

# Soil Biology and Biochemistry

## Deepened snow enhances gross nitrogen cycling among Pan-Arctic tundra soils during both winter and summer

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To the editor of Soil Biology and Biochemistry,

This letter accompany a renewed version of the revised manuscript (SBB17374R1) previously submitted for review. Due to an unfortunate omission of key data in the previous revised version this was withdrawn, and the current version amended with the missing soil moisture data of the Svalbard experimental site (see Table 1). The manuscript is entitled “Deepened snow enhances gross nitrogen cycling among Pan-Arctic tundra soils during winter and summer”, authored by Xu et al., and has been revised carefully and thoroughly in accordance with comments given by reviewers as outlined in the detailed response actions taken. The main topic, observations and conclusion from the manuscript reads below:

**1. What is the scientific question we are addressing?**

How are potential N cycling rates among Pan-Arctic tundra soils affected by deepened snow cover? The study investigates effects in late winter (after several months of continuous snow cover) and late growing season (after ca. three months of snow-free condition) and addresses site-specific changes among contrasting soil and vegetation conditions.

**2. What is/are the key finding(s) that answers this question?**

Despite contrasting site conditions, we do observe universal deepened snow effects on several N cycling rates (significantly increased) in both late winter and summer, and we conclude that increased availability of soluble carbon and nitrogen (DOC and DON) are the main drivers for these increases across sites. A notable observation is that deepened snow increases N cycling processes most in wet tundra ecosystems compared to dry tundra.

**3. Why is this work important and timely?**

This is the first study to systematically compare impacts of climate change on tundra soil N cycling at contrasting sites in the northern circumpolar region in combination with seasonality. The combination of multi-season and multi-site investigations have important projections for annual N cycling in the contrasting Arctic landscapes.

## HIGHLIGHTS:

- Deepened snow enhanced winter nitrification,  $\text{NO}_3^-$ -N immobilization, denitrification
- Deepened snow enhanced summer gross mineralization and denitrification
- The increases in N cycling rates were driven by the availability of DOC and DON
- The effects of deepened snow were most pronounced in wet tundra ecosystems

1 Deepened snow enhances gross nitrogen cycling among Pan-Arctic  
2 tundra soils during both winter and summer

3

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## 17 Abstract

18 Many Arctic regions currently experience an increase in winter snowfall as a result of climate  
19 change. Deepened snow can enhance thermal insulation of the underlying soil during winter,  
20 resulting in warmer soil temperatures that promote soil microbial nitrogen (N)-cycle processes  
21 and the availability of N and other nutrients. We conducted an *in vitro* study comparing the  
22 effects of deepened snow (using snow fences that have been installed for 3-13 years) on  
23 microbial N-cycle processes in late summer (late growing season) and winter (late snow-covered  
24 season) among five tundra sites in three different geographic locations across the Arctic  
25 (Greenland (dry and wet tundra), Canada (mesic tundra), and Svalbard, Norway (heath and  
26 meadow tundra)). Soil gross N cycling rates (mineralization, nitrification, immobilization of  
27 ammonium ( $\text{NH}_4^+$ ) and nitrate ( $\text{NO}_3^-$ ), and denitrification) were determined using a  $^{15}\text{N}$  pool  
28 dilution. Potential denitrification activity (PDA) and nitrous oxide reductase activity (N2OR)  
29 were measured to assess denitrifying enzyme activities.

30 The deepened snow treatment across all sites had a significant effect of the potential soil capacity  
31 of accelerating N cycling rates in late winter, including quadrupled gross nitrification, tripled  
32  $\text{NO}_3^-$ -N immobilization, and doubled denitrification as well as significantly enhanced late  
33 summer gross N mineralization, denitrification (two-fold) and  $\text{NH}_4^+$ -N availability. The increase  
34 in gross N mineralization and nitrification rates were primarily driven by the availability of  
35 dissolved organic carbon (DOC) and nitrogen (DON) across sites. The largest increases in winter  
36 DOC and DON concentrations due to deepened snow were observed at the two wetter sites (wet  
37 and mesic tundra), and N cycling rates were also more strongly affected by deepened snow at  
38 these two sites than at the three other drier sites. Together, these results suggest that the potential  
39 effects of deepened winter snow in stimulating microbial N-cycling activities will be most  
40 pronounced in relatively moist tundra ecosystems. Hence, this study provides support to prior

41 observations that growing season biogeochemical cycles in the Arctic **are** sensitive to snow depth  
42 with altered nutrient availability for microorganisms and vegetation. It can be speculated that on  
43 the one hand growing season N availability will increase and promote plant growth, but on the  
44 other hand foster increased water- and gaseous (e.g. N<sub>2</sub> and N<sub>2</sub>O) N-losses with implications for  
45 overall nutrient status.

46 **Keywords:** soil nitrogen, gross mineralization, gross nitrification, immobilization, denitrification,  
47 snowfence, moist tundra

## 48 Introduction

49 Arctic ecosystems are characterized by short growing seasons and long cold winters with frozen  
50 and snow-covered soils generally resulting in low nutrient availabilities and primary productivity  
51 (M. H. Jones, Fahnestock, & Welker, 1999; Nadelhoffer, Giblin, Shaver, & Linkins, 1992).

52 Meanwhile, in spite of these harsh conditions, soil microbial activity continues through the winter  
53 (Mikan, Schimel, & Doyle, 2002; Nobrega & Grogan, 2007). Despite low CO<sub>2</sub> emission rates,  
54 previous work has confirmed that soil respiration during the long winter accounts for an  
55 important component of annual carbon (C) budget of Arctic tundra ecosystems (Morgner,  
56 Elberling, Strebel, & Cooper, 2010; Natali et al., 2019). Accordingly, significant plant litter  
57 decomposition and soil nitrogen (N) mineralization occur under such conditions and a large  
58 proportion of annual labile N in Arctic tundra soils may be produced during winter (Bokhorst,  
59 Bjerke, Melillo, Callaghan, & Phoenix, 2010; Schimel, Bilbrough, & Welker, 2004).

60 Both measurements and climate model projections consistently show that air temperature across  
61 the Arctic has increased during the past few decades and will continue to increase beyond the  
62 global average (Pörtner et al., 2019; Screen & Simmonds, 2010; Stocker et al., 2013). However,  
63 warming rates differ markedly between seasons (Cohen et al., 2014) and regions (Westergaard-

64 Nielsen, Karami, Hansen, Westermann, & Elberling, 2018). The extent of winter warming is  
65 expected to be at least four times as great as summer warming by the end of this century (Richard  
66 Bintanja & Van der Linden, 2013). In addition, increased winter temperature may trigger an  
67 increase of more than 50% in peak-regional precipitation as snowfall in some Arctic regions (R.  
68 Bintanja & Selten, 2014). Snow cover can thermally insulate the soil from the extremely cold  
69 winter air temperatures in the Arctic, hence dampening soil temperature fluctuations, and  
70 moderating the severity, depth and extent of winter soil freezing (Sharratt, Baker, Wall, Skaggs,  
71 & Ruschy, 1992), and facilitating warming of underlying permafrost (Biskaborn et al., 2019). For  
72 instance, experimental snowfences that increased snow depth from 30 to 150 cm, led to an  
73 increase in soil surface temperature of 6 °C during late winter in the two common tundra types in  
74 high Arctic Svalbard (Morgner et al., 2010). This may enhance microbial decomposition of soil  
75 organic matter and N mineralization rates during winter (Borner, Kielland, & Walker, 2008;  
76 Schimel et al., 2004; Semenchuk et al., 2019). The consequent increase in N availability due to  
77 higher N mineralization may in turn alleviate N-limitation on plant growth in the subsequent  
78 growing season (Larsen, Michelsen, Jonasson, Beier, & Grogan, 2012; Semenchuk et al., 2015),  
79 promoting assimilation of CO<sub>2</sub> from the atmosphere (Hobbie & Chapin, 1996). Nevertheless,  
80 deepened snow cover will delay snowmelt in spring and lead to colder and wetter soils in early  
81 growing season, thus negatively affecting the growth and reproductive success of early-growing  
82 plants (Cooper, Dullinger, & Semenchuk, 2011; Morgner et al., 2010).

83 Along with soil temperature limitation, the presence of liquid water has been identified as both a  
84 prerequisite for biological activity and the primary control on soil C and nutrient cycles in winter  
85 (Öquist et al., 2009). As soil freezes, water availability is reduced to micro-films, inhibiting  
86 diffusion and mass transport of substrates, enzymes and microorganisms, and thus limiting

87 microbial activities (Ostroumov & Siegert, 1996; J. P. Schimel & Mikan, 2005). Furthermore, the  
88 reduction in liquid water content may be accompanied by a decrease in air-filled pore space due  
89 to expansion of H<sub>2</sub>O during freezing, resulting in reduced diffusion of oxygen and to microbial  
90 depletion of the remnant oxygen within those pore spaces, together and thus inhibition of aerobic  
91 respiration (Tucker, 2014).

92 Arctic terrestrial ecosystems generally receive low amounts of atmospheric nitrogen (N)  
93 deposition (<2 kg N ha<sup>-1</sup> y<sup>-1</sup>) (Dentener et al., 2006) and N is one of the most important growth-  
94 limiting nutrients in most tundra ecosystems (Elser et al., 2007; Tamm, 2012). This leads to  
95 strong competition for both bioavailable organic and mineral N between plants and free-living  
96 microorganisms (Kuzyakov & Xu, 2013). Microbial N mineralization, nitrification,  
97 immobilization and denitrification are key soil N transformation processes as they drive the  
98 turnover of inorganic ammonium and nitrate, while also regulating N losses along hydrological as  
99 well as gaseous pathways (Butterbach-Bahl et al., 2011). Dissolved organic nitrogen (DON) is  
100 considered as one of the most mobile and labile organic N forms. The DON pool may directly  
101 regulate the rates of mineralization and nitrification in soil as it provides the initial substrate for  
102 these N transformation pathways (D. L. Jones, Shannon, V. Murphy, & Farrar, 2004). For  
103 example, Cookson and Murphy (2004) found that gross N mineralization and nitrification rates  
104 were significantly decreased after removal of the DON pool and suggested that the rate of  
105 insoluble soil organic N conversion to DON limited gross N transformation rates.

106 The Pan-Arctic tundra is heterogeneous and encompasses a mosaic of distinct vegetation types  
107 that show diverse patterns of plant growth, C sequestration, N requirements and storage.  
108 Additionally, variation in plant species composition among tundra sites drives the variability of  
109 litter and soil organic matter quality (such as C:N ratio), resulting in variability in soil N cycling



110 rates and thus ecosystem N retention (Christopher, Shibata, Ozawa, Nakagawa, & Mitchell,  
111 2008). Small- and large-scale topographic heterogeneity can influence hydrology, nutrient  
112 movement, microclimate, and snow distribution, and thus may alter plant productivity as well as  
113 microbial communities and activities related to C and N turnover processes (DeMarco, Mack, &  
114 Bret-Harte, 2011). Several studies have reported progressively increased gross N mineralization  
115 and inorganic N availability along transects from upland to low-lying areas across three  
116 contrasting Arctic ecosystems (Christiansen et al., 2017; Paré & Bedard-Haughn, 2012; Welker,  
117 Fahnestock, Henry, O'Dea, & Chimner, 2004). This pattern was generally attributed to the  
118 redistribution of soluble nutrients with water, wind and snow, as well as increasingly thick snow  
119 accumulation, lowered soil temperature variation and increased soil water status when moving  
120 down-hill.

121 Changing winter climate and snow cover can have legacy effects on soil N turnover and plant  
122 growth in specific Arctic locations even during the snow-free growing season. For instance,  
123 higher growing-season soil and plant N concentrations arising from multi-year increased snow  
124 depth have been reported in Alaska (Schimel et al., 2004; Welker, Fahnestock, Sullivan, &  
125 Chimner, 2005) and high Arctic Svalbard (Mörsdorf et al., 2019; Semenchuk et al., 2015).  
126 However, no study has systematically compared: i) contrasting tundra sites in the northern  
127 circumpolar region; or ii) samples obtained in summer with corresponding samples obtained  
128 during frozen conditions in winter.

129 In this study, we investigated the effects of deepened snow cover on soil nutrients pools and N  
130 cycling activities in five distinct tundra ecosystems of contrasting moisture regime across the  
131 Arctic. At each of the study sites snow fences had been established to manipulate snow-depths  
132 for at least 3 years (and up to 13 years). Soil samples were collected in late winter (to investigate

133 the effects of several months of continuous snow cover), and late in the growing season (to  
134 investigate the legacy effects after ca. three months of snow-free conditions), and shipped at  
135 temperature conditions resembling those in the field for subsequent laboratory work. Overall, our  
136 research objectives were to unravel the effects of deepened snow on soil gross N mineralization,  
137 nitrification, immobilization and denitrification, as well as potential denitrification and nitrous  
138 oxide (N<sub>2</sub>O) reductase activity, and soil N nutrient pools, and to identify environmental factors  
139 explaining responses of N-cycle processes to deepened snow. We hypothesized that: (H1)  
140 deepened snow enhances gross soil N transformation rates across tundra sites in winter and  
141 summer; (H2) increased availability of soil organic N drives the responses of gross N  
142 transformation rates to deepened snow; and (H3) the extent of the increase in gross N  
143 transformation rates mediated by deepened snow is linked to ecosystem moisture regime.

## 144 Material and methods

### 145 Site description

146 The study included five contrasting tundra sites with markedly different vegetation, climate,  
147 moisture regimes and soil types from three different geographical locations: Disko Island of West  
148 Greenland; Daring Lake region, Northwest Territories, Canada; and Svalbard, Norway (Table 1).  
149 The Disko Island sites were at Blæsedalen Valley (69°16'N, 53°27'W), West Greenland, in the  
150 low Arctic. This valley has a mean annual air temperature of -3 °C (mean monthly temperature  
151 ranging from 8 °C in July to -14 °C in March) and an annual mean precipitation of 400 mm (40%  
152 as snow) (Hansen, Elberling, Humlum, & Nielsen, 2006; Hollesen et al., 2015). The mean annual  
153 soil temperature at 5 cm depth is -1.9 °C and frozen soil conditions prevail from October to late  
154 May. The sites lie within the discontinuous permafrost zone. One site (denoted 'Disko Dry') is a  
155 dry shrub heath tundra with low (<10 cm) shrubs of deciduous dwarf birch (*Betula*

156 *nana*) and gray willow (*Salix glauca*), evergreens including mountain cranberry (*Vaccinium vitis-*  
157 *idaea*), black crowberry (*Empetrum nigrum*) and Arctic bell-heather (*Cassiope tetragona*), with a  
158 mixture of mosses and lichens covering the ground. The other site ('Disko Wet') is a wet fen  
159 dominated by water sedge (*Carex aquatilis* ssp. *stans*), looseflower alpine sedge (*Carex*  
160 *rariflora*), common cottongsedge (*Eriophorum angustifolium*), mosses (*Paludella squarrosa* and  
161 *Tomentypnum nitens*) and the deciduous shrub northern willow (*Salix arctophila*). The soil in  
162 Blæsedalen is formed on volcanic basalt. Disko Dry site has a shallow O-horizon (1-5 cm) atop  
163 the mineral A-horizon. The Disko Wet soil has a 20 cm deep peat layer , with the water table  
164 fluctuating from 20 cm below its surface to 15 cm above (Nielsen et al., 2017).

165 The other low Arctic site is located near the Tundra Ecological Research Station at Daring Lake  
166 (64°52'N, 111°34'W), approximately 300 km northeast of Yellowknife in the Northwest  
167 Territories, Canada. The area is underlain by continuous permafrost to a depth of 160 m (Dredge,  
168 Kerr, & Wolfe, 1999) and has a shallow active layer forming to ca. 77 cm depth in the thaw  
169 season. The Daring Lake Weather Station (S. Kokelj, Water Management and Monitoring  
170 Division of the Department of Environment and Natural Resources, GNWT) records an annual  
171 average temperature of -8.4 °C, with average monthly temperature ranging from -26.4°C in the  
172 coldest month (January) to 13.5 °C in the warmest month (July), and an annual precipitation  
173 ranging between 200 and 300 mm, of which 75-125 mm is received in summer (June-August).  
174 First snowfall usually happens in mid-October and snow depth is generally not more than 10 cm  
175 until the beginning of November, reaching an average peak of 37 cm (10-year range: 20-59 cm)  
176 in exposed areas by later winter. Our study focused on the birch hummock ecosystem-type which  
177 is located in a slightly sloping wide valley, and is characterized by 10-30 cm high hummocks and  
178 10-40 cm tall deciduous birch (*Betula glandulosa*) shrubs that attain 10-30% of the areal

179 coverage. The remaining cover is a mixture of mostly ericaceous shrubs including bog rosemary  
180 (*Andromeda polifolia*), *V. vitis-idaea*, bog bilberry (*Vaccinium uliginosum*), and labrador tea  
181 (*Rhododendron subarcticum* [formerly *Ledum decumbens*]), and sedges, mosses, lichens. The  
182 soil in this ecosystem is characterized as Orthic Dystric Turbic Cryosols and consists of an  
183 organic horizon 3-20 cm deep above cryoturbated silt-sand mineral horizons (Buckeridge, Cen,  
184 Layzell, & Grogan, 2009).

185 The high Arctic study site is located in Adventdalen (78°10'N, 16°04'E), a flat valley located in  
186 the western part of Svalbard, Norway. The climate records from the nearby weather station at  
187 Svalbard Airport report a mean annual temperature of -2.5 °C with mean monthly temperature  
188 ranging from -10.7°C in the coldest month (March) to 7.4 °C in the warmest month (July), and a  
189 mean annual precipitation of 228 mm, with most precipitation (120 mm) as snow during  
190 November to May (2009-2018). Our study used two different vegetation types at this site: a heath  
191 ('Svalbard Heath') and a mesic meadow ('Svalbard Meadow'). The heath has rougher stony soils  
192 and topography, whereas the meadow is flat, and most vegetation is below 10 cm in height. The  
193 heath vegetation is dominated by *C. tetragona* and mountain avens (*Dryas octopetala*), with  
194 polar willow (*Salix polaris*) throughout. The mesic meadow is mainly composed of *D.*  
195 *octopetala*, polar fox tail (*Alopecurus ovatus*), northern wood rush (*Luzula arcuata* subsp.  
196 *Confuse*), *S. polaris*, alpine bistort (*Bistorta vivipara*) and bryophytes. A typical soil profile  
197 consists of an upper O-horizon in the range of 0.2 to 6 cm with slightly decomposed organic  
198 matter and plant roots, a dark brown A-horizon of 1 to 5 cm, and B/C horizons composed of grey  
199 silt (Strebel, Elberling, Morgner, Knicker, & Cooper, 2010).

