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

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ABSTRACT

Many Arctic regions currently experience an increase in winter snowfall as a result of climate change. Deepened snow can enhance thermal insulation of the underlying soil during winter, resulting in warmer soil temperatures that promote soil microbial nitrogen (N)-cycle processes and the availability of N and other nutrients. We conducted an *ex situ* study comparing the effects of deepened snow (using snow fences that have been installed for 3–13 years) on microbial N-cycle processes in late summer (late growing season) and winter (late snow-covered season) among five tundra sites in three different geographic locations across the Arctic (Greenland (dry and wet tundra), Canada (mesic tundra), and Svalbard, Norway (heath and meadow tundra)). Soil gross N cycling rates (mineralization, nitrification, immobilization of ammonium (NH_4^+) and nitrate (NO_3^-), and denitrification) were determined using a ^{15}N pool dilution. Potential denitrification activity (PDA) and nitrous oxide reductase activity (N2OR) were measured to assess denitrifying enzyme activities.

The deepened snow treatment across all sites had a significant effect of the potential soil capacity of accelerating N cycling rates in late winter, including quadrupled gross nitrification, tripled NO_3^--N immobilization, and doubled denitrification as well as significantly enhanced late summer gross N mineralization, denitrification (two-fold) and NH_4^+-N availability. The increase in gross N mineralization and nitrification rates were primarily driven by the availability of dissolved organic carbon (DOC) and nitrogen (DON) across sites. The largest increases in winter DOC and DON concentrations due to deepened snow were observed at the two wetter sites (wet and mesic tundra), and N cycling rates were also more strongly affected by deepened snow at these two sites than at the three other drier sites. Together, these results suggest that the potential effects of deepened winter snow in stimulating microbial N-cycling activities will be most pronounced in relatively moist tundra ecosystems. Hence, this study provides support to prior observations that growing season biogeochemical cycles in the Arctic are sensitive to snow depth with altered nutrient availability for microorganisms and vegetation. It can be speculated that on the one hand growing season N availability will increase and promote plant growth, but on the other hand foster increased water- and gaseous (e.g. N_2 and N_2O) N-losses with implications for overall nutrient status.

1. Introduction

Arctic ecosystems are characterized by short growing seasons and long cold winters with frozen and snow-covered soils generally resulting in low nutrient availabilities and primary productivity (Nadelhoffer et al., 1992; Jones et al., 1999). Meanwhile, in spite of these harsh conditions, soil microbial activity continues through the winter (Mikan et al., 2002; Nobrega and Grogan, 2007). Despite low CO_2 emission rates, previous work has confirmed that soil respiration during the long winter accounts for an important component of annual carbon (C) budget of Arctic tundra ecosystems (Morgner et al., 2010; Natali et al., 2019). Accordingly, significant plant litter decomposition and soil

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nitrogen (N) mineralization occur under such conditions and a large proportion of annual labile N in Arctic tundra soils may be produced during winter (Schimel et al., 2004; Bokhorst et al., 2010).

Both measurements and climate model projections consistently show that air temperature across the Arctic has increased during the past few decades and will continue to increase beyond the global average (Screen and Simmonds, 2010; Stocker et al., 2013; Pörtner et al., 2019). However, warming rates differ markedly between seasons (Cohen et al., 2014) and regions (Westergaard-Nielsen et al., 2018). The extent of winter warming is expected to be at least four times as great as summer warming by the end of this century (Bintanja and Van der Linden, 2013). In addition, increased winter temperature may trigger an increase of more than 50% in peak-regional precipitation as snowfall in some Arctic regions (Bintanja and Selten, 2014). Snow cover can thermally insulate the soil from the extremely cold winter air temperatures in the Arctic, hence dampening soil temperature fluctuations, and moderating the severity, depth and extent of winter soil freezing (Sharratt et al., 1992), and facilitating warming of underlying permafrost (Biskaborn et al., 2019). For instance, experimental snowfences that increased snow depth from 30 to 150 cm, led to an increase in soil surface temperature of 6 °C during late winter in the two common tundra types in high Arctic Svalbard (Morgner et al., 2010). This may enhance microbial decomposition of soil organic matter and N mineralization rates during winter (Schimel et al., 2004; Borner et al., 2008; Semenchuk et al., 2019). The consequent increase in N availability due to higher N mineralization may in turn alleviate N-limitation on plant growth in the subsequent growing season (Larsen et al., 2012; Semenchuk et al., 2015), promoting assimilation of CO_2 from the atmosphere (Hobbie and Chapin, 1996). Nevertheless, deepened snow cover will delay snowmelt in spring and lead to colder and wetter soils in early growing season, thus negatively affecting the growth and reproductive success of early-growing plants (Morgner et al., 2010; Cooper et al., 2011).

Along with soil temperature limitation, the presence of liquid water has been identified as both a prerequisite for biological activity and the primary control on soil C and nutrient cycles in winter (Öquist et al., 2009). As soil freezes, water availability is reduced to micro-films, inhibiting diffusion and mass transport of substrates, enzymes and microorganisms, and thus limiting microbial activities (Ostroumov and Siegert, 1996; Schimel and Mikan, 2005). Furthermore, the reduction in liquid water content may be accompanied by a decrease in air-filled pore space due to expansion of H_2O during freezing, resulting in reduced diffusion of oxygen and to microbial depletion of the remnant oxygen within those pore spaces, together and thus inhibition of aerobic respiration (Tucker, 2014).

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

The Pan-Arctic tundra is heterogeneous and encompasses a mosaic of distinct vegetation types that show diverse patterns of plant growth, C sequestration, N requirements and storage. Additionally, variation in plant species composition among tundra sites drives the variability of litter and soil organic matter quality (such as C:N ratio), resulting in variability in soil N cycling rates and thus ecosystem N retention (Christopher et al., 2008). Small- and large-scale topographic heterogeneity can influence hydrology, nutrient movement, microclimate, and snow distribution, and thus may alter plant productivity as well as microbial communities and activities related to C and N turnover processes (DeMarco et al., 2011). Several studies have reported progressively increased gross N mineralization and inorganic N availability along transects from upland to low-lying areas across three contrasting Arctic ecosystems (Welker et al., 2004; Paré and Bedard-Haughn, 2012; Christiansen et al., 2017). This pattern was generally attributed to the redistribution of soluble nutrients with water, wind and snow, as well as increasingly thick snow accumulation, lowered soil temperature variation and increased soil water status when moving down-hill.

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

In this study, we investigated the effects of deepened snow cover on soil nutrients pools and N cycling activities in five distinct tundra ecosystems of contrasting moisture regime across the Arctic. At each of the study sites snowfences had been established to manipulate snow-depths for at least 3 years (and up to 13 years). Soil samples were collected in late winter (to investigate the effects of several months of continuous snow cover), and late summer (to investigate the legacy effects after ca. three months of snow-free conditions), and shipped at temperature conditions resembling those in the field for subsequent laboratory work. Overall, our research objectives were to unravel the effects of deepened snow on soil gross N mineralization, nitrification, immobilization and denitrification, as well as potential denitrification and nitrous oxide (N_2O) reductase activity, and soil nutrient pools, and to identify environmental factors explaining responses of N-cycle processes to deepened snow. We hypothesized that: (H1) deepened snow enhances gross soil N transformation rates across tundra sites in winter and summer; (H2) increased availability of soil organic N drives the responses of gross N transformation rates to deepened snow; and (H3) the extent of the increase in gross N transformation rates mediated by deepened snow is linked to ecosystem moisture regime.

2. Material and methods

2.1. Site description

The study included five contrasting tundra sites with markedly different vegetation, climate, moisture regimes and soil types from three different geographical locations: Disko Island of West Greenland; Daring Lake region, Northwest Territories, Canada; and Svalbard, Norway (Table 1). The Disko Island sites were at Blæsedalen Valley (69°16′N, 53°27′W), West Greenland, in the transition zone between low and high Arctic. This valley has a mean annual air temperature of −3 °C (mean monthly temperature ranging from 8 °C in July to −14 °C in March) and an annual mean precipitation of 400 mm (40% as snow) (Hansen et al., 2006; Hollesen et al., 2015). The mean annual soil temperature at 5 cm depth is −1.9 °C and frozen soil conditions prevail from October to late May. The sites lie within the discontinuous permafrost zone. One site (denoted 'Disko Dry') is a dry shrub heath tundra with low (<10 cm) shrubs of deciduous dwarf birch (*Betula nana*) and grey willow (*Salix glauca*), evergreens including mountain cranberry (*Vaccinium vitis-idaea*), black crowberry (*Empetrum nigrum*) and Arctic bell-heather (*Cassiope tetragona*), with a mixture of mosses and lichens covering the

Table 1
Climatic conditions at study sites.

Site		Disko		Daring Lake	Svalbard	
		Dry	Wet		Heath	Meadow
Air temperature (°C)[a]		−14/8		−26.4/13.5	−10.4/7.6	
Precipitation (mm)[b]		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)[c]	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)[d]	Control	5.6	7.2	6.7	5.2	5.7
	Snowfence	5.5	6.4**	ND[e]	4.3**	4.7**
Soil moisture (Vol %)[f]	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)[g]	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[f]	Control	October to May		October to May	October to May	
Growing season period[f]	Snowfence	October to mid-June		October to mid-June	October to mid-June	
	Control	June to early September		June to early September	June to early September	
	Snowfence	Mid-June to early September		Mid-June to early September	Mid-June to early September	

Significant effects of deepened snow treatment (snowfence) in each site are shown $^{**}p \leq 0.01$.
[a] Mean monthly temperature in the coldest month/in the warmest month.
[b] Mean annual total precipitation/precipitation as snowfall.
[c, d] 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 Health and Meadow sites (n = 4–6 for each treatment; 2014–2018).
[e] No data due to lack of observations.
[f] 5 cm depth, mean daily volumetric 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.
[g] Maximum active layer depth, at Disko Dry and Wet (2017–2019), Daring Lake (2012–2013), and Svalbard Health and Meadow sites (2011).
[f] The snow-covered period generally covers mid-to late autumn, winter and spring, and the growing season period generally covers summer and early autumn.

ground. The other site ('Disko Wet') is a wet fen dominated by water sedge (*Carex aquatilis* ssp. *stans*), looseflower alpine sedge (*Carex rariflora*), common cottonsedge (*Eriophorum angustifolium*)), mosses (*Paludella squarrosa* and *Tomentypnum nitens*) and the deciduous shrub northern willow (*Salix arctophila*). The soil in Blæsedalen is formed on volcanic basalt. Disko Dry site has a shallow O-horizon (1–5 cm) atop the mineral A-horizon. The Disko Wet soil has a 20 cm deep peat layer, with the water table fluctuating from 20 cm below its surface to 15 cm above (Nielsen et al., 2017).

The low Arctic site is located near the Tundra Ecological Research Station at Daring Lake (64°52′N, 111°34′W), approximately 300 km northeast of Yellowknife in the Northwest Territories, Canada. The area is underlain by continuous permafrost to a depth of 160 m (Dredge et al., 1999) and has a shallow active layer forming to ca. 77 cm depth in the thaw season. The Daring Lake Weather Station (S. Kokelj, Water Management and Monitoring Division of the Department of Environment and Natural Resources, GNWT) records an annual average temperature of −8.4 °C, with average monthly temperature ranging from −26.4 °C in the coldest month (January) to 13.5 °C in the warmest month (July), and an annual precipitation ranging between 200 and 300 mm, of which 75–125 mm is received in summer (June–August). First snowfall usually happens in mid-October and snow depth is generally not more than 10 cm until the beginning of November, reaching an average peak of 37 cm (10-year range: 20–59 cm) in exposed areas by later winter. Our study focused on the birch hummock ecosystem-type which is located in a slightly sloping wide valley, and is characterized by 10–30 cm high hummocks and 10–40 cm tall deciduous birch (*Betula glandulosa*) shrubs that attain 10–30% of the areal coverage. The remaining cover is a mixture of mostly ericaceous shrubs including bog rosemary (*Andromeda polifolia*), *V. vitis-idaea*, bog bilberry (*Vaccinium uliginosum*), and labrador tea (*Rhododendron subarcticum* [formerly *Ledum decumbens*]), and sedges, mosses, lichens. The soil in this ecosystem is characterized as Orthic Dystric Turbic Cryosols and consists of an organic horizon 3–20 cm deep above cryoturbated silt-sand mineral horizons (Buckeridge et al., 2009a).

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

2.2. Experimental setup and design

At Disko: In July 2012, we established the snowfences oriented perpendicular to the prevailing winter wind direction at Disko Dry (n = 5), whereas the snowfences in Disko Wet (n = 5) were established in July 2013. Each snowfence is 14.7 m long and 1.5 m high. Control plots were located between 6 and 11 m from the windward side of the fence. The maximum snow depth (140–150 cm) occurred between 3 and 8 m from the fence on the leeward side, usually 2–3 times the depth at the control plots (Christiansen et al., 2017). Snow cover typically remains at the snowfence plots until mid-June, which is one-two weeks later than the control plots. Soil temperature at 2 cm depth (n = 3–4 for each

treatment) was logged hourly using thermistors connected to data-loggers (Gemini Data Loggers; Tinytag, Chichester, West Sussex, UK) since the start of the experiment. Volumetric soil moisture content at the 0–5 cm depth (n = 3–4 for each treatment) was recorded every half hour (HOBO, Onset Computer Corporation, MA, USA).

At Daring Lake: In summer 2004, snowfences (15 m long and 1.2 m high; n = 5) were established within the birch hummock tundra near Daring Lake. Control plots were established parallel with the fences in similar patches of vegetation at least 30 m apart from the nearest fence to ensure sufficient separation from enhanced snow cover areas. The snowfences, oriented perpendicular to the prevailing winter wind direction, created snowdrifts of around 20 m from both sides of each fence, with typical peak uniform snow depth of 90–100 cm within at least 3 m from each fence (Christiansen et al., 2018). By comparison, snow depth in the ambient control plots typically reaches a maximum depth of 30–40 cm. In addition, the snowfences usually delayed the complete snowmelt date by 7–10 days each spring (Buckeridge and Grogan, 2008). Soil temperature at 2 cm depth (n = 2 for each treatment) was measured hourly and logged as 4-hourly means using thermocouple probes connected to data loggers (CR10X, Campbell Scientific, Logan, UT, USA) since the start of experiment. Volumetric soil moisture content was recorded at the same frequency, using dielectric permittivity probes (CSC16, Campbell Scientific, Logan, UT, USA) that measured soil moisture across the 0–5 cm depth interval (n = 2 for each treatment). Due to the problems of the temperature probes, the data were available until July 2016. Considering soil N-cycle processes should be affected by the long-term snowfence treatment (not only the specific sampling year), we used soil temperature data of 2015–2016 as a representative to show the typical sonwfence effect at Daring Lake.

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

2.3. Soil sampling

Soil samples were collected on September 1st, 2016 and again in mid-April 2017 at the Disko sites, on August 20th 2016 and again on May 8th 2017 at the Daring Lake site, and on September 1st, 2017 and June 5th, 2018 at the Svalbard sites. In each of the snowfence and control plots at Disko (both dry and wet sites), Daring Lake, and Svalbard (both heath and meadow sites), we collected soil samples from the 0–5 cm depth interval after removing the litter layer. For sampling under non-frozen conditions, we used soil augers of 5–6 cm diameter and collected 3–5 samples in each plot that subsequently were mixed into one composite sample representing the plot. For sampling under frozen conditions in late winter, snow was removed and the soil excavated by chiseling to the desired depth. The snow depth on the day of sampling in April 2017 at Disko was ca. 150 cm with no differences between control and snowfence plots. We did not measure snow depth at Daring Lake on the sampling day, but the typical snow depth during the past years was indicated above. During winter sampling at the Svalbard sites snow depth at the snowfence plots was ca. 150 cm and 10–20 cm higher than that at the control plots. The mean sampling distance to the west of snowfences at the Svalbard sites was 847 ± 50 cm (mean ± 1 SE; defined in other publications as the 'Deep regime'). Active layer depth was measured in each site (D'Imperio et al., 2017; Christiansen et al., 2018). The samples were shipped immediately to the laboratory in Copenhagen at temperature conditions resembling those in the field (*i.e.*, either in frozen condition for the late winter samples, or at 5 °C for the late summer samples) and kept under the same conditions until the biogeochemical analyses which occurred within ca. two weeks. The frozen samples were thawed at 5 °C for three days prior to use. All samples were gently mixed, and woody and coarse roots and stones were removed by hand.

2.4. Incubation experiments

Soil gross N transformation rates were measured using a ^{15}N pool dilution technique. Soil samples (15 g moist soil) were transferred to 100-mL plastic cups and gently mixed with 1 mL of ^{15}N solution (30 mg N L^{-1} at 3.37 atom% excess ^{15}N; ca. 2.5 mg N kg^{-1} DW), and the cup covered with pierced Para-film. According to the IRMS analysis associated with the diffusion method, a total of 50 μg N is required for optimal analysis at ^{15}N. However, due to the low inherent mineral N pool in some soils, the amount of added N should be low to avoid fertilizer effects. Hence, the amount of added tracer is a compromise between potential tracer addition effects and optimal analysis. The solution was either ammonium chloride ($^{15}NH_4Cl$) for determination of gross N mineralization or potassium nitrate ($K^{15}NO_3$) for determination of gross nitrification. Incubation took place at constant room temperature, following a 24 h thermal equilibrium period. We prepared three analytical replicates for each combination of soil sample and ^{15}N solution. Soils were extracted immediately, 2 days and 7 days after labelling by suspension in 75 mL of 0.5 M K_2SO_4 solution (1 h on horizontal shaker) (Stark and Hart, 1996). All soil suspensions were filtered through ash-less quartz filter (Whatman GF/D, Maidstone, UK). The total amount of inorganic N in each soil extract (after the addition of tracer) exceeded 50 μg N (data not shown).

Potential denitrification activity (PDA) was implemented as a proxy of concentration of denitrifying enzymes in a soil sample (Page et al., 1982). Ten grams of moist soil was immersed in 30 mL of solution containing 1 mM KNO_3, 0.5 mM glucose, 0.5 mM sodium acetate and 0.5 mM sodium succinate in a 100-mL glass bottle. The bottle was flushed with N_2 for 30 s and sealed with a rubber stopper and aluminum crimp cap. After 15 mL of acetylene was added, the bottles were shaken on a horizontal shaker at a moderate shaking level at 5 °C and 3 mL of the headspace was collected at 60, 120, 180, 240 and 300 min for N_2O analysis. Headspace samples were transferred to 3-mL pre-evacuated Exetainers (Labco Scientific, High Wycombe, UK) for analysis of N_2O on a GC (HP7890A, Agilent, Wilmington, USA) equipped with an Electron Capture Detector (μECD).

Nitrous oxide reductase activity (N2OR) is an indicator for the concentration of soil N_2O reductase that reduces microbial and atmospheric N_2O into dinitrogen (N_2) (Wallenstein and Weintraub, 2008). Soil samples (10 g of moist soil) were transferred to 50-mL centrifuge tubes and suspended in 25 mL phosphate-buffered saline (PBS) by stirring with a spatula and vigorously shaking the tube. Subsequently the tubes were centrifuged for 10 min at 12000×*g* at 5 °C and the supernatant decanted. This step was repeated three times to deplete the soil of NO_3^- that would otherwise lead to formation of N_2O during the anaerobic incubation and hence interfere with the measurement of N2OR activity. Finally, 25-mL of a solution containing 0.5 mM glucose, 0.5 mM sodium acetate and 0.5 mM sodium succinate was added and the soil suspension transferred to 100-mL glass bottles. The bottles were flushed with N_2 to make them anaerobic and sealed as explained above except that we added 15 mL of 200 ppm N_2O in N_2 to a final concentration of ca. 25 ppm N_2O, and incubated them on a horizontal shaker (200 rpm) at 5 °C. Three-mL headspace samples were collected at 10, 70, 130, 190 and 250 min for N_2O analysis. During incubation, the concentration of N_2O

decreases over time, which affects the rates of N2OR activity. For calculation of N2OR activity rates in individual incubation bottles, the N_2O concentration at the different time points was fitted to a model assuming first order enzyme kinetics and rates were calculated at a standardized N_2O concentration of 20 ppm.

For estimation of substrate induced respiration (SIR), 5 g of moist soil from each sample was immersed in 10 mL of 20 mM glucose in a 100-mL incubation bottle. Each bottle was sealed with a butyl rubber stopper and aluminium crimp and then pressurized with 14 mL of air to avoid development of sub-atmospheric pressure associated with subsequent headspace sampling. The bottle was incubated on a shaker (200 rpm) at 5 °C and 3 mL of the headspace was collected at 10 min, 60 min, 110 min and 160 min for CO_2 analysis by GC (HP7890A, Agilent, Wilmington, USA) equipped with a methanizer and a Flame Ionization Detector (FID).

Respiration was measured as described for SIR above, but without glucose addition. Three mL of the headspace was collected at 10 min, ca. 6 h, ca. 22 h and ca. 28 h for CO_2 analysis. Anaerobic respiration was estimated as CO_2 production under the conditions described for PDA above.

2.5. Laboratory analyses

Soil moisture was determined upon oven drying (105 °C for 24 h). Soil pH was measured using a moist soil to water ratio of 1:2.5. Ammonium and NO_3^- concentrations in the filtered extracts described above were determined using flow-injection analysis (Tecator 5000 FIAStar, Höganäs, Sweden). To determine ^{15}N enrichments of extractable NH_4^+-N, soil extracts were amended with magnesium oxide (MgO) to raise the pH above 13 in order to convert NH_4^+-N into ammonia (NH_3–N) that is subsequently caught on acidified filter papers (Sørensen and Jensen, 1991). For analysis of $^{15}NO_3^-$-N, NH_4^+-N was removed from the extracts as described above, and then Devarda alloy was added to convert NO_3^--N into NH_4^+-N, followed by further conversion into NH_3 at pH > 13 as described above for NH_4^+-N (Sørensen and Jensen, 1991). Due to low molarity and to avoid swelling of the acid traps, potassium chloride (KCl) was added to the extract prior to diffusion to bring the ionic strength of the solution (1.5 M) closer to that of acid trap (2 M) (Stark and Hart, 1996). Soil total C and N contents and the ^{15}N contents in soil and acid traps was determined by elemental analysis (CE1110, Thermo Electron, Milan, Italy) coupled in continuous flow mode to a Finnigan MAT Delta PLUS isotope ratio mass spectrometer (IRMS; Thermo Scientific, Bremen, Germany). Prior to analysis, 20–30 mg subsamples of finely ground soil material was weighed into tin combustion cups and freeze-dried acid filters likewise wrapped in tin cups. Soil dissolved organic C (DOC) and total dissolved N (TDN) in K_2SO_4 extracts were measured using a TOC-TN analyzer (Shimadzu, Kyoto, Japan). Dissolved organic N (DON) was calculated as the difference between TDN and (NO_3^--N + NH_4^+-N).

2.6. Calculations and statistics

Gross N transformation rates were calculated by the ^{15}N-isotope pool dilution method using the tracing model FLUAZ81, which combines a numerical model for solving the mass balance equations and a non-linear fitting program for optimizing the N rate parameters (Mary et al., 1998). Specifically, gross mineralization, gross nitrification, immobilization of NH_4^+ and NO_3^-, and denitrification were estimated by this model. The input data were amount of NH_4^+-N and NO_3^--N, and ^{15}N excess of NH_4^+-N, NO_3^--N and organic-N (five variables), and the model was run for each plot (10–12 plots per site).

The data distributions were tested for normality using Shapiro-Wilk tests. Due to generally non-normal distribution or heterogeneous variances, they were analyzed by Kruskal-Wallis H test (non-parametric). Differences in soil characteristics, N cycling rates, PDA, N2OR, SIR and respiration between treatments in each season within site or across sites, seasonal differences in each site within treatment or across treatments, and site differences in each season across treatments were tested, and a p value lower than 0.05 was considered to be significant. Relationships between soil characteristics (soil moisture content, pH, C, N, DOC, DON, NH_4^+-N and NO_3^--N) and N cycling rates as well as PDA and N2OR were identified using multiple stepwise linear regression analysis based on the coefficient of determination (R^2) and Akaike Information Criterion (AIC) (Ziegel, 2003). The Variance Inflation Factor (VIF) values (should be close to 1) were calculated to avoid collinearity, and normality and homoscedasticity of residuals were tested by diagnostic plots in each regression model. Principal component analysis (PCA) was applied to evaluate the extents of separations among the sites, and to quantify the comprehensive relationship between soil characteristics and N cycling rates as well as PDA and N2OR within each treatment or season by using the prcomp package (Mankin, 2008). The coefficient of variation (CV) is a relative measure of variability that is defined as the ratio of the standard deviation to the mean. It helps to compare groups that have means of very different magnitudes and characteristics that use different units of measurements. We calculated the CV of N-cycle and C-cycle rates within each site to compare snowfence-induced changes (variability) in N-cycle and C-cycle rates. All statistical analyses were conducted using R 3.6.1 (Team, 2019).

3. Results

3.1. Soil characteristics

Basic soil physical and chemical properties are shown in Table 2. In general, the measured parameters were not impacted by the snowfence treatment and season, with soil NH_4^+-N and NO_3^--N as notable exceptions to this. Considering different measuring ranges of volumetric moisture probes, we compared soil moisture among sites based on gravimetric water content (GWC). Soil moisture content (GWC) ranged widely among the sites (40.2–81.5%) with peak values at Disko Wet and Daring Lake. The pH varied between 3.7 and 7.0 with the most acidic conditions at Daring Lake, intermediate pH at Disko Dry, and slightly acidic to neutral conditions at Disko Wet and the Svalbard sites. The Daring Lake soil was purely organic with C contents of around 45%, and the Disko Wet soil also had a high C% (30%), while Svalbard Meadow had the lowest C% of 7%. At Daring Lake, the soil C:N ratio was the highest (>28) among all sites and the ratio in deepened snow plots significantly exceeded the control in summer ($p = 0.04$), but not in winter.

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

Across all sites and treatments, NH_4^+ was generally the predominant form of inorganic N, except for Disko Wet in summer, where NO_3^--N exceeded NH_4^+-N 5-fold (Table 2). The deepened snow treatment across sites significantly increased NH_4^+-N concentrations in summer ($p = 0.0088$), but not in winter. For specific sites, NH_4^+-N concentrations were significantly or tended to be higher in the deepened snow treatment at Svalbard Heath and Meadow in summer ($p = 0.032$ and $p = 0.063$, respectively) and winter ($p = 0.093$ and $p = 0.004$, respectively). Moreover, significantly higher NH_4^+-N concentrations were observed in Disko Wet and Svalbard Meadow in winter than in summer ($p < 0.01$ and $p = 0.013$, respectively).The deepened snow treatment significantly enhanced NO_3^--N concentrations across sites in summer ($p = 0.014$), and for specific sites, NO_3^--N concentrations were significantly increased at Daring Lake ($p = 0.016$), Svalbard Heath ($p = 0.016$) and Meadow ($p = 0.045$) in summer, whereas no effects were observed in winter. In contrast, NO_3^--N concentrations were significantly reduced in winter

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	**28.6 ± 0.6**	17.8 ± 0.6	15.9 ± 0.5	
	Summer, Snowfence	24.4 ± 1.4	15.9 ± 0.7	**32.6 ± 2.5***	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^{-1})	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^{-1})	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	**41.6 ± 3.6**	**14.6 ± 1.5**	9.3 ± 1.0	7.8 ± 2.0	
	Winter, Snowfence	9.0 ± 2.7	**74.6 ± 12.5§**	**24.6 ± 5.0§**	15.4 ± 6.3	7.8 ± 1.3	
	Season	**					
NH_4^+-N (mg kg^{-1})	Summer, Control	1.2 ± 0.7	2.8 ± 0.8	0.4 ± 0.0	**0.8 ± 0.1**	**0.7 ± 0.2**	
	Summer, Snowfence	1.6 ± 0.5	5.3 ± 1.1	0.5 ± 0.1	**2.3 ± 0.4***	**3.0 ± 1.1§**	**
	Winter, Control	0.7 ± 0.1	57.0 ± 7.1	0.4 ± 0.1	**2.2 ± 0.5**	**2.2 ± 0.5**	
	Winter, Snowfence	0.5 ± 0.0	86.9 ± 17.9	0.5 ± 0.1	**16.4 ± 11.0§**	**9.4 ± 2.1***	
	Season		**			*	
NO_3^--N (mg kg^{-1})	Summer, Control	0.06 ± 0.06	24.35 ± 9.73	**0**	**0.10 ± 0.06**	**0.03 ± 0.00**	
	Summer, Snowfence	0.05 ± 0.04	19.46 ± 7.99	**0.24 ± 0.11***	**9.11 ± 2.15***	**0.96 ± 0.57***	*
	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 § ≤ 0.1; *$p \leq 0.05$ and **$p \leq 0.01$. GWC: Gravimetric water content, ND: no data.

compared to summer across all sites under increased snow conditions ($p = 0.015$).

3.2. Gross N cycling rates

Gross N-cycle results indicated significant site-to-site variation with highest activity generally observed at the Disko Wet site (Fig. 1). Across all sites, increased snow depth enhanced gross N mineralization rates in summer ($p = 0.044$), but not in winter (Fig. 1a). The stimulation of gross N mineralization rates by deepened snow was particular apparent at Svalbard Heath in summer ($p = 0.008$) and Svalbard Meadow in winter ($p = 0.041$; Fig. 1a). Similar tendencies were observed also for Disko Dry and Svalbard Meadow during summer ($p = 0.064$ and $p = 0.065$, respectively; Fig. 1a). The gross N mineralization rates in Disko Wet snow fence plots were significantly increased in winter relative to summer ($p = 0.016$) and similar tendencies were also observed for the control plots ($p = 0.095$; Fig. 1a). In contrast, N mineralization rates in Disko Dry snowfence plots were significantly lower in winter compared to controls ($p = 0.016$; Fig. 1a).

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

In accordance with the high mineralization rates, Disko Wet also showed significantly higher NH_4^+-N immobilization rates than the other sites in both seasons ($p < 0.01$; Fig. 1c). Moreover, the deepened snow treatment tended to increase NH_4^+-N immobilization rates in winter ($p = 0.056$), resulting in significantly higher immobilization under deepened snow in winter relative to the summer ($p = 0.032$; Fig. 1c).

The NO_3^--N immobilization rates varied substantially across sites with peak rates observed in the Daring Lake samples (Fig. 1d), which is in contrast to the low NH_4^+-cycle activity observed at that site (Fig. 1a–b). The deepened snow treatment significantly enhanced (3-fold) overall NO_3^--N immobilization rates across sites in winter ($p = 0.007$), which was largely driven by the significant increases at Disko Wet and Daring Lake ($p = 0.025$ and $p = 0.031$, respectively; Fig. 1d). At Daring Lake, this increase was perhaps primarily a result of significantly

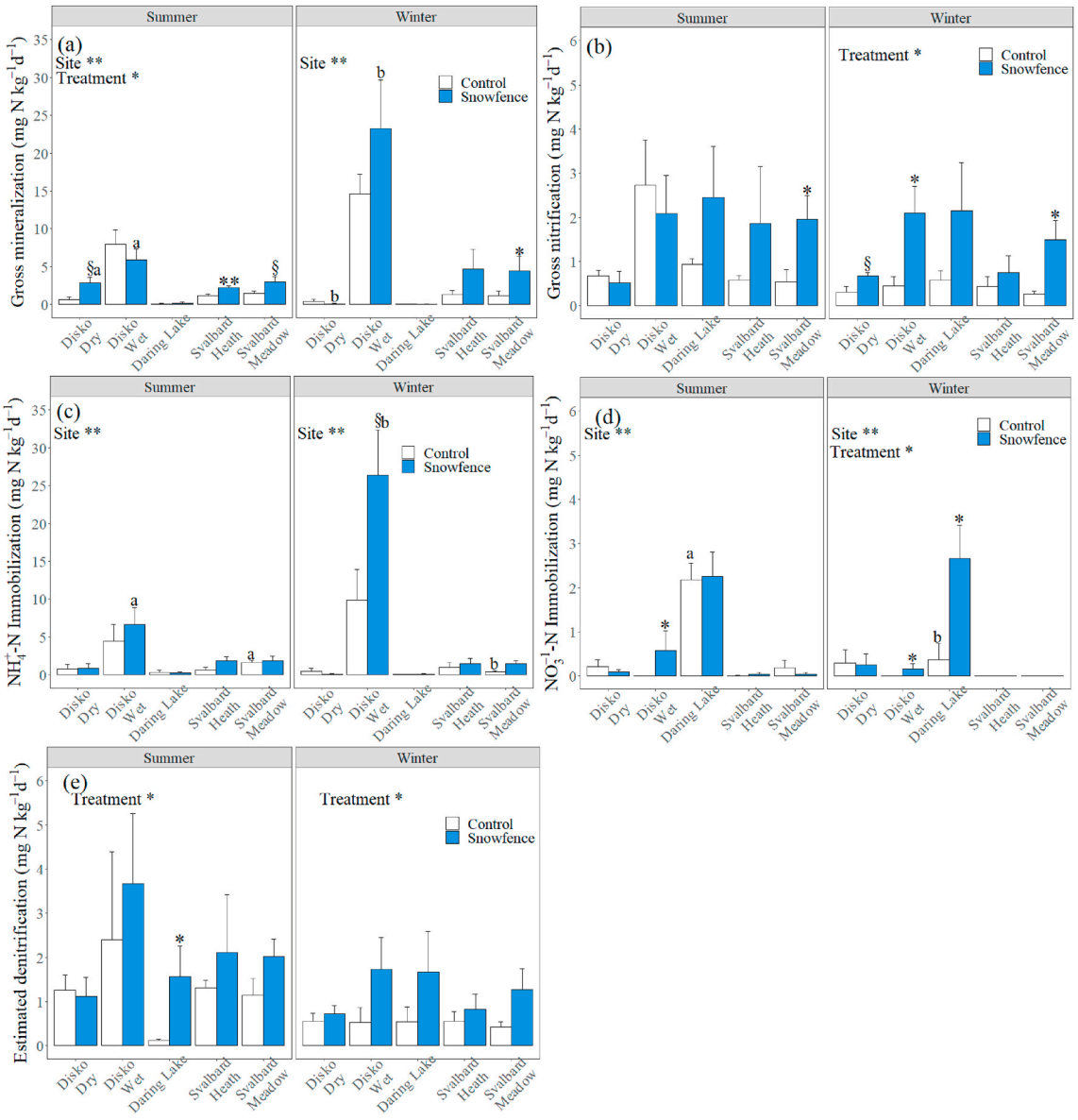

Fig. 1. Effects of the deepened snow treatment (snowfence) on gross rates of N mineralization (a), nitrification (b), NH_4^+-N immobilization (c), NO_3^--N immobilization (d) and estimated denitrification (e) in summer and winter (mg N kg_{dw}^{-1} d^{-1}). Note that different rates cover different scales on the y-axis. Significant differences between the treatments at each site and across sites, and significant effects of site in each season are shown as § ≤ 0.1; *$p \leq 0.05$ and **$p \leq 0.01$. Lowercase letters indicate significant differences between seasons within each treatment in each site.

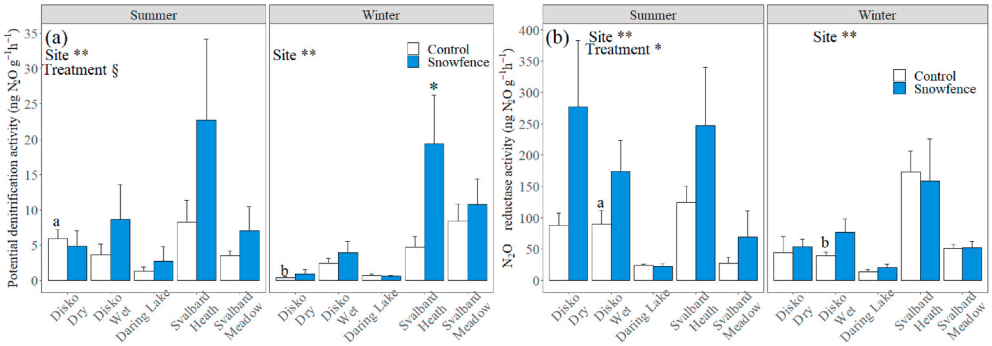

Fig. 2. Effects of the deepened snow treatment (snowfence) on potential denitrification activity (a) and N_2O reductase activity (b), in the summer and winter (ng N_2O g_{dw}^{-1} h^{-1}). Significant differences between the treatments at each site, across sites, and significant effect of site in each season are shown as § ≤ 0.1; *$p \leq 0.05$ and **$p \leq 0.01$. Lowercase letters indicate significant differences between seasons within each treatment in each site.

decreased NO_3^--N immobilization rates in the control plots in winter compared to summer ($p = 0.031$; Fig. 1d). A significant increase in NO_3^--N immobilization rates under deepened snow was also observed in summer ($p = 0.045$), where the control plots barely showed any activity (Fig. 1d).

The estimated total gaseous losses by denitrification did not vary among the sites (Fig. 1e). However, the deepened snow treatment significantly increased denitrification (2-fold) across sites in both seasons ($p = 0.038$ and $p = 0.02$ for summer and winter, respectively), although this snow effect was only significant for Daring Lake in summer ($p = 0.016$; Fig. 1e). Denitrification under deepened snow conditions also tended to be higher in summer than in winter across all sites ($p = 0.076$; Fig. 1e).

3.3. Potential denitrification and nitrous oxide reductase activity

Potential denitrification activity (PDA) varied among sites with rates up to 25-fold higher at Svalbard Heath compared to Daring Lake (Fig. 2a). The deepened snow treatment tended to increase summertime and wintertime PDA across sites ($p = 0.098$ and $p = 0.12$, respectively). However, for the specific sites, this deepened snow effect was only significant at Svalbard Heath, where activities in the snowfence plots exceeded controls in winter ($p = 0.032$) and a large enhancement by deepened snow (3-fold; $p = 0.283$) was observed in summer (Fig. 2a).

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

3.4. Correlations between soil characteristics and N cycling rates

Separate principal component analyses (PCA) were used to determine the clustering in the control and snowfence plot data of all response variables (soil characteristics, N cycling rates and denitrification enzyme activities) across seasons (Fig. 3a and b). Under control snow conditions, the two wetter sites Daring Lake and Disko Wet clearly separated in two distinct groups, whereas the three drier sites appeared to cluster. The Daring Lake site was associated with high C:N ratio and NO_3^--N immobilization, and Disko Wet with high DON and NH_4^+-N concentrations, mineralization and NH_4^+-N immobilization. Among the drier sites, Svalbard Heath correlated with high denitrification enzyme activities (PDA and N2OR). Under deepened snow conditions, a similar distribution could be observed except that the two Svalbard sites were congruent. Disko Wet site exhibited wider variation along PC1-axis explained by soil moisture, DOC, DON and total N contents, which indicates that the degree of explanation changes with snow depth in particular for the Disko Wet site.

A stepwise regression analysis was performed with the above-mentioned variables (Table 3). Gross mineralization was explained mainly by DON (83.4%) in control plots, and by DON (42.8%) and DOC (31.3%) in snowfence plots. Gross nitrification was dominantly explained by DON (84.0%) in the control plots, whereas under deepened snow soil moisture was the main driver. Ammonia immobilization was mainly driven by gross mineralization (50.4%) and NH_4^+-N (32.7%), and to a low extent by DOC (6.9%) in the control plots, but to a higher extent (17.9%) in the snowfence plots. Nitrate immobilization was equally controlled by the soil bulk C (52.2%) and soil pH (47.8%) in the snowfence plots. The explanation of PDA was generally poor in the control plots across sites (23.4% of variance), and was mainly related to water content (79.8%) and DON (20.2%). There was a significant relationship between denitrification and PDA across sites and treatments in summer ($p = 0.006$, $R^2 = 0.172$; Fig. S1). The N2OR could also only be explained in the control plots, and mainly by soil pH (90.6%).

4. Discussion

4.1. Effects of deepened snow on soil nutrients and N cycling

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

Despite contrasting climate conditions, vegetation types and soil characteristics responses to snowfence treatments were similar on different aspects across the sites. Both duration of snow-cover and soil temperature during the snow-covered period were increased by the deepened snow treatment (Table 1), as were also soil nutrient concentrations and N-cycle processes. We observed significantly higher gross nitrification, NO_3^--N immobilization and denitrification rates in the deepened snow compared to control plots across all sites during winter. This is probably due to higher abundance and diversity of N-cycle microbial communities (Jusselme et al., 2016), as a result of significantly warmer winter soil temperatures under the deepened snow (Table 1, Figs. S2a–e). For instance, Xue et al. (2016) found that a snowfence treatment increased the abundance of key genes involved in N mineralization (*ureC*), nitrification (*amoA*), and denitrification (*narG*, *nirS/nirK* and *nosZ*) in the active layer of Alaskan tundra soils. In our study, no significant correlations were observed between soil temperature and N-cycling rates across sites and seasons (data not shown), probably due

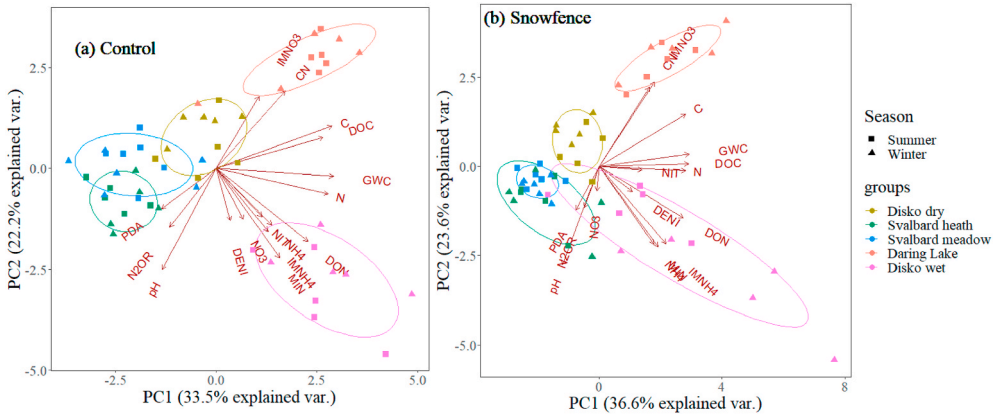

Fig. 3. Principal component analyses (PCA) for the extent of separations among the sites in control (a) and snowfence (b) plots. GWC: gravimetric water content, MIN: gross mineralization, NIT: gross nitrification, IMNH4: NH_4^+-N immobilization, IMNO3: NO_3^--N immobilization, DENI: denitrification, PDA: potential denitrification activity, and N2OR: N_2O 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	83.4	<0.001 ***	57.48
		pH	16.6	0.004 **	
	NIT	DON	84.0	<0.001 ***	50.82
		NH_4^+-N	16.0	0.0003 **	
	IMNH4	MIN	50.4	<0.001 ***	75.69
		NH_4^+-N	32.7	0.038 *	
		C	9.9	<0.001 ***	
		DOC	6.9	<0.001 ***	
	PDA	GWC	79.8	<0.001 ***	23.41
		DON	20.2	0.018 *	
	N2OR	pH	90.6	<0.001 ***	37.82
		NH_4^+-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	MIN	44.4	<0.001 ***	89.39
		NH_4^+-N	29.6	0.004 **	
		DOC	17.9	<0.009 **	
		pH	8.1	0.025 *	
	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_4^+-N immobilization, IMNO3: NO_3^--N immobilization, DENI: denitrification, PDA: potential denitrification activity, and N2OR: N_2O reductase activity. Models that explained proportion of variance less than 20% were excluded.

to indirect effects of soil temperature via changing soil characteristics, or to long-term ecosystem acclimation such as microbial community change. Meanwhile, soil handling and substrate addition may be exaggerating N immobilization and denitrification as calculated with the isotope dilution method, which could influence the correlations between soil temperature and N-cycling rates, but these methodological issues do not detract from the large differences found between treatments.

The generally higher wintertime gross N mineralization rates under deepened snow led to an increase in soil NH_4^+-N concentrations in most of the sites. In contrast, soil NO_3^--N concentrations were unaffected by the deepened snow, even though higher gross nitrification was observed across sites (Fig. 1b). This is likely because potentially increased NO_3^--N due to higher nitrification was rapidly depleted by the higher NO_3^--N immobilization (Daring Lake and Disko Wet) and denitrification rates under deepened snow conditions (Fig. 2d and e). These observations are parallel to negligible net nitrification rates observed by Chu and Grogan (2009) at Daring Lake. In addition, the increased NO_3^--immobilization rates under deepened snow conditions in winter at that site is in agreement with a previous study there reporting that deepened snow increased N accumulation in microbial biomass during late winter (Buckeridge and Grogan, 2008). At Disko, Christiansen et al. (2017) conducted *in situ* litterbag incubations and observed enhanced fungal abundance in both the Wet and Dry sites, and more litter mass loss in the Dry site in response to deepened snow, which could help explain the increased gross N mineralization (summer, Dry site) and NO_3^--immobilization rates (both seasons, Wet site) we observed there.

We found enhanced summer gross N mineralization rates due to the deepened snow treatment across all sites (Fig. 1a), which was associated with increased concentrations of soil NH_4^+ and NO_3^- (Table 2). Hence, the pattern of higher inorganic N from winter processes under deepened snow was repeated in late summer, consistent with findings in a previous study at the same Svalbard sites indicating that labile N from the winter period exceeds the early summer demands of both microbes and plants (Semenchuk et al., 2015). Large fluctuations in microbial community composition and abundances occurred between winter and summer in the Arctic tundra (e.g. Schostag et al., 2015), and thus deepened snow is expected to mainly affect diversity or abundance of soil microorganisms that dominate during winter. Indeed, Moriana-Armendariz et al. (in press) and Mundra et al. (2016) demonstrated effects of deepened snow on plant pathogenic and soil fungi in our Svalbard sites. Moreover, the potential legacy effect of deepened snow on the subsequent growing season has been shown to depend on the severity of local winter microclimate (McMahon et al., 2011; Buckeridge et al., 2013). In this study, PDA and N2OR were both increased across sites by the deepened snow treatment in summer (Fig. 2a and b), indicating that denitrifying enzyme activities were enhanced by deepened snow. The changes in PDA and N2OR may result from altered substrate availability, denitrifier abundance and/or denitrifier community composition (Attard et al., 2011), and denitrification was significantly correlated with PDA across sites ($p = 0.006$, $R^2 = 0.172$; Fig. S1), which altogether may explain the snowfence-enhanced summertime denitrification rates across sites (Fig, 1e).

Nevertheless, contrasting seasonal patterns of NH_4^+-N and NO_3^--N were observed in most sites. This is likely because nitrification is constrained under low temperature (<5 °C) leading to an accumulation of NH_4^+-N via N mineralization during winter (Smith et al., 2010), whereas in the warmer growing season, NH_4^+-N is taken up by microbes and plants as well as transformed into NO_3^--N via nitrification resulting in a strong decreasing trend for NH_4^+-N concentrations across sites in summer compared to winter ($p = 0.06$; Table 2). Additionally, differences in vegetation-type may influence soil inorganic N during the growing season. For example, the biomass of plants with high inorganic N sink strength, such as graminoids that have a strong ability to exploit additional inorganic N, is higher in the Svalbard Meadow than Heath (Mörsdorf et al., 2019). This may partly explain the much lower NO_3^--N concentrations during summer in the Meadow compared to the Heath site, especially in the deepened snow plots. However, this does not appear to be the case at Disko Wet, where soil NO_3^--N concentrations were higher in late summer despite a presumed higher inorganic N sink strength of the vegetation there compared to Disko Dry. Nitrate is easily leached into deeper soil profiles and the differential extent of NO_3^- leaching could help to explain contrasting soil NO_3^--N concentrations between these two sites. This is further supported by observations from Rasmussen et al. (2020), who found that overall higher NO_3^--N concentrations of soil water at the depth of 10 and 20 cm throughout the growing season in the dry site as compared to the wet site. All these observations above, however, have to be interpreted with caution, since concentrations of soil NH_4^+-N and NO_3^--N as well as water content may vary during the growing season, as reported by Buckeridge et al. (2013), Semenchuk et al. (2015) and Mörsdorf et al. (2019).

According to coefficients of variation for each C-cycle and N-cycle process across treatments and seasons in each site, C-cycle processes were much less affected by the snowfence treatment and season compared to N-cycle processes (Table S1). The substrate induced

respiration (SIR) method estimates the potentially active microbial biomass (Anderson and Domsch, 1978). The SIR as well as basic aerobic respiration and anaerobic respiration was unaffected by the snowfence treatment or season (Fig. S3) indicating that deepened snow did not affect the potentially active microbial biomass and microbial CO_2 production across our five sites. However, previous studies have reported either increased or reduced *in situ* soil respiration by deepened snow treatment at these sites in either summer or winter (Morgner et al., 2010; Björkman et al., 2016; Christiansen et al., 2018). This inconsistency between laboratory incubations and field flux measurement may result from alteration of autotrophic respiration due to the absence of plants in our incubations, and other differences from *in situ* environmental factors (e.g. soil temperature, soil moisture and/or thaw depth) that we know are significantly influenced by the snowfence treatment.

4.2. Soil characteristics correlate to responses of N cycling to deepened snow over a wider geographical region

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. They also suggest an important role of C availability for the response of several N cycling rates to deepened snow. Many previous studies in the Arctic tundra ecosystems have demonstrated the importance of soil characteristics such as soil organic matter (Weintraub and Schimel, 2003; DeMarco et al., 2011; Schnecker et al., 2014), C:N ratio (Chu and Grogan, 2009; Maslov and Makarov, 2016) and soil moisture (Chapin, 1996; DeMarco et al., 2011) for N-cycle processes. Despite significant site differences in many soil characteristics and N-cycle processes, we did observe strong broad scale correlations between soil characteristics and N-cycle processes. In this study, the availability of DOC was a predictor for N mineralization at the snowfence but not control plots across sites, with a relatively high importance (31.3%). This suggests that the snowfence-induced increase in gross N mineralization is linked to elevated availability of organic C substrates that complement microbial C demand (Schmidt et al., 2011). The elevated organic substrate availability for N mineralization under deepened snow conditions could originate from either increased breakdown of soil organic macromolecules or input of labile C and N through damaged and killed roots or microbial cells turnover (Brooks et al., 1998; Larsen et al., 2002; Schimel et al., 2004). Generally, it is well known that organic C substrates play an important role in regulating N mineralization (Booth et al., 2005). 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 (Buckeridge et al., 2009b). Hence, it seems that the chemical quality is more important than the quantity of soil C as the principal driver of N mineralization. Furthermore, the variability of NH_4^+-N immobilization was explained to a greater extent by DOC availability in the snowfence plots (17.9%) than in the control plots (6.9%), which indicates that C availability induced by the deepened snow may favor not only N mineralization but also NH_4^+-N immobilization. This is in agreement with a previous study implying that microbial N immobilization is facilitated by active heterotrophic microbes stimulated by high C availability (Montaño et al., 2007).

According to the regression analysis, gross nitrification was explained by DON and NH_4^+-N in the control plots, whereas soil moisture was the main driver in the snowfence plots. This indicates that nitrification is limited by other soil environmental factors rather than labile N substrates due to increased N mineralization by deepened snow. It is noticeable that gross N mineralization at the Daring Lake was negligible in both treatments, but it displayed high gross N nitrification, especially in the snowfence plots (gross nitrification exceeded mineralization), indicating that heterotrophic nitrification at this site was predominantly by direct NO_2^-/NO_3^--N formation from oxidation of organic N. Previous studies have shown that heterotrophic nitrification dominates over autotrophic nitrification in a range of terrestrial ecosystems (Huygens et al., 2008; Rütting et al., 2008; Müller et al., 2009; Wang et al., 2016). We found rather low soil pH (~3.9), high C:N ratio and high total soil C content at Daring Lake, which could explain why relatively high heterotrophic nitrification occurred at this site compared to the other sites. This conclusion is consistent with some earlier studies indicating that heterotrophic nitrification may be an important, and possibly dominant pathway for NO_3^--N production in environments where pH is low and organic C content high (Perakis et al., 2005; Islam et al., 2007; Zhang et al., 2011). In general, a wide range of microorganisms possesses the potential for heterotrophic nitrification, especially fungi, which may be less prone to low soil pH (even at pH 3) (De Boer and Kowalchuk, 2001; Zhu et al., 2012). A recent review related to nitrification and nitrifiers in acidic soils concluded that soils with lower pH from the same sites tended to have a higher proportion of heterotrophic nitrification but other factors such as substrates availability, moisture content and soil temperature have equal or even greater effect (Li et al., 2018). For instance, Banerjee and Siciliano found that heterotrophic nitrification comprised a considerable proportion of the overall nitrification potential (47%) in a Canadian tundra soil with high water content and organic matter. We suggest the increased nitrification under deepened snow conditions in winter observed in Daring Lake, Svalbard Heath and Disko Wet was likely due to increased availability of labile organic substrates as supported by the tendency for increased DON concentration in snowfence plots at these sites.

The growth and activity of denitrifiers are regulated by the availability of substrates (i.e. NO_3^--N as an electron acceptor and simple organic C as an electron donor). Generally, soil total C:N ratio is an indicator of substrate quality and a measure of recalcitrant organic matter in relation to organically-bound N (Dodla et al., 2008). In this study, we observed high aerobic and anaerobic respiration at Disko Dry and Daring Lake (Figs. S3b and S3c), suggesting high organic C quality and availability in these two sites even though they had relatively high C:N ratios. Kandeler et al. (2006) observed that soil organic C was the most important factor explaining denitrifier abundance. Similarly, Attard et al. (2011) reported that soil organic C influenced PDA partially through a build-up of denitrifier abundance, and therefore a higher C availability could enhance the abundance of denitrifiers. However, this does not appear to be the case at these two sites, where PDA in winter was lower though soil organic C was higher than the other sites, probably due to limited winter NO_3^--N availability (Table 1).

Although the regression analysis clearly pointed out some universal drivers explaining the variability across sites, the enhancement of N cycle was additionally affected by the strength of soil temperature and moisture effect under deepened snow. 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 no 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, probably in part due to higher soil moisture under deepened snow conditions in summer (Table 1, Fig. S4d) and thus increased mobility of solutes, enzymes and microorganisms (Manzoni et al., 2012). Similarly, the highest enhancement of summertime PDA activity also occurred in this site.

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

Daring Lake and Disko Wet were characterized by relatively high soil moisture and organic matter contents, and deepened snow led to more pronounced increases in wintertime DOC and DON concentrations than at the three other sites. These results suggest that soil moisture content is

an important control of labile organic C and N formation under elevated winter temperatures, and thus microbial decomposition processes (Manzoni et al., 2012). The phase transition of water to the solid state leads to dramatic reduction in liquid H_2O during winter (Brooks et al., 2011), leading to low soil volumetric moisture content observed during winter (Figs. S4a–c). The reduction in soil moisture in winter was stronger in Disko Dry compared to Disko Wet. Thus, deepened snow as a result of future climate change may affect soil N cycling more in mesic and wet tundra than in dry tundra. Consequently, freezing-induced drought stress and limitations in substrate diffusion and mass transport may be more important in dry soils, such as Disko Dry (ca. 5% moisture content in winter) and lead to generally low N cycling rates during winter. Furthermore, the high energy demand associated with the phase shift between water and ice generally leads to lower temperatures in dry soils compared to wet soils in winter (Christiansen et al., 2017), as supported by lower winter temperatures at the Disko Dry site compared to Disko Wet (Fig. 2a and b). Accordingly, N cycling rates were generally enhanced by deepened snow cover to a greater extent in these two wetter sites (Disko Wet and Daring Lake) than in the three other sites, associated with high labile organic C and N concentrations, 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. The regression analysis revealed soil moisture content as one of the primary predictors for PDA across sites. This is consistent with Jusselme et al. (2016) showing that variation in N-related microbial abundances and enzyme activities along a snow depth gradient in subalpine grassland was driven primarily by soil moisture, indicating that access to substrates for the microbes is mainly controlled by water availability in partly frozen soils. According to the PCA, the two wetter sites clearly separated in distinct groups, whereas the three drier sites appeared to cluster (Fig. 3), indicating that soil moisture is an important controlling variable for other soil characteristics and N-cycle processes, as also supported by the strong positive correlation between water content and principal component 1 (of highest explained variance). This is in accordance with observations by Rasmussen et al. (2020), who studied the effects of shrub removal, warming, and snow addition on soil water chemistry in the Disko Dry and found strong influence of summer soil moisture as an independent physical variable in determining treatment effects across years. In winter across treatments, the wettest site (Disko Wet) showed wider variation along PC1-axis as a result of snow effect compared to the other sites, further suggesting N-cycle microbial activities in soils with high water contents can respond more strongly to increased snow depth (Fig. S5b). Our results therefore support previous studies in Alaska and the same Svalbard sites proving that deepened-snow-induced increases in soil N availability and foliar N content is more pronounced in moist tundra than in dry tundra (Schimel et al., 2004; Welker et al., 2005; Semenchuk et al., 2015). It is still not clear why the lowest gross N mineralization occurred at the Daring Lake, even though it had both high moisture content and respiration rates (Fig. S3). A previous study from Daring Lake concluded that low N mineralization and high CO_2 emissions, corresponding to our study, likely resulted from the combined effect of low soil pH and high C:N ratio (Paré and Bedard-Haughn, 2012).

5. Conclusion

To the best of our knowledge, this is the first study to compare both winter and summer responses of N-cycle processes to projected changes in winter climate among several Pan-Arctic tundra ecosystems. We conclude that deepened snow enhanced wintertime gross N nitrification (4-fold), denitrification (2-fold) and NO_3^--N immobilization (3-fold) rates across the five investigated sites. Gross N mineralization rates were also enhanced by deepened snow in summer, associated with increased NH_4^+-N availability. There was an increase of denitrification rates (2-fold) across sites by deepened snow in summer, likely due to increased denitrifying enzyme activities (PDA and N2OR). Furthermore, DOC and DON were the main drivers for several enhanced gross N cycling rates in response to deepened snow across all sites, suggesting that microbial N-cycle activities promoted by deepened snow are primarily due to the increased availability of dissolved organic matter. Interestingly, the differential effects of deepened snow on winter N cycling rates among the Pan-Arctic tundra ecosystems are likely governed by contrasting moisture regimes. Accordingly, our results suggest that in a warmer future, winter soil N cycling rates are likely to be most enhanced in tundra locations where the soil is relatively moist, and that consequent increases in soil nutrient availability and thus N assimilation of plants may be most pronounced in moist tundra during the following growing season. It can also be speculated that these particular conditions may enhance N losses by leaching or gaseous emissions (e.g. N_2 and N_2O), pointing at altered N balances and climate feedbacks associated with wet Arctic tundra. The combination of multi-season and multi-site studies are important for understanding future annual N cycling in contrasting Arctic landscapes and this study strongly ask for supplementary *in situ* investigations to clarify such consequences.

Declaration of competing interest

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.

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Appendix A. Supplementary data

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