

Article (refereed) - postprint

Oulehle, F.; Rowe, E.C.; Myška, O.; Chuman, T.; Evans, C.D. 2016. **Plant functional type affects nitrogen use efficiency in high-Arctic tundra.**

© 2015 Elsevier Ltd.

This manuscript version is made available under the CC-BY-NC-ND 4.0 license <http://creativecommons.org/licenses/by-nc-nd/4.0/>



This version available <http://nora.nerc.ac.uk/512938/>

NERC has developed NORA to enable users to access research outputs wholly or partially funded by NERC. Copyright and other rights for material on this site are retained by the rights owners. Users should read the terms and conditions of use of this material at <http://nora.nerc.ac.uk/policies.html#access>

NOTICE: this is the author's version of a work that was accepted for publication in *Soil Biology and Biochemistry*. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in *Soil Biology and Biochemistry* (2016), 94. 19-28. [10.1016/j.soilbio.2015.11.008](https://doi.org/10.1016/j.soilbio.2015.11.008)

www.elsevier.com/

Contact CEH NORA team at
noraceh@ceh.ac.uk

1 Plant functional type affects nitrogen use efficiency in High- 2 Arctic tundra

3
4 F. Oulehle^{1,2}, E.C. Rowe¹, O. Myška³, Tomáš Chuman² and C.D. Evans¹

5 ¹Centre for Ecology and Hydrology, Bangor, LL57 2UW, UK

6 ²Biogeochemistry Department, Czech Geological Survey, Klárov 3, 118 21, Prague, Czech Republic

7 ³Global Change Research Centre, Academy of Sciences of the Czech Republic, Bělidla 986/4a, 603 00,
8 Brno, Czech Republic

9 Corresponding author: Filip Oulehle, Tel: +420251085431, Fax: +420251818748

10 e-mail: filip.oulehle@geology.cz

11 Highlights

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

16 Abstract

17 To unravel the potential effects of climate warming on soil N availability in a high Arctic tundra
18 ecosystem we studied temperature effects on soil mineralization, and N uptake from different soil
19 depths (-3, -10 and -30 cm) by tundra plants. Uptake was assessed using ¹⁵N tracer injected directly
20 into mineral soil as ¹⁵NH₄Cl solution to specifically mimic altered N availability from enhanced
21 mineralization. Net N mineralization rates were very low, suggesting that N is strongly limiting in this
22 system. There was no apparent temperature effect (-2°, 5°, 10°C) on mineralization, but net
23 nitrification was strongly limited by temperature – under the -2°C treatment no nitrification

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

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

37

38 1. Introduction

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

48 Besides other environmental changes, increased nutrient availability is of key concern for future
49 change in arctic vegetation (Dormann and Woodin, 2002). For example, it has been postulated that
50 snow-shrub interactions have created a positive feedback whereby warming increases nutrient
51 availability, leading to shrub growth and expansion, which in turn leads to deeper snow cover over
52 the shrub canopy, raising winter temperatures and causing further nutrient release (Sturm et al.,
53 2005). Recently, Myers-Smith and Hik (2013) found that abiotic influences of shrub canopy cover
54 alone on nutrient dynamics were weaker than previously asserted. However, increases in
55 temperatures predicted for high latitudes may not necessarily cause greater rates of nitrogen (N)
56 mineralization (Nadelhoffer et al., 1991; Robinson, 2002). Despite generally lower net N
57 mineralization in the Arctic compared to temperate ecosystems, N mineralization varies widely
58 across different types of arctic ecosystems (Robinson et al., 1995). Thus understanding climate
59 effects (altered soil temperature, moisture) on N availability is of great importance in strongly N-
60 limited arctic ecosystems.

61 Based on a recent synthesis of warming experiments in the Arctic, Elmendorf et al. (2012) have
62 shown that shrubs are expanding most in warmer tundra regions, whilst graminoids and forbs are
63 expanding predominantly in colder tundra (areas with a mean July temperature < 7 °C). They
64 hypothesise that this might be due to the fact that the tallest growth forms in colder tundra areas
65 tend to be herbs, which can easily prostrate dwarf shrubs, whereas the tallest growth forms in
66 warmer tundra areas are woody (low and tall) shrubs. However, competition for light is only one
67 aspect of interspecific plant competition, and the balance between plant functional types may be
68 affected by availability of other resources. There is evidence for different and complementary
69 strategies to meet N demand by different plant functional groups (Kahmen et al., 2006). Hitherto
70 little attention has been paid to the potential separation of N acquisition niches in high Arctic soils, in
71 contrast to studies in warm tundra (Grogan and Jonasson, 2003; McKane et al., 2002), or tropical and
72 temperate ecosystems (Göransson et al., 2008; Houle et al., 2014; Rowe et al., 2001). The depth at
73 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 ¹⁵N added into the
95 mineral soil at different depths by tundra plants, both in the short term (10 days after ¹⁵N 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

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

103

104 2. Materials and methods

105

106 2.1 Site description

107 Experiments were done in a high Arctic semi-desert tundra ecosystem surrounding the Kongsfjorden,
108 approximately 2 km west from Ny Ålesund, Svalbard, at the site Leirhaugen (78° 55' N, 11° 49' E, 55
109 m a.s.l.). The area is underlain by continuous permafrost and the mineral soil, developed over
110 limestone, consists of silty clay with interspersed stones. This is overlaid by a thin and discontinuous
111 organic layer. Soil pH increases from 5.71 in the organic horizon to 7.36 in the mineral soil at 20 – 30
112 cm depth with mean C/N of 18 in the organic soil and 15 in the mineral soil. Mean annual air
113 temperature over last two decades was -4.5 °C, with July temperatures ranging from 4.6 to 6.9 °C.
114 Annual precipitation is ≈370 mm, which mostly falls as snow between September and May, with the
115 driest month in May (17 mm) and wettest month in September (46 mm). Soil thaw depth is
116 approximately 1 m during the growing season (Roth and Boike, 2001). Tundra vegetation is exposed
117 to reindeer grazing. Reindeer in Ny-Ålesund are descended from animals introduced to the area in
118 1978, since which time the population has fluctuated with densities up to 0.89 individuals km⁻²
119 (Aanes et al., 2002; Hayashi et al., 2014).

