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Plant functional type affects nitrogen use efficiency in High-Arctic tundra

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Highlights

- Limited effect of soil temperature on net N mineralization.
- Soil freeze limits net N nitrification, thus prevent N leaching during the winter.
- Plant functional types vary in the soil depth from which they take up N.
- Nitrogen added above-ground will have different effects to N mineralised within the soil.

Abstract

To unravel the potential effects of climate warming on soil N availability in a high Arctic tundra ecosystem we studied temperature effects on soil mineralization, and N uptake from different soil depths (-3, -10 and -30 cm) by tundra plants. Uptake was assessed using ^{15}N tracer injected directly into mineral soil as $^{15}NH_4Cl$ solution to specifically mimic altered N availability from enhanced mineralization. Net N mineralization rates were very low, suggesting that N is strongly limiting in this system. There was no apparent temperature effect (-2°, 5°, 10°C) on mineralization, but net nitrification was strongly limited by temperature – under the -2°C treatment no nitrification

occurred. As a consequence of ongoing mineralization and limited nitrification under freezing conditions, mineral NH_4 may accumulate during the winter season and be available for plant uptake without risk of loss via NO_3^- leaching immediately after snowmelt. Nitrogen uptake niches were clearly stratified by depth. Graminoids (*Carex misandra* and *Luzula arctica*) were most effective at taking up N from deep soil horizons, and recovery in graminoid biomass after one year was independent of ^{15}N injection depth. Recovery of N by the dwarf shrub *Salix polaris* was significantly higher following shallow application (-3 cm) compared to deeper treatments (-10 and -30 cm). Lichens and mosses also showed a decline in N uptake with application depth, and very little N was recovered by lichens and mosses even from -3 cm, in contrast to the strong uptake that has been observed in mosses when N is applied to the vegetation surface. The ability of graminoids to access nutrients from deeper mineral soil may give them an advantage over mosses and dwarf shrubs in warmer high Arctic tundra in acquiring limited available nutrient resources.

Keywords: Arctic, nitrogen, isotope, mineralization, nitrification, tundra

1. Introduction

Among the Earth's major biomes, the Arctic is responding most rapidly to global warming (Chapin et al., 2005; Spielhagen et al., 2011). Rising temperatures may cause perturbation in the terrestrial carbon balance due to permafrost thawing (Schuur and Abbott, 2011) and/or increased mineralization of organic matter, releasing plant growth limiting nutrients and thereby increasing the productivity of tundra plants (Chapin et al., 2005; Schimel et al., 2004; Sturm et al., 2001). The Arctic supports globally important biodiversity and has a major influence on the global climate, so it is important to understand how its ecosystems are likely to change in terms of soil carbon, plant cover and vegetation structure. Predicting plant responses to these changes depends on understanding the dynamics of N mineralization, uptake and transport during the short Arctic growing season.

Besides other environmental changes, increased nutrient availability is of key concern for future change in arctic vegetation (Dormann and Woodin, 2002). For example, it has been postulated that snow-shrub interactions have created a positive feedback whereby warming increases nutrient availability, leading to shrub growth and expansion, which in turn leads to deeper snow cover over the shrub canopy, raising winter temperatures and causing further nutrient release (Sturm et al., 2005). Recently, Myers-Smith and Hik (2013) found that abiotic influences of shrub canopy cover alone on nutrient dynamics were weaker than previously asserted. However, increases in temperatures predicted for high latitudes may not necessarily cause greater rates of nitrogen (N) mineralization (Nadelhoffer et al., 1991; Robinson, 2002). Despite generally lower net N mineralization in the Arctic compared to temperate ecosystems, N mineralization varies widely across different types of arctic ecosystems (Robinson et al., 1995). Thus understanding climate effects (altered soil temperature, moisture) on N availability is of great importance in strongly N-limited arctic ecosystems.

Based on a recent synthesis of warming experiments in the Arctic, Elmendorf et al. (2012) have shown that shrubs are expanding most in warmer tundra regions, whilst graminoids and forbs are expanding predominantly in colder tundra (areas with a mean July temperature < 7 °C). They hypothesise that this might be due to the fact that the tallest growth forms in colder tundra areas tend to be herbs, which can easily prostrate dwarf shrubs, whereas the tallest growth forms in warmer tundra areas are woody (low and tall) shrubs. However, competition for light is only one aspect of interspecific plant competition, and the balance between plant functional types may be affected by availability of other resources. There is evidence for different and complementary strategies to meet N demand by different plant functional groups (Kahmen et al., 2006). Hitherto little attention has been paid to the potential separation of N acquisition niches in high Arctic soils, in contrast to studies in warm tundra (Grogan and Jonasson, 2003; McKane et al., 2002), or tropical and temperate ecosystems (Göransson et al., 2008; Houle et al., 2014; Rowe et al., 2001). The depth at which N uptake occurs is likely to have considerable effects on system-level N use efficiency

74 (Jónsdóttir et al., 1995). Nitrogen availability near the surface will be relatively high during the spring
75 thaw, as a result of N inputs from ice and mineralisation and because water is available (Figure 1).
76 However, near-surface water and N availability tend to decline rapidly in the dry Arctic spring. Later
77 in the growing season the inorganic N remaining in the system will mainly be deeper in the soil, from
78 where it can only be recycled into the terrestrial ecosystem by deeper-rooting plants.
79 Ongoing changes in tundra plant composition may have further direct consequences for soil organic
80 matter (SOM) accumulation due to altered litter production and quality, and consequent changes in
81 SOM decomposition. After twenty years of a warming experiment in a moist acidic tussock tundra
82 ecosystem, plant carbon stocks had increased by 50%, without changes in net soil carbon storage
83 (Sistla et al., 2013). On the other hand, another fertilization experiment on the same type of
84 ecosystem similarly stimulated plant productivity, but also stimulated decomposition of soil organic
85 matter, leading to net loss of carbon from the ecosystem after 20 years of fertilization with N and
86 phosphorus (Mack et al., 2004). A common motive of the fertilization experiments is to mimic the
87 higher availability of limiting nutrients expected under changing climate due to higher mineralization
88 rates, or in the case of N to increased deposition. However, whilst surface N fertilisation may provide
89 a reasonable representation of the effects of N deposition, it will not reflect the effects of N
90 mineralization in deeper soil, where the balance of N acquisition structures between plant functional
91 types is different. Surface applications may also lead to a proliferation of roots towards the soil
92 surface, thus disadvantaging deep-rooted species such as graminoids (Mack et al., 2004).
93 We conducted two sets of experiments specifically designed to: i) study *ex situ* temperature effects
94 on N mineralization in soil profile samples; and ii) track the *in situ* uptake of ^{15}N added into the
95 mineral soil at different depths by tundra plants, both in the short term (10 days after ^{15}N addition)
96 and longer term (one year after addition). We used studies of temperature effects on mineralization
97 and of N uptake from different soil depths to explore how these factors may determine ecosystem
98 responses to warming. Specifically we tested whether soil net N mineralization rates are
99 temperature-dependent over a temperature range from -2°C to +10°C. Furthermore, based on

previous work (Elmendorf et al., 2012) we predicted that graminoids in the high Arctic may have advantages in a warming climate over other functional groups (lichens, bryophytes and dwarf shrub) in competition for mineral N in the soil.

2. Materials and methods

2.1 Site description

Experiments were done in a high Arctic semi-desert tundra ecosystem surrounding the Kongsfjorden, approximately 2 km west from Ny Ålesund, Svalbard, at the site Leirhaugen (78° 55' N, 11° 49' E, 55 m a.s.l.). The area is underlain by continuous permafrost and the mineral soil, developed over limestone, consists of silty clay with interspersed stones. This is overlaid by a thin and discontinuous organic layer. Soil pH increases from 5.71 in the organic horizon to 7.36 in the mineral soil at 20 – 30 cm depth with mean C/N of 18 in the organic soil and 15 in the mineral soil. Mean annual air temperature over last two decades was -4.5 °C, with July temperatures ranging from 4.6 to 6.9 °C. Annual precipitation is ≈370 mm, which mostly falls as snow between September and May, with the driest month in May (17 mm) and wettest month in September (46 mm). Soil thaw depth is approximately 1 m during the growing season (Roth and Boike, 2001). Tundra vegetation is exposed to reindeer grazing. Reindeer in Ny-Ålesund are descended from animals introduced to the area in 1978, since which time the population has fluctuated with densities up to 0.89 individuals km^{-2} (Aanes et al., 2002; Hayashi et al., 2014).

2.2 Net mineralization experiment

For the net N mineralization and potential net nitrification experiments, five replicate mineral soil horizons were sampled using a cylindrical soil corer (diameter 4.6 cm) on 4th July 2011, shortly after snow melt. The mineral soil profile was divided into separate layers of 0 - 10 cm, 10 - 20 cm and 20 -

30 cm. Each replicate consisted of three bulked soil samples retrieved by the corer. The organic soil was sampled using a 10 x 10 cm plastic frame. Organic and mineral soil was sieved (5 mm and 2 mm, respectively), dried (105 °C) and homogenized samples analysed for total organic carbon and N (Flash 2000, Thermo Scientific).

Within one week after soil sampling, fresh sieved soil samples were transported at 2 °C to the UK for further laboratory soil incubation. Moist soil (60 – 70 % of water holding capacity) was incubated in 100 ml flasks sealed with perforated parafilm at -2 °C, 5 °C and 10 °C without substrate addition for 6 weeks. Soil moisture was checked weekly and distilled water was added when necessary to maintain the original soil moisture. Soil samples were extracted with 2 M KCl (extractant/soil ratio was 5 : 1 for organic and 2 : 1 for mineral soil, v/w) after shaking for 1 h; then, the soil slurry was centrifuged (4,000 *g*, 10 min) and the supernatant filtered through a 0.45 µm cellulose filter. The extract was analysed for NH_4^+ and NO_3^- contents by automated discrete spectrophotometer (AQ2, Seal Analytical). The net ammonification and nitrification rates were calculated after two and six weeks as the difference in extractable NH_4^+ and NO_3^-, respectively, between the measurement date and the start date of the incubation, divided by the number of days. The net N mineralization rate was calculated as the sum of net ammonification and net nitrification rates (Santruckova et al., 2009).

In addition to the soil sampling for mineralization incubations, three soil pits were dug with a spade and samples for root density calculations were retrieved by excavating laterally from the pit, to minimise disturbance of the sample. Organic soil samples (n = 3; average volume = 35 cm^3) and mineral soil samples in 0 - 10 cm, 10 - 20 cm, 20 - 30 cm, 30 - 40 cm horizons (n = 3; average volume = 145 cm^3) were used for root length density assessment. Roots were carefully separated from soil by gently flushing with water, and then distributed over squared paper. Horizontal and vertical grid intersections were counted and root density calculated according to the line intercept method (Tennant, 1975).

2.3 Subsurface ^{15}N addition experiment

151 Labelled ^{15}N (98 atom %) was applied to the plots in a solution of $^{15}NH_4Cl$ at a rate of 0.4 g N m^{-2} on
152 24th July 2010. Applications consisted of three treatments, with injection of the solution directly into
153 the mineral soil at depths of -3 cm, -10 cm and -30 cm relative to the mineral soil surface. Altogether
154 15 small plots (5 replicates for each treatment depth) were established, and NH_4Cl solution was
155 injected as follows: plots (900 cm^2) were divided into 9 small subplots (100 cm^2) and in the centre of
156 each subplot a plastic tube was installed to the respective depth (Figure 2b). NH_4Cl solution (1 ml)
157 was then injected into each of the 9 tubes per plot, carefully to prevent overflow. The method aimed
158 to distribute solution as evenly as possibly across the plot area, at the defined depth.
159 Vegetation for soil sampling was retrieved from two replicate subplots (10 cm x 10 cm) placed
160 crosswise within the 30 cm x 30 cm plot. Although the organic layer is discontinuous across the
161 landscape, the subplots were positioned to include areas with an organic layer and with all four plant
162 functional types. Vegetation samples were collected from the main taxa, representing four functional
163 groups, which were present in all plots. These were Lichens (all species present), Mosses (all species
164 present), Dwarf shrub (*Salix polaris* Wahlenb.) and Graminoid species (*Carex misandra* R. Br. and
165 *Luzula arctica* Blytt). *Salix polaris* is extremely short-growing and is overtopped by graminoids in this
166 system. Other flowering plant species (*Saxifraga sp., Polygonum viviparum* L.*, Oxyria digyna* (L.) Hill.)
167 were present in some plots, but as they were absent from other plots and accounted for a relatively
168 small part of the overall plant cover, they were not included in the analysis.
169 To assess short-term (10 days) assimilation of ^{15}N in aboveground plant biomass, and to avoid
170 excessive disturbance to the vegetation, only parts of aboveground tissues (leaves, stem and spike
171 for graminoid spp.; leaves, stem and buds for *Salix polaris*; and aboveground tissue of mosses) were
172 taken for qualitative analysis. Samples were collected on 3rd August 2010 (Figure 2a).
173 On 8th August 2011, one year after ^{15}N application, vegetation was harvested from the 10 x 10 cm
174 subplots by removal of the thin organic layer with all the plant material still in place. Plant material
175 was sorted into four fractions (Lichens, Mosses, Dwarf shrubs and Graminoids, as above) and divided
176 into the above-ground and below-ground parts. The remaining organic soil (humus and litter

177 fraction) was sieved (5 mm) and stones removed. From each plot, mineral soil was retrieved by soil
178 corer as described above, and divided into 0 – 10 cm, 10 – 20 cm and 20 – 30 cm layers. All
179 vegetation and soil fractions were weighed and sub-sampled for further analysis. Plant material was
180 dried at 60 °C and soil samples were dried at 105 °C. Total N and C were analyzed in plant samples,
181 and total N and total organic C in soil samples, using an elemental analyser (Flash 2000, Thermo
182 Scientific). Exchangeable soil NH_4^+ and NO_3^- were determined by the same procedure as in soils taken
183 for mineralization analysis.
184 Plant fractions were finely ground and samples were analysed for total N and atom percentage ^{15}N
185 using a mass spectrometer (20 - 20 stable isotope analyser, PDZ Europa, Northwich, UK). The amount
186 of ^{15}N in plant fractions was determined by comparing control and enriched samples (Powlson and
187 Barraclough, 1993):

$$F = \frac{T(As-Ab)}{Af}$$

188 Where *F* is the weight of N derived from the ^{15}N application, *T* is the total weight of N in the sample
189 and *As*, *Ab* and *Af* are the atom % of ^{15}N in the sample, control and added label, respectively.
190
191 2.4. Statistical evaluation
192
193 We used separate one-way analysis of variance (ANOVA) to compare rates of N mineralization (net
194 ammonification, net nitrification) under the different temperatures (-2 °C, 5 °C and 10 °C) within each
195 soil depth (organic, 0 – 10 cm, 10 – 20 cm and 20 – 30 cm). The Tukey-Kramer multiple comparison
196 test was used when data followed a normal probability distribution. The F-ratio was used to
197 determine statistical significance at $p < 0.05$. If data violated the normal distribution, the non-
198 parametric Kruskal-Wallis one-way ANOVA on ranks was used, and differences among groups were
199 assessed by Kruskal-Wallis multiple comparison Z value test (Dunn´s test) with Bonferroni corrections
200 for multiple tests. Instead of using means, this multiple comparison procedure uses average ranks.
201 The H value was used to determine statistical significance at $p < 0.05$. The same procedure was

applied to test the differences among plant functional types in their ability to take up ^{15}N after 10 days and after 1 year.

3. Results

3.1 Soil nitrogen, carbon pools and root length distribution

The measured soil organic C pool in the organic horizon was 560 g m^{-2} and the N pool was 32 g m^{-2} in soil samples recovered for the soil incubation experiment (after snowmelt on 2nd July 2011). In the mineral soil (to 30 cm depth) the C and N pools averaged 3094 g m^{-2} and 206 g m^{-2} respectively. The C/N ratio (g g^{-1}) decreased from 18 in the organic soil to 15 in the mineral soil (Table 1). The exchangeable NH_4^+ pool was highest in the organic soil (100 mg N m^{-2}), and declined steadily to 69 mg N m^{-2} in the 0 - 10 cm and 28 mg N m^{-2} in 20 - 30 cm mineral layers. The exchangeable NO_3^- pool was lowest in organic soil (8.9 mg N m^{-2}), peaked in the upper mineral soil (54 mg N m^{-2} in the 0 - 10 cm), and then declined to 18 mg N m^{-2} in the 20 - 30 cm mineral layer. Thus the NH_4^+ / NO_3^- ratio was highest in the organic soil (≈ 11) and declined towards 1 in the mineral soil (Table 1). Towards the end of the growing season, the pool of exchangeable NH_4^+ gradually decreased; 20 mg N m^{-2} was measured in the organic horizon and between 13 and 20 mg N m^{-2} in the mineral soil in August (Table S1).

Root length density was greatest in the organic horizon (32 cm cm^{-3}). In the mineral soil, the greatest root length density was measured in the top soil (7.6 cm cm^{-3}), with a sharp decrease to 1 cm cm^{-3} in the 10-20 cm layer and a further decline to 0.09 cm cm^{-3} at 30-40 cm depth (Table 1).

3.2 Soil incubations

Across the five replicate samples collected, we observed high variability in the amount of exchangeable NH_4^+ in the organic horizon at the beginning of the incubation (100 ± 43 mg N m^{-2}). The

amount of exchangeable NH_4^+ in mineral soil horizons decreased with depth (Table 1). The size of the initial NH_4^+ pool was positively related to % C in the organic horizon (R^2 = 0.99, P < 0.001), and a similar (albeit weaker) relationship was also observed in the mineral soil samples (R^2 = 0.39, P = 0.013). Later in the growing season (samples from 17th August 2011, Figure 2) the relationship between exchangeable NH_4^+ and % C in the organic horizon was weaker (R^2 = 0.15, P = 0.032) suggesting depletion of the available NH_4^+ soil pool.

3.2.1 Rates of net ammonification

The rate of net ammonification in the organic layer significantly differed among temperature treatments. The rate of net ammonification was significantly higher at - 2°C (2.67 ± 0.42 mg N m^{-2} day^{-1}) compared to 5°C and 10°C after 6 weeks of incubation, where rates were close to zero (Table 2, Figure 3, Table S2). In the mineral layers, mean net ammonification rates were negative for all soil layers and all temperatures except for the 0 – 10 cm, -2°C incubation (0.56 ± 0.35 mg N m^{-2} day^{-1}). Significant differences in net ammonification among temperature treatments were detected in 0 – 10 cm and in 10 – 20 cm (Table S2); no significant differences were detected in 20 – 30 cm. In both mineral horizons (0 – 20 cm) a higher ammonification rate was detected at – 2 °C (0.56 ± 0.35 mg N m^{-2} day^{-1} in 0 – 10 cm and -0.01 ± 0.13 mg N m^{-2} day^{-1} in 10 – 20 cm) compared to 5°C and 10°C.

3.2.2 Rates of net nitrification

A significant temperature effect was observed for net nitrification in the organic horizon (Table 2, Figure 3, Table S2). The only significant difference was detected between - 2°C (-0.10 ± 0.03 mg N m^{-2} day^{-1}) and 5°C (1.47 ± 0.60 mg N m^{-2} day^{-1}). In the mineral soil, net nitrification significantly differed among temperature treatments (0 - 10 cm and 10 – 20 cm, Table S2). However, despite high rates of net nitrification at 5°C and 10°C (without significant differences between them), no accumulation of NO_3^- was observed at - 2°C incubation in either the organic and mineral soil over the 6 week incubation, with slightly negative net nitrification rates recorded in all soil layers (Table 2, Figure 3).

In contrast, average net nitrification was positive in all 5°C and 10°C incubations. However no significant differences in net nitrification as a function of temperature were detected in the 20 – 30 cm depth.

3.2.3 Rates of net mineralization

Positive potential net mineralization rates were recorded under all three temperatures in the organic soil. A slightly higher net mineralization rate (not significant) was detected for - 2°C (2.57 ± 0.39 mg N m^{-2} day^{-1}) compared to 5°C (1.45 ± 0.61 mg N m^{-2} day^{-1}) and 10°C (1.26 ± 1.00 mg N m^{-2} day^{-1}). Higher incubation temperatures had a positive effect on net mineralization rates in the upper mineral soil (0 - 10 cm) compared to the - 2°C treatment (Table 2), albeit not significant ($P = 0.056$; $F_{2,12} = 3.7$). Deeper in the soil net mineralization rates decreased, with negative rates measured at all incubation temperatures in the deepest horizon. No significant differences in mineral soil mineralization rates were detected under different temperature regimes (Table 2, Table S2).

Based on the 6 week incubation experiment, accumulation of mineral N occurred under all three temperature regimes in the organic soil, with the dominant form being NH_4^+ in the - 2°C treatment, and NO_3^- in the 5°C and 10°C treatments. Accumulation of mineral N was also detected in the upper mineral soil (0 - 10 cm), under all temperature treatments. Depletion of the mineral N pool was observed in the lower mineral soil (10 - 30 cm) (Table 2).

3.3 Fate of ^{15}N added to high Arctic soils

3.3.1 Fate of ^{15}N after 10 days

Analysis of ^{15}N assimilation into above-ground biomass ten days after tracer addition showed distinct variability related to i) plant functional type and ii) depth of tracer injection. Plant functional types had significantly differing tissue biomass ^{15}N concentrations when tracer was applied at depths of 3 cm and 10 cm (Table S3). Tissue ^{15}N concentration in graminoids significantly differed from mosses and dwarf shrubs following application at 3 cm depth, and from mosses following application at 10 cm depth (Figure 4a). Note that ^{15}N levels in the (small) lichen biomass pool were not measured.

The depth of tracer injection had a significant effect on ^{15}N tissue concentration for each functional type. Significant variance in ^{15}N concentration in mosses was detected as a consequence of application depth (Table S4), with higher ^{15}N concentration measured after tracer injection at 3 cm compared to 10 cm and 30 cm. Concentrations of ^{15}N in dwarf shrubs significantly differed between the 3cm and 30cm injection depths (Table S4). Only graminoids recovered ^{15}N from all three depths, demonstrating the ability of graminoid root systems to access available N in the deep mineral soil, although there were significant differences among depths (Table S4). Concentrations of ^{15}N close to background were observed for mosses at 10cm and 30cm and for dwarf shrubs at 30cm. Overall, significantly higher ^{15}N recovery was observed under 3 cm injection treatment compared to the 10 cm and 30 cm injection treatments (Figure 4b).

3.3.2 Fate of ^{15}N after one year

One year after ^{15}N addition, we harvested aboveground vegetation biomass and divided this into the four plant types described above. Plant functional types had significantly differing ^{15}N biomass concentrations. If tracer was applied at 3 cm depth, significant differences between plant functional types were detected (Table S3); ^{15}N in lichens and mosses were significantly lower compared to graminoids. Dwarf shrubs did not significantly differ from lichens, mosses or graminoids (Figure 5a). Tracer application at 10 cm resulted in significant differences among plant functional types (Table

S3); and significant higher ^{15}N biomass concentration were detected in graminoids compared to lichens/mosses (Figure 5a). For the 30 cm injection depth, plant functional types differed significantly in their ^{15}N concentration (Table S3); differences were similar to those observed for the 3 cm injection (Figure 5a).

The depth of tracer injection had a significant effect on ^{15}N biomass concentration after one year in lichens, mosses and *Salix polaris* (Table S4). In all cases ^{15}N concentration was significantly higher in the 3 cm compared to the 30 cm injection depth. Dwarf shrubs also significantly differed between 3 cm and 10 cm injection depths (Figure 5b). However, one year after the original ^{15}N application no significant differences in ^{15}N biomass concentration were detected in graminoids as a function of tracer injection depth. This suggested rather uniform N uptake in the whole soil profile by graminoids (Figure 5b, Table S3).

3.3.3 ^{15}N recovery in plant biomass after one year since tracer application

Full sampling of above-ground vegetation at this time allowed us to calculate biomass pools and elemental ratios. The highest biomass pool was measured for mosses (488 g m^{-2}), which had a C/N ratio of 44 g g^{-1}, followed by dwarf shrubs with an aboveground biomass of 118 g m^{-2} and a C/N ratio of 31. Graminoid aboveground biomass constituted 47 g m^{-2}, with an average C/N ratio of 42. Lichen formed the smallest biomass pool, on average 19 g m^{-2}, with a C/N ratio of 94 (Table 3). Based on the pools of plant above-ground biomass and their recovery of ^{15}N as a fraction of the total application, we calculated the proportional recovery in above-ground tissue of ^{15}N injected at the different depths of the soil profile, one year after addition. In the 3 cm treatment plots, Dwarf shrubs accumulated 9.8 % of added N, followed by graminoids with 6.4 % and mosses with 0.6 %. Lichens did not substantially contribute to the recovery of added N. Based on the non-parametric Kruskal-Wallis test, significant differences were only detected between lichens and dwarf shrubs/graminoids (Figure 6a, Table S3). Altogether, plant aboveground biomass contained 16.8% of the ^{15}N tracer that

had been injected at 3 cm. For the 10 cm ^{15}N addition, we recovered 5.1 % in above-ground graminoid biomass, 2.6 % in dwarf shrubs, a negligible amount (< 0.1%) in mosses, and none in lichens, giving a total above-ground recovery of 7.8 %. For the 30 cm treatment, 3.4 % was recovered in graminoids, 1.8 % in dwarf shrubs, and none in mosses and lichens, giving a total above-ground recovery of 5.2 %. Total tracer recovery appeared to be greatest in the 3 cm injection treatment plots but this difference was not significant ($P = 0.055$; $F_{2,12} = 3.72$) compared to 10 cm and 30 cm applications. Tracer application depth significantly affected ^{15}N recovery in lichens and mosses (Figure 6b, Table S4) and in dwarf shrubs (Table S4) where 3 cm plots significantly differed from 10 cm and 30 cm (Figure 6b).

4. Discussion

4.1 Temperature controls on soil N cycling

It has been hypothesised that increasing air temperature may stimulate higher N mineralization, thus increasing N availability and providing a positive feedback on further plant productivity (Sturm et al., 2005). Nadelhoffer et al. (1991) suggested that C and N mineralization rates were insensitive to temperature between 3°and 9°C, but increased by factor of 2 or more between 9°and 15°C. These observations are in agreement with our results, to the extent that we did not see any temperature effect between 5° and 10°C on net N mineralization after the 6 week incubation, in either organic or mineral soil (Figure 3). However, a positive temperature effect on mineralization between - 2°and 5°C was observed in the top mineral soil (albeit not significant, $P = 0.055$), leading to the accumulation of mineral N in soil. Deeper in the soil profile, net N mineralization rates declined and were even negative at 20 – 30 cm depth, and appeared insensitive to temperature. Low or negative net N mineralization rates are a common feature of arctic soils (Robinson, 2002; Schmidt et al., 1999), indicating strong nutrient limitation in these soils. The high demand for N by microbes demonstrated by our *ex situ* experiment does not necessarily mean that plants in the field are unable to access

346 mineral N from gross mineralization, however; Schmidt et al. (2002) have shown that plants compete
347 well with microbes for nutrients in arctic ecosystems.
348 The absence of a significant observed temperature effect on organic soil N mineralization rates
349 between -2°and 5°C might be partly due to the fact that samples were collected after the spring
350 thaw, then stored for one week at 2°C before freezing to -2°C at the beginning of the incubation. Soil
351 physical disturbance together with nutrient release from lysed cells of dying microbes can release
352 both inorganic and labile organic N, thus overestimating the net N mineralization rate that would
353 occur under more sustained freezing conditions. On the other hand, despite the high variability in the
354 initial pool of exchangeable NH_4^+ (from 23 to 264 mg N m^{-2}) in organic soil, measured rates of N
355 mineralization varied only by factor of 2.6 (from 1.4 to 3.7 mg N m^{-2} day^{-1}) and mineralization rates
356 were not related to % N in the soil, which suggests that NH_4^+ release by soil physical disruption was
357 not likely to have been the main control on N mineralization rates. Moreover, soil particles continue
358 to have liquid water films around them down to freezing temperatures well below 0°C (Romanovsky
359 and Osterkamp, 2000), enabling microbial activity to continue.
360 Soil nitrification may have profound implications for arctic ecosystems, partly because it is an
361 acidifying process, but also because the nitrate produced is more mobile than ammonium in sols and
362 so more susceptible to leaching, as well as loss through denitrification. Nitrification has been
363 detected in river water in the nearby glacial catchment Midtre Lovénbreen (Ansari et al., 2012) and
364 elevated nitrate concentrations have been measured in the stream closest to our experimental plots
365 (Nowak and Hodson, 2014). Nowak and Hodson (2014) also measured low $\delta^{18}O$ values in stream NO_3^-
366 over the entire summer, indicating effective microbial nitrification over the vegetation period.
367 Nitrification and denitrification losses may thus partly balance the atmospheric N input, which is very
368 low in this part of the Arctic ≈ 0.07 g N m^{-2} yr^{-1} (Kühnel et al., 2013). At our site, there is high potential
369 for a temperature-related increase in nitrification, which was found to increase strongly between -2
370 and 5 °C at all depths. The absence of a further increase in nitrification between 5 and 10 °C suggests
371 that the temperature-sensitivity of this process may be greatest at or just above the freezing point,

372 implying that changes in the length of the ice-free period, as opposed to increases in peak summer
373 temperatures, may have the most profound consequences for the N cycle. Our results also have
374 implications for the overall availability of mineral N during the spring thaw.
375 Based on our observation that mineralization took place in the organic layer even at -2°C, it seems
376 likely that NH_4^+ accumulates during the autumn/early spring season, and supports plant growth after
377 snowmelt. There is considerable potential for loss during the thaw period, when water fluxes are
378 large and temperatures are likely to be too low for plant uptake. However if, as our results suggest,
379 nitrification is delayed, this leaching may be limited by the lower mobility of NH_4^+. Accumulation of
380 NH_4^+ during the winter season and depletion during the growing season is also shown by a higher
381 pool of extractable NH_4^+ at the beginning of the growing season (early July) than towards the end
382 (mid August). Also, the ratio of NH_4^+-N to NO_3^--N in the mineral soil changed from 1.3 to 0.5 during
383 the vegetation season. Despite the dominance of NH_4^+ over NO_3^- in the organic soil over the whole
384 season, NO_3^-may thus become the dominant form of N in the mineral soil later in the year. Alteration
385 of the NH_4^+ / NO_3^- ratio in soil may have further implications for plant composition, as plant taxa
386 differ in their ability to utilize different forms of available N (Atkin et al., 1993; Smirnoff and Stewart,
387 1985).
388
389 Positive net N mineralization rates were detected in organic horizons under all three temperature
390 regimes, and at 5°and 10°C in the upper mineral soil. The apparent lack of significant temperature
391 effects on net N mineralization rates may indicate fairly conservative N soil cycling. On the other
392 hand, there were significant effects of temperature on the individual constituents of measured N
393 mineralization in the upper soil (a negative effect of temperature on net ammonification in the
394 organic layer, and a positive effect of temperature on net nitrification in the 0 – 10 cm mineral soil)
395 suggesting that individual N transformation processes are more temperature-sensitive than the
396 overall net mineralization rate. Furthermore, as net N mineralization represents the balance of gross
397 mineralization and immobilization (both biotic and abiotic), it may not reflect true N availability, as

simultaneous increases in both gross mineralization and gross immobilisation (i.e. an increase in both N supply and N demand) would not be reflected in the net mineralization measurement.

4.2 The fate of ^{15}N added to the tundra mineral soil

Our experimental application of 0.4 g ^{15}N m^{-2} into the mineral soil represented an approximate doubling of the amount of extractable mineral N in the soil profile, to the depth of 30 cm, at the time of addition in early July (Table 1). Although this is a substantial increase, the effects are likely to have been short-lived due to the rapid turnover of the soil ammonium pool. Addition towards the end of the vegetation season (Figure 2) may mimic the effect of soil warming, which is likely to extend the season during which N is mineralised. One year after treatment, in August, the total amount of soil extractable mineral N was 0.16 g N m^{-2} in the treated plots, a lower value than the pool of mineral N pool in untreated soil from July, suggesting a minor contribution of the added N to the exchangeable pool of soil mineral N.

4.3 Short-term ^{15}N recovery in vegetation

Short-term ^{15}N partitioning in aboveground biomass of three plant fractions (Moss, Dwarf shrub and Graminoid) was measured 10 days after ^{15}N application. Application of ^{15}N directly into the mineral soil demonstrated clear differences in the capability of different plant groups to utilize available N from different depths. Mosses and lichens were able to take up little if any of the ^{15}N injected below-ground, even from the -3 cm injection, reflecting their lack of structures for acquiring N from mineral soil and consequent reliance on atmospheric inputs and meltwaters as sources of nutrients. This observation contrasts with those obtained from conventional ^{15}N tracer studies, where N is added to the surface vegetation, which typically show mosses and lichens to be effective scavengers of above-ground N inputs (Bilbrough et al., 2000; Tye et al., 2005). Taken together, these observations are consistent with the expectation that increased N mineralization rates due to rising temperatures would (if observed) favour the growth of vascular plants, possibly at the expense of bryophytes and

423 lichens (Malmer et al., 1994, Jónsdóttir et al., 1995). Conversely, changes in the amount and timing
424 of snowmelt (Maturilli et al., 2014), as well as episodic inputs associated with polluted rain events
425 (Björkman et al., 2013; Kühnel et al., 2013) are likely to have a greater influence on lower plants.
426 Of the vascular species present, the most efficient in recovering soil ^{15}N in aboveground biomass
427 were graminoids, which were able to access N also from the deepest application depth. In contrast,
428 the Dwarf shrub species present, *Salix polaris*, was only able to recover a comparatively small part of
429 the added ^{15}N, and only from the -3 cm and -10 cm additions. This suggests firstly (as expected) that
430 the deep-rooted graminoids (primarily sedges) present at this site have greater capability to source N
431 from deep within the mineral soil than the shallower-rooted *Salix polaris*. Secondly, the greater
432 capture of ^{15}N by graminoids from all depths (particularly relative to their comparatively small above-
433 ground biomass, Table 3) suggests either that they are more effective in capturing N from the
434 mineral soil in general, or alternatively that they continue to assimilate available N until later in the
435 growing season (Larsen et al., 2012). This might have important consequences for vegetation
436 development under increasing air temperatures, which may also stimulate higher evapotranspiration
437 and water stress in polar semi-desert regions such as Svalbard. Annual totals of evaporation are low
438 in the Arctic, but evaporation is concentrated in the summer months, when total solar energy levels
439 can be as high as in lower latitudes. Precipitation is also low and, although a considerable amount of
440 water is made available by the spring snow melt, there is the potential for summer water stress, as
441 for most tundra ecosystems significant biological activity is confined to a thin active layer of soil
442 which supports at most a dwarf plant community (Hodkinson et al., 1999). Eddy covariance-based
443 modelling of CO_2 exchange has also highlighted the importance of snowmelt timing, the frequency
444 and duration of precipitation events during the summer, and soil temperatures in regulating the
445 overall C balance of high Arctic semi-desert tundra (Lloyd, 2001). However *Salix polaris* exhibits a
446 high photosynthetic rate only when well supplied with water (Barták et al., 2012). Increasing N
447 availability in deep soil during the end of vegetation season thus may favour graminoids over *Salix*

448 both directly (via nutrient uptake) and indirectly (by increasing evapotranspiration rates and thus
449 water stress for the shallower rooting *Salix*).
450
451 ### 4.4 ^{15}N partitioning one year after addition
452 One year after N addition into the mineral soil, the observed recovery of ^{15}N in aboveground biomass
453 replicated some features of N uptake already apparent ten days after ^{15}N injection. Graminoids were
454 most successful at assimilating the tracer ^{15}N into aboveground biomass. Lichens and mosses
455 recovered very little ^{15}N in their biomass (0.02 - 0.05 % in the -3 cm treatment), similar to the trend
456 after short-term assessment, implying that movement of N in water or biomass did not enable these
457 plants to assimilate tracer ^{15}N during the following year. The lack of significant treatment effects (in
458 terms of depth of ^{15}N application) on N uptake by graminoids highlighted the importance of deeper
459 mineral soil as a niche for N acquisition by this functional type (Figure 5B). High ^{15}N concentrations in
460 graminoid biomass may partly reflect that this does not include long lived tissue, whereas the woody
461 stems of *Salix polaris* grow slowly over decades. Inclusion of woody biomass in dwarf shrub samples
462 certainly partly diluted the ^{15}N signal, although this should not have affected the measurement of
463 total ^{15}N recovery. On the other hand, the bulk C/N ratio of dwarf shrub biomass was actually lower
464 (31) than that of graminoids (42), suggesting that the latter are more efficient in terms of N
465 requirement per unit of C growth. Of the 0.4 g ^{15}N m^{-2} added to the experimental plots, 16.8 % was
466 recovered in above-ground biomass of harvested vegetation in the shallowest treatment (-3 cm).
467 Despite a lower ^{15}N concentration in *Salix* biomass, its higher biomass pool per unit area led to more
468 ^{15}N recovery overall in the shallow treatment compared to the graminoids. The 30 cm application
469 resulted in recovery of 5.2% after one year, of which around two thirds was in graminoid biomass
470 and one third in dwarf shrub biomass. This suggests that both the graminoids present and *Salix* have
471 the capacity to utilize deep mineral nutrient resources. The sustained differences in ^{15}N assimilation
472 between plant groups as a function of tracer injection depth, a full year after ^{15}N addition, suggests a

high degree of vertical stratification within the rooting system between these two key components of tundra vegetation.

The absence of enhanced exchangeable mineral N concentration in the treated plots indicates that the rest of the ^{15}N added was transferred into other pools, most likely into unmeasured root biomass, microbial biomass and (subsequently) soil organic matter, and/or has been lost from the system by denitrification and/or leaching. The fate of the remaining added ^{15}N is unknown. The possibility that N is lost through leaching is partly supported by observations of a nearby stream, where nitrate concentrations were fairly high during the growing season (Nowak and Hodson, 2014). However ^{15}N addition experiments in moist arctic tundra (Nordin et al., 2004) and in an ecosystem similar (and close) to ours (Tye et al., 2005) have shown that soil biota can act as a major N sink, rapidly sequestering a large proportion of the labelled N.

4.5 Conclusions

Based on our experiments, we conclude that the response of Arctic tundra ecosystems to rising temperatures may differ from that previously predicted in a number of key respects. Firstly, it appears that higher temperatures may not invariably lead to an increase in net N mineralization (although this does not preclude an increase in gross mineralization, counterbalanced by an increase in plant and microbial N uptake). We did however observe a clear temperature-dependence of net nitrification, which could lead to increased nitrate leaching (and thus depletion of N pools) from warming tundra ecosystems. Our results also suggest that the ecological impacts of any increase in gross N mineralization rates could have markedly different ecological consequences than those suggested by conventional above-ground fertilisation experiments, which have typically shown an enhancement of bryophyte growth. In our below-ground additions, 5 – 15% of the added N was captured by vascular plants in aboveground biomass, with deep-rooted graminoids outcompeting shallow-rooted dwarf shrubs for N applied in deeper mineral soils. It therefore seems likely that

499 increasing N deposition and increasing temperature, despite both enhancing N availability, could
500 have opposing impacts on vegetation. The net impact of multiple anthropogenic pressures acting
501 simultaneously on tundra ecosystems remains hard to predict, and more evidence is needed to
502 disentangle the spatiotemporal dynamics of temperature and N availability in Arctic soils.
503

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511

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Figures

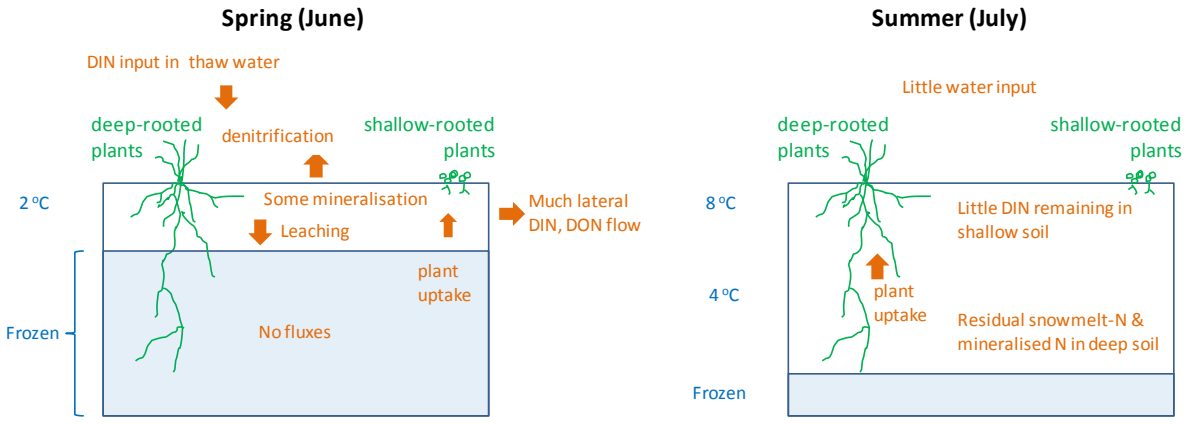


Figure 1. Conceptual schema of major N flows in Arctic tundra ecosystems, during the spring melt and later in the growing season.

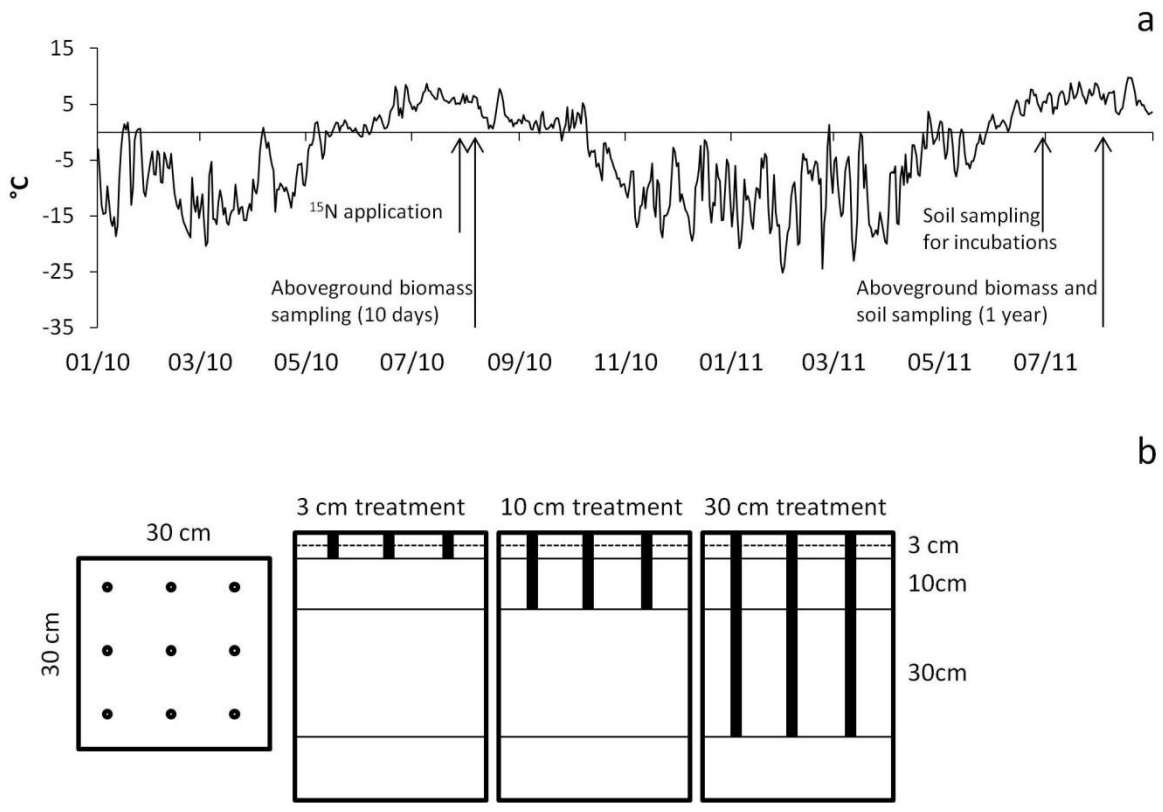
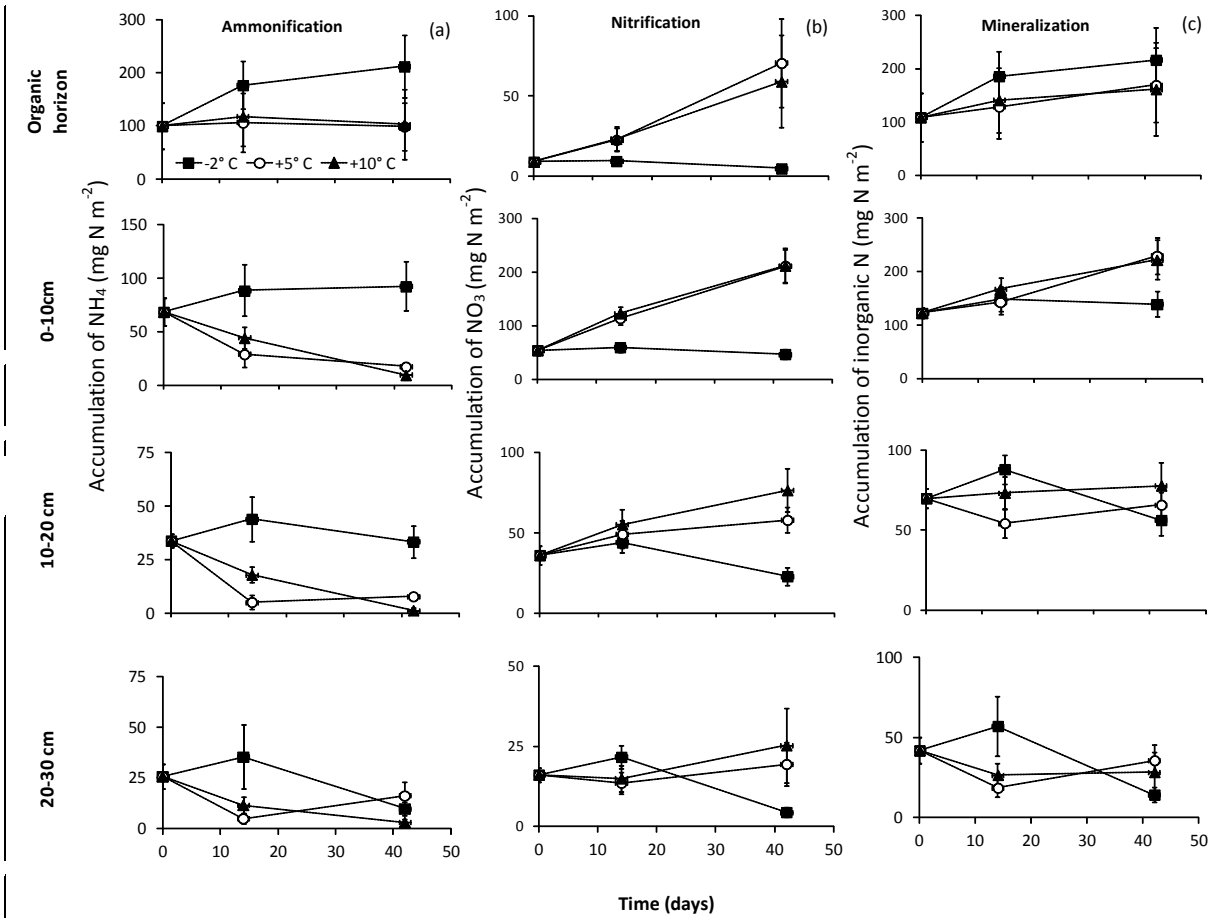


Figure 2. Timing of soil and vegetation sampling and ^{15}N application together with the course of air temperature in Ny Ålesund (Maturilli et al., 2013) **(a) and scheme of ^{15}N application into the mineral soil at different depths of the soil profile (b).**

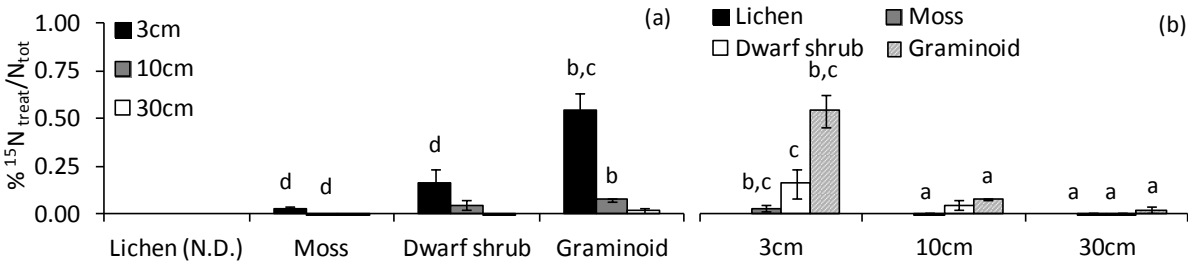


660

661 **Figure 3. Accumulation of NH_4^+ (net ammonification; a), NO_3^- (net nitrification; b) and mineral N**

662 **(net mineralization; c) in organic soil (top panel) and mineral subsoil (0 – 10 cm, 10 – 20 cm and 20**

663 **– 30 cm) over two and six week incubation under - 2°C, 5°C and 10°C, with standard errors.**



664

Figure 4. ^{15}N as a proportion of total N in aboveground biomass of each plant functional type, 10 days after ^{15}N application at 3, 10 or 30 cm depth. Figure a represents significant differences among plant functional types and figure b represents significant differences for each functional type as a consequence of tracer application depth. Columns that do not share the same superscript letters are significantly different ($p < 0.05$). Error bars represent +/- one standard error.

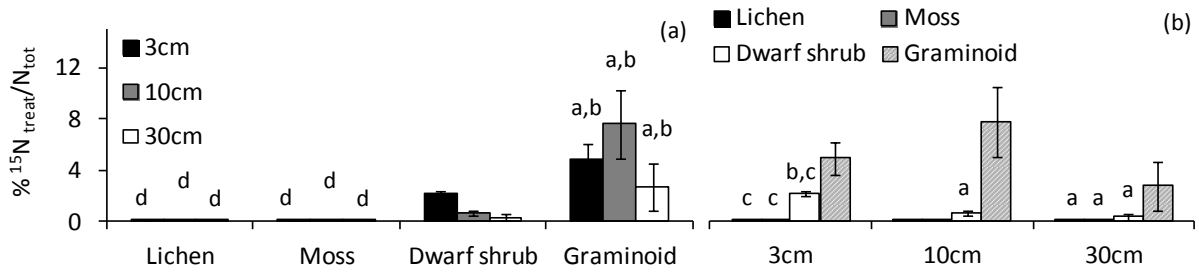


Figure 5. ^{15}N as a proportion of total N in aboveground biomass of each plant functional type, one year after ^{15}N application at 3, 10 or 30 cm depth. Figure a represents significant differences among plant functional types and figure b represents significant differences for each functional type as a consequence of tracer application depth. Columns that do not share the same superscript letters are significantly different ($p < 0.05$). Error bars represent +/- one standard error.

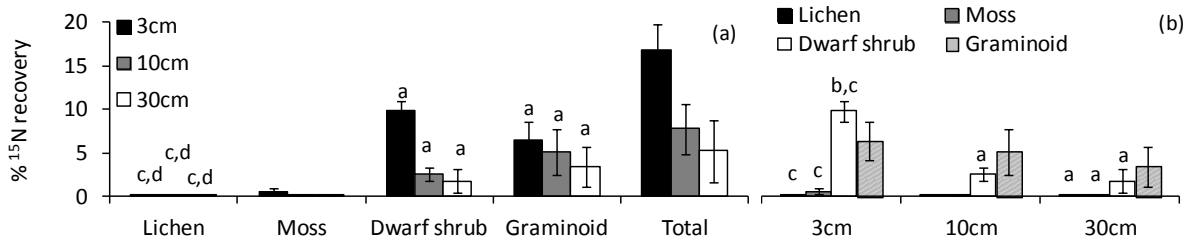


Figure 6. Recovery of applied ^{15}N one year after application in four plant functional types (a) under different treatments (b). Total represents sum of % ^{15}N recovery for all functional types. Columns that do not share the same superscript letters are significantly different ($p < 0.05$). Error bars represent +/- one standard error.

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668 Tables

669 *Table 1. Root distribution and pools of total and exchangeable C and N in soil profiles used for*

670 *mineralization assessment.*

Horizon (cm)	Root length density	Dry soil matter	pH (H_2O)	C	N	NH_4-N	NO_3-N
	cm cm^{-3}	kg m^{-2}		g m^{-2}	g m^{-2}	mg m^{-2}	mg m^{-2}
Organic (2 cm)	32.1 ± 6.20	2.6 ± 0.20	5.71 ± 0.24	560 ± 75	32 ± 4.9	100 ± 43	8.9 ± 2.4
0-10	7.6 ± 2.30	55 ± 3.8	6.16 ± 0.21	1126 ± 61	84 ± 5.0	69 ± 13	54 ± 9.2
10-20	1.0 ± 0.29	69.3 ± 3.7	6.90 ± 0.23	1148 ± 79	76 ± 3.0	34 ± 3.1	36 ± 5.8
20-30	0.31 ± 0.15	58.1 ± 5.6	7.36 ± 0.22	820 ± 121	45 ± 6.0	28 ± 5.1	18 ± 2.2
30-40	0.09 ± 0.04						

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Table 2. Net N mineralization rates (mg N m^{-2} day^{-1} ± standard error) calculated after 6 weeks under different temperature regimes. Significant differences among temperature treatments are highlighted with upper index.

	-2°C	**5°C**	**10°C**
	mgN m^{-2} day^{-1}	mgN m^{-2} day^{-1}	mgN m^{-2} day^{-1}
	Net Ammonification		
Organic	$2.67^{b,c}$ ± 0.42	-0.02^{a} ± 0.17	0.07^{a} ± 0.59
0-10	$0.56^{b,c}$ ± 0.35	-1.21^{a} ± 0.25	-1.40^{a} ± 0.22
10-20	$-0.01^{b,c}$ ± 0.13	-0.62^{a} ± 0.07	-0.77^{a} ± 0.09
20-30	-0.38 ± 0.08	-0.23 ± 0.20	-0.54 ± 0.14
	Net Nitrification		
Organic	-0.10^{b} ± 0.03	1.47^{a} ± 0.60	1.18 ± 0.63
0-10	$-0.17^{b,c}$ ± 0.11	3.75^{a} ± 0.86	3.76^{a} ± 0.91
10-20	$-0.31^{b,c}$ ± 0.05	0.52^{a} ± 0.16	0.96^{a} ± 0.37
20-30	-0.28 ± 0.04	0.08 ± 0.12	0.22 ± 0.24
	Net Mineralization		
Organic	2.57 ± 0.39	1.45 ± 0.61	1.26 ± 1.00

0-10	0.36 ± 0.45	2.54 ± 0.64	2.36 ± 0.73
10-20	-0.32 ± 0.17	-0.10 ± 0.19	0.19 ± 0.42
20-30	-0.66 ± 0.11	-0.15 ± 0.18	-0.32 ± 0.18

672 *Table 3. Mean dry matter pools (g m^{-2}) and C and N concentrations (%), ± standard error in above-*
673 *ground biomass of four plant functional types, on plots to which the isotopic N was added at*
674 *different depths.*

	Dry matter g m^{-2}	C %	N %	C/N
Lichen	19 ± 5	38 ± 0.3	0.42 ± 0.02	94 ± 5
Moss	488 ± 40	39 ± 0.3	0.89 ± 0.03	44 ± 1.7
Dwarf shrub	118 ± 7	47 ± 0.2	1.55 ± 0.04	31 ± 0.9
Graminoid	47 ± 8	42 ± 0.4	1.03 ± 0.04	42 ± 1.7

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