200 [Experimental setup and design](#)

201 At Disko: In July 2012, we established the snowfences oriented perpendicular to the prevailing  
202 winter wind direction at Disko Dry (n=5), whereas the snowfences in Disko Wet (n=5) were  
203 established in July 2013. Each snow fence is 14.7 m long and 1.5 m high. Control plots were  
204 located between 6 and 11 m from the windward side of the fence. The maximum snow depth  
205 (140 to 150 cm) occurred between 3 and 8 m from the fence on the leeward side, usually 2-3  
206 times the depth at the control plots (Christiansen et al., 2017). Snow cover typically remains at  
207 the snowfence plots until mid-June, which is one-two weeks later than the control plots. Soil  
208 temperature at 2 cm depth (n=3-4 for each treatment) was logged hourly using thermistors  
209 connected to dataloggers (Gemini Data Loggers; Tinytag, Chichester, West Sussex, UK) since  
210 the start of the experiment. Volumetric soil moisture content at the 0-5 cm depth (n=3-4 for each  
211 treatment) was recorded every half hour (HOBO, Onset Computer Corporation, MA, USA).

212 At Daring Lake: In summer 2004, snowfences (15 m long and 1.2 m high; n=5) were established  
213 within the birch hummock tundra near Daring Lake. Control plots were established parallel with  
214 the fences in similar patches of vegetation at least 30 m apart from the nearest fence to ensure  
215 sufficient separation from enhanced snow cover areas. The snowfences, oriented perpendicular to  
216 the prevailing winter wind direction, created snowdrifts of around 20 m from both sides of each  
217 fence, with typical peak uniform snow depth of 0.9-1.0 m within at least 3 m from each fence  
218 (Christiansen, Lafreniere, Henry, & Grogan, 2018). By comparison, snow depth in the ambient  
219 control plots typically reaches a maximum depth of 0.3-0.4 m. In addition, the snowfences  
220 usually delayed the complete snowmelt date by 7-10 days each spring (Buckeridge & Grogan,  
221 2008). Soil temperature at 2 cm depth (n=2 for each treatment) was measured hourly and logged  
222 as 4-hourly means using thermocouple probes connected to data loggers (CR10X, Campbell  
223 Scientific, Logan, UT, USA) since the start of experiment. Volumetric soil moisture content was

224 recorded at the same frequency, using dielectric permittivity probes (CSC16, Campbell  
225 Scientific, Logan, UT, USA) that measured soil moisture across the 0-5 cm depth interval (n=2  
226 for each treatment).

227 At Svalbard: In autumn 2006, snowfences (6.2 m long and 1.5 m high) were established in  
228 Svalbard Heath (n=5) and Svalbard Meadow (n=6). Control plots with natural snow cover were  
229 adjacent to each snowfence. The snow depth of control plots was 32 cm in Svalbard Heath and 21  
230 cm in Svalbard Meadow (Cooper et al., 2011). The snowfences were installed perpendicular to  
231 the prevailing south-eastern winter wind and increased snow accumulation to a distance of at  
232 least 20 m behind on the leeward side, with the greatest snow depth of 150 cm between 3 to 12 m  
233 from the fence. The snowfence plots experienced snow cover for 2-4 weeks longer relative to  
234 control plots (7-8 months of the year). Soil temperature at 2 cm depth (n=4-6 for each treatment)  
235 was recorded hourly using thermistors connected to data loggers (Gemini Data Loggers; Tinytag,  
236 Chichester, West Sussex, UK) since the start of the experiment. Soil moisture at 0-5 cm depth  
237 was measured manually using a Theta meter soil moisture probe (Theta Probe ML2x; Delta-T  
238 Devices, Cambridge, UK) during the growing season in 2015.

### 239 [Soil sampling](#)

240 Soil samples were collected on September 1<sup>st</sup> 2016 and again in mid-April 2017 at the Disko  
241 sites, on August 20<sup>th</sup> 2016 and again on May 8<sup>th</sup> 2017 at the Daring Lake site, and on September  
242 1<sup>st</sup> 2017 and June 5<sup>th</sup>, 2018 at the Svalbard sites. In each of the snowfence and control plots at  
243 Disko (both dry and wet sites), Daring Lake, and Svalbard (both heath and meadow sites), we  
244 collected soil samples from the 0-5 cm depth interval after removing the litter layer. For sampling  
245 under non-frozen conditions, we used soil augers of 5-6 cm diameter and collected 3-5 samples in  
246 each plot that subsequently were mixed into one composite sample representing the plot. For

247 sampling under frozen conditions in the late winter, snow was removed and the soil excavated by  
248 chiseling to the desired depth. The snow depth on the day of sampling in April 2017 at Disko was  
249 ca. 150 cm with no differences between control and snowfence plots, while during winter  
250 sampling at the Svalbard sites snow depth was 10-20 cm lower at the control plots compared to  
251 the plots affected by the snowfences. The mean sampling distance to the west of snowfences at  
252 the Svalbard sites was  $847 \pm 50$  cm (mean  $\pm$  1 SE; defined in other publications as the ‘Deep  
253 regime’). The samples were shipped immediately to the laboratory in Copenhagen, either in  
254 frozen condition for the late winter samples, or at 5 °C for the late summer samples, and kept  
255 under these conditions until the biogeochemical analyses which occurred within ca. two weeks.  
256 The frozen samples were thawed at 5 °C for three days prior to use. All samples were gently  
257 mixed, and woody and coarse roots and stones were removed by hand.

#### 258 Incubation experiments

259 Soil gross N transformation rates were measured using a  $^{15}\text{N}$  pool dilution technique. Soil  
260 samples (15 g moist soil) were transferred to 100-mL plastic cups and gently mixed with 1 mL of  
261  $^{15}\text{N}$  solution (30 mg N L<sup>-1</sup> at 3.37 atom% excess  $^{15}\text{N}$ ; ca. 2.5 mg N kg<sup>-1</sup> DW), and the cup covered  
262 with pierced Para-film. The solution was either ammonium chloride ( $^{15}\text{NH}_4\text{Cl}$ ) for determination  
263 of gross N mineralization or potassium nitrate ( $\text{K}^{15}\text{NO}_3$ ) for determination of gross nitrification.  
264 Incubation took place at constant room temperature, following a 24 h thermal equilibrium period.  
265 We prepared three analytical replicates for each combination of soil sample and  $^{15}\text{N}$  solution.  
266 Soils were extracted immediately, 2 days and 7 days after labelling by suspension in 75 mL of  
267 0.5 M  $\text{K}_2\text{SO}_4$  solution (one hour on horizontal shaker). All soil suspensions were filtered through  
268 ash-less quartz filter (Whatman GF/D, Maidstone, UK).

269 Potential denitrification activity (PDA) was implemented as a proxy of concentration of  
270 denitrifying enzymes in a soil sample (Page, Miller, & Keeney, 1982). Ten g of moist soil was  
271 immersed in 30 mL of solution containing 1 mM KNO<sub>3</sub>, 0.5 mM glucose, 0.5 mM sodium acetate  
272 and 0.5 mM sodium succinate in a 100-mL glass bottle. The bottle was flushed with N<sub>2</sub> for 30  
273 seconds and sealed with a rubber stopper and aluminum crimp cap. After 15 mL of acetylene was  
274 added, the bottles were shaken on a horizontal shaker at a moderate shaking level at 5 °C and 3  
275 mL of the headspace was collected at 60, 120, 180, 240 and 300 min for N<sub>2</sub>O analysis. Headspace  
276 samples were transferred to 3-mL pre-evacuated Exetainers (Labco Scientific, High Wycombe,  
277 UK) for analysis of N<sub>2</sub>O on a GC (HP7890A, Agilent, Wilmington, USA) equipped with an  
278 Electron Capture Detector (μECD).

279 Nitrous oxide reductase activity (N<sub>2</sub>OR) reduces microbial and atmospheric N<sub>2</sub>O into dinitrogen  
280 (N<sub>2</sub>) and is an indicator for the N<sub>2</sub>O reductase concentration in soil (Wallenstein & Weintraub,  
281 2008). Soil samples (10 g of moist soil) were transferred to 50-mL centrifuge tubes and  
282 suspended in 25 mL phosphate-buffered saline (PBS) by stirring with a spatula and vigorously  
283 shaking the tube. Subsequently the tubes were centrifuged for 10 min at 12000 x g at 5 °C and the  
284 supernatant decanted. This step was repeated three times to deplete the soil of NO<sub>3</sub><sup>-</sup> that would  
285 otherwise lead to formation of N<sub>2</sub>O during the anaerobic incubation and hence interfere with the  
286 measurement of N<sub>2</sub>OR activity. Finally, 25-mL of a solution containing 0.5 mM glucose, 0.5  
287 mM sodium acetate and 0.5 mM sodium succinate was added and the soil suspension transferred  
288 to 100-mL glass bottles. The bottles were flushed with N<sub>2</sub> to make them anaerobic and sealed  
289 as explained above except that we added 15 mL of 200 ppm N<sub>2</sub>O in N<sub>2</sub> to a final concentration of  
290 ca. 25 ppm N<sub>2</sub>O, and incubated them on a horizontal shaker (200 rpm) at 5 °C. Three-mL  
291 headspace samples were collected at 10, 70, 130, 190 and 250 min for N<sub>2</sub>O analysis. During



292 incubation, the concentration of N<sub>2</sub>O decreases over time, which affects the rates of N<sub>2</sub>OR  
293 activity. For calculation of N<sub>2</sub>OR activity rates in individual incubation bottles, the N<sub>2</sub>O  
294 concentration at the different time points was fitted to a model assuming first order enzyme  
295 kinetics and rates were calculated at a standardized N<sub>2</sub>O concentration of 20 ppm.

296 For estimation of substrate induced respiration (SIR), 5 g of moist soil from each sample was  
297 immersed in 10 mL of 20 mM glucose in a 100-mL incubation bottle. Each bottle was sealed with  
298 a butyl rubber stopper and aluminium crimp and then added 14 mL air. The bottle was incubated  
299 on a shaker (200 rpm) at 5 °C and 3 mL of the headspace was collected at 10 min, 60 min, 110  
300 min and 160 min for CO<sub>2</sub> analysis of a GC (HP7890A, Agilent, Wilmington, USA) equipped  
301 with a methanizer and a Flame Ionization Detector (FID).

302 Respiration was measured as described for SIR above, but without glucose addition. Three mL of  
303 the headspace was collected at 10 min, ca. 6 hours, ca. 22 hours and ca. 28 hours for CO<sub>2</sub>  
304 analysis. Anaerobic respiration was estimated as CO<sub>2</sub> production under the conditions described  
305 for PDA above.

### 306 [Laboratory analyses](#)

307 Soil moisture was determined upon oven drying (105 °C for 24 h). Soil pH was measured using a  
308 moist soil to water ratio of 1:2.5. Ammonium and NO<sub>3</sub><sup>-</sup> concentrations in the filtered extracts  
309 described above were determined using flow-injection analysis (Tecator 5000 FIAStar, Höganäs,  
310 Sweden). To determine <sup>15</sup>N enrichments of extractable NH<sub>4</sub><sup>+</sup>-N, soil extracts were amended with  
311 magnesium oxide (MgO) to raise pH above 13 in order to convert NH<sub>4</sub><sup>+</sup>-N into ammonia (NH<sub>3</sub>-  
312 N) that is subsequently caught on acidified filter papers. (Sørensen & Jensen, 1991). For analysis  
313 of <sup>15</sup>NO<sub>3</sub><sup>-</sup>-N, NH<sub>4</sub><sup>+</sup>-N was removed from the extracts, and then Devarda alloy was added to  
314 convert NO<sub>3</sub><sup>-</sup>-N into NH<sub>4</sub><sup>+</sup>-N, followed by further conversion into NH<sub>3</sub> at pH >13 as described

315 above for  $\text{NH}_4^+\text{-N}$  (Sørensen & Jensen, 1991). Due to low molarity and to avoid swelling of the  
316 acid traps, potassium chloride (KCl) was added to the extract prior to diffusion to bring the ionic  
317 strength of the solution (1.5 M) closer to that of acid trap (2 M) (Stark & Hart, 1996). Soil total C  
318 and N contents and the  $^{15}\text{N}$  contents in soil and acid traps was determined by elemental analysis  
319 (CE1110, Thermo Electron, Milan, Italy) coupled in continuous flow mode to a Finnigan MAT  
320 Delta PLUS isotope ratio mass spectrometer (IRMS; Thermo Scientific, Bremen, Germany).  
321 Prior to analysis, 20-30 mg subsamples of finely ground soil material was weighed into tin  
322 combustion cups and freeze-dried acid filters likewise wrapped in tin cups. Soil dissolved organic  
323 C (DOC) and total dissolved N (TDN) in  $\text{K}_2\text{SO}_4$  extracts were measured using a TOC-TN  
324 analyzer (Shimadzu, Kyoto, Japan). Dissolved organic N (DON) was calculated as the difference  
325 between TDN and ( $\text{NO}_3^-\text{-N} + \text{NH}_4^+\text{-N}$ ).

#### 326 Calculations and statistics

327 Gross N transformation rates were calculated by the  $^{15}\text{N}$ -isotope pool dilution method using the  
328 calculation model FLUAZ81, which combines a numerical model for solving the mass balance  
329 equations and a non-linear fitting program for optimizing the N rate parameters (Nelson &  
330 Sommers, 1996). Specifically, gross mineralization, gross nitrification, immobilization of  $\text{NH}_4^+$   
331 and  $\text{NO}_3^-$ , and denitrification were estimated by this model.

332 The data distributions were tested for normality using Shapiro-Wilk tests. Due to generally non-  
333 normal distribution or heterogeneous variances, they were analyzed by Kruskal-Wallis H test  
334 (non-parametric). Differences in soil characteristics, N cycling rates, PDA,  $\text{N}_2\text{OR}$ , SIR and  
335 respiration between treatments in each season within site or across sites, seasonal differences in  
336 each site within treatment or across treatments, and site differences in each season across  
337 treatments were tested, and a  $p$  value lower than 0.05 was considered to be significant.

338 Relationships between soil characteristics (soil moisture content, pH, C, N, DOC, DON,  $\text{NH}_4^+\text{-N}$   
339 and  $\text{NO}_3^-\text{-N}$ ) and N cycling rates as well as PDA and N2OR were identified using multiple  
340 stepwise linear regression analysis based on the coefficient of determination ( $R^2$ ) and Akaike  
341 Information Criterion (AIC) (Ziegel, 2003). The Variance Inflation Factor (VIF) values (should  
342 be close to 1) were calculated to avoid collinearity, and normality and homoscedasticity of  
343 residuals were tested by diagnostic plots in each regression model. Principal component analysis  
344 (PCA) was applied to evaluate the extents of separations among the sites, and to quantify the  
345 comprehensive relationship between soil characteristics and N cycling rates as well as PDA and  
346 N2OR within each treatment or season by using the prcomp package (Mankin, 2008). All  
347 statistical analyses were conducted using R 3.6.1 (Team, 2019).

## 348 Results

### 349 Soil characteristics

350 Basic soil physical and chemical properties are shown in Table 2. In general, the measured  
351 parameters were not impacted by the snowfence treatment and season, with soil  $\text{NH}_4^+\text{-N}$  and  
352  $\text{NO}_3^-\text{-N}$  as notable exceptions to this. Soil moisture content ranged widely among the sites (40.2-  
353 81.5%) with peak values at Disko Wet and Daring Lake. The pH varied between 3.7 and 7.0 with  
354 the most acidic conditions at Daring Lake, intermediate pH at Disko Dry, and slightly acidic to  
355 neutral conditions at Disko Wet and the Svalbard sites. The Daring Lake soil was purely organic  
356 with C contents of around 45%, and the Disko Wet soil also had a high C% (30%), while  
357 Svalbard Meadow was the least organic with around 7% C. At Daring Lake, the soil C:N ratio  
358 was the highest (>28) among all sites and the ratio in deepened snow plots significantly exceeded  
359 the control in summer ( $p=0.04$ ), but not in winter.

360 The highest DOC concentrations in summer and winter were observed in Daring Lake and Disko  
361 Wet, respectively, and Disko Wet also showed significantly higher concentrations across  
362 treatments in winter relative to summer ( $p=0.045$ ). For Disko Wet and Daring Lake, there was a  
363 tendency for increased wintertime DON as a consequence of the deepened snow treatment  
364 ( $p=0.083$  and  $p=0.063$ , respectively). Disko Dry showed generally lower DON concentrations in  
365 winter than in summer ( $p<0.01$ ).

366 Across all sites and treatments,  $\text{NH}_4^+$  was generally the predominant form of inorganic N, except  
367 for Disko Wet in summer, where  $\text{NO}_3^-$ -N exceeded  $\text{NH}_4^+$ -N 5-fold (Table 2). The deepened snow  
368 treatment across sites significantly increased  $\text{NH}_4^+$ -N concentrations in summer ( $p=0.0088$ ), but  
369 not in winter. For specific sites,  $\text{NH}_4^+$ -N concentrations were significantly or tended to be higher  
370 in the deepened snow treatment at Svalbard Heath and Meadow in summer ( $p=0.032$  and  
371  $p=0.063$ , respectively) and winter ( $p=0.093$  and  $p=0.004$ , respectively). Moreover, significantly  
372 higher  $\text{NH}_4^+$ -N concentrations were observed in Disko Wet and Svalbard Meadow in winter than  
373 in summer ( $p<0.01$  and  $p=0.013$ , respectively). The deepened snow treatment significantly  
374 enhanced  $\text{NO}_3^-$ -N concentrations across sites in summer ( $p=0.014$ ), and for specific sites,  $\text{NO}_3^-$ -N  
375 concentrations were significantly increased at Daring Lake ( $p=0.016$ ), Svalbard Heath ( $p=0.016$ )  
376 and Meadow ( $p=0.045$ ) in summer, whereas no effects were observed in winter. In contrast,  $\text{NO}_3^-$   
377 -N concentrations were significantly reduced in winter compared to summer across all sites under  
378 increased snow conditions ( $p=0.015$ ).

### 379 Gross N cycling rates

380 Gross N-cycle results indicated significant site-to-site variation with highest activity generally  
381 observed at the Disko Wet site (Fig. 1). Across all sites, increased snow depth enhanced gross N  
382 mineralization rates in summer ( $p=0.044$ ), but not in winter. The stimulation of gross N

383 mineralization rates by deepened snow was particular apparent at Svalbard Heath in summer  
384 ( $p=0.008$ ) and Svalbard Meadow in winter ( $p=0.041$ ). Similar tendencies were observed also for  
385 Disko Dry and Svalbard Meadow during summer ( $p=0.064$  and  $p=0.065$ , respectively; Fig. 1a).  
386 The gross N mineralization rates in Disko Wet snow fence plots were significantly increased in  
387 winter relative to summer ( $p=0.016$ ) and similar tendencies were also observed for the control  
388 plots ( $p=0.095$ ; Fig. 1a). In contrast, N mineralization rates in Disko Dry snowfence plots were  
389 significantly lower in winter compared to controls ( $p=0.016$ ; Fig. 1a).