120

121 2.2 Net mineralization experiment

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

125 30 cm. Each replicate consisted of three bulked soil samples retrieved by the corer. The organic soil
126 was sampled using a 10 x 10 cm plastic frame. Organic and mineral soil was sieved (5 mm and 2 mm,
127 respectively), dried (105 °C) and homogenized samples analysed for total organic carbon and N (Flash
128 2000, Thermo Scientific).

129 Within one week after soil sampling, fresh sieved soil samples were transported at 2 °C to the UK for
130 further laboratory soil incubation. Moist soil (60 – 70 % of water holding capacity) was incubated in
131 100 ml flasks sealed with perforated parafilm at -2 °C, 5 °C and 10 °C without substrate addition for 6
132 weeks. Soil moisture was checked weekly and distilled water was added when necessary to maintain
133 the original soil moisture. Soil samples were extracted with 2 M KCl (extractant/soil ratio was 5 : 1 for
134 organic and 2 : 1 for mineral soil, v/w) after shaking for 1 h; then, the soil slurry was centrifuged
135 (4,000 g, 10 min) and the supernatant filtered through a 0.45 µm cellulose filter. The extract was
136 analysed for NH_4^+ and NO_3^- contents by automated discrete spectrophotometer (AQ2, Seal
137 Analytical). The net ammonification and nitrification rates were calculated after two and six weeks as
138 the difference in extractable NH_4^+ and NO_3^- , respectively, between the measurement date and the
139 start date of the incubation, divided by the number of days. The net N mineralization rate was
140 calculated as the sum of net ammonification and net nitrification rates (Santruckova et al., 2009).

141 In addition to the soil sampling for mineralization incubations, three soil pits were dug with a spade
142 and samples for root density calculations were retrieved by excavating laterally from the pit, to
143 minimise disturbance of the sample. Organic soil samples ($n = 3$; average volume = 35 cm³) and
144 mineral soil samples in 0 - 10 cm, 10 - 20 cm, 20 - 30 cm, 30 - 40 cm horizons ($n = 3$; average volume =
145 145 cm³) were used for root length density assessment. Roots were carefully separated from soil by
146 gently flushing with water, and then distributed over squared paper. Horizontal and vertical grid
147 intersections were counted and root density calculated according to the line intercept method
148 (Tennant, 1975).

149

150 2.3 Subsurface ¹⁵N addition experiment

151 Labelled ^{15}N (98 atom %) was applied to the plots in a solution of $^{15}\text{NH}_4\text{Cl}$ 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 possible 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

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

204 3. Results

205 3.1 Soil nitrogen, carbon pools and root length distribution

206 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
207 soil samples recovered for the soil incubation experiment (after snowmelt on 2nd July 2011). In the
208 mineral soil (to 30 cm depth) the C and N pools averaged 3094 g m^{-2} and 206 g m^{-2} respectively. The
209 C/N ratio (g g^{-1}) decreased from 18 in the organic soil to 15 in the mineral soil (Table 1). The
210 exchangeable NH_4^+ pool was highest in the organic soil (100 mg N m^{-2}), and declined steadily to 69
211 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
212 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
213 cm), and then declined to 18 mg N m^{-2} in the 20 - 30 cm mineral layer. Thus the $\text{NH}_4^+ / \text{NO}_3^-$ ratio was
214 highest in the organic soil (≈ 11) and declined towards 1 in the mineral soil (Table 1). Towards the
215 end of the growing season, the pool of exchangeable NH_4^+ gradually decreased; 20 mg N m^{-2} was
216 measured in the organic horizon and between 13 and 20 mg N m^{-2} in the mineral soil in August (Table
217 S1).

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

221

222 3.2 Soil incubations

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

225 amount of exchangeable NH_4^+ in mineral soil horizons decreased with depth (Table 1). The size of the
226 initial NH_4^+ pool was positively related to % C in the organic horizon ($R^2 = 0.99$, $P < 0.001$), and a
227 similar (albeit weaker) relationship was also observed in the mineral soil samples ($R^2 = 0.39$, $P =$
228 0.013). Later in the growing season (samples from 17th August 2011, Figure 2) the relationship
229 between exchangeable NH_4^+ and % C in the organic horizon was weaker ($R^2 = 0.15$, $P = 0.032$)
230 suggesting depletion of the available NH_4^+ soil pool.

231

232 3.2.1 Rates of net ammonification

233 The rate of net ammonification in the organic layer significantly differed among temperature
234 treatments. The rate of net ammonification was significantly higher at -2°C ($2.67 \pm 0.42 \text{ mg N m}^{-2}$
235 day^{-1}) compared to 5°C and 10°C after 6 weeks of incubation, where rates were close to zero (Table 2,
236 Figure 3, Table S2). In the mineral layers, mean net ammonification rates were negative for all soil
237 layers and all temperatures except for the 0 – 10 cm, -2°C incubation ($0.56 \pm 0.35 \text{ mg N m}^{-2} \text{ day}^{-1}$).
238 Significant differences in net ammonification among temperature treatments were detected in 0 – 10
239 cm and in 10 – 20 cm (Table S2); no significant differences were detected in 20 – 30 cm. In both
240 mineral horizons (0 – 20 cm) a higher ammonification rate was detected at -2°C ($0.56 \pm 0.35 \text{ mg N}$
241 $\text{m}^{-2} \text{ day}^{-1}$ in 0 – 10 cm and $-0.01 \pm 0.13 \text{ mg N m}^{-2} \text{ day}^{-1}$ in 10 – 20 cm) compared to 5°C and 10°C .

242

243 3.2.2 Rates of net nitrification

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

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

254

255 3.2.3 Rates of net mineralization

256 Positive potential net mineralization rates were recorded under all three temperatures in the organic
257 soil. A slightly higher net mineralization rate (not significant) was detected for - 2°C (2.57 ± 0.39 mg N
258 $\text{m}^{-2} \text{day}^{-1}$) compared to 5°C (1.45 ± 0.61 mg N $\text{m}^{-2} \text{day}^{-1}$) and 10°C (1.26 ± 1.00 mg N $\text{m}^{-2} \text{day}^{-1}$). Higher
259 incubation temperatures had a positive effect on net mineralization rates in the upper mineral soil (0
260 - 10 cm) compared to the - 2°C treatment (Table 2), albeit not significant ($P = 0.056$; $F_{2,12} = 3.7$).
261 Deeper in the soil net mineralization rates decreased, with negative rates measured at all incubation
262 temperatures in the deepest horizon. No significant differences in mineral soil mineralization rates
263 were detected under different temperature regimes (Table 2, Table S2).

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

269 3.3 Fate of ¹⁵N added to high Arctic soils

270 3.3.1 Fate of ¹⁵N after 10 days

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

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

287

288 3.3.2 Fate of ¹⁵N after one year

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

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

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

306

307 3.3.3 ^{15}N recovery in plant biomass after one year since tracer application

308

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

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

330

331 4. Discussion

332 4.1 Temperature controls on soil N cycling

333 It has been hypothesised that increasing air temperature may stimulate higher N mineralization, thus
334 increasing N availability and providing a positive feedback on further plant productivity (Sturm et al.,
335 2005). Nadelhoffer et al. (1991) suggested that C and N mineralization rates were insensitive to
336 temperature between 3°and 9°C, but increased by factor of 2 or more between 9°and 15°C. These
337 observations are in agreement with our results, to the extent that we did not see any temperature
338 effect between 5° and 10°C on net N mineralization after the 6 week incubation, in either organic or
339 mineral soil (Figure 3). However, a positive temperature effect on mineralization between - 2°and 5°C
340 was observed in the top mineral soil (albeit not significant, $P = 0.055$), leading to the accumulation of
341 mineral N in soil. Deeper in the soil profile, net N mineralization rates declined and were even
342 negative at 20 – 30 cm depth, and appeared insensitive to temperature. Low or negative net N
343 mineralization rates are a common feature of arctic soils (Robinson, 2002; Schmidt et al., 1999),
344 indicating strong nutrient limitation in these soils. The high demand for N by microbes demonstrated
345 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 soils 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 $\delta^{18}\text{O}$ 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 $\approx 0.07 \text{ g N m}^{-2} \text{ yr}^{-1}$ (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 $\text{NH}_4^+ / \text{NO}_3^-$ 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; Smirnov 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°C 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

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

400

401 4.2 The fate of ¹⁵N added to the tundra mineral soil

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

411 4.3 Short-term ¹⁵N recovery in vegetation

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

452 One year after N addition into the mineral soil, the observed recovery of ¹⁵N in aboveground biomass
453 replicated some features of N uptake already apparent ten days after ¹⁵N injection. Graminoids were
454 most successful at assimilating the tracer ¹⁵N into aboveground biomass. Lichens and mosses
455 recovered very little ¹⁵N 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 ¹⁵N during the following year. The lack of significant treatment effects (in
458 terms of depth of ¹⁵N 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 ¹⁵N 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 ¹⁵N signal, although this should not have affected the measurement of
463 total ¹⁵N 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 ¹⁵N 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 ¹⁵N concentration in *Salix* biomass, its higher biomass pool per unit area led to more
468 ¹⁵N 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 ¹⁵N assimilation
472 between plant groups as a function of tracer injection depth, a full year after ¹⁵N addition, suggests a

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

475

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

485

486 4.5 Conclusions

487 Based on our experiments, we conclude that the response of Arctic tundra ecosystems to rising
488 temperatures may differ from that previously predicted in a number of key respects. Firstly, it
489 appears that higher temperatures may not invariably lead to an increase in net N mineralization
490 (although this does not preclude an increase in gross mineralization, counterbalanced by an increase
491 in plant and microbial N uptake). We did however observe a clear temperature-dependence of net
492 nitrification, which could lead to increased nitrate leaching (and thus depletion of N pools) from
493 warming tundra ecosystems. Our results also suggest that the ecological impacts of any increase in
494 gross N mineralization rates could have markedly different ecological consequences than those
495 suggested by conventional above-ground fertilisation experiments, which have typically shown an
496 enhancement of bryophyte growth. In our below-ground additions, 5 – 15% of the added N was
497 captured by vascular plants in aboveground biomass, with deep-rooted graminoids outcompeting
498 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

504 **Acknowledgements**

505 This research was primarily funded by a Marie Curie Initial Stage Training Network (NSINK-Sources,
506 sinks and impacts of atmospheric nitrogen deposition in the Arctic, project number R/123386).
507 Additional support was provided by Global Change Research Centre (CZ.1.05/1.1.00/02.0073). We
508 thank Sonal Choudhary and Aimeric Blaud for field assistance, the UK Natural Environment Research
509 Council for providing accommodation in Arctic research station, and Nick Cox for support during the
510 fieldwork in Ny-Ålesund.

511

512 References

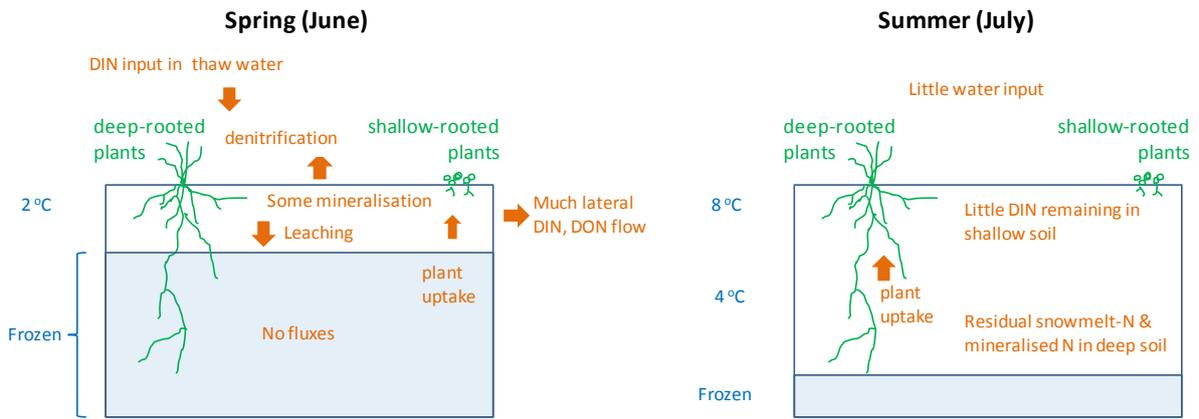
- 513 Aanes, R., Saether, B.-E., Smith, F.M., Cooper, E.J., Wookey, P.A., Oritsland, N.A., 2002. The Arctic
514 Oscillation predicts effects of climate change in two trophic levels in a high-arctic ecosystem.
515 *Ecol. Lett.* 5, 445–453. doi:10.1046/j.1461-0248.2002.00340.x
- 516 Ansari, A.H., Hodson, A.J., Heaton, T.H.E., Kaiser, J., Marca-Bell, A., 2012. Stable isotopic evidence for
517 nitrification and denitrification in a High Arctic glacial ecosystem. *Biogeochemistry* 113, 341–
518 357. doi:10.1007/s10533-012-9761-9
- 519 Atkin, O.K., Villar, R., Cummins, W.R., 1993. The ability of several high arctic plant species to utilize
520 nitrate nitrogen under field conditions. *Oecologia* 96, 239–245. doi:10.1007/BF00317737
- 521 Barták, M., Váczi, P., Hájek, J., 2012. Photosynthetic activity in three vascular species of Spitsbergen
522 vegetation during summer season in response to microclimate. *Polish Polar Res.* 33, 443–462.
523 doi:10.2478/v10183-012-0018-z
- 524 Bilbrough, C.J., Welker, J.M., Bowman, W.D., 2000. Early spring nitrogen uptake by snow-covered
525 plants: A comparison of arctic and alpine plant function under the snowpack. *Arctic, Antarct.*
526 *Alp. Res.* 32, 404–411.
- 527 Björkman, M.P., Kühnel, R., Partridge, D.G., Roberts, T.J., Aas, W., Mazzola, M., Viola, A., Hodson, A.,
528 Ström, J., Isaksson, E., 2013. Nitrate dry deposition in Svalbard. *Tellus B* 65.
529 doi:10.3402/tellusb.v65i0.19071
- 530 Chapin, F.S., Sturm, M., Serreze, M.C., McFadden, J.P., Key, J.R., Lloyd, A.H., McGuire, A.D., Rupp,
531 T.S., Lynch, A.H., Schimel, J.P., Beringer, J., Chapman, W.L., Epstein, H.E., Euskirchen, E.S.,
532 Hinzman, L.D., Jia, G., Ping, C.L., Tape, K.D., Thompson, C.D.C., Walker, D.A., Welker, J.M., 2005.
533 Role of land-surface changes in Arctic summer warming. *Science* (80-.). 310, 657–660.
534 doi:10.1126/science.1117368
- 535 Dormann, C.F., Woodin, S.J., 2002. Climate change in the Arctic: using plant functional types in a
536 meta-analysis of field experiments. *Funct. Ecol.* 16, 4–17. doi:10.1046/j.0269-
537 8463.2001.00596.x
- 538 Elmendorf, S.C., Henry, G.H.R., Hollister, R.D., Björk, R.G., Bjorkman, A.D., Callaghan, T. V, Collier, L.S.,
539 Cooper, E.J., Cornelissen, J.H.C., Day, T.A., Fosaa, A.M., Gould, W.A., Grétarsdóttir, J., Harte, J.,
540 Hermanutz, L., Hik, D.S., Hofgaard, A., Jarrad, F., Jónsdóttir, I.S., Keuper, F., Klanderud, K., Klein,
541 J.A., Koh, S., Kudo, G., Lang, S.I., Loewen, V., May, J.L., Mercado, J., Michelsen, A., Molau, U.,
542 Myers-Smith, I.H., Oberbauer, S.F., Pieper, S., Post, E., Rixen, C., Robinson, C.H., Schmidt, N.M.,
543 Shaver, G.R., Stenström, A., Tolvanen, A., Totland, O., Troxler, T., Wahren, C.-H., Webber, P.J.,
544 Welker, J.M., Wookey, P.A., 2012. Global assessment of experimental climate warming on
545 tundra vegetation: heterogeneity over space and time. *Ecol. Lett.* 15, 164–75.
546 doi:10.1111/j.1461-0248.2011.01716.x
- 547 Göransson, H., Ingerslev, M., Wallander, H., 2008. The vertical distribution of N and K uptake in
548 relation to root distribution and root uptake capacity in mature *Quercus robur*, *Fagus sylvatica*
549 and *Picea abies* stands. *Plant Soil* 306, 129–137. doi:10.1007/s11104-007-9524-x

- 550 Grogan, P. and Jonasson, S., 2003. Controls on annual nitrogen cycling in the understory of a sub-
551 arctic birch forest. *Ecology* 84(1): 202-218.
- 552 Hayashi, K., Cooper, E.J., Loonen, M.J.J.E., Kishimoto-Mo, A.W., Motohka, T., Uchida, M., Nakatsubo,
553 T., 2014. Potential of Svalbard reindeer winter droppings for emission/absorption of methane
554 and nitrous oxide during summer. *Polar Sci.* 8, 196–206. doi:10.1016/j.polar.2013.11.002
- 555 Hodkinson, I.D., Webb, N.R., Bale, J.S., Block, W., 1999. Hydrology, water availability and tundra
556 ecosystem function in a changing climate: the need for a closer integration of ideas? *Glob.*
557 *Chang. Biol.* 5, 359–369. doi:10.1046/j.1365-2486.1999.00229.x
- 558 Houle, D., Moore, J.-D., Ouimet, R., Marty, C., 2014. Tree species partition N uptake by soil depth in
559 boreal forests. *Ecology* 95, 1127–1133. doi:10.1890/14-0191.1
- 560 Jónsdóttir, I.S., Callaghan, T.V., Lee, J.A., 1995. Fate of added nitrogen in a moss-sedge Arctic
561 community and effects of increased nitrogen deposition. *Sci. Total Env.* 160/161, 677-685.
- 562 Kahmen, A., Renker, C., Unsicker, S.B., Buchmann, N., 2006. Niche complementarity for nitrogen: An
563 explanation for the biodiversity and ecosystem functioning relationship? *Ecology* 87 (5), 1244-
564 1255. doi: 10.1890/0012-9658
- 565 Kühnel, R., Björkman, M.P., Vega, C.P., Hodson, A., Isaksson, E., Ström, J., 2013. Reactive nitrogen and
566 sulphate wet deposition at Zeppelin Station, Ny-Ålesund, Svalbard. *Polar Res.* 32.
567 doi:10.3402/polar.v32i0.19136
- 568 Larsen, K.S., Michelsen, A., Jonasson, S., Beier, C., Grogan, P., 2012. Nitrogen uptake during fall,
569 winter and spring differs among plant functional groups in a subarctic heath ecosystem.
570 *Ecosystems* 15 (6): 927-939.
- 571 Lloyd, C.R., 2001. The measurement and modelling of the carbon dioxide exchange at a high Arctic
572 site in Svalbard. *Glob. Chang. Biol.* 7, 405–426. doi:10.1046/j.1365-2486.2001.00422.x
- 573 Mack, M.C., Schuur, E.A.G., Bret-Harte, M.S., Shaver, G.R., Chapin, F.S., 2004. Ecosystem carbon
574 storage in arctic tundra reduced by long-term nutrient fertilization. *Nature* 431, 440–443.
575 doi:10.1038/nature02887
- 576 Malmer, N., Svensson, B.M., Wallén, B., 1994. Interactions between Sphagnum mosses and field layer
577 vascular plants in the development of peat-forming systems. *Folia Geobot. Phytotaxon.* 29,
578 483–496. doi:10.1007/BF02883146
- 579 Maturilli, M., Herber, A., König-Langlo, G., 2013. Climatology and time series of surface meteorology
580 in Ny-Ålesund, Svalbard. *Earth Syst. Sci. Data* 5, 155–163. doi:10.5194/essd-5-155-2013
- 581 Maturilli, M., Herber, A., König-Langlo, G., 2014. Surface radiation climatology for Ny-Ålesund,
582 Svalbard (78.9° N), basic observations for trend detection. *Theor. Appl. Climatol.*
583 doi:10.1007/s00704-014-1173-4
- 584 McKane, R.B., Johnson, L.C., Shaver, G.R., Nadelhoffer, K.J., Rastetter, E.B., Fry, B., Giblin, A.E.,
585 Kielland, K., Kwiatkowski, B.L., Laundre, J.A., Murray, G., 2002. Resource-based niches provide a
586 basis for plant species diversity and dominance in arctic tundra. *Nature* 415, 68–71.
587 doi:10.1038/415068a

- 588 Myers-Smith, I.H., Hik, D.S., 2013. Shrub canopies influence soil temperatures but not nutrient
589 dynamics: An experimental test of tundra snow-shrub interactions. *Ecol. Evol.* 3, 3683–700.
590 doi:10.1002/ece3.710
- 591 Nadelhoffer, K.J., Giblin, A.E., Shaver, G.R., Laundre, J.A., 1991. Effects of temperature and substrate
592 quality on element mineralization in six Arctic soils, *Ecology*.
- 593 Nordin, A., Schmidt, I.K., Shaver, G.R., 2004. Nitrogen uptake by arctic soil microbes and plants in
594 relation to soil nitrogen supply. *Ecology* 85, 955–962.
- 595 Nowak, A., Hodson, A., 2014. On the biogeochemical response of a glacierized High Arctic watershed
596 to climate change: revealing patterns, processes and heterogeneity among micro-catchments.
597 *Hydrol. Process.* n/a–n/a. doi:10.1002/hyp.10263
- 598 Powlson, D.A., Barraclough, D., 1993. Mineralization and assimilation in soil-plant systems., in:
599 Knowles, R., Blackburn, H. (Eds.), *Nitrogen Isotope Techniques*. Academic Press, San Diego, pp.
600 209–221.
- 601 Robinson, C.H., 2002. Controls on decomposition and soil nitrogen availability at high latitudes. *Plant
602 Soil* 242, 65–81.
- 603 Robinson, C.H., Wookey, P.A., Parsons, A.N., Potter, J.A., Callaghan, T. V., Lee, J.A., Press, M.C.,
604 Welker, J.M., 1995. Responses of plant litter decomposition and nitrogen mineralization to
605 simulated environmental change in a high arctic polar semi-desert and a subarctic dwarf shrub
606 heath. *Oikos* 74, 503–512.
- 607 Romanovsky, V.E., Osterkamp, T.E. 2000. Effects of unfrozen water on heat and mass transport
608 processes in the active layer and permafrost. *Permafrost and Periglacial Processes* 11, 219–
609 239.
- 610 Roth, K., Boike, J., 2001. Quantifying the thermal dynamics of a permafrost site near Ny-Ålesund,
611 Svalbard. *Water Resour. Res.* 37, 2901–2914. doi:10.1029/2000WR000163
- 612 Rowe, E.C., Van Noordwijk, M., Suprayogo, D., Hairiah, K., Giller, K.E., Cadisch, G., 2001. Root
613 distributions partially explain 15N uptake patterns in *Gliricidia* and *Peltophorum* hedgerow
614 intercropping systems. *Plant Soil* 235, 167–179. doi:10.1023/A:1011961409353
- 615 Santruckova, H., Tahovska, K., Kopacek, J., 2009. Nitrogen transformations and pools in N-saturated
616 mountain spruce forest soils. *Biol. Fertil. SOILS* 45, 395–404. doi:10.1007/s00374-008-0349-4
- 617 Schimel, J.P., Bilbrough, C., Welker, J.M., 2004. Increased snow depth affects microbial activity and
618 nitrogen mineralization in two Arctic tundra communities. *Soil Biol. Biochem.* 36, 217–227.
619 doi:10.1016/j.soilbio.2003.09.008
- 620 Schmidt, I.K., Jonasson, S., Michelsen, A., 1999. Mineralization and microbial immobilization of N and
621 P in arctic soils in relation to season, temperature and nutrient amendment. *Appl. Soil Ecol.* 11,
622 147–160. doi:10.1016/S0929-1393(98)00147-4
- 623 Schmidt, I.K., Jonasson, S., Shaver, G.R., Michelsen, A., Nordin, A., 2002. Mineralization and
624 distribution of nutrients in plants and microbes in four arctic ecosystems: Responses to
625 warming. *Plant and Soil*. pp. 93–106. doi:10.1023/A:1019642007929

- 626 Schuur, E.A.G., Abbott, B., 2011. Climate change: High risk of permafrost thaw. *Nature* 480, 32–3.
627 doi:10.1038/480032a
- 628 Sistla, S.A., Moore, J.C., Simpson, R.T., Gough, L., Shaver, G.R., Schimel, J.P., 2013. Long-term
629 warming restructures Arctic tundra without changing net soil carbon storage. *Nature* 497, 615–
630 8. doi:10.1038/nature12129
- 631 Smirnoff, N., Stewart, G.R., 1985. Nitrate assimilation and translocation by higher plants:
632 Comparative physiology and ecological consequences. *Physiol. Plant.* 64, 133–140.
633 doi:10.1111/j.1399-3054.1985.tb02326.x
- 634 Spielhagen, R.F., Werner, K., Sørensen, S.A., Zamelczyk, K., Kandiano, E., Budeus, G., Husum, K.,
635 Marchitto, T.M., Hald, M., 2011. Enhanced modern heat transfer to the Arctic by warm Atlantic
636 Water. *Science* 331, 450–3. doi:10.1126/science.1197397
- 637 Ste-Marie, C., Paré, D., 1999. Soil, pH and N availability effects on net nitrification in the forest floors
638 of a range of boreal forest stands. *Soil Biol. Biochem.* 31, 1579–1589.
- 639 Sturm, M., McFadden, J.P., Liston, G.E., Stuart Chapin, F., Racine, C.H., Holmgren, J., 2001. Snow-
640 shrub interactions in Arctic Tundra: A hypothesis with climatic implications. *J. Clim.* 14, 336–
641 344.
- 642 Sturm, M., Schimel, J., Michaelson, G., Welker, J.M., Oberbauer, S.F., Liston, G.E., Fahnestock, J.,
643 Romanovsky, V.E., 2005. Winter biological processes could help convert arctic tundra to
644 shrubland. *Bioscience* 55, 17–26.
- 645 Tennant, D., 1975. A Test of a Modified Line Intersect Method of Estimating Root Length. *J. Ecol.* 63,
646 995. doi:10.2307/2258617
- 647 Tye, A.M., Young, S.D., Crout, N.M.J., West, H.M., Stapleton, L.M., Poulton, P.R., Laybourn-Parry, J.,
648 2005. The fate of 15N added to high Arctic tundra to mimic increased inputs of atmospheric
649 nitrogen released from a melting snowpack. *Glob. Chang. Biol.* 11, 1640–1654.
650 doi:10.1111/j.1365-2486.2005.01044.x

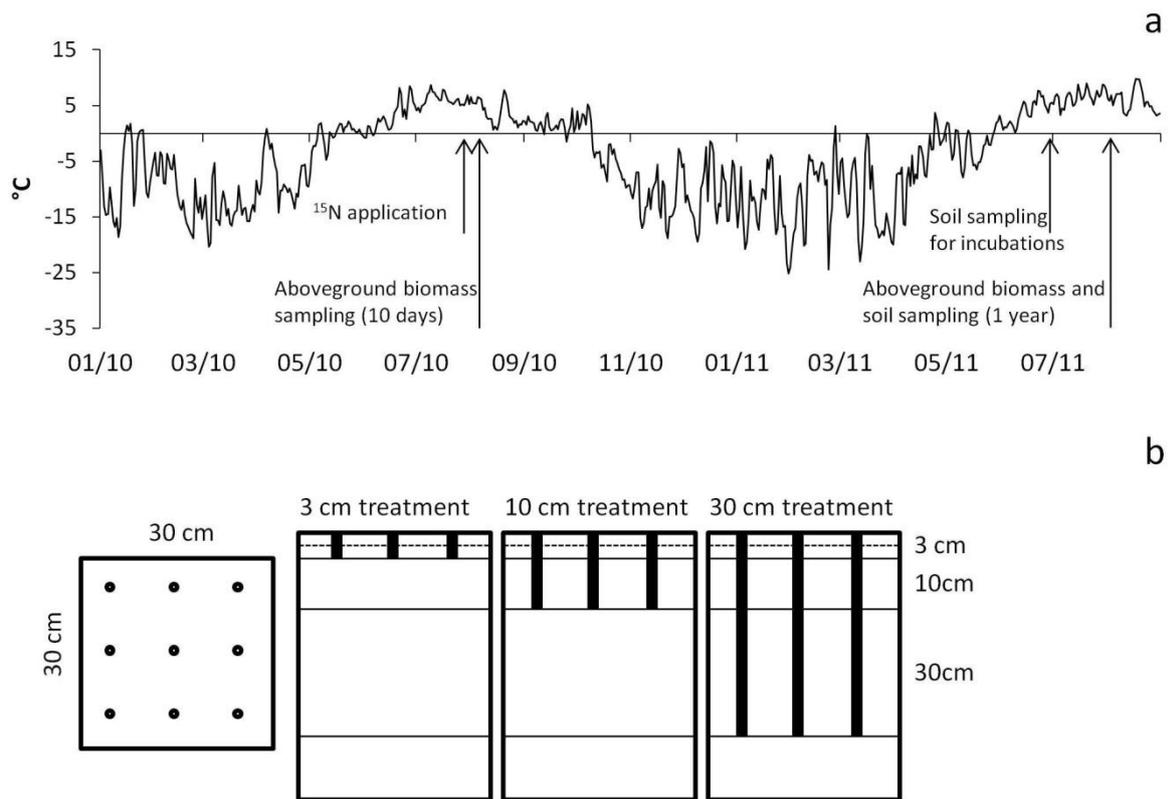
651



653

654 **Figure 1. Conceptual schema of major N flows in Arctic tundra ecosystems, during the spring melt**

655 **and later in the growing season.**

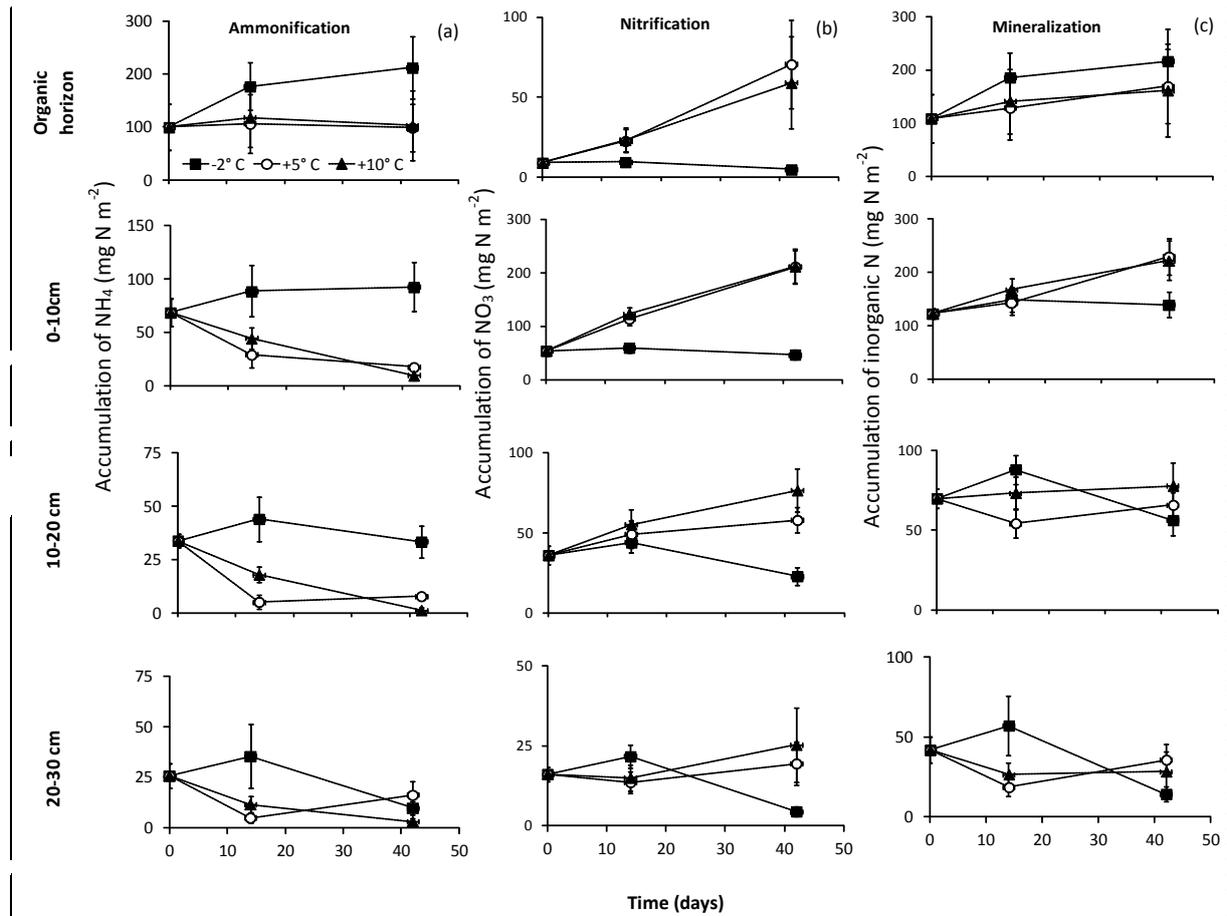


656

657 **Figure 2. Timing of soil and vegetation sampling and ¹⁵N application together with the course of air**

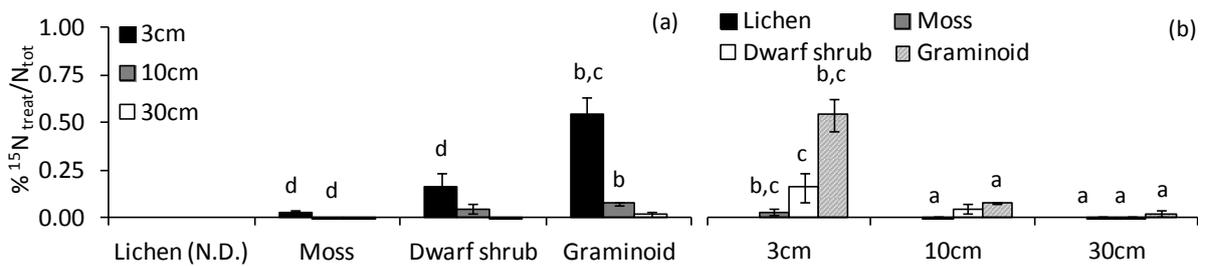
658 **temperature in Ny Ålesund (Maturilli et al., 2013) (a) and scheme of ¹⁵N application into the mineral**

659 **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.

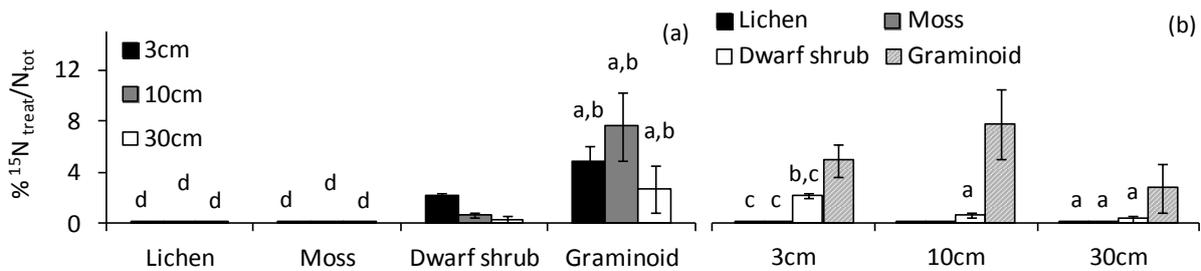


Figure 5. ^{15}N as a proportion of total N in aboveground biomass of each plant functional type, one year after ^{15}N application at 3, 10 or 30 cm depth. Figure a represents significant differences among plant functional types and figure b represents significant differences for each functional type 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.

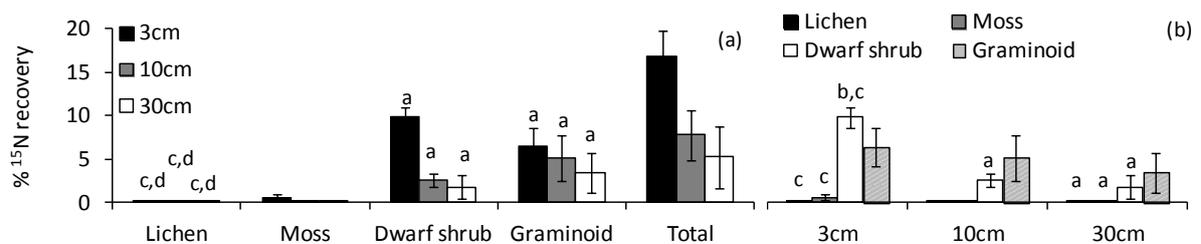


Figure 6. Recovery of applied ^{15}N one year after application in four plant functional types (a) under different treatments (b). Total represents sum of % ^{15}N recovery for all functional types. Columns that do not share the same superscript letters are significantly different ($p < 0.05$). Error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

665

666

667

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 ₂ O)	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						

671

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

675

676