Biogeochemistry (2017) 136:213–222
DOI 10.1007/s10533-017-0393-y

Nitrogen fixation in the High Arctic: a source of 'new' nitrogen?

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Received: 21 April 2017 / Accepted: 7 October 2017 / Published online: 23 October 2017

Abstract Biological nitrogen (N_2) fixation performed by diazotrophs (N_2 fixing bacteria) is thought to be one of the main sources of plant available N in pristine ecosystems like arctic tundra. However, direct evidence of a transfer of fixed N_2 to non-diazotroph associated plants is lacking to date. Here, we present results from an in situ ^{15}N–N_2 labelling study in the High Arctic. Three dominant vegetation types (organic crust composed of free-living cyanobacteria, mosses, cotton grass) were subjected to acetylene reduction assays (ARA) performed regularly throughout the growing season, as well as ^{15}N–N_2 incubations. The ^{15}N-label was followed into the dominant N_2 fixer associations, soil, soil microbial biomass and non-diazotroph associated plants three days and three weeks after labelling. Mosses contributed most to habitat N_2 fixation throughout the measuring campaigns, and N_2 fixation activity was highest at the beginning of the growing season in all plots. Fixed ^{15}N–N_2 became quickly (within 3 days) available to non-diazotroph associated plants in all investigated vegetation types, proving that N_2 fixation is an actual source of available N in pristine ecosystems.

Keywords Acetylene reduction · Arctic · Bryophytes · Nitrogen isotopes · Cyanobacteria · Nitrogen fixation · Stable isotopes · Tundra

Responsible Editor: Chris D. Evans.

Electronic supplementary material The online version of this article (doi:10.1007/s10533-017-0393-y) contains supplementary material, which is available to authorized users.

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Introduction

Nitrogen (N) is usually the limiting nutrient for plant growth. This is especially true in pristine ecosystems that receive low amounts of atmospheric N deposition like arctic and subarctic tundra. In arctic ecosystems, N_2 fixation performed by free-living N_2 fixing bacteria (diazotrophs) and by N_2 fixing cyanobacteria associated with mosses and lichens is a major source of plant available N (Stewart et al. 2011; Zielke et al. 2005; Rousk and Michelsen 2017). For instance, organic crusts composed of free-living cyanobacteria, mosses and lichens in the High Arctic can fix between 3 and 7 kg N ha^{-1} $year^{-1}$ (Stewart et al. 2011), and 1 kg N ha^{-1} $year^{-1}$ in subarctic tundra (Rousk et al. 2016). In wetlands, such as alluvial meadows in the

boreal biome, N_2 fixation by free-living cyanobacteria can be as high as 29 kg N ha^{-1} $year^{-1}$ (DeLuca et al. 2013). Nitrogen fixation rates by moss-associated cyanobacteria vary between 1 and 3 kg N ha^{-1} $year^{-1}$ in subarctic habitats (Rousk et al. 2015; Rousk and Michelsen 2017). In addition to their role as hosts of diazotrophs, mosses cover 50–100% in most subarctic and arctic ecosystems, contributing more than half to ecosystem productivity (Martin and Adamson 2001; see also Elbert et al. 2012) and exert significant controls over soil temperature and moisture (e.g. Gornall et al. 2007; Turetsky et al. 2012), highlighting their importance for these ecosystems.

While N_2 fixation rates in different N_2 fixer associations can be high, how much of the fixed N_2 is actually available to non-diazotroph associated plants in High Arctic settings remains unknown. Thus, the role of N_2 fixation as a source of new N for the ecosystem N pool is ambiguous. For instance, mosses retain acquired N for several months, if not years (e.g. Rousk et al. 2014), and hardly any fixed N_2 is transferred from mosses to the underlying soil and other ecosystem components in subarctic tundra within 5 weeks (Rousk et al. 2016). On the other hand, fixed N_2 can be released from free-living cyanobacteria or crusts in other ecosystems within days or weeks, thereby becoming available to the ecosystem N pool (Belnap 2001; Rousk et al. 2016). This warrants the question if and which diazotroph associations are short-term sources of 'new' N in High Arctic tundra and hence, slowly leak a fraction of the fixed N_2 within days or weeks, or rather keep the fixed N_2 over longer time periods, hence serving mainly as sinks of N during summer. To elucidate this question, we conducted a field experiment in which we measured N_2 fixation throughout the growing season using the acetylene reduction assay (ARA) in situ in High Arctic tundra. We measured N_2 fixation in three land cover types typical for the Arctic: organic crust, a dense moss surface and a water logged fen dominated by cotton grass and mosses. We further performed a ^{15}N–N_2 labelling experiment in situ in the same vegetation types and followed the ^{15}N label into N_2 fixer associations, plants not associated with diazotrophs, as well as into soil and soil microbes. We hypothesized that fixed ^{15}N–N_2 becomes available to other ecosystem components within several weeks in sites dominated by free-living cyanobacteria, while no transfer to any other ecosystem component occurs in moss dominated sites within the same time frame.

Materials and methods

Site description

The experimental site is located in a dry heath at Zackenberg, Greenland, near the Zackenberg Research Station (74°30′N, 21°00′W). The climate is continental High Arctic with an average air temperature of −20, 7.0 and −9.0 °C in January, July and whole year, respectively. The total annual precipitation is 211 mm, ranging between 93 and 310 mm (data 1997–2014; Jensen et al. 2016), with the most precipitation falling as snow (see also Online Resource 1, 2). The bedrock is characterized by non-calcareous sandy fluvial sediments, and the soil is classified as a Typic Psammoturbels with a pH between 5 and 7. The permafrost is continuous and the active layer is less than 1 m deep.

Vegetation types and plot selection

The labelling experiment was established in plots with three common arctic vegetation types within an overall area of 50 m × 50 m: (1) organic crust, (2) a moss dominated snow bed with *Scorpidium cossonii*, *Polytrichum piliferum* and *Ditrichum sp*., (3) a cotton grass (*Eriophorum angustifolium*) fen dominated by graminoids, mosses (*Aulacomnium turgidum* and *Scorpidium cossonii*, with some *Polytrichum piliferum* and *Dicranum sp.*) and the liverwort *Ptilidium ciliare* (Fig. 1; Online Resource 3). Six replicate plots of 0.5 m × 0.5 m were randomly selected within each vegetation type and were at least 0.5 m apart from each other. The three vegetation types were subjected to N_2 fixation measurements using the acetylene reduction assay as in Rousk et al. (2015) and ^{15}N labelling as in Rousk et al. (2016) (see below).