390 Gross nitrification rates were overall about half of the gross mineralization rates, and did not vary  
391 among the different sites (Fig. 1b) even though Daring Lake had particularly low N  
392 mineralization rates (Fig. 1a). Gross nitrification rates were generally increased 4-fold by the  
393 deepened snow treatment in winter, but not summer ( $p<0.01$ ; Fig. 1b), even though a snow effect  
394 was observed at Svalbard Meadow in summer ( $p=0.015$ ; Fig. 1b). Enhanced gross nitrification  
395 was significant in Disko Wet and Svalbard Meadow ( $p=0.036$  and  $p=0.037$ , respectively) in  
396 winter, with a similar tendency for Disko Dry ( $p=0.095$ ; Fig. 1b). There was no obvious  
397 seasonality in gross nitrification rates at any site.

398 In accordance with the high mineralization rates, Disko Wet also showed significantly higher  
399  $\text{NH}_4^+$ -N immobilization rates than the other sites in both seasons ( $p<0.01$ ; Fig. 1c). Moreover, the  
400 deepened snow treatment tended to increase  $\text{NH}_4^+$ -N immobilization rates in winter ( $p=0.056$ ),  
401 resulting in significantly higher immobilization under deepened snow in winter relative to the  
402 summer ( $p=0.032$ ; Fig. 1c).

403 The  $\text{NO}_3^-$ -N immobilization rates varied substantially across sites with peak rates observed in the  
404 Daring Lake samples (Fig. 1d), which is in contrast to the low  $\text{NH}_4^+$ -cycle activity observed at  
405 that site (Figs. 1a-b). The deepened snow treatment significantly enhanced (3-fold) overall  $\text{NO}_3^-$ -

406 N immobilization rates across sites in winter ( $p=0.007$ ; Fig. 1d), especially at Daring Lake  
407 ( $p=0.031$ ), although this response was perhaps primarily a result of significantly decreased  $\text{NO}_3^-$ -  
408 N immobilization rates in the control plots ( $p=0.031$ ; Fig. 1d) in winter compared to summer. A  
409 significant increase in  $\text{NO}_3^-$ -N immobilization rates under deepened snow was observed in both  
410 summer and winter at Disko Wet, where the control plots barely showed any activity (Fig. 1d).

411 The estimated total gaseous losses by denitrification did not vary among the sites (Fig. 1e).

412 However, the deepened snow treatment significantly increased denitrification (2-fold) across sites  
413 in both seasons ( $p=0.038$  and  $p=0.02$  for summer and winter, respectively), although this snow  
414 effect was only significant for Daring Lake in summer ( $p=0.016$ ; Fig. 1e). Denitrification under  
415 deepened snow conditions also tended to be higher in summer than in winter across all sites  
416 ( $p=0.076$ ; Fig. 1e).

#### 417 Potential denitrification and nitrous oxide reductase activity

418 Potential denitrification activity (PDA) varied among sites with rates up to 25-fold higher at  
419 Svalbard Heath compared to Daring Lake (Fig. 2a). The deepened snow treatment tended to  
420 increase summertime and wintertime PDA across sites ( $p=0.098$  and  $p=0.12$  respectively).

421 However, for the specific sites, this deepened snow effect was only apparent at Svalbard Heath,  
422 where activities in the snowfence plots exceeded controls in winter ( $p=0.032$ ; Fig. 2a) and a large  
423 enhancement by deepened snow (3-fold;  $p=0.283$ ) was observed in summer.

424 The nitrous oxide reductase activity (N2OR) varied among the study sites, with maximum  
425 activities observed at Disko Dry and Svalbard Heath in summer and winter, respectively (Fig.  
426 2b). The deepened snow treatment generally increased N2OR across sites in summer ( $p=0.023$ ),  
427 although there was no snowfence effect at any specific site.

## 428 Correlations between soil characteristics and N cycling rates

429 Separate principal component analyses (PCA) were used to determine the clustering in the  
430 control and snowfence plot data of all response variables (soil characteristics, N cycling rates and  
431 denitrification enzyme activities) across seasons (Figs. 3a and 3b). Under control snow  
432 conditions, the two wetter sites Daring Lake and Disko Wet clearly separated in two distinct  
433 groups, whereas the three drier sites appeared to cluster. The Daring Lake site was associated  
434 with high C:N ratio and  $\text{NO}_3^-$ -N immobilization, and Disko Wet with high DON and  $\text{NH}_4^+$ -N  
435 concentrations, mineralization and  $\text{NH}_4^+$ -N immobilization. Among the drier sites, Svalbard  
436 Heath correlated with high denitrification enzyme activities (PDA and N2OR). Under deepened  
437 snow conditions, a similar distribution could be observed except that the two Svalbard sites were  
438 congruent. Disko Wet site exhibited wider variation along PC1-axis explained by soil moisture,  
439 DOC, DON and total N contents, which indicates that the degree of explanation changes with  
440 snow depth in particular for the Disko Wet site.

441 A stepwise regression analysis was performed with the above-mentioned variables (Table 3).  
442 Gross mineralization was explained mainly by  $\text{NH}_4^+$ -N (44.7%) and DON (43.3%) in control  
443 plots, and by DON (42.8%) and DOC (31.3%) in snowfence plots. Gross nitrification was equally  
444 and dominantly explained by DON (46.7%) and  $\text{NO}_3^-$ -N (44.4%) in the control plots, whereas  
445 under deepened snow soil moisture was the main driver. Ammonia immobilization was mainly  
446 driven by  $\text{NH}_4^+$ -N (80.2%), and to a low extent by DOC (7.4%) in the control plots, but to a  
447 higher extent (22.9%) in the snowfence plots. Nitrate immobilization was equally controlled by  
448 the soil bulk C (52.2%) and soil pH (47.8%) in the snowfence plots. The explanation of PDA was  
449 generally poor in the control plots across sites (23.4% of variance), and was mainly related to  
450 water content (79.8%) and DON (20.2%). There was a significant relationship between

451 denitrification and PDA across sites and treatments in summer ( $p=0.006$ ,  $R^2=0.172$ ; Fig. S4). The  
452 N2OR could also only be explained in the control plots, and mainly by soil pH (90.6%).

## 453 Discussion

### 454 Effects of deepened snow on soil nutrients and N cycling

455 Our data show that several N cycling rates were enhanced by experimentally deepened snow and  
456 hence partly support hypothesis H1. The N-cycle processes were enhanced by the snowfence  
457 treatment both in late winter (i.e. upon several months of continuous snow cover), and at the end  
458 of the active growing season (i.e. after ca. three months of snow-free conditions).

459 Despite contrasting climate conditions, vegetation types and soil characteristics responses to  
460 snowfence treatments were similar on different aspects across the sites. Both duration of snow-  
461 cover and soil temperature during the snow-covered period were driven in the same direction by  
462 the deepened snow treatment (Table 1), as were also soil nutrient concentrations and N-cycle  
463 processes. We observed significantly higher gross nitrification,  $\text{NO}_3^-$ -N immobilization and  
464 denitrification rates in the deepened snow compared to control plots across all sites during winter.  
465 This is probably due to higher abundance and diversity of N-cycle microbial communities  
466 (Jusselme et al., 2016), as a result of significantly warmer winter soil temperatures under the  
467 deepened snow (Table 1, Figs. S2a-e). For instance, Xue et al. (2016) found that a snowfence  
468 treatment increased the abundance of key genes involved in N mineralization (*ureC*), nitrification  
469 (*amoA*), and denitrification (*narG*, *nirS/nirK* and *nosZ*) in the active layer of Alaskan tundra  
470 soils. In our study, no significant correlations were observed between soil temperature and N-  
471 cycling rates across sites and seasons (data not shown), probably due to indirect effects of soil  
472 temperature via changing soil characteristics, or to long-term ecosystem acclimation such as  
473 microbial community change.



474 The generally higher wintertime gross N mineralization rates under deepened snow led to an  
475 increase in soil  $\text{NH}_4^+$ -N concentrations in most of the sites. In contrast, soil  $\text{NO}_3^-$ -N  
476 concentrations were unaffected by the deepened snow, even though higher gross nitrification was  
477 observed across sites (Fig. 1b). This is likely because excess amounts of  $\text{NO}_3^-$ -N were rapidly  
478 depleted by the higher  $\text{NO}_3^-$ -N immobilization (Daring Lake and Disko Wet) and denitrification  
479 rates under deepened snow conditions (Figs. 2d and 2e). These observations are parallel to  
480 negligible net nitrification rates observed by Chu and Grogan (2009) at Daring Lake. In addition,  
481 the increased  $\text{NO}_3^-$ -immobilization rates under deepened snow conditions in winter at that site is  
482 in agreement with a previous study there reporting that deepened snow increased N accumulation  
483 in microbial biomass during late winter (Buckeridge & Grogan, 2008). At Disko, Christiansen et  
484 al. (2017) conducted *in situ* litterbag incubations and observed enhanced fungal abundance in  
485 both the Wet and Dry sites, and more litter mass loss in the Dry site in response to deepened  
486 snow, which could help explain the increased gross N mineralization (summer, Dry site) and  
487  $\text{NO}_3^-$ -immobilization rates (both seasons, Wet site) we observed there.

488 We found enhanced summer gross N mineralization rates due to the deepened snow treatment  
489 across all sites (Fig. 1a), which was associated with increased concentrations of soil  $\text{NH}_4^+$  and  
490  $\text{NO}_3^-$  (Table 2). Hence, the pattern of surplus inorganic N from winter processes under deepened  
491 snow was repeated in the late growing season, consistent with findings in a previous study at the  
492 same Svalbard sites indicating that labile N from the winter period exceeds the early summer  
493 demands of both microbes and plants (Semenchuk et al., 2015). Large fluctuations in microbial  
494 community composition and abundances occurred between winter and summer in the Arctic  
495 tundra (e.g. Schostag et al., 2015), and thus deepened snow is expected to mainly affect diversity  
496 or abundance of soil microorganisms that dominate during winter. Indeed, Mundra et al. (2016)

497 demonstrated effects of deepened snow on plant pathogenic and soil fungi in our Svalbard sites.  
498 Hence, the potential legacy effect of deepened snow on the subsequent growing season will  
499 depend on the severity of local winter microclimate (Buckeridge, Banerjee, Siciliano, & Grogan,  
500 2013; McMahon, Wallenstein, & Schimel, 2011). In this study, PDA and N2OR were both  
501 increased across sites by the deepened snow treatment in summer (Figs. 2a and 2b), indicating  
502 that denitrifying enzyme activities were enhanced by deepened snow. The changes in PDA and  
503 N2OR may result from altered denitrifier abundance and/or denitrifier community composition  
504 (Attard et al., 2011), and denitrification was significantly correlated with PDA across sites  
505 ( $p=0.006$ ,  $R^2=0.172$ ; Fig. S4), which altogether may explain the snowfence-enhanced  
506 summertime denitrification rates across sites (Fig. 1e). During denitrification,  $\text{NO}_3^-$  is reduced to  
507  $\text{N}_2\text{O}$  by denitrifiers in an anaerobic respiratory pathway, which can be further reduced into  $\text{N}_2$ ,  
508 largely depending on  $\text{O}_2$  status. Since  $\text{N}_2\text{O}$  is a strong greenhouse gas, whereas  $\text{N}_2$  is  
509 atmospherically inert, the ratio of the two gaseous end products of denitrification can have an  
510 impact on ecosystem feedback to climate change (Voigt et al., 2020).

511 Nevertheless, contrasting seasonal patterns of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  were observed in most sites.  
512 This is likely because nitrification is constrained under low temperature ( $<5^\circ\text{C}$ ) leading to an  
513 accumulation of  $\text{NH}_4^+\text{-N}$  via N mineralization during winter (Smith, Wagner-Riddle, & Dunfield,  
514 2010), whereas in the warmer growing season,  $\text{NH}_4^+\text{-N}$  is taken up by microbes and plants as  
515 well as transformed into  $\text{NO}_3^-\text{-N}$  via nitrification resulting in a strong decreasing trend for  $\text{NH}_4^+\text{-N}$   
516 concentrations across sites in summer compared to winter ( $p=0.06$ ; Table 2). Additionally,  
517 differences in vegetation-type may influence soil inorganic N during the growing season. For  
518 example, the biomass of plants with high inorganic N sink strength, such as graminoids that have  
519 a strong ability to exploit additional inorganic N, is higher in the Svalbard Meadow than Heath

520 (Mörsdorf et al., 2019). This may partly explain the much lower  $\text{NO}_3^-$ -N concentrations during  
521 summer in the Meadow compared to the Heath site, especially in the deepened snow plots.  
522 However, this does not appear to be the case at Disko Wet, where soil  $\text{NO}_3^-$ -N concentrations  
523 were higher during the growing season despite a presumed higher inorganic N sink strength of  
524 the vegetation there compared to Disko Dry. Nitrate **is** easily leached into deeper soil profiles and  
525 the differential extent of  $\text{NO}_3^-$  leaching could help to explain contrasting soil  $\text{NO}_3^-$ -N  
526 concentrations between these two sites. This is further supported by observations from  
527 Rasmussen et al. (2020), who found that overall higher  $\text{NO}_3^-$ -N concentrations of soil water at the  
528 depth of 10 and 20 cm throughout the growing season in the dry site as compared to the wet site.  
529 All these observations above, however, have to be interpreted with caution, since concentrations  
530 of soil  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N as well as water content may vary during the growing season, as  
531 reported by Buckeridge et al. (2013), Semenchuk et al. (2015) and Mörsdorf et al. (2019).

532 According to coefficients of variation for each C-cycle and N-cycle process across treatments and  
533 seasons in each site, C-cycle processes were much less affected by the snowfence treatment and  
534 season compared to N-cycle processes (Table S1). The substrate induced respiration (SIR)  
535 method estimates the potentially active microbial biomass (Anderson & Domsch, 1978). SIR as  
536 well as basic aerobic respiration and potential anaerobic respiration was unaffected by the  
537 snowfence treatment or season (Fig. S1) indicating that deepened snow did not affect the  
538 potentially active microbial biomass and microbial  $\text{CO}_2$  production across our five sites.

539 However, previous studies have reported either increased or reduced *in situ* soil respiration by  
540 deepened snow treatment at these sites in either summer or winter (Björkman et al., 2016;  
541 Christiansen et al., 2018; Morgner et al., 2010). This inconsistency between laboratory  
542 incubations and field flux measurement may result from alteration of autotrophic respiration due

543 to the absence of plants in our incubations, and other differences from *in situ* environmental  
544 factors (e.g. soil temperature, soil moisture and/or thaw depth) that we know are significantly  
545 influenced by the snowfence treatment.

546 [Soil characteristics correlate to responses of N cycling to deepened snow over a wider  
547 geographical region](#)

548 [Our observations show that increased availability of soil organic N drives the responses of gross](#)

549 [mineralization rates to deepened snow cover, and hence partly support H2.](#) They also suggest an

550 important role of C availability for the response of several N cycling rates to deepened snow.

551 Many previous studies in the Arctic tundra ecosystems have demonstrated the importance of soil  
552 characteristics such as soil organic matter (DeMarco et al., 2011; Schnecker et al., 2014;

553 Weintraub & Schimel, 2003), C:N ratio (Chu & Grogan, 2009; Maslov & Makarov, 2016) and

554 soil moisture (Chapin, 1996; DeMarco et al., 2011) for N-cycle processes. Despite significant site

555 differences in many soil characteristics and N-cycle processes, we did observe strong broad scale

556 correlations between soil characteristics and N-cycle processes. In this study, the availability of

557 DOC was a predictor for N mineralization at the snowfence but not control plots across sites,

558 with a relatively high importance (31.3%). This suggests that the snowfence-induced increase in

559 gross N mineralization is linked to elevated availability of organic C substrates that complement

560 microbial C demand (Schmidt et al., 2011). The elevated organic substrate availability for N

561 mineralization under deepened snow conditions could originate from either increased breakdown

562 of soil organic macromolecules or input of labile C and N through damaged and killed roots or

563 microbial cells turnover (Brooks, Williams, & Schmidt, 1998; Larsen, Jonasson, & Michelsen,

564 2002; Schimel et al., 2004). Generally, it is well known that organic C substrates play an

565 important role in regulating N mineralization (Booth, Stark, & Rastetter, 2005). [A comparative](#)

566 [study of two ecosystems in low Arctic tundra also showed that tall birch soils had higher labile C](#)

567 concentrations and faster N mineralization relative to birch hummock soil, but lower soil total C  
568 contents (Buckeridge, Zufelt, Chu, & Grogan, 2009). Hence, it seems that the chemical quality is  
569 more important than the quantity of soil C as the principal driver of N mineralization.  
570 Furthermore, the variability of  $\text{NH}_4^+$ -N immobilization was explained to a greater extent by DOC  
571 availability in the snowfence plots (22.9%) than in the control plots (7.4%), which indicates that  
572 C availability induced by the deepened snow may favor not only N mineralization but also  $\text{NH}_4^+$ -  
573 N immobilization. This is in agreement with a previous study implying that microbial N  
574 immobilization is facilitated by active heterotrophic microbes stimulated by high C availability  
575 (Montaño, García-Oliva, & Jaramillo, 2007).

576 According to the regression analysis, gross nitrification was equally explained by DON and  
577  $\text{NO}_3^-$ -N in the control plots, whereas soil moisture was the main driver in the snowfence plots.  
578 This indicates that nitrification is limited by other soil environmental factors rather than labile N  
579 substrates due to increased N mineralization by deepened snow. It is noticeable that gross N  
580 mineralization at the Daring Lake was negligible in both treatments, but it displayed high gross N  
581 nitrification, especially in the snowfence plots (gross nitrification exceeded mineralization),  
582 indicating that heterotrophic nitrification at this site was predominantly by direct  $\text{NO}_2^-$ / $\text{NO}_3^-$ -N  
583 formation from oxidation of organic N. Previous studies have showed that heterotrophic  
584 nitrification dominating over autotrophic nitrification in a range of terrestrial ecosystems  
585 (Huygens et al., 2008; Müller et al., 2009; Rütting et al., 2008; Wang et al., 2016). We found  
586 rather low soil pH (~3.9), high C:N ratio and high total soil C content at Daring Lake, which  
587 could explain why relatively high heterotrophic nitrification occurred at this site compared to the  
588 other sites. This conclusion is consistent with some earlier studies indicating that heterotrophic  
589 nitrification may be an important, and possibly dominant pathway for  $\text{NO}_3^-$ -N production in

590 environments where pH is low and organic C content high (Islam, Chen, & White, 2007; Perakis,  
591 Compton, & Hedin, 2005; Zhang, Müller, Zhu, Cheng, & Cai, 2011). In general, a wide range of  
592 microorganisms possesses the potential for heterotrophic nitrification, especially fungi, which  
593 may be less prone to low soil pH (even at pH 3) (De Boer & Kowalchuk, 2001; Zhu et al., 2012).  
594 A recent review related to nitrification and nitrifiers in acidic soils concluded that soils with  
595 lower pH from the same sites tended to have a higher proportion of heterotrophic nitrification but  
596 other factors such as substrates availability, moisture content and soil temperature have equal or  
597 even greater effect (Li, Chapman, Nicol, & Yao, 2018). For instance, Banerjee and Siciliano  
598 found that heterotrophic nitrification comprised a considerable proportion of the overall  
599 nitrification potential (47%) in a Canadian tundra soil with high water content and organic matter.  
600 We suggest the increased nitrification under deepened snow conditions in winter observed in  
601 Daring Lake, Svalbard Heath and Disko Wet was likely due to increased availability of labile  
602 organic substrates as supported by the tendency for increased DON concentration in snowfence  
603 plots at these sites.

