem types within a typical low arctic tundra landscape; b) and to estimate N input via N_2-fixation over the growing season using microclimatically driven models based upon incubation studies of the ecophysiological

responses of individual N_2-fixing associations to moisture, temperature and light conditions over the growing season.

METHODS

Study Site

The study area was located in a low arctic tundra region at the Tundra Ecosystem Research Station, Daring Lake, Northwest Territories (64°52'N, 111°35'W, 414-470 m a.s.l.) (Fig. 1), approximately 90 km northeast of the northern limit of continuous trees within the physiographic zone of the Bear-Slave Upland of the Canadian Shield (Obst, 2008). Landscape features include eskers, boulder fields, exposed bedrock, upland and lowland tundra, wetlands and various sizes of lakes, ponds and streams. Continuous permafrost is present at the site with a soil active layer ranging from 0.3-2 m (Obst, 2008).

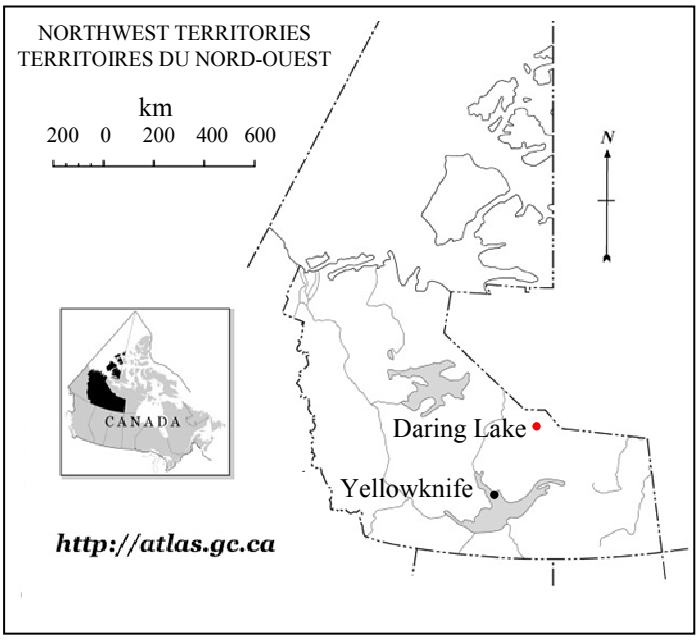

Figure 2. Location of the study site at Daring Lake, Northwest Territories, Canada (adapted from http://www.enr.gov.nt.ca).

Mean monthly air temperature in January is -30^{o}C and +13^{o}C in July (INAC 2007; Obst, 2008). Mean monthly precipitation from May to October as rain is 25 mm. Snow accumulation is highly variable across the landscape, but usually ranges from 15-60 cm in low-lying heath vegetation by mid to late May (1996-2008; Bob Reid, INAC, unpublished data). Snowmelt usually starts after mid-May ending in early June, with some snow-beds persisting on slopes until late June or early July. The plant growing season generally begins in late May or early June and ends by late August (Nobrega & Grogan, 2008; Lafleur & Humphreys, 2008).

The landscape study area encompasses the East Daring Lake Basin (26.7 km^2). Ecosystem mapping and distribution of landscape units for the landscape study area follows Obst 2008. A 1-m resolution IKONOS image provided detailed information on 15 classes (plus unclassified areas) of land covers, vegetation communities and ecosystem types present in the study area (Obst, 2008). We focused our study on the dominant ecosystem types that together occupy a total of 68% of the study area: Heath-Lichen /Heath-Mat Tundra (42%), Birch Hummock (13%), Wet Sedge Meadow (8%) and Xerophytic Herb Tundra (5%). The distribution of these four ecosystem types is largely driven by esker topography (Table 2).

Table 2. The principal topographic position, substrate, drainage and characteristic plant species for each ecosystem type included in the landscape study at Daring Lake, NWT (follows Obst, 2008).

Ecosystem type	Topographic position	Substrate/Drainage	Characteristic plant species
Xerophytic Herb Tundra	Esker tops and plateaus	Sand, gravel, rocks and boulders/Well-drained	*Saxifraga tricuspidata* Rothb.**,** *Empetrum nigrum* Böcher.**,** *Arctostaphylos alpina* L. *Vaccinium* spp., Lichens
Heath-Lichen/ Heath-Mat Tundra	Esker upper sides and slopes/ lower slopes and base	Sand, gravel, loam and some organic /Well-drained to moderately well-drained	*Betula glandulosa* Michx., *Ledum decumbens* Ait., *E. nigrum*, *Salix* spp., *A. alpina*, *Vaccinium* spp., Lichens
Birch Hummock	Gentle lower slopes and hummock-hollow complexes	Silts, silt loam, fine sandy loam and organic /Moderately well-drained to poorly drained	*B. glandulosa*, *Rubus chamaemorus* L., *Salix* spp., *L. decumbens*, *Eriophorum vaginatum* L., Mosses
Wet Sedge Meadow	Low-lying depressions and valley base	Well-developed organic /Saturated	*Carex chordorrhiza* Ehrh., *Carex rotundata* Wahlenb., *Eriophorum russeolum* Fr.ex Hartm., *Sphagnum* spp., *Salix* spp., *L. decumbens.*

N_2-Fixing Associations

Four predominant cyanobacterial associations were identified within the selected landscape study area at Daring Lake: Biological Soil Crusts (BSC) in hollows, BSC on mineral soil mounds, *Sphagnum* spp. and *Stereocaulon paschale* sensu lato. Each cyanobacterial association was found in all of the ecosystem types included in the landscape study, however, the abundance of each association varied between ecosystem types. Vascular plant species

with N_2-fixing associations, such as *Oxytropis nigrescens* (Pall.) Fisch. ex DC. and *Alnus crispa* (Aiton) Pursh., do occur in the area but are rare (Obst, 2008).

Two major BSC communities were found in association with hummock-hollow microtopography. Hollow BSCs were found mainly in the depressions between hummocks and were composed of liverworts growing in dense mats generally underlain by varying depths of organic matter. The main components of Hollow BSCs were *Anastrophyllum minutum* Schreb. and *Cephalloziella* spp. including *C. rubella* (Nees) Warnst. and *C. hampeana* complex (O. Lee, unpublished data). Cyanobacteria on Hollow BSCs were mostly the filamentous and heterocystous cyanobacterium *Stigonema* cf. *turfaceum* (Berk.) Cooke (B. Büdel, unpublished data). However, on some samples filamentous and heterocystous *Tolypothrix* sp., and the filamentous, non-heterocystous *Schizothrix* cf. *cuspidata* W. et G.S. West, were found growing in between the leafy liverworts and on the *Stigonema* filaments. *Stigonema minutum* (C. Agardh) Hass. and *Calothrix* sp. were also found on Hollow BSC samples. Hummock BSCs were cohesive well-developed crusts (1-2 cm thick) found on cryoturbated mineral soil mounds. Small, less well-developed patches of Hummock BSC also occurred in sandy well-drained areas on ridge tops. Hummock BSCs were complex communities made up of lichens, mosses and liverworts. Lichen species included *Placynthiella uliginosa* Schrader., *Bryocaulon divergens* Ach., *Bryoria tenuis* (E. Dahl) Brodo & D. Hawksw., *Cladonia* spp., *Japewia tornoensis* Nyl., *Ochrolechia frigida* Sw., and *Solorina crocea* L (C. Bjork, unpublished data). Moss species (*Funaria* sp. *Pohlia* sp. *Ditrichum* sp. *and Polytricum piliferum* Hedwig.) and liverwort species (*Cephalozia* sp., *Cephaloziella* sp., *Anastrophyllum* sp., *Anthelia* sp., *Lophozia* sp. and *Lophozia incise* Schrad.) were also key components of these diverse communities. *Stigonema turfaceum*, *S.*

minutum and *S. hormoides* (Kutz.) Born. & Flah. dominated the cyanobacteria of Hummock BSC, however, *Gloeocapsa decorticans* A. Braun., *G. novacekii, S. cuspidata, Anabaena* sp. and *Chroococcidiopsis* sp. were also present.

Sphagnum spp. were the dominant ground cover in Wet Sedge Meadows and were found scattered in damp depressions throughout the landscape. The majority of *Sphagnum* spp. samples were composed of *Sphagnum aongstroemii* C. Hartm., and *S. subsecundum* complex, with occasional fine strands of *S. balticum* (Russ.) C. Jens. (O. Lee, unpublished data). In addition, other moss (*Drepanocladus aduncus* (Hedw.) Warnst.) and liverwort (*Gymnocolea inflate* Huds.) species were found intermingled within *Sphagnum* spp. samples. The cyanobacteria *G. decorticans* was found in association with *Sphagnum* spp. samples (B. Büdel, unpublished data).

Stereocaulon paschale was predominantly found in small continuous mats on high/mid slope positions, often in areas where late lying snow patches occurred. Patchy distribution of *S. paschale* also occurred on well-drained ridge tops and in hummock-hollow complexes.

N_2-Fixation Rates

Measurements of N_2-fixation were made using acetylene reduction assays (Stewart et al., 1967). Acetylene gas (C_2H_2) was generated on-site from CaC_2 and water, with incubations injected with 10% (v/v) acetylene. Ethylene concentrations were measured in the field with a portable gas chromatograph (SRI 8610A, Wennick Scientific Corporation, Ottawa, ON, Canada) fitted with a Porapak column (Alltech Canada, Guelph, ON, Canada) and a flame ionization detector. A Stand-Alone Hydrogen Generator (SRI H_2-50, Alltech Canada, Guelph, ON, Canada) provided hydrogen as the carrier gas, which was held at a constant

pressure of 26 psi. Column temperature was held at 65°C. The gas chromatograph was calibrated for each incubation with ethylene (BOC Canada Ltd., Mississauga, ON, Canada, C_2H_4, 98%+) that was kept at the same temperature as incubation gas samples.

Cores of Hollow BSC (n=12), Hummock BSC (n=12) and *S. paschale* (n=12) were randomly sampled for each incubation from an area of ~5 km^2 that was representative of the larger landscape study area. Samples were taken from multiple positions on both north and south facing slopes of the main east-west oriented esker. BSC samples were trimmed to an area of 19 cm^2 and 0.75 cm depth such that each sample had a thin underlying soil substrate. *Stereocaulon paschale* was trimmed to an area of 19 cm^2 and 2 cm depth, but no underlying soil substrate was included. Samples were enclosed in 250 ml glass canning jars with modified lids containing a rubber septum. The mean headspace of ARA incubations was 235.75 ml (250 ml jar volume minus 14.25 ml sample) for Hollow and Hummock BSCs and 212 ml for *S. paschale* (250 ml jar volume minus 38 ml sample). *Sphagnum* spp. cores (n=12) were sampled from an area of ~0.5 km^2 within the Wet Sedge Meadow ecosystem type only. Samples were trimmed to an area of 56 cm^2 and 6 cm depth and included both live (green) and underlying decaying stems. *Sphagnum* spp. samples were incubated in 1 L canning jars with modified lids containing rubber septa and had a mean headspace of 664 ml (1000 ml jar volume minus 336 ml sample). All *Sphagnum* spp. jars were incubated *in situ* in the Wet Sedge Meadow with the glass bottom facing up and the *Sphagnum* spp. sample level with the surrounding vegetation. For each set of incubations, one sample for each N_2-fixing association was used as a control, which served as both a temperature control and a blank not injected with acetylene. Control samples did not show any natural evolution of

ethylene. Contamination of generated acetylene with ethylene was monitored and corrections were made for each set of incubations, as required.

Daytime ARA incubations occurred between 10:00-16:00 hr (6 hours) and night-time ARA incubations between 21:00-7:00 hr (10 hours). A pilot study conducted in 2007 indicated respiration in both Hollow BSC (108 μL $L^{-1}CO_2$/hr) and Hummock BSC (162 μL $L^{-1}CO_2$/hr) under average light conditions, suggesting that CO_2 limitation was unlikely to limit N_2-fixation despite longer incubation periods. However, we injected the *S. paschale* incubations with 1% (v/v) CO_2 after 3 hours for daytime incubations and after 1 hour for night-time incubations because the lichen samples lacked an underlying soil substrate to provide a CO_2 source.

Destructive sampling was used for each incubation with new samples of each cyanobacterial association (n=12) collected per incubation. Nine consecutive sets of *in situ* incubations under ambient field conditions were conducted over a 6 day period (5 night and 4 daytime) in each growing season month for each N_2-fixing association, with the exception of *Sphagnum* spp. in 2007. Incubations for Hollow BSC and Hummock BSC were conducted from June 19^{th}-24^{th}, July 6^{th}-11^{th}, August 9^{th}-13^{th} in 2007 and from June 12^{th}-17^{th}, July 1^{st}-6^{th}, August 5^{th}-10^{th} in 2008. *Sphagnum* spp. were incubated for a 24 hr period over 5 consecutive days between June 25^{th}-29^{th}, July 9^{th}-13^{th} and August 17^{th}-22^{nd} of 2007 due to logistical constraints. In 2008 *Sphagnum* spp. were incubated in the same manner as other N_2-fixing associations from June 18^{th}-23^{rd}, July 7^{th}-12^{th} and August 17^{th}- 22^{nd}. *Sphagnum* spp. N_2-fixation rates were not significantly different between 2007 and 2008; therefore, the difference in incubation length likely had little influence on the overall rate estimation. *Stereocaulon paschale* was incubated only in 2008 from June 4^{th}-9^{th}, July 7^{th}-12^{th} and August

11^{th}-16^{th}. Over the 2007-2008 growing seasons a total of 571 Hollow BSCs, 572 Hummock BSCs, 794 *Sphagnum* spp. and 294 *S. paschale* samples were incubated *in situ* under ambient field conditions.

With the exception of *Sphagnum* spp., all *in situ* samples were incubated outdoors near the research station laboratory under ambient field conditions. Incubation chambers were placed in water baths and bath temperature was altered to ensure that incubation temperatures reflected ambient conditions. Photosynthetically Active Radiation (PAR), air temperature, incubation temperature and ambient temperature of Hollow BSC, Hummock BSC and *S. paschale* were monitored every 30 minutes during daytime ARA incubations. On average the surface temperature of incubation samples were within 1.5^{o}C of the surface temperature of the respective N_2-fixing associations under ambient conditions. Heating of incubation chambers via solar radiation was not a concern for night-time incubations where microclimate was monitored for the first and last hour only. Moisture of Hollow BSC, Hummock BSC and *S. paschale* were determined both pre- and post- incubation to ensure that drying of specimens did not occur during the incubation period. Average loss of moisture during incubations was less than <1.8% for all N_2-fixing associations. Following incubation all samples were weighed, air dried and then re-weighed to determine moisture content. Moisture content of samples over the growing season was later used for modeling N_2-fixation potential.

In addition to *in situ* incubations under ambient field conditions, N_2-fixing associations were also incubated *in situ* under optimal environmental conditions (200 µmol PAR $m^{-2}s^{-1}$, 20^{o}C) at the end of each set of incubations in June, July and August 2007/2008. For each of our N_2-fixing associations we likely had several different cyanobacterial species present with

varying optimal operating environments. However, an optimal temperature of ~20°C has been demonstrated for several species/environments (Basilier & Granhall, 1978; Chapin et al., 1991; Liengen, 1999a) and light saturation has been demonstrated at ~100 μmol PAR $m^{-2}s^{-1}$ (Coxson & Kershaw, 1983a; Chapin et al., 1991). Samples (n=12) were treated in the same way as field incubations, with the exception of a 24 hr wetting pretreatment at optimal hydration levels.

N_2-fixation rates for both *in situ* incubations under ambient and optimal conditions were calculated as micromoles of ethylene reduced per hour per m^2 based upon the length of incubation and area of each sample (19 cm^2 BSCs and *S. paschale*, 56 cm^2 *Sphagnum* spp.). Conversion ratios determined for each N_2-fixing association (see below) were used to convert ethylene reduced to N_2 reduced. ARA values were corrected for differences in incubation jar volume, mean sample volume and for differences in area. Different N_2-fixing associations were allowed to vary in sample depth (i.e. 0.75 cm BSCs, 2 cm *S. paschale*, 6 cm *Sphagnum* spp.) to help ensure sampling units were kept intact and that N_2-fixing surfaces were representative of the different associations under natural conditions.

^{15}N-Incubations

Samples of each cyanobacterial association were collected from the Daring Lake landscape in August 2008 to determine conversion ratios for each of the N_2-fixing associations following the methods of Liengen (1999b). Samples were kept cool (~4°C) and shipped to the laboratory at the University of Northern British Columbia. Prior to incubation, samples were kept at optimal hydration in a growth chamber for 72 hours under a 17/7 hr light (200 μmol PAR $m^{-2}s^{-1}$)/dark cycle with temperatures at 15°C during light hours and 5°C during

dark hours. Cores for each N_2-fixing association (n=8) were similar in area (19 cm^2) and depth (0.75-2 cm) to those used for field incubations.

In order to achieve detection of ^{15}N enrichment it was determined that 48hr laboratory incubations (200 µmol PAR $m^{-2}s^{-1}$, 20°C) were required. Air (10% v/v) was replaced with 10% (v/v) ^{15}N gas (Cambridge Isotope Laboratories Inc., Andover, MA, USA, $^{15}N_2$, 98%+). To reduce the potential for CO_2 limitation due to the long incubation period, each chamber was injected with 5% (v/v) CO_2. After the 48hr incubation samples were immediately dried at 105°C. Dry samples were ground in a ball mill and sent for ^{15}N and total N analysis (Stable Isotope Facilities, University of Saskatchewan, Saskatoon, SK, Canada). Control samples (n=8 for each N_2-fixing association) treated in the same manner but incubated with C_2H_2 were used to determine the natural abundance ^{15}N and the acetylene reduction rate. The amount of N fixed was calculated using (Liengen, 1999b, p.224):

$$Y = \left(\frac{atom\,\%\,15_{N_{excess}}}{100}\right) \times \left(\frac{\text{total } N_{\text{sample}} \text{ x } 10^9}{t \times 28}\right) \times \left(\frac{100}{\%\,15_{\text{N air}}}\right) \quad (1)$$

where Y (nmol $N \cdot gdw^{-1} \cdot h^{-1}$) are the amounts of N_2 fixed during the experiment, atom% $^{15}N_{excess}$ is the difference between atom% $^{15}N_{sample}$ and atom% $^{15}N_{control}$, total N is the total amount of nitrogen in the sample ($g \cdot 100\ gdw^{-1}$), *t* is the incubation time, 28 is the molecular weight of N_2 (g/mol), and $\%^{15}N$ air is the percentage of ^{15}N out of the total amount of N gas in each incubation chamber. Conversion ratios varied among the different N_2-fixing associations.

Estimation of Landscape Level N Inputs

Microclimatic monitoring

Hollow and Hummock BSC microclimatic conditions were monitored in several different hollow-hummock complexes within the study landscape in 2007 (Julian days 169-257) and 2008 (Julian days 154-235). PAR was measured with quantum sensors (n=2-3) (LI-190 Quantum Sensors, LI-COR, Lincoln, Nebraska ,USA) installed at ground level in separate hummocks and hollows and connected to a multiplexer (AM416, Campbell Scientific Inc, Edmonton, AB, Canada). Soil surface temperature was monitored with fine-wire copper constantan thermocouples (n=7-23) connected to a multiplexer (AM25T, Campbell Scientific Inc). Impedance clips (n=4-19) were inserted at the surface of Hollow and Hummock BSC to monitor moisture conditions (after Coxson, 1991). All multiplexers and impedance clips were connected to a datalogger (CR23X, Campbell Scientific Inc.) and hourly means recorded. Impedance measurements were calibrated in the lab by simultaneously monitoring clip values and gravimetric moisture of Hollow BSC and Hummock BSC samples from a saturated to desiccated state. Both Hollow BSC and Hummock BSC % moisture were best explained by exponential relationships with impedance clip values ($f = 25.55*\exp(1.18*x)$, adjusted $R^2= 0.65$; and ($f= \exp (3.65*x)$, adjusted $R^2=0.75$).

Sphagnum spp. temperature was monitored with a pair copper constantan thermocouples installed at a depth of 2 cm. One thermocouple was connected to a multiplexer (AM25T, Campbell Scientific Inc.) and datalogger (21X, Campbell Scientific Inc.) recording hourly means in 2007/2008. The other thermocouple was connected to an additional datalogger (CR10X, Campbell Scientific Inc.) recording 4 hour mean temperatures in 2007/2008.

Modelling N_2-fixation potential

Models of N_2-fixation potential were constructed for BSCs using N_2-fixation rates and microclimatic data recorded in the *in situ* ambient incubations. The *Sphagnum* spp. N_2-fixation model was based on N_2-fixation rates under controlled laboratory conditions, and the *S. paschale* model was based on N_2-fixation rates recorded during *in situ* ambient incubations and macroclimatic data recorded at a local Daring Lake weather station (~500 m from the research station) (2007-2008; Bob Reid, INAC, unpublished data). Spearman correlations were determined between mean N_2- fixation rate, mean light (PAR), mean incubation temperature, and mean % moisture for each incubation across all *in situ* ambient incubations (2007 & 2008) for Hollow (n= 54) and Hummock BSC (n =54). Temperature had the highest correlation with N_2-fixation for both Hollow (r=0.78) and Hummock BSCs (r=0.64). Light and temperature had a high covariance for Hollow BSC (r=0.84) and Hummock BSC (r=0.81), therefore; only temperature and moisture were used in the models. Separate models were determined for high and low moisture conditions. High and low moisture classes were based on % moisture values above ('high') and below ('low') the median % moisture detected for Hollow or Hummock BSCs incubated in the field over the growing season in 2007 and 2008.

Sphagnum spp. were sampled from the field site in early August 2009, kept cool (~4^{o}C) and shipped to the laboratory at the University of Northern British Columbia. The samples were incubated under a range of laboratory conditions reflecting field conditions in 2007-2008 to determine the response of N_2-fixation to temperature and light. Moisture was not included because >70% of *Sphagnum* spp. within the landscape occurred in Wet Sedge Meadows where moisture remains relatively high throughout the growing season and is likely not

limiting. *Sphagnum* spp. N_2-fixation rates were significantly correlated with temperature (r=0.62). N_2-fixation rates under light conditions ranging from 0 to 1000 μmol PAR $m^{-2}s^{-1}$ were not significantly different (ANOVA, Tukey post hoc, p= 0.09), therefore only temperature was included in the model.

Mean N_2-fixation by *S. paschale* under *in situ* ambient incubations (n=27) was highly correlated with mean % moisture (r=0.92). Days since precipitation was used as the moisture variable for *S. paschale* since direct measurements of field moisture content were not possible. A considerable lag period often occurs following saturation of *Stereocaulon* mats from rainfall events before steady nitrogenase activity is recovered (Crittenden & Kershaw, 1978). Therefore, a 24 hour lag was incorporated into the days since precipitation variable. The 24 hours following a precipitation event (≥ 1mm) was coded as 0 and every subsequent day without a precipitation event coded with an increasing value of 1. Days since precipitation was highly correlated with mean % moisture (r=-0.76) and with N_2-fixation (r=-0.84).

The above models were used to estimate hourly N_2-fixation rates for each association over a growing season based upon microclimate and macroclimatic monitoring in the study landscape. Hourly N_2-fixation rates were summed to provide daily and seasonal totals. We defined the start of the growing season as the first set of three or more consecutive days with no snow cover and mean air temperature above 0^oC, and the end of the growing season as the first occurrence of three or more consecutive days with mean air and soil surface temperature $<0^oC$. In 2007 and 2008 these conditions occurred between Julian days 152 and 257 and 144 and 259 respectively. Estimates of N_2-fixation for all of the above growing season days were not possible for every N_2-fixing association due to unavailable microclimatic data.

Therefore, the total mean N input for each association was based on the average of 2007 and 2008 estimates over a 103 day growing season from 154 to 257 in both years. Air temperature, snow depth and precipitation were determined from macroclimatic data from the local Daring Lake weather station (2007-2008; Bob Reid, INAC, unpublished data).

Quantification of N_2-fixing associations in the landscape

The areal extents of each of the N_2-fixing associations within each ecosystem type (Xerophytic Herb Tundra, Heath-Lichen/Heath-Mat Tundra, Birch Hummock, Wet Sedge Meadows) in the study area were determined using line transects in June 2007. Ten parallel transects (~50 m apart, and ~1 km in length) were run from an esker ridge down across a valley and up to an elevated boulder field plateau within the East Daring Lake drainage basin. N_2-fixation samples were collected from within the area where transects were located. The variation in topography and therefore of vegetation types within the transect area is typical of the Barrenlands region and representative of the landscape study area. Percent cover of each N_2-fixing association was visually estimated within all 25 x 25 cm^2 subsections of 1 m^2 quadrats that were placed every 10 m along each line transect. The dominant ecosystem type in each quadrat was noted, and then the mean percent cover of each of the four principal N_2-fixing associations was visually estimated by two independent observers. The total area of each N_2-fixing association within the landscape was estimated based upon its mean % cover within each ecosystem type (determined from transect data) and the total area occupied by each ecosystem type within the 26.7 km^2 landscape study area (determined from Obst, 2008).

The total mean growing season N input (kg $ha^{-1}yr^{-1}$) for each N_2-fixing association was estimated by averaging the 2007 and 2008 model outputs for Julian days 154-257 in both

years. The total N input by each N_2-fixing association within each ecosystem type was determined by multiplying the mean total growing season N input for each association (kg $ha^{-1}yr^{-1}$) by the area (ha) occupied by each association. Total N input for each ecosystem type is the sum of N inputs from all N_2-fixing associations within a given ecosystem type. Total landscape N input over the growing season was determined by summing N input from all N_2-fixing associations in each of the ecosystem types over the growing season and dividing by the total landscape study area (26.7 km^2).

Statistical Analyses

Comparisons of mean N_2-fixation rates by the principal N_2-fixing associations over the growing season (June-August) under field and optimal conditions were analysed using separate factorial analyses of variance (ANOVA) (N_2-fixing type, month, and their interaction). Logistic regressions were used to develop the models of N_2-fixation potential based on microclimate. Data from both 2007 and 2008 were used in comparisons of N_2-fixation rates by the principal N_2-fixing associations and in the models of N_2-fixation potential based on microclimate. N_2-fixation rates were log transformed prior to all statistical analyses (SYSTAT 8.0, Systat Software, Inc.).

RESULTS

N_2- Fixation Rates of the Principal Cyanobacterial Associations

Mean monthly N_2-fixation rates under field conditions differed significantly among N_2-fixing associations ($F_{(3,2232)}$=156.51, $p<0.01$) and were significantly different between months ($F_{(2,2232)}$= 3.40, p=0.03) (Table 3). The interaction of month and N_2-fixing association was also significant ($F_{(6,2232)}$ =27.67, $p<0.01$) with patterns of N_2-fixation over the growing

season (June-August) varying among the different associations. The highest rates of N_2-fixation for all of the associations with the exception of *S. paschale* occurred in July; however, Hollow BSC had lower rates in June compared with July and August and *Sphagnum* spp. had lower rates in August compared with June and July.

Table 3. Mean monthly N_2-fixation rates (μmol N $m^{-2}hr^{-1}$) in incubations under field and optimised environmental conditions for each of the principal N_2-fixing cyanobacterial associations in the low arctic tundra landscape near Daring Lake, NWT, Canada. Acetylene reduction conversion ratios based on optimal conditions are included for each N_2-fixing association. Parentheses indicate standard errors.

N_2-Fixing Association	Incubation condition	Mean Monthly N_2- Fixation rate (μmol N $m^{-2}hr^{-1}$)			Conversion ratio C_2H_4/N_2
		June	July	August	
Hollow BSC	Field	4.28 (0.47)	13.01 (1.30)	11.00 (1.23)	3.49 (0.85)
	Optimal	11.40 (2.26)	25.87 (3.57)	25.05 (4.45)	
Hummock BSC	Field	11.69 (0.90)	13.70 (0.91)	10.70 (0.91)	1.33 (0.40)
	Optimal	28.12 (3.63)	37.08 (4.27)	19.34 (1.43)	
Sphagnum spp.	Field	31.05 (1.87)	33.11 (2.37)	20.69 (1.34)	0.85 (0.12)
	Optimal	n/a	n/a	n/a	
Stereocaulon paschale	Field	56.97 (7.08)	43.45 (8.89)	59.98 (7.24)	1.78 (0.20)
	Optimal	192.10 (23.74)	303.06 (29.20)	217.38 (25.24)	

N_2-fixation rates under optimal conditions (200 μmol PAR $m^{-2}s^{-1}$, 20°C) differed among cyanobacterial associations ($F_{(2,154)}$=181.15, p<0.01) and between months ($F_{(2,154)}$=8.17, P<0.01), and there was a significant interaction between these two factors ($F_{(4,154)}$=2.70, P=0.03). The highest N_2-fixation rates under optimal conditions for all associations were in

July (Table 3). The lowest rates under optimal conditions occurred in June for both Hollow BSC and *S. paschale,* while the lowest rates occurred in August for Hummock BSC. Comparison of N_2-fixation rates under field and optimal conditions clearly indicated that adverse *in situ* environmental factors severely curtailed N_2-fixation, and that the extent of this constraint varied substantially among cyanobacterial associations. BSC associations had N_2-fixation rates under optimal conditions that were 2-3 times higher than those observed under field conditions while rates in *S. paschale* were 4-7 times higher under optimal conditions (Table 3).

Landscape-scale Patterns of N Input

Microclimatic models of potential N_2-fixation for each cyanobacterial association

Our simple models of N_2-fixation rates in relation to either temperature and/or moisture explained at least 50% of the variation in the field incubation data (Table 4). N_2-fixation rates were correlated with temperature in the high moisture and low moisture category samples ($R^2 = 0.77$, $p<0.001$; $R^2 = 0.78$, $p<0.001$, respectively) of Hollow BSC associations (Table 4). Similarly, N_2-fixation rates were correlated with temperature in the high moisture and low moisture category samples ($R^2=0.56$, $p<0.001$; $R^2=0.50$, $p<0.001$, respectively) of Hummock BSC associations (Table 3). N_2-fixation rates for the *Sphagnum* spp. cyanobacterial associations were also correlated with temperature ($R^2= 0.72$, $p<0.001$) while *S. paschale* rates were significantly correlated with days since precipitation ($R^2 = 0.69$, $p<0.001$) (Table 4).

Table 4. Potential N_2-fixation rate logistic regression models based on acetylene reduction (AR) rates in field incubations for each of the principal N_2-fixing associations. Hollow and Hummock BSC data were each separated into two moisture classes as indicated. Environmental variables included in models are surface temperature of Hollow (T_{ho}) and Hummock (T_{hu}) BSCs, *Sphagnum* spp. temperature at 2 cm depth (T_s) and Days since precipitation (D_{sp}). The dependent variable for all models is log acetylene reduction (µmol $C_2H_4\, m^{-2}hr^{-1}$).

N_2-fixing Association	Moisture class	Model	N	F	R^2
Hollow BSC	High (>80%)	(0.07 X T_{ho}) + 0.37	25	80.39	0.77
	Low (<80%)	(0.05 X T_{ho}) + 0.69	25	84.67	0.78
Hummock BSC	High (>35%)	(0.05 X T_{hu}) + 0.55	22	28.05	0.56
	Low (<35%)	(0.04 X T_{hu}) + 0.60	28	28.46	0.50
Sphagnum spp.	None	(0.04 X T_s) + 0.99	14	34.68	0.72
Stereocaulon paschale	None	(-0.21 X D_{sp}) +2.21	23	49.46	0.69

(All models presented statistically significant, $p<0.01$)

Seasonal trends of N_2-fixation estimated by using full growing season field microclimatic records in the models indicated that each N_2-fixing association had similar patterns of activity in 2007 and 2008 (Fig. 3). N_2-fixation inputs in Hollow BSC, Hummock BSC and *Sphagnum* spp. associations fluctuated dynamically during the first half of the season but tended to generally increase toward peak values in mid to late July, and then to decline fairly steadily afterwards. No clear seasonal trend could be observed for *S. paschale* because estimates were based solely on days since precipitation.

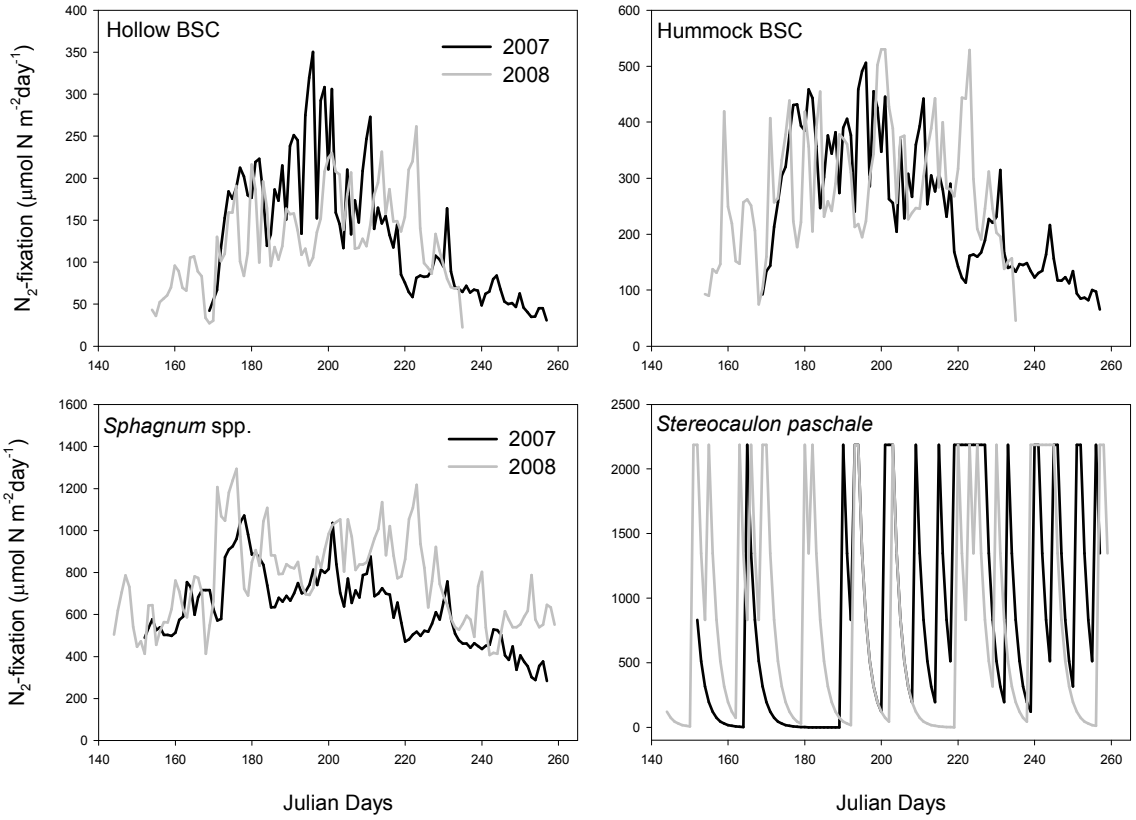

Figure 3. Seasonal trends in N_2-fixation rate (µmol N $m^{-2}day^{-1}$) estimated from potential N_2-fixation rate models and field environmental data records for each N_2-fixing association for 2007 and 2008 at Daring Lake, NWT. See Table 4 for further model details. ARA to N_2-fixation conversion ratios (Table 3) were applied for each N_2-fixing association.

The model estimates of mean total N input across the growing season (June 3rd to September 13th) for each cyanobacterial association ranged from 3.4 kg N $ha^{-1}yr^{-1}$(Hollow BSC) to 24.9 kg N $ha^{-1}yr^{-1}$ (*S. paschale*) for a 103 day growing season (Table 5).

Table 5. Mean total N fixed over the growing season (June 3rd to September 13th) based on estimates of N_2-fixation by Hollow BSC, Hummock BSC, *Sphagnum* spp. and *Stereocaulon paschale* at Daring Lake, NWT in 2007 and 2008. Microclimatic models were used to predict hourly acetylene reduction rates per m^2 (See Table 4). ARA to N_2-fixation conversion ratios (Table 3) were applied for each N_2-fixing association. Rates were summed to give total mg N $m^{-2}yr^{-1}$ based on the 2007 and 2008 growing seasons indicated by Julian days. The mean of 2007 and 2008 estimates based on a 103 day growing season (154-257) in both years was used to determine mean total N.

N_2-Fixing Association	Year (Julian Days)	Total mg N $m^{-2}yr^{-1}$	Mean Total N $kg^{-1}ha^{-1}yr^{-1}$ (Julian Days 154-257)
Hollow BSC	2007 (169-257)	334	3.4
	2008 (154-235)	292	
Hummock BSC	2007 (169-257)	622	7.1
	2008 (154-235)	645	
Sphagnum spp.	2007 (152-257)	1865	20.5
	2008 (144-259)	2460	
Stereocaulon	2007 (152-257)	3150	24.9
paschale	2008 (144-259)	3204	

N input by N_2-fixing associations and by ecosystem types

No single N_2-fixing association dominated N inputs across all of the ecosystem types. *Stereocaulon paschale* was the largest source of N input in both Xerophytic Herb Tundra and Heath-Lichen/Mat-Lichen Tundra followed by Hummock BSC (Table 6). *Sphagnum* spp. was the largest source of N input in both Birch Hummock and Wet Sedge Meadow ecosystems followed by Hollow BSC. Despite having the highest mean N_2-fixation rate, *S. paschale* did not have the highest overall landscape N input (549.84 kg; Fig. 4). *Sphagnum* spp. had the highest N input (1030.72 kg) due to its relatively high mean N_2-fixation rate and greater area within the landscape (50.28 ha) compared with *S. paschale* (22.11 ha) (Fig. 4).

Table 6. The contributions of individual N_2-fixing associations to total N input across the selected landscape study area and to N inputs per unit area for each of the major ecosystem types at Daring Lake, NWT over the growing season (Julian days 154 to 257).

Ecosystem type	Total area (ha) (and proportion) of each ecosystem type within the study landscape	N_2-fixing association	Mean cover of N_2-fixing association within each ecosystem type (%)	Area of each N_2-fixing association (ha)	N input by each N_2-fixing association within each ecosystem type (kg N	Total N input within each ecosystem type (kg N yr^{-1})	Total N input per unit area for each ecosystem type (kg N $ha^{-1}yr^{-1}$)
Xerophytic Herb Tundra	74.58 (5.5%)	Hollow BSC	0.02	0.01	0.03	54.48	0.73
		Hummock BSC	4.44	3.31	23.54		
		Sphagnum spp.	0.28	0.21	4.31		
		Stereocaulon sp.	1.44	1.07	26.61		
Heath-Lichen/ Heath-Mat Tundra	568.87 (42.0%)	Hollow BSC	0.36	2.05	7.07	777.19	1.37
		Hummock BSC	4.19	23.84	169.52		
		Sphagnum spp.	0.82	4.66	95.53		
		Stereocaulon sp.	3.57	20.31	505.07		
Birch Hummock	171.29 (12.6%)	Hollow BSC	4.12	7.06	24.35	235.39	1.37
		Hummock BSC	0.53	0.91	6.47		
		Sphagnum spp.	5.33	9.13	187.16		
		Stereocaulon sp.	0.41	0.70	17.41		
Wet Sedge Meadow	68.9 (5.1%)	Hollow BSC	2.39	1.65	5.69	750.24	10.89
		Hummock BSC	0.02	0.01	0.07		
		Sphagnum spp.	52.65	36.28	743.73		
		Stereocaulon sp.	0.04	0.03	0.75		

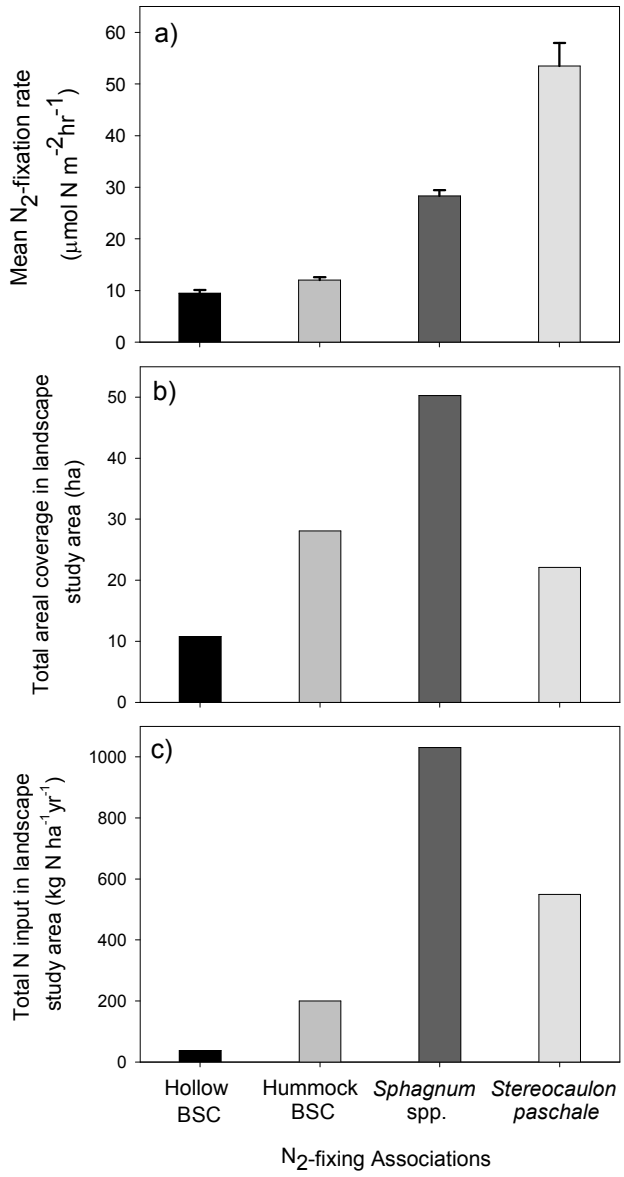

Figure 4. a) Mean N_2-fixation rate measured over the growing season 2007-2008 (see Table 3), b) total area of N_2-fixing associations in landscape study area, and c) total N input for Hollow BSC, Hummock BSC, *Sphagnum* spp. and *Stereocaulon paschale* determined by potential N_2-fixation models in landscape study area at Daring Lake NWT. Total area of N_2-fixing associations and total N input in landscape study area were calculated from values in Table 6. Error bars in a) indicate standard error.

We used the model estimates of growing season N_2-fixation by each cyanobacterial association along with the mapping data of the distribution of ecosystem types in our landscape study area to estimate overall N inputs in each ecosystem type. Total N input per unit area was ~10 times higher in the Wet Sedge Meadow than in any other ecosystem type (Table 6). Our spatially explicit analyses indicate that this effect can be explained by particularly high inputs by *Sphagnum* spp. cyanobacterial associations due to relatively high fixation rates (Table 5) in combination with relatively large proportional cover of this association in the Wet Sedge Meadow (Table 6). Heath-Lichen/Heath-Mat Tundra fixed relatively small quantities of N per unit area but made the largest total N input in our selected landscape study area because of its abundant coverage in the landscape. Birch Hummock tundra had similar total N_2-fixation rates per unit area to Heath-Lichen/Heath-Mat Tundra, but its coverage was low in the study area, resulting in low total N inputs. Finally, N_2-fixation rates per unit area within Xerophytic Herb Tundra were lowest, and its coverage was also low, resulting in relatively small N inputs into the selected landscape study area. Total N input for 68% the Daring Lake landscape study area over the 103 day growing season was 0.68 kg $ha^{-1}yr^{-1}$.

DISCUSSION

Our study demonstrates that biological N_2-fixation across a low arctic landscape is both temporally and spatially heterogeneous due to the presence of distinct cyanobacterial associations that varied in their responses to seasonal environmental changes, and in their distribution among vegetation types. Our study design integrated individual N_2-fixing association responses to seasonal microclimatic conditions, the abundance of each N_2-fixing association within different ecosystem types and the prevalence of the ecosystem types

within the landscape. By employing this multi-scale approach we not only provided a landscape-level estimate of N input via N_2-fixation (0.68 kg $ha^{-1}yr^{-1}$), but also identified the ecosystem type (Wet Sedge Meadow), cyanobacterial association (*Sphagnum* spp.) and microclimatic controls (moisture and temperature) that are key to understanding biological N inputs. In addition, we found a significant interaction between growing season month and type of N_2-fixing association, indicating that changes in seasonal progression of N_2-fixation activity vary among cyanobacterial associations. Further, our results highlight the importance of considering both the abundance cover and average N_2-fixation rate of each N_2-fixing association in characterising the controls on patterns of N input across the landscape, and in estimating the total magnitude of N inputs. For example, the primary importance of *Sphagnum* spp. associations to total landscape N inputs was due to their relatively high rates of N_2-fixation, as well as their high percent cover compared to the other N_2-fixing associations. By contrast, the lichen *S. paschale* was relatively infrequent on the landscape but made the second largest contribution to total N inputs because it had particularly high rates of N_2-fixation (Fig. 4). Together these results provide substantial insights into the principal factors causing both temporal and spatial heterogeneity in biological N_2-fixation inputs in the low Arctic.

Microclimatic Controls on Seasonal and Spatial Variation in N_2-Fixation

Several studies have detected distinct seasonal patterns in N_2-fixation rates in the Arctic (Alexander & Schell, 1973**;** Henry & Svoboda, 1986; Chapin et al., 1991; Zielke et al., 2005)**.** Our data (Table 2 and Fig. 2) are consistent with the general pattern of detectable N_2-fixation rates soon after snowmelt (March-June) followed by the highest rates coinciding with the peak growing season and declining rates in late July to August depending on

latitude. Our field measurements of N_2-fixation demonstrate that seasonal patterns vary among the vegetation communities, which may account for the lack of seasonal variation detected in studies that average across both topography and vegetation type (Hobara et al., 2006).

Moisture appears to be one of the most important environmental factors controlling N_2-fixation across various arctic environments (Chapin & Bledsoe, 1992; Nash & Olafsen, 1995; Zielke et al., 2002, 2005). Moisture can affect seasonal patterns of N_2-fixation within individual N_2-fixing communities, and spatial heterogeneity in N_2-fixation is often a reflection of differences in cyanobacterial biomass due to the long-term characteristics of the community moisture regime (Chapin et al., 1991). *Stereocaulon paschale*, which was primarily located on xeric esker tops and well-drained upper esker slopes, had the highest mean rate of N_2-fixation over the growing season, but demonstrated a strong sensitivity to desiccation. Lichens are often established on drier exposed habitats where nitrogenase activity may be reduced to a few comparatively short episodes when moisture conditions are suitable (Crittenden & Kershaw, 1979). Rainfall in July 2008 was 6.7 mm compared to 24.6 mm and 28.1 mm in June and August respectively. Accordingly, the average percent moisture of *S. paschale* incubated in July was 26% as compared to June (56%) and August (45%), perhaps explaining the relatively low July N_2-fixation rates. The relatively high and consistent N_2-fixation rates associated with *Sphagnum* spp. over the growing season are likely due to the consistently high moisture conditions of the low-lying Wet Sedge Meadow where *Sphagnum* spp. are the dominant vegetation.

Temperature has also been significantly correlated with N_2-fixation rates in the Arctic (Smith, 1984; Lennihan et al., 1994; Liengen & Olsen 1997b; Zielke et al., 2002). Our strong

correlations between N_2-fixation and temperature for Hollow BSC, Hummock BSC and *Sphagnum* spp. indicate that seasonal temperature fluctuations are important in determining seasonal rates of N_2-fixation. Like Zielke et al., (2005), we also found that temperature was a good predictor of N_2-fixation provided different models were used depending on moisture condition. Hollow BSC had lower field and optimal rates of N_2-fixation in June compared with other cyanobacterial associations (Table 3). Mean Hollow BSC temperature in June was 6.8^{o}C compared to 9.1^{o}C for Hummock BSC. Therefore, the lower rates of N_2-fixation for Hollow BSC are likely due to the persistence of snow in these depressions resulting in relatively low temperatures, as well as, restricted light inputs that together may impede the recovery and development of cyanobacterial communities in the early growing season (Fig. 5).

Figure 5. Hummock-hollow complexes at Daring Lake, NWT June 17, 2008. Differences in early season fixation rates between Hollow and Hummock BSC may result from snow covering hollows of hummock-hollow complexes whereas Hummock BSC is exposed.

Some studies have found N_2-fixation to be light-dependent (Granhall & Lid-Tosvik, 1975; Alexander et al., 1978) while others have found little light dependence as photosynthetic rates tend to saturate at relatively low light levels (<500 μmol PAR $m^{-2}s^{-1}$) (Coxson & Kershaw, 1983b; Smith, 1984; Chapin & Bledsoe, 1992; Nash & Olafsen, 1995; Zielke et al., 2002). Varying light conditions (0 to 1000 μmol PAR $m^{-2}s^{-1}$) didn't affect *Sphagnum* spp. N_2-fixation rates in our study, supporting the concept that stored energy for N_2-fixation, combined with continuous or near continuous daylight and a limited plant canopy, reduce the potential for light to act as a controlling factor on N_2-fixation in the Arctic (Chapin & Bledsoe, 1992). Nevertheless, remote sensing, repeat photography, and warming experiments in combination with nutrient addition studies all suggest that current warming trends in the low Arctic may be promoting shrub growth and expansion within various topographic positions (Chapin et al., 1995; Sturm et al., 2001; Goetz et al., 2005; Walker et al., 2006). Declining macrolichen abundance in warming sub- and mid-arctic environments may be a function of increased growth and abundance of deciduous shrubs, which may inhibit lichen performance and persistence through shading (Cornelissen et al., 2001). N_2-fixation rates and persistence of other N_2-fixing associations in these environments may also be similarly influenced by reduced light availability.

The higher N_2-fixation rates detected under optimal conditions for all N_2-fixing associations indicate that microclimatic conditions in the field are limiting N_2-fixation for all of the principal N_2-fixing associations. We conclude that climate change scenarios that result in warmer surface temperatures without increased surface desiccation are likely to lead to higher rates of N_2-fixation (Chapin & Bledsoe, 1992).

The Significance of Biological N_2-Fixation to N Cycling in a Low Arctic Landscape

Since N availability is commonly a major limitation on tundra plant growth, our results provide important insights to understanding the functioning of low arctic terrestrial ecosystems. Our estimate of mean seasonal N_2-fixation in Birch Hummock (Table 5– 1.4 $kg^{-1}ha^{-1}yr^{-1}$ over 103 days) is ~$1/300^{th}$ the late summer rate of gross N mineralisation by soil microbes in the same ecosystem type (Buckeridge et al., 2010). Therefore, internal recycling of N from soil organic matter is undoubtedly the critical N supply process within the Birch Hummock ecosystem type at least. Nevertheless our data suggest a significant influence of N_2-fixation on N cycling and carbon uptake at a larger scale. N_2-fixation during the growing season was highest in the Wet Sedge Meadow, which is also the ecosystem with the largest annual plant primary production in this landscape (Nobrega & Grogan, 2008). Nutrient inputs associated with run-off and leachates from higher elevation ecosystems toward the valley floor where wet sedge ecosystems predominate may facilitate the high rates of primary production there. Our data here suggest that in addition to that process, *in situ* N_2-fixation inputs may be an important pathway supplying N to support the high primary productivity of this ecosystem type.

Total biological N_2-fixation input across the study landscape area was estimated at 0.68 kg N $ha^{-1}yr^{-1}$. Previous estimates of arctic N_2-fixation inputs range from 0.06 to 3 kg N $ha^{-1}yr^{-1}$, with the majority of estimates ranging from 0.10 to 1.20 kg N $ha^{-1}yr^{-1}$ (Alexander & Schell, 1973; Barsdate & Alexander, 1975; Chapin & Bledsoe, 1992; Hobara, 2006). Summertime mean atmospheric N inputs from wet deposition at the nearest monitoring station (Snare Rapids, 63.52°N 116.00°W) to Daring Lake (~240 km away) were 0.39 kg N $ha^{-1}yr^{-1}$ (1991-2006; CAPMon, Environment Canada, unpublished data). Wintertime atmospheric N

deposition inputs as total inorganic N accumulation in ambient snow packs (0.3 m) at Daring Lake in 2007 were 0.05 kg N ha^{-1} (Buckeridge & Grogan, 2010). Together, these numbers suggest that total biological N_2-fixation input for the landscape study area at Daring Lake is approximately twice the amount of N deposited via atmospheric deposition. While some studies have found N_2-fixation contributed 80% or higher to total landscape N inputs (Hobara et al., 2006; Solheim et al., 2006), other studies, including ours, have found the contribution of N_2-fixation to ecosystem N inputs is approximately 50%-70% (Chapin & Bledsoe, 1992; Henry & Svoboda, 1986).

We found N_2-fixation across a low arctic tundra landscape was concentrated in the Wet Sedge Meadow ecosystem type where N_2- fixation per unit area was ~10 times higher than in any of the other ecosystem types (Table 5). Of the four principal N_2-fixing associations, *Sphagnum* spp., which had the highest percent cover in Wet Sedge Meadows made the largest contribution (55.2%) to total N input. Several other studies have also found the highest rates of N_2-fixation in arctic landscapes are associated with cyanobacteria moss associations (Alexander & Schell, 1973; Henry & Svoboda, 1986; Solheim et al., 1996). Five methodological constraints may have affected our landscape estimates of biological N_2-fixation inputs. Firstly, conversion ratios can vary depending on the operating environment of a given N_2-fixing association (Millbank, 1981; Gunther, 1989), and seasonal variation in conversion ratios have been detected for free-living cyanobacteria from high arctic habitats (Liengen, 1999b). Secondly, we used visual estimates of abundance for the N_2-fixing associations without accounting for variations in cyanobacterial biomass that can impact rates of N_2-fixation. Thirdly, the ecosystem types in our analysis account for only 68 % of the Daring Lake study area. Some excluded ecosystem types (Exposed Sand and Gravel and

Rocky Outcrops) probably contribute little to landscape N input. However, other ecosystem types such as Dry Sedge Meadows (8.2%) may contain considerable *Sphagnum* spp. cyanobacterial associations and therefore may make significant contributions to landscape N input, albeit for a limited duration due to less favourable microclimatic conditions. Fourthly, our estimates of modelled N inputs would have been improved by more accurate quantification of spatial variability in soil surface microclimate by using a much larger number of climate sensors. This is a limitation that is common to many studies of arctic and subarctic ecosystems (Rouse, 1976; Young et al., 1997). Fortunately, the type of conditions that favour N_2-fixation by most cyanobacterial associations (i.e. during or immediately following growing season precipitation events) will tend to minimize between site variability in soil surface microclimate, reducing the impact of this factor on our estimates. Fifthly, we used a 103 day growing season as the basis for yearly N input. However, N_2-fixation likely occurs outside of this period whenever microclimatic conditions are favourable (Davey, 1983; Liengen, 1999a; Zielke et al., 2002; Hobara et al., 2006). In summary, we conclude that provided our conversion ratios, percent cover of cyanobacterial associations and models of potential N_2-fixation are sufficiently accurate, then our estimates of ecosystem N_2-fixation inputs and of total landscape-level N input over the growing season are minimum values.

CHAPTER 3: SMALL-SCALE SPATIAL PATTERNS IN N2-FIXATION AND NUTRIENT AVAILABILITY IN AN ARCTIC HUMMOCK-HOLLOW ECOSYSTEM

ABSTRACT

Atmospheric nitrogen that is fixed by associative cyanobacteria can be released into the surrounding soil environment providing a key source of N for terrestrial arctic ecosystems. Yet, little is known about nitrogen fixation by Biological Soil Crusts (BSCs) within hummock-hollow complexes that are typical of many arctic tundra environments. In this study, we examined spatial and temporal patterns in N_2-fixation, dinitrogenase reductase (*nif*H) gene abundance and release of N in a low arctic hummock-hollow ecosystem. The impacts of cyanobacteria on N status in soil were evaluated by assessing soil nitrogen in relation to the cyanobacterial associations found on Hummock and Hollow BSCs. In addition, potential P limitation of N_2-fixation by cyanobacteria was assessed for Hummock and Hollow BSCs. The tops of hummocks and the bottoms of hollows were areas of high N_2-fixation, whereas minimal N_2-fixation occurred on the sides of hummock-hollow complexes. Compared with Hummock BSCs, Hollow BSCs had a higher mean growing season N_2-fixation rate, a higher mean growing season *n*ifH abundance, a higher mean total %N and δ15N values closer to that of atmospheric N_2. Soil N status was linked to rates of N_2-fixation by BSCs indicating that these N_2-fixing associations act as important point sources of soil N in this low arctic ecosystem. Over the course of a growing season temporal variation in N_2-fixation and *n*ifH abundance were weakly linked suggesting that N_2-fixation was carried out by complex communities of diazotrophic microorganisms and that factors such as nutrient availability may limit N_2-fixation to a greater extent than *nif*H abundance.

INTRODUCTION

Atmospheric N_2-fixation is a main source of N input in arctic ecosystems (Bazely & Jefferies, 1989, Chapin & Bledsoe, 1992; Hobara et al., 2006). Up to 70% of the N_2 fixed by associative cyanobacteria can be released into the surrounding soil environment providing a key source of N for soil ecosystems (Alexander & Schell, 1973; Harper & Belnap, 2001; Mayland & MacIntosh, 1966; Stewart, 1967). In arctic environments N_2 fixed by cyanobacteria can provide readily available N (Alexander et al., 1978; Chapin & Bledsoe, 1992) but there is high spatial variability in N_2-fixation (Gold et al., 2001), often due to landscape topography (Biasi et al., 2005; Mueller et al., 1999; Walker et al., 2004). Hummock-hollow complexes are common features in tundra ecosystems and provide a model system for investigating the influence of microtopography on N_2-fixation and the subsequent distribution of soil nutrients. Well-developed Biological Soil Crusts (BSCs) on hummocks and in hollows are important point sources of nitrogen within the landscape (Stewart et al., unpublished data). However, the small-scale spatial patterns of nutrient availability associated with these point sources are not well understood.

*Nif*H is the gene that encodes for the Fe protein subunit of nitrogenase, the enzyme responsible for nitrogen fixation (Deslippe et al., 2005). Since, *nif*H is highly conserved among all diazotrophic groups it is an ideal molecular marker for N_2-fixing organisms. Assessment of the *nif*H abundance associated with BSCs in hummock-hollow complexes can provide important insights into temporal and spatial variability in N_2-fixation and the subsequent patterns of nutrient availability.

Most soil nutrients do not have a homogeneous spatial distribution across an ecosystem and soil chemistry varies among plant types and between microsites (Biasi et al., 2005; Housman

et al., 2007). The role that some N_2-fixing associations play in altering nutrient availability remains controversial (Belnap, 2001; Johnson et al., 2005; Knowles et al., 2006; Lagerstrom et al., 2007). A variety of factors can affect the concentration of available nitrogen in the soil, including uptake by plants, immobilisation by microbes, soil temperatures, microtopography and time of year (Biasi et al., 2005; Veluci et al., 2006). Furthermore, rainfall intensity alters the influence of BSCs on soil N concentrations. Higher intensity rainfall events (i.e. high volume of precipitation over short time) result in greater amounts of N or C being released because organisms are not able to reassimilate losses (Crittenden, 1983; Wilson & Coxson, 1999). In general the greatest release of leachates occurs upon initial rewetting after a prolonged period of desiccation. Thus, a comparison of N availability below BSCs in hummock-hollow complexes and the release of nutrients upon rewetting can be used as an indication of the importance of N_2-fixing association type, microtopography and N cycling processes that can influence the N status of soils.

Although N limitation is often cited as the main factor limiting ecosystem productivity, the fixation of N may in turn be limited by phosphorus availability (Cole & Heil, 1981; Crews, 1993; Eisele et al., 1989; Smith, 1992). Phosphate is a limiting factor for N_2-fixation by cyanobacteria in arctic habitats (Basilier & Granhall, 1978; Chapin et al., 1991; Liengen, 1999a). Cole & Heil (1981) suggest that close linkages between P and N cycling processes are related because of the large energy requirements of N transformations. Low phosphorus availability may reduce rates of photosynthesis, which in turn may inhibit nitrogenase by reducing photosynthate supplies and in particular the supply of ATP (Crews, 1993; Hartley and Schlesinger, 2002; Layzell, 1990).

The objective of this study was to examine spatial and temporal patterns in N_2-fixation, *nif*H abundance and release of N in a hummock-hollow low arctic environment. We hypothesized that BSC N_2-fixation rates would be linked to soil N status and that the effect of BSC on soil fertility would differ between BSC type and location. Furthermore, we hypothesized that BSC N_2- fixation activity would be limited by P supply.

METHODS

Study Site and N_2-Fixing Associations

The study area was located in a low Arctic tundra region at the Tundra Ecosystem Research Station, Daring Lake, Northwest Territories, Canada (64°52'N, 111°35'W) (Fig. 2). Elevation ranges from 414-470 m a.s.l. and landscape features include eskers, boulder fields, exposed bedrock, upland and lowland tundra, wetlands and various sizes of lakes, ponds and streams. Continuous permafrost is present at the site with a soil active layer ranging from 0.3-2 m (Obst, 2008).

Mean monthly air temperature in January is -30°C and +13°C in July (INAC, personal communication; Obst, 2008). Snow-melt usually starts after mid-May ending in early June, leaving some snow beds on slopes until late June or early July. The growing season usually occurs between the end of May or early June and ends after mid-August.

Located within the physiographic zone of the Bear-Slave Upland of the Canadian Shield, approximately 90 km northeast of the northern limit of continuous trees, the Daring Lake study site is classified as low Arctic (Obst, 2008). Several ecosystem types including Xerophytic Herb Tundra, Heath-Lichen Tundra, Heath-Mat Tundra and Birch Hummock are present in the landscape and classification follows Obst (2008). The hummock-hollow

complexes investigated were formed from cryoturbated mineral soil mounds approximately 30-50 cm in height and adjoining depressions of approximately the same depth. Complexes are often in groupings of several hummocks and hollows occupying an area of 1-5m^2 occurring mainly within the Birch Hummock ecosystem type. Birch Hummock occurs in moderately to poorly-drained terrain on gentle lower esker slopes (Obst, 2008). Soils in the Birch Hummock ecosystem are classified as Orthic Dystic Turbic Cryosols (Soil Classification Working Group, 1998), which consist of an organic layer above a silt-sand mineral layer (Buckeridge et al., 2009). Vegetation was characterized by scattered shrubs (0.2 – 0.5 m tall) of Dwarf Birch (*Betula glandulosa* Michx.), Cloudberry (*Rubus chamaemorus* L.), Willows (*Salix* spp.), Labrador Tea (*Ledum decumbens* Ait.) and tussock-forming Sheathed Cotton-grass (*Eriophorum vaginatum* L.). Mosses (*Sphagnum* spp*., Ditrichum* sp*., Polytricum piliferum* Hedwig.)*,* liverworts (*Anastrophyllum minutum* Schreb. and *Cephalloziella* spp.) and lichens (*S. paschale*, *Bryoria tenuis* (E. Dahl) Brodo & D. Hawksw*., Placynthiella uliginosa* Schrader. and *Cladonia* spp.) are also common.

Two major BSC communities were found in association with hummock-hollow microtopography. Hollow BSCs were found mainly in the depressions between hummocks and were composed of liverworts growing in dense mats generally underlain by varying depths of organic matter. The main components of Hollow BSCs were *Anastrophyllum minutum* Schreb. and *Cephalloziella* spp. including *C. rubella* (Nees) Warnst. and *C. hampeana* complex. Cyanobacteria on Hollow BSCs mostly the filamentous and heterocyst containing cyanobacterium *Stigonema* cf. *turfaceum* (Berk.) Cooke. However, on some samples filamentous and heterocystous *Tolypothrix* sp., and the filamentous, non-heterocystous *Schizothrix* cf. *cuspidata* W. et G.S. West., were found growing in between the

leafy liverworts and on the *Stigonema* filaments. *Stigonema minutum* (C. Agardh) Hass. and *Calothrix* sp. were also found on Hollow BSC samples. Hummock BSCs were cohesive well-developed crusts (1-2 cm thick) found on cryoturbated mineral soil mounds. Small less well-developed patches of Hummock BSC also occurred in sandy well-drained areas on ridge tops. Hummock BSCs were complex communities made up of lichens, mosses and liverworts. Lichen species included *P. uliginosa*, *Bryocaulon divergens* Ach., *B. tenuis*, *Cladonia* spp., *Japewia tornoensis* Nyl., *Ochrolechia frigida* Sw., and *Solorina crocea* L. Moss species (*Funaria* sp. *Pohlia* sp. *Ditrichum* sp. *and P. piliferum*) and liverwort species (*Cephalozia* sp., *Cephaloziella* sp., *Anastrophyllum* sp., *Anthelia* sp., *Lophozia* sp. and *Lophozia incise* Schrad.) were also key components of these diverse communities. *Stigonema turfaceum*, *S. minutum* and *S. hormoides* (Kutz.) Born. & Flah. were found on Hummock BSCs, however, *Gloeocapsa decorticans* (A. Braun) Ritcher., *G. novacekii* (Komárek & Anagnotid.), *S. cuspidata, Anabaena* sp. and *Chroococcidiopsis* sp. were also present.

N_2-Fixation Rates

Measurements of N_2-fixation were made using acetylene reduction assays (ARAs) (Stewart et al. 1967). Acetylene gas (C_2H_2) was generated on-site from CaC_2 and water, with incubations injected with 10% (v/v) acetylene. Ethylene concentrations were measured in the field with a portable gas chromatograph (SRI 8610A, Wennick Scientific Corporation, Ottawa, ON, Canada) fitted with a Porapak column (Alltech Canada, Guelph, ON, Canada) and a flame ionization detector. A stand-alone hydrogen generator (SRI H_2-50, Alltech Canada, Guelph, ON, Canada) provided hydrogen as the carrier gas, which was held at a constant pressure of 26 psi. Column temperature was held at 65^{o}C.

Cores (19cm^2, 0.75 cm depth) of Hollow BSC (n=12) and Hummock BSC (n=12) were randomly selected at independent hummocks and hollows in the study site over the growing season (June 15th -16th, July 3rd-4th, August 7th-8th 2008). Samples were enclosed in 250 ml glass canning jars with rubber septa placed through a modified lid. For each set of incubations one sample for each N_2-fixing association was used as a control, which served as both a temperature control fitted with a thermocouple and a blank not injected with acetylene. Control samples did not show any natural evolution of ethylene.

Acetylene reduction assay (ARA) incubations were run for 6 hours between 10:00-16:00 h. Photosynthetically active radiation (PAR), air temperature, incubation temperature and ambient temperature of Hollow and Hummock BSC were monitored every 30 minutes during ARA incubations. Incubation chambers were placed in water baths and bath temperature was controlled to ensure that incubation temperatures reflected ambient conditions. On average the surface temperature of incubation samples were within 1.5^{o}C of the surface temperature of the respective N_2-fixing associations under ambient conditions. Following incubation all samples were weighed to determine percent moisture and immediately frozen at approximately -20^{o}C.

In addition, N_2-fixation was assessed at distances down from hummocks and up from hollows respectively. Surface cores (19 cm^2, 0.75 cm depth) on hummocks were taken at the lower edge of the mound with BSC (0 cm) and at 5, 15 and 30 cm in a downwards direction (Fig. 7, Insert a). Surface cores in hollows were taken at the very bottom of the hollow with BSC (0cm) and at 5, 15 and 30 cm in an upwards direction (Fig. 7, Insert b). All cores were incubated under optimal light and temperature conditions (200µmolm^{-2}s^{-1}, 20^{o}C) at the Daring Lake study site in 2007/2008. Samples at each distance from hollows (n=10) and

hummocks (n=10) were treated in the same way as field incubations, with the exception of a 24 hr wetting pretreatment at optimal hydration levels.

DNA Extraction and *nif*H Quantitative PCR

Frozen samples from ARA incubations were homogenized and a 0.5 g sub-sample was collected for DNA extraction. The extraction procedure followed Griffiths et al. (2000) with the exception that DNA was purified overnight in PEG. Extracted DNA was stored at -20^{o}C. The amplification employed a forward primer (5'-TGGTCCTGAGCCTGGAGTTG) and reverse primer (5'-TCTTCTAGGAAGTTGATGGAGGTGT) to amplify a 359 bp fragment of the *nif*H gene from a diluted extract (Church et al., 2005). Primers were synthesized by Invitrogen (Burlington, ON, Canada). The reaction mixtures consisted of approximately 10 ng genomic DNA, 10 µM of each primer, 10 µl of SsoFast™ EvaGreen® Supermix (Bio-Rad, Mississauga, ON, Canada) and 6 µl of dH_2O for a final volume of 20 µl. A 7500 Real Time PCR System (Applied Biosystems, Foster City, CA, USA) was used for quantitative detection of amplified PCR products using the following thermal cycling conditions: 50^{o}C for 2 min, 97^{o}C for 15 min, and 45 cycles of 94^{o}C for 15 s, 58^{o}C for 40 s, 72^{o}C for 30 s and 78^{o}C for 45 s, followed by 95^{o}C for 15 s, 60^{o}C for 1 min and 95^{o}C for 15 s. *Nif*H gene copies were quantified relative to a standard curve. Standards were made from serial dilution of a positive control providing a range of *nif*H targets containing between 10^{1} and 10^{10} *nif*H gene copies that were repeated in triplicate. PCR amplifications were repeated at least twice for each environmental sample. Least-squares linear regression analyses of Ct values of the standards versus log10 *n*ifH gene copies were used to quantify target genes in the environmental samples.

Total N and Natural Abundance ^{15}N

Soil samples for determination of total N and natural abundance ^{15}N were collected from hummocks (n=10) and hollows (n=10) July 13th-14th 2008. Hummock samples were collected at the lower edge of the mound with BSC (0cm) and at 5, 15 and 30cm in a downward direction (Fig. 7, Insert a). Hollow samples were collected at the very bottom of the hollow with BSC (0cm). At each sampling location for both hummocks and hollows the surface (top 1 cm) and the 2 cm below the surface sample were collected and termed upper and lower respectively. All samples were air dried and dry samples were ground in a ball mill and sent for total nitrogen and natural abundance ^{15}N ($\delta^{15}N$) analysis (Stable Isotope Facilities, University of Saskatchewan, Saskatoon, SK, Canada).

Nutrient Release

Seasonal nutrient availability

Nutrient and mineral availability over the growing season was assessed as nutrient supply rate to ion exchange resins under Hummock BSC and Hollow BSC at 10 different randomly selected locations in the study area. Supply rates of NO_3-N, NH_4-N, Ca, Mg, K, P, Fe, Mn, Cu, Zn, B, S, Pb, Al and Cd over the growing season were determined with Plant Root Simulator (PRS^{TM}) probes (Western AG Innovations Inc., Saskatoon, SK, Canada). PRS^{TM}-probes have an ion exchange membrane encapsulated in a plastic probe (15 cm X 3 cm). For each N_2-fixing association at each sampling location the average of 4 anion PRS^{TM}-probes and 4 cation PRS^{TM}-probes were used to determine nutrient availability. Probes were inserted at a shallow angle (<20^{o}) below (~2 cm depth) N_2-fixing associations on June 1st 2008. Since we were interested in the nutrient supply rate under normal field conditions including the influence of soil water content, water was not added during the experiment nor

was soil pre-wetted. Control probes that were not buried but placed in sample bags and refrigerated (~5^{o}C) for the duration of the burial were used to ensure contamination of probes had not occurred. Buried probes were immediately rinsed with deionized water and placed in the refrigerator upon removal from the soil on August 24th 2008. PRS^{TM}-probes were eluted using 0.5N HCl for 1 hour, following which the eluent was analyzed for NO_3^--N and NH_4^+-N by colourimetry using a Technicon Autoanalyzer II (TIC 1977). Analysis for other nutrients was done using Inductively Coupled Plasma-Plasma Emission Spectroscopy. All analyses were done by Western AG Innovations Inc, Saskatoon, SK, Canada.

Throughflow upon rewetting

Surface cores (10.5 cm diameter, 0.5 cm depth, 86 cm^2) of Hummock BSC (n=22) and Hollow BSC (n=22) were randomly collected from the study area on June 5th and June18th-19th. In the laboratory, samples were air dried and placed on acid washed mesh covered funnels with collection bags beneath. Using deionized water each core was misted from a height of 10 cm at a rate of approximately 0.67 ml min^{-1} over the 3 hr experiment. Throughflow captured in collection bags was removed at 3 time intervals: 1 hr, 2 hrs and 3 hrs after wetting. Control samples (n=8) collected in the same manner but without BSCs present were used to control for contamination. Samples were immediately filtered through Nalgene Nylon Membrane Filters (47mm diameter, pore size 0.45µm) and kept frozen (-20^{o}C) until analysis. For the 1 hr collection time interval inorganic N, total soluble N, organic N and total dissolved C were analyzed. Only inorganic N was analyzed for the 2 and 3 hr time intervals. Prior to analysis samples were filtered again through 0.45µm membrane filters. Inorganic N (NH_4-N and NO_3-N) was determined on an OI-Analytical Alpkem FSIV instrument (EZkem, Oregon, USA). Total soluble N was measured on the same instrument

after an autoclave facilitated alkaline-persulphate digestion. Organic N was reported as the difference between the total soluble and inorganic fractions. Total dissolved carbon was determined using liquid capsules on a Leco Truspec CNS combustion elemental analyzer (LECO corp., Michigan, USA). All throughflow sample analysis was completed by Analytical Chemistry Services B.C. Ministry of Forests and Range, Research Branch Laboratory, Victoria, BC, Canada.

Phosphorus Addition

Hummock and Hollow BSCs were collected from the study site on August 24th 2008 and transported to the laboratory where they were placed in a growth chamber under a 17/7 hr light (200 $\mu molm^{-2}s^{-1}$)/dark cycle with temperatures at 15^{o}C during light hours and 5^{o}C during dark hours. Cores (19 cm^2, 0.75 cm depth) of each type of BSC were sampled for phosphorus addition. Control samples (n=10) were treated with 5 ml of deionized water 4 times per week over a 4 week period and P addition samples (n=10) were treated over the same period with 5 ml of 10 $\mu mol\ l^{-1}$ P (as a $Na_2HPO_4 \cdot 7H_20$ solution). Total addition of P over the month long experiment was equivalent to 0.13 kg ha^{-1}. ARA incubations (4 hrs at 20^{o}C and 200 $\mu molm^{-2}s^{-1}$) were conducted at the end of week 2 and week 4 following the above protocol on both control and P addition samples for all BSCs. In a pilot study we found an increase in N_2-fixation for both control and the P addition treatments, however significant increases were not detected until after 2 weeks. Therefore, in this study the influence of P addition on N_2-fixation was determined by examining differences in the percent change in N_2-fixation rates between week 2 and week 4 for control and P addition samples.

Statistical Analyses

All statistical analyses were done using SYSTAT software (SYSTAT 8.0, Systat Software, Inc.). All N_2-fixation rates were log transformed and comparisons of N_2-fixation among summer months, growing season and distance from hollows and hummocks were done using ANOVA with Tukey post-hoc. *N*ifH copy numbers were log transformed and comparisons of growing season and monthly copy numbers done using t-tests and ANOVA with Tukey post-hoc respectively. Total % N was log transformed. Hollow and hummock total % N and $\delta^{15}N$ were compared using ANCOVA with upper and lower soil sample positions used as the covariate. Upper and lower total % N and $\delta^{15}N$ were compared at hummocks using an ANCOVA with distance from the hummock included as a covariate. Spatial patterns in total % N and $\delta^{15}N$ of upper soils at hummocks only were compared using ANOVA with Tukey post-hoc. Seasonal nutrient and mineral availability was compared between the two BSCs using t-tests. A log transformation was done on total N, NO_3-N, NH_4-N, Fe, Ca, S and Al. Concentration of nutrients in throughflow between hollows and hummock were compared using t-tests and comparison of time intervals was done using ANOVA with Tukey post-hoc. Comparison of percent increase in N_2-fixation between control and P addition samples were done using t-tests.

RESULTS

ARA and *nif*H Abundance

Over the growing season Hollow BSC had more *nif*H (6.8×10^8 *nif*H copies g^{-1} soil) and a greater acetylene reduction rate (134 nmol C_2H_4 $g^{-1}h^{-1}$) compared to Hummock BSC (8.4×10^7 *nif*H copies g^{-1} soil and 8.7 nmol C_2H_4 $g^{-1}h^{-1}$) (t-tests, $p = 0.04$, $p<0.01$ respectively). Hollow BSC had more *n*ifH in July compared to both June and August (ANOVA, Tukey

post-hoc, June p=0.01, August p=0.04) (Fig. 6a). Hummock BSC also had more *nif*H in July but only in comparison with August (ANOVA, Tukey post-hoc, June p=0.60, August p=0.03) (Fig. 6b). N_2-fixation in both hollows and hummocks was significantly lower in June compared to other summer months (ANOVAs, Tukey post-hoc, hollows July $p < 0.01$, August $p = 0.02$; hummocks July and August $p < 0.01$) (Fig. 6).

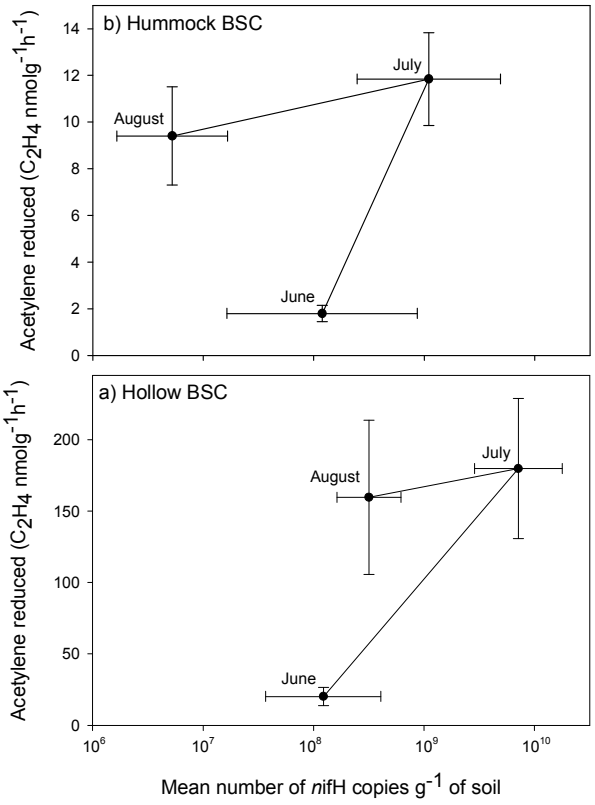

Figure 6. The relation between acetylene reduction (nmol $C_2H_4g^{-1}h^{-1}$) and mean number of *nif*H copies per g of soil for Hollow and Hummock BSC in June, July and August 2008 at Daring Lake, NWT. Data represents mean with standard error.

Spatial Patterns of N_2-Fixation and Natural Abundance ^{15}N

Rates of N_2-fixation declined rapidly with distance upwards from the bottom of hollows (ANOVA, Tukey post-hoc, $p<0.01$ for 15 and 30 cm) and downwards from the lower edge of hummocks ($p<0.01$ for 5, 15 and 30 cm) such that the side of the hummocks can be considered as an area of minimal N_2-fixation (Fig. 7). Although rates tended to be higher at the bottom of the hollow than at 5 cm upslope there was no significant difference in N_2-fixation over this short distance ($p=0.14$).

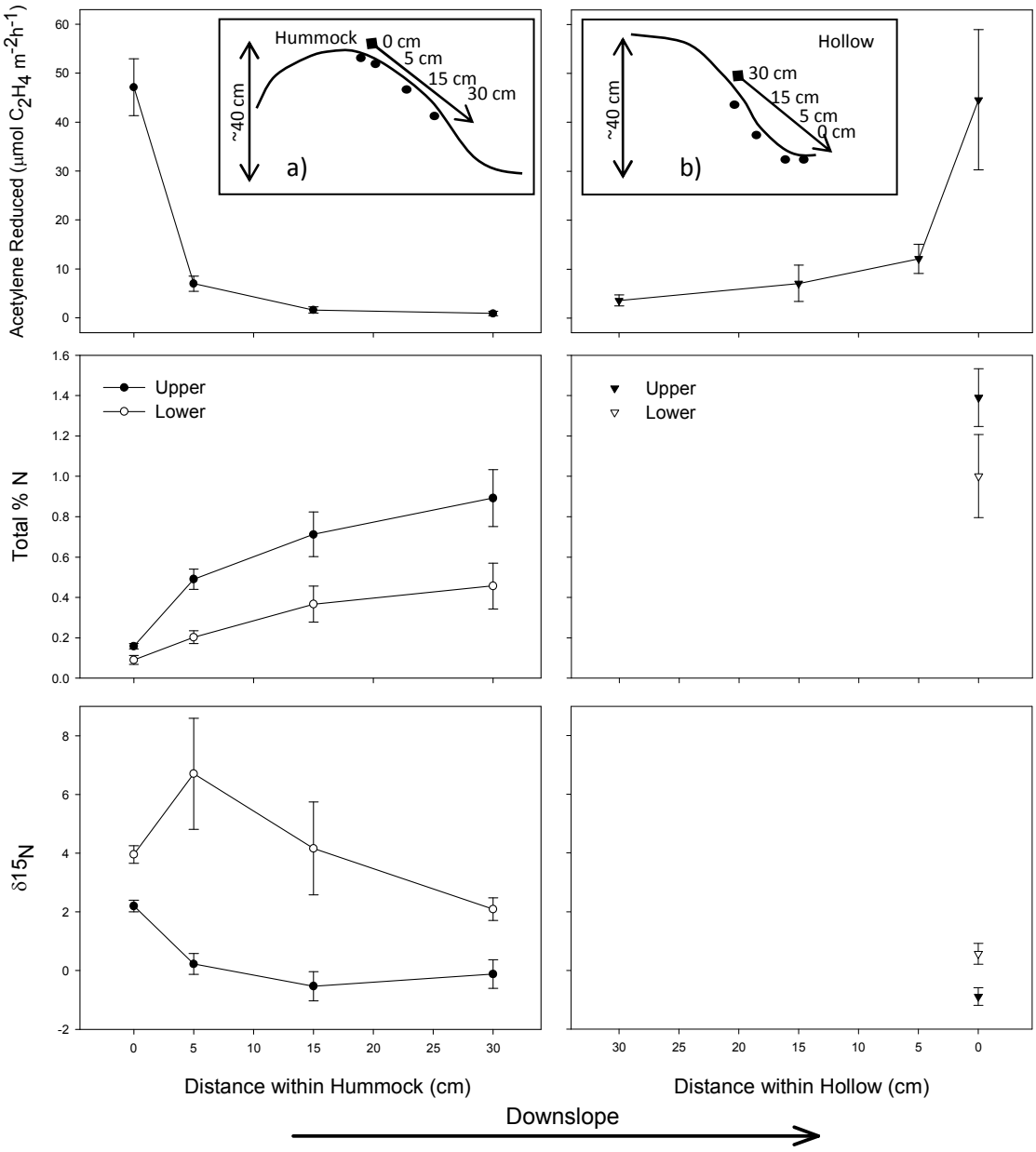

Figure 7. Mean acetylene reduction of surface samples at 0, 5, 15 and 30 cm in hummocks (insert a), (●) and hollows (insert b), (▼), mean total % N and mean $\delta^{15}N$ in upper (top 1 cm) and lower (2 cm below) soils at 0, 5, 15 and 30 cm in hummocks and at 0 cm in hollows at Daring Lake NWT. Data represents mean with $\pm$ SE.

The mean growing season acetylene reduction rate was over ten times higher for Hollow

BSCs, therefore, it is not surprising that we detected significantly higher total % N and $\delta^{15}N$

values closer to atmospheric N_2 in soils below hollows (0 cm) than in soils below hummocks (0 cm) (ANCOVAs, covariate = upper (top 1 cm) or lower position (2 cm below), $p< 0.01$) (Fig. 7). The mean upper hummock $\delta^{15}N$ value (2.20) had significantly higher ^{15}N enrichment compared with the mean upper hollow $\delta\ ^{15}N$ value (-0.89).

Total % N and $\delta^{15}N$ at hummocks were also investigated in upper and lower soil layers at 0, 5, 15 and 30 cm (Fig. 7). The upper soils layer of hummocks had significantly higher total % N and significantly lower $\delta^{15}N$ values compared with the lower soil samples (ANCOVA, covariate = distance from hummock, $p<0.01$) suggesting that this N was derived from N_2-fixation at the surface. Comparison of spatial patterns in the upper soils of hummocks alone revealed lower total % N on the hummock at 0 cm than at 15 and 30 cm (ANOVA, Tukey post-hoc, $p<0.01$ for all comparisons), but no significant difference between 0 cm and 5 cm ($p = 0.06$). $\delta^{15}N$ indicated significantly higher ^{15}N enrichment on the hummock (0 cm) compared with 5, 15 and 30 cm (ANOVA, Tukey, post-hoc, 5 cm $p=0.01$, $p< 0.01$ for all other comparisons), which did not have significantly different δ15N values from each other.

Nutrient Availability and N_2-Fixation

Between the two types of BSC, the supply rate of total N, NH_4-N, NO_3-N and P were not significantly different (Table 7). Soils immediately below Hollow BSC had significantly higher supply rates of Ca, Mg, Fe and Al compared with Hummock BSC, while soils immediately below Hummock BSC had significantly higher supply rates of K (t-test, $p <0.01$ for all comparisons).

Table 7. Nutrient availability determined by mean PRS™-probe supply rate (µg/10 cm^2/June 1st -August 24th 2008) with standard error of Total N, NO_3-N, NH_4-N, Ca, Mg, K, P, Fe, Mn, Zn, B, S, Al immediately below Hollow BSC and Hummock BSC and at Daring Lake, NWT. Significantly different mean supply rates for a given nutrient are indicated by different superscript letters (a,b).

	Type of Biological Soil Crust	
Mean PRS™-probe supply rate µg/10 cm^2/June 1st - August 24th 2008 (SE)	Hollow BSC	Hummock BSC
Total N	11 (2.6)[a]	9.0 (0.61)[a]
NO_3-N	2.9 (1.3)[a]	2.7 (0.52)[a]
NH_4-N	8.8 (1.6)[a]	6.3 (0.71)[a]
Ca	1015 (127)[a]	214 (38)[b]
Mg	462 (56)[a]	201 (38)[b]
K	33 (10)[a]	171 (14)[b]
P	0.56 (0.19)[a]	0.66 (0.20)[a]
Fe	103 (43)[a]	11 (2.2)[b]
Mn	4.8 (0.64)[a]	1.0 (0.51)[b]
Zn	3.2 (0.31)[a]	1.4 (0.22)[b]
B	1.3 (0.08)[a]	1.6 (0.09)[a]
S	124 (33)[a]	90 (41)[a]
Al	66 (5.4)[a]	37 (5.4)[b]

Throughflow collected upon rewetting desiccated samples of Hummock and Hollow BSC revealed different relationships than nutrient availability over the growing season. Mean NH_4-N and NO_3-N concentrations were significantly higher for Hummock BSC (T-test, p= 0.02 and p= 0.03 respectively) when samples were pooled for the three time intervals (1, 2 and 3 hrs). In addition, soluble carbon was significantly higher for Hummock BSC (184 µg ml^{-1}) than for Hollow BSC (72 µg ml^{-1}) (t-test, $p < 0.01$) (Table 8).

Table 8. Mean NH_4-N, NO_3-N, total soluble N, organic N and total soluble C (µg/ml) concentrations in throughflow collected under desiccated Hollow and Hummock BSCs following rewetting over 3 time intervals (1, 2 and 3 hrs). Significantly different means for each time interval and overall mean (three time intervals pooled) are indicated by different superscript letters (a,b). Data represent means with ± SE.

Collection Interval	Throughflow µg/ml (SE)	Type of Biological Soil Crust	
		Hollow BSC	Hummock BSC
1 hr	NH_4-N	0.29 (0.08)[a]	0.29 (0.07)[a]
	NO_3-N	0.07 (0.01)[a]	0.10 (0.02)[a]
	Total soluble N	10 (2.7)[a]	5.3 (1.4)[a]
	Organic N	9.7 (2.6)[a]	5.0 (1.3)[a]
	Total soluble C	71 (7.4)[a]	184 (17)[b]
2 hrs	NH_4-N	0.40 (0.11)[a]	0.70 (0.19)[a]
	NO_3-N	0.16 (0.03)[a]	0.19 (0.03)[a]
3 hrs	NH_4-N	0.27 (0.07)[a]	0.43 (0.11)[a]
	NO_3-N	0.10 (0.02)[a]	0.17 (0.03)[a]
Mean	NH_4-N	0.32 (0.05)[a]	0.48 (0.08)[b]
	NO_3-N	0.12 (0.01)[a]	0.15 (0.01)[b]

BSC samples collected from the Daring Lake study site in 2008 showed higher rates of N_2-fixation after 4 weeks of P addition compared to control samples. The mean ARA rate increased from 14.8 to 21.6 nmol C_2H_4 $g^{-1}h^{-1}$ for Hummock BSC and 64.0 to 119.2 nmol C_2H_4 $g^{-1}h^{-1}$ for Hollow BSC with P addition. However, only Hummock BSC (n=10) had significantly higher rates of N_2-fixation compared with controls (n= 10) (t-test, $p < 0.01$).

DISCUSSION

Small-scale patterns of N_2-fixation in hummock-hollow complexes revealed areas of high N_2-fixation occurring on the lower edge of hummocks and in the bottom of hollows. N_2-fixation dropped rapidly within 5 cm downward of a hummock or upward of a hollow. BSCs were not common in intermediate positions within hummock-hollow complexes, which likely accounts for the significant decrease in N_2-fixation. In hummock-hollow tundra

ecosystems bryophytes tend to dominate the interhummock areas, which are expected to exhibit low growth rates, slow decomposition rates, relatively high C/N ratios and long nutrient turnover times (Biasi et al., 2005; Hobbie, 1995). In contrast hummocks are expected to support higher growth rates, and have lower C/N ratios with more rapid nutrient turnover (Biasi et al., 2005; Chapin et al., 1995; Hobbie, 1995). Despite the rapid turnover in nutrients associated with hummocks, some studies have found higher rates of N_2-fixation in lower lying trough and interhummock areas (Henry & Svoboda, 1986; Schell & Alexander, 1973).

In our study, there was a significantly higher mean growing season N_2-fixation rate and abundance of *nif*H associated with Hollow BSC compared to Hummock BSC. The proximity of permafrost to the soil surface, the ability of moss-mats to retain moisture and obtain moisture from the soil via capillary action helps to create a moist environment in these low lying areas, which is likely crucial in maintaining a greater abundance of cyanobacteria and higher rates of N_2-fixation (Chapin & Bledsoe, 1992). Moisture is often cited as the most important environmental factor controlling N_2-fixation across various arctic environments (Alexander, 1974; Alexander et al., 1978; Line, 1992; Zielke et al., 2002, 2005).

Soils below Hollow BSC did have significantly higher Ca, Mg, Fe and Al over the growing season. A positive correlation between the amount of extractable Mg and Ca and N_2-fixation has been found in the high Arctic (Liengen & Olsen, 1997a, 1997b). In addition, Mo and Fe are two micronutrients that may limit N_2-fixation as both are essential components of the nitrogenase enzyme (Hartley & Schlesinger, 2002; Smith, 1992). Higher availability of these nutrients may be an important factor supporting the higher N_2-fixation rates in hollows. The

higher supply rate of K in soils below Hummock BSC may be due to differences in soil type below Hummock and Hollow BSC (i.e. mineral versus organic respectively). However, these data should be interpreted cautiously as differences in soil moisture between hollows and hummocks likely had a strong influence on the nutrient supply rate detected over the growing season. Soil water content has a significant effect on ion movement and mineralization with drier soils demonstrating slower ion movement. Average percent moisture near the surface of hollows (77%) over the growing season was much higher than that near the surface of hummocks (35%).

Patterns of total % N and $\delta^{15}N$ values were reflective of differences in N_2-fixation between hummocks and hollows. Hollow BSCs had higher N_2-fixation and *nif*H abundance, as well as, higher total % N and $\delta^{15}N$ values (-0.89‰) that were significantly closer to that of atmospheric N_2 (0‰) compared with Hummock BSCs (2.20‰). In addition to N inputs via fixation, lateral flow of water may contribute to the observed pattern. In hummock-hollow tundra lateral flow of water from elevated hummocks to lower-situated hollows occurs (Biasi et al., 2005; Quniton, 2000). Leaching from high mounded areas may increase dissolved organic and inorganic N in depressions and/or increase inputs of phosphate that could stimulate N_2-fixation (Biasi et al., 2005). We found Hummock BSCs had a significantly greater loss of NH_4-N, NO_3-N and C upon rewetting after desiccation. Higher efflux of nutrients from hummocks could partially account for the lower total % N on hummocks, as well as, provide an additional source of N in hollows. Green et al. (2008) found that point sources of organic and inorganic N can be dispersed over approximately 1 m^2 at rates up to 100 cm day^{-1} during periods of active growth. Higher total % N in hollows, therefore, may

not only be the result of higher N_2-fixation rates but also the result of N inputs leached from hummocks.

Upper soil samples (top 1 cm) at both hummocks and hollows had $\delta^{15}N$ values that were significantly closer to that of atmospheric N_2 compared with lower soil samples (2 cm below), likely due to the influence of surface N_2-fixers supplying soil N. $\delta^{15}N$ at downslope distances from the lower edge of hummocks indicate sources of N via N_2-fixation, which contradict the spatial trends of N_2-fixation we detected. Although, lateral flow may account for some of these discrepancies it is also likely that denitrification and nitrification may be occurring at higher rates on hummocks resulting in higher $\delta^{15}N$ values. Chapin (1996) found that differences in $\delta^{15}N$ values of soils and plants from two arctic sites were consistent with the differences in denitrification rates. In the reaction leading to denitrification and nitrification there is usually discrimination against the heavier ^{15}N isotope in favour of the lighter ^{14}N isotope (Chapin 1996; Mariotti et al., 1981; Turner et al., 1983). Greater denitrification, therefore would lead to an enrichment of the ^{15}N isotope and higher $\delta^{15}N$ values in remaining soil N. Nitrification would also result in higher $\delta^{15}N$ values; however the rapid and continued uptake of mineral N in N-deficient arctic ecosystems may limit the importance of fractionation during nitrification (Chapin, 1996; Fry 2006).

Like others, we observed seasonal variation in N_2-fixation (Alexander & Schell, 1973**;** Chapin et al., 1991; Henry & Svoboda, 1986; Zielke et al., 2005; Stewart et al., unpublished data). The highest rates of N_2-fixation occurred in July; however, due to a large variation in rates these were not significantly different than rates in August. We did detect significantly higher *n*ifH copy numbers in July compared with other growing season months. However, seasonal variation in *n*ifH copy number was not clearly linked with seasonal variation in N_2-

fixation rates. Our inability to detect linkages between seasonal *nif*H copy number and N_2-fixation may be due to the use of a single *nif*H primer targeting a specific group of *nif*H genes that only reflects a portion of our complex BSC communities composed of many different diazotrophic microorganisms. In addition, detection of genomic *nif*H gene copies does not unequivocally indicate that the organisms were actively fixing (Steppe & Pearl, 2005) and persistence of non-viable *nif*H copies in the environment may also confound our results. Deslippe et al. (2005) found a poor relationship between *nif*H community structure and nitrogenase activity in the Arctic and suggested that the factors controlling the distribution of *nif*H genotypes in soil may not be directly related to expression of *nif*H genes. Furthermore, nutrient status and microclimatic conditions may play equally important roles in determining variation in N_2-fixation. For example, we found that N_2-fixation rates on hummocks were P limited and moisture is a well known controller of N_2-fixation activity.

Over the growing season we did not detect any differences in total N, NH_4-N, NO_3-N and P below hollows and hummocks despite detecting higher rates of N_2-fixation and higher % N in samples of Hollow BSC. Liverwort mats, the main component of Hollow BSC, may be more effective at retaining nutrients than the Hummock BSC making them less available to soils below. Bryophytes have adapted to nutrient-poor environments and are extremely efficient both in their use of N and their ability to retain N and may exert control over the N retention efficiency of the ecosystem (Aldous, 2002; Bowden, 1991; Phuyal et al., 2008). N released from bryophytes may be in less biologically available forms and bryophytes may reduce nutrient turnover rates through the production of acidic nutrient-poor organic matter, retention of N in recalcitrant compounds and by reducing soil temperatures and hence lowering decomposition rates (Eckstein, 2000; Lagerstrom et al., 2007; Turetsky, 2003).

We found increased rates of N_2-fixation with P addition for Hummock BSC. Several studies have found evidence to suggest that N_2-fixing organisms increase in both abundance and fixation rate when P supply is high, especially in ecosystems with a relatively low N supply (Benner et al., 2007; Benner & Vitousek, 2007; Chapin et al., 1991; Crews, 1993; Davidson et al., 2002; Eisele et al., 1989; Kurina & Vitousek, 1999; Smith 1992; Vitousek & Howarth, 1991; Vitousek et al., 2002; Weiss et al. 2005). The absolute increase in N_2-fixation with P addition was greater for Hollow than Hummock BSCs, however this increase was not significantly different than the increase in Hollow control samples. P may be limiting for both hummock and hollow N_2-fixation, but may be less important for Hollow BSCs if they are already fixing N_2 near maximal rates. Alternatively, liverwort mats that are the primary plant component of Hollow BSCs may be effective at capturing and retaining P, but P may not be available to the associative cyanobacteria; whereas N_2-fixing lichen species found in Hummock BSCs, such as *Solorina crocea*, may be more sensitive to P addition. Hummock BSCs also tended to have higher cyanobacteria richness and species, such as *Anabaena* sp., were only found in Hummock BSCs. These cyanobacteria have shown significantly higher rates of N_2-fixation with P addition in other studies (Liengen, 1999a). Therefore, while it appears that P limitation may play an important role in structuring spatial patterns of N_2-fixation, further studies that separate host and cyanobacterial components of these N_2-fixing associations are needed to clarify the response of these organisms to P addition.

Soil N status was linked to rates of N_2-fixation by BSCs indicating that these N_2-fixing associations may act as important point sources of soil N. While small-scale patterns of nutrient availability were influenced by microtopography, the type of N_2-fixing association

present and other nutrient cycling processes, it is clear that spatial differences in N_2-fixation are associated with patterns of soil fertility in N limited low arctic ecosystems.

CHAPTER 4: BRYOPHYTE-CYANOBACTERIAL ASSOCIATIONS AS A KEY FACTOR IN N2-FIXATION ACROSS THE CANADIAN ARCTIC

ABSTRACT

Nitrogen inputs via biological N_2-fixation are extremely important in arctic environments where N often limits plant productivity. An understanding of the direct and indirect theoretical causal relationships between key intercorrelated variables that drive the process of N_2-fixation is essential to understanding current and future N input. An exploratory multi-group Structural Equation Modeling (SEM) approach was used to examine the direct and indirect effects of soil moisture, plant community functional composition, and bryophyte and lichen abundance on rates of nitrogen fixation at a low arctic ecosystem, two high arctic oases and a high arctic polar desert in the Canadian Arctic. Increasing soil moisture was strongly associated with an increasing presence of bryophytes and increasing bryophyte abundance was a major factor determining higher N_2-fixation rates at all sites. Surprisingly there was no soil moisture, lichen and N_2-fixation pathway at any of the sites, suggesting that for our data, lichens were not linked to soil moisture or to N_2-fixation. Shrubs had a negative effect on bryophyte abundance at all sites with the exception of the polar desert site at Alexandra Fiord highland, where both shrubs and graminoids had a positive influence. The importance of competition from vascular plants, potentially through shading, appears to be greater in more productive sites and may increase at lower latitudes. Moisture availability may have an indirect effect on ecosystem development by affecting N input into the system with bryophytes-cyanobacterial associations playing an important intermediary role in the process. Warmer temperatures and changes in moisture availability due to climate change may increase N_2-fixation, however, increased shrub and graminoid cover may counter-act this increase in N_2-fixation.

INTRODUCTION

Nitrogen inputs via N_2-fixation are extremely important in arctic environments where N often limits plant productivity. The role of vegetation and environmental conditions in determining N_2-fixation rates for specific cyanobacterial species or N_2-fixing associations at a given site have been extensively studied (Schell & Alexander, 1973; Crittenden & Kershaw, 1978; Gunther, 1989; Henry & Svoboda, 1986; Chapin et al., 1991; Solheim et al., 1996; Dickson, 2000; Zielke et al., 2002, 2005; Hobara et al., 2006). Few studies, however, have simultaneously examined the relationships between environmental conditions, vascular plant communities, N_2-fixing associations and rates of N_2-fixation across several arctic sites varying widely in latitude and with diverse vegetation communities. In this study, approximately 400 samples with roughly 100 samples each taken from a high arctic polar oasis (Alexandra Fiord lowland), a high arctic polar desert (Alexandra Fiord highland), a high arctic wetland polar oasis (Truelove Lowlands) and a low arctic esker ecosystem (Daring Lake) were evaluated.

Cyanobacteria are ubiquitous in the Arctic where they are the primary source of newly fixed nitrogen (Alexander & Schell, 1973; Alexander, 1974; Granhall & Lid-Torsvik, 1975; Henry & Svoboda, 1986; Chapin et al., 1991; Chapin & Bledsoe, 1992; Liengen, 1999a; Hobara et al., 2005; Solheim et al., 2006). Cyanobacteria form many associations with vegetation including epiphytic and endophytic facultative associations with bryophytes (Turetsky, 2003) and the lichen symbioses and soil surface colonies that are components of biological soil crusts (Belnap et al., 2001). Bryophyte-associated cyanobacteria can provide 2-58% of N in arctic ecosystems (Dodds et al., 1995; Solheim et al., 2006) and while variation is often high within and between bryophyte species, the highest rates of N_2-fixation in arctic landscapes

are often associated with cyanobacteria-bryophyte associations (Alexander & Schell, 1973; Henry & Svoboda, 1986; Solheim et al., 1996). Cyanobacterial symbioses with lichens are also are a major source of fixed N as they often have N_2-fixation rates exceeding that of other cyanobacterial symbioses (Schell & Alexander, 1973; Kallio & Kallio, 1975; Crittenden & Kershaw, 1978; Gunther, 1989; Hobara et al., 2006). Finally, the prevalence of biological soil crusts in many arctic ecosystems ensure that the cyanobacteria associated with those crusts are major contributors to arctic N inputs (Alexander & Schell, 1973, Alexander et al., 1978).

The interactions between plant communities and environmental factors such as soil moisture can be important in determining both the establishment and survival of N_2-fixing associations and the rates at which they fix N_2. Soil moisture is not only important in structuring vegetation communities in the Arctic (Sohlberg & Bliss, 1984; Oberbauer & Dawson, 1992; Bliss et al., 1994; Gold & Bliss 1995a; Walker, 2000), but is one of the most important environmental factors controlling N_2-fixation across many arctic environments (Alexander, 1974; Alexander et al., 1978; Davey, 1983; Chapin & Beldsoe, 1992; Line, 1992; Nash & Olafsen, 1999; Zielke et al., 2002, 2005; Convey & Smith, 2006).

Vegetation functional types can play a major role in determining the moisture, light and temperature regimes under which N_2-fixing associations operate. Differences in the capacity of vegetation types to retain moisture and make it accessible to cyanobacteria have been correlated with rates of N_2-fixation (Zielke et al., 2002; 2005). Shading vegetation can reduce the light intensities available to a N_2-fixing association, and can limit the persistence of some lichens into later successional stages (Kershaw, 1976; Foster, 1985; Kurina & Vitousek, 1999). Shading of N_2-fixing associations by shrubs may be particularly important

given that remote sensing, repeat photography and experimental warming studies all suggest that current warming trends may be promoting shrub growth and expansion (Chapin et al., 1995; Sturm et al., 2001; Goetz et al., 2005; Walker et al., 2006).

An understanding of the contribution made by different N_2-fixing associations to N input across arctic environments is important. However, an understanding of the direct and indirect theoretical causal relationships between key intercorrelated variables that drive the process of N_2-fixation is essential to understanding current and future N input. In this study we used an exploratory multi-group Structural Equation Modeling (SEM) approach to examine the direct and indirect effects of soil moisture, plant community functional composition, and bryophyte and lichen abundance on rates of nitrogen fixation at four sites varying in latitude and vegetation type. The effects of these factors and the networks of interactions among them were compared across sites to determine the influence of different N_2-fixing associations on fixation and key interactions driving N_2-fixation across the Arctic.

METHODS

Site Descriptions

The four study sites located across the Canadian Arctic ranged in latitude from 79°53'N to 64°52'N. Two high arctic sites were located ~3km apart at Alexandra Fiord, Ellesmere Island, Nunavut Territory, one in a lowland polar oasis (78°53'N, 75°55'W) and the other in a highland polar desert (78°51'N, 76°06'W). A third high arctic site was located at Truelove Lowlands, Devon Island, Nunavut Territory (75°67'N, 84°58'W) and a low arctic site was located at Daring Lake, Northwest Territories (64°52'N, 111°35'W) (Fig. 8).

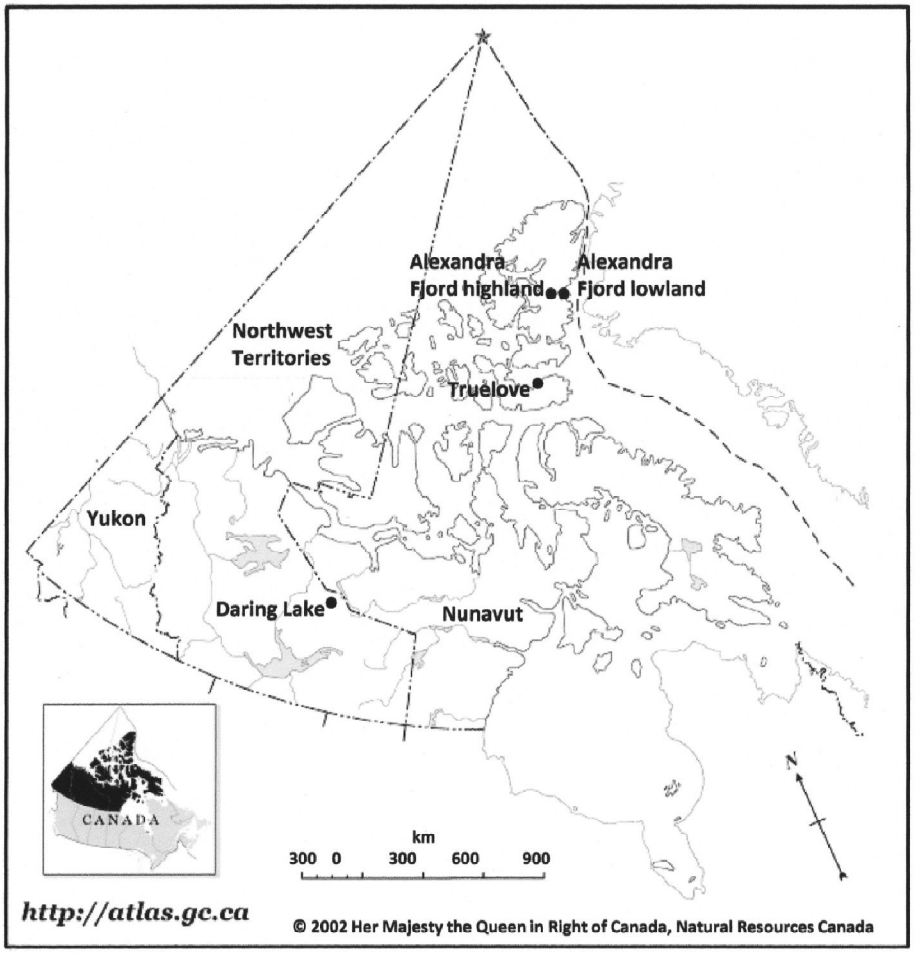

Figure 8. Location of study sites in the Canadian high and low Arctic. High arctic sites were at Alexandra Fiord highland and lowland, Ellesmere Island Nunavut Territory and at Truelove Lowlands, Devon Island, Nunavut Territory. The low arctic site was at Daring Lake, Northwest Territories.

The Alexandra Fiord lowland site was in an 8 km^2 lowland oasis on the eastern side of central Ellesmere Island. The oasis is a deglaciated lowland delimited by a glacier to the south, cliffs and talus slopes (ca. 500 m) to the west and east and by the Fiord waters to the north (Muc et al., 1989). Lowland soils are predominantly Regosolic Static Cryosols (Soil Classification Working Group, 1998) that are generally coarse textured with variable concentrations of organic matter. Average air temperature is -15^{o}C and mean monthly air

temperature in July is 4.5°C (Labine, 1994). Annual precipitation at Alexandra Fiord lowland is < 60 mm with < 10 mm falling during the growing season from mid-June to August (Muc et al., 1989). The lowland has an extensive vegetation cover dominated by deciduous dwarf shrubs, heaths, cushion plants and hydric sedges. Transects were placed over relatively flat terrain with the presence of some hummocky areas where dwarf shrub-cushion plant communities were dominant (follows Muc et al., 1989).

The Alexandra Fiord highland study site was a polar desert located on the western plateau (ca. 500 m a.s.l.) approximately 3 km to the west of Alexandra Fiord lowland. The highlands of Alexandra Fiord are within the Churchill Structural Province of the Canadian Shield Geological Region (Batten & Svoboda, 1994). Upland soils are predominantly Regosolic Turbic Cryosols with both granitic and dolomitic parent materials. Air temperature in the upland tends to be cooler than the central area of the lowland, though the mean monthly air temperature in July (4.4°C) is comparable (Labine, 1994). The upland has only 40% of the vascular species found in the lowland. Polygonal ground creates microrelief in the polar desert that impacts the distribution of plant species (Batten & Svoboda, 1994). Transects were placed over relatively flat terrain with some polygons present. *Saxifraga oppositifolia-Luzula*, *Salix arctica-Cassiope tetragona* dwarf-shrub, *Dryas*-barrens and *Dryas-Carex* complex were the dominant plant communities along the transects (follows Batten & Svoboda, 1994).

The Truelove Lowland site was located in a 43 km^2 lowland oasis on Devon Island. The lowland was bordered by shoreline to the north, west and part of the south and by steep cliffs (ca. 300 m) to the east and remaining south (Bliss, 1987). Pleistocene age deposits that overlay a Precambrian complex of granulites and granitic gneisses are present. Soils were

predominantly Regosolic Static Cryosols and better-developed Brunisolic Eutric Static Cryosols. Mean annual temperature averages -16 to -19^{o}C with summer temperatures averaging 3 to 6^{o}C (Bliss et al., 1994). Mean annual precipitation in the area ranges from 150 to 200 mm with approximately 36 mm of precipitation at Truelove during the summer. Transects were placed over a series of beach ridges. Ridges were dominated by cushion plant–lichen communities and the intervening lowlands by hummocky sedge-moss meadows (follows Muc & Bliss, 1987).

The Daring Lake study site was located at the Tundra Ecosystem Research Station, Northwest Territories. The site was in a low arctic tundra region within the physiographic zone of the Bear-Slave Upland of the Canadian Shield, approximately 90 km northeast of the northern limit of continuous trees (Obst, 2008). Elevation ranges from 414-470 m a.s.l. and landscape features include eskers, boulder fields, exposed bedrock, upland and lowland tundra, wetlands and various sizes of lakes, ponds and streams. Mean monthly air temperature in January is -30^{o}C and +13^{o}C in July (Obst, 2008). Transects were placed perpendicular to an east-west oriented esker with sample plots in upper slope areas predominately in Xerophytic Herb Tundra and Heath-Lichen Tundra, back slope plots in Heath-Mat Tundra and Birch Hummock and lower slope plots in Birch Hummock and Sedge Meadows (follows Obst, 2008).

Transect Sampling

At Alexandra Fiord, samples were collected at 31 points (0, 0.1, 0.2, 0.5,1, 2, 5, 10, 20, 50, 100, 100.1, 100.2, 100.5, 101, 102, 105, 110, 120, 150, 200, 200.1, 200.2, 200.5, 201, 202, 205, 210, 220, 250, 300 m) along three parallel transects 2 m apart for a total of 93 samples. In the Alexandra Fiord lowland, the transects were perpendicular to the slope of the lowland.

In the Alexandra Fiord highland polar desert, transects were positioned such that the first 100 m was in the dolomitic desert and the remainder was in granitic desert. At Truelove lowland, samples were collected at 128 points located every 2 m on a 256 m transect. This transect crossed two raised beach crests and thus, captured the majority of the soil types present at Truelove, Raised Beach Crest, Upper Fore Slope, Lower Fore Slope and Sedge Meadow. At Daring Lake 3 parallel transects 2 m apart were placed perpendicular to an east-west oriented esker. Samples were collected at 34 points on each transect including upslope (0, 0.1, 0.2, 0.5,1, 2, 5, 10, 15, 20 m), back slope (0, 0.1, 0.2, 0.5,1, 2, 5, 10, 15, 20 m) and lower slope (0, 0.1, 0.2, 0.5,1, 2, 5, 10, 15, 20, 40, 60, 80, 100 m) positions for a total of 102 samples. A GPS unit (Trimble^{TM} GPS Systems, California, USA) was used to identify spacing (+/- 8 cm) between samples.

Vascular plant and cryptogam functional composition was assessed in 0.5 m by 0.5 m quadrats at each sampling point. The percent cover of each vascular species was assessed individually by eye as was the total cover of bryophytes and lichens and bare ground (rocks, gravel, and finer materials). A soil sample of approximately 10 cm depth was collected directly below each N_2-fixation surface sample and gravimetric moisture was determined.

N_2-Fixation Rates

Surface samples (~38 cm^2, 2 cm depth) from high arctic sites including Alexandra Fiord lowland, Alexandra Fiord highland and Truelove (July 2008) were collected at each transect position. Samples were kept cool (~4^oC) and shipped to the laboratory at the University of Northern British Columbia.

Measurements of N_2-fixation were made using acetylene reduction assays (ARA) (Stewart et al., 1967). Acetylene gas (C_2H_2) was generated from CaC_2 and water, with incubations injected with 10% (v/v) acetylene. Ethylene concentrations were measured with a portable gas chromatograph (SRI 8610A, Wennick Scientific Corporation, Ottawa, ON, Canada) fitted with a Porapak column (Alltech Canada, Guelph, ON, Canada) and a flame ionization detector. A Stand-Alone Hydrogen Generator (SRI H_2-50, Alltech Canada, Guelph, ON, Canada) provided hydrogen as the carrier gas, which was held at a constant pressure of 26 psi. Column temperature was held at 65^{o}C.

High arctic surface samples (19cm^2, 1 cm depth) from each transect position were given a wetting pretreatment at optimal hydration levels in a growth chamber for 72 hours under a 17/7 hr light (200 µmol PAR $m^{-2}s^{-1}$)/dark cycle with temperatures at 15^{o}C during light hours and 5^{o}C during dark hours. Samples were then enclosed in 500 ml glass canning jars with modified lids containing a rubber septum and 6 hour ARAs were conducted under optimal environmental conditions (200 µmol PAR $m^{-2}s^{-1}$, 20^{o}C).

Surface samples (19cm^2, 1 cm depth) at Daring Lake were collected and incubated in the field (August 20th-30th, 2008) under the same optimal environmental conditions following the same procedure with the exception of a 24 hr wetting pretreatment. Photosynthetically Active Radiation (PAR) and temperature in incubation chambers were monitored every 30 minutes during ARA incubations. In the laboratory the environmental chamber was adjusted as necessary to maintain optimal conditions in the incubation chambers and in the field incubation chambers were placed in water baths and bath temperature was altered.

To determine if the shipping of surface samples had a significant detrimental effect on the N_2-fixation rates detected we re-sampled the Alexandra Fiord highland transects *in situ* on July 11th-15th 2009. Samples were treated and ARAs were conducted in the same manner as those at Daring Lake in 2008. N_2-fixation rates at Alexandra Fiord highland were higher for samples incubated in the field in 2009 than after shipping in 2008 (0.68 and 0.46 mg N $m^{-2}h^{-1}$ respectively), however, the difference was not significant (t-test, ,t= -1.94, df=181, p=0.05 for data analyzed in log units).

Statistical Analysis

We used multi-group Structural Equation Modeling (SEM) with observed variables to separate the direct and indirect effects of soil moisture, plant community functional composition, and potential N_2-fixing association abundance on rates of nitrogen fixation. SEM allows the direct and indirect theoretical causal relationships between a series of intercorrelated variables to be tested (Shipley 2000, Grace 2006). In a SEM figure each single-headed arrow represents a causal relationship such that the variable at the tail of the arrow is believed to be a direct cause of the variable at the head, while a double-headed arrow indicates an unresolved correlation between two variables. An initial SEM is specified based on prior theoretical knowledge, and is then tested to determine whether the covariance structures implied by the model adequately fit the actual covariance structures of the data. An initial theory-based model that adequately fits the data is a powerful confirmation of the validity of the theory used to construct the initial model. If the initial model does not adequately fit the data then model modification indices provide a strong tool for data exploration and hypothesis generation (Grace 2006). In a multi-group SEM the same initial path model is fit to each group (in this case study site) with all model parameters constrained

to be equal between groups. Model fitting involved the progressive relaxation of parameter constraints allowing particular parameters to differ between two or more groups. The identification of cases where parameter values differ between groups is an indication that the process represented by that path coefficient is operating differently at each site.

The initial structural equation model (Fig. 9) was developed to describe how the effects of vascular plant community functional composition on bryophyte and lichen abundance indirectly influence N_2-fixation. The continuous variables included in the model are described in Appendix I. Vascular plant functional composition was incorporated into the model by separating the total % cover of vascular plants into shrubs, graminoids, and forbs. Due to strong differences in plant communities between sites there was little overlap in species composition between sites and thus a more detailed classification of functional types was not possible. Direct paths from potential N_2-fixing cyanobacteria associations (Bryophyte, Lichen and Bare ground) to N_2-fixation were included. Bare ground was included as a potential N_2-fixing association because biological soil crusts, which are communities composed of bacteria, cyanobacteria, algae, mosses, liverworts, fungi and small lichens, were not explicitly included and where present would have been recorded as bare ground. Each of the potential N_2-fixing associations received a direct path from each of the plant community functional groups (Shrubs, Graminoids and Forbs) and Bryophytes, Lichens, Shrubs, Graminoids and Forbs all received a direct path from Soil Moisture. The interactions between plant communities and environmental factors such as soil moisture can be important in determining both the ability of N_2-fixers to survive and the rates at which they can fix N_2. Soil moisture is a key environmental factor determining the distribution of vegetation types in arctic environments (Oberbauer & Dawson, 1992) and vegetation

functional types can play a major role in determining the operating environment of N_2-fixing associations (Zielke et al., 2002; 2005).

The SEM models were fit using Amos 17.0 (Amos Development Corporation, Crawfordville, FL, USA). An initial multigroup SEM was fit with all model parameters constrained to be equal between sites. This model did not have adequate fit (χ^2_{113}=2750.703, p<0.0001). Parameter constraints were progressively relaxed in subsequent models with a drop in the CMIN statistic (Grace 2006) used as justification to retain a new model. The parameter constraint to relax in each model was selected based on examination of the matrix of standardized residuals for large values. Model fitting continued until an adequate χ^2 test was achieved (final model χ^2_{59}= 54.235, p=0.651).

RESULTS

The final model adequately fit these data (χ^2_{59}= 54.235, p=0.651), and explained 0, 11, 5 and 12% of the variation in N_2-fixation at the Alexandra Fiord highland, Alexandra Fiord lowland, Truelove and Daring Lake sites, respectively (Fig. 9). Unstandardized path coefficients, t-test results and total direct and indirect effects are summarized in Appendix S1 in supporting information. Despite the low r^2 values the SEM revealed important and consistent patterns of N_2-fixation across the Arctic. The zero r^2 value for N_2-fixation at Alexandra Fiord highland indicates that, with the exception of a very small contribution from bryophytes, all of the important factors controlling fixation at this site are missing from the model. Increasing soil moisture was strongly associated with an increasing presence of bryophytes and increasing bryophyte abundance was a major factor determining higher N_2-fixation rates at all arctic sites (Fig. 9). Standardized path coefficients for the bryophyte–N_2-fixation relationship varied between sites. However, the unstandardized coefficient was the

same for all high arctic sites, indicating that the bryophyte–N_2-fixation relationship is likely consistent across the entire Arctic. Surprisingly lichen abundance had no effect on rates of N_2-fixation at any of the sites. Bare ground had a positive influence on N_2-fixation at Alexandra Fiord lowland (0.34), but bare ground had no effect on N_2-fixation at the other sites.

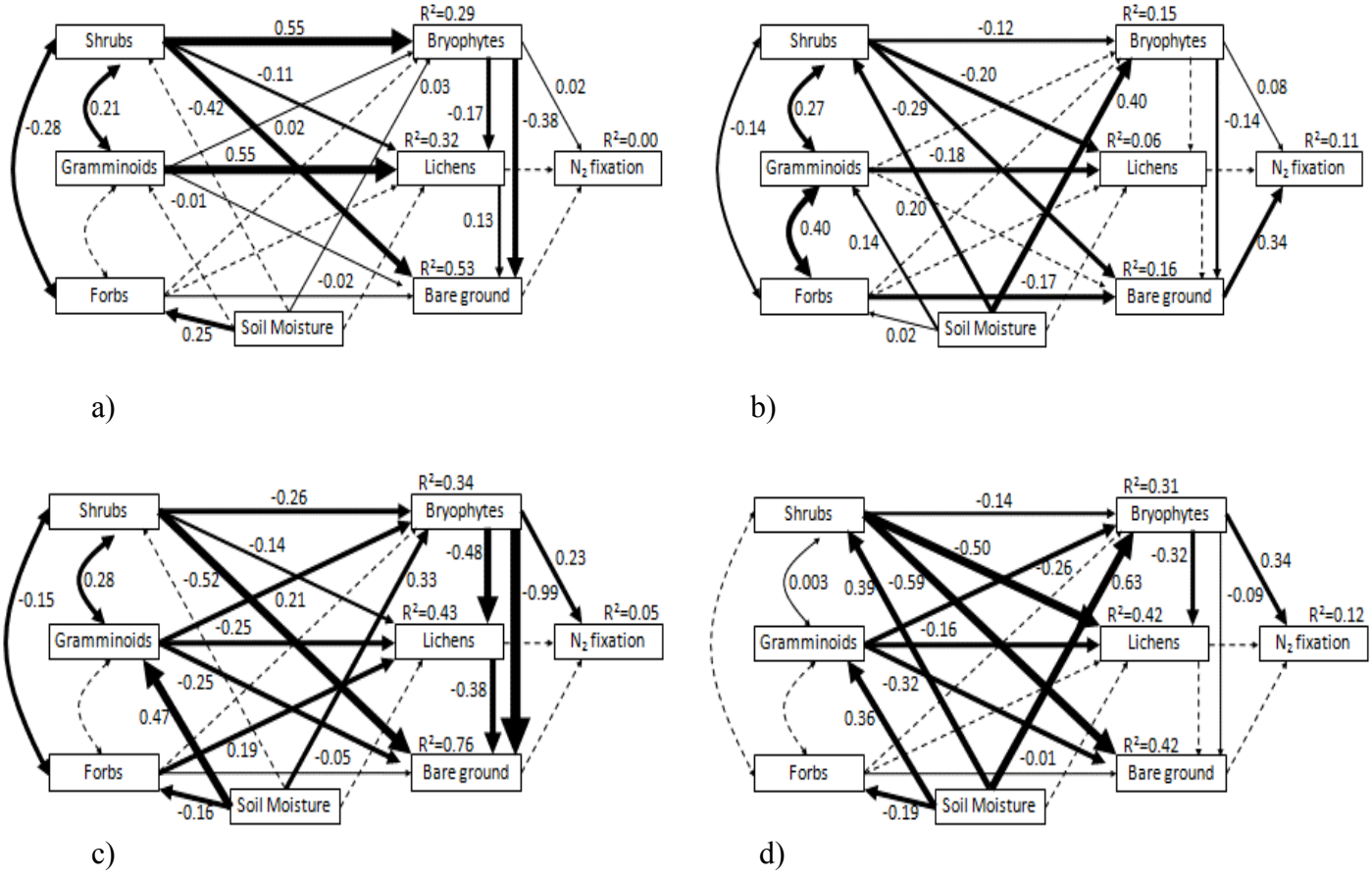

Figure 9. Final structural equation models for a) Alexandra Fiord highland, Ellesmere Island NT b) Alexandra Fiord lowland, Ellesmere Island NT, c) Truelove Lowlands, Devon Island NT and d) Daring Lake, NWT. Significant paths are indicated by solid arrows of varying thickness that reflect the magnitude of the standardized SEM coefficients given beside each path. Nonsignificant paths are indicated by dotted arrows.

The relationships between soil moisture and vascular plant community functional groups varied between sites, but the influence of soil moisture on bryophyte and lichen abundance was consistent. At all sites increasing soil moisture had a direct positive effect on bryophyte abundance (0.03 Alexandra Fiord highland; 0.40 Alexandra Fiord lowland; 0.33 Truelove; 0.63 Daring Lake), while soil moisture had no effect on lichen abundance at any of the sites. In fact, there was no soil moisture, lichen and N_2-fixation pathway at any of the sites, suggesting that for our data, lichens were not linked to soil moisture or to N_2-fixation.

Increasing soil moisture appears to directly promote bryophyte abundance. However, at sites such as Alexandra Fiord lowland (0.20) and Daring Lake (0.39) where higher soil moisture also promotes shrub abundance, soil moisture had indirect negative effects on bryophyte abundance (-0.02 and -0.15 respectively) (Appendix I). With the exception of the Alexandra Fiord highland site, we found consistent negative effects of the vascular plant community on N_2-fixing association abundance that suggest exclusion of N_2-fixers, likely via shading. Higher shrub abundance had a direct negative influence on bryophyte and lichen abundance at all sites, except at Alexandra Fiord highland where bryophyte abundance was increased (0.55). Similarly, increasing graminoids abundance led to lower abundance of lichens at all sites, except Alexandra Fiord highland were lichen abundance was higher.

The importance of bryophytes in determining N_2-fixation in arctic environments may be linked to their ability to dominant moist areas to the exclusion of other N_2-fixing associations. Increasing bryophyte abundance led to lower abundances of both lichens and bare ground at all sites, except for no effect on lichens at Alexandra Fiord lowland (Fig. 9).

DISCUSSION

Our model revealed a strong consistent relationship of increasing soil moisture positively influencing bryophyte abundance and increasing bryophyte abundance positively influencing N_2-fixation rates. The direct and indirect influences of soil moisture are perhaps the most important factors in structuring plant species distribution at a local level within arctic landscapes (Webber, 1978; Bliss, 1987; Walker et al., 1989; Bliss et al., 1994; Oberbauer & Dawson, 1992; Gold & Bliss, 1995b). The strong positive influence of soil moisture on bryophyte abundance at all of our sites likely reflects the high sensitivity of bryophyte communities to moisture conditions due to their poikilohydric nature. Due to a lack of roots, bryophytes and lichens are often considered to be less tightly associated with soil properties than vascular plants. While soil moisture did not have a significant influence on lichen abundance at any of our sites, soil moisture does appear to have an important influence on bryophyte abundance across the Canadian Arctic. Lichens are often established on drier exposed habitats and due to their sensitivity to desiccation N_2-fixation by lichens is often tightly coupled with precipitation events (Crittenden & Kershaw, 1979). Patches of bryophytes, however, are often associated with permanent desiccation cracks and/or microtopographical depressions in the landscape where soil moisture content is higher (Sohlberg & Bliss, 1984). In addition, bryophytes tend to form thick mats that can hold water and nutrients from snowflush runoff or precipitation and remain moist throughout the growing season due to reduced soil evaporation (Bliss & Gold, 1994, Gold & Bliss 1995b). Even with limited precipitation inputs high soil moisture can be maintained by the upward wicking of permafrost meltwater from the thaw front at the base of the active layer.

In N limited arctic environments N_2-fixation is a major source of N (Chapin & Bledsoe, 1992). Cyanobacteria are often closely associated with bryophytes where moisture conditions are more favourable (Alexander et al., 1978; Arndal, 2009) and the cyanobiont may receive carbohydrates from the host (Turetsky, 2003). The dense packing of stems and leaves provides protection from desiccation and enables water translocation to the cyanobacterial zone (Line, 1992). The ability of moss-mats to retain moisture and obtain moisture from the soil via capillary action makes them an ideal habitat for N_2-fixing cyanobacteria (Chapin & Bledsoe, 1992). Moisture enhances the metabolic activity of N_2-fixing associations directly by increasing carbon and energy supplies for N_2-fixation and indirectly by stimulating net primary production (Wierenga et al., 1987; Hartley & Schlesinger, 2002).

A general indirect relationship between bryophyte biomass and gross ecosystem productivity has been observed and is likely due to larger cyanobacterial biomass developing on moss-mats that in turn enhance N_2-fixation and N availability (Billings, 1992; Zielke et al., 2005, Arndal et al., 2009; Hudson & Henry, 2009). Therefore, moisture availability may have an indirect effect on ecosystem development by affecting N input into the system (Dickson, 2000) with bryophytes-cyanobacterial associations playing an important intermediary role in the process. Sorensen et al., (2006) found N_2-fixation by bryophyte–covered surfaces was approximately 2.7 times the annual plant N demand and a correlation between ethylene production (a proxy for N_2-fixation) and soil moisture was found only for bryophyte covered surfaces. Our findings suggest that a moisture-bryophyte-N_2-fixation relationship is found across the Canadian Arctic in many different vegetation types and at different latitudes.

Given the importance of bryophyte N to gross ecosystem productivity, the role of bryophytes in arctic nutrient availability needs to be further explored.

Despite detection of similar relationships across sites, differences among the sites reveal important variations in processes occurring in the Arctic. Bare ground made an important contribution to N_2-fixation only at the Alexandra Fiord lowland site, likely due to well-developed Biological Soil Crusts (BSCs) that were actively fixing N_2 at this site (unpublished data, K. Stewart). However, since BSCs were not directly quantified this interpretation is tentative. Differences in the relationships between vegetation functional groups and bryophytes indicate differences between vegetation communities and latitudinal variation. Shrubs had a negative effect on bryophyte abundance at all sites with the exception of Alexandra Fiord highland, where both shrubs and graminoids had a positive influence. At Daring Lake in the low arctic both shrubs and graminoids had a negative effect on bryophyte abundance.

Polar deserts are particularly important considering most of the ice-free terrestrial environments within the Canadian High Arctic are polar desert (44%) or semidesert (49%) (Bliss & Gold, 1999). Polar deserts tend to have a patchy distribution of the most productive areas with desiccation-cracks, the margins of soil polygons and stripes and other slight concavities being important sites for seed germination and seedling establishment (Sohlberg & Bliss, 1984; Bliss &Gold, 1994; Gold & Bliss, 1995b). Vascular plant cover and succession in polar deserts appears to be tightly linked to these sites where a greater cover of cryptogams is also found (Bliss et al., 1994; Dickson, 2000; Breen & Lévesque, 2008). These microsites tend to have higher temperature, lower wind speeds, greater soil moisture and higher nitrate levels (Sohlberg & Bliss, 1984). Nitrogen supplied through cyanobacterial

N_2-fixation is a significant source in polar desert communities with total soil N below well-developed cryptogamic crusts (0.09%) doubles that of non-crusted sites (0.04%) (Gold & Bliss, 1995b). The influence of soil water content on vascular plants, therefore, is mediated through alterations in nutrient availability (Chapin et al., 1988; Gold & Bliss, 1995b). The positive relationships between shrubs, graminoids and bryophytes found only at Alexandra Fiord highland likely reflects the influence of an extreme polar desert environment where abiotic factors play a more important role in structuring vegetation distribution. Vegetation in polar deserts appears to be distributed by their abiotic tolerances with no evidence of incipient niche differentiation and no competitive exclusion of species from vegetated sites (Sohlberg & Bliss, 1984).

The direct influence of water availability as a limiting factor and determinant of vegetation structure, productivity and composition, declines with decreasing latitude from high to low Arctic (Oberbauer & Dawson, 1992). We found soil moisture played an important role in both high arctic polar oases and in the low Arctic; where higher soil moisture led to a higher abundance of vascular plant types, such as shrubs. However, increasing shrub abundance appears to have a negative influence on N_2-fixation rates in less extreme arctic environments. Nitrogen fixation tends to decrease with increasing vegetation development, advancing succession and increasing plant cover (Crocker & Major, 1955; Liengen & Olsen, 1997a; Sorensen et al. 2006). While shrubs had a negative influence on N_2-fixation at Alexandra Fiord lowland, Truelove and Daring Lake, graminoids had a negative influence on N_2-fixation only at Daring Lake. Therefore, the importance of competition from vascular plants, potentially through shading, may increase at lower latitudes. In warming experiments a stronger vegetative growth response has been observed in the low Arctic, whereas, in colder

high arctic sites a greater reproductive response associated with the colonization of unvegetated ground may occur (Arft et al., 1999). It has been suggested that declining macrolichen abundance in warming sub- and mid-arctic environments may be a function of increased growth and abundance of shrubs, which may inhibit lichen performance through shading (Cornelissen et al., 200; Walker et al., 2006). Rates of N_2-fixation and persistence of other N_2-fixing associations such as bryophyte-cyanobacterial associations may be similarly influenced by reduced light availability. Climatic changes may directly affect N_2-fixation rates as a result of warmer temperatures and changes in moisture availability, however as our study suggests N_2-fixation may also be indirectly affected by alterations in the distribution and abundance of vegetation types. Many of these changes however, will differ depending on both latitude and site-to-site variability.

The zero r^2 value for N_2-fixation at Alexandra Fiord highland indicates that there are other important factors that affect N_2-fixation in this extreme environment that were not included in the model. The colonization frequencies, abundance, and distribution of N_2-fixing organisms and/or rates of N_2-fixation in arctic landscapes can be affected by microtopography (Schell & Alexander, 1973; Henry & Svoboda, 1986), microaspect (George et al., 2000; Davidson et al., 2002), soil texture (Kleiner & Harper, 1977; Anderson et al., 1982; Verrecchia et al., 1995; Harper & Belnap, 2001; Gold et al., 2001), soil pH (Ponzetti & McCune, 2001; Smith et al., 2002; Turetsky, 2003), nutrient availability (Chapin & Bledsoe, 1992; Vitousek et al., 2002), surface moisture (Dickson, 2000; Breen & Lévesque, 2008) and disturbance history (Belnap, 2002). Since N_2-fixation occurs in response to this large suite of intercorrelated variables inclusion of additional factors would likely increase r^2 values.

In our relatively simple model the importance of bryophytes in N_2-fixation across the Canadian Arctic is evident. Soil moisture is a major factor that indirectly influences N_2-fixation by directly influencing the presence of vegetation, such as bryophytes that form cyanobacterial associations. The role of bryophytes needs be examined further, especially in the light of climatic changes currently occurring across the Arctic.

CHAPTER 5: N_2-FIXATION AND NITROGEN CYCLING IN HIGH ARCTIC WET SEDGE MEADOW AND DRYAS HEATH VEGETATION COMMUNITIES

ABSTRACT

Nutrient limitation, especially Nitrogen (N), is a key factor limiting plant growth in most arctic regions. Climatic changes in temperature, light and CO_2 concentrations can alter plant productivity; however, the indirect influences of climate change on nutrient cycling processes may ultimately be of greater importance in determining plant productivity. To fully understand the implications of climate change on N availability the interactions among the processes that drive the N cycle in these arctic environments need to be more clearly understood. At Alexandra Fiord lowland, Ellesmere Island, Canada we took paired measurements of N_2-fixation rates and inorganic soil N with surface greenhouse gas fluxes, including CO_2, N_2O and CH_4, in both Wet Sedge Meadow and Dryas Heath vegetation communities. The Wet Sedge Meadow had higher N_2-fixation rates, higher soil NH_4-N concentrations, higher rates of photosynthesis and higher CH_4 gas efflux over the 2009 growing season. NO_3-N concentrations were significantly lower in the Wet Sedge Meadow compared with the Dryas Heath. Both the higher soil temperature and lower soil moisture conditions at the Dryas Heath site may lead to higher rates of nitrification. Loss of N through denitrification does not appear to be a significant factor in the N cycling within either vegetation communities. In these N limited communities, higher rates of N_2-fixation occurred in areas with greater moisture availability and higher Carbon inputs. Higher rates of nitrification may be associated with warmer and drier vegetation types; however, increasing NO_3-N availability does not appear to increase rates of denitrification. Differences in nutrient cycling processes between vegetation communities may be largely

driven by patterns of moisture. Therefore, patterns of moisture may be a key factor in understanding the response of high arctic vegetation to climate change.

INTRODUCTION

Plant productivity in many arctic regions is constrained both by low soil temperature and low soil moisture content, but during the growing season soil nitrogen (N) is believed to be the primary factor limiting plant growth (Shaver & Chapin, 1980; Nadelhoffer et al., 1992; Liengen & Olsen, 1997a; Zielke et al., 2005). While climate warming undoubtedly has direct influences on arctic communities, several multifactor experiments have shown that tundra ecosystems are more responsive to additions of N and phosphorus than to changes in temperature, light or CO_2 (Henry et al., 1986; Chapin et al., 1995; Hobbie & Chapin, 1998; Shaver et al., 1998; Van Wijk et al., 2002; Hill & Henry, 2011). The indirect influences of climate change, therefore, may be of greater importance in determining plant productivity. Atmospheric N_2-fixation is a key N input to arctic terrestrial ecosystems (Barsdate & Alexander, 1972; Bazely & Jefferies, 1989; Chapin & Bledsoe, 1992; Hobara et al., 2006). N_2-fixation is able to provide enough N to meet the needs of these low biomass systems and may even be able to sustain additional plant growth (Dickson, 2000; Sorenson et al., 2006). Changes in temperature and moisture may have strong direct effects on N_2-fixation rates. Chapin & Bledsoe (1992) estimate a net increase in N_2-fixation of 65-85% depending on different precipitation scenarios; however, indirect effects associated with feedbacks from other processes, such as mineralization and nutrient cycling will also influence N_2-fixation rates. To fully understand the implications of climate change on N availability the interactions among the processes that drive the nitrogen cycle in these arctic environments need to be more clearly understood.

Increases in temperature and precipitation may lead to deeper active layers, higher rates of chemical transformations, and greater nutrient availability (Berendse & Jonasson, 1992; Shaver et al., 2000, Rolph, 2003; Walker et al., 2008). Increasing rates of litter decomposition, litter nitrogen release and soil mineralization have been demonstrated in warming experiments in the arctic tundra (Hobbie, 1996; Rolph, 2003). In addition, higher inorganic N availability and soluble organic N have been observed in some vegetation communities in the high Arctic (Rolph, 2003). Higher N availability may act as a negative feedback and inhibit N_2-fixation directly. Higher rates of mineralization may promote nitrification, which may in turn stimulate denitrification and increase nitrous oxide (N_2O) production (Nadelhoffer et al., 1992; Paul & Clark, 1996; Walker et al., 2008). Increasing production of N_2O gas is a concern not only because N_2O is an important greenhouse gas, but also because this could lead to a net loss of N from arctic ecosystems. Since the conditions that promote N_2-fixation are also favourable for denitrification the potential for loss of N may be high, and ecosystem gain of N even under high rates of N_2-fixation may be reduced (Chapin, 1996; Sorensen et al., 2006). However, low or negligible rates of denitrification have been detected in several different arctic environments and these low rates are often attributed to a limited by availability of inorganic N substrate (Nadelhoffer et al., 1992; Sorensen et al., 2006; Buckeridge et al., 2009).

Lower rates of nitrification have been associated with N_2-fixing soils, which could reduce N_2O production and corresponding losses (Smith et al., 2002). However, N limitations may not be the result of slow cycling within soils. Low rates of N accumulation can also result where rates of N transformations and loss are relatively fast (Peterjohn & Schlesinger, 1991; Evans & Belnap, 1999). Rapid cycling of N and higher turnover, however, may offset

reduced N_2O. While rates of N transformations are generally thought to be quite slow in arctic soils, nitrogen cycling through litter and tundra soils may increase due to warming climatic conditions (Hobbie, 1996).

In regions where rainfall dominates during warm periods, most N_2-fixation and release is likely to occur when N leaching is also greatest. Denitrification processes could simultaneously compete with plants and microbes for newly released N (Belnap, 2001; Veluci et al., 2006). Higher summer temperatures could lead to increased denitrification rates, but N_2O fluxes may be minimal if most of the N fixed is consumed by plants and microbes during these times (Veluci et al., 2006). We examined the linkages between N and C cycling processes in arctic systems through paired measurements of N_2-fixation, inorganic soil N with surface greenhouse gas fluxes, including CO_2, N_2O and CH_4, in two high arctic vegetation communities. Our ability to accurately predict long-term changes in arctic vegetation communities in response to climate change is reliant upon our understanding of N cycling within these systems.

METHODS

Sites Descriptions and Field Sampling

Two sites were located ~0.5km apart in a lowland polar oasis at Alexandra Fiord, Ellesmere Island, Nunavut Territory (78°53'N, 75°55'W). Located on the eastern side of central Ellesmere Island, the 8 km^2 oasis is a deglaciated lowland delimited by a glacier to the south, cliffs and talus slopes (ca. 500 m) to the west and east and by the Fiord waters to the north (Muc et al., 1989). Lowland soils are predominantly Regosolic Static Cryosols (Soil Classification Working Group, 1998) that are generally coarse textured with variable

concentrations of organic matter. Average air temperature is -15°C and mean monthly air temperature in July is 4.5°C (Labine, 1994). Annual precipitation at Alexandra Fiord lowland is < 100 mm with < 10 mm falling during the growing season from mid-June to August (Muc et al., 1989). The lowland has an extensive vegetation cover dominated by deciduous dwarf shrubs, heaths, cushion plants and hydric sedges.

We sampled in two distinct plant communities within the polar oasis, including a Wet Sedge Meadow and a Dryas Heath community. The Wet Sedge Meadow is characterized as a hydric sedge-cushion plant-dwarf shrub community and the Dryas Heath as a xeric-mesic lichen-cushion plant-dwarf shrub community (follows Muc et al., 1989). Soils at the Wet Sedge Meadow site were saturated and poorly developed with a variably thick (5-20cm) organic layer overlaying undifferentiated sand and silt (Henry and Svoboda, 1986; Henry et al., 1990; Muc et al., 1994a). Soils at the Dryas Heath site were moderately well-drained with a shallow organic soil layer (3-5 cm) underlain by coarse mineral soils. The Wet Sedge Meadow was located on a stream margin and was wet throughout the growing season with hydric sedges *Eriophorum angustifolium* (Honck.), *Carex stans* ((Drej.) Hult.) and *C. membranacea* (Hook.) dominating. Scattered shrubs including *Salix arctica* (Pall.), *Vaccinium uliginosum* (L.) and *Cassiope tetragona* ((L.) D. Don) were also present. Compared to other vegetation communities within the lowland the Wet Sedge Meadow has the highest vascular plant cover (67%) and the highest bryophyte cover (23%) with an abundance of the moss *Drepanocladus* spp. ((Hedw.) Warnst.) (Muc et al., 1989). The N_2-fixing cyanobacterium *Nostoc* spp. was found extensively within the Wet Sedge Meadow. Largely unvegetated raised-center frost boils were found within the Dryas Heath site, however, we did not sample directly on frost boils. The depressions and relatively stable and

protected margins of frost boils, where we did sample, were sparsely populated by *Dryas integrifolia* (M. Vahl), *Cassiope tetragona* ((L.) D. Don) *Saxifraga oppositifolia* (L.). Dryas Heath had a relatively large lichen cover (~30-40%) and poorly to well-developed black cryptogamic crusts similar to those described by Dickson, (2000).

The Wet Sedge Meadow (June 30th, July 1st, 5th,6th, 19th, 23rd, 24th, 28th, 29th, August 2nd, 3rd, 7th, 8th and 12th) and Dryas Heath (July 2nd, 3rd, 7th,8th, 20th, 21st, 25th, 26th, 30th, 31st, August 4th, 5th, 9th, 10th and 13th) sites were sampled over the 2009 growing season. Twenty-four hours before each sampling date 5 plastic collars (0.0314 m^2) were inserted to a depth of ~7.3 cm at the given site. Greenhouse gas flux, N_2-fixation and soil samples were then taken for each of the 5 replicates (i.e. plastic collars) on the same sampling day. Over the growing seasons a total of 72 Wet Sedge Meadow and 73 Dryas Heath samples were assessed for greenhouse gas flux, surface N_2-fixation and soil NH_4-N and NO_3-N at ~3-5 cm below the surface.

Greenhouse Gas Flux Measurements

Measurements of the GHG flux at the soil:atmosphere interface were measured by connecting a Fourier Transform InfraRed-Thermogravimetric Analyzer (FTIR-TGA) to a Li-Cor long-term monitoring chamber (Model 8100-104; Li- Cor). Two different types of chambers, one transparent (with an internal volume of 5.3 L) and one opaque (with an internal volume of 4.5 L) were used to monitor GHG emissions from the collar area. Flux measurements for each chamber were obtained by closing the chamber and monitoring the change in gas concentration over a 10 min period. The transparent chamber was deployed first followed by the opaque chamber with a short period (≥ 10 min) in between to allow greenhouse gas concentrations to return to ambient. The FTIR-TGA collected one spectral

sample every 100 ms, with the on-board software recording gas concentrations averaged over 60 s intervals. Greenhouse gas fluxes were calculated by plotting the change in concentration *vs*. time and using standard curve fitting techniques to determine the slope of the curve at time zero. Preliminary studies showed that instrument precision during a 60 s sampling interval was 0.006% for CO_2, 0.20% for CH_4, and 0.21% for N_2O.

N_2-Fixation Rates

Measurements of N_2-fixation were made using acetylene reduction assays (Stewart et al., 1967). Acetylene gas (C_2H_2) was generated on-site from CaC_2 and water, with incubations injected with 10% (v/v) acetylene. Ethylene concentrations were measured in the field with a portable gas chromatograph (SRI 8610A, Wennick Scientific Corporation, Ottawa, ON, Canada) fitted with a Porapak column (Alltech Canada, Guelph, ON, Canada) and a flame ionization detector. A Stand-Alone Hydrogen Generator (SRI H_2-50, Alltech Canada, Guelph, ON, Canada) provided hydrogen as the carrier gas, which was held at a constant pressure of 26 psi. Column temperature was held at 65^{o}C. The gas chromatograph was calibrated for each incubation with ethylene (BOC Canada Ltd., Mississauga, ON, Canada, C_2H_4, 98%+) that was kept at the same temperature as incubation gas samples.

Surface samples of Wet Sedge Meadow and Dryas Heath (314 cm^2 and ~2 cm deep) were sampled from the entire surface area of the collar. Samples were enclosed in clear 4.5 L plexiglass incubation chamber with a rubber septum injection port. The mean headspace of ARA incubations was 3.87 L (4.5 L incubation chamber volume minus 628 ml sample) for all samples. Control samples not injected with acetylene were included for some sets of incubations and these controls did not show any natural evolution of ethylene.

Contamination of generated acetylene with ethylene was monitored and corrections were made for each set of incubations, as required.

All incubations were 5 hours in length and occurred between the hours of 10:30-16:00 directly following greenhouse gas measurements. All surface samples were incubated outdoors near the research station laboratory under ambient field conditions. Temperature of surface samples within the incubation chambers was monitored. Incubation chambers were moated and moats were filled with ice and/or ocean water to ensure that incubation temperatures reflected ambient conditions. Photosynthetically Active Radiation (PAR), air temperature and incubation temperature were monitored every hour during daytime ARA incubations. The percent moisture of each N_2-fixing surface sample was determined gravimetrically.

N_2-fixation rates were calculated as micromoles of ethylene reduced per hour per m^2 based upon the length of incubation and area of each sample. A 3:1 conversion ratio was used to convert ethylene reduced to N_2 reduced for all samples.

Soil Sampling

Soil moisture (VWC) and temperature for each collar were determined with a ProCheck digital sensor (Decagon Devices, Pullman, WA, USA) equipped with an ECH_2O-TE combined moisture-temperature probe that was inserted into the soil and allowed to reach thermal equilibrium (~2 min.). Soil samples (~3-5 cm below the surface) were taken directly below the N_2-fixing surface (~ 2cm depth) once the surface sample had been removed. Soil samples were extracted into water, filtered (11µm pore size, Grade 1, Whatman Ltd) and immediately frozen (-20^{o}C). Frozen samples were shipped to the laboratory at University of

Saskatchewan where they were thawed and inorganic N (NH_4-N (µg/g dry soil) and NO_3-N (µg/ g dry soil)) extracted by water were determined on a SmartChem^{TM} instrument (Mandel Scientific Company Inc., Ontario, Canada). Since local water sources were used for the extractions, control water samples were also taken and corrections made as required.

Statistical Analyses

All statistical analyses were done using SYSTAT software (SYSTAT 8.0, Systat Software, Inc.). N_2-fixation rates and NH_4-N values were log transformed. Comparison of mean N_2-fixation, NH_4-N, NO_3-N, and gas flux values between Wet Sedge Meadow and Dryas Heath were done with t-tests. Comparison of gas flux measured in transparent versus opaque chambers were also done with t-tests. Correlations between N_2-fixation and all other variables were determined using Spearman's correlations.

RESULTS

Compared to the Dryas Heath vegetation community, the Wet Sedge Meadow community had higher mean N_2-fixation rates (t-test, $p<0.01$), soils with higher mean NH_4-N concentrations (t-test, $p<0.01$), higher mean CO_2 gas flux from the surface in transparent (t-test, $p<0.01$) and opaque chambers (t-test, $p=0.03$) and higher mean CH_4 gas flux (t-test, $p=0.01$) over the 2009 growing season at Alexandra Fiord (Table 9). Gas flux measured in transparent versus opaque chambers was significantly different for CO_2 flux only (t-tests, CO_2 $p<0.01$, N_20 $p=0.92$, CH_4 $p=0.42$). Soils at Dryas Heath had significantly higher mean NO_3-N concentrations than those at Wet Sedge Meadows (t-test, $p<0.01$). N_2O flux was very low over the growing season at both sites and not significantly different between the two (t-test, $p= 0.88$).

Table 9. Mean N_2-fixation, soil NH_4-N and NO_3-N concentrations and greenhouse gas flux in transparent (T) and opaque (Op) chambers over the 2009 growing season in Wet Sedge Meadow and Dryas Heath vegetation communities at Alexandra Fiord Lowland, Ellesmere Island. Significantly different means are indicated by * ($p<0.05$) or ** ($p<0.01$). Data represent mean with +/- SE.

Vegetation Type	n	N_2-fixation (µmol N_2 $m^{-2}h^{-1}$)	NH_4-N (µg/g dry soil)	NO_3-N (µg/g dry soil)	Greenhouse Gas Flux			
					CO_2 (T) (µmol $m^{-2}h^{-1}$)	CO_2 (Op) (µmol $m^{-2}h^{-1}$)	N_2O (Op) (µmol $m^{-2}h^{-1}$)	CH_4 (Op) (µmol $m^{-2}h^{-1}$)
Wet Sedge Meadow	72	30 (2.1) **	4.57 (0.65)**	4.36 (0.24)	-1389 (126)**	698 (109)*	-0.12 (0.2)	5.7 (0.25)*
Dryas Heath	73	2.4 (0.29)	2.09 (0.44)	6.17 (0.41)**	- 83 (96)	396 (78)	-0.17 (0.19)	-0.24 (2.1)

Soil moisture and surface moisture of the N_2-fixing sample layer were significantly higher in the Wet Sedge Meadow and soil temperature was significantly higher in the Dryas Heath vegetation community (t-tests, $p<0.01$for all comparisons) (Table 10).

Table 10. Mean soil temperature, soil moisture and surface moisture of the N_2-fixing layer over the 2009 growing season in Wet Sedge Meadow and Dryas Heath vegetation communities at Alexandra Fiord Lowland, Ellesmere Island. Significantly different means are indicated by ** ($p<0.01$). Data represent mean with +/- SE.

Vegetation Type	n	Soil Temperature (^oC)	Soil Moisture (%)	N_2-fixing Surface Moisture (%)
Wet Sedge Meadow	72	7.5 (0.24)**	85 (0.76)**	84.57 (1.42)**
Dryas Heath	73	8.9 (0.32)	19 (1.1)	30.31 (2.17)

Soil moisture had the highest correlation ($r=0.75$) with N_2-fixation compared with all other variables investigated. Moisture of the N_2-fixing layer had the second highest correlation ($r=0.70$), but was closely associated ($r=0.83$) with soil moisture (Table 11). Differences in soil moisture and N_2-fixation rates between Wet Sedge Meadow and Dryas Heath

communities appear to be a major factor driving the soil moisture N_2-fixation relationship within the polar oasis (Figure 10).

Table 11. Spearman's correlations between mean N_2-fixation, soil NH_4-N and NO_3-N concentrations, greenhouse gas flux in transparent (T) and opaque (Op) chambers, soil temperature, soil moisture and surface moisture of the N_2-fixing layer over the 2009 growing season across both vegetation communities at Alexandra Fiord Lowland, Ellesmere Island.

	N_2-fixation	NH_4-N	NO_3-N	CO_2 (T)	CO_2 (Op)	N_2O (Op)	CH_4 (Op)	Soil Temp.	Soil Moist.	Surface Moist.
N_2-fixation	1.00									
NH_4-N	0.25	1.00								
NO_3-N	-0.32	0.13	1.00							
CO_2 (T)	-0.53	-0.20	0.05	1.00						
CO_2 (Op)	0.15	0.23	0.02	-0.06	1.00					
N_2O (Op)	0.02	0.04	0.11	0.02	0.22	1.00				
CH_4 (Op)	0.31	0.15	-0.09	-0.28	0.14	-0.16	1.00			
Soil Temp.	-0.20	-0.18	0.05	0.12	0.18	-0.01	-0.12	1.00		
Soil Moist.	0.75	0.40	-0.15	-0.52	0.16	0.002	0.29	-0.33	1.00	
Surface Moist.	0.70	0.51	-0.14	-0.53	0.25	0.10	0.34	-0.30	0.83	1.00

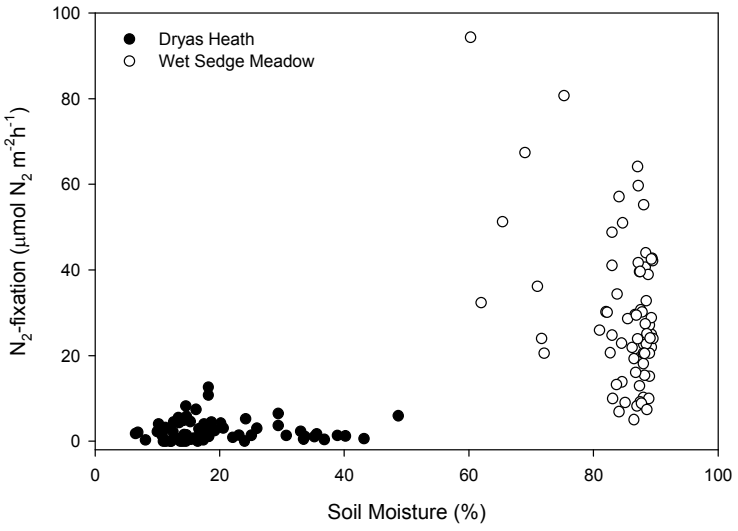

Figure 10. Relationship between N_2-fixation rate and soil moisture across the Wet Sedge Meadow and Dryas Heath vegetation communities within the polar oasis at Alexandra Fiord Lowland, Ellesmere Island over the 2009 growing season.

Both N_2O flux and respiration (CO_2 flux in opaque chamber) had very weak correlations (r=0.02 and 0.15, respectively) with N_2-fixation rate and CH_4 flux had a weak correlation (r=0.31) with fixation (Table 11). Despite detection of denitrification on some sampling dates we found no net N_2O emissions over the growing season and no significant correlation between N_2O flux and N_2-fixation. Photosynthesis (CO_2 flux in transparent chamber), however had the second highest correlation with N_2-fixation (r=-0.53) after soil and N_2-fixing layer moisture (Table 11). Differences in photosynthesis and N_2-fixation rates between Wet Sedge Meadow and Dryas Heath communities appear to be a major factor driving the photosynthesis N_2-fixation relationship within the polar oasis (Figure 11). NH_4-N and NO_3-N concentrations were both significantly correlated with N_2-fixation rate (r = 0.25 and -0.32, respectively).

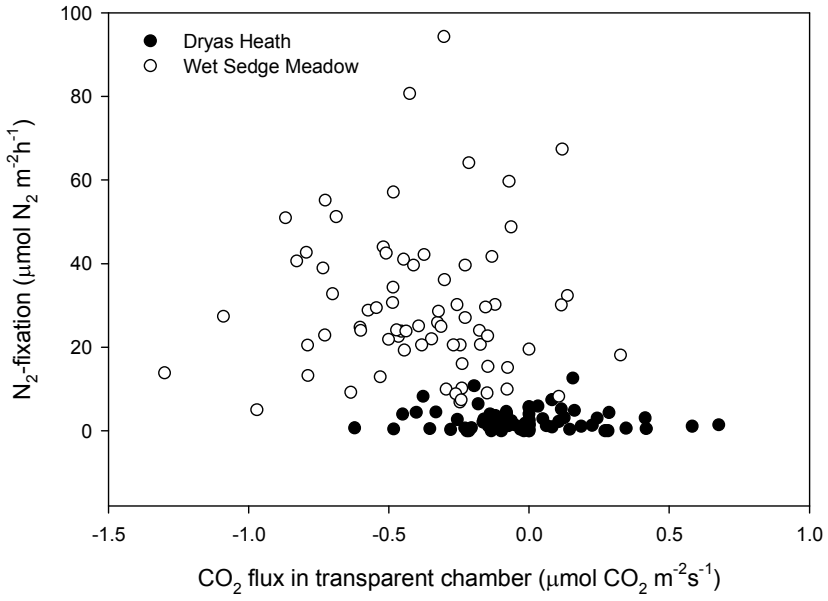

Figure 11. Relationship between N_2-fixation rate and CO_2 flux in the transparent chamber across the Wet Sedge Meadow and Dryas Heath vegetation communities within the polar oasis at Alexandra Fiord Lowland, Ellesmere Island over the 2009 growing season.

DISCUSSION

Abiotic conditions, such as soil moisture and moisture of the N_2-fixing layer appear to be the most important factors in determining N_2-fixation in this high arctic oasis. Several studies have found moisture to be one of the most important factors in determining rates of N_2-fixation in the Arctic (Chapin & Bledsoe, 1992; Nash & Olafsen, 1999; Zielke et al., 2002, 2005). Higher input of water can affect N_2-fixers indirectly by stimulating net primary production. Greater moisture availability in the Wet Sedge Meadow has likely led to greater N_2-fixing biomass, resulting in the detection of higher N_2-fixation rates. The Wet Sedge Meadow also had higher rates of net photosynthesis and respiration, and greater fluxes of CH_4 than the Dryas Heath.

The flux of N_2O was not significantly different between the vegetation communities and both communities acted as net sinks of N_2O gas over the growing season. Although, N_2-fixation and concentrations of NH_4-N were higher in the Wet Sedge Meadow, NO_3-N concentrations were significantly lower than in Dryas Heath leading to reduced substrate availability for denitrification. In addition, Walker et al. (2008) found the Wet Sedge Meadow at Alexandra Fiord lowland had significantly lower *nos*Z genotype richness compared with a heath vegetation type. N_2O flux was not significantly correlated with N_2-fixation indicating that denitrification likely plays a very limited role in N cycling within these communities and may not act as a pathway for N loss from this ecosystem even under higher rates of N_2-fixation.

The negative correlation between NO_3-N and N_2-fixation most likely reflects differences in the rates of nitrogen transformations occurring at the two vegetation communities. The Wet Sedge Meadow had a much higher N_2-fixation rate and not surprisingly also had significantly

higher NH_4-N concentration in the soil layer directly below the N_2-fixers. Several studies have found higher concentrations of NH_4^+ and/or potentially mineralizable N in association with higher rates of N_2-fixation (Gunther, 1989; Knowles et al., 2006; Veluci et al., 2006). However, NO_3-N concentrations were significantly lower in the Wet Sedge Meadow compared with the Dryas Heath. Both the higher soil temperature and lower soil moisture conditions at the Dryas Heath site may lead to higher rates of nitrification and hence higher concentrations of NO_3-N. Low temperatures and anoxic conditions have been suggested as reasons for low nitrification rates in arctic soils (Flint & Gersper, 1974; Nadelhoffer et al., 1992). At the high arctic site of Truelove lowland, Devon Island, Chapin (1996) found lower nitrification rates in a sedge meadow vegetation community compared to a willow-herb hummock vegetation community. While temperature did not appear to influence nitrification rates at Truelove, strong differences in soil moisture between the vegetation communities was the primary environmental factor controlling nitrification rates. Similarly, the Wet Sedge Meadow community at Alexandra Fiord may have lower nitrification rates due to saturated soils with anoxic conditions resulting in a lower abundance of nitrifiers and reduced nitrifier activity. Anoxic conditions in the Wet Sedge Meadow may also account for the significantly higher CH_4 emissions detected there.

Net photosynthesis appears to play an important role in determining N_2-fixation rates, demonstrating strong linkages between carbon availability and N cycling. The energetic costs of fixing N_2 are often higher than that of absorbing and assimilating ammonium or nitrate. Electrons for N_2-fixation are ultimately derived from photosynthesis with photophosphorylation being a major source of ATP (Stal, 1995). Symbiotic N_2-fixers must expend 8-12g of glucose to acquire 1g of N via fixation, not including the construction or

maintenance costs of specialized structures, such as heterocysts (Gutschick, 1981; Vitousek & Howarth, 1991). For free-living diazotrophic bacteria acquiring N may be even more energetically expensive requiring the utilization of 100g of C to fix 1 to 5 g of N (Marschner, 1995; Kurina & Vitousek, 2001). Significant increases in soil N_2-fixation have been demonstrated with the addition of C (glucose) and water (Hartley & Schlesinger, 2002). Patterns of total N pool size often resemble those of C pool size and biomass across different vegetation communities in the Arctic (Arndal et al., 2009). The Dryas Heath community, which had much lower rates of N_2-fixation and lower total soil inorganic N also acted a net CO_2 source and has lower biomass (Muc et al., 1994b). While the Wet Sedge Meadow which acted as a net CO_2 sink over the growing season had higher rates of N_2-fixation, higher total soil inorganic N and much higher biomass.

The Wet Sedge Meadow at Alexandra Fiord lowland has been extensively studied. Compared to other vegetation communities at Alexandra Fiord, Walker et al. (2008) found that the Wet Sedge Meadow was most consistently affected by experimental warming with a reduction in both *nos*Z and *nif*H genotype richness. In addition, Rolph (2003) found the responses of nitrogen transformations to warming were also greatest in the Wet Sedge Meadow compared with other lowland vegetation communities. In 2005 the average aboveground biomass (158% increase) and the average belowground biomass (root biomass 67% increase and rhizome biomass 139% increase) of the sedge community at Alexandra Fiord were much greater than the mean biomass measured in the early 1980s (Hill & Henry, 2011). This increase in both above and belowground biomass is attributed to the indirect effects of increased temperature on nutrient availability and subsequent nutrient acquisition. Not only do our measurements of CO_2 flux lend support for accumulation of carbon in the

Wet Sedge Meadow, but our measurements of N_2-fixation may also support increasing N inputs. Henry & Svoboda (1986) measured a mean N_2-fixation rate of 6.3 μmol N_2 $m^{-2}h^{-1}$ in the Wet Sedge Meadow over the growing season in 1983. Our estimates of N_2-fixation within the Wet Sedge Meadow (30 μmol $m^{-2}h^{-1}$) based on a similar methodology is ~5 times greater in 2009.

Warming has resulted in higher above and belowground biomass, altered *nif*H and *nos*Z microbial communities and increased rates of some N transformations in the Alexandra Fiord lowland (Rolph, 2003; Walker et al., 2008; Hill & Henry, 2011). These responses, however, appear to be strongly related to vegetation community type. Nutrient inputs, especially via biological means, and nutrient cycling vary greatly between different vegetation communities in the high Arctic, even within a single polar oasis. Vegetation communities in the high Arctic will not necessarily respond in a similar manner to climatic changes. Areas with high moisture may sequester more CO_2 due to higher rates of photosynthesis, but may act as greater sources of CH_4 emissions. In these nitrogen limited communities, N_2-fixation appears to be associated with areas of greater moisture availability and higher C inputs. Higher rates of nitrification may be associated with warmer and drier vegetation types, however, increasing NO_3-N availability does not appear to increase rates of denitrification. Loss of N through denitrification does not appear to be a significant factor in the N cycling within either Wet Sedge Meadow or Dryas Heath vegetation communities. Differences in nutrient cycling processes between vegetation communities may be largely driven by patterns of moisture. Therefore, patterns of moisture may be a key factor in understanding the response of high arctic vegetation to climate change.

CHAPTER 6: SUMMARY AND CONCLUSIONS

Despite human alteration of the nitrogen cycle in many habitats globally, many arctic ecosystems still provide an opportunity to examine nitrogen cycling under relatively pristine conditions. An understanding of the magnitude of N inputs via N_2-fixation, the temporal and spatial variation in N_2-fixation and the role of N_2-fixers within nutrient cycling in arctic environments is essential and may assist in predicting the impact of future climatic changes.

We examined temporal and spatial variation in N_2-fixation by the principal cyanobacterial associations (hummock and hollow biological soil crusts, *Sphagnum* spp. associations, and *Stereocaulon paschale*) in a wide range of ecosystems within a Canadian low arctic tundra landscape, and estimated N input via N_2-fixation over the growing season based upon microclimatic conditions. We approached landscape-level estimation of N_2-fixation from multiple scales by simultaneously considering the key N_2-fixing associations present, the representation of different N_2-fixers within the main ecosystem types and the extent of the main ecosystem types within the landscape.

Total growing season (June 3rd-September 13th) N_2-fixation input from measured components across a carefully mapped landscape study area (26.7 km^2) was estimated at 0.68 kg ha^{-1}yr^{-1}, which is approximately twice the estimated average N input via wet deposition. Our estimate of N input via N_2-fixation is within the range of estimates determined for other arctic sites (Table 1- 0.10 to 1.20 kg N ha^{-1}y^{-1}). While some arctic studies have found N_2-fixation contributed 80% or higher to total landscape N inputs (Hobara et al., 2006; Solheim et al., 2006), other studies, including ours, have found the contribution of N_2-fixation to

ecosystem N inputs is approximately 50%-70% (Chapin & Bledsoe, 1992; Henry & Svoboda, 1986).

The most common pattern of seasonal N_2-fixation is an increase in rates soon after snowmelt (March-June) followed by the highest rates coinciding with the peak growing season (maximal plant biomass) and declining rates in late July to August depending on latitude. Our field measurements of N_2-fixation and our models of potential N_2-fixation both showed this seasonal pattern for most N_2-fixing associations. Moisture and temperature had a strong influence on the seasonal patterns of N_2-fixation within individual N_2-fixing communities.

Spatial variation in N_2-fixation activity appears to be closely related to moisture patterns associated with topography and microtopography. Topography is the primary determinant of soil moisture patterns across the tundra landscape, and therefore plays a major role in determining the distribution of vegetation types (Walker, 2000) and their cyanobacterial associations. We found the highest N input occurred in the low-lying Wet Sedge Meadow and the lowest N input occurred in the Xerophytic Herb Tundra located on the upper most esker slopes at Daring Lake. Our investigation of small-scale spatial patterns of N_2-fixation in hollow-hummock tundra revealed higher mean percent moisture in hollows. Hollow Biological Soil Crusts (BSCs) also had a higher mean growing season N_2-fixation rate, a higher mean growing season *nif*H abundance, a higher mean total %N and $\delta^{15}N$ values closer to that of atmospheric N_2.

Our landscape estimates of biological N_2-fixation inputs for Daring Lake provide a highly detailed account of N_2-fixation by the principal N_2-fixing associations. However, there are some methodological constraints that may have affected our landscape estimates: i) The use

of a single conversion ratio for each N_2-fixing association under all operating conditions across the growing season, ii) the use of visual estimates for determining the abundance of N_2-fixing associations, iii) limited quantification of the spatial variability in soil surface microclimate, and iv) inclusion of only 103 days of the year and only 68% of the landscape study area. However, the multi-scale approach employed in our study still provided a comprehensive way to more accurately estimate ecosystem and landscape N inputs via N_2-fixation, and to understand the controls on that process.

Using an exploratory multi-group Structural Equation Modeling (SEM) approach we examined the direct and indirect effects of soil moisture, plant community functional composition, and bryophyte and lichen abundance on rates of nitrogen fixation at a low arctic ecosystem, two high arctic oases and a high arctic polar desert in the Canadian Arctic. Increasing soil moisture was strongly associated with an increasing presence of bryophytes and increasing bryophyte abundance was a major factor determining higher N_2-fixation rates at all sites. In our landscape study at Daring Lake, *Sphagnum* spp. made the largest contribution (55.2%) of all of the N_2-fixing associations to total N input. In the hollow-hummock tundra ecosystem we examined, Hollow BSCs that were primarily composed of liverwort-cyanobacterial associations, had higher rates of N_2-fixation in comparison to Hummock BSCs. Therefore, N_2-fixation by bryophyte-cyanobacterial associations are likely important across the Canadian Arctic. Several other studies have also found the highest rates of N_2-fixation in arctic landscapes are associated with cyanobacteria moss associations (Alexander & Schell, 1973; Henry & Svoboda, 1986; Solheim et al., 1996).

Soil N status was linked to rates of N_2-fixation by different cyanobacterial associations in both the high and low Arctic indicating that these N_2-fixing associations may act as

important point sources of soil N. However, nitrogen availability in arctic ecosystems is not only dependent on N_2-fixation, but is also the result of different N and C cycling processes. We found nutrient inputs via biological means, and nutrient cycling varied greatly between different vegetation communities in the Arctic. Annual plant primary production is high in the Wet Sedge Meadows at both Alexandra Fiord and Daring Lake in comparison to other ecosystem types within the given landscapes. At Alexandra Fiord, we found significantly higher rates of net photosynthesis in the Wet Sedge Meadow compared with Dryas Heath and a strong correlation between rates of net photosynthesis and N_2-fixation. In these nitrogen limited communities, N_2-fixation appears to be associated with that have greater moisture availability and higher C inputs. Moisture availability may have an indirect effect on ecosystem development by affecting N input into the system with bryophytes-cyanobacterial associations playing an important intermediary role in the process.

Warmer temperatures and changes in moisture availability due to climate change may increase N_2-fixation, however, increased shrub and graminoid cover may counter-act this increase in N_2-fixation. Shrubs had a negative effect on bryophyte abundance at all sites with the exception of the polar desert site at Alexandra Fiord highland, where both shrubs and graminoids had a positive influence. The importance of competition from vascular plants, potentially through shading, appears to be greater in more productive sites and may increase at lower latitudes. Remote sensing, repeat photography, and warming experiments in combination with nutrient addition studies all suggest that current warming trends in the Arctic may be promoting shrub growth and expansion in some areas (Chapin et al., 1995; Sturm et al., 2001; Goetz et al., 2005; Walker et al., 2006). N_2-fixation rates, and persistence

of N_2-fixing associations in these environments, may be influenced by reduced light availability, however further research is needed.

Alteration of the rates of different N transformations due to climate warming may be crucial in determining the long-term N availability and the response of arctic ecosystems. Higher rates of nitrification may be associated with warmer and drier vegetation types; however, increasing NO_3-N availability does not appear to increase rates of denitrification. Loss of N through denitrification does not appear to be a significant factor in the N cycling within high arctic Wet Sedge Meadow or Dryas Heath vegetation communities at Alexandra Fiord. Differences in nutrient cycling processes between vegetation communities may be largely driven by patterns of moisture. Therefore, patterns of moisture may be a key factor in understanding the response of arctic vegetation to climate change.

The importance of biological N_2-fixation by the many cyanobacterial associations present in Canadian Arctic landscapes is clear. Our study has found that many factors control both the temporal and spatial variability of N_2-fixation, including topography, microtopography, vegetation characteristics, microclimatic conditions, *nif*H abundance and availability of other nutrients, such as phosphorus. Moisture, in particular, appears to be a key factor not only in determining N_2-fixation but also by influencing related nutrient cycling processes. Further research efforts are needed that address long-term changes in N_2-fixation and N cycling, particularly in areas of high moisture. In addition, due to the apparent importance of bryophyte-cyanobacterial associations in arctic ecosystems further studies addressing the physiological processes that underlie these associations are needed. The release, recycling and availability of N directly related to these bryophyte-cyanobacterial associations also requires further investigation.

COMMUNICATIONS WITH THE PUBLIC

There is a growing need to effectively communicate the findings of scientific studies with the public, especially with members of northern communities that will continue to be directly impacted by climate change. In addition to public presentations that allow for the opening of dialogue between researchers and stakeholders, brief documents that convey important scientific findings in an accessible manner are needed. The document included here is intended as an educational tool to aid in communicating the findings of this thesis with the public (Appendix II).

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APPENDIX I

Appendix I. Description of variables included in the structural equation model and full model results including direct and indirect effects and unstandardized path coefficients.

Table 1. Description of variables included in the structural equation model.

Variable		Description
Soil Moisture		Percent moisture of soil determined gravimetrically from soil samples collected directly below each N_2-fixation surface sample.
Vascular plant community functional composition	Shrubs	Percent cover of all vascular plant species in 0.5 X 0.5 m square quadrats in July (Alexandra Fiord and Truelove) and August (Daring) 2008. The total % cover of vascular plants was divided into shrubs, graminoids, and forbs. There was relatively little species overlap between sites and a more detailed classification of functional types was not possible.
	Graminoids	
	Forbs	
Potential N_2-fixing associations	Bryophytes	Percent cover of all mosses, liverworts and hornworts summed as total bryophytes in 0.5 X 0.5 m plots in July-August 2008
	Lichens	Percent cover of all foliose, fruiticose, and crustose lichens summed as total lichens in 0.5 X 0.5 m plots in July-August 2008.
	Bare ground	Percent cover of rocks, gravel and finer material summed as bare ground in 0.5 X 0.5 m plots in July-August 2008. Bare ground was included as a potential N_2-fixing association because biological soil crusts, which are communities composed of bacteria, cyanobacteria, algae, mosses, liverworts, fungi and small lichens, were not explicitly included in the survey and where present would have been recorded as bare ground.

Table 2. Unstandardized and standardized path coefficients, the standard error of the unstandardized coefficients and t-test results from the N_2-fixation structural equation models for each site. Sites are indicated by superscript letters (Alexandra Fiord Highland = AH, Alexandra Fiord Lowland,= AL, Truelove = T and Daring Lake = DL). Paths are from the variables in lower case to the variable in bold at the top of each section in the table.

	Unstand. path coefficients	Std Error	t value	P value	Standard. coefficients
Shrubs					
Soil moisture^{AH}	-0.018	0.014	-1.33	0.183	-0.005
Soil moisture^{AL}	0.188	0.094	1.991	0.046	0.196
Soil moisture^{T}	-0.018	0.014	-1.33	0.183	-0.116
Soil moisture^{DL}	0.642	0.174	3.683	<0.001	0.393
Graminoids					
Soil moisture^{AH}	0.002	0.015	0.113	0.91	0.012
Soil moisture^{AL}	0.067	0.011	6.121	<0.001	0.139
Soil moisture^{T}	0.067	0.011	6.121	<0.001	0.465
Soil moisture^{DL}	0.302	0.09	3.339	<0.001	0.361
Forbs					
Soil moisture^{AH}	0.207	0.082	2.523	0.012	0.245
Soil moisture^{AL}	-0.007	0.003	-2.472	0.013	-0.021
Soil moisture^{T}	-0.007	0.003	-2.472	0.013	-0.160
Soil moisture^{DL}	-0.007	0.003	-2.472	0.013	-0.187
Bryophytes					
Soil moisture^{AH}	0.09	0.022	4.008	<0.001	0.029
Soil moisture^{AL}	0.514	0.128	4.027	<0.001	0.396
Soil moisture^{T}	0.09	0.022	4.008	<0.001	0.326
Soil moisture^{DL}	1.026	0.176	5.842	<0.001	0.634
Shrubs^{AH}	0.439	0.072	6.138	<0.001	0.547
Shrubs^{AL}	-0.143	0.083	-1.721	0.085	-0.105
Shrubs^{T}	-0.455	0.132	-3.448	<0.001	-0.262
Shrubs^{DL}	-0.143	0.083	-1.721	0.085	-0.144
Graminoids^{AH}	0.404	0.161	2.501	0.012	0.020
Graminoids^{AL}	-0.348	0.273	-1.276	0.202	-0.130
Graminoids^{T}	0.404	0.161	2.501	0.012	0.212
Graminoids^{DL}	-0.506	0.2	-2.525	0.012	-0.261
Forbs^{AH}	0.154	0.217	0.711	0.477	0.042
Forbs^{AL}	0.154	0.217	0.711	0.477	0.043
Forbs^{T}	0.154	0.217	0.711	0.477	0.026
Forbs^{DL}	0.154	0.217	0.711	0.477	0.004
Lichens					
Soil moisture^{AH}	-0.008	0.013	-0.628	0.53	-0.002
Soil moisture^{AL}	-0.008	0.013	-0.628	0.53	-0.011
Soil moisture^{T}	-0.008	0.013	-0.628	0.53	-0.049
Soil moisture^{DL}	-0.008	0.013	-0.628	0.53	-0.005

Shrubs^{AH}	-0.146	0.049	-2.971	0.003	-0.112
Shrubs^{AL}	-0.146	0.049	-2.971	0.003	-0.189
Shrubs^{T}	-0.146	0.049	-2.971	0.003	-0.142
Shrubs^{DL}	-0.446	0.078	-5.688	<0.001	-0.503
Graminoids^{AH}	18.546	2.889	6.42	<0.001	0.554
Graminoids^{AL}	-0.276	0.071	-3.904	<0.001	-0.181
Graminoids^{T}	-0.276	0.071	-3.904	<0.001	-0.245
Graminoids^{DL}	-0.276	0.071	-3.904	<0.001	-0.160
Forbs^{AH}	-0.104	0.194	-0.535	0.593	-0.017
Forbs^{AL}	-0.104	0.194	-0.535	0.593	-0.051
Forbs^{T}	0.662	0.24	2.762	0.006	0.189
Forbs^{DL}	-0.104	0.194	-0.535	0.593	-0.003
Bryophytes^{AH}	-0.282	0.038	-7.347	<0.001	-0.174
Bryophytes^{AL}	0.019	0.057	0.33	0.741	0.033
Bryophytes^{T}	-0.282	0.038	-7.347	<0.001	-0.478
Bryophytes^{DL}	-0.282	0.038	-7.347	<0.001	-0.316
Bare ground					
Shrubs^{AH}	-0.949	0.193	-4.925	<0.001	-0.42
Shrubs^{AL}	-0.136	0.048	-2.848	0.004	-0.285
Shrubs^{T}	-0.697	0.064	-10.823	<0.001	-0.52
Shrubs^{DL}	-0.342	0.061	-5.578	<0.001	-0.594
Graminoids^{AH}	-0.362	0.061	-5.931	<0.001	-0.006
Graminoids^{AL}	-0.098	0.099	-0.985	0.325	-0.104
Graminoids^{T}	-0.362	0.061	-5.931	<0.001	-0.246
Graminoids^{DL}	-0.362	0.061	-5.931	<0.001	-0.321
Forbs^{AH}	-0.213	0.11	-1.935	0.053	-0.021
Forbs^{AL}	-0.213	0.11	-1.935	0.053	-0.168
Forbs^{T}	-0.213	0.11	-1.935	0.053	-0.046
Forbs^{DL}	-0.213	0.11	-1.935	0.053	-0.009
Bryophytes^{AH}	-1.055	0.242	-4.361	<0.001	-0.375
Bryophytes^{AL}	-0.051	0.029	-1.758	0.079	-0.144
Bryophytes^{T}	-0.764	0.044	-17.304	<0.001	-0.99
Bryophytes^{DL}	-0.051	0.029	-1.758	0.079	-0.087
Lichens^{AH}	0.23	0.126	1.826	0.068	0.133
Lichens^{AL}	0.082	0.061	1.337	0.181	0.132
Lichens^{T}	-0.467	0.072	-6.476	<0.001	-0.357
Lichens^{DL}	-0.097	0.071	-1.366	0.172	-0.149
N_2-fixation					
Bryophytes^{AH}	0.139	0.074	1.873	0.061	0.015
Bryophytes^{AL}	0.139	0.074	1.873	0.061	0.078
Bryophytes^{T}	0.139	0.074	1.873	0.061	0.229
Bryophytes^{DL}	0.612	0.196	3.123	0.002	0.343
Lichens^{AH}	-0.028	0.091	-0.307	0.759	-0.005
Lichens^{AL}	-0.028	0.091	-0.307	0.759	-0.009
Lichens^{T}	-0.028	0.091	-0.307	0.759	-0.027

Lichens^{DL}	-0.028	0.091	-0.307	0.759	-0.014
Bare ground^{AH}	0.039	0.088	0.442	0.659	0.012
Bare ground^{AL}	1.702	0.5	3.402	<0.001	0.335
Bare ground^{T}	0.039	0.088	0.442	0.659	0.049
Bare ground^{DL}	0.039	0.088	0.442	0.659	0.013

Table 3. Unstandardized and standardized path coefficients, the standard error of the unstandardized coefficients and t-test results for covariances in the N_2-fixation structural equation models for each site. Sites are indicated by superscript letters (Alexandra Fiord Highland = AH, Alexandra Fiord Lowland,= AL, Truelove = T and Daring Lake = DL).

Covariance	Unstand. path coefficients	Std Error	t value	P value	Standard. coefficients
Shrubs-Graminoids^{AH}	0	0	1.944	0.052	0.206
Shrubs-Graminoids^{AL}	-0.002	0.001	-2.657	0.008	-0.265
Shrubs-Graminoids^{T}	-0.004	0.001	-3.09	0.002	-0.281
Shrubs-Graminoids^{DL}	0	0	1.944	0.052	0.003
Shrubs-Forbs^{AH}	-0.001	0	-3.397	<0.001	-0.281
Shrubs-Forbs^{AL}	-0.001	0	-3.397	<0.001	-0.144
Shrubs-Forbs^{T}	-0.001	0	-3.397	<0.001	-0.147
Shrubs-Forbs^{DL}	0	0	-0.261	0.794	-0.03
Graminoids-Forbs^{AH}	0	0	-0.94	0.347	-0.097
Graminoids-Forbs^{AL}	0.001	0	3.698	<0.001	0.4
Graminoids-Forbs^{T}	0	0	-0.94	0.347	-0.002
Graminoids-Forbs^{DL}	0	0	-0.94	0.347	-0.01

Table 4. Total direct and indirect effects in the N_2-fixation model at each site. These effects were calculated using standardized path coefficients. Nonsignificant effects and paths not included in the model are indicated by ns and – respectively.

	Direct	Indirect	Total
Shrubs			
Soil moisture^{AH}	ns	-	ns
Soil moisture^{AL}	0.196	-	0.196
Soil moisture^{T}	ns	-	ns
Soil moisture^{DL}	0.393	-	0.393
Graminoids			
Soil moisture^{AH}	ns	-	ns
Soil moisture^{AL}	0.139	-	0.139
Soil moisture^{T}	0.465	-	0.465
Soil moisture^{DL}	0.361	-	0.361
Forbs			
Soil moisture^{AH}	0.245	-	0.245
Soil moisture^{AL}	-0.021	-	-0.021
Soil moisture^{T}	-0.16	-	-0.16
Soil moisture^{DL}	-0.187	-	-0.187
Bryophytes			
Soil moisture^{AH}	0.029	-	0.029
Soil moisture^{AL}	0.396	-0.021	0.375
Soil moisture^{T}	0.326	0.099	0.425
Soil moisture^{DL}	0.634	-0.151	0.438
Shrubs^{AH}	0.547	-	0.547
Shrubs^{AL}	-0.105	-	-0.105
Shrubs^{T}	-0.262	-	-0.262
Shrubs^{DL}	-0.144	-	-0.144
Graminoids^{AH}	0.02	-	0.02
Graminoids^{AL}	ns	-	ns
Graminoids^{T}	0.212	-	0.212
Graminoids^{DL}	-0.261	-	-0.261
Forbs^{AH}	ns	-	ns
Forbs^{AL}	ns	-	ns
Forbs^{T}	ns	-	ns
Forbs^{DL}	ns	-	ns
Lichens			
Soil moisture^{AH}	ns	-0.005	-0.005
Soil moisture^{AL}	ns	-0.062	-0.062
Soil moisture^{T}	ns	-0.300	-0.347
Soil moisture^{DL}	ns	-0.408	-0.408
Shrubs^{AH}	-0.112	-0.095	-0.207
Shrubs^{AL}	-0.189	ns	-0.189
Shrubs^{T}	-0.142	0.125	-0.017

Shrubs^{DL}	-0.503	0.046	-0.457
Graminoids^{AH}	0.554	-0.003	0.551
Graminoids^{AL}	-0.181	ns	-0.181
Graminoids^{T}	-0.245	-0.101	-0.346
Graminoids^{DL}	-0.160	0.082	-0.078
Forbs^{AH}	ns	ns	ns
Forbs^{AL}	ns	ns	ns
Forbs^{T}	0.189	ns	0.189
Forbs^{DL}	ns	ns	ns
Bryophytes^{AH}	-0.174	-	-0.174
Bryophytes^{AL}	ns	-	ns
Bryophytes^{T}	-0.478	-	-0.478
Bryophytes^{DL}	-0.316	-	-0.316
Bare ground			
Soil moisture^{AH}	-	0.003	0.003
Soil moisture^{AL}	-	-0.001	-0.001
Soil moisture^{T}	-	-0.042	-0.042
Soil moisture^{DL}	-	0.013	0.013
Shrubs^{AH}	-0.42	-0.220	-0.640
Shrubs^{AL}	-0.285	0.015	-0.270
Shrubs^{T}	-0.52	0.310	-0.21
Shrubs^{DL}	-0.594	0.013	-0.581
Graminoids^{AH}	-0.594	0.066	-0.581
Graminoids^{AL}	ns	ns	ns
Graminoids^{T}	-0.104	-0.123	-0.227
Graminoids^{DL}	-0.246	0.023	-0.223
Forbs^{AH}	-0.021	ns	-0.021
Forbs^{AL}	-0.168	ns	-0.168
Forbs^{T}	-0.046	-0.067	-0.113
Forbs^{DL}	-0.009	ns	-0.009
Bryophytes^{AH}	-0.375	-0.023	-0.398
Bryophytes^{AL}	-0.144	ns	-0.144
Bryophytes^{T}	-0.990	0.171	-0.819
Bryophytes^{DL}	-0.087	ns	-0.087
Lichens^{AH}	0.133	-	0.133
Lichens^{AL}	ns	-	ns
Lichens^{T}	-0.357	-	-0.357
Lichens^{DL}	ns	-	ns
N_2-fixation			
Bryophytes^{AH}	0.015	ns	0.015
Bryophytes^{AL}	0.078	-0.048	0.030
Bryophytes^{T}	0.229	ns	0.229
Bryophytes^{DL}	0.343	ns	0.343
Lichens^{AH}	ns	ns	ns
Lichens^{AL}	ns	ns	ns

Lichens^{T}	ns	ns	ns
Lichens^{DL}	ns	ns	ns
Bare ground^{AH}	ns	-	ns
Bare ground^{AL}	0.335	-	0.335
Bare ground^{T}	ns	-	ns
Bare ground^{DL}	ns	-	ns

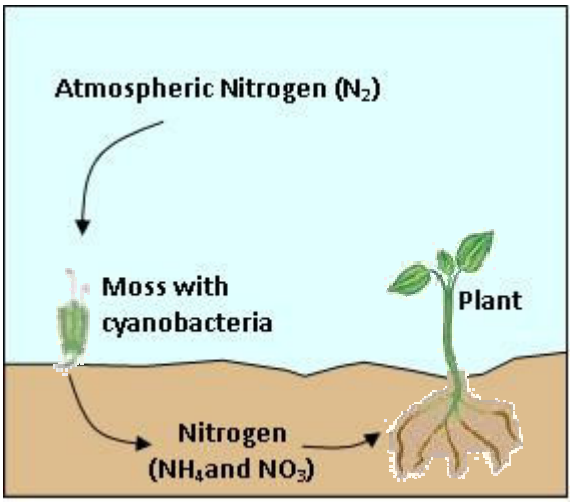
APPENDIX II

Nitrogen in the North

We often think of the Arctic as a cold, snow covered land, but did you know that during the growing season (June–August) the Arctic is full of colourful and interesting plants.

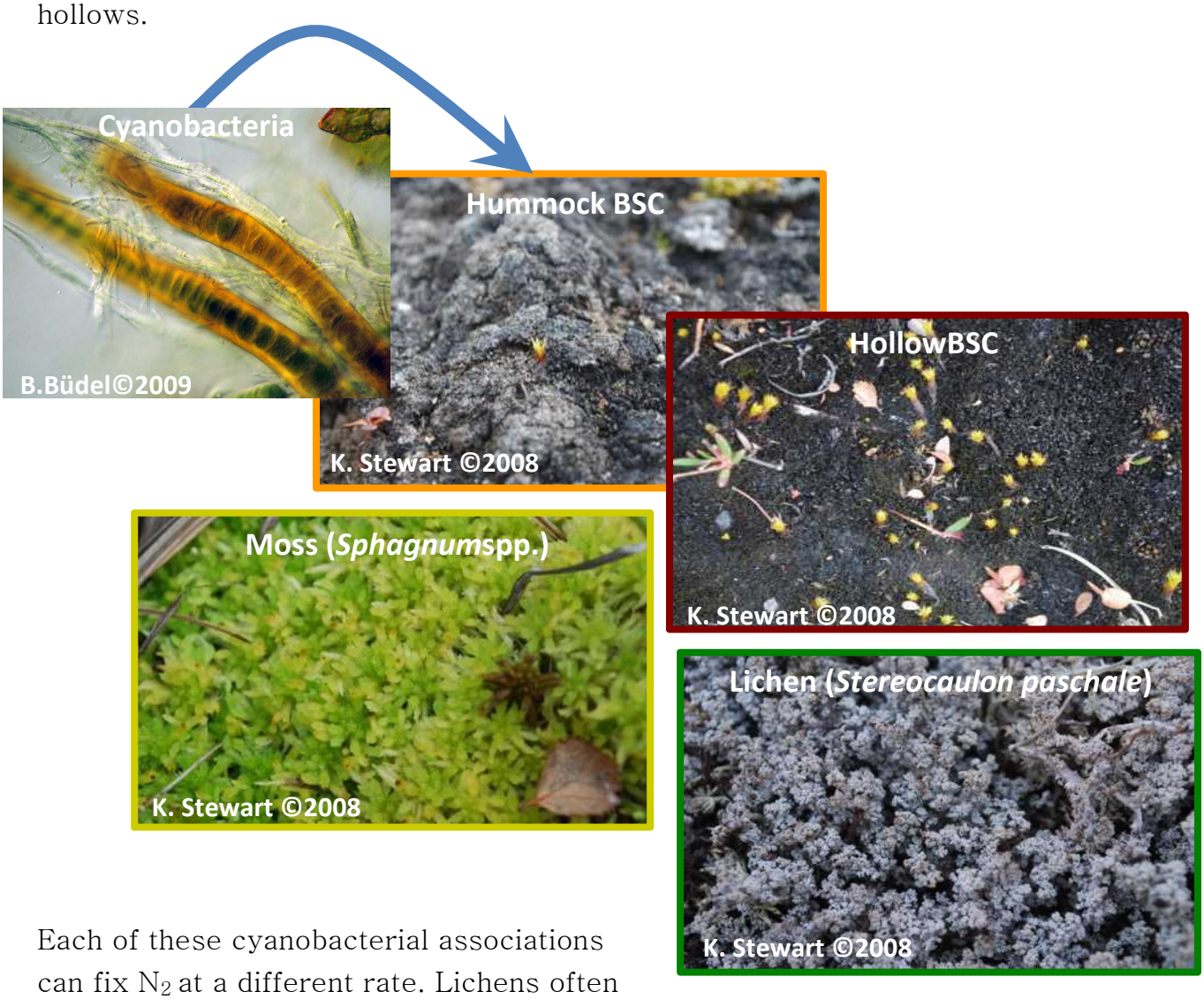
Arctic plants are often small, low to the ground and clumped, which helps them survive the cold and often dry environment in which they grow. Not only does the cold and dry environment make it hard for plants to grow, but their growth is also limited by low amounts of nitrogen in Arctic soils.

All plants need nitrogen to grow, which they take up through their roots. Most of the nitrogen on earth is found in the atmosphere. Nitrogen can get into the soil by weathering, wet deposition in rain or snow, dry deposition or by a process called biological nitrogen fixation. In the Arctic blue-green algae or cyanobacteria are everywhere and they perform biological nitrogen fixation, taking in nitrogen from the atmosphere that is later released into the soil.

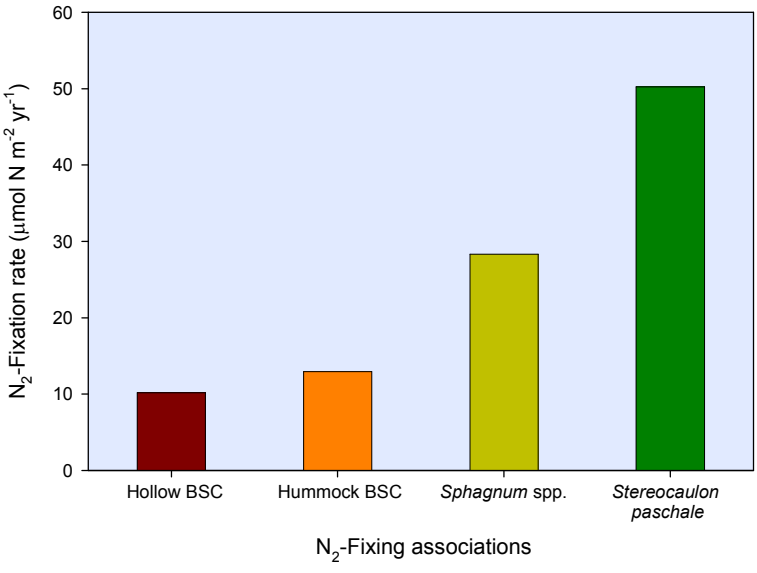


Cyanobacteria are found in combination or association with biological soil crusts (BSCs), mosses and lichens. BSCs are communities composed of

bacteria, cyanobacteria, algae, mosses, liverworts, fungi and lichens, and are found on soil mounds, or hummocks and in adjoining shallow depressions, or hollows.

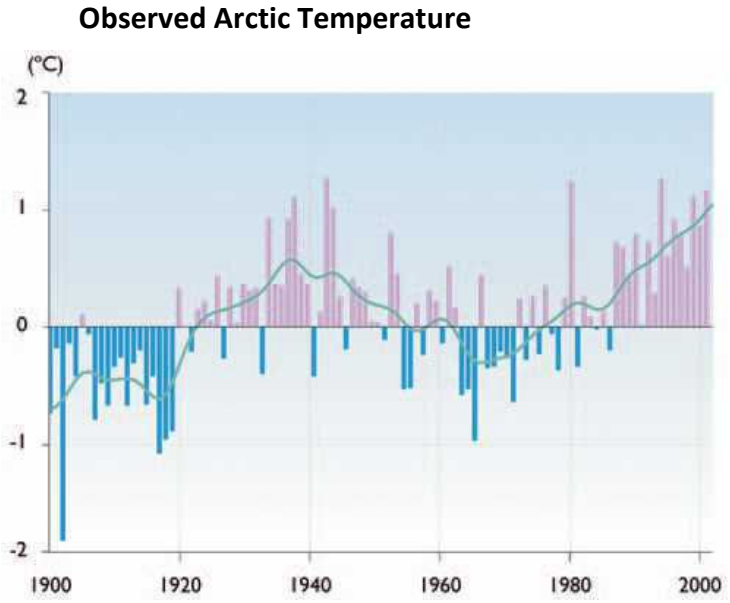


Each of these cyanobacterial associations can fix N_2 at a different rate. Lichens often fix N_2 at a higher rate, while BSCs fix N_2 at a lower rate.



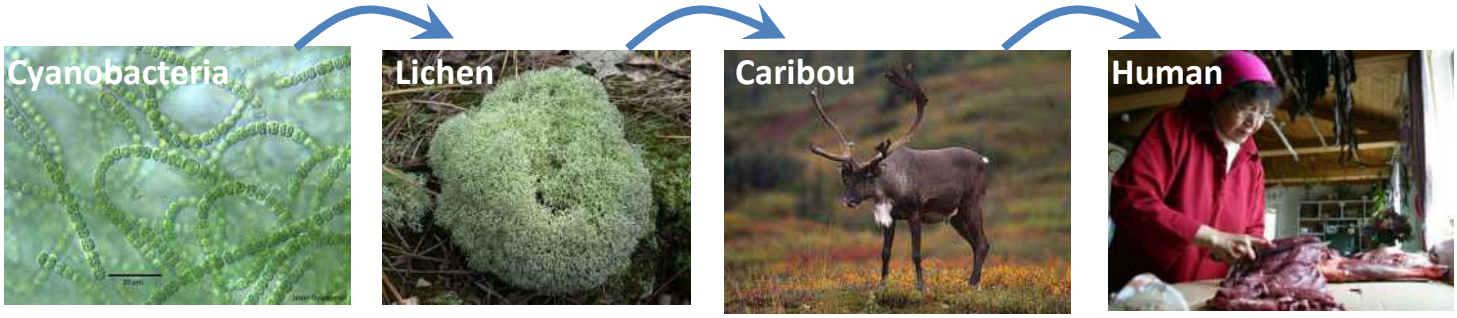
This graph shows the average N_2-fixation rate for the above cyanobacterial associations measured over the growing season 2007-2008 at Daring Lake NWT.

N_2-fixation is affected by the moisture, temperature and light conditions in the arctic environment. In general, higher rates of N_2-fixation occur under wet and warm conditions. Due to global climate change the environment in the Arctic is changing.



Annual average change in near surface air temperature from stations on land relative to the average for 1961-1990, for the region from 60 to 90°N. ©2004, ACIA

As the climate warms we might expect higher rates of N_2-fixation with warmer temperatures. Changes in the patterns of precipitation and soil moisture are a bit harder to predict, but we might also expect higher rates of N_2-fixation under wetter conditions. Scientists are still trying to understand how cyanobacterial associations will respond to a changing climate and how this might affect the amount of nitrogen available for arctic plants.



When we think about climate change we often think of big changes like melting ice caps, rising sea level and the extinction of polar bears. At the heart of many of the most important processes on earth, however, are the small things like cyanobacteria. So next time you think about climate change don't forget these tiny and magnificent organisms that help make everything grow.