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ORIGINAL PAPER

Short- and long-term plant and microbial uptake of ^{15}N-labelled urea in a mesic tundra heath, West Greenland

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Abstract

Terrestrial animals are key elements in the cycling of elements in the Arctic where nutrient availability is low. Waste production by herbivores, in particular urine deposition, has a crucial role for nitrogen (N) recycling, still, it remains largely unexplored. Also, experimental evidence is biased toward short-term studies and Arctic regions under high herbivore pressure. In this study, we aimed to examine the fate of N derived from urine in a nutrient poor tundra heath in West Greenland, with historical low level of herbivory. We performed a pulse labelling with ^{15}N-urea over the plant canopy and explored ecosystem N partition and retention in the short-term (2 weeks and 1 year) and longer-term (5 years). We found that all vascular plants, irrespective of their traits, could rapidly take up N-urea, but mosses and lichens were even more efficient. Total ^{15}N enrichment was severely reduced for all plants 5 years after tracer addition, with the exception of cryptogams, indicating that non-vascular plants constituted a long-term sink of ^{15}N-urea. The ^{15}N recovery was also high in the litter suggesting high N immobilization in this layer, potentially delaying the nutrients from urine entering the soil compartment. Long-term ^{15}N recovery in soil microbial biomass was minimal, but as much as 30% of added ^{15}N remained in the non-microbial fraction after 5 years. Our results demonstrate that tundra plants that have evolved under low herbivory pressure are well adapted to quickly take advantage of labile urea, with urine having only a transient effect on soil nutrient availability.

Keywords Arctic tundra · Ecosystem N retention · Microbial N immobilization · ^{15}N labelling · Plant nitrogen uptake · Urine

Introduction

Permafrost-affected soils are large reservoirs of organic matter and nitrogen (N) (Harden et al. 2012; Hugelius et al. 2014; Voigt et al. 2020). Although recent evidence suggests hotspots of N availability in some Arctic ecosystems such as in nearshore coastal zones (Sanders et al. 2022) and permafrost peatlands (Ramm et al. 2022), most Arctic soils are strongly N limited with small fractions of labile inorganic N (Templer et al. 2012; Maslov and Makarov 2016). Long cold winter season with below-zero temperatures, cool summer and high soil acidity constrain microbial activity and limit N mineralization in tundra soils (Robinson 2001; Weintraub and Schimel 2003; Morsdorf et al. 2019). Despite reports of direct uptake of low molecular weight amino acids or proteins by tundra plants (Chapin et al. 1993; Schimel and Chapin 1996; Hill et al. 2011; Ravn et al. 2017), most soil N in Arctic soils are bound into complex organic compounds and are not directly available for plant uptake (Kielland 1994; Neff et al. 2003; Read and Perez-Moreno 2003). In addition, a large proportion of N is also immobilized in soil microbial biomass and plant–microbe competition for soil available N is fierce (Jonasson et al. 1996; Michelsen et al. 1999; Liu et al. 2018). In ecosystems with such constraints on nutrient availability, any mechanism acting on N sources

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and N recycling would profoundly affect plant productivity and soil biogeochemical cycles.

Herbivores play a key role in a large range of ecosystems by strongly influencing plant community composition and nutrient cycling via three main mechanisms occurring simultaneously; selective foraging, vegetation and soil trampling and excreta deposition (Olff and Ritchie 1998; Bardgett and Wardle 2003; Pastor and Cohen 2006). While senescent vegetation enters the soil as slowly-decomposing litter, herbivores convert vegetation into labile and nutrient rich dung and urine from which nitrogen is released rapidly into the soil thus stimulating microbial activity, soil nutrient availability, and plant productivity (Bazely and Jefferies 1985; Day and Detling 1990; Hobbs 1996; Frank et al. 2004). Plants can take up urea $CO(NH_2)_2$, the main N compound in urine, directly through foliage absorption (Uscola et al. 2014) or in the soil by root absorption of either intact molecule of urea (Fujii and Hayakawa 2022) with the presence of high and low affinity transporters in plant cells (Wang et al. 2008) or in mineralized forms (Mugasha and Pluth 1994). Both in plants and in the soil, urea is rapidly hydrolyzed into ammonium by urea degrading enzymes and in plant cells N compounds derived from urea are later assimilated by glutamine synthetase (Wang et al. 2008). Experimental evidence of the role of urine for N cycling in natural ecosystems is scarce, particularly at high latitude environments, with most evidence emerging from highly managed agricultural systems where urea is applied as a fertilizer at high doses.

Barthelemy et al. (2018) explored the short-term fate of urea, in a tundra heath and a graminoid dominated vegetation in northern Norway, with both sites under a high herbivore pressure by a large population of reindeer (albeit a lower density in the tundra health) and many species of rodents with cyclic population outbreaks. Urea was applied as trace amounts of ^{15}N-urea over the plant canopy to simulate urine deposition, a method to follow the fate of ^{15}N-urea without altering its natural behavior in the ecosystem (Robinson 2001). A quick and high ^{15}N recovery in plant canopy was observed shortly after tracer addition, with low ^{15}N-recovery in soil and microbial communities (Barthelemy et al. 2018). This study demonstrated that tundra plants under a high herbivore pressure seem to be able to rapidly benefit from N compounds derived from urine and that urea is an important element in the N budget of naturally grazed tundra ecosystems. The fate of N-urea is unclear for ecosystems with low herbivore density where plants could be less adapted to take advantage of N derived from urine excretion (i.e. lower urea transport activity). However, it is also possible that plant uptake of N-urea could be greater for tundra plants experiencing lower levels of herbivore disturbance (i.e. less defoliation and trampling) which can reduce foliar and root nutrient absorption. Tundra ecosystems are highly sensible to the ongoing rapid and dramatic climate change (IPCC 2013) and changes in Arctic herbivore activity, density and distribution have been reported (Koltz et al. 2022). As the Arctic warms, shifts in geographical range for small and large mammal populations are expected (Baltensperger and Huettmann 2015; Mallory and Boyce 2018; Cuyler et al. 2020) and can give herbivores access to new territories with previously minor herbivore pressure or no mammal herbivory history with consequences for biogeochemical cycles, particularly N recycling.

Tundra ecosystems host a large variety of plant functional groups with strikingly different traits and life strategies (Bjorkman et al. 2018). Plant coexistence in nutrient-poor environments is achieved by a strong resource partitioning of available nutrients in space (i.e. root depth), in time (i.e. differential uptake during the growing season) and chemical forms (i.e. organic and inorganic forms) (McKane et al. 2002). Mosses and lichens, ubiquitous in tundra, take up most of their nutrients from the atmosphere and from wet or dry deposition (Jonsdottir et al. 1995; Eckstein and Karlsson 1999; Dahlman et al. 2004; Ayres et al. 2006; Krab et al. 2008), decompose slowly and show high internal nutrient recycling (Eckstein 2000; Lang et al. 2009). Hence, they likely capture most of the incoming N compounds from urine and retain it for a long time thus reducing N uptake by microbes and vascular plants. In tundra, a large proportion of vascular plants are associated with mycorrhizal fungi (Michelsen et al. 1996, 1998; Barthelemy et al. 2017) that provide an advantage for N uptake from the dominating organic N sources compared to non-mycorrhizal vascular plants, which rely mainly on inorganic N sources (Read and Perez-Moreno 2003; Schimel and Bennett 2004). In the short term, different tundra plant species seem to be equally efficient in the uptake and retention of ^{15}N-urea (Barthelemy et al. 2018). However, in the long term, ^{15}N compounds derived from urine may eventually enter the soil compartment through decomposition of ^{15}N-enriched litter and become available for uptake by vascular plants and soil microorganisms. Then, differences in partitioning of ^{15}N from urine should be observed between mycorrhizal plants with shallow root systems close to the surface layer and non-mycorrhizal plants with deeper root systems. Soil microbial communities might also efficiently compete for incoming N derived from urine.

Ecosystem N retention, the ability of plant-soil-microbe pools to retain N, is an important function of terrestrial ecosystems (de Vries and Bardgett 2016; Wang et al. 2018). Understanding at which proportion incoming N can be lost to the surroundings by leaching or volatilization is vital in tundra ecosystems where microbial decomposition and mineralization activities are strongly limited. In the Arctic, belowground biomass is proportionally high and a large fraction the ecosystem N pool is found in microbial biomass and decomposing litter (Jonasson et al. 1999; Poorter et al. 2012;

Iversen 2014; Zhu et al. 2016). Hence, there is a crucial need for studies exploring N retention in aboveground and belowground part of the system. Interactions between plants and soil microbes are likely to be vital in controlling retention and loss of incoming nutrients from urine. Further, urea volatilization rates following urine excretion and nutrient leaching toward deeper soil layers are also important factors in determining N-urea retention in the system. Retention of urine-derived N may be large in ecosystems dominated by slow growing and resource conservative plant species and their associated fungal microbial communities and provide a long-term source of nutrients supporting plant productivity. Long-term ecosystem N retention studies in the Arctic are rare, and to our knowledge, the fate of N from urine in the plant–microbe-soil compartments of tundra ecosystem in longer terms has never been documented.

In this study, we explored the N availability and ecosystem stocks by analyzing ^{15}N natural abundance ($\delta^{15}N$) and N pool sizes in a mesic, nutrient-poor tundra heath in Greenland. We furthermore analyzed the partitioning and retention of ^{15}N labelled urea in aboveground and belowground N pools in both the short-term (2 weeks and 1 year) and the long-term (5 years). Trace amounts of ^{15}N labelled urea was applied above the plant canopy to mimic the natural return of nutrients in the form of urine. The study was performed at Disko Island, West Greenland with historically low herbivore pressure, where no large herbivores are present and only a low density of small herbivores. We hypothesized that: (1) Most of the ^{15}N-urea applied above the vegetation is rapidly assimilated into the plant canopy. We expect ^{15}N plant enrichment in our system to differ from ^{15}N enrichment reported for tundra under high herbivore pressure, with higher uptake under low herbivore disturbance due to less defoliation and trampling; (2) Mosses and lichens are the most efficient plant groups to capture ^{15}N-urea; (3) In the long term (5 years), ^{15}N recovery in soil and microbial biomass will increase and plant species with different traits and mycorrhizal status will differ in ^{15}N recoveries. Plants with acquisitive traits are expected to show larger decline in ^{15}N over time compared to plants with more conservative traits; (4) ^{15}N retention is high due to low soil nutrient availability and a dominance of resource-conservative plant species.

Materials and methods

Study site

The study was conducted in a mesic tundra heath in Blæsedalen Valley at Disko Island, West-Greenland (69° 15′ N 53° 34′ W) (Fig. 1). The climate is characteristic for low Arctic tundra, with mean annual air temperature − 3.0 °C (1991–2011), July the warmest month with a mean temperature at 7.9 °C and March the coldest month with a mean temperature at − 14.0 °C. The mean annual precipitation is 400 mm with approximately 60% falling as rain (1994–2006), with data from the closest climatic station, 3 km from the study site (Hollesen et al. 2015). The soil type can be classified as a Haplic Cryosol (FAO)/Orthic Gelisol (USDA) and soils consist of a thin (5–10 cm) organic layer overlaying glacially rebedded basaltic sediments of a volcanic origin. The vegetation, a mesic tundra heath (Fig. 1b), is dominated by deciduous and evergreen dwarf shrubs with the most common vascular plant species being *Betula nana*, *Salix glauca*, *Vaccinium uliginosum, Empetrum hermaphroditium* and *Cassiope tetragona*, while grasses and forbs

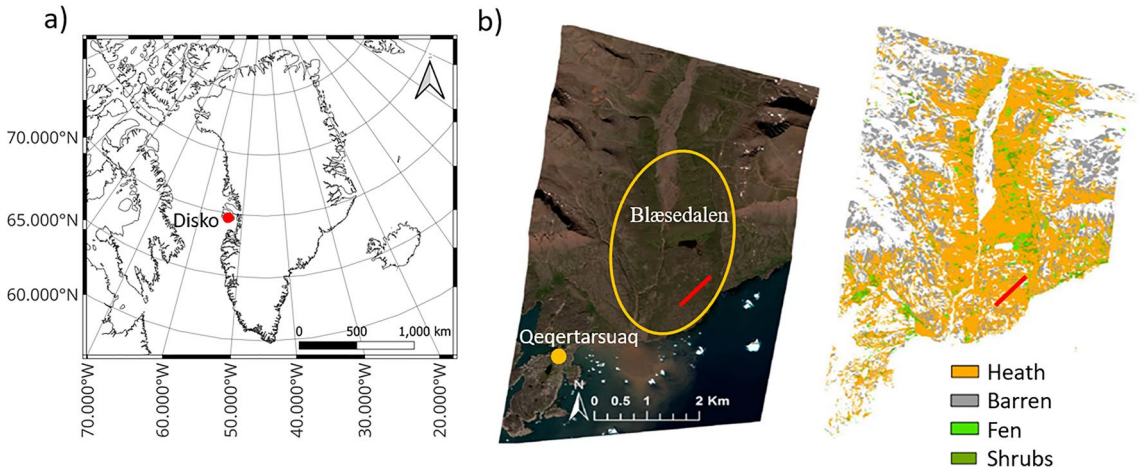

Fig. 1 Location of Disko Island in West Greenland (coordinate reference system: WGS 84 / NSIDC Sea Ice Polar Stereographic North) (**a**) and satellite scene of the southern part of Disko Island with Blæsedalen valley where the ^{15}N-urea study was conducted (left panel) and corresponding terrestrial cover classes (right panel) (**b**). The ten experimental plots are located along the red line. Modified from D'Imperio et al. (2017) with permission

are rare (Blok et al. 2016). Bryophytes and lichens are also an important part of the total plant biomass at the research site. The vegetation is interspaced with patches of exposed bare ground. Disko Island lacks large vertebrate herbivores, and small-sized vertebrate herbivores, such as the Arctic hare (*Lepus arcticus*) and the rock ptarmigan (*Lagopus muta*), have very low densities. Soil pH measured as Milli-Q H_2O to dry soil in a 1: 10 ratio is 5.08 ± 0.09 at 0–5 cm and 5.81 ± 0.03 at 5–10 cm depth, and carbon to nitrogen ratio (C:N) is 22.50 (± 1.15) at 0–5 cm and 15.15 (± 0.40) at 5–10 cm depth (means ± SE, $n = 5$) (D'Imperio et al. 2017). Atmospheric N deposition at the site is low, < 0.1 g m^{-2} (Kanakidou et al. 2016).

Experimental design

At the peak of the growing season, in July 2012, 10 experimental sites at least 10 m apart were established at Blæsedalen. Sites were selected to have similar topography, plant community composition, and percentage of bare ground. Within each site, two plots (1 × 1 m) were established and randomly allocated to control or to the ^{15}N enriched urea addition manipulation. The ^{15}N-labelled urea (Urea-^{15}N, 98% ^{15}N, Cambridge Isotope Laboratories, Inc., USA) was diluted in 2 L of water from a nearby lake and sprayed uniformly over the vegetation at a dose of 0.1 g N m^{-2}, on the 14 of July 2012. Control plots received the equivalent amount of non-^{15}N enriched lake water. In this study, we aimed to mimic natural urine deposition and the ^{15}N enriched urea plots did not receive any additional non-enriched water to wash off the added tracer from the vegetation. The lake was located in a remote location and nutrient poor, with a concentration of 0.312 mg L^{-1} total N and 0.024 mg L^{-1} total P (on 22 July 2017). Each plot received a negligible 0.000624 g N m^{-2} with water, but 0.1 g N m^{-2} with the ^{15}N addition. Applying this trace amount of urea over the vegetation, which is ^{15}N-enriched relative to any occurring natural level, allowed us to trace the N-urea in the different ecosystem N pools but precluded fertilization effect (Barthelemy et al. 2018).

Sampling

Aboveground biomass (standing plant biomass including foliage and twigs) was collected 10 days (24 July 2012, short-term recovery), and 5 years (20 July 2017, long-term recovery) after the ^{15}N-urea addition from 25 cm × 25 cm subplots in each experimental plot. To estimate mid-term ^{15}N-urea recovery in the vegetation, we harvested aboveground part of plants in 19 cm × 19 cm subplots in 6 randomly selected sites 1 year after the tracer addition (20 July 2013). All fresh plant material was stored at 4 °C until further processing. The harvested plants were sorted by species, except for graminoids, mosses and lichens that were treated collectively. The litter layer (dead leaves, dead moss pieces and twigs and fragments thereof, inevitably mixed with some soil) was collected in the same sub-plots and included both fresh litter and older plant remains that were not separated. The litter layer was differentiated from the organic horizon below, the latter here defined as finely fragmented dead plant material and decaying organic matter. The above-ground plant biomass was divided into the following nine compartments; Graminoids, Forbs, *B. nana*, Other deciduous shrub species, *E. hermaphroditum*, Other evergreen shrub species, Lichens, Mosses and Litter. The complete species composition and abundance for the Forbs, Other deciduous shrubs and Other evergreen shrubs, are presented in the Online Resource 1. After sampling, all the aboveground plant parts were washed with water to remove any remaining ^{15}N-urea deposition on the surface of the plants, oven-dried (60 °C, 48 h) and weighed.

Belowground plant biomass was collected in all experimental plots 10 days and 5 years after tracer addition. After 10 days, root biomass was sampled at 0–5 cm depth, which corresponds to the most active root zone at the site (one soil core, area 44 cm^2) while after 5 years, root biomass was sampled at 0–5 cm depth (two soil cores, area 14 cm^2) and also at 5–10 cm and 10–20 cm (three soil cores, area 21 cm^2). All samples were kept at 4 °C until further processing in the laboratory. Roots were separated from soil, washed to remove remaining organic and mineral matter, oven-dried (60 °C, 48 h) and weighed. After 5 years, the collected roots were sorted into fine (< 1 mm) and coarse (> 1 mm) roots.

To estimate ^{15}N-urea recovery in the soil and microbial N pools and soil nutrient availability at the site, soil samples were collected in each experimental plot 10 days and 5 years after tracer addition. Ten days after tracer addition, two or three soil cores (area 14 cm^2) were taken at 0–5 cm and 5 years after tracer addition, soil was retrieved from the same cores from which also root biomass was sampled at 0–5 cm, 5–10 cm and 10–20 cm depth. Undecomposed dead plant material were removed and the fresh samples separated in roots and soil by sieving (mesh 2 mm). The soil was kept at 4 °C until further processing.

Chemical analysis

Above- and belowground plant samples were ground to fine powder and subsamples encapsulated into preweighed tin capsules. The $\delta^{15}N$ and elemental N concentration was measured on an Isoprime isotope ratio mass spectrometer (Isoprime Ltd., Cheadle Hulme, UK) coupled to a CN elemental analyser (Eurovector, Milan, Italy), at the Stable

Isotope Facility at Department of Biology, University of Copenhagen.

For each soil sample, soil water content was measured gravimetrically (105 °C, 12 h) and organic matter content was determined by loss-on-ignition (475 °C, 12 h). A 10 g soil subsample was shaken with 70 ml of demineralized water for 1h and filtered through glass fibre filters (Whatman No 1). Another 10 g soil subsample was fumigated with chloroform ($CHCl_3$) vapor (50 ml chloroform in glass vial in vacuum desiccator) for 24 h in order to release N contained in the microbial biomass before shaking with water (Brookes et al. 1985; Clemmensen et al. 2008). The soil and fumigated extracts were kept frozen until further analyses. Total extractable N (N_e) was determined by persulfate digestion by oxidizing all N to NO_3^- (Williams et al. 1995) and then analyzed by flow injection analysis (FIASTAR 5000 analyzer, FOSS Analytical; application note AN 5201from the manufacturer). The microbial N content (N_{mic}) was calculated by subtracting the total extractable N in the non-fumigated extracts (N_e) from the N in the fumigated extracts (N_f) with a microbial correction factor K_{EN} of 0.4 (Jonasson et al. 1996; Clemmensen et al. 2008). In 2017, NH_4^+, NO_3^- and PO_4^{3-} were determined for the non-fumigated soil extracts using FIASTAR 5000 analyzer application notes AN 5220, AN 5201 and AN 5241 from the manufacturer.

To determine extractable $\delta^{15}N$ ($\delta^{15}N_e$) and microbial $\delta^{15}N$ ($\delta^{15}N_{mic}$), a subsample of 10 ml of digested extracts collected 10 days after tracer addition (in 2012) were prepared for isotope analysis following a modification of the acid trap diffusion technique (Barthelemy et al. 2018). Five years after tracer addition (in 2017), fumigated extracts (30–50 ml) and soil extracts (70 ml) were freeze-dried for isotope analysis as in Ravn et al. (2017); and Pedersen et al. (2020). The acidified diffusion discs were analysed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the Stable Isotope Facility, University of California, Davis. The freeze-dried extracts were analysed at the Stable Isotope Facility at Department of Biology, University of Copenhagen.

Isotopic calculations

Enrichment and natural abundance of ^{15}N was measured as $^{15}N/^{14}N$ ratio in plant and soil fractions (Barthelemy et al. 2018). The natural abundance of ^{15}N was expressed as

$$\delta^{15}N(‰) = 1000 \times \left(\frac{R\mathit{sample} - R\mathit{standard}}{R\mathit{standard}} \right)$$

where Rsample is the isotope ratio of the sample ($^{15}N/^{14}N$) relative to a standard calibrated against atmospheric N_2.

The urea-^{15}N enrichment in each ecosystem N pools was calculated as the ^{15}N atomic frequency in excess as

$$\text{Atom}^{15}N\%\text{excess} = \text{Atom}\%\,\mathit{tracer} - \text{Atom}\%\,\mathit{background}$$

where Atom % tracer is the ^{15}N atomic frequency of a sample collected in the ^{15}N urea addition plot and the Atom % background is the ^{15}N atomic frequency of the corresponding sample collected in the non-labelled plot.

The percentage tracer recovery in each ecosystem N pool was estimated as

$$\%\text{Tracer recovery}^{15}N = \frac{(\mathit{Atom}^{15}N\,\mathit{excess} \times N \times \mathit{Mass})/10^4}{\mathit{Total\ amount\ of\ tracer\ added}} * 100$$

where Atom ^{15}N excess is the urea-^{15}N enrichment (%), N is the N content (%), Mass is the total mass of each pool of biomass or soil (g m^{-2}) and the total amount of the tracer added corresponds to the 0.1 g m^{-2} of the isotopic labelled urea. Tracer recovery was corrected for the fractional abundance of ^{15}N in the tracer (98 Atom % ^{15}N). We assumed negligible N isotope fractionation during tracer movement in the different ecosystem pools (Robinson 2001) and no difference in ^{15}N natural abundance within the N pools from 2 weeks, 1 year and 5 years after tracer addition.

Mycorrhizal colonization analysis

Mycorrhizal colonization analysis was conducted for the 10 most common plant species at the site; the graminoids *Carex bigelowii*, the forbs *Tofieldia pusilla*, *Pyrola grandiflora*, *Pedicularis flammea*, *Bistorta vivipara*, the deciduous shrubs *S. glauca*, *B. nana*, *V. uliginosum* and the evergreen shrubs *E. hermaphroditum* and *C. tetragona*. Root samples were harvested 5 years after tracer addition (in 2017) from 10 individuals close to the experimental plots by tracing the fine roots from the stem and main roots down into the soil. Root samples were thoroughly washed with water and stored in 70% ethanol until further processing. Root sample preparation for mycorrhizal analysis and colonization rates of fungi can be found in the Online Resource 2.

Statistics

All variables were log-transformed prior analysis to fulfill the assumptions of normality and homoscedasticity, with the exception of soil nutrient availability, which fulfilled the model's assumption. We performed one-way analysis of variance (ANOVA) followed by post hoc Tukey's HSD (Honestly Significance Difference) to investigate differences between soil depth for soil nutrient (NH_4^+, NO_3^- and

PO_4^{3-}) concentration, root biomass, and root, bulk soil N, soil extractable N, microbial N pool size, ^{15}N natural abundance, ^{15}N enrichment and ^{15}N recovery. To test differences in ^{15}N natural abundance between aboveground plant groups and plant litter we conducted one-way ANOVA followed by post hoc Tukey's HSD test. Differences in soil nutrient availability, biomass and N pool size between control and ^{15}N enriched plots were tested with one-way ANOVA. Differences in plant biomass, plant, soil and microbial N pool sizes, ^{15}N enrichment and ^{15}N recovery between years were tested using linear mixed effect model with time following tracer addition as fixed factor and plot identity as a random effect. This analysis avoids temporal pseudoreplication by taking into account the sampling of the same plots at 2 weeks, 1 year and 5 years after tracer addition. Linear contrasts were used to examine the differences between years. For each linear model, Akaike's information criteria and residual plots were used to assess the fit of the models. All statistical analyses were performed using the statistical package R (R Core Team 2022).

Results

Biomass and N pool size

Plant litter, deciduous shrubs and mosses were the largest N pools and contained collectively 80 to 85% of the total aboveground N pool (Fig. 2a), which did not differ between sampling years. The N pool of the different plant groups remained unchanged across the 5 years period except that the N pool was significantly lower after 2 weeks compared to 1 year in deciduous shrubs (Tukey's HSD, $p = 0.034$). Larger amount of N was stored in plant aboveground than in plant belowground biomass (Fig. 2a, b). Both roots and microbial N had larger N pools in 0–5 cm compared to deeper

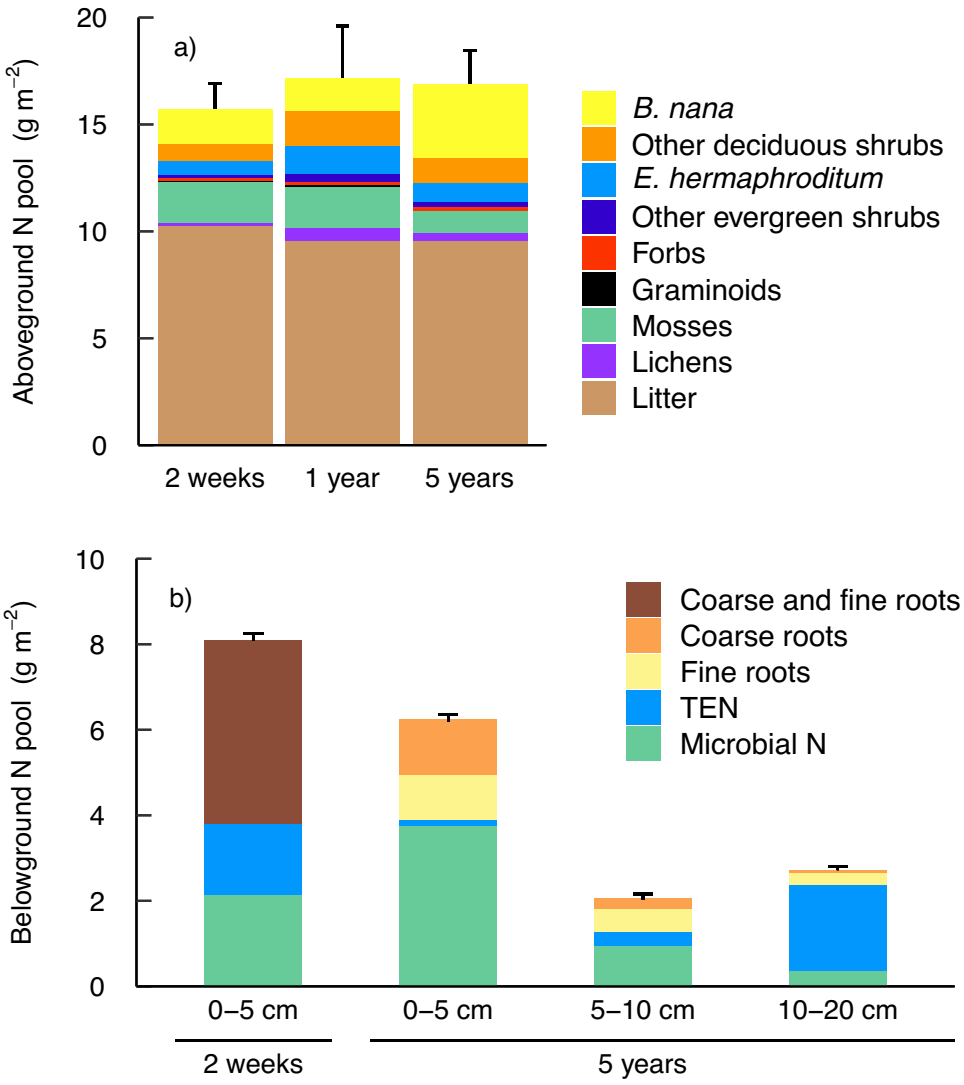

Fig. 2 Nitrogen pool (g m^{-2}) in each ^{15}N-enriched plot for aboveground ecosystem pools 2 weeks, 1 year and 5 years after tracer addition (**a**) and for belowground ecosystem pools 2 weeks and 5 years after tracer addition (**b**). TEN: Total extractable N. Three soil horizons were considered in 2017 (5 years after tracer addition). Bars represent means for each ecosystem N pool and the error bars account for the standard error of the total N pool for each sampling year. $n = 10$ in each ecosystem compartment except for aboveground N pools at 1 year ($n = 6$)

horizons (Tukey's HSD, $p < 0.001$) while the opposite pattern was found for soil extractable N and total N (Tukey's HSD, $p < 0.001$) (Fig. 2b and Online Resource 3). In 0–5 cm, total extractable N differed between the two sampling years (Tukey's HSD, $p < 0.001$) while roots and microbial N pools remained unchanged (Fig. 2b). Detailed analysis of soil nutrient availability is presented in the Online Resource 3 and aboveground and belowground plant biomass is presented in the Online Resource 1.

^{15}N natural abundance

Graminoids had significantly higher ^{15}N natural abundance (δ^{15}N) than all other plant functional groups (Fig. 3 and Online Resource 4), and the δ^{15}N of evergreen shrubs and *E. hermaphroditum* were significantly lower than the δ^{15}N of lichens, mosses, and forbs (Fig. 3 and Online Resource 4). The δ^{15}N of mixed litter was significantly lower than that of live graminoids (Tukey's HSD, $p < 0.001$). Fine roots had a higher δ^{15}N than coarse roots (Fig. 3, ANOVA, $F_{1,43}$, $p = 0.008$), and δ^{15}N in fine roots was higher at 5–10 cm depth compared to 0–5 cm (Tukey's HSD, $p = 0.004$) and 10–20 cm (Tukey's HSD, $p < 0.001$) whereas δ^{15}N of coarse roots was similar in all depths. Soil and microbial N pools had higher δ^{15}N