604 The growth and activity of denitrifiers are regulated by the availability of substrates (i.e.  $\text{NO}_3^-$ -N  
605 as an electron acceptor and simple organic C as an electron donor). Generally, soil total C:N ratio  
606 is an indicator of substrate quality and a measure of recalcitrant organic matter in relation to  
607 organically-bound N (Dodla, Wang, DeLaune, & Cook, 2008). In this study, we observed high  
608 aerobic and anaerobic respiration at Disko Dry and Daring Lake (Figs. S1b and S1c), suggesting  
609 high organic C quality and availability in these two sites even though they had relatively high  
610 C:N ratios. Kandeler et al. (2006) observed that soil organic C was the most important factor  
611 explaining denitrifier abundance. Similarly, Attard et al. (2011) reported that soil organic C  
612 influenced PDA partially through a build-up of denitrifier abundance, and therefore a higher C

613 availability could enhance the abundance of denitrifiers. However, this does not appear to be the  
614 case at these two sites, where PDA in winter was lower though soil organic C was higher than the  
615 other sites, probably due to limited winter  $\text{NO}_3^-$ -N availability (Table 1).

616 Although the regression analysis clearly pointed out some universal drivers explaining the  
617 variability across sites, the enhancement of N cycle was additionally affected by the strength of  
618 soil temperature and moisture effect under deepened snow. For example, the highest  
619 enhancement of wintertime soil temperature by deepened snow occurred at Svalbard Meadow  
620 site (2.5 °C; Table 1) where the most notably increased wintertime gross N mineralization and  
621 nitrification were observed. There were also significant summertime cooling effects in Disko Wet  
622 (Table 1), which may explain no effects by snowfence treatment on summertime gross N  
623 mineralization and nitrification rates despite relatively high soil DOC and DON concentrations.  
624 A significant effect on summertime gross N mineralization was only observed at Svalbard Heath  
625 (Table 1, Fig. S6d), probably in part due to higher soil moisture under deepened snow conditions  
626 in summer and thus increased mobility of solutes, enzymes and microorganisms (Manzoni,  
627 Schimel, & Porporato, 2012). Similarly, the highest enhancement of summertime PDA activity  
628 also occurred in this site.

629 Does N cycling in tundra ecosystems with contrasting moisture regimes respond  
630 differently to deepened snow?

631 Daring Lake and Disko Wet were characterized by relatively high soil moisture and organic  
632 matter contents, and deepened snow led to more pronounced increases in wintertime DOC and  
633 DON concentrations than at the three other sites. These results suggest that soil moisture content  
634 is an important control of labile organic C and N formation under elevated winter temperatures,  
635 and thus microbial decomposition processes (Manzoni et al., 2012). Tundra soils tend to dry over  
636 winter as a result of the vapor pressure gradient from relatively warm soils to the cold overlying

637 snow that has a relatively low saturated vapor pressure (Kane, Hinkel, Goering, Hinzman, &  
638 Outcalt, 2001), and the phase transition of water to the solid state leads to dramatic reduction in  
639 liquid H<sub>2</sub>O during winter (Brooks et al., 2011), altogether leading to low soil volumetric moisture  
640 content observed during winter (Figs. S3a-c). This reduction in soil moisture in winter was  
641 stronger in Disko Dry compared to Disko Wet. Thus, deepened snow as a result of future climate  
642 change may affect soil N cycling more in mesic and wet tundra than in dry tundra. Consequently,  
643 freezing-induced drought stress and limitations in substrate diffusion and mass transport may be  
644 more important in dry soils, such as Disko Dry (ca. 5% moisture content in winter) and lead to  
645 generally low N cycling rates during winter. Furthermore, the high energy demand associated  
646 with the phase shift between water and ice generally leads to lower temperatures in dry soils  
647 compared to wet soils in winter (Christiansen et al., 2017), as supported by lower winter  
648 temperatures at the Disko Dry site compared to Disko Wet (Figs. 2a and 2b). Accordingly, N  
649 cycling rates were generally enhanced by deepened snow cover to a greater extent in these two  
650 wetter sites (Disko Wet and Daring Lake) than in the three other sites, associated with high labile  
651 organic C and N concentrations, supporting our hypothesis H3 that the extent of the increase in  
652 gross N transformation rates across seasons mediated by deepened snow is linked to ecosystem  
653 moisture regime. The regression analysis revealed soil moisture content as one of the primary  
654 predictors for PDA across sites. This is consistent with Jusselme et al. (2016) showing that  
655 variation in N-related microbial abundances and enzyme activities along a snow depth gradient in  
656 subalpine grassland was driven primarily by soil moisture, indicating that access to substrates for  
657 the microbes is mainly controlled by water availability in partly frozen soils. According to the  
658 PCA, the two wetter sites clearly separated in distinct groups, whereas the three drier sites  
659 appeared to cluster (Fig. 4), indicating that soil moisture is an important controlling variable for  
660 other soil characteristics and N-cycle processes, as also supported by the strong positive



661 correlation between water content and principal component 1 (of highest explained variance).  
662 This is in accordance with observations by Rasmussen et al. (2020), who studied the effects of  
663 shrub removal, warming, and snow addition on soil water chemistry in the Disko Dry and found  
664 strong influence of summer soil moisture as an independent physical variable in determining  
665 treatment effects across years. In winter across treatments, the wettest site (Disko Wet) showed  
666 wider variation along PC1-axis as a result of snow effect compared to the other sites, further  
667 suggesting N-cycle microbial activities in soils with high water contents can respond more  
668 strongly to increased snow depth (Fig. S5b). Our results therefore support previous studies in  
669 Alaska and the same Svalbard sites proving that deepened-snow-induced increases in soil N  
670 availability and foliar N content is more pronounced in moist tundra than in dry tundra (Schimel  
671 et al., 2004; Semenchuk et al., 2015; Welker et al., 2005). It is still not clear why the lowest gross  
672 N mineralization occurred at the Daring Lake, even though it had both high moisture content and  
673 respiration rates (Fig. S1). A previous study from Daring Lake concluded that low N  
674 mineralization and high CO<sub>2</sub> emissions, corresponding to our study, likely resulted from the  
675 combined effect of low soil pH and high C:N ratio (Paré & Bedard-Haughn, 2012). Moreover,  
676 the duration of the snow manipulation experiment varied among the study sites and this could  
677 have affected the responses of N cycles via ecosystem acclimation e.g. vegetation and microbial  
678 community change and stabilization to new conditions upon longer term manipulations  
679 (Christiansen et al., 2018; Mundra et al., 2016). This may help explain the negligible response of  
680 gross N mineralization at Daring Lake where the duration of the snowfence treatment has been  
681 relatively long (13 years; Table 1) compared to the other sites.

## 682 Conclusion

683 To the best of our knowledge, this is the first study to compare both winter and summer  
684 responses of N-cycle processes to projected changes in winter climate among several Pan-Arctic  
685 tundra ecosystems. We conclude that deepened snow enhanced wintertime gross N nitrification  
686 (4-fold), denitrification (2-fold) and  $\text{NO}_3^-$ -N immobilization (3-fold) rates across the five  
687 investigated sites. Gross N mineralization rates were also enhanced by deepened snow in  
688 summer, associated with increased  $\text{NH}_4^+$ -N availability. There was an increase of denitrification  
689 rates (2-fold) across sites by deepened snow in summer, likely due to increased denitrifying  
690 enzyme activities (PDA and N2OR). Furthermore, DOC and DON were the main drivers for  
691 several enhanced gross N cycling rates in response to deepened snow across all sites, suggesting  
692 that microbial N-cycle activities promoted by deepened snow are primarily due to the increased  
693 availability of dissolved organic matter. Interestingly, the differential effects of deepened snow  
694 on winter N cycling rates among the Pan-Arctic tundra ecosystems are likely governed by  
695 contrasting moisture regimes. Accordingly, our results suggest that in a warmer future, winter  
696 soil N cycling rates are likely to be most enhanced in tundra locations where the soil is relatively  
697 moist, and that consequent increases in soil nutrient availability and thus N assimilation of plants  
698 may be most pronounced in moist tundra during the following growing season. It can also be  
699 speculated that these particular conditions may enhance N losses by leaching or gaseous  
700 emissions (e.g.  $\text{N}_2$  and  $\text{N}_2\text{O}$ ), pointing at altered N balances and climate feedbacks associated  
701 with wet Arctic tundra. The combination of multi-season and multi-site studies are important for  
702 understanding future annual N cycling in contrasting Arctic landscapes and this study strongly  
703 ask for supplementary *in situ* investigations to clarify such consequences.

704

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Site	Disko		Daring Lake	Svalbard		
	Dry	Wet		Heath	Meadow	
Air temperature (°C) <sup>a</sup>	-14/8		-26.4/13.5	-10.4/7.6		
Precipitation (mm) <sup>b</sup>	400/160		250/100	228/120		
Soil type	Haplic Cryosol		Orthic Dystric Turbic Cryosols	Tubic Cryosols		
Snowfence installed (year)	2012	2013	2004	2006		
Soil temperature (snow-covered; °C) <sup>c</sup>	Control	-5.5	-3.2	-4.0	-5.4	-5.5
	Snowfence	-3.8**	-1.6**	-2.7**	-3.3**	-3.0**
Soil temperature (non-snow-covered; °C) <sup>d</sup>	Control	5.6	7.2	6.7	5.2	5.7
	Snowfence	5.5	6.4**	ND <sup>e</sup>	4.3**	4.7**
Soil moisture (Vol %) <sup>f</sup>	Control	13.3	49.7	29.0	46.5	49.4
	Snowfence	12.7	50.2	29.7	50.8**	47.5
Maximum active layer depth (cm) <sup>g</sup>	Control	>300	85	60	106	130
	Snowfence	>300	91	62	110	ND
Maximum Snow depth (cm)	Control	60		37	40	
	Snowfence	145		110	150	
Snow-covered period	Control	October to May		October to May		
	Snowfence	October to early June		October to early June		

Table 1 Climatic conditions at study sites

Significant effects of snowfence treatment in each site are shown  $**p \leq 0.01$ .

<sup>a</sup> Mean monthly temperature in the coldest month/in the warmest month

<sup>b</sup> Mean annual total precipitation/precipitation as snowfall

<sup>c, d</sup> 2 cm depth, mean daily soil temperature in snow-covered (October to May) and non-snow-covered period (late June to September) at Disko Dry and Wet sites (n=3-4 for each treatment; 2012-2017), Daring Lake (n=2 for each treatment; 2015-2016), and Svalbard Heath and Meadow sites (n=4-6 for each treatment; 2014-2018)

<sup>e</sup> No data due to lack of observations

<sup>f</sup> 5 cm depth, mean daily moisture in the growing season at Disko Dry and Wet (n=3-4 for each treatment; 2016), Daring Lake (n=2 for each treatment; 2016), Svalbard Heath and Meadow sites (n=4-6 for each treatment; 2015). The measurement range of soil moisture sensors used in two Svalbard sites was 0-100%, while 0-55% in the other three sites

<sup>g</sup> Maximum active layer depth, at Disko Dry and Wet (2017-2019), Daring Lake (2012-2013), and Svalbard Heath and Meadow sites (2011)

Table 2 Soil characteristics of study sites

Site		Disko		Daring lake	Svalbard		Treatment effect
		Dry	Wet		Heath	Meadow	
GWC (%)	Summer, Control	52.6±3.5	80.6±3.5	73.9±1.5	40.5±6.1	43.1±3.4	
	Summer, Snowfence	55.3±2.3	75.7±6.4	74.7±0.8	46.9±4.6	40.2±3.8	
	Winter, Control	63.2±4.2	80.8±5.0	76.6±4.6	55.3±2.0	48.2±6.6	
	Winter, Snowfence	59.7±2.5	81.5±5.1	81.6±2.7	57.5±7.1	51.1±2.2	
pH	Summer, Control	5.2±0.2	6.6±0.1	3.7±0.1	7.0±0.3	5.8±0.1	
	Summer, Snowfence	5.6±0.2	6.5±0.1	3.9±0.2	6.7±0.2	6.1±0.1	
	Winter, Control	5.4±0.3	6.3±0.2	3.9±0.1	ND	ND	

	Winter, Snowfence	5.7±0.2	6.5±0.1	3.9±0.1	ND	ND	
Soil C (%)	Summer, Control	26.1±3.1	29.6±1.9	45.3±0.5	11.0±2.0	7.6±0.8	
	Summer, Snowfence	26.3±1.8	25.7±4.2	44.8±2.7	12.1±1.1	7.3±1.0	
	Winter, Control	21.6±3.2	31.3±4.8	42.2±6.2	9.1±1.2	7.7±2.4	
	Winter, Snowfence	21.2±2.2	33.6±6.2	47.1±3.1	9.5±3.2	4.8±0.8	
Soil N (%)	Summer, Control	1.0±0.1	2.0±0.1	1.6±0.0	0.6±0.1	0.5±0.1	
	Summer, Snowfence	1.1±0.1	1.6±0.3	1.4±0.1	0.7±0.1	0.5±0.1	
	Winter, Control	0.9±0.1	1.6±0.2	1.2±0.2	0.7±0.1	0.6±0.2	
	Winter, Snow fence	0.8±0.1	1.7±0.2	1.3±0.1	0.7±0.2	0.4±0.1	
C:N ratio	Summer, Control	25.6±1.3	15.1±0.6	<b>28.6±0.6</b>	17.8±0.6	15.9±0.5	
	Summer, Snowfence	24.4±1.4	15.9±0.7	<b>32.6±2.5*</b>	17.0±0.2	15.8±0.3	
	Winter, Control	22.6±1.6	20.5±3.6	37.9±6.8	12.5±0.6	12.5±0.9	
	Winter, Snowfence	25.3±1.1	19.2±3.0	35.8±3.3	14.0±1.3	11.2±0.4	
DOC (mg kg <sup>-1</sup> )	Summer, Control	197.8±32.7	235.7±76.0	395.1±28.3	59.1±24.4	39.0±6.6	
	Summer, Snowfence	180.6±16.0	164.8±39.7	437.8±98.3	80.9±18.5	63.6±15.4	
	Winter, Control	147.3±30.3	317.8±25.4	425.2±121.9	101.8±6.8	91.0±36.8	
	Winter, Snowfence	137.2±35.6	694.5±222.9	591.0±159.6	134.0±60.4	52.7±7.0	
	Season		*				
DON (mg kg <sup>-1</sup> )	Summer, Control	21.9±1.8	57.6±12.1	21.9±2.4	5.8±1.8	5.4±1.1	
	Summer, Snowfence	22.5±2.7	41.7±7.1	24.0±2.8	8.3±1.4	8.3±0.7	
	Winter, Control	9.9±1.2	<b>41.6±3.6</b>	<b>14.6±1.5</b>	9.3±1.0	7.8±2.0	
	Winter, Snowfence	9.0±2.7	<b>74.6±12.5§</b>	<b>24.6±5.0§</b>	15.4±6.3	7.8±1.3	
	Season	**					
NH <sub>4</sub> <sup>+</sup> -N (mg kg <sup>-1</sup> )	Summer, Control	1.2±0.7	2.8±0.8	0.4±0.0	<b>0.8±0.1</b>	<b>0.7±0.2</b>	
	Summer, Snowfence	1.6±0.5	5.3±1.1	0.5±0.1	<b>2.3±0.4*</b>	<b>3.0±1.1§</b>	**
	Winter, Control	0.7±0.1	57.0±7.1	0.4±0.1	<b>2.2±0.5</b>	<b>2.2±0.5</b>	
	Winter, Snowfence	0.5±0.0	86.9±17.9	0.5±0.1	<b>16.4±11.0§</b>	<b>9.4±2.1*</b>	
	Season		**			*	
NO <sub>3</sub> <sup>-</sup> -N (mg kg <sup>-1</sup> )	Summer, Control	0.06±0.06	24.35±9.73	<b>0</b>	<b>0.10±0.06</b>	<b>0.03±0.00</b>	
	Summer, Snowfence	0.05±0.04	19.46±7.99	<b>0.24±0.11*</b>	<b>9.11±2.15*</b>	<b>0.96±0.57*</b>	*
	Winter, Control	0	0.61±0.11	0.08±0.02	0.17±0.10	0.28±0.13	
	Winter, Snowfence	0	0.84±0.15	0.08±0.02	0.24±0.10	0.18±0.07	
	Season		**				

All values are means ±1 SE ( $n = 5$  except Svalbard Meadow where  $n=6$ ). Significant differences between the treatments in each season within each site (in bold) and across sites, and significant effect of season across treatment in each site are shown as §  $\leq 0.1$ ; \* $p \leq 0.05$  and \*\* $p \leq 0.01$ . GWC: Gravimetric water content, ND: no data.

