Nitrogen accumulation and partitioning in High Arctic tundra ecosystem from extreme atmospheric N deposition events

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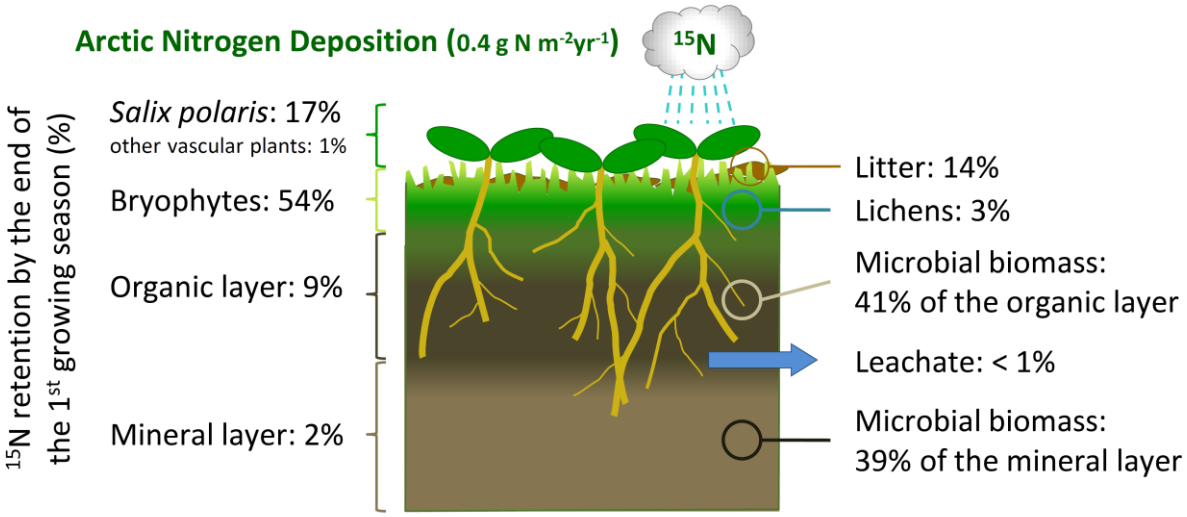
Running Headline: Extreme N deposition event fate in High Arctic Tundra

Graphical abstract



Highlights

- High Arctic tundra demonstrated a very high (90–95%) N pollutant retention capacity.
- Non-vascular plants and soil microbial biomass were important short-term N sinks.
- Vascular plants and soil showed capacity to be efficient longer-term N sinks.
- Deposition rich in nitrate can alter almost all ecosystem N pools.
- Extreme N depositions may already be contributing to the N enrichment of tundra.

Abstract

Arctic ecosystems are threatened by pollution from recently detected extreme atmospheric nitrogen (N) deposition events in which up to 90% of the annual N deposition can occur in just a few days. We undertook the first assessment of the fate of N from extreme deposition in High Arctic tundra and are presenting the results from the whole ecosystem ^{15}N labelling experiment. In 2010, we simulated N depositions at rates of 0, 0.04, 0.4 and 1.2 g N m^{-2} yr^{-1}, applied as $^{15}NH_4{}^{15}NO_3$ in Svalbard (79°N), during the summer. Separate

applications of $^{15}NO_3^-$ and $^{15}NH_4^+$ were also made to determine the importance of N form in their retention.

More than 95% of the total ^{15}N appliedwas recovered after one growing season (~90% after two), demonstrating a considerable capacity of Arctic tundra to retain N from these deposition events. Important sinks for the deposited N, regardless of its application rate or form, were non-vascular plants N vascular plants N organic soil N litter N mineral soil, suggesting that non-vascular plants could be the primary component of this ecosystem to undergo measurable changes due to N enrichment from extreme deposition events. Substantial retention of N by soil microbial biomass (70% and 39% of ^{15}N in organic and mineral horizon, respectively) during the initial partitioning demonstrated their capacity to act as effective buffers for N leaching. Between the two N forms, vascular plants (*Salix polaris*) in particular showed difference in their N recovery, incorporating four times greater $^{15}NO_3^-$ than $^{15}NH_4^+$, suggesting deposition rich in nitrate will impact them more. Overall, these findings show that despite the deposition rates being extreme in statistical terms, biologically they do not exceed the capacity of tundra to sequester pollutant N during the growing season. Therefore, current and future extreme events may represent a major source of eutrophication.

Key-words

Extreme nitrogen deposition, arctic tundra N pools, N immobilization, ^{15}N tracer, plant–soil interactions, ecosystem N dynamics

1. Introduction

Atmospheric nitrogen deposition is one of the top three threats to global biodiversity (Sala et al., 2000; Phoenix et al., 2006, 2012). Since the industrial revolution, there has been a marked increase in nitrogen (N) deposition across many regions of the world, including supposedly pristine remote locations such as the Arctic (Jónsdóttir et al., 1995; Forsius et al., 2010).

Despite this, arctic ecosystems still typically receive relatively low rates of atmospheric N deposition (ranging from <0.1 g N m^{-2} yr^{-1} in Svalbard and other arctic regions to ~1 g N m^{-2} yr^{-1} in the Taymyr Peninsular in Russia and parts of northern Alaska; Woodin, 1997; Fischer et al., 1998; Simoẽs and Zagorodnov, 2001; Kühnel et al., 2011). Therefore, even relatively modest increases in current and future N inputs may represent a significant additional supply of N. This is of particular concern given that arctic ecosystems are sensitive to increased N supply (Gordon et al., 2001; Arens et al., 2008; Street et al., 2015) due to the inherent low N availability in these nutrient-limited systems (Shaver and Chapin, 1980; Henry et al., 1986; Chapin et al., 1995).

While chronic rates of N deposition in the Arctic are low, a greater threat may arise from extreme N deposition events that have been discovered relatively recently (Hodson et al., 2005, 2010; Kühnel et al., 2011, 2013). The extremeness of the atmospheric N deposition event refers to its high intensity (or concentration) as well as its low frequency i.e. a statistically rare occurrence (Jentsch, 2006; Kühnel et al., 2011, 2013). Such extreme events result from polluted air masses that arise from industrialised countries at lower latitudes and are transported to high latitude regionswith minimal dispersal. As an example, a single extreme event was observed to supply 40% of the total annual deposition in just oneweek (Hodson et al., 2005), and recent research suggests that despite their low frequency, because of the large quantities of N deposited annual atmospheric N deposition in High Arctic

Svalbard can be dominated by such episodic events with these forming 10–90% of the annual atmospheric N input (Hodson et al., 2005; Kühnel et al., 2011, 2013). It is of further concern that increasing cyclonic activity over the North Atlantic and predicted increases in precipitation over the Arctic may lead to more extreme N deposition events (Klonecki et al., 2003; Kühnel et al., 2011). Furthermore, the changing arctic climate (Førland et al., 1997; Cassano et al., 2006) and intensification of shipping activities (Serreze et al., 2007; Peters et al., 2011) may lead especially to particularly greater increases in deposition events during the summer, the time of year when arctic ecosystems will have the greatest biological capacity to sequester the N, potentially exacerbating N enrichment impacts. To date, however, the fate and impacts of N from these extreme deposition events remains unknown, despite the potential for these to affect arctic ecosystems by loading a large proportion of the annual atmospheric N input in just a few days. The effects of such extreme depositions will depend in part on the amount of N retained in the system and its partitioning among different compartments of the ecosystem(plant, soil, microbial, leachate pools) cross time. Quantification of the fate of N may also allow for a better understanding of the mechanisms underlying shifts in species composition of an ecosystem.

Many nitrogen manipulation experiments in tundra have used NPK fertiliser additions rather than simulating atmospheric N deposition (e.g., Shaver and Chapin, 1995; Robinson et al., 1998; Schmidt et al., 2000) to study the effects of N availability. Other N addition studies have either used large N applications (ranging from 5 to 25 g m^{-2} yr^{-1}) or investigated chronic N loading but to date have not simulated extreme atmospheric N deposition events (e.g., Gordon et al., 2001; Madan et al., 2007; Arens et al., 2008).While a limited number of studies have focused on the fate of atmospheric N deposition in the Arctic, these studied mainly snowpack N inputs during spring melt (Bilbrough et al., 2000; Tye et al., 2005; Templer et al., 2012). Moreover, none of the studies have demonstrated a comprehensive

understanding of atmospheric N partitioning and retention of different N forms (e.g. $NH_4^+NO_3^-$, only-NH_4^+ and only-NO_3^-) within different ecosystem pools.

With these concerns in mind, we used a field simulation of extreme deposition events, with ^{15}N (applied over 4 days in summer at 0, 0.04, 0.4 and 1.2 g N m^{-2} yr^{-1}) to determine the fate (accumulation and partitioning) of acutely deposited N over two growing seasons. Our objectives were (1) to identify major sinks for atmospheric N, deposited in extreme events, and therefore, determining the most sensitive component of the ecosystem to be affected by such depositions, (2) to determine the fate of different species of reactive N (NO_3^- and NH_4^+) in different ecosystem compartments, and therefore, investigating if there is any preference for either of the N forms by any of the compartments of the ecosystem as well as the form of nitrogen that could impact this ecosystem more. Finally, we (3) analysed the capacity of different doses and forms of ^{15}N in enriching the total N pool of the different ecosystem compartments to understand the eutrophication potential of the deposited N. We report results from the whole ecosystem for both the initial partitioning and recovery of ^{15}N label in different pools: tundra plants, soil, litter, microbial biomass and leachate as well as the cycling of ^{15}N over the short (7–21 days) and medium terms (388 days).