Vegetation ground cover

The vegetation cover in each plot was estimated on 23 July 2010 in ten randomly placed 0.5 m × 0.5 m frames in each of the three vegetation types, hence a total of 30 frames were analyzed. In each frame, the presence of each species (vascular plant species,

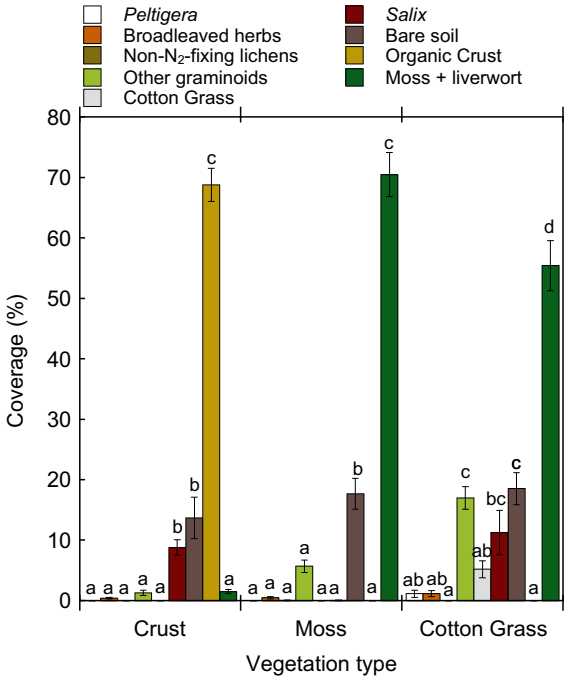


Fig. 1 Vegetation coverage in the three investigated vegetation types (crust, moss, cotton grass) in the High Arctic. Given are mean ± SE (n = 10). Within vegetation types, different lower case letters indicate significant differences between the ecosystem components

mosses and lichens), and bare soil and cryptogamic crust was noted in 100 points (2 cm spaced).

Acetylene reduction assay

Nitrogen fixation was assessed using the acetylene reduction assay (ARA) in situ in the three vegetation types described above. ARA was performed six times throughout the main growing season in the High Arctic (16th of July until the 7th of August). For this, we used cylindrical cores of each of the vegetation types (5 cm diameter × 7 cm height), placed them into nylon meshbags (50 nm mesh size, Sintab, Oxie, Sweden), and put them carefully back from where the cores originated when not performing the assay. For the ARAs, the nylon meshbags with the cores were placed into transparent plastic containers with a volume of 0.5 l. The cores were weighed prior to ARA to obtain the soil moisture content, and the soil temperature at 2 cm depth was measured (Online Resource 4). The same core was used in each of the six measurement rounds. Calcium carbide (0.175 g) was placed in a 20 ml vial in the container, and 10 ml water was added with a syringe through a rubber septum. This resulted in the development of 10% (vol.) acetylene in the containers. A gas sample of 20 ml was taken after 1 min and after 2 h and 1 ml was analysed for acetylene and ethylene on a gas chromatograph (Shimadzu GC-14B, Tokyo, Japan). To estimate N_2 fixation at habitat level, we divided the acetylene reduction rates by the ground cover of the respective N_2 fixer association in the cores obtained by a visual estimation and multiplied this by their cover in the different habitats.

In situ ^{15}N labelling and harvest

To investigate the short-term fate of fixed N_2 in High Arctic tundra, we used in situ ^{15}N–N_2 pulse labelling the 15th of July 2010 in the vegetation types described above, and followed the label into different pools of the ecosystem. In each of the three vegetation types, 12 carefully cut mesocosm cores containing the intact vegetation and 2–3 cm soil were placed in transparent polycarbonate containers, and were kept in the hole from where the plant-soil mesocosms originated. The containers had a diameter of 10 cm, and a volume of 0.5 l or in a few instances 1 l, depending on the plant biomass in the cores. Fourty milliliter (or 80 ml for the 1 l containers) air was taken from the headspace of the containers and replaced with 40 ml ^{15}N–N_2 (98% enriched) (or 80 ml for the 1 l containers). The mesocosms were incubated for 24 h in the field, after which the lids were removed until harvest. Three days (18th of July 2010) and 3 weeks (4th of August 2010) after the pulse labelling, six cores per vegetation type and sampling date were harvested by separating aboveground plant parts from the soil. Aboveground parts were carefully sorted into different species, dried and weighed. In addition, the soils were sampled and roots separated by hand sorting. In the organic crust vegetation type, crust was separated from soil. The soil was homogenized and subjected to chloroform-fumigation extraction and analysis as in Larsen et al. (2012). In brief, 10 g of the sorted, fresh soil was fumigated with chloroform for 24 h to release N and C in the soil microbial biomass, after which the soil was extracted for 1 h in 50 ml demineralized H_2O. The extracts were filtered through Whatman GF-D filters and frozen until analyses. Another 10 g fresh soil was treated as above, but without the chloroform

fumigation to recover total dissolved N (TDN) and dissolved organic carbon (DOC). To obtain microbial N, 5 ml of fumigated and unfumigated, demineralized H_2O-extracts were digested in persulphate and analysed by Fiastar 5000 Flow Analyzer. Microbial N and C contents were calculated by subtracting the N and C in digested, unfumigated extracts from that in the digested, fumigated extracts. The microbial N and C content were calculated assuming an extractability of 0.4 for N and 0.45 for C.

All plant and soil material was dried, milled and analysed for $^{15}N/^{14}N$ and $^{13}C/^{12}C$ as well as for total nitrogen and carbon content using an Isoprime isotope ratio mass spectrometer (Isoprime Ltd., UK) coupled to an Eurovector CN analyzer. The amount of ^{15}N in the microbial biomass was analysed by IRMS after freeze-drying the fumigated and non-fumigated extracts. Plant and soil samples for analyses of ^{15}N natural abundances were collected in separate, non-$^{15}N_2$ labelled plots 8 days after the first harvest (26th of July 2010) to calculate ^{15}N enrichment and ^{15}N recovery (% of added label that potentially could be fixed). Recovery of ^{15}N includes fixation and recovery of the label.

Statistical analyses

To test for differences in vegetation coverage between the vegetation types and the plant functional groups/species, we ran 2-way ANOVAs with vegetation type and plant functional group/species as factors. We ran 1-way ANOVAs to test for differences between N_2 fixation on vegetation cover basis, and repeated measures ANOVA for the change in N_2 fixation through the growing season. Three-way ANOVAs were run to test for differences in ^{15}N enrichment, ^{15}N recovery, soil and plant measures between the vegetation types, individual ecosystem components within each vegetation type, and time (3 days after labelling, July, and 3 weeks after labelling, August). All ANOVAs were followed by Tukey's posthoc test. All analyses were performed with R 3.0.3. (R Development Core Team 2014).

Results

Vegetation cover

The overall vegetation coverage was higher in the cotton grass than in the crust and moss plots ($p = 0.016$; $F_{2,242} = 4.20$). Organic crust covered most of the ground in the crust plots, and mosses covered most in the moss and cotton grass plots ($p < 0.0001$; $F_{16,242} = 100.73$; Fig. 1).

Acetylene reduction assay

Mosses had the highest acetylene reduction rates when accounted for groundcover ($p = 0.023$; $F_{2,15} = 4.89$; inset Fig. 2), as well as throughout the measuring period ($p < 0.0001$; $F_{10,89} = 7.10$), and ARA decreased with progressing growing season in all vegetation types ($p < 0.0001$; $F_{5,89} = 10.75$; Fig. 2).