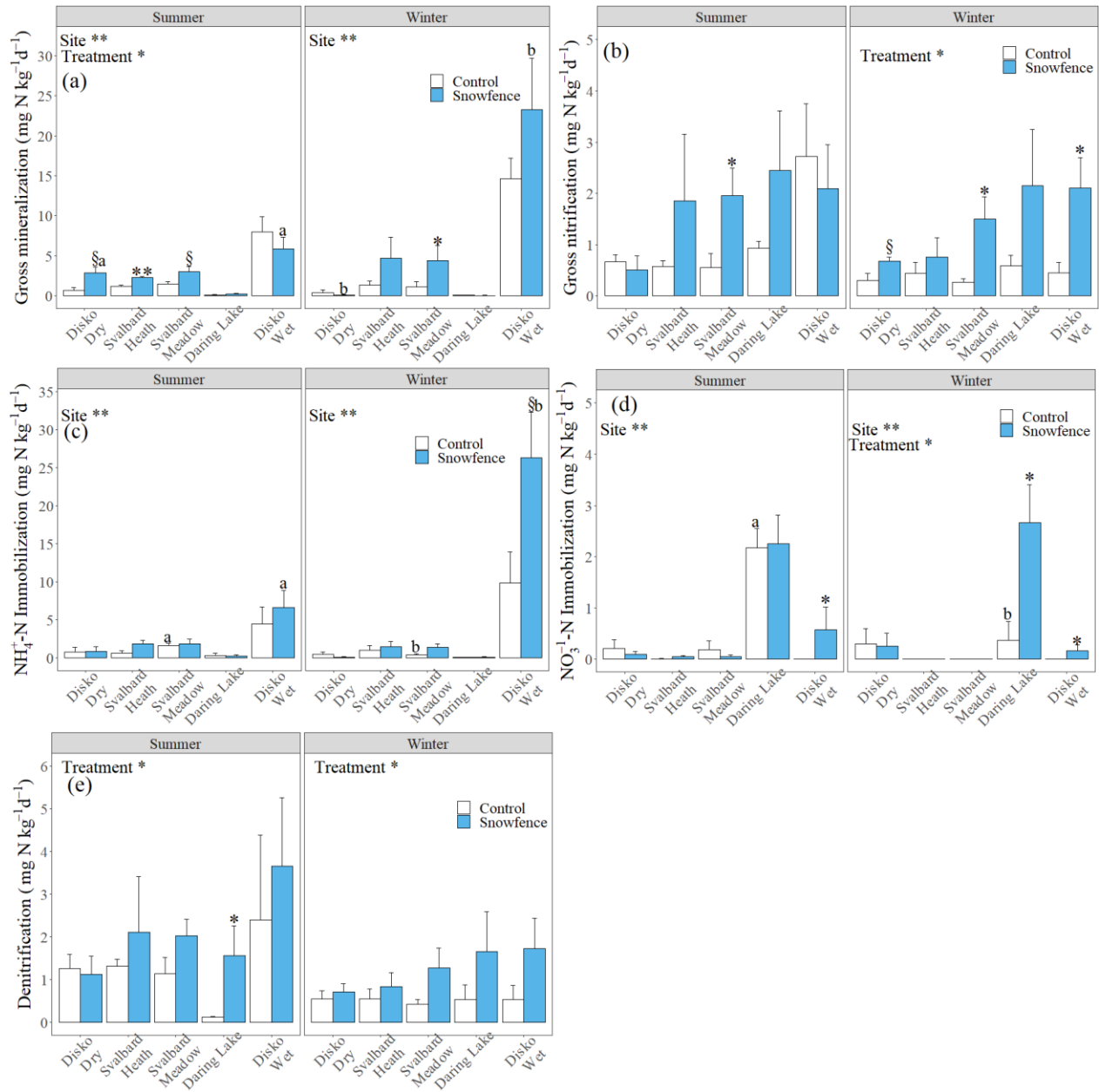


Fig. 1 Effects of the deepened snow treatment on gross rates of N mineralization (a), nitrification (b), NH<sub>4</sub><sup>+</sup>-N immobilization (c), NO<sub>3</sub><sup>-</sup>-N immobilization (d) and denitrification (e) in summer and winter (mg N kg<sub>dw</sub><sup>-1</sup> d<sup>-1</sup>). The sites are ordered by gravimetric water content from left (driest) to right (wettest). Significant differences between the treatments at each site and across sites, and significant effects of site in each season are shown as § ≤ 0.1; \*p ≤ 0.05 and \*\*p ≤ 0.01. Lowercase letters indicate significant differences between seasons within each treatment in each site.

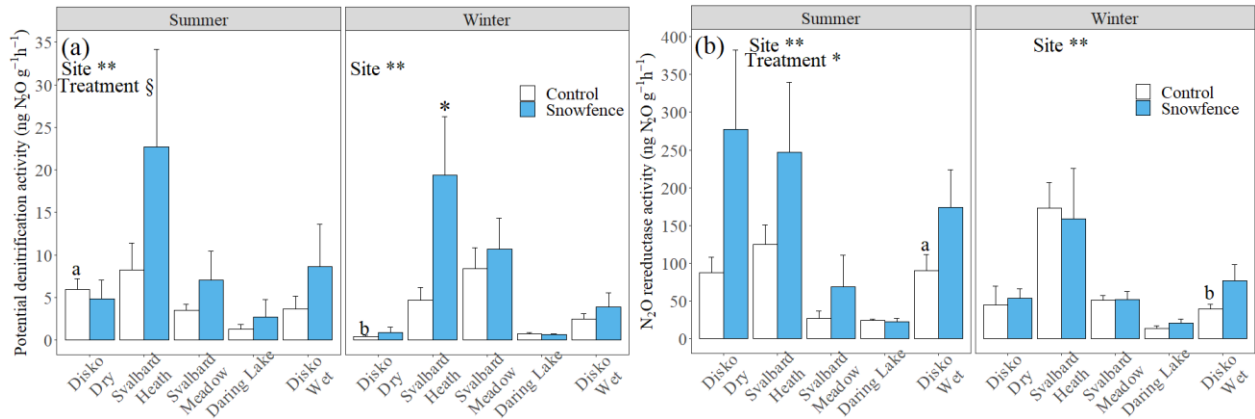


Fig. 2 Effects of the deepened snow treatment on potential denitrification activity (a) and N<sub>2</sub>O reductase activity (b), in the summer and winter (ng N<sub>2</sub>O g<sub>dw</sub><sup>-1</sup> h<sup>-1</sup>). Significant differences between the treatments at each site, across sites, and significant effect of site in each season are shown as  $\leq 0.1$ ; \* $p \leq 0.05$  and \*\* $p \leq 0.01$ . Lowercase letters indicate significant differences between seasons within each treatment in each site.

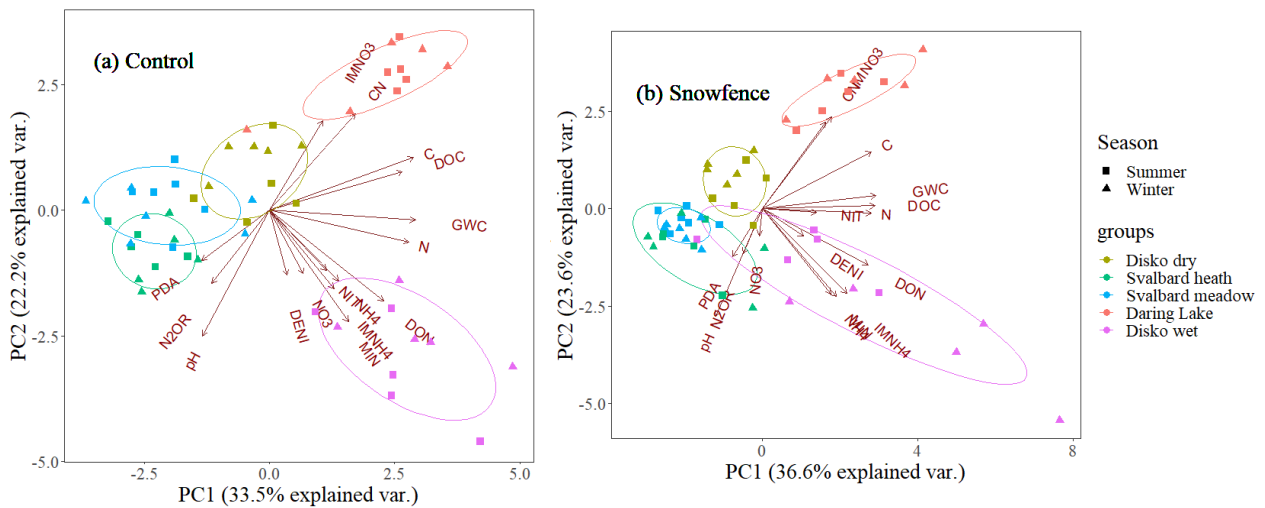


Fig. 3 Principal component analyses (PCA) for the extent of separations among the sites in control (a) and snowfence (b) plots. GWC: gravimetric water content, MIN: gross mineralization, NIT: gross nitrification, IMNH4: NH<sub>4</sub><sup>+</sup>-N immobilization, IMNO3: NO<sub>3</sub><sup>-</sup>-N immobilization, DENI: denitrification, PDA: potential denitrification activity, and N2OR: N<sub>2</sub>O reductase activity.

Table 3 Multiple stepwise regression analysis of the relationship between soil characteristics and N cycling rates in control and snowfence plots in combined seasons across sites.

Treatment	Response variable	Predictor variables	Relative importance of the variable (%)	P value	Proportion of variance explained by model (%)
Control	MIN	DON	47.7	<0.001 ***	78.30
		NH <sub>4</sub> <sup>+</sup> -N	43.3	<0.001 ***	
		pH	8.9	0.007 **	
	NIT	DON	46.7	<0.001 ***	64.87
		NO <sub>3</sub> <sup>-</sup> -N	44.4	<0.001 ***	
		NH <sub>4</sub> <sup>+</sup> -N	8.9	0.002 **	
	IMNH4	NH <sub>4</sub> <sup>+</sup> -N	80.2	<0.001 ***	58.10
		C	12.3	0.002 **	
		DOC	7.4	0.002 **	
	PDA	GWC	79.8	<0.001 ***	23.41
		DON	20.2	0.018	
	N2OR	pH	90.6	<0.001 ***	37.82
NH <sub>4</sub> <sup>+</sup> -N		9.4	0.039 *		
Snowfence	MIN	DON	42.8	0.001 **	72.71
		DOC	31.3	0.004 **	
		pH	25.9	<0.001 ***	
	NIT	GWC	-	0.022 *	33.73
	IMNH4	NH <sub>4</sub> <sup>+</sup> -N	46.6	<0.001 ***	81.54
		DOC	22.9	0.020 *	
		pH	19.0	<0.001 ***	
		C	11.6	0.005 **	
	IMNO3	C	52.2	0.004 **	56.98
		pH	47.8	0.001 **	

GWC: gravimetric water content, MIN: gross mineralization, NIT: gross nitrification, IMNH4: NH<sub>4</sub><sup>+</sup>-N

immobilization, IMNO3: NO<sub>3</sub><sup>-</sup>-N immobilization, DENI: denitrification, PDA: potential denitrification activity, and

N2OR: N<sub>2</sub>O reductase activity. Models that explained proportion of variance less than 20% were excluded.

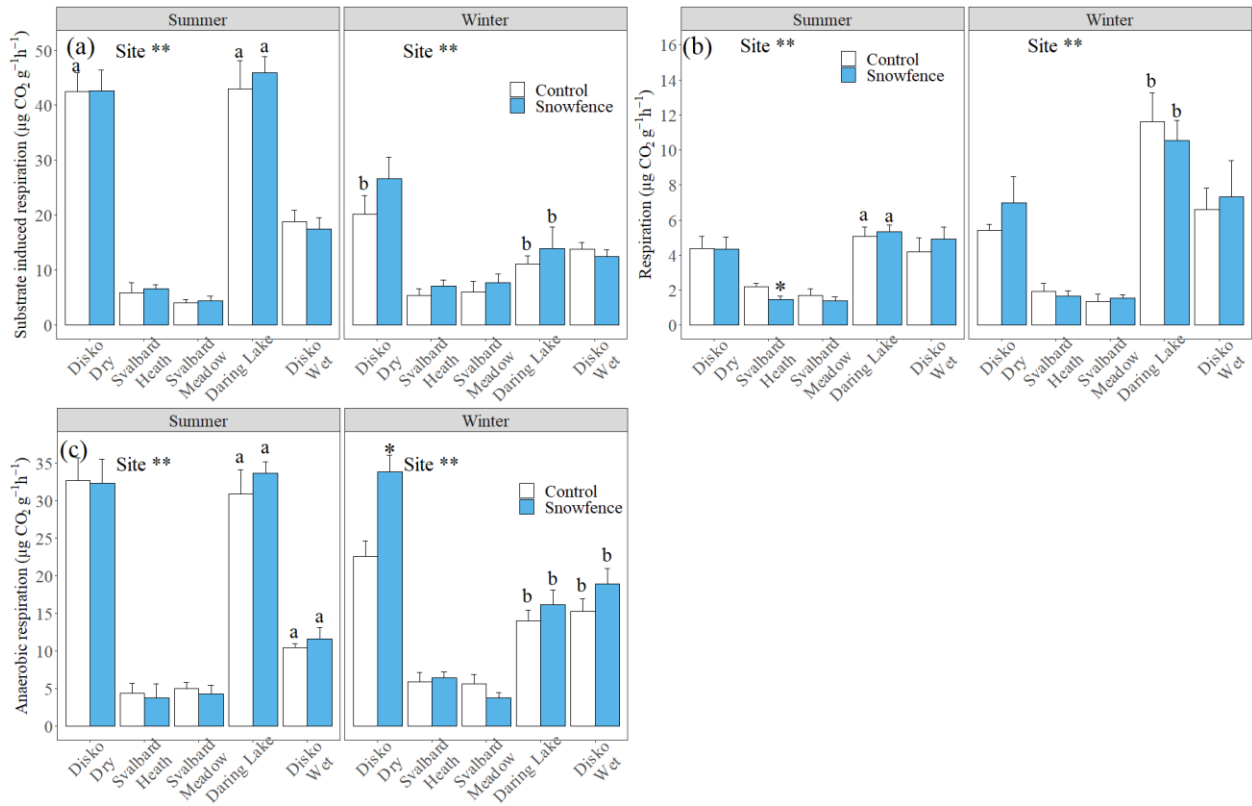
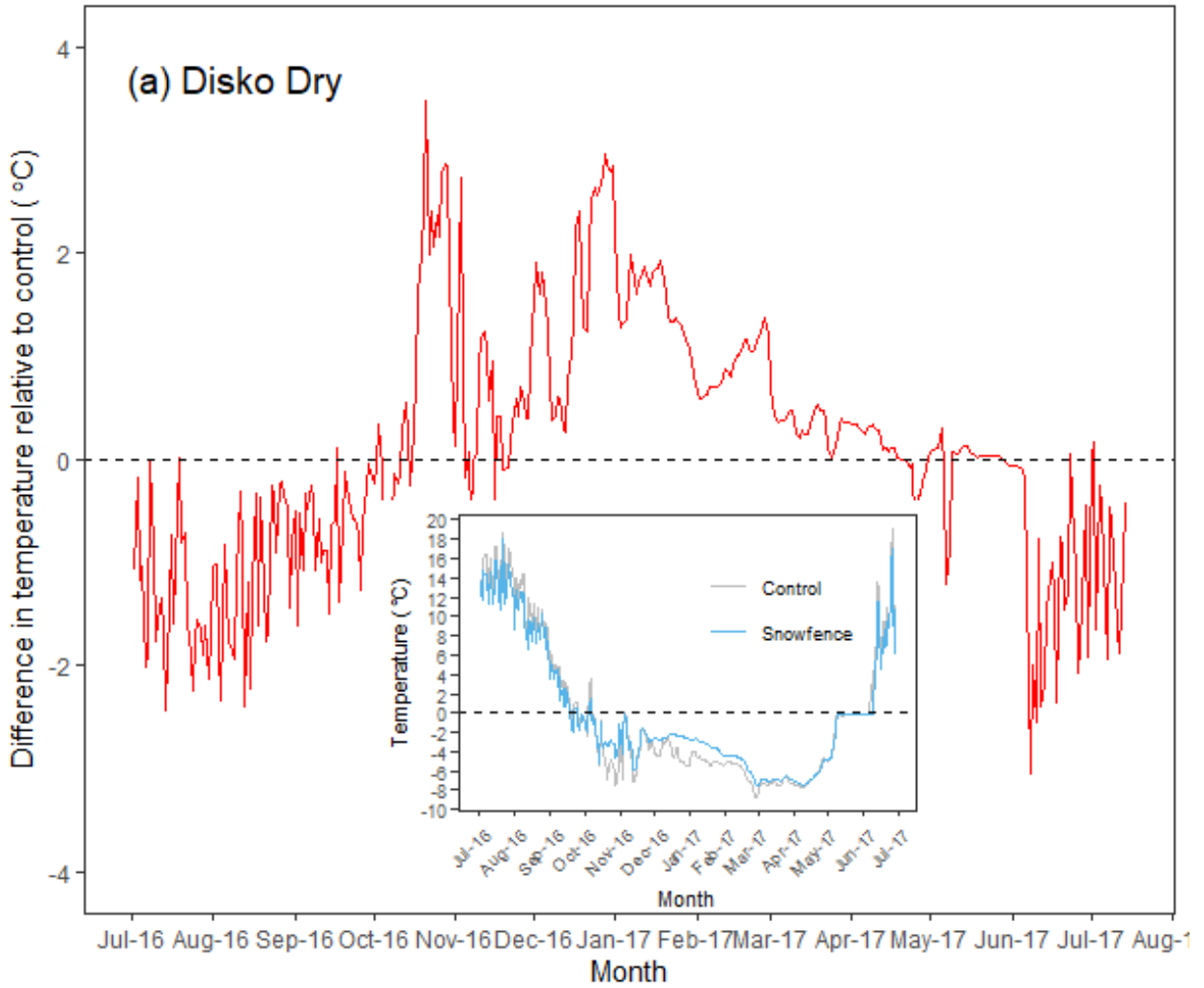
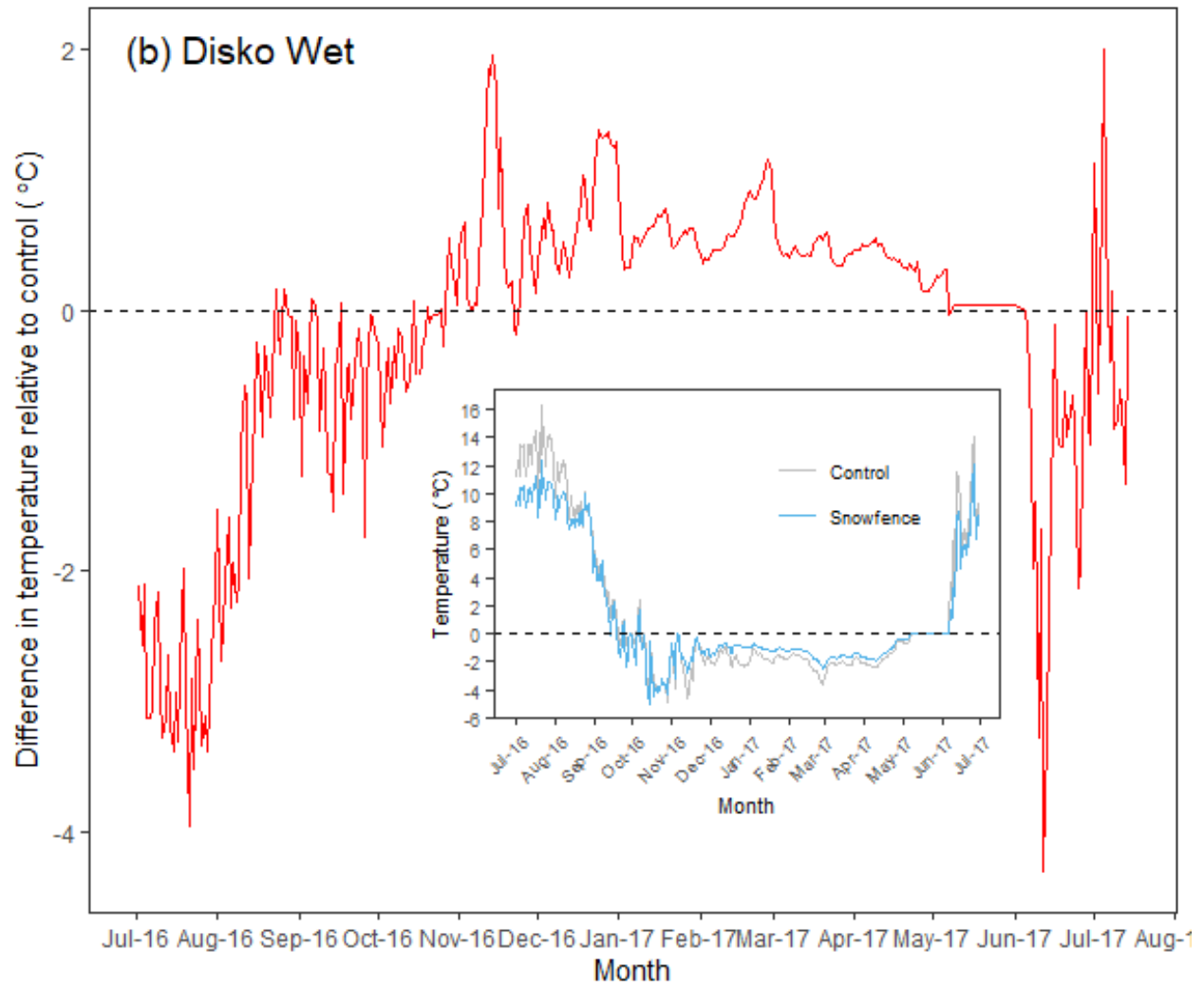


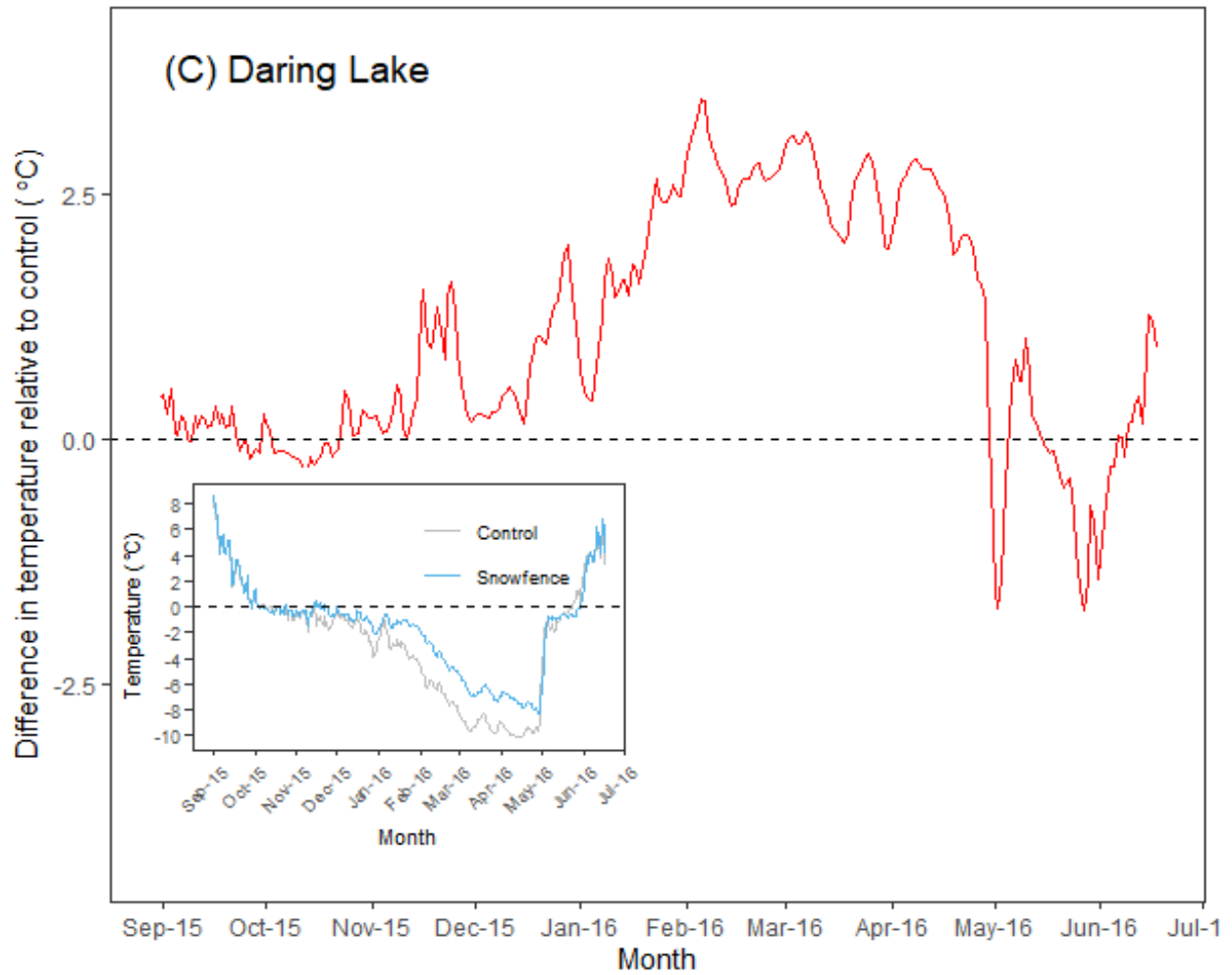
Fig. S1 Effects of the deepened snow treatment on substrate induced respiration (a), aerobic respiration (b) and anaerobic respiration (c) in summer and winter ( $\mu\text{g CO}_2 \text{ g}_{\text{dw}}^{-1} \text{ h}^{-1}$ ). Significant differences between the treatments at each site, across sites and significant effect of site in each season are shown as  $\S \leq 0.1$ ;  $*p \leq 0.05$  and  $**p \leq 0.01$ .

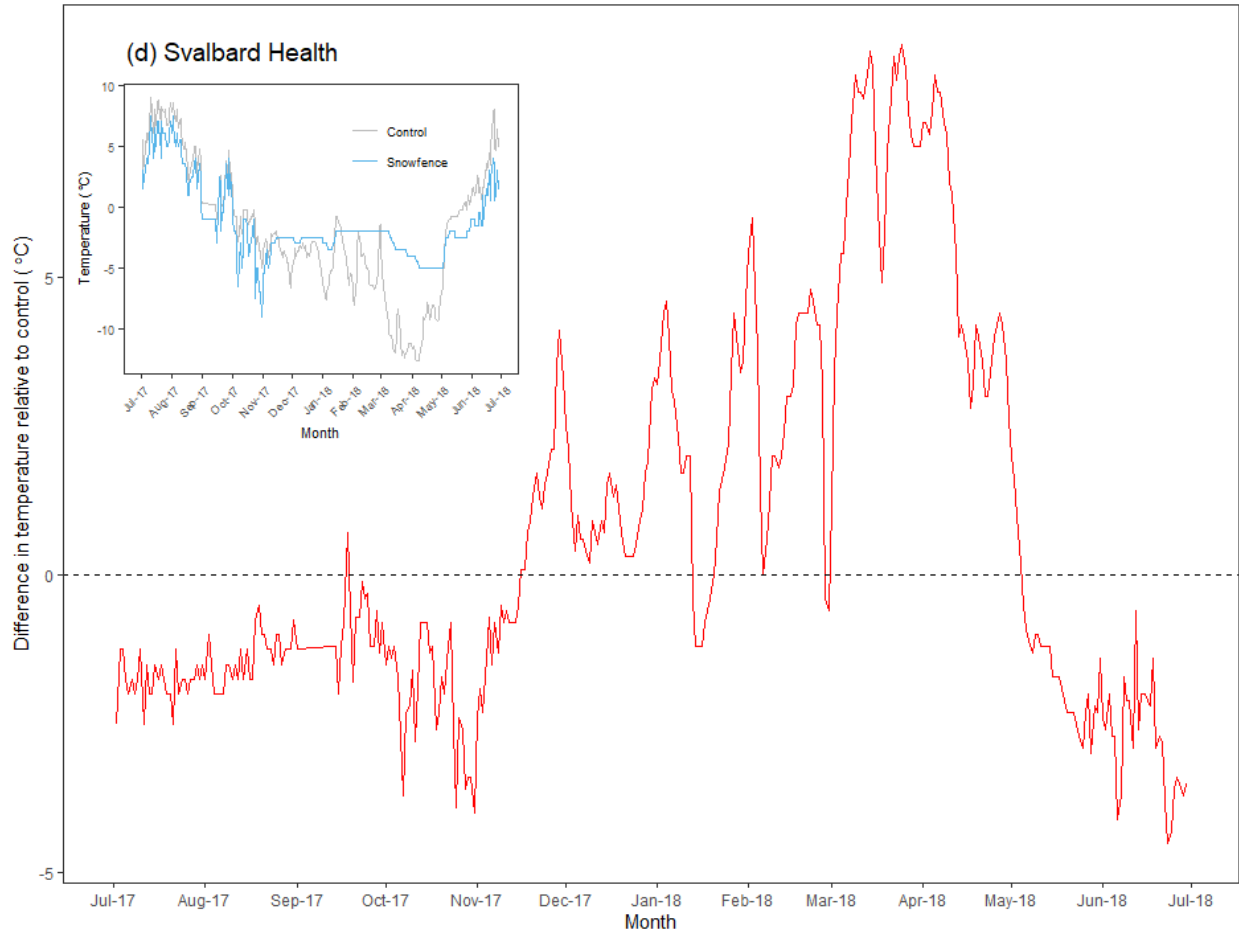
Lowercase letters indicate significant differences between seasons within each treatment in each site.











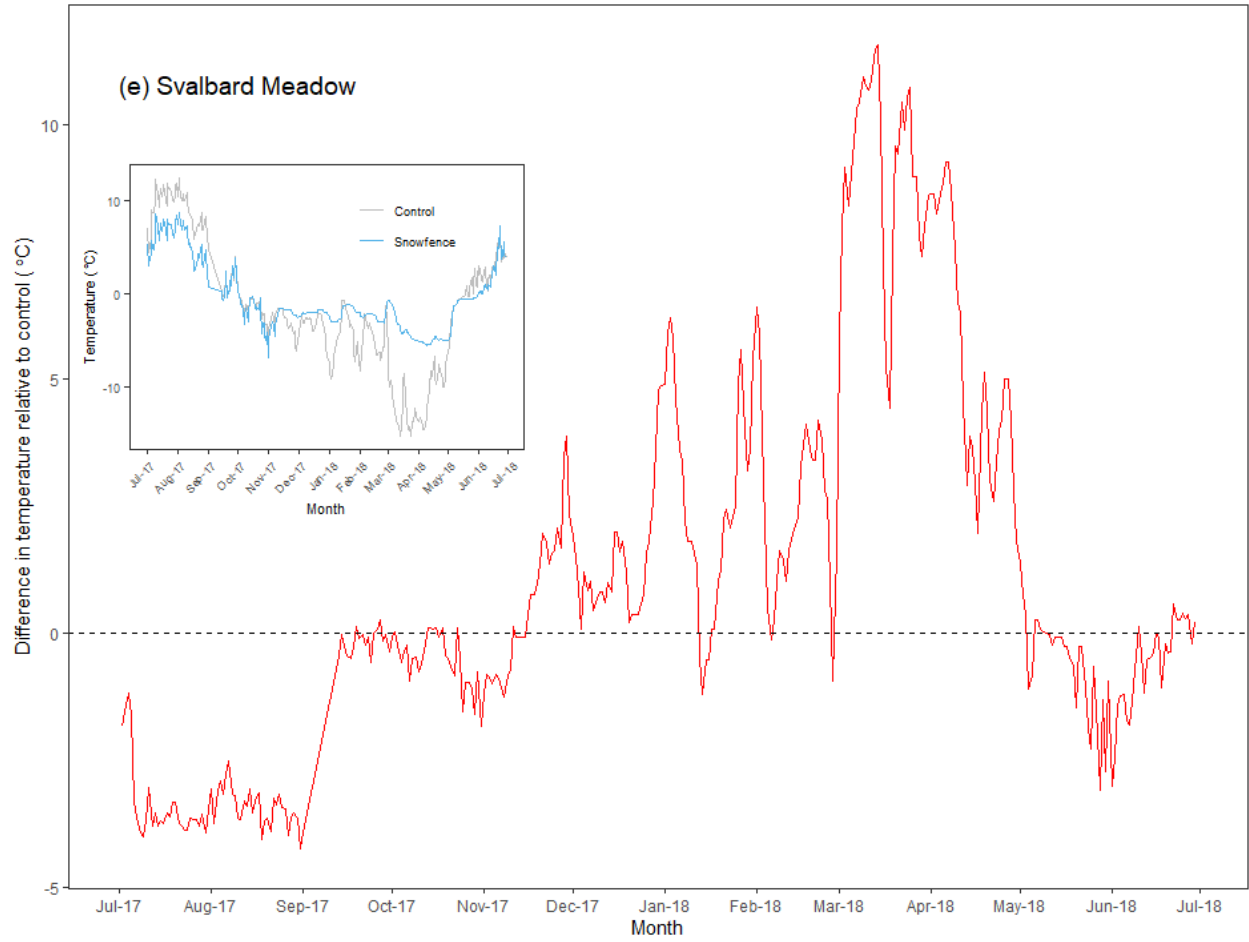
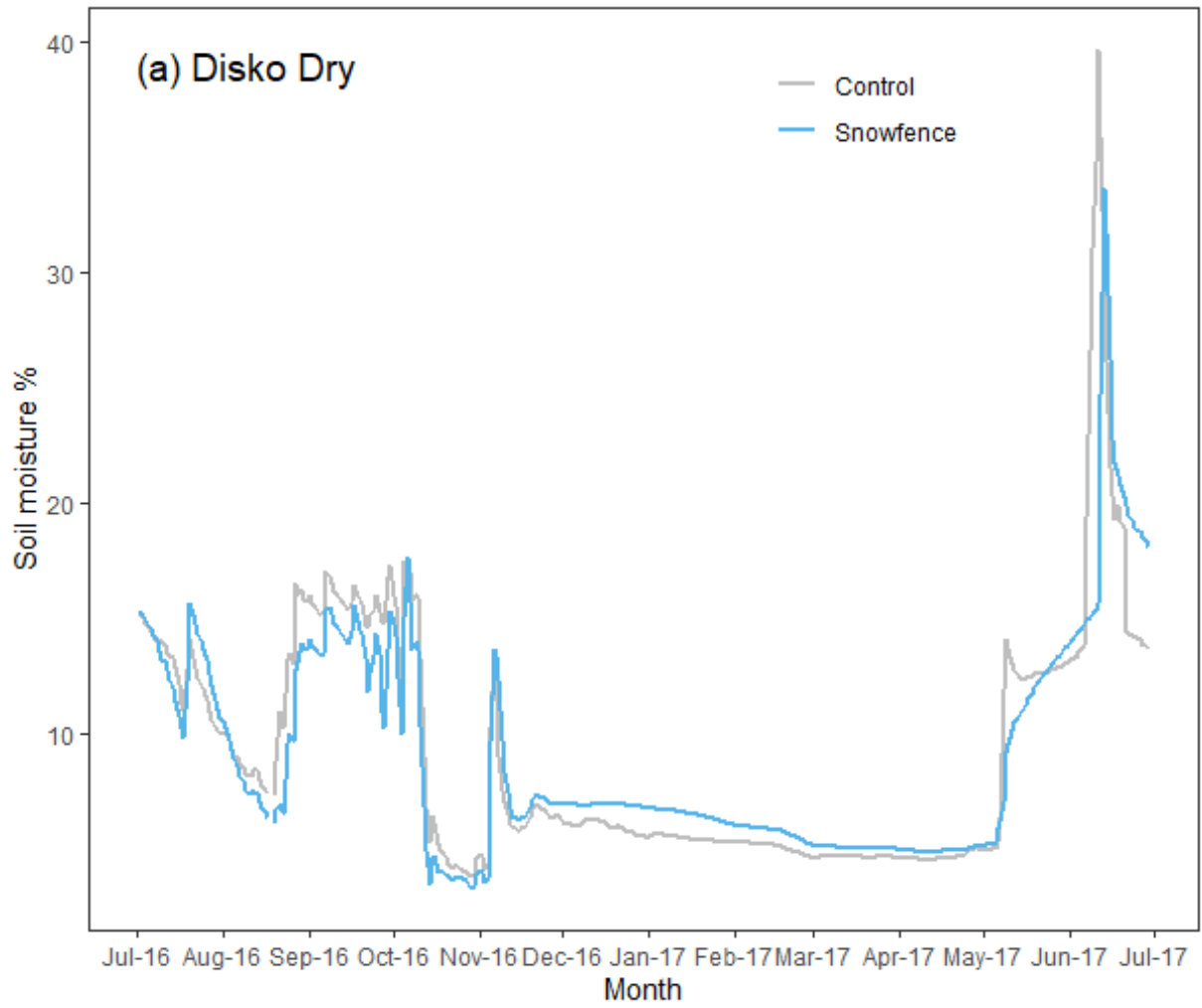
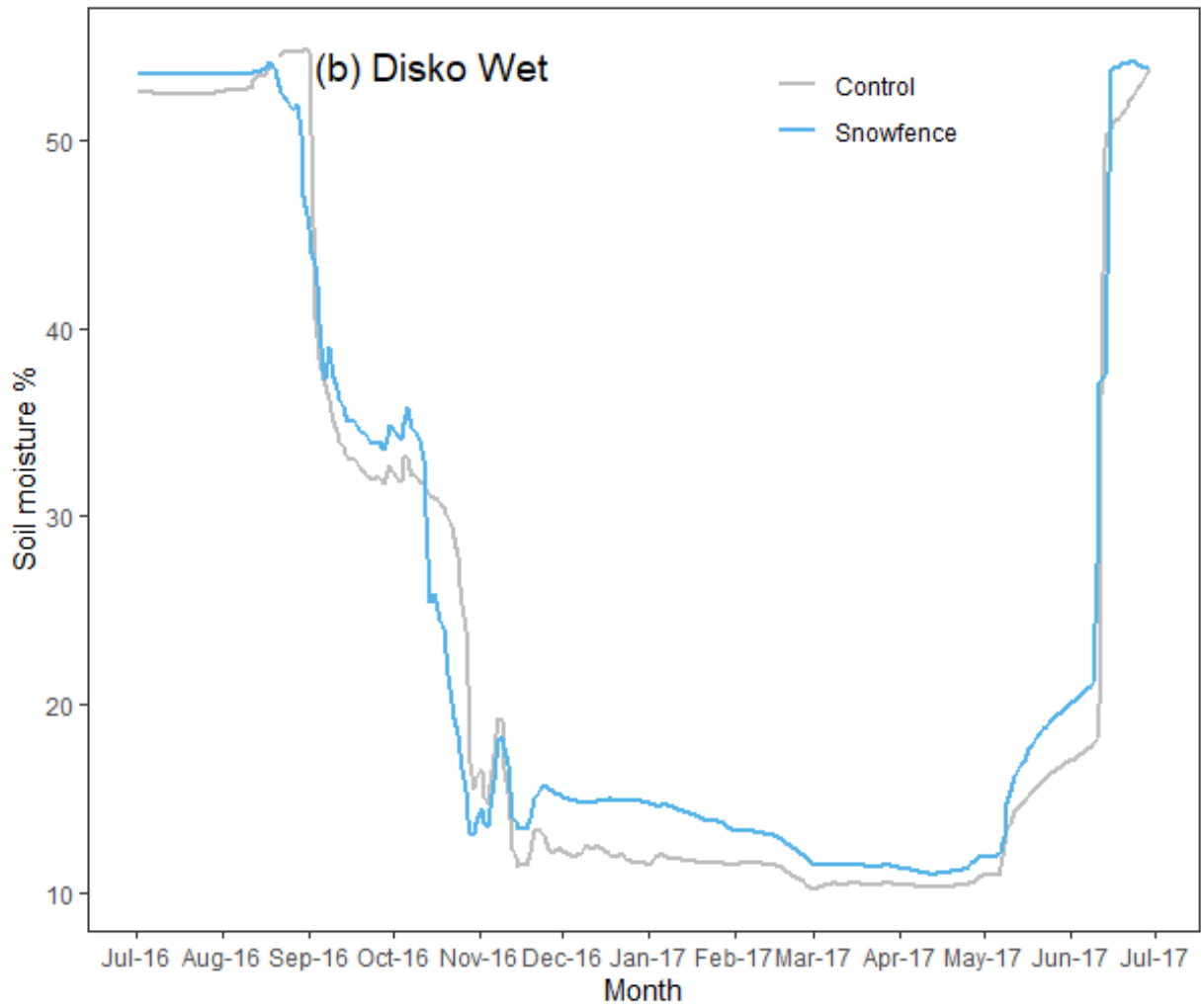


Fig. S2 Effects of deepened snow on diel ground surface temperature (plotted as differences relative to their corresponding controls) at Disko Dry (a) and Wet (b), Daring Lake (c), Svalbard Meadow (d) and Heath (e) sites, respectively. Positive values (above the zero line) indicate warmer surface temperatures in the deepened snow plots.





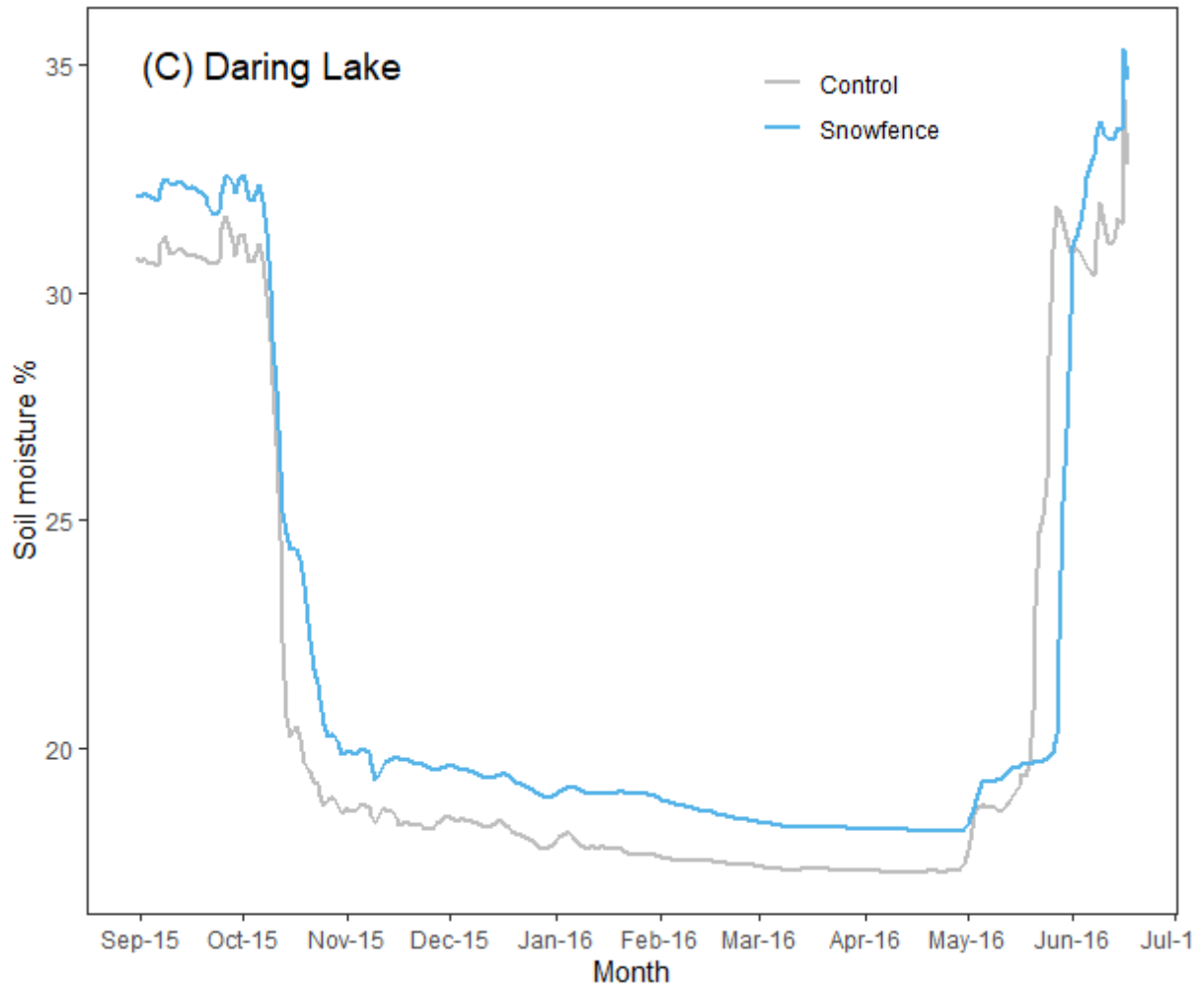


Fig. S3 Diel soil moisture between the control and snow fence plots at 5 cm depth at Disko Dry (a) and Wet (b), Daring Lake (c), respectively, (no data for Svalbard Heath and Meadow sites due to lack of observations for the entire year).

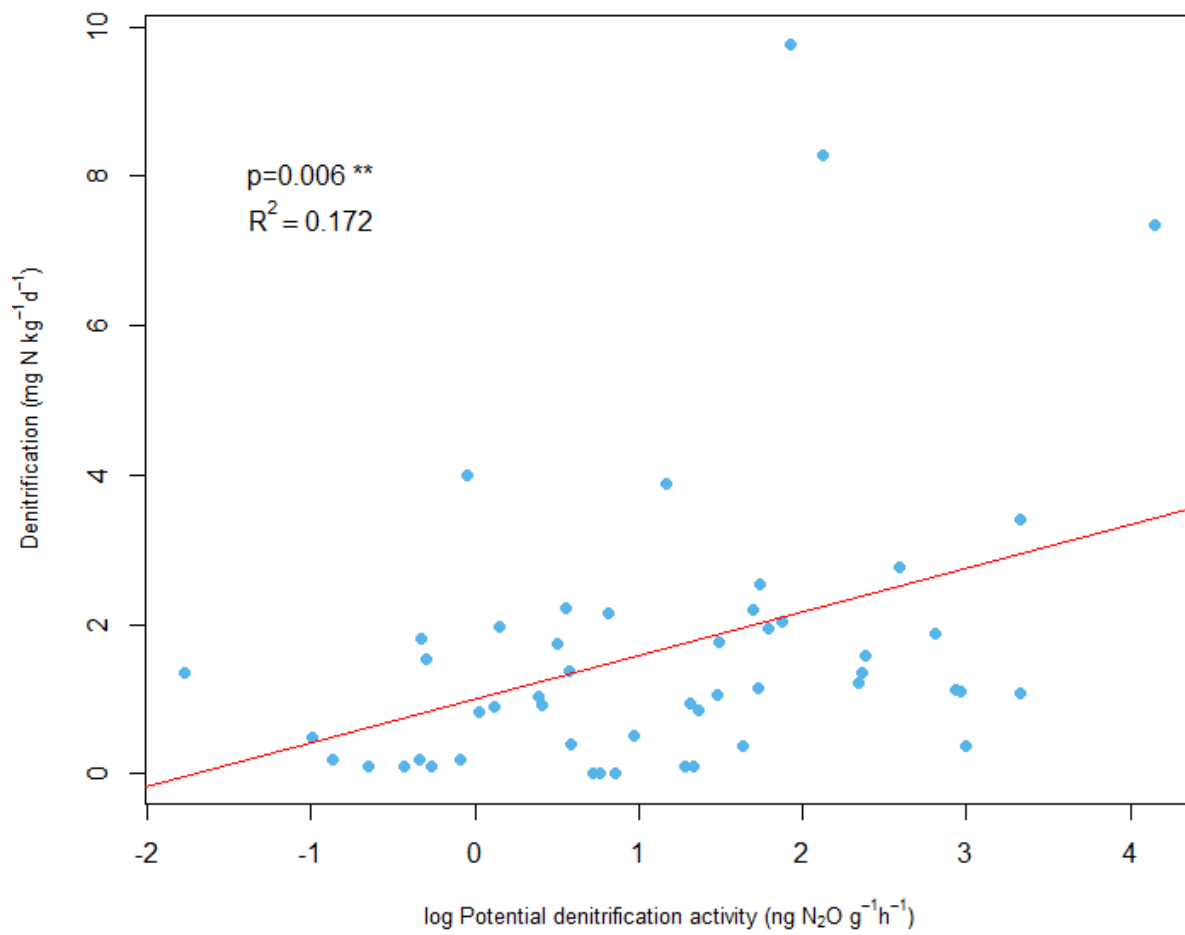


Fig. S4 Relationship between denitrification rates and potential denitrification activity across sites and treatments in summer.



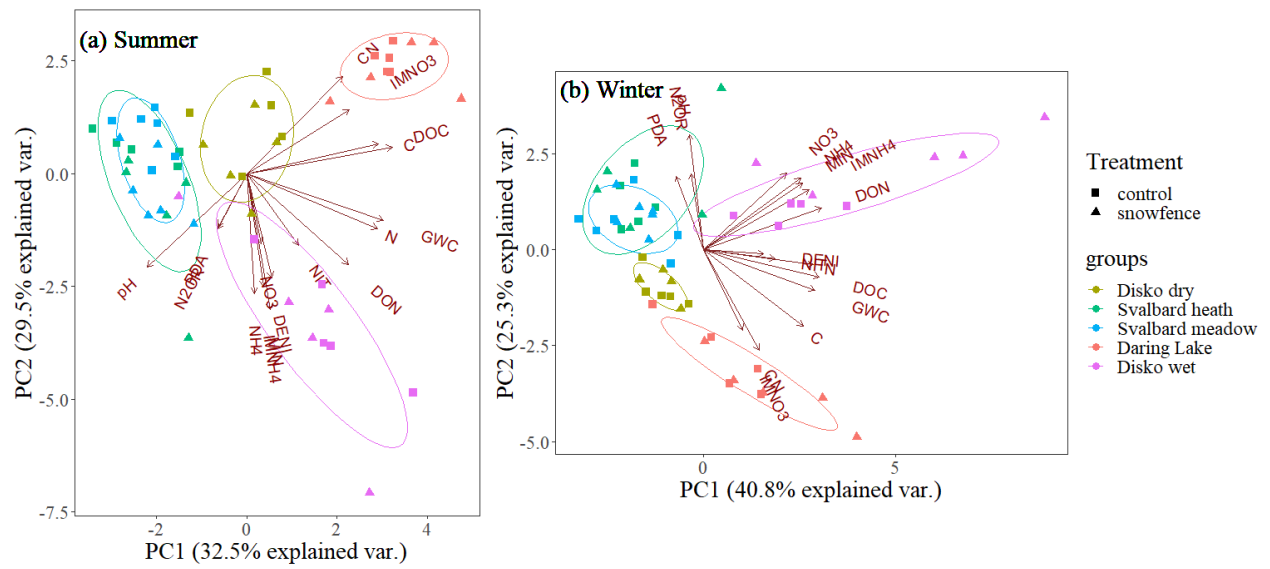
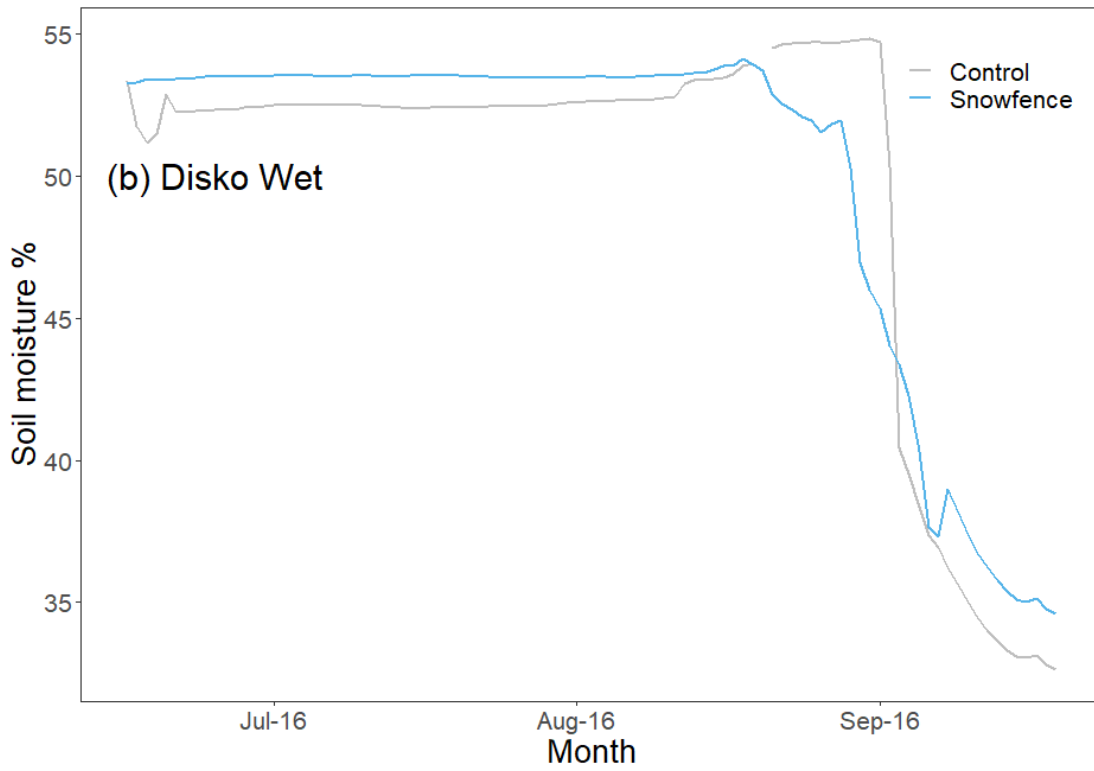
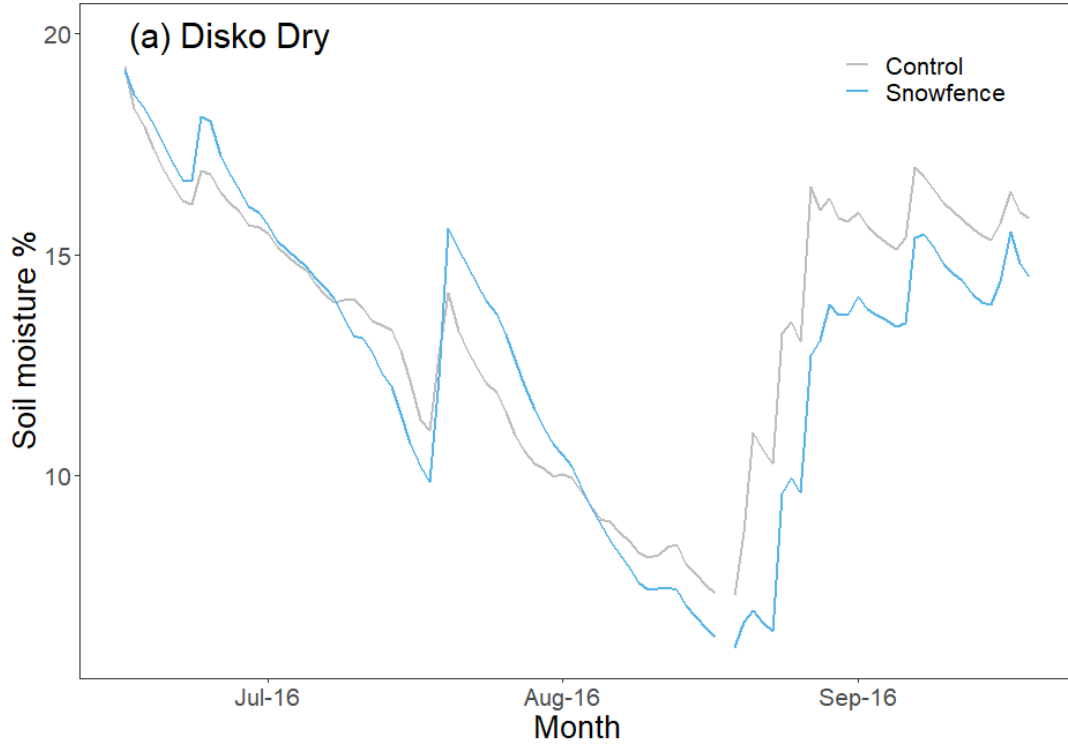
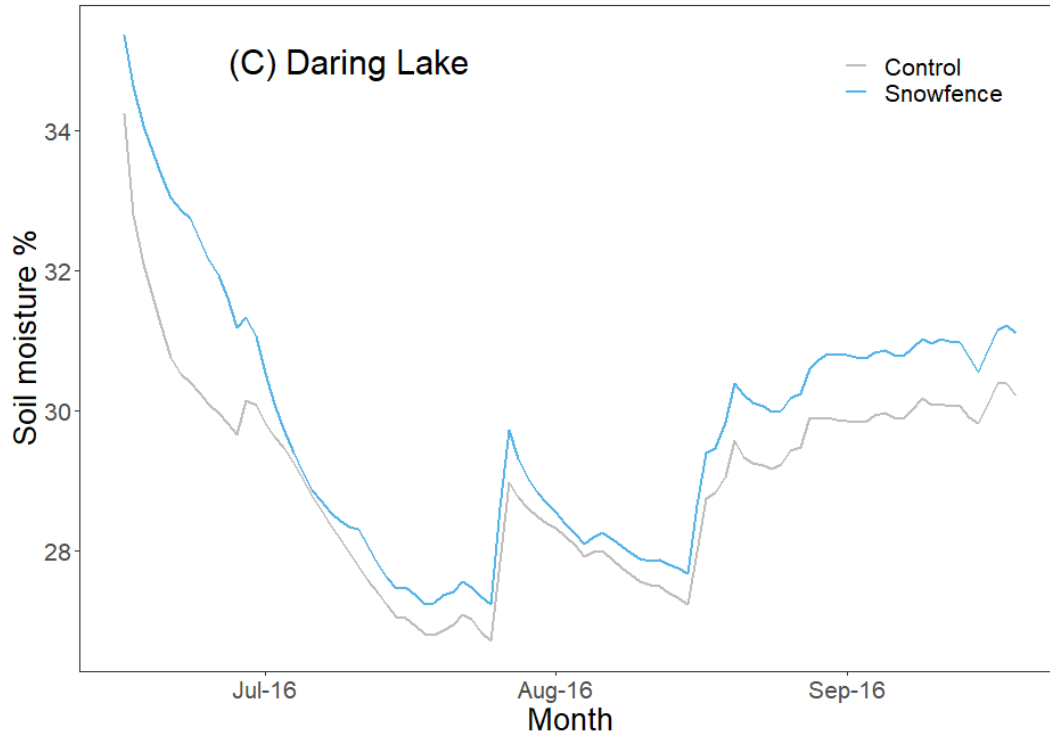


Fig. S5 Principal component analyses (PCA) for the extent of separations among the sites across treatments in summer (a) and winter (b). GWC: gravimetric water content, MIN: gross mineralization, NIT: gross nitrification, IMNH4:  $\text{NH}_4^+$ -N immobilization, IMNO3:  $\text{NO}_3^-$ -N immobilization, DENI: denitrification, PDA: potential denitrification activity, and N2OR:  $\text{N}_2\text{O}$  reductase activity.





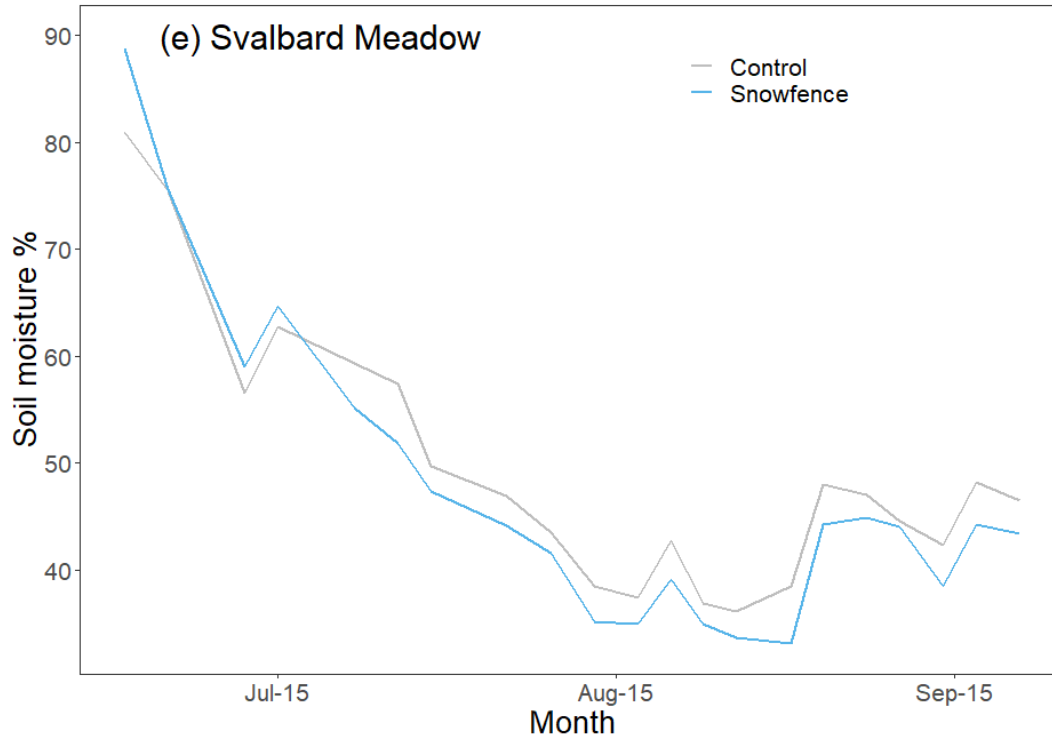


Fig. S6 Diel soil moisture between the control and snow fence plots at 5 cm depth at Disko Dry (a) and Wet (b), Daring Lake (c), Svalbard Heath (d) and Meadow (e) sites during the growing season.

Table S1 Coefficient of variation for N-cycle and C-cycle processes across treatments and seasons in each site.

Site	Disko Dry	Disko Wet	Daring Lake	Svalbard Heath	Svalbard Meadow
MIN	1.44	0.79	1.44	1.32	1.11
NIT	0.71	0.95	1.20	1.68	1.03
IMNH4	1.69	1.01	1.63	1.01	0.87
IMNO3	2.10	2.91	0.76	2.62	3.58
DENI	0.75	1.30	1.46	1.29	0.82
PDA	1.21	1.31	1.74	1.23	0.93
N2OR	1.30	0.82	0.42	0.85	0.64
SIR	0.38	0.29	0.64	0.44	0.61
R	0.42	0.53	0.46	0.42	0.46
NR	0.24	0.33	0.42	0.45	0.65

MIN: gross mineralization, NIT: gross nitrification, IMNH4:  $\text{NH}_4^+$ -N immobilization, IMNO3:  $\text{NO}_3^-$ -N

immobilization, DENI: denitrification, PDA: potential denitrification activity, N2OR:  $\text{N}_2\text{O}$  reductase activity, SIR:

substrate induced respiration, R: respiration and NR: anaerobic respiration.

### Reply to reviewers' comments:

Dear editor and reviewers. We thank you all very much for being in support of our work and for providing such helpful and constructive comments. We agree with the points raised, have made revisions to the manuscript accordingly and believe that we now have an improved manuscript.

Reviewer #1: This article, named Deepened snow enhances gross nitrogen cycling among Pan-Arctic tundra soils during winter and summer, investigated the effects of y depth of snow on gross N cycling in Pan-Arctic tundra soils. It is a very interesting topic. In this study, authors conducted an in-vitro study comparing the effects of deepened snow using snow fences in long-term field experiments in five tundra sites and measured the gross N transformation rate using a  $^{15}\text{N}$  pool dilution. The results showed that deepened snow enhanced winter nitrification,  $\text{NO}_3^-$ -N immobilization, denitrification, summer gross mineralization and denitrification. The increases in N cycling rates were driven by the availability of DOC and DON. The effects of deepened snow were most pronounced in wet tundra ecosystems. These results could provide support to prior observations that growing season biogeochemical cycles in the Arctic is sensitive to snow depth with altered nutrient availability for microorganisms and vegetation.

Generally, the article is well written. My major concern is the method of measurement of  $^{15}\text{N}$  enrichments of extractable  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in soil extracts.

The method that authors used is that soil extracts were processed for diffusion of these components on acidified filter papers. However, the concentration of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in soil extracts could significantly affect the results of  $^{15}\text{N}$  enrichments measured using diffusion. Generally, when the concentration of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in extract was lower than 0.5 mg N /L, the diffusion method can not accurately determine  $^{15}\text{N}$  enrichments. Authors should provide more detail descriptions of the method.

We thank the reviewer for pointing this out and we have added a more detailed description of our protocol, which is optimized for analysis of low concentration of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ . Please see the lines 308-315 in the revised manuscript. According to the IRMS analysis associated with the diffusion method, a total of 50  $\mu\text{g N}$  is required for optimal analysis at  $^{15}\text{N}$ . Also, considering the low molarity of extracts, additional salt was added to increase ionic strength of solution and thus to avoid  $\text{H}_2\text{O}$  diffusion into the traps and swelling. Due to the low inherent mineral N pool in some soils (at least these two time points), the amount of added N should be low to avoid fertilizer effects.

Reviewer #2: REVIEWER REPORT on manuscript titled 'Deepened snow enhances gross nitrogen cycling among Pan-Arctic tundra soils during winter and summer' by Xu et al.

### GENERAL COMMENTS

This manuscript reports effects of deepened snow on N pools and processes across five arctic study sites, representing variable climate and soil characteristics. The universal trends across study sites (Table 3) together with the contrast between seasons (winter vs. summer) make this study particularly valuable and comprehensive. It adds significantly our knowledge about regulation of N cycling processes in permafrost-affected soils - a field that has not gained sufficiently attention considering its important consequences for ecosystem functioning and climatic feedbacks. I really enjoyed reading this carefully designed and conducted and very well written piece of work that is well connected to the previous work on the same topic via thorough literature review and tight integration of current results to the literature. I would like to see a clearer presentation of the treatment effects on soil temperature and moisture (see below), but besides that I have only minor comments on the manuscript.

### MAJOR COMMENT

The figs. S2 and S3 shows how the snow manipulation experiment has affected the physical soil conditions

temperature and moisture. Both these factors are crucial for microbial activities, and were affected differently across sites and seasons (much larger winter warming effect at Svalbard site compared to other sites; variable summer cooling effect). This calls for a clear and compact summary of the treatment effects and statistical significances in the main manuscript, e.g. in Table 1 but even better as a graph similar to those in Figs. 1 and 2. Although the regression analysis in Table 3 clearly points out some universal drivers explaining the variability across sites, the enhancement of N cycle could be additionally affected by the strength of temperature and soil moisture effect. For example, gross mineralization was higher in Svalbard meadow, where the winter warming was particularly strong.

We thank reviewer for these suggestions and we have statistically analyzed treatment effects on soil temperature (during snow-covered and non-snow-covered period) and moisture. Since soil temperature and moisture were based on several years in all sites, except for Daring Lake due to problems with the probes, these data were presented in a summary table and treatment differences were also indicated. We have done the linear regression analysis between soil temperature and N cycling rates across sites and seasons, but observed no significant correlations, probably due to indirect effects of soil temperature via changing soil characteristics such as DOC and DON, or to long-term ecosystem acclimation such as microbial community change. In that case, DOC and DON were the main universal drivers for the variability across sites, but soil temperature effect was still mentioned in the Introduction and the first subsection of Discussion. We have also indicated that the strength of soil temperature and moisture enhancement by deepened snow can additionally affect N cycle. The sentences now read that “For example, the highest enhancement of wintertime soil temperature by deepened snow occurred at Svalbard Meadow site (2.5 °C; Table 1) where the most notably increased wintertime gross N mineralization and nitrification were observed. There were also significant summertime cooling effects in Disko Wet (Table 1), which may explain the lack of effects by snowfence treatment on summertime gross N mineralization and nitrification rates, despite relatively high soil DOC and DON concentrations. A significant effect on summertime gross N mineralization was only observed at Svalbard Heath (Table 1, Fig. S6d), probably in part due to higher soil moisture under deepened snow conditions in summer and thus increased mobility of solutes, enzymes and microorganisms. Similarly, the highest enhancement of summertime PDA activity also occurred in this site”. Please see lines 469-472 and 615-627.

## MINOR COMMENTS

line 41: Change to ...cycles are sensitive to...

We thank the reviewer for pointing this out and we have amended the sentence. Please see line 41 in the revised manuscript.

line 69: It would be good to add here also the effect of snow accumulation on permafrost, see Biskaborn et al. 2019 (<https://doi.org/10.1038/s41467-018-08240-4>). Related to this, I recommend reporting max thaw depths separately for control and treatment in Table 1, and add related text to the discussion section.

We thank the reviewer for this suggestion and we have reported max thaw depths separately for control and snowfence plots (Table 1) and observed no significant treatment differences. Because of that, and also considering the *in vitro* soil incubation (from 0-5 cm depth) conducted in this study, effects of snow accumulation on thaw depth were not discussed further. Meanwhile general effect has been included in the Introduction section. Please see line 71 in the revised manuscript.

line 133-134: It is quite unlikely that winter in situ temperatures (= mild freezing temperatures) were achievable, in that case it would be better to say shipped '...at temperature conditions resembling those in the field...', or something similar.

We thank the reviewer for this suggestion and we have changed to “shipped at temperature conditions resembling those in the field for subsequent laboratory work”. Please see lines 134-135 in the revised manuscript.

line 153: The description of permafrost conditions is confusing, would be better to simply state which permafrost zone the region belongs to.

We thank the reviewer for this suggestion and we have changed to “The sites lie within the discontinuous permafrost zone”. Please see line 154 in the revised manuscript.

lines 156 and 179: Use systematically an English name for *Vaccinium vitis-idaea*; according to my knowledge crowberry is not used but lingonberry, cowberry or mountain cranberry.

We thank the reviewer for pointing this out and we have revised its English name. Please see lines 156 and 180 in the revised manuscript.

lines 230-234: For similar description of the study sites, you should describe also the snow conditions at Daring lake site.

We thank the reviewer for pointing this out. Due to lack of observations, the snow depth at Daring Lake during winter sampling was not reported, but the typical snow conditions at both control and snowfence plots have been included in the revised manuscript on lines 215-218.

lines 241-242: Please report how large was the mineral N addition in mg kg<sup>-1</sup> DW to allow comparison with mineral N levels reported in Table 2. It seems N addition for the pool dilution experiment exceeded the inherent mineral N pools at most of the sites. I believe this was necessary to ensure measurable mineral N pools, but I would like to see some justification/discussion on how this might have affected the gross rates. Generally, the mineral N label addition is kept at some fraction of the inherent mineral N pool, not more.

We thank the reviewer for pointing this out and we have reported the amount of added tracer based on dry weight (approximately 2.5 mg N kg<sup>-1</sup>). Please see lines 261 and 310-317 in the revised manuscript. Importantly, according to this micro-diffusion method, each filter needs to catch ca. 50 µg N for optimal analysis of <sup>15</sup>N abundance. Hence, the amount of added tracer is a compromise between tracer addition effects and optimal analysis. Also, the added tracer is the end product of either mineralization and nitrification, and the deployed amounts are considered too low to inhibit these processes.

line 260-261: Explain here or another suitable occasion the functional importance of N<sub>2</sub>O reductase - reduction of microbial or atmospheric N<sub>2</sub>O, and thus, reduction of N<sub>2</sub>O release or N<sub>2</sub>O consumption. Also, it would be good to comment the association between PDA and N<sub>2</sub>O. Since N<sub>2</sub>O is a strong GHG and N<sub>2</sub> is atmospherically inert, this has impact on ecosystem-climate feedbacks (see Voigt et al. 2020 <https://doi.org/10.1038/s43017-020-0063-9>).

We thank the reviewer for these suggestions and we have added relevant context. The sentences now read that “During denitrification, NO<sub>3</sub><sup>-</sup> is reduced to N<sub>2</sub>O by denitrifiers in an anaerobic respiratory pathway, which can be further reduced into N<sub>2</sub>, largely depending on O<sub>2</sub> status. Since N<sub>2</sub>O is a strong greenhouse gas, whereas N<sub>2</sub> is atmospherically inert, the ratio of the two gaseous end products of denitrification can have an impact on ecosystem feedbacks to climate change (Voigt et al., 2020)”. Please see lines 279-280 and 505-509 in the revised manuscript.

line 318: Did you scale the variable prior to PCA to equalize the variances?

Yes, since our input variables are on different scales, we scale to unit variance by using package `prcomp` in R with `argument scale=TRUE`.

line 433-437: Please check this sentence, might need some rewording.

We thank reviewer for pointing this out, and we have deleted “soil moisture” since soil moisture was not

affected by deepened snow in four of our study sites. Please see lines 460-462 in revised manuscript.

line 468-470 and 475-477: Could these increases in PDA and N<sub>2</sub>OR, and nitrification be driven by moisture, and to which extent? For checking this, it would be great to see where moisture and temperature were significantly changed, see my major comment.

We thank the reviewer for this suggestion. We observed no treatment differences in soil volumetric moisture in growing season at four of the sites (except for Svalbard Heath site). The soil moisture of the two Svalbard sites were not directly comparable with the other three, due to different measurement ranges of moisture sensors used (see Table 1). Also, given that there were only 2 replicates for each treatment at Daring lake and 3 replicates at Disko sites, we put soil gravimetric moisture (GWC; 5-6 replicates for each treatment at all the sites) in the multiple regression model instead, and observed that nitrification at snowfence plots across sites were driven by GWC.

line 488: Change to 'Nitrate is easily leached...'

We thank the reviewer for pointing this out and we have revised the sentence. Please see line 523 in the revised manuscript.

line 512: Writing H2 open here will improve fluent reading.

We thank the reviewer for this suggestion and we have changed to “Our observations show that increased availability of soil organic N drives the responses of gross mineralization rates to deepened snow cover and hence partly support H2”. Please see lines 547-548 in the revised manuscript.

line 528-530: May need some rewording - first and second phrase are repetitive?

We thank the reviewer for point this out and now the sentence reads that “A comparative study of two ecosystems in low Arctic tundra also showed that tall birch soils had higher labile C concentrations and faster N mineralization relative to birch hummock soil, but lower soil total C contents”. Please see lines 564-567.

line 602: Also here it would be good to write the hypothesis H3 open.

We thank the reviewer for this suggestion and we have changed to “supporting our hypothesis H3 that the extent of the increase in gross N transformation rates across seasons mediated by deepened snow is linked to ecosystem moisture regime”. Please see lines 650-652 in the revised manuscript.

Table 1. The duration of the snow manipulation experiment at the sampling occasion was quite variable - could this have affected the results via ecosystem acclimation in long-term (vegetation change, microbial community change and stabilization to new conditions) in contrast to short-term imbalance in N cycles? Please comment this in appropriate place of the discussion section.

We thank the reviewer for this suggestion and we have added relevant discussion. Now it reads that “Moreover, the duration of the snow manipulation experiment varied among the study sites and this could have affected the responses of N cycles via ecosystem acclimation, e.g. vegetation and microbial community change and stabilization to new conditions upon longer term manipulations (Christiansen et al., 2018; Mundra et al., 2016). This may help explain the negligible response of gross N mineralization at Daring Lake that had the longest duration of snow manipulation treatments (13 years; Table 1)”. Please see lines 674-680.

Figs. 1 and 2: Please add site names under each subplot to improve readability. This is particularly important since the site order here is different to what you use in the Tables and the text. Considering also increasing the font size (also for Supplementary figs), they should be readable at 100% zoom.

We thank the reviewer for pointing this out and the font size of all the figures have been increased and they can be readable at 100% zoom.



**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: