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Nitrogen Inputs by Associative Cyanobacteria across a Low Arctic Tundra Landscape

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Abstract

Available soil N is a key factor limiting plant productivity in most low arctic terrestrial ecosystems. Atmospheric N_2-fixation by cyanobacteria is often the primary source of newly fixed N in these nutrient-poor 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 (3 June–13 September) 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.

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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 and Chapin, 1980; Nadelhoffer et al., 1992; Liengen and 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 and Schell, 1973; Schell and Alexander, 1973; Bazely and Jefferies, 1989; Gunther, 1989; Chapin and 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 and Lid-Torsvik, 1975; Chapin and 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 and 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 and 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 and 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 (Goetz et al., 2005; Sturm et al., 2001) may shade out lichens and possibly other N_2-fixing associations in mesic tundra. Evaluation of the

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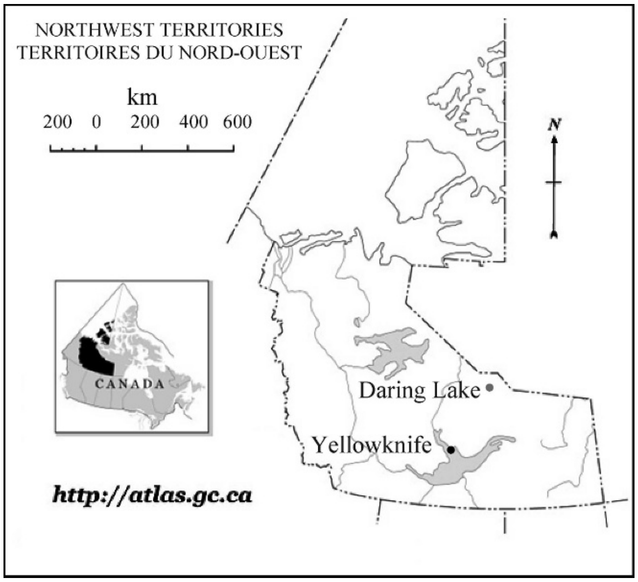

FIGURE 1. Location of the study site at Daring Lake, Northwest Territories, Canada (64°52′N, 111°35′W) (adapted from http://www.enr.gov.nt.ca/_live/pages/wpPages/Tundra_Ecosystem_Research_Station.aspx).

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; and (b) estimate N input via N_2-fixation over the growing season using microclimatically driven models based on incubation studies of the ecophysiological responses of individual N_2-fixing associations to moisture, temperature, and light conditions over the growing season.

Materials and 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 to 2 m (Obst, 2008).

Mean monthly air temperature is −30 °C in January and +13 °C in July (INAC, 2007; Obst, 2008). Mean monthly precipitation from May to October is 25 mm as rain. Snow accumulation is highly variable across the landscape, but usually ranges from 15 to 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 and Grogan, 2008; Lafleur and 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 1).

N_2-FIXING ASSOCIATIONS

Four predominant cyanobacterial associations were identified within the selected landscape study area at Daring Lake: Biological Soil Crusts (BSCs) in hollows, BSCs 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).

TABLE 1

The principal topographic position, substrate, drainage, and characteristic plant species for each ecosystem type included in the landscape study at Daring Lake, Northwest Territories, Canada (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.*

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 BSCs 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. *Stiognema turfaceum*, *S. minutum*, and *S. hormoides* (Kutz.) Born. & Flah. dominated Hummock BSCs; however, *Gloeocapsa decorticans* A. Braun., *G. novacekii* (Komárek & Anagnotid), *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, Ontario, Canada) fitted with a Porapak column (Alltech Canada, Guelph, Ontario, Canada) and a flame ionization detector. A Stand-Alone Hydrogen Generator (SRI H_2-50, Alltech Canada, Guelph, Ontario, 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, Ontario, Canada; C_2H_4, 98%+) that was kept at the same temperature as incubation gas samples.

Cores of Hollow BSCs ($n = 12$), Hummock BSCs ($n = 12$), 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 and 16:00 hr (6 hours) and nighttime ARA incubations between 21:00 and 7:00 hr (10 hours). A pilot study conducted in 2007 indicated net respiration in both Hollow BSCs (108 $\mu L\ L^{-1}\ CO_2$/hr) and Hummock BSCs (162 $\mu L\ L^{-1}\ CO_2$/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 nighttime 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 BSCs and Hummock BSCs were conducted on 19–24 June, 6–11 July, and 9–13 August in 2007; and on 12–17 June, 1–6 July, and 5–10 August in 2008. *Sphagnum* spp. were incubated for a 24 hr period over 5 consecutive days on 25–29 June, 9–13 July, and 17–22 August in 2007 due to logistical constraints. In 2008, *Sphagnum* spp. were incubated in the same manner as other N_2-fixing associations on 18–23 June, 7–12 July, and 17–22 August. *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 on 4–9 June, 7–12 July, and 11–16 August. Over the 2007–

2008 growing seasons a total of 571 (Hollow BSCs), 572 (Hummock BSCs), 794 (*Sphagnum* spp.), and 294 (*S. paschale*) individual 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 BSCs, Hummock BSCs, and *S. paschale* were monitored every 30 minutes during daytime ARA incubations. On average, the surface temperature of incubation samples were within 1.5 °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 nighttime incubations where microclimate was monitored for the first and last hour only. Moisture of Hollow BSCs, Hummock BSCs, 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 °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 and Granhall, 1978; Chapin et al., 1991; Liengen, 1999a), and light saturation was been demonstrated at ~100 µmol PAR m^{-2} s^{-1} (Coxson and 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 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 lab 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 48 hr 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, Massachusetts, U.S.A.; $^{15}N_2$, 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 48 hr 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, Saskatchewan, 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 of ^{15}N and the acetylene reduction rate. The amount of N fixed was calculated using (Liengen, 1999b, p. 224):

$$Y = \left(\frac{atm\%^{15}N_{excess}}{100}\right) \times \left(\frac{totalN_{sample} \times 10^9}{t \times 28}\right) \times \left(\frac{100}{\%^{15}N_{air}}\right), \quad (1)$$

where Y (nmol N·gdw^{-1}·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·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 (Table 2).

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, U.S.A.) installed at ground level in separate hummocks and hollows and connected to a multiplexer (AM416, Campbell Scientific Inc., Edmonton, Alberta, 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 BSCs 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 of 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.

TABLE 2

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

N_2-Fixing Association	Incubation condition	Mean Monthly N_2-Fixation rate (μmol N m^{-2} hr^{-1}) June	July	August	Conversion ratio C_2H_4/N_2
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)	

Modeling N_2-Fixation Potential

Models of N_2-fixation potential were determined 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 and 2008) for Hollow (n = 54) and Hummock BSCs (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 BSCs (r = 0.84) and Hummock BSCs (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 percent 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 °C), and shipped to the lab 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 and Kershaw, 1978). Therefore, a 24 hour lag was incorporated into the days since precipitation variable. The 24 hours following a precipitation event (≥1 mm) 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 (Fig. 2) and seasonal totals (Table 4). 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 °C, 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 °C. 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. 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 × 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 on its mean % cover

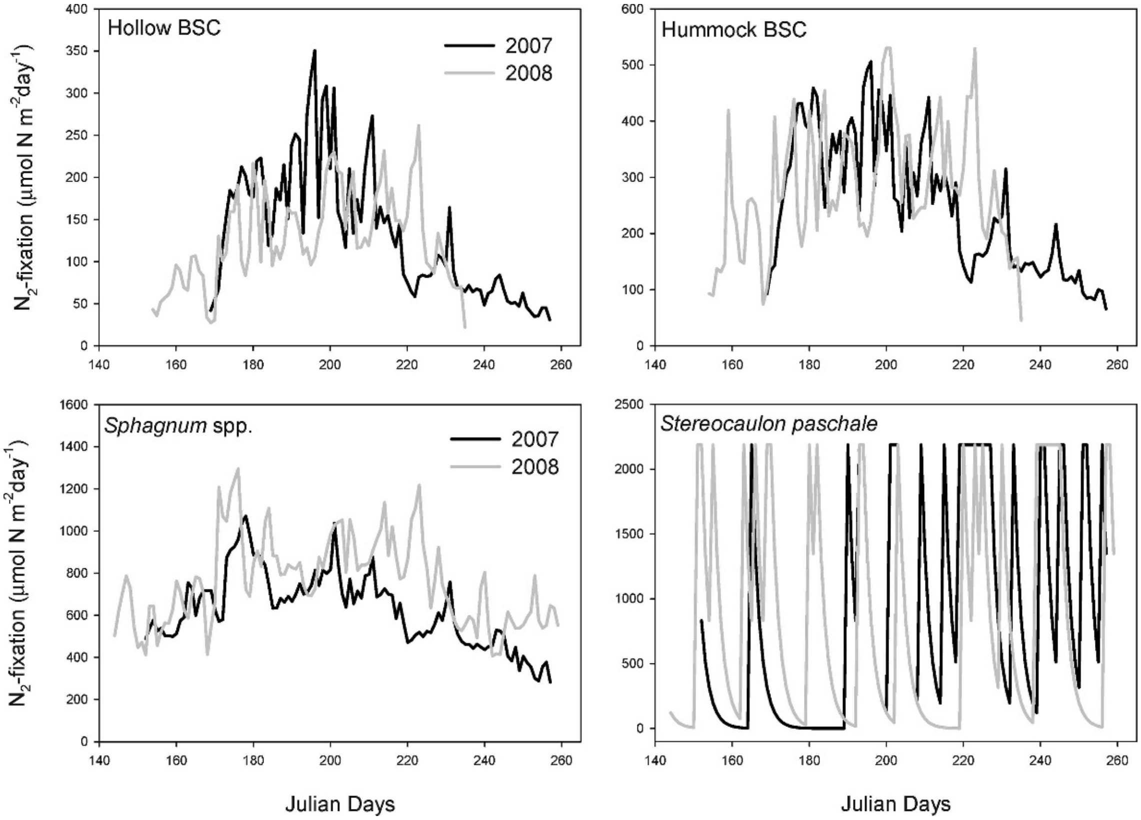

FIGURE 2. Seasonal trends in N_2-fixation rate (μmol N m^{-2} day^{-1}) estimated from potential N_2-fixation rate models and field environmental data records for each N_2-fixing association for 2007 and 2008 at Daring Lake, NWT. See Table 3 for further model details. ARA to N_2-fixation conversion ratios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

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 analyzed 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 2). 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 BSCs had lower rates in June compared with July and August, and *Sphagnum* spp. had lower rates in August compared with June and July.

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 2). The lowest rates under optimal conditions occurred in June for both Hollow BSCs and *S. paschale*, while the lowest rates occurred in August for Hummock BSCs.

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.

TABLE 3

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%)	$(0.07 \times T_{ho}) + 0.37$	25	80.39	0.77
	Low (<80%)	$(0.05 \times T_{ho}) + 0.69$	25	84.67	0.78
Hummock BSC	High (>35%)	$(0.05 \times T_{hu}) + 0.55$	22	28.05	0.56
	Low (<35%)	$(0.04 \times T_{hu}) + 0.60$	28	28.46	0.50
Sphagnum spp.	None	$(0.04 \times T_s) + 0.99$	14	34.68	0.72
Stereocaulon paschale	None	$(-0.21 \times D_{sp}) + 2.21$	23	49.46	0.69

Note: All models statistically significant, $P < 0.01$.

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 2).

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 3). 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 3). 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 3).

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. 2). 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. The model estimates of mean total N input across the growing season (3 June to 13 September) for each cyanobacterial association ranged from 3.4 kg N ha^{-1} yr^{-1} (Hollow BSCs) to 24.9 kg N ha^{-1} yr^{-1} (*S. paschale*) for a 103 day growing season (Table 4).

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/Heath-Mat Tundra followed by Hummock BSC (Table 5). *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. 3). *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. 3).

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 5). Our spatially explicit analyses indicate that this effect can be explained by particularly high inputs

TABLE 4

Mean total N fixed over the growing season (3 June to 13 September) based on estimates of N_2-fixation by Hollow BSC, Hummock BSC, *Sphagnum* spp., and *Stereocaulon paschale* at Daring Lake, Northwest Territories, in 2007 and 2008. Microclimatic models were used to predict hourly acetylene reduction rates per m^2 (See Table 3). ARA to N_2-fication conversion ratios (Table 2) 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 paschale	2007 (152–257)	3150	24.9
	2008 (144–259)	3204	

TABLE 5

The contributions of individual N_2-fixing associations to total biological 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, Northwest Territories, over the growing season (Julian days 154 to 257).

Ecosystem type	Total area (and proportion) of each ecosystem type within the study landscape (ha)	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 yr^{-1})	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 paschale	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 paschale	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 paschale	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 paschale	0.04	0.03	0.75		

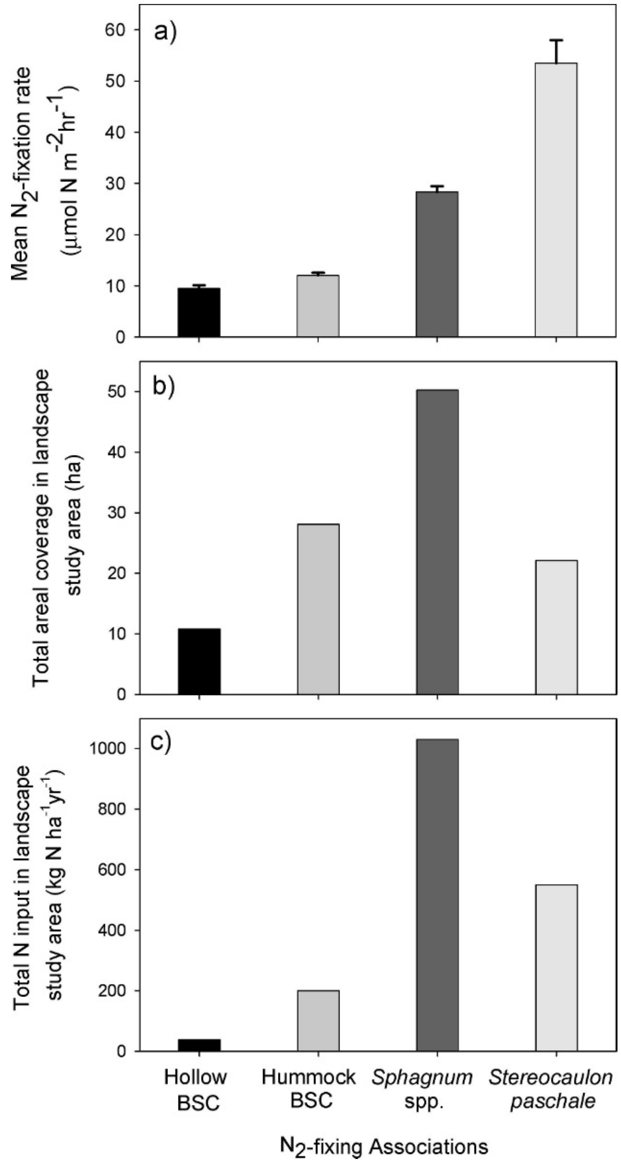

FIGURE 3. (a) Mean N_2-fixation rate measured over the growing seasons of 2007 and 2008 (see Table 2), (b) total area of N_2-fixing associations in landscape study area, and (c) total N input for Hollow biological soil crusts (BSCs), Hummock BSCs, *Sphagnum* spp., and *Stereocaulon paschale* determined by potential N_2-fixation models in the 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 5. Error bars in (a) indicate standard error.

by *Sphagnum* spp. cyanobacterial associations due to relatively large fixation rates (Table 4) in combination with relatively high proportional cover of this association in the Wet Sedge Meadow (Table 5). Heath-Lichen/Heath-Mat Tundra had relatively low N inputs per unit area but had 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}.

FIGURE 4. Hummock-hollow complexes at Daring Lake, NWT, 17 June 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.

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 multiscale 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 characterizing 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. 3). 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 and Schell, 1973; Henry and 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 and Bledsoe, 1992; Nash and 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 and 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 and 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 2). Mean Hollow BSC temperature in June was 6.8 °C compared to 9.1 °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. 4).

Some studies have found N_2-fixation to be light dependent (Granhall and Lid-Torsvik, 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 and Kershaw, 1983b; Smith, 1984; Chapin and Bledsoe, 1992; Nash and Olafsen, 1995; Zielke et al., 2002). Varying light conditions (0 to 1000 μmol PAR m^{-2} s^{-1}) did not 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 and Bledsoe, 1992). Nevertheless, remote sensing, repeat photography, and experimental nutrient addition studies all suggest that current warming trends in the low Arctic may be promoting shrub growth and expansion within various topographic positions (Goetz et al., 2005; Sturm et al., 2001;

Chapin et al., 1995). 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 and 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 ~1/300th the late summer rate of gross N mineralization 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 and 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 and Schell, 1973; Barsdate and Alexander, 1975; Chapin and Bledsoe, 1992; Hobara et al., 2006). Summertime mean atmospheric N inputs from wet deposition at the nearest monitoring station (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 and 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 and Bledsoe, 1992; Henry and 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 and Schell, 1973; Henry and 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 has 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 or nothing 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 favorable microclimatic conditions. Fourthly, our estimates of modeled 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 favor 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 favorable (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, our estimates of ecosystem N_2-fixation inputs and of total landscape-level N input over the growing season are minimum values.

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