^{15}N enrichment in the ecosystem components

Enrichment of ^{15}N differed significantly between the different ecosystem components in each vegetation

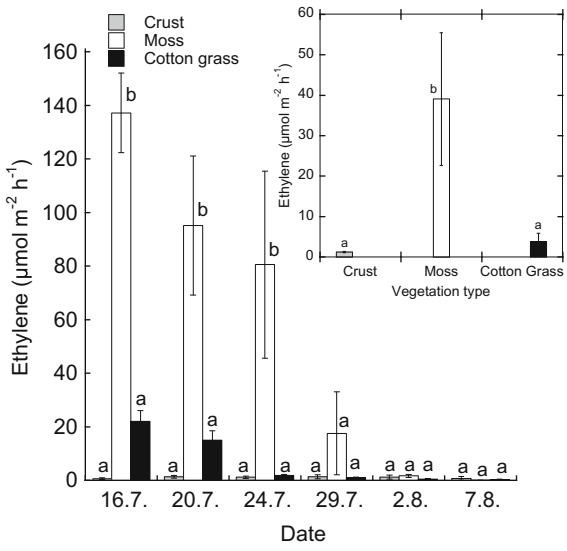


Fig. 2 Acetylene reduction (µmol m^{-2} h^{-1}) measured in three vegetation types throughout the growing season in the High Arctic. The inset shows the mean acetylene reduction rates across the measurement campaigns accounted for the groundcover of the respective N_2 fixer association. Given are mean ± SE (n = 6) for both figures. Different lower case letters indicate significant differences between the vegetation types

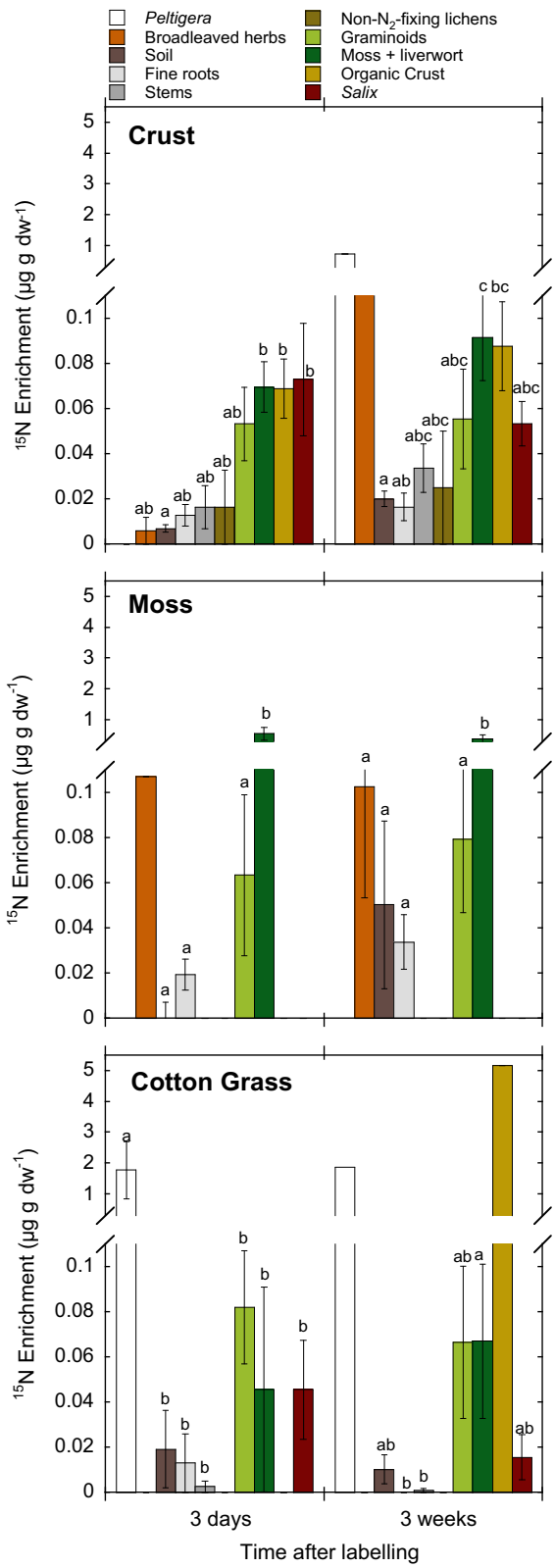


Fig. 3 ^{15}N enrichment (µg ^{15}N g dw^{-1}) in the ecosystem components in the three investigated vegetation types (crust, moss, cotton grass) 3 days and 3 weeks after ^{15}N labelling in the High Arctic. Given are mean ± SE (n = 6). Different lower case letters indicate significant differences between the ecosystem components. In case the ecosystem component was absent, no letters are given

type ($p_{crust,3d.} = 0.001$; $F_{9,33} = 4.23$, $p_{crust,3weeks} < 0.0001$; $F_{9,32} = 41.32$, $p_{moss,3days} = 0.008$; $F_{4,20} = 4.64$, $p_{moss,3weeks} = 0.004$; $F_{4,22} = 5.28$, $p_{cottongrass,3days} = 0.005$; $F_{7,33} = 3.68$; $p_{cotton-grass,3weeks.} = 0.02$; $F_{5,30} = 5.3$; time n.s.; Fig. 3; Online Resource 5). Further, graminoids and mosses were ^{15}N enriched in all three vegetation types only 3 days after labelling. While ^{15}N enrichment in the crust vegetation type was found in all ecosystem components at both sampling times, the enrichment pattern in the moss vegetation type changed between the sampling campaigns, albeit not significant. For instance, 3 weeks after labelling, high ^{15}N enrichment was found in broadleaved herbs and soils, which were not enriched 3 days after labelling. However, the distribution of ^{15}N enrichment of individual ecosystem components did not differ significantly between the sampling dates. Enrichment of the microbial biomass by ^{15}N was lowest in the moss plots ($p < 0.008$; $F_{2,30} = 5.72$; Fig. 4), and ^{15}N recovery in the microbial biomass was lower in the moss than in the cotton grass vegetation type ($p = 0.02$; $F_{2,30} = 4.7$; Table 1). No differences in ^{15}N enrichment or ^{15}N recovery in the microbial biomass between the sampling campaigns were found.

Total N_2 fixed in the three vegetation types summed up to 0.30 ± 0.03; 1.26 ± 0.45; 0.31 ± 0.13 kg N ha^{-2} for the crust, moss and cotton grass, respectively, calculated for a 3 months period, reflecting the length of the growing season. These numbers are based on the ^{15}N recovery data 3 days after labelling, multiplied by 90 days.

^{15}N recovery in the ecosystem components

Between vegetation types, recovery of ^{15}N was higher in the moss than in the crust and cotton grass vegetation types ($p < 0.0001$; $F_{2,265} = 13.56$; Fig. 5; Online Resource 6). Within vegetation types, the highest ^{15}N recovery was found in the organic crust in

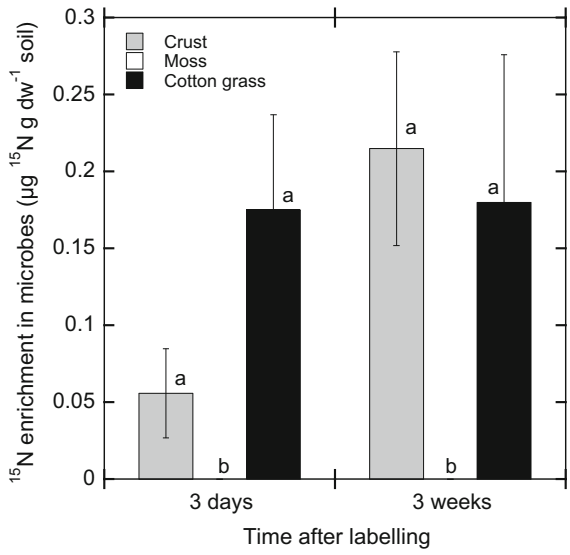


Fig. 4 ^{15}N enrichment (µg ^{15}N g dw^{-1}) in the microbial biomass in the three investigated vegetation types 3 days and 3 weeks after labelling. Given are mean ± SE (n = 6). Different lower case letters indicate significant differences between the vegetation types

the crust vegetation type and in the moss in the moss vegetation type. In the cotton grass vegetation type, no significant differences were found in ^{15}N recovery between the different ecosystem components. Recovery in the soil was similar to recovery in other ecosystem components such as graminoids, fine roots and moss in the crust vegetation type, already three days after labelling. Soils in the moss plots were the second largest pool of recovered ^{15}N. No differences were found in ^{15}N recovery between the sampling times (Fig. 5). Total ^{15}N recovery after 3 days was 0.79 ± 0.1, 3.3 ± 1.2 and 0.82 ± 0.3, and 0.82 ± 0.06, 4.8 ± 0.9 and 0.75 ± 0.1% ^{15}N recovered after 3 weeks for the crust, moss and cotton grass vegetation type, respectively.

Plant, soil and microbial measures

Significant differences in soil TN and C/N ratios between crust, moss and cotton grass plots were found, with generally higher soil N content in the cotton grass plots ($p < 0.0001$; $F_{3,73} = 12.90$; Table 1), and also higher N concentration (%) here in many of the different ecosystem components as fine roots and moss ($p < 0.0001$; $F_{9,89} = 7.16$), and overall higher N concentration in the cotton grass vegetation type ($p < 0.0001$; $F_{2,109} = 13.28$; Online Resource 7). Other microbial and soil measures (TN and TC in microbes, TDN, DOC) were also different between the vegetation types, with the highest C and N availability in the cotton grass and lowest in the crust vegetation type (all $p < 0.001$; Table 1).

Discussion

Importance of N_2 fixation as a source of 'new' N in the High Arctic

Our results stand in sharp contrast to our hypothesis and to the so far only reported study in arctic or subarctic settings on the transfer of fixed N_2 from different N_2 fixer associations to the rest of the ecosystem (Rousk et al. 2016). This previous study in subarctic tundra did not find any evidence for a transfer of fixed N_2 from mosses (and lichens) to any other ecosystem component within 5 weeks. In our study, we found a very different pattern: quick transfer of fixed N_2 to other ecosystem components in all investigated vegetation types. This hints to significant differences in N utilization strategies by plants between high and subarctic ecosystems. Plants in the High Arctic have to make quick and efficient use of the little N that is available during the 2–3 months growing season (Billings and Mooney 1968; Bliss 1971; Arndal et al. 2009). Alternatively, differences in environmental conditions during the experimental period such as rainfall and dry episodes, may lead to differences in nutrient transfer between ecosystem components.

The almost immediate enrichment and recovery of ^{15}N in the soil microbial biomass in the crust vegetation type is in line with previous studies (Belnap 2001), as well as the finding that hardly any ^{15}N was recovered in soil microbes in the moss plots (Rousk et al. 2016). However, despite a dominance of mosses in the cotton grass plots, ^{15}N recovery in soil microbes was higher here than in the moss plots, and similar to recovery in the crust plots. This indicates that either different moss species may differ in their N release, or that the very moist conditions in the cotton grass plots (Online Resource 4) might have created conditions in which nutrients are easier leached from moss and/or the N_2 fixing lichen *Peltigera* to soil, and hence more readily available.

Table 1 Selected soil and plant characteristics in the ^{15}N field labelling experimental plots in High Arctic tundra, Greenland

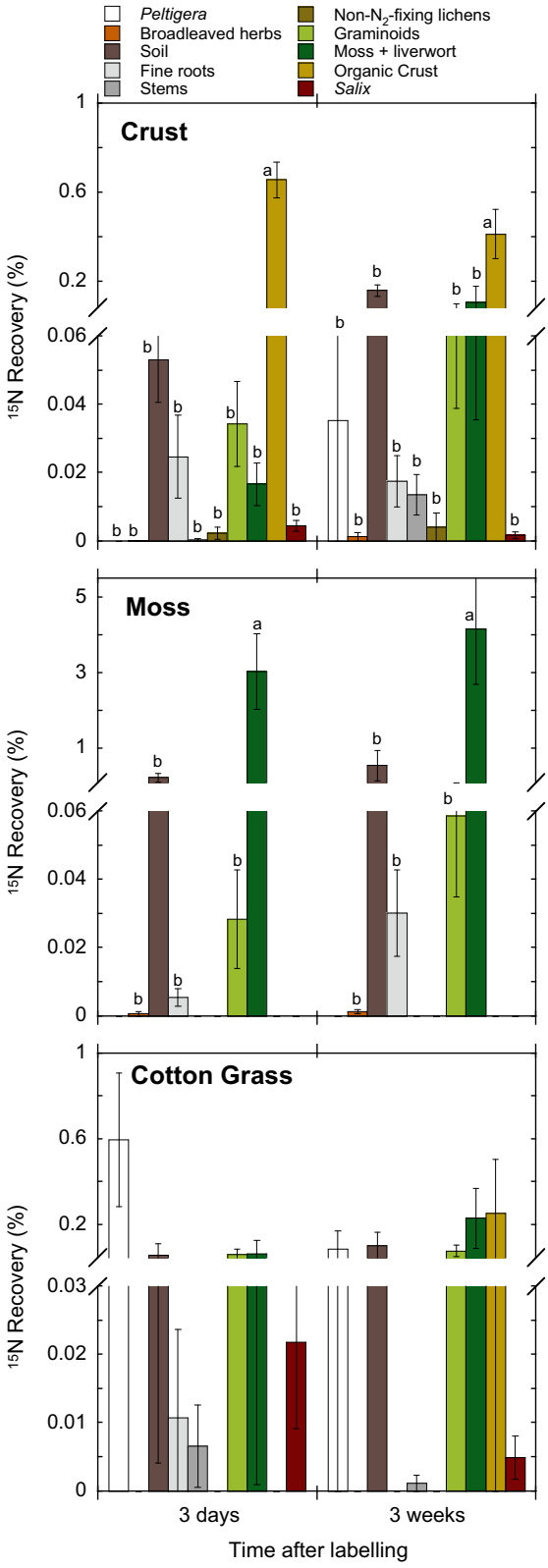
	Crust		Moss		Cotton grass	
	3 days	3 weeks	3 days	3 weeks	3 days	3 weeks
TN crust	3.8 ± 0.4a	5.6 ± 0.7b	NA	NA	NA	NA
TN soil	1.6 ± 0.2a	2.3 ± 0.2a	5.1 ± 0.9b	4.6 ± 1.0b	15.2 ± 2.9c	8.5 ± 0.5c
TN moss	5.6 ± 0.7	7.5 ± 1.1	8.9 ± 1.5	6.9 ± 0.9	7.5 ± 0.4	9.1 ± 0.8
TN *Peltigera*	NA	12.18	NA	NA	7.1 ± 0.3A	18.9 ± 1.2B
C:N crust	21.7 ± 0.3A	26.5 ± 0.3B	NA	NA	NA	NA
C:N soil	18.8 ± 0.6a	19.3 ± 0.4a	17.1 ± 1.1aA	24.6 ± 1.9aB	47.2 ± 6.2b	45.6 ± 5.7b
C:N moss	31.7 ± 2.4a	35.8 ± 2.2a	26.7 ± 1.2a	33.9 ± 2.1a	53.4 ± 2.5b	45.8 ± 4.3b
C:N microbes	12.1 ± 0.5	10.4 ± 0.4	13.2 ± 1.3	12.6 ± 1.3	12.8 ± 0.9	13.4 ± 1.2
TN microbes	0.02 ± 0.007a	0.02 ± 0.01a	0.08 ± 0.009b	0.04 ± 0.02b	2.5 ± 0.2c	2.7 ± 0.2c
TC microbes	0.21 ± 0.07a	0.22 ± 0.1a	0.91 ± 0.08a	0.51 ± 0.2a	28.8 ± 3.1b	32.1 ± 2.5b
DOC	0.05 ± 0.004a	0.08 ± 0.03a	0.05 ± 0.01a	0.06 ± 0.01a	0.34 ± 0.07b	0.45 ± 0.09b
TDN	0.01 ± 0.002a	0.02 ± 0.004a	0.04 ± 0.004a	0.02 ± 0.007a	1.0 ± 0.07b	1.1 ± 0.1b
^{15}N recovery microbes	0.007 ± 0.005ab	0.03 ± 0.01ab	0b	0b	0.02 ± 0.01a	0.02 ± 0.01a

The labelling was performed in three vegetation types: organic crust ("Crust"), moss and cotton grass. Given are mean ± SE (n = 6). Lower case letters indicate significant differences between the vegetation types (within sampling times), and upper case letters indicate significant differences between the sampling times, within vegetation types ($P < 0.05$)

TN Total nitrogen (mg g dw^{-1}), *TC* Total carbon (mg g dw^{-1}), *DOC* Dissolved organic carbon (mg g soil dw^{-1}), *TDN* Total dissolved nitrogen (mg g soil dw^{-1}), *NA* Ecosystem component not present

The ^{15}N recovery data of the ecosystem components revealed a different pattern than the enrichment data. For instance, soil, crust and moss had high ^{15}N recovery due to high mass, while ^{15}N enrichment was low in e.g. soil and higher in *Salix* (Figs. 3 vs. 5). However, the overall conclusions also hold for the recovery data: a quick transfer of fixed ^{15}N from all investigated N_2 fixer associations to other ecosystems components, including soil. The recovery of ^{15}N reflects N transformation processes at habitat level by including N pools of the ecosystem components and N transfers subsequent to $^{15}N_2$ fixation. Our ^{15}N recovery data (mean ± SE: 0.79 ± 0.1, 3.3 ± 1.2, 0.82 ± 0.3% ^{15}N recovered 3 days after labelling for crust, moss and cotton grass vegetation type, respectively) are higher than ^{15}N recovery reported in Rousk et al. 2016 (0.002–0.014% ^{15}N recovered 3 days after labelling). In this previous report from the Subarctic, no ^{15}N was recovered in soil under organic crust and moss 3 days after labelling, while our results from the Arctic suggest transfer of fixed ^{15}N from organic crust and mosses to soil. However, we cannot exclude that free-living diazotrophs in soil contributed to habitat N_2 fixation. Thus, ^{15}N recovery in soil may be a combination of transfer of fixed ^{15}N from moss, organic crust and lichen to the soil, and heterotrophic N_2 fixation in soil. Yet, previous assessments show only very little N_2 fixation in soil-only plots from the Arctic, and those rates are close to detection limit and negligible compared to N_2 fixation rates measured in e.g. moss (K Rousk, unpublished data).

The high contribution to habitat N_2 fixation by cyanobacteria associated with mosses (Fig. 2), confirms previous studies from the High Arctic (Solheim et al. 1996; Stewart et al. 2011). Along those lines, ^{15}N enrichment in the compartments of the cotton grass vegetation type was often higher compared to that in the moss and crust vegetation types, for instance in the lichen *Peltigera* and organic crust (Fig. 3), whereas overall ^{15}N recovery was highest in the moss plots, driven by high recovery in the moss (Fig. 5), showing that individual N_2 fixer associations can contribute significantly to habitat N_2 fixation by maintaining high nitrogenase activity (see e.g. Hobara et al. 2006; Stewart et al. 2011). Localized high N_2 fixation rates can therefore contribute to the patchiness of N availability in arctic landscapes, resulting in specific distribution and occurrence of plant communities



◀**Fig. 5** ^{15}N recovery (% of added label) in the ecosystem components in the three investigated vegetation types (crust, moss, cotton grass) 3 days and 3 weeks after ^{15}N labelling in the High Arctic. Given are mean ± SE (n = 6). Different lower case letters indicate significant differences between the ecosystem components, within sampling periods. In case the ecosystem component was absent, no letters are given. Please note the different y-axes between the habitat types

(Stewart et al. 2014). All soil chemical parameters in the cotton grass plots were several folds higher than in the other vegetation types (Table 1), likely due to downslope movement of water and nutrient to the wetter cotton grass areas, further confirming the large spatial variation in soil nutrients within a small area in the High Arctic.

The moss vegetation type had the highest acetylene reduction activity at habitat level (Fig. 2), while ^{15}N enrichment was higher in the cotton grass vegetation type (Fig. 3). However, while ARA assesses the nitrogenase enzyme activity, ^{15}N enrichment both indicates incorporation and subsequent turnover of the added ^{15}N-N_2, and does not account for biomass. Yet, total plot ^{15}N recovery is representative of habitat N_2 fixation and was highest in the moss vegetation type, reflecting our ARA measurements.

The ^{15}N enrichment and recovery in graminoids only 3 days after labelling could either hint to a quick transfer of fixed N_2 from diazotrophs, or to diazotrophs that live epiphytically on the graminoids themselves. Graminoid associated diazotrophs have been documented previously in High Arctic ecosystems (Henry and Svoboda 1986; Solheim et al. 1996) and should not be disregarded as potential N sources. The ^{15}N enrichment in *Salix arctica* could be the result of acquired N_2 fixed by free-living diazotrophs being active in the rhizosphere of *S. arctica* or in organic crusts. Nitrogen uptake from soil can differ significantly between plant functional types in the High Arctic. For instance, the dwarf shrub *Salix polaris* has been found to recover most of added ^{15}N ($^{15}NH_4Cl$) from shallow soil depths (3 cm) compared to graminoids, which could take up N from deeper soil depths (3-30 cm) (Oulehle et al. 2016). Thus, *Salix* shrubs may preferably take up N that is available in the first few centimetres along the soil profile that has been previously fixed by diazotrophs, while other vascular plants may have different N uptake strategies such as

hosting diazotrophs, and investing in an extended root system for increased soil N uptake.

Nitrogen fixation rates

Previous N_2 fixation estimates for High Arctic ecosystems range from e.g. 0.73 kg N ha^{-1} $year^{-1}$ in a dry heath to 10.89 kg N ha^{-1} $year^{-1}$ in a wet sedge meadow, calculated for a 103 day long growing season (Stewart et al. 2011). At N_2 fixer level, *Sphagnum* mosses (*S. aongstremii* (C. Hartm.) and *S. subsecundum* complex) and the lichen *Stereocaulon paschale* fixed more than 20 kg N ha^{-1} $year^{-1}$; and wet crust fixed more than twice as much as dry crust (7.1 vs. 3.4 kg N ha^{-1} $year^{-1}$). Those estimates are based on conversion ratios between ARA and $^{15}N_2$ fixation, which ranged in that study from 0.85 to 3.49 (Stewart et al. 2011). The conversion ratios are strongly dependent on nutrient availability (Rousk et al. 2017), and can therefore vary not only between diazotrophs, but also between measuring campaigns. The use of different conversion ratios could explain the large differences in estimated N_2 fixation rates between studies and sites.

Most estimates of N_2 fixation rates for the Arctic are scaled up to the entire year (despite a period of temperatures above 0 °C of only 3 months), which would translate to 1.2 in the crust, 5.0 kg in the moss, and 1.2 kg N ha^{-1} in the cotton grass vegetation type in our study. Further, we measured N_2 fixation 3 days after ^{15}N labelling during which fixed N_2 might have been lost already via leaching or increased microbial activity. Gaseous loss via denitrification is unlikely given the limited availability of inorganic N in these ecosystems. Our estimates highlight the importance of N_2 fixation as a main source of 'new' N for these ecosystems considering other potential pathways of N input. Atmospheric N deposition in these systems is below 2 kg N ha^{-1} $year^{-1}$ (Van Cleve and Alexander 1981), and soil N mineralization rates are between 0.1 and 0.2 g N m^{-2} per growing season (e.g. Schmidt et al. 2002; Oulehle et al. 2016). Thus, N_2 fixation contributes at least as much to total ecosystem N input as N deposition and N mineralization.

Our N_2 fixation estimates (1.2–5.0 kg N ha^{-2} - $year^{-1}$) are comparable to rates previously reported from the High Arctic (0.65–10.89 kg ha^{-1} $year^{-1}$; Henry and Svoboda 1986; Hobara et al. 2006; Stewart et al. 2011). The large range of reported rates may be due to the strong dependence of N_2 fixation on past and prevailing moisture and temperature conditions (Rousk et al. 2015; Rousk and Michelsen 2017). Further, N_2 fixation in lichens and mosses show strong seasonal variation (Rousk et al. 2015) and may also occur outside the main growing season in arctic ecosystems (Lett and Michelsen 2014). Our ARA estimates (0–160 μmol m^{-2} h^{-1}) are within the range of previously reported rates in subarctic tundra (0–300 μmol m^{-2} h^{-1}; Rousk et al. 2015) and in Arctic settings for lichens and mosses (0–100 μmol m^{-2} h^{-1}; Henry and Svoboda 1986; Hobara et al. 2006; Stewart et al. 2011).

When using the theoretical conversion ratio of 3 to convert our acetylene reduction rates (inset Fig. 2) to N_2 fixation rates (Hardy et al. 1973), the two measures of N_2 fixation are of the same magnitude. Estimates of N_2 fixation using ARA are 0.25, 7.88, 0.76 kg N ha^{-1} per 90 days vs. 0.30, 1.26, 0.31 kg N ha^{-1} per 90 days for ^{15}N–N_2 fixation for crust, moss and cotton grass vegetation type, respectively. The estimates are comparable, except for the moss vegetation type. Here, ARA yields several fold higher N_2 fixation rates compared to the ^{15}N–N_2 assay. This may be due to differences in ground cover of the N_2 fixer associations, or small-scale differences in ecosystem components in the ARA field assessments compared to the samples we used for our ^{15}N–N_2 assay. Nevertheless, ARA remains a useful tool to assess N_2 fixation, especially in field settings where ^{15}N labelling can be unfeasible.

Conclusions

Fixed N_2 by various diazotrophs was shown to be more quickly available to other ecosystem compartments in High Arctic settings, as compared to a similar study in subarctic settings. In the High Arctic, processes like dry-rewet and freeze–thaw cycles may more strongly impact diazotrophs and their release of fixed N_2, with implications for N movement within and between landscape types. Hence, comparisons in regard to N cycling processes following N_2 fixation between subarctic and High Arctic habitats cannot be made forthrightly. Our results further demonstrate that N derived from N_2 fixation is a significant and actual source of available N in the High Arctic also in the shorter term, and not just a potential one.

Acknowledgements Funding was provided by the Danish Council for Independent Research (Grant ID: DFF – 6108-00089), the Carlsberg Foundation (Grant ID: 2009_01_0570), and the Danish National Research Foundation (Center for Permafrost, CENPERM DNRF100). We thank G. Sylvester for assistance with laboratory analyses at the University of Copenhagen. We thank Flemming Ekelund and Irina Goldberg for their great help with moss identification. Climate data were provided by the Greenland Ecosystem Monitoring Programme.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Electronic Supplementary Material for:

Nitrogen fixation in the High Arctic: A source of 'new' nitrogen?

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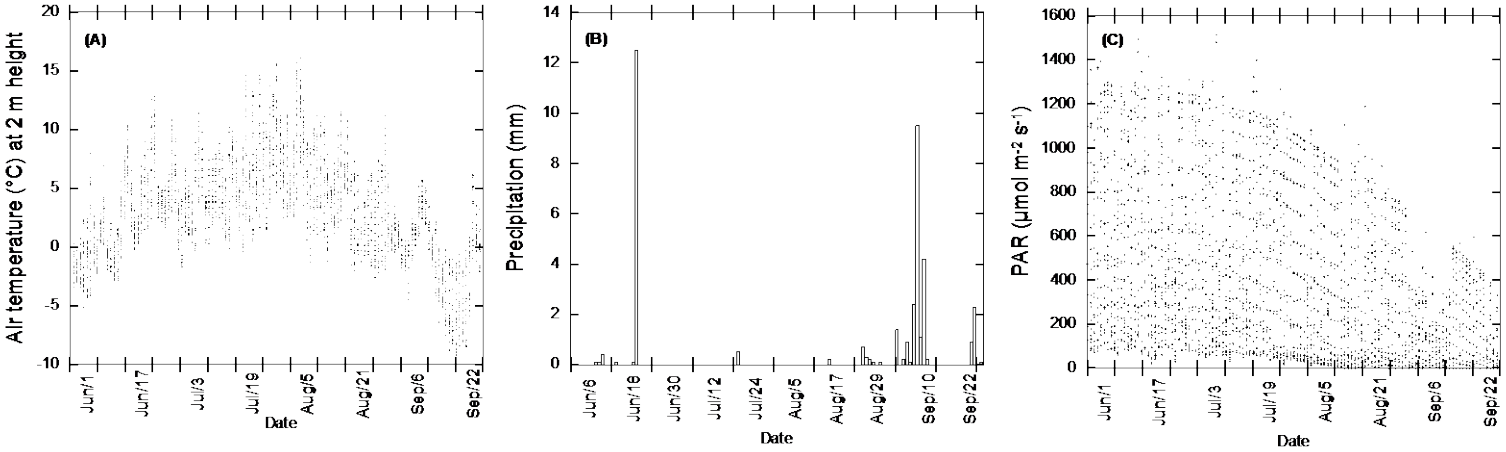
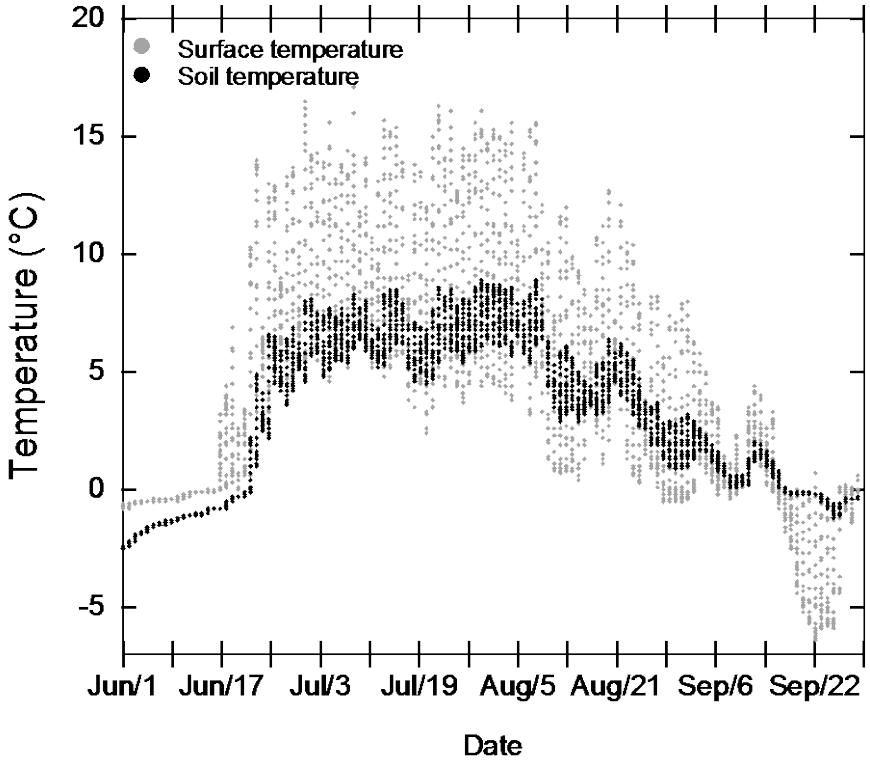
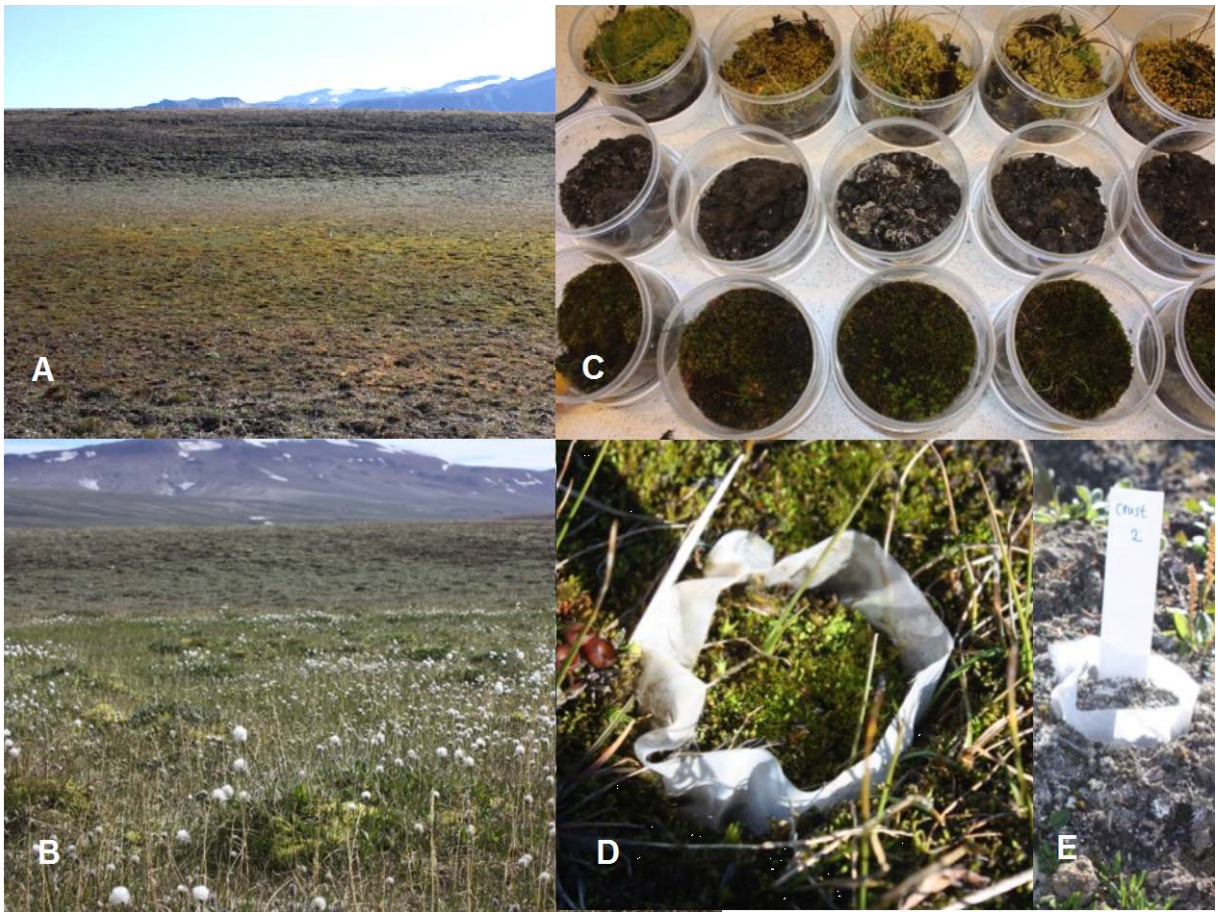