2. Material and methods

2.1. Site description

The field site was located in the High Arctic on Svalbard, at Leirhaugen Kolhaugen (78°55′231″N; 11°49′819″E), 25 m above mean sea level on the Brøggerhalvöya peninsula, 2 km southwest of NyÅlesund. The mean annual temperature at Ny-Ålesund is −5.2 °C, with a summer (July–August) average of +5 °C. The vegetation was dominated by bryophytes (~40%; mainly: *Sanionia uncinata, Ptilidium ciliare, Dicranum laevidens, Dicranum spadiceum* and *Oncophorus wahlenbergii*) and *Salix polaris* (a deciduous dwarf shrub:

~30%) and lichens (~16%) with a thin layer of litter (mainly Salix) on the surface underlain by an organic (O) layer (~5 cm) over amineral (A) layer. Further details on climatic conditions during the experiment, soils and vegetation C and N stocks are provided in Appendix A (Tables A.1 & A.2).

2.2. Experimental design

In July 2009, 25 plots (1.5 m × 1.5 m) were established in approximately a 600 m2 area of tundra (see Blaud et al., 2015 and Appendix A for details). In the following year (15–18 July 2010), N applications were made using dual labelled $^{15}NH_4{}^{15}NO_3$ (99% labelled; SerCon, Crewe, UK), applied at rates of 0 (controls with distilled water only, referred to as Cw), 0.04, 0.4 g Nm^{-2} yr^{-1} and 1.2 g Nm^{-2} yr^{-1} (referred to as 0.04N, 0.4N and 1.2N, respectively) with five replicated plots each, to quantify the fate of the pollutant N.

N treatments were applied using a watering can in 10 l of distilled water per plot (adjusted to pH 4 with HNO_3, in line with the pH of extreme deposition events). These treatments were applied over 4 days in 2010, with one-quarter of the total amount applied per day to simulate wet deposition events previously observed (Hodson et al., 2005; Kühnel et al., 2011). The lowest N addition (0.04N) simulated one of the previously recorded extreme N deposition events where 0.04 g N m^{-2} yr^{-1} (~40% of the annual atmospheric N input) was deposited with rainfall in less than a week (Hodson et al., 2005). The highest N treatment of 1.2N was undertaken to allow comparison with other past studies that simulated chronic (rather than extreme) N deposition in the High Arctic (Baddeley et al., 1994; Woodin, 1997; Gordon et al., 2001; Street et al., 2015).

Since the long-range transported air masses in the Arctic have been observed to consist of nitrate or ammonium aerosol which could sometimes result in deposition dominated by one or the other form of N (Dickerson, 1985; Hole et al., 2009; Aas et al.,

2011), a further five plots were split in two and used to determine the fate of separate NO_3^- and NH_4^+ N depositions. One half of each split plot (1.5 m × 0.75 m) received single labelled $^{15}NO_3^-$ ($Na^{15}NO_3$ solution in 5 l water; hereafter referred to as 'only $^{15}NO_3^-$') and the other half received single labelled $_{15}NH_4^+$ ($^{15}NH_4Cl$ solution; hereafter referred to as 'only $^{15}NH_4^+$') at a rate of 0.4 g N m^{-2} yr^{-1}. Identical N treatments were then repeated in 2011 from 1 to 4 July with non-labelled NH_4NO_3, $NaNO_3$ and NH_4Cl.

2.3. Fate of ^{15}N: field sampling

Soil and plant samples were taken from each plot after 7 (25 July 2010), 21 (8 August 2010) and 388 (10 August 2011) days of the ^{15}N treatment. At each harvest in each plot, a knife was used to cut a 10 × 10 cm intact turf to a soil-depth of 10 cm. The knife was thoroughly cleaned between each soil sampling to avoid ^{15}N contamination. Two extra soil samples of 2 × 2 cm of 10 cm depth were also taken from each plot for further estimation of ^{15}N in the soil. Each sample was stored in separate plastic bags in a cold bag with ice packs and transported to the NERC Arctic Research Station (Ny-Ålesund), and stored at 4 °C prior to processing within 24 h.

Leachate samples were collected using two mini-rhizon soil samplers (10 cm length, 2.5 mm diameter; Van Walt, Surrey, U.K.) per plot installed below the main rooting zone and just above the mineral soil layer. Samples were collected after 7, 21 days in the ^{15}N application season, and after 347 days in the subsequent growing season (with rhizon samplers for that collection installed on day 344). Samples were frozen and returned to the UK for further analysis.

2.4. Sample processing and analysis

From each 10 × 10 cm plant-soil monolith, the above ground plant material was separated from the soil and the soil organic and mineral layers were weighed and divided into sub-samples for further analyses. The (very) few stones present in both horizons were removed. Fresh soil sub-samples were used for quantification of soil moisture, pH, inorganic N and microbial C and N content. Gravimetric soil moisture was determined by drying sub-samples (10–20 g) in the oven at 60 °C for three days. These oven-dried sub-samples were then returned to the UK for determination of total soil N and ^{15}N. "Plant available" inorganic N(NO_3^- and NH_4^+) in soil was measured in fresh sub-samples by KCl extraction (Allen, 1989). These KCl extracts were frozen and returned to the UK for further analysis.

Microbial C and N extraction was measured by chloroform fumigation using 0.5 M K_2SO_4 (Vance et al., 1987), determined from the difference in C and N released between fumigated (~48 h with ethanol free chloroform) and non-fumigated soils. Fumigation extracts were prepared for ^{15}N analysis by the diffusion method (Brooks et al., 1989).

Above-ground plant material was oven-dried at 80 °C prior to separation into different plant fractions and analyses of above-ground plant biomass, total N and ^{15}N content. Above-ground plant material was separated into bryophytes (mosses and liverworts), lichens, *Salix polaris* (the dominant vascular plant), other vascular plants (largely *Saxifraga*, *Oxyria* and *Polygonum* species) and litter (a mix of dead *Salix* leaves and stems, and dead mosses). Below-ground stems of *Salix polaris* were removed from the 10 × 10 cm soil samples and roots from a 5 × 5 cm section down the full soil depth. Samples were washed and dried in an oven at 60 °C for three days for analyses of biomass, total N and ^{15}N content.

Dried plant and soil samples were ground to a fine powder and total N content and ^{15}N enrichment were determined by isotope ratio mass spectrometry (ANCA GSL 20-20, PDZ Europa, Crewe, Cheshire, UK). Calculations of ^{15}N recovered in plant and soil pools were then determined using the standard equation (Powlson and Barraclough, 1993):

$$F = T (A_S - A_B) / A_F \quad (1)$$

where F is N recovered from the labelled addition (^{15}N (g)/g of sample), T is the total weight of N in the sample (N (g)/g of sample), and AS, AB and AF are the at.% ^{15}N in the treated sample, unlabelled control and added label, respectively. The percentage of ^{15}N treatment that was recovered in each pool was then calculated.

Total inorganic N concentrations of KCl extracts were determined by flow injection analysis (FIAflow2, Burkard Scientific, Uxbridge, UK), using sodium salicylate and sulphanilimide colorimetric reactions to determine soil NH_4^+ and NO_3^- content respectively. C, N and ^{15}N content of microbial biomass was determined as the difference between fumigated and non-fumigated soil extracts that were freeze-dried and subsequently analysed using the isotope ratio mass spectrometer. To correct for incomplete extraction, a conversion factor (KEC) of 0.35 was used for microbial C (Cheng and Virginia, 1993) and a factor of 0.4 for microbial N (KEN) (Jonasson et al., 1996).

Contribution of deposited N from extreme events to existing ecosystem N pools was also measured by expressing ^{15}N as a percentage of the existing total N pool in each fraction. All the above- and below-ground plant materials for *Salix* were added together for calculation of total ^{15}N retention and contribution of ^{15}N to total N in the *Salix* pool.

2.5. Statistical analyses

Statistical analyses were carried out using SPSS 17.0 (SPSS Inc., Chicago, Illinois, USA). Repeated-measures ANOVA (with date as the within-subject factor and treatment as the between-subject factor) were used to determine overall N treatment effects and whether there were significant changes in total ^{15}N recovery and ^{15}N retention in the different pools with time. For each pool at individual samplings, data were compared using Tukey's HSD tests to determine differences between treatment levels (0.04N, 0.4N and 1.2N) in ^{15}N

retention and ^{15}N proportion in the total N pool. A t-test was used to test differences between $^{15}NO_3^-$ and $^{15}NH_4^+$ retention and their proportion of the total N for each pool at individual sampling. Homogeneity of variances was tested with Levene's test and, where necessary, the appropriate square root- or log-transformations were performed.

3. Results

3.1. Recovery and partitioning of ^{15}N

3.1.1. Application of $^{15}NH_4^{15}NO_3$

Total % recovery of ^{15}N was consistently high across all treatments with average total recovery of 81±3.9%, 96±4.9% and 92±5.4% at day 7, 21 and 388, respectively (days and treatments not significantly different from each other) (Table B.1, Appendix B).

***Non-vascular plants*:** Bryophytes were the largest single pool for ^{15}N, retaining between 32 and 62% (Fig. 1a). Percentage ^{15}N retention showed a marginally significant decline with increasing N input (repeated measures ANOVA: d.f. = 2, 12, F = 3.42, P = 0.08), with % retention 30% smaller overall under the 1.2N treatment compared to 0.04N (repeated measures, Tukey, $P < 0.05$). Retention of ^{15}N peaked at day 21 (retentions of 62, 54 and 43% in the 0.04, 0.4 and 1.2N) and showed a decline (to 41, 35 and 32%) by day 388 (marginally significant time effect, repeated measures ANOVA: d.f.= 2, 11, F = 2.95, P = 0.08).

Lichens (which constituted just 4% of plant biomass) immobilised 2–6% of the ^{15}N (Fig. 1d). Unlike bryophytes, % retention of ^{15}N was relatively similar across N treatments over the two growing season, with average retentions of 3.5, 3 and 3% under 0.04, 0.4 and 1.2N, respectively.

***Vascular plants*:** *Salix polaris* (the dominant vascular plant) was the second greatest pool for ^{15}N, accumulating between 5 and 30% of applied ^{15}N in its leaves, stems and roots (Fig. 1b). This % retention remained relatively constant over the two growing seasons for all the treatments. Unlike non-vascular plants, % retention of ^{15}N increased with the increasing N input (repeated measures ANOVA: d.f. = 2, 12, F = 11.43, $P < 0.01$) with average retention of ^{15}N being 6, 15 and 24% of the 0.04, 0.4 and 1.2N treatments respectively. Overall, % ^{15}N retention under 1.2N treatment was 75% greater than that of the 0.04N treatment (repeated measures, overall Tukey, $P < 0.01$). Other vascular plants which were a mix of *Saxifraga*, *Oxyria* and *Polygonum* sp. Retained between 1 and 4% of the applied ^{15}N. These plants were not widely present in all plots and there were no significant differences in % ^{15}N recovery among the treatments (Fig. 1c).

***Litter*:** The litter fraction represented the third greatest retention pool for ^{15}N, accumulating between 6 and 15%, irrespective of the treatment levels (Fig. 1e) with no significant differences among the treatment levels.

***Organic Soil*:** The organic soil fraction retained similar amounts of ^{15}N to the litter fraction (4–15%). Retention was similar across N treatments with a modest increase in % ^{15}N retention from day 21 to day 388 (not significant) (Fig. 1f).

Due to time constraints, microbial biomass could only be obtained for 0.4N and 1.2N plots. At day 7, 70% of the ^{15}N in the organic soil was immobilised by the microbial biomass irrespective of the treatment dose. This retention significantly decreased to 41 and 52% of the soil ^{15}N by day 21 and day 388, respectively (time effect, repeated measures ANOVA: d.f. = 2, 11, F = 7.11, $P < 0.01$).

***Mineral Soil*:** The mineral soil was the fifth greatest pool for ^{15}N, holding between 2 and 8% (Fig. 1g). By the end of the second season (day 388), ^{15}N % retention under 1.2N was

300 and 90% greater than under 0.04N and 0.4N (repeated measures ANOVA: d.f. = 2, 12, F = 3.828, $P < 0.05$).

Of the ^{15}N in the mineral soil, 39% of this was immobilised by the microbial biomass with no significant differences in the amount of ^{15}N recovered over time.

Soil water: The ^{15}N in soil water increased proportionally with the dose of N applied, leaching 0.005, 0.5 and 4.8 mg $^{15}N\ L^{-1}$ soil water on day 7 (see Table 1 for Tukey's test) which decreased by 75, 98 and 100% on day 347 under the 0.04, 0.4 and 1.2N treatments, respectively (time∗ treatment interaction, repeated measures ANOVA, d.f. = 6, 30, F = 3.02, $P < 0.05$) (Table 1). Loss of ^{15}N via leaching only represents <5% of the total ^{15}N applied even if it is assumed that all applied water is leached.

3.1.2. Separate application of $^{15}NO_3^-$ and $^{15}NH_4^+$

Overall, both $^{15}NO_3^-$ and $^{15}NH_4^+$ were highly retained in the system. The main sinks of NO_3^- were both bryophytes and *Salix polaris*, retaining between 33 and 49% and 31–39% of N applied as $^{15}NO_3^-$, respectively while ^{15}N from $^{15}NH_4^+$ was mainly present in bryophytes (35–65%). Differences between retention of ^{15}N from the separate additions of NO_3^- and NH_4^+ for each fraction are described in detail in the Appendix B. In brief, bryophytes, lichens, litter and microbial biomass showed no difference in overall retention of the ^{15}N fromNO_3^- and NH_4^+ (Fig. 2), except for bryophytes at day 7 with a significant greater retention of ^{15}N from NH_4^+ (d.f.= 7, $t = -5.06$, $P < 0.01$). In contrast, there were large significant differences in the 15N retention of the two N forms in *Salix polaris* at all of the sampling days, with much greater retention of ^{15}N from NO_3^- (31 to 39%) compared to ^{15}N from NH4+ (6 to 8%) (repeated measures ANOVA: d.f. = 1, 8, F = 95.73, $P < 0.001$) (Fig. 2b). Organic and mineral soil also showed greater retention of $^{15}NO_3^-$ compared to $_{15}NH_4^+$ for all of the sampling days (repeated measures ANOVA: $P < 0.05$) (Fig. 2f & g). There was ~20

319 times more $^{15}NO_3^-$ (1 mg ^{15}N L^{-1} soil water) than $^{15}NH_4^+$ in the soil water by day 7 (repeated
320 measures ANOVA, d.f. = 2, 11, F = 6.19, P < 0.05; Tukey, P b 0.05), and this difference
321 decreased to 3 times more $^{15}NO_3^-$ (0.02 mg ^{15}N L^{-1} water) than $^{15}NH_4^+$ by day 347
322 (time∗treatment, repeated measures ANOVA, d.f.=4, 22, F=4.06, P < 0.05) (Table 1).
323 Approximations suggest a maximum loss of 2% of the applied $^{15}NO_3^-$ and <0.1% of the
324 applied $^{15}NH_4^+$ at day 7.
325

326 ## 3.2. Pollutant ^{15}N enrichment of existing N pools

327 ### 3.2.1. $^{15}NH_4{}^{15}NO_3$ enrichment

328 Contribution of the ^{15}N to existing N pools showed very similar patterns across the
329 day 7, 21 and 388 harvests, so here we focus on day 388 data to describe the longest-term
330 enrichment (Fig. 3a).

331 The contribution of the applied ^{15}N to the total N pools of all the fractions increased
332 proportionally with the increasing level of N treatments except for *Salix polaris*, where the
333 contribution of applied ^{15}N under the 1.2N treatment was much (~85 times) greater than for
334 the 0.04N treatment (Tukey, P b 0.001). The contribution of the ^{15}N to existing N pools was
335 greatest in lichens N bryophytes N *Salix polaris* N microbial biomass N litter N organic soil
336 N mineral soil.

337 In the non-vascular plant pool, treatment ^{15}N made up 0.41, 4.3 and 9.3% of the total
338 bryophyte N pool under the 0.04N, 0.4N and 1.2N treatments respectively, (ANOVA: d.f.= 2,
339 12, F = 53.50, P < 0.001) with all treatment levels being significantly different from each
340 other (Tukey, P < 0.01 between 0.04N and 0.4N, P < 0.001 for all other pairwise
341 comparisons). In lichens, these contributions were slightly greater at 0.59, 5.7 and 11.5%
342 (ANOVA: d.f. = 2, 12, F = 345.18, P < 0.001), again with all treatment levels being
343 significantly different (Tukey, P < 0.001 among all).

In *Salix polaris*, the ^{15}N contribution was lower when compared to non-vascular plants, comprising 0.05, 0.99 and 4.7% of the existing N pools under the 0.04N, 0.4N and 1.2N treatments, respectively (ANOVA: d.f.= 2, 12, F = 84.01, P < 0.001; all treatment levels were significantly different from each other, Tukey, P b 0.01).

In litter ^{15}N contributed 0.08, 0.87 and 2.1% of the total litter N pool (ANOVA: d.f. = 2, 12, F = 47.68, P < 0.001; all treatment levels were significantly different from each other, Tukey, P b 0.01). While in the organic soil, these contributions were only 0.01, 0.07 and 0.32% (ANOVA: d.f.= 2, 12, F = 23.41, P < 0.001; Tukey, P < 0.01 for all comparisons), and 0.01 × 10−2, 0.01 and 0.05% in the mineral soil, under the 0.04N, 0.4N and 1.2N treatments, respectively (ANOVA: d.f.= 2, 12, F = 24.76, P < 0.001; Tukey, P < 0.001 between 0.04N and 1.2N and between 0.4N and 1.2N).

In the microbial N pool, ^{15}N constituted 0.82 and 3.4% in the organic soil (d.f. = 8, t = −2.940, P < 0.05) and 0.15 and 0.69% in the mineral soil, under the 0.4N and 1.2N treatments (d.f. = 8, t = −2.88, P < 0.05), respectively.

3.2.2. Separate enrichment by $^{15}NO_3^-$ and $^{15}NH_4^+$

At day 388, ^{15}N fromNO_3^- and NH_4^+ contributed similar proportions to each of the N pools with the exceptions of the vascular plant and soil pools (Fig. 3b). In the *Salix* pool, ^{15}N from NO_3^- contributed significantly more (1.8%) of the N pool compared to ^{15}N fromNH_4^+ (0.51%) (d.f.= 8, t = 3.48, P < 0.01) (Fig. 3b). In the organic soil horizon, ^{15}N fromNO_3^- contributed more to the existing N pool (0.14%) than N from NH_4^+ (0.06%), (d.f.= 8, t = 2.95, P < 0.05) (Fig. 3b). There were similar differences in the mineral soil horizon, with ^{15}N from NO_3^- contributing 0.04% compared to 0.002% from NH_4^+ (d.f. = 8, t = 2.27, P = 0.05) (Fig. 3b).

4. Discussions

4.1. Recovery of deposited $^{15}NH_4^{15}NO_3$ in plants, soil and microbial fractions

This study shows that High Arctic tundra has considerable capacity to accumulate the pollutant N deposited in an extreme event, retaining ~95% across all treatments by the end of the first growing season with ~90% still retained after the second growing season. This is of further concern, given that tundra may be slow to recover from N deposition impacts (Street et al., 2015). Other work using ^{15}N in arctic and alpine tundra habitats has also demonstrated the ability of these ecosystems to act as rapid sinks for N (Bilbrough et al., 2000; Nordin et al., 2004; Tye et al., 2005; Templer et al., 2012). Given the different aims and methods of ^{15}N application in those studies, however, they cannot be used to estimate N accumulation from summer extreme N deposition events (being performed either at snowmelt or using soil injections, or not including doses or N recovery time-scales that relate to understanding extreme N deposition impacts). Most relevant to our study was that of Tye et al. (2005), which used snow-pack applied ^{15}N to trace its fate immediately after snowmelt. In that study, much lower retention of ^{15}N was observed (~60% compared to 90% or greater in this current study) which might indicate the importance of the timing of N deposition for N accumulation (e.g. more active plant and microorganisms, soil thaw), although sites differences between this and our study (e.g. plant cover, soil texture) may also explain the difference. The high retention of N at our site was largely due to the ability of the bryophytes to retain incoming NH_4NO_3 (Longton, 1997; Hyvarinen and Crittenden, 1998; Kotanen, 2002). This was likely facilitated by their abundance, since they represented 47–61% of plant biomass and their high N assimilation capacity (Choudhary, 2013). Our results also showed with increasing N dose, the decreasing ^{15}N retention in the bryophytes was balanced largely by increasing ^{15}N

retention in *Salix polaris* and in both the soil horizons. This suggests that bryophytes might reach their N-saturation capacity at deposition rates between 0.4 and 1.2 g N m^{-2} yr^{-1} and is consistent with previous work which has reported N-saturation in mosses under 1 g Nm^{-2} yr^{-1} on Svalbard, as indicated by reduced nitrate reductase activity (Gordon et al., 2001). Moreover, high retention after two growing seasons suggests N is absorbed by the bryophytes rather than retained on the bryophytes surface.

There were some differences in short- (day 7 and 21) to medium term (day 388) ^{15}N retention in some of the pools but this did not affect the total recovery of ^{15}N over time, suggesting a tight ecosystem N cycling (Grogan et al., 2004). Microbial biomass in the organic horizon showed the greatest changes in its ^{15}N retention over time, retaining ~70% of the ^{15}N present in the soil during the initial partitioning (day 7) and decreasing later in the season. This indicates a rapid microbial ^{15}N turnover into both soil extractable and non-extractable pools in the organic horizon (Tye et al., 2005; Clemmensen et al., 2008; Templer et al., 2012). In contrast, the % ^{15}N retention by the microbial biomass in the lower mineral horizon that was able to immobilise ~30–40% of the ^{15}N in the soil, remained unchanged with time. This suggests a quick uptake of the ^{15}N released from the dying microbial biomass by new microbial biomass.

Furthermore, ^{15}Nanalyses suggest in the organic soil horizon, the released ^{15}N on microbial turnover could be largely taken up by vascular plants, roots of which were mostly found in that upper layer (Choudhary, 2013). *Salix*, thus appeared to have outcompeted microbes for ^{15}N after the initial partitioning (day 7), indicating its capacity to be an efficient longer-term N sink together with the organic soil and bryophytes. In contrast, previous studies have reported greater competitive ability of microbes compared to plants for nutrients in arctic ecosystems (Nordin et al., 2004; Clemmensen et al., 2008). This may again be attributed to the soil injection method (with N applied often below 10 cm) used in some past

work, which bypasses the moss layer and the main rooting zone that was the principal location of N retention in our study. Our findings of surface applied N during the summer months have greater parallels with the snowmelt period where there is transition from microbial based N retention to plant-based retention by root uptake in the organic layer (Brooks et al., 1998; Lipson and Monson, 1998). Microbial biomass in both the soil horizons, thus, act as effective buffers for N leaching and help retain the deposited N in the soil by either forming part of the extractable N pool for plants and microbes or the non-extractable N pool that contributes to the total soil N pool.

4.2. Importance of N form: fate of N from NO_3^- and NH_4^+

Our results showed that bryophytes were the dominant sink for both the applied $^{15}NO_3^-$ and $^{15}NH_4^+$. They showed only an initial preference for NH_4^+, though this initial preference could be the cause of their well-known sensitivity to reduced N (Mäkipää, 1995). In the longer term they had no preference for either of the N forms. Many bryophyte species have been previously reported to efficiently scavenge NH_4^+ (Yano et al., 2010) and NO_3^- either by absorption across their entire surface (Turetsky, 2003) or through capillary movement from the soil beneath (Press and Lee, 1982, Turetsky, 2003). Lichens and microbial biomass also did not showany preference for $^{15}NO_3^-$ or $^{15}NH_4^+$ and previous work also suggest that lichens are efficient nutrient immobiliser for NO_3^- and NH_4^+ (Tye et al., 2005).

In contrast, vascular plants and especially *Salix*, generally competed better for deposited NO_3^- than for NH_4^+. This result agrees with earlier field studies of N uptake using N-form mixtures in arctic ecosystems, which showed a greater uptake of NO_3^- than of NH_4^+ and amino acid N (Atkins et al., 1993; Nordin et al., 2004; Clemmensen et al., 2008; but see McKane et al., 2002). The relatively higher mobility of NO_3^- in the soil, may also have

444 contributed to high plant access to NO_3^- in the rooting zone (data not shown) compared to
445 NH_4^+, given that the diffusion rate of NO_3^- is fivefold higher than that of NH_4^+ (Jones et al.,
446 2005). Thus, deposition rich in nitrates could impact almost all the pools of this ecosystem
447 and may represent a major source of eutrophication.

448 We have not studied all form of N depositions, particularly organic N and particulate
449 matter. It is likely that these would show greater retention within the system, and through
450 microbial transformation end up in the same pools as the inorganic N that we have studied.
451 Empirical work is needed to test this.

452

453 4.3. Pollutant N (^{15}N) contribution to existing N pools

454 The % contribution of ^{15}N at all treatment levels to existing N pools was greatest in
455 the non-vascular plants, showing the capacity of such acute and low rates of N deposition to
456 enrich tissue N of these fractions. This also means non-vascular plants may be the first and
457 main component of this ecosystem to undergo measureable changes due to N enrichment
458 from extreme deposition events. This is consistent with other long-term studies that have
459 shown high sensitivity of bryophytes and lichens to N fertilisation (Gordon et al., 2001;
460 Madan et al., 2007).

461 ^{15}N contribution to the existing N pool of the vascular plants (mainly *Salix*) under the
462 1.2N treatment was ~85 times more than the 0.04N treatment (despite the lesser 30-fold
463 difference in dose between treatments). Similarly, the proportion of ^{15}N in the total microbial
464 N pool also increased with the increasing N load to a similar extent as seen for *Salix*. This
465 probably arises from the fact that most of the ^{15}N at the lowest N treatment is captured by the
466 non-vascular plants leaving less available for vascular plants or the microbial pool. At higher
467 N deposition rates, the non-vascular pool saturates and ^{15}N more readily breaks through to the
468 soil and rooting zone. None-the-less even the low ^{15}N treatments made modest contributions

to the vascular plant and microbial N pools, suggesting that multiple acute N deposition events could gradually enrich these N pools.

High and equal contributions of ^{15}N from $^{15}NO_3^-$ and $^{15}NH_4^+$ into the non-vascular plants- and microbial-N pools confirm that either of these N forms could enrich these pools and may affect their structure and functions in a longer term. In contrast, the considerable preference by *Salix* for NO_3^- over NH_4^+ resulted in ~4 times greater proportion of the applied $^{15}NO_3^-$ than of the applied $^{15}NH_4^+$ in their total N pool. These findings are consistent with Tye et al. (2005) who also reported a significantly greater proportion of ^{15}N in the *Salix* total N pool from $^{15}NO_3^-$ compared with $^{15}NH_4^+$ in a snowmelt N application study.

5. Conclusions

This work shows that atmospheric N deposited in extreme events during summer months is largely retained within the tundra regardless of the treatment rates and N forms, and remains so over two growing seasons as a result of conservative N cycling. Although there are differences in amount of N retained within different ecosystem compartments at lower and higher N treatments, the loss of N from one pool (e.g. non-vascular plants) is largely balanced by other pools (e.g. vascular plants and soil horizons). This also suggests that non-vascular plants were important short-term sinks and were N saturated below 1.2 g N m^{-2} yr^{-1}, while vascular plants and soil are important long term sinks. Microbial biomass in both the soil horizons help retain the deposited N in the soil during the initial partitioning and were, thus, effective buffers for N leaching. Between the two N forms, deposition rich in ammonium would primarily affect non-vascular plants whereas nitrate could affect both non-vascular and vascular plants. Substantial % contribution of ^{15}N to existing N pools suggested that extreme deposition events may already be driving eutrophication of arctic tundra ecosystems, which is of further concern given that these events are predicted to increase in

494 frequency in the future and that tundra may recover only slowly from N enrichment. In
495 conjunction with warming in this region, this may also have important implications for
496 primary productivity and hence the carbon balance of High Arctic tundra. Further studies are
497 needed to better understand long-term N retention as well as responses of plant and microbial
498 communities in tundra to such extreme N deposition events. Such studies can also improve
499 our understanding of critical N load of tundra ecosystem.
500

501 ## Acknowledgement

502 This work was funded by a Marie Curie EU Initial Training Network Grant (FP7/MC
503 ITN/ 215503) to GKP, AMO and MCP as a part of the international project NSINK (Sources,
504 sinks and impacts of atmospheric nitrogen deposition in the High Arctic). Field work was
505 based at the NERC Arctic Research Station, Ny-Ålesund, and we thank Nick Cox, Tom
506 Marshall and Ali Messy for their logistical support. Analytical and technical support at the
507 University of Sheffield was provided by Heather Walker, Irene Johnson, Dave Johnson and
508 Victoria Sloan. We also thank Filip Oulehle, Fiona Moore, Philip Blaen, Parminder Ranhotra
509 and Rajneesh Dwevedi for their field assistance.

References

Allen, S.E. (1989) Chemical analysis of ecological materials. pp 368, Blackwell Scientific, Oxford, UK.

Arens, S.J.T., Sullivan, P.F. & Welker, J.M. (2008) Nonlinear responses to nitrogen and strong interactions with nitrogen and phosphorus additions drastically alter the structure and function of a High Arctic ecosystem. *Journal of Geophysical Research*, **113** (3), Article ID G03S09.

Atkins, O.K., Villar, R. & Cummins W.R. (1993) The ability of several High Arctic plant species to utilize nitrate nitrogen under field conditions. *Oecologia*, **96**, 239-245

Baddeley, J.A., Woodin, S.J. & Alexander, I.J. (1994) Effects of increased nitrogen and phosphorus availability on the photosynthesis and nutrient relations of three Arctic dwarf shrubs from Svalbard. *Functional Ecology*, **8**, 676-685.

Beine, H.J., Engardt, M., Jaffe, Da, Hov, Ø., Holmén, K. & Stordal, F. (1996) Measurements of NOx and aerosol particles at the Ny-Ålesund Zeppelin mountain-station on svalbard: influence of local and regional pollution sources. *Atmospheric Environment* **30** (7), 1067-1079.

Bilbrough, C.J., Welker, J.M. & Bowman, W.D. (2000) Early spring nitrogen uptake by snow covered plants: a comparison of Arctic and alpine plant function under the snow pack. *Arctic, Antartic and Alpine Research*, **32**, 404-411.

535 Blaud A, Phoenix GK, Osborn AM. Variation in bacterial, archaeal and fungal community
536 structure and abundance in High Arctic tundra soil. Polar Biol 2015 **38**, 1009–1024.
537
538 Bokhorst, S., Bjerke, J.W., Street, L.E., Callaghan, T.V. & Phoenix, G.K. (2011) Impacts of
539 multiple extreme winter warming events on sub-Arctic heathland: phenology,
540 reproduction, growth, and CO2 flux responses. *Global Change Biology* **17**, 2817–
541 2830
542
543 Brooks, P.D., Stark, J.M., McInteer, B.B. *et al*. (1989) Diffusion method to prepare soil
544 extracts for automated nitrogen-15 analysis. *Soil Science Society of America Journal*,
545 53, 1701-1711.
546
547 Cassano, J.J., Uotila, P. & Lynch, A.H. (2006) Changes in synoptic weather patterns in the
548 polar regions in the twentieth and twenty-first centuries, part 1: Arctic. *International*
549 *Journal of Climatology*, **26** (8), 1027–1049.
550
551 Chapin, F.S., Shaver, G.R., Giblen, A.E. *et al*. (1995) Responses of Arctic tundra to
552 experimental and observed changes in climate. Ecology, **76**, 694-711.
553
554 Cheng, W. & Virginia, R.A. (1993) Measurements of microbial biomass in arctic tundra soils
555 using fumigation-extraction and substrate-induced respiration procedures. *Soil*
556 *Biology Biochemistry*, **25**, 135-141
557
558 Choudhary, S. (2013) Fate and impacts of acute atmospheric nitrogen depostions on High
559 Arctic tundra ecosystems. PhD thesis, University of Sheffield, UK

Clemmensen, K.E., Sorensen, P.L., Michelsen, A. *et al*. (2008) Site-dependent N uptake from N-form mixtures by arctic plants, soil microbes and ectomycorrhizal fungi. *Oecologia*, **155**, 771-783

Fischer, H., Wagenbach, P. & Kipfstuhl, J. (1998) Sulfate and nitrate firn concentrations on the Greenland ice sheet – 2. Temporal anthropogenic deposition changes. *Journal of Geophysical Research–Atmospheres*, **103**, 21935-21942.

Førland, E.J., Hanssen-Bauer, I. & Nordli, P.Ø. (1997) Climate statistics & longterm series of temperature and precipitation at Svalbard and Jan Mayen. Technical Report, The Norwegian Meteorological Institute.

Forsius, M., Posch, M., Aherne, J., Reinds, G.R., Christensen, J. & Hole, L. (2010) Assessing the impacts of long-range sulfur and nitrogen deposition on arctic and sub-arctic ecosystems. *Ambio*, **39**, 136-147

Gordon, C., Wynn, J.M. & Woodin, S.J. (2001) Impacts of increased nitrogen supply on High Arctic heath: the importance of bryophytes and phosphorus availability. *New Phytologist*, **149** (3), 461-471.

Grogan, P., Michelsen, A., Ambus, P. & Jonasson, S. (2004) Freeze–thaw regime effects on carbon and nitrogen dynamics in sub-arctic heath tundra mesocosms. *Soil Biology & Biochemistry*, **36**, 641-654

585 Henry, G.H.R., Freedman, B. & Svoboda, J. (1986) Effects of fertilization on three tundra
586 plant communities of a polar desert oasis. *Canadian Journal of Botany*, **64**, 2502-
587 2507.
588
589 Hodson, A.J., Mumford, P.N., Kohler, J. & Wynn, P.M. (2005) The High Arctic glacial
590 ecosystem: new insights from nutrient budgets. *Biochemistry*, 72, 233-256.
591
592 Hodson, A.J., Roberts, T.J., Engvall, A., Holmén, K. & Mumford, P. (2010). Glacier
593 ecosystem response to episodic nitrogen enrichment in Svalbard, European High
594 Arctic. *Biogeochemistry*, 98, 171-184.
595
596 Hyvarinen, M. & Crittenden, P.D. (1998) Relationships between atmospheric nitrogen inputs
597 and the vertical nitrogen and phosphorus concentration gradients in the lichen
598 Cladonia portentosa. *New Phytologist*, **140**, 519–530.
599
600 Jentsch, A. (2006) Extreme Climatic Events in Ecological Research. *Frontiers in Ecology*
601 *and the Environment*, **4** (5), 235-236
602
603 Jentsch, A., Kreyling, J. & Beierkuhnlein, C. (2007) A new generation of climate-change
604 experiments: events, not trends. *Frontiers in Ecology and the Environment*, 5 (7),
605 365-374
606
607 Jentsch, A., Kreyling, J., Elmer, M. *et al*., (2011) Climate extremes initiate ecosystem-
608 regulating functions while maintaining productivity. *Journal of Ecology*, **99** (3), 689–
609 702.

Jonasson, S., Michelsen, A., Schmidt, I.K., Nielsen, E.V. & Callaghan, T.V. (1996) Microbial biomass C, N and P in two arctic soils and responses to addition of NPK fertilizer and sugar: implications for plant nutrient uptake. *Oecologia*, **106**, 507-515.

Jónsdóttir, I.S., Callaghan, T.V. & Lee, J.A. (1995) Fate of added nitrogen in a moss-sedge Arctic community and effects of increased nitrogen deposition. *The Science of the Total Environment*, **160/161**, 677-685

Jung, V., Cécile H.A., Cyrille V., Georges K., Grégory L. & Thomas S. (2014) Intraspecific Trait Variability Mediates the Response of Subalpine Grassland Communities to Extreme Drought Events'. *Journal of Ecology*, **102** (1), 45–53.

Klonecki, A., Hess, P., Emmons, L., Smith, L., Orlando, J. & Blake, D. (2003) Seasonal changes in the transport of pollutants into the Arctic troposphere-model study. *Journal of Geophysical Research*, **108** (4), 21.

Kotanen, P.M. (2002) Fates of added nitrogen in freshwater arctic wetlands grazed by snow geese: the role of mosses. *Arctic, Antarctic, and Alpine Research*, **34**, 219-225.

Kühnel, R., Roberts, T.J., Björkman, M.P., Isaksson, E., Aas, W., Holmén. K. & Ström, J. (2011) 20-year climatology of NO_3^- and NH_4^+ wet deposition at Ny-Ålesund, Svalbard. *Advances in Meteorology*, Article ID 406508

634 Kühnel, R., Björkman, M.P., Vega, C., Hodson, A., Isaksson, E. & Ström, J. (2013) Reactive
635 nitrogen and sulphate wet deposition at Zeppelin Station, Ny-Ålesund, Svalbard.
636 *Polar Research*, **32**, Article ID 19136
637
638 Longton, R.E. (1997) The role of bryophytes and lichens in polar ecosystems. *Ecology of*
639 *Arctic Environments*, 69-96.
640
641 Madan, N.J., Deacon, L.J. & Robinson, C.H. (2007) Greater nitrogen and/or phosphorus
642 availability increase plant species' cover and diversity at a High Arctic polar
643 semidesert. *Polar Biology*, **30**, 559-570.
644
645 Mäkipää, R. (1995) Sensitivity of forest-floor mosses in boreal forests to nitrogen and
646 sulphur deposition. Water, Air, and Soil Pollution, **85** (3), 1239-1244
647
648 McKane, R.B., Johnson, L.C., Shaver, G.R. *et al*. (2002) Resource-based niches provide a
649 basis for plant species diversity and dominance in arctic tundra. *Nature*, **415**, 68–71.
650
651 Nordin, A., Schmidt, I.K. & Shaver, G.R. (2004) Nitrogen uptake by arctic soil microbes and
652 plants in relation to soil nitrogen. *Ecology*, **85** (4), 955-962.
653
654 Peters, G.P., Nilssen, T.B., Lindholt, L. *et al*. (2011) Future emissions from shipping and
655 petroleum activities in the Arctic. *Atmospheric Chemistry and Physics*, **11** (11), 5305-
656 5320.
657

658 Phoenix, G.K., Hicks, W.K., Cinderby, S. *et al.* (2006) Atmospheric nitrogen deposition in
659 world biodiversity hotspots: the need for a greater global perspective in assessing N
660 deposition impacts. *Global Change Biology*, **12**, 470-476.
661
662 Phoenix, G.K., Emmett, B.A., Caporn, S.J.M. *et al.* (2012) Impacts of atmospheric nitrogen
663 deposition: responses of multiple plant and soil parameters across contrasting
664 ecosystems in long-term field experiments. *Global Change Biology*, **18**, 1197-1215.
665
666 Powlson, D.A. & Barraclough, D. (1993) Mineralisation and assimilation in soil–plant
667 systems. In: Nitrogen Isotope Techniques (eds Knowles R, Blackburn H), pp. 209-
668 221. Academic Press, San Diego.
669
670 Press, M.C. & Lee, J.A. (1982) Nitrate reductase activity of sphagnum species in the south
671 pennines. *New Phytologist*, **92**, 487-494.
672
673 Rahn, K.A., Borys, R.D. & Shaw, G.E. (1977) The Asian source of Arctic haze bands.
674 *Nature*, **268**, 713-715
675
676 Sala, O.E., Chapin, III F.S., Armesto, J.J. *et al*. (2000) Global biodiversity scenarios for the
677 year 2100. *Science*, **287**, 1770-1774.
678
679 Schmidt, I.K., Ruess, L., Baath, E., Michelsen, A., Ekelund, E. & Jonasson, S. (2000). Long-
680 term manipulation of the microbial community and microfauna of two contrasting
681 subarctic heaths by addition of fungicide, bactericide, carbon and fertilizer. *Soil*
682 *Biology and Biochemistry*, **32**, 707-720.

683

684 Serreze, M.C., Holland, M.M. & Stroeve, J. (2007) Perspectives on the Arctic's shrinking
685 sea-ice cover. *Science*, **315**,1533 –1536.

686

687 Shaver, G.R. & Chapin, III F.S. (1980) Response to fertilization by various plant growth
688 forms in an Alaskan tundra: nutrient accumulation and growth. *Ecology*, **61**, 662-675.

689

690 Shaver, G.R., & Chapin, III F.S. (1995). Long-term responses to factorial, NPK fertilizer
691 treatment by Alaskan wet and moist tundra sedge species. *Ecography*, **18**, 259-275.

692

693 Simoẽs, J.C. & Zagorodnov, V.S. (2001) The record of anthropogenic pollution in snow and
694 ice in Svalbard, Norway. *Atmospheric Environment*, **35**, 403-413.

695

696 Smith, M.D. (2011) An ecological perspective on extreme climatic events: a synthetic
697 definition and framework to guide future research. *Journal of Ecology*, 99(3), pp.656–
698 663.

699

700 Street, L., Burns, N. & Woodin, S. (2015) Slow recovery of High Arctic heath communities
701 from nitrogen enrichment. *New Phytologist*, doi: 10.1111/nph.13265

702

703 Templer, P.H., Mack, M.C., Chapin, III F.S., *et al*. (2012) Sinks for nitrogen inputs in
704 terrestrial ecosystems: a meta-analysis of 15N tracer field studies. *Ecology*, **93** (8),
705 1816–1829

706

707 Turetsky, M.R. (2003) The role of bryophytes in carbon and nitrogen cycling. *The*

Bryologist, **106**, 395-409.

Tye, A.M., Young, S.D., Crout, N.M.J., West, H.M., Stapleton, L.M., Poulton, P.R. & Laybourn-Parry, J. (2005) The fate of ^{15}N added to High Arctic tundra to mimic increased inputs of atmospheric N released from a melting snowpack. *Global Change Biology*, **11**, 1640–1654.

Vance, E.D., Brookes, P.C. & Jenkinson, D.S. (1987) An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, **19**, 703–707.

Woodin, S.J. (1997) Effects of acid deposition on Arctic vegetation. In: *Ecology of Arctic Environments* (edsWoodin SJ, Marquiss M), 19–239. Blackwell Science, Oxford.

Yano, Y., Shaver, G.R., Giblin, A.E., Rastetter, E.B. & Nadelhoffer, K.J. (2010) Nitrogen dynamics in a small arctic watershed: retention and downhill movement of 15N. *Ecological Monographs*, **80**, 331-351

733 **Table 1.** Mg ^{15}N per L soil water (means ± SE) from the plots treated with different doses
734 and forms of N after 7, 21 and 347 days of ^{15}N application. Applications of $^{15}NH_4{}^{15}NO_3$ at
735 rates of 0 g N $m^{-2}yr^{-1}$ (Control), 0.04 g N $m^{-2}yr^{-1}$ (0.04N), 0.4 g N $m^{-2}yr^{-1}$ (0.4N), and 1.2 g
736 N $m^{-2}yr^{-1}$ (1.2N), and $Na^{15}NO_3$ (NO_3^-) and $^{15}NH_4Cl$ (NH_4^+) at rates of 0.4 g N $m^{-2}yr^{-1}$ rates
737 were applied in July 2010 and sampled after 7, 21 and 347 days of the treatment. Values
738 sharing the same letter within a date are not significantly different (Tukey HSD, $P < 0.05$). ±
739 are standard errors (N = 5).
740

	Days after ^{15}N application		
Treatment	**7**	**21**	**347**
Control	0.004 ± 0.001[a]	0.004 ± 0.001	0.002 ± 0.0002
0.04N	0.005 ± 0.003[a]	0.002 ± 0.0003	0.001 ± 0.0004
0.4N	0.48 ± 0.31[ab]	0.08 ± 0.05	0.01 ± 0.007
1.2N	4.85 ± 2.23[b]	3.22 ± 2.21	0.02 ± 0.01
NO_3^-	1.14 ± 0.57[b]	0.19 ± 0.13	0.005 ± 0.002
NH_4^+	0.06 ± 0.05[a]	0.006 ± 0.003	0.001 ± 0.0003

741
742

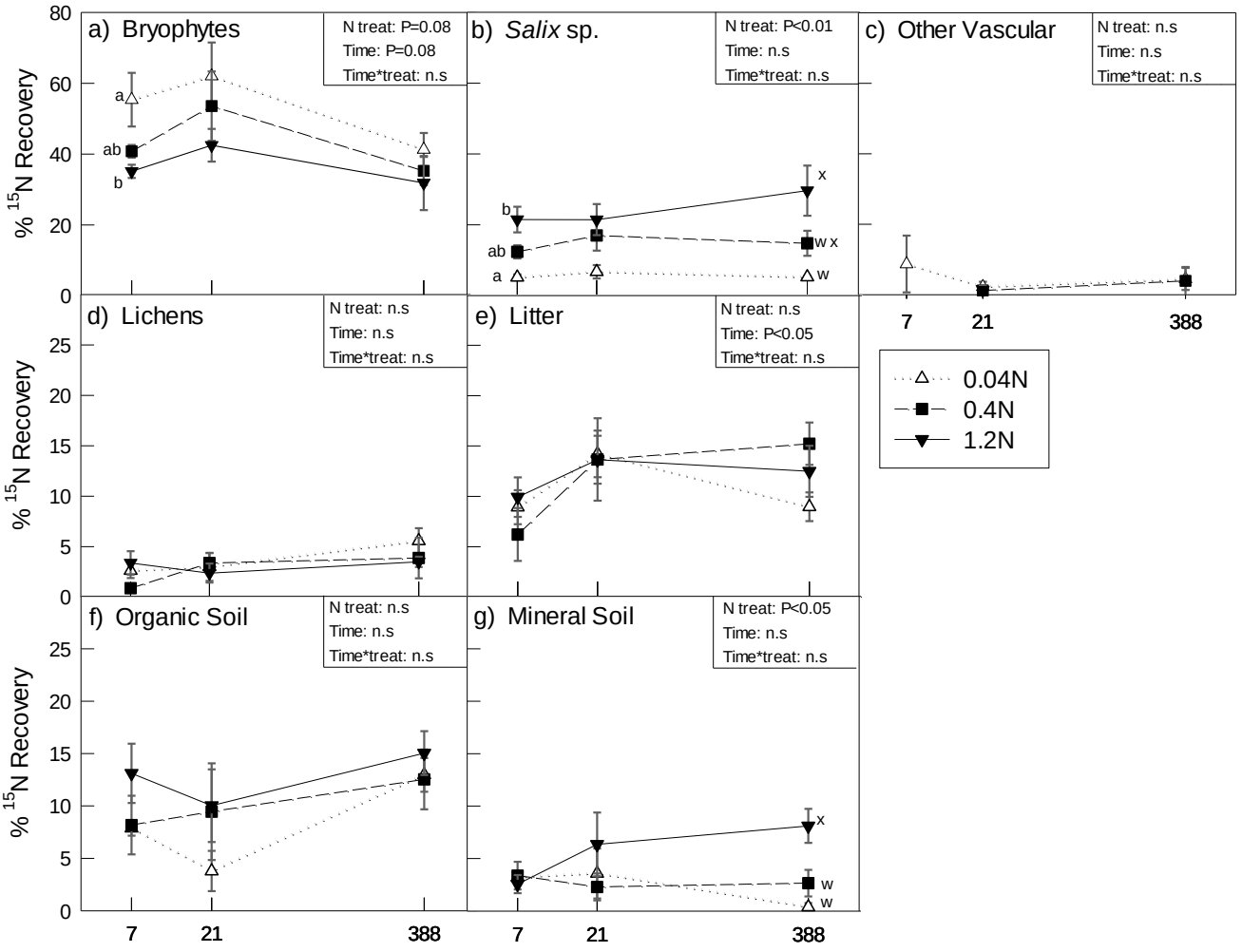


Figure 1. Mean % retention of applied ^{15}N (from $^{15}NH_4{}^{15}NO_3$) at 7, 21 and 388 days after treatment with applications of 0.04 g N $m^{-2}yr^{-1}$ (0.04N), 4 g N $m^{-2}yr^{-1}$ (0.4N), and 1.2 g N $m^{-2}yr^{-1}$ (1.2N). Dates of 7, 21 and 388 days correspond to the initial partitioning of ^{15}N, and recovery at the end of the first and second growing season respectively. Error bars represent one standard error and have been greyed for the clarity. Repeated measures ANOVA results shown for: N treat = N treatments, Time = time, Time*treatment = Time-treatment interaction; n.s = not significant. Different letters indicate significance within the same time point (Tukey HSD). Note the different scales of Y axes: 0-80 for a-c, 0-30 for d-g.

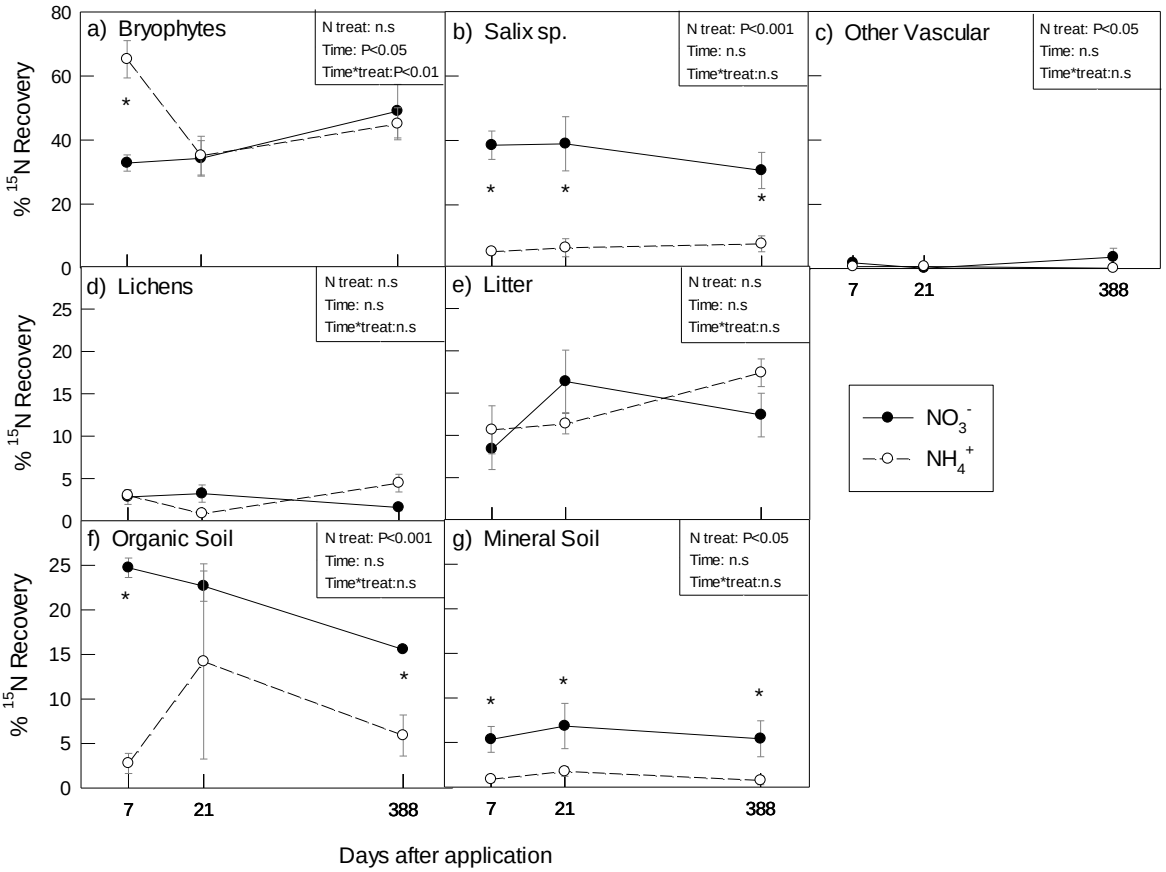
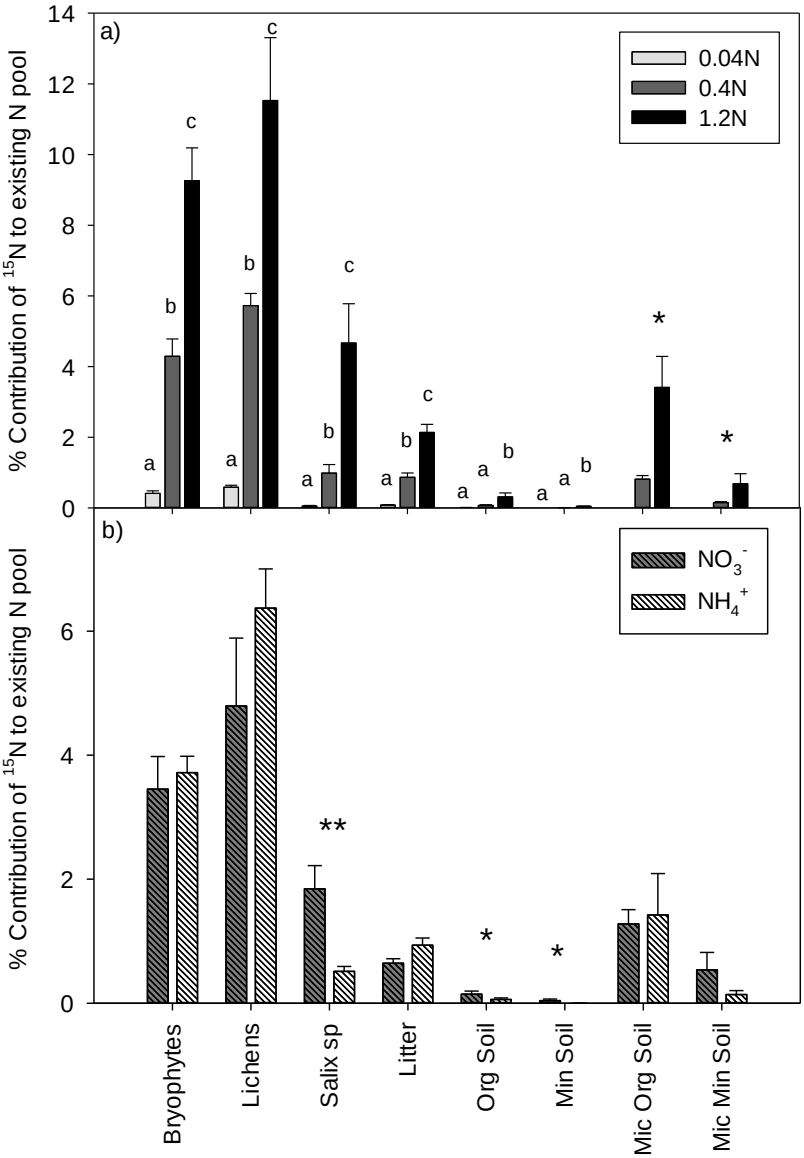


Figure 2. Mean % retention of applied ^{15}N from separate applications of $Na^{15}NO_3$ (NO_3^-) and $^{15}NH_4Cl$ (NH_4^+) at a rate of 0.4 g N $m^{-2}yr^{-1}$. Samples taken at 7, 21 and 388 days following treatment. Error bars represent one standard error (N = 5) and have been greyed for clarity. Repeated measures ANOVA results shown with abbreviations as for Fig 1. * indicates significant difference between $^{15}NO_3^-$ and $^{15}NH_4^+$ (t-test). Note the different scales of Y axes.



765

766 **Figure 3.** Percentage ^{15}N contribution to the total N content of each pool after 388 days
767 following application of (a) $^{15}NH_4{}^{15}NO_3$, and (b) $Na^{15}NO_3$ (NO_3^-) and $^{15}NH_4Cl$ (NH_4^+).
768 Applications rates are for $^{15}NH_4{}^{15}NO_3$: 0.04 g N $m^{-2}yr^{-1}$ (0.04N), 0.4 g N $m^{-2}yr^{-1}$ (0.4N), and
769 1.2 g N $m^{-2}yr^{-1}$ (1.2N), and 0.4 g N $m^{-2}yr^{-1}$ for the separate $Na^{15}NO_3$ (NO_3^-) and $^{15}NH_4Cl$
770 (NH_4^+) applications. Error bars represent one standard error (N = 5). Error bars in (a) that
771 share the same letter are not significantly different (Tukey HSD, $P < 0.05$) and in (b)
772 statistically significant differences shown as ** $P < 0.01$; * $P < 0.05$ (t-test). A t-test was
773 performed for microbial fractions (Mic) in organic soil (Org soil) and mineral soil (Min soil)
774 in graph (a) as there were just two treatments (0.4N and 1.2N) sampled.

Appendix A

Supplementary material and methods

Experimental design

In July 2009, 25 plots (1.5m × 1.5m) were established in approximately a 600 m^2 area of tundra (Blaud *et al.*, 2015). On 21 and 22 August 2009, non-labelled NH_4NO_3 solution was applied at rates of 0 (controls with distilled water only, referred to as "Cw"), 0.04 and 0.4 g N m^{-2} yr^{-1} ("0.04N" and "0.4N", five replicate plots each), with half of the total amount applied per day. ^{15}N applications were made in the following year (July 2010).

Precipitation and atmospheric N deposition data of Ny-Ålesund were obtained from the Norwegian Institute for Air Research (NILU, Eklima, http://ebas.nilu.no) and air temperature data were obtained from the Norwegian Meteorological Institute. In 2011, air and soil temperatures were also measured at the experimental site throughout the growing season using dataloggers (Tinytag Transit, Gemini, Chichester, UK).

Soil pH was determined in a 1:5 soil:water suspension.

Site N deposition, climate and vegetation

Cumulative precipitation during the summer (July-August) was 17.5 mm and 57.7 mm in 2010 and 2011, respectively. Average inorganic N inputs during the summer precipitation in 2010 and 2011 were low (0.0044 g NO_3^- m^{-2} and 0.0040 g NH_4^+ m^{-2}, respectively), with total annual N deposition of 0.064 and 0.112 g N m^{-2} yr^{-1} in those years, respectively. Mean air temperatures during the growing season (July-August) at Ny-Ålesund were 4.6 (±2.1) ºC and 6.0 (±2.2) ºC for 2010 and 2011, respectively. Mean pH of the organic and mineral horizon was 6.68 and 7.19, respectively. Vegetation cover at our experimental site was dominated by bryophytes (~40%), *Salix polaris* (~30%), lichens (~16%) and other

vascular plants. Further details on soils and vegetation N stocks are provided in Tables A.1 & A.2.

Table A.1. Soil pH, N and C content, and C/N ratios (means ± SE) in control plots. Extractability factors of 0.40 for microbial N (N_{mic}) and 0.35 for microbial C (C_{mic}) were assumed. DW represents dry weight of the soil and BD is below detection limit. N_{total} is the total bulk soil N, N_{inorg} are the KCl extractable fractions, C_{total} is the total soil carbon; C_{CaCO3} is the soil inorganic carbon.

Parameter	Organic Layer (O)	Mineral Layer (A)
Soil pH in H_2O	6.65 ± 0.05	7.21 ± 0.07
N_{total} (mg g^{-1} DW)	8.98 ± 0.66	4.75 ± 0.62
N_{inorg} (NO_3-N mg g^{-1} DW)	0.0007 ± 0.0001	0.0005 ± 0.0001
N_{inorg} (NH_4-N mg g^{-1} DW)	0.0073 ± 0.0005	0.0025 ± 0.0003
N_{mic} (mg g^{-1} DW)	0.48 ± 0.09	0.06 ± 0.01
C_{total} (mg g^{-1} DW)	168.09 ± 9.76	49.21 ± 6.00
C_{CaCO3} (mg g^{-1} DW)	BD	BD
C_{mic} (mg g^{-1} DW)	3.67 ± 0.62	1.56 ± 0.28
C_{total} / N_{total}	20.10 ± 1.91	10.54 ± 0.28
C_{mic} / N_{mic}	7.15 ± 0.70	18.54 ± 3.28

Table A.2. Mean dry matter and C and N stocks (gm^{-2}) of the soil and plant fractions. Values are means (± SE) of 20 plots and all samplings as there were no significant changes.

Fractions	Dry matter ($g\ m^{-2}$)	N ($g\ m^{-2}$)	C ($g\ m^{-2}$)
Soil Organic Layer	6323 ± 111	63 ± 1.77	1011 ± 26.39
Range	12287-4362	98-25	1993-492
Soil Mineral Layer	19788 ± 559	113 ± 5.38	1100 ± 46.61
Range	32418-10046	230-31	2050-345
Bryophytes	549 ± 27	4.07 ± 0.20	198 ± 10
Range	1554-112	10-0.98	581-37
Lichens	42 ± 3.44	0.19 ± 0.02	16 ± 1.41
Range	160-1.70	0.72-0.01	83-0.60
Salix sp.	398 ± 13	5 ± 0.19	187 ± 6.78
Range	737-184	11-1.72	379-83
Other vascular plants	69 ± 13	0.79 ± 0.13	29 ± 5.82
Range	420-3	4-0.06	173-1.46
Equisetum	3 ± 0.57	0.03 ± 0.01	1.21 ± 0.29
Range	6.3-0.20	0.07-0.01	3.47-0.11
Graminoids	9 ± 5.70	0.07 ± 0.04	3.22 ± 2.11
Range	26-1.40	0.20-0.01	10-0.62
Litter	454 ± 21	7 ± 0.35	179 ± 8.93
Range	1125-136	16-1.07	443-47

Appendix B

Supplementary results & discussions

Fate of nitrate ($^{15}NO_3^-$) and ammonium ($^{15}NH_4^+$)

Total recovery of ^{15}N from $^{15}NO_3^-$ in plant, soil and litter pools exceeded 100% (109 to 115%) whereas recovery from $^{15}NH_4^+$ ranged between 71 and 96%. Overall $^{15}NO_3^-$ was taken up by all of the plant species (total "all-plant" fractions retained between 65% and 94%

826 of N from $^{15}NO_3^-$), while ^{15}N from $^{15}NH_4^+$ was mainly present in bryophytes (42-68%) with
827 total plant fractions retaining between 50 and 76%. of N from $^{15}NH_4^+$.

828 ***Non-vascular plants*:** Bryophytes showed no difference in overall retention of the ^{15}N
829 from NO_3^- and NH_4^+, retaining between 33 and 49% of N applied as $^{15}NO_3^-$, and 35 and 65%
830 applied as $^{15}NH_4^+$ (Fig. 2a). The only difference was a significant greater retention of ^{15}N
831 from NH_4^+ at day 7 (d.f=7, t=-5.06, P<0.01). Likewise, lichens did not show any differences
832 in ^{15}N retention from either form, retaining between 2 and 3% from NO_3^- and 1 and 5% from
833 NH_4^+ (Fig. 2d).

834 ***Vascular plants***: In contrast, there were large significant differences in the ^{15}N
835 retention of the two N forms in *Salix polaris* at all of the sampling days, with much greater
836 retention of ^{15}N from NO_3^- (31 to 39%) compared to ^{15}N from NH_4^+ (6 to 8%) (repeated
837 measures ANOVA: df=1,8, F=95.73, P<0.001) (Fig. 2b). For all the other vascular plants
838 (Fig 2c), there were not enough plants present on the day 7 and 21 harvests for comparison
839 but at day 388, recovery from NO_3^- was ~5 times greater than from NH_4^+ (no statistical test
840 performed due to limited samples).

841 ***Litter*:** As with non-vascular plants, there were no differences between the retention
842 of N from $^{15}NO_3^-$ and $^{15}NH_4^+$, with an average retention of ~13% of the applied ^{15}N for either
843 form (Fig. 2e).

844 ***Organic and mineral soil*:** Organic soil showed greater retention of $^{15}NO_3^-$ (16 to
845 25%) compared to $^{15}NH_4^+$ (3 to 14%) for all of the sampling days (repeated measures
846 ANOVA: d.f=1,8, F=155.57, P<0.001) (Fig. 2f). Microbial biomass in the organic layer
847 showed no preference for either of the N forms applied and retained between 37 and 70% of
848 the ^{15}N pools in the organic soil applied in either form. As with the organic soil, the mineral
849 soil showed greater recovery of the applied ^{15}N from NO_3^- (5 to 7%) compared to ^{15}N from
850 NH_4^+ (1 to 2%) across all sampling days (repeated measures ANOVA: d.f=1,8, F=8.29,

P<0.05) (Fig. 2g). Microbial biomass in the mineral soil did not show any preference for either N form and immobilised between 16 and 51% of the ^{15}N in the mineral soil pool.

Soil water: There was ~20 times more $^{15}NO_3^-$ (1 mg ^{15}N L^{-1} soil water) than $^{15}NH_4^+$ in the soil water by day 7 (repeated measures ANOVA, d.f.=2,11, F=6.19, P<0.05; Tukey, P<0.05), and this difference decreased to 3 times more $^{15}NO_3^-$ (0.02 mg ^{15}N per L water) than $^{15}NH_4^+$ by day 347 (time*treatment, repeated measures ANOVA, d.f.=4, 22, F=4.06, P<0.05) (Table 1). Approximations suggest a maximum loss of 2% of the applied $^{15}NO_3^-$ and <0.1% of the applied $^{15}NH_4^+$ at day 7.

Table B.1. Total % recovery of ^{15}N (means ± SE). Applications of $^{15}NH_4{}^{15}NO_3$ at 0.04 g N $m^{-2}yr^{-1}$ (0.04N), 0.4 g N $m^{-2}yr^{-1}$ (0.4N), and 1.2 g N $m^{-2}yr^{-1}$ (1.2N) rates, and $Na^{15}NO_3$ (NO_3^-) and $^{15}NH_4Cl$ (NH_4^+) at 0.4 g N $m^{-2}yr^{-1}$ rates were applied from 15-19 July 2010 and sampled after 7, 21 and 388 days of the treatment. No statistically significant differences found between the treatments or within the days. ± are the standard errors (N = 5).

Treatments	D+7	D+21	D+388
0.04N	85 ± 7.6	95 ± 9.6	80 ± 4.7
0.4N	67 ± 2.2	100 ± 6.7	88 ± 3.2
1.2N	86 ± 6.8	98 ± 11.1	107 ± 13
NO_3^-	109 ± 3.7	116 ± 5.1	111 ± 10.3
NH_4^+	96 ± 6	71 ± 11.8	81 ± 3.3

Total recovery of nitrate ($^{15}NO_3^-$) and ammonium ($^{15}NH_4^+$)

Unexpectedly, total recovery of the applied $^{15}NO_3^-$ (109-115%) was greater than that of $^{15}NH_4^+$ (71-96%). However, a smaller loss of the more mobile NO_3^- seems unlikely since

lower concentrations of $^{15}NH_4^+$ than $^{15}NO_3^-$ were found in the leachate, and there would be little NH_3 volatilization given the cool temperatures and wet conditions with rainfall following the N applications (Nömmik & Vahtras 1982). Similar results were observed by Templer *et al*., (2012) in a meta-analysis of six tundra (combined arctic and alpine) sites. However, the reasons for such higher recovery of NO_3^- compared to NH_4^+, where soil conditions do not support NH_3 volatilization, are still not well known. Nevertheless, given total recovery of both N forms were high and not greatly different, deposition rich in either forms have a capacity to affect different compartments of the tundra ecosystem. Deposition rich in nitrates could impact almost all the compartments of this ecosystem whereas ammonium deposition would primarily affect the non-vascular plants.