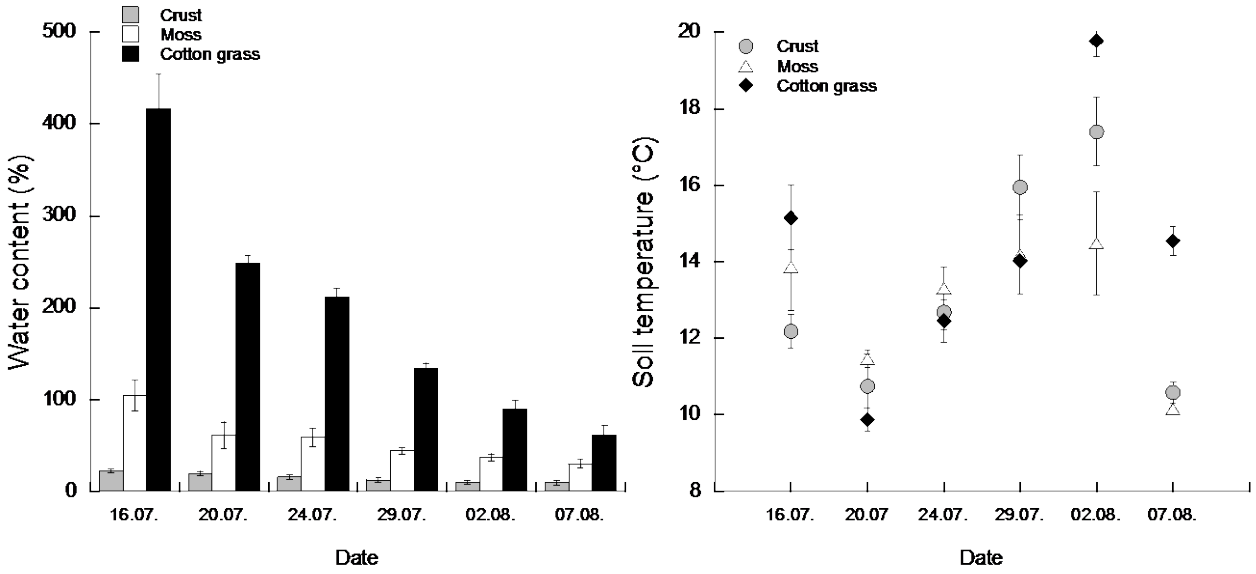
Online Resource 1 (A) Hourly air temperature (2 m height, °C), (B) precipitation (mm day^{-1}) and (C) hourly photosynthetic active radiation (PAR; µmol m^{-2} s^{-1}) 1 June to 30 September 2010 at Zackenberg Research Station, NE Greenland.



Online Resource 2 Hourly surface temperature (grey circles) and hourly soil temperature at 10 cm depth (black circles) in willow tundra heath at Zackenberg, NE Greenland in 2010.



Online Resource 3 Landscape photos of the investigated vegetation types, **(A)** crust (upper left slope) and moss (center) and **(B)** cotton grass. **(C)** Plant-soil cores harvested 3 days after *in situ* ^{15}N labelling, shown are the three investigated vegetation types (top: cotton grass, middle: crust, bottom: moss). **(D)** Moss and **(E)** crust-core from the *in situ* acetylene reduction incubations, Zackenberg, NE Greenland.



Online Resource 4 Soil water content (% of dry weight) and soil temperature (°C; at 2 cm depth) throughout the growing season in the plant-soil-cores used for ARA in the three investigated vegetation types (crust, moss, cotton grass) in the High Arctic. n = 6 ±SE.

Online Resource 5 ^{15}N enrichment (µg ^{15}N g dw^{-1}) of the ecosystem components in the three investigated vegetation types (crust, moss, cotton grass) 3 days and 3 weeks after ^{15}N labelling in the High Arctic. Given are means ± SE (n = 6). Different lower case letters indicate significant differences between the ecosystem components. NA = Ecosystem component not present

	Crust		Moss		Cotton grass	
Ecosystem component	3 days	3 weeks	3 days	3 weeks	3 days	3 weeks
Peltigera	NA	0.7390	NA	NA	1.7865±0.9274a	1.8679
Broadleaved herbs	0.0060 ±0.006ab	0.1178	0.1077	0.1028±0.0494a	NA	NA
Soil	0.0069±0.0016a	0.0200±0.0035a	0a	0.0503±0.0372a	0.0192±0.0173b	0.0101±0.0064ab
Fine roots	0.0127 ±0.005ab	0.0164±0.0062ab	0.0194±0.007a	0.0338±0.0122a	0.0129±0.0129b	0b
Stems	0.0162±0.0096ab	0.0337±0.0107abc	NA	NA	0.0025±0.0025b	0.0009±0.0009b
Non-N_2-fixing lichens	0.0165±0.0165ab	0.0250±0.025abc	NA	NA	NA	NA
Graminoids	0.0533±0.0164ab	0.0554±0.022abc	0.0635±0.0357a	0.0794±0.0326a	0.0820±0.0251b	0.0666±0.0337ab
Moss	0.0696±0.0112b	0.0917±0.0192c	0.5656±0.2075b	0.3933±0.1359b	0.0456±0.0454b	0.0670±0.0341a
Organic Crust	0.0689±0.0131b	0.0878±0.0198bc	NA	NA	NA	5.1785
Salix	0.0730±0.0249b	0.0533±0.0099abc	NA	NA	0.0455±0.0220b	0.0155±0.0100ab

Online Resource 6 ^{15}N recovery (%) in the ecosystem components in the three investigated vegetation types (crust, moss, cotton grass) 3 days and 3 weeks after ^{15}N labelling in the High Arctic. Given are means ± SE (n = 1-6). Different lower case letters indicate significant differences between the ecosystem components.

	Crust		Moss		Cotton grass	
Ecosystem component	3 days	3 weeks	3 days	3 weeks	3 days	3 weeks
Peltigera	0b	0.0354 ±0.0354b	NA	NA	0.598±0.312a	0.086±0.086
Broadleaved herbs	0.0001±0.0001b	0.0013 ±0.0013b	0.0007 ±0.0007b	0.0013±0.0006b	NA	NA
Soil	0.0531±0.0124b	0.1582±0.0265b	0.225 ±0.118b	0.5406±0.402b	0.059±0.054b	0.101±0.065
Fine roots	0.0247±0.0122b	0.0175±0.0075b	0.0055±0.0026b	0.0301±0.0107b	0.011±0.011b	0
Stems	0.0004± 0.0003b	0.0136±0.006b	NA	NA	0.0066±0.0060b	0.0012±0.0012
Non-N_2-fixing lichens	0.0023±0.0017b	0.0041±0.0041b	NA	NA	NA	NA
Graminoids	0.0343±0.0124b	0.0683±0.0294b	0.0284±0.0145b	0.0587±0.0237b	0.0618±0.023b	0.0779±0.0276
Moss	0.0167±0.0063b	0.1057 ±0.0702b	3.036±1.005a	4.163±1.46a	0.0648±0.0638b	0.2297±0.1409
Organic Crust	0.6560 ±0.0804a	0.4118 ±0.1108a	NA	NA	0a	0.2525±0.2525
Salix	0.0045±0.0015b	0.0018±0.001b	NA	NA	0.0218±0.0127b	0.0049±0.0032

NA = Ecosystem component not present

Online Resource 7 Total N (%) in the ecosystem components in the three investigated vegetation types (crust, moss, cotton grass) in the High Arctic. n = 6 ±SE. Significant differences in N concentration between the vegetation types are indicated with different lower case. NA = Ecosystem component not present.

Ecosystem component	Crust	Moss	Cotton Grass
Graminoids	0.66 ±0.04a	1.64 ±0.21b	1.43 ±0.06b
Broadleaved herbs	0.76 ±0.03ab	1.02 ±0.06bc	1.54 ±0.14c
Salix	1.43 ±0.08a	NA	1.97 ±0.11b
Moss	0.49 ±0.04a	0.57 ±0.08a	1.01 ±0.10b
Organic crust	0.46 ±0.03	NA	NA
Peltigera lichen	NA	NA	1.42 ±0.17
Non-fixing lichens	0.57 +0.03	NA	NA
Stems	0.91 ±0.04	NA	0.77 ±0.02
Fine roots	0.52 ±0.05a	0.56 ±0.06a	1.02 ±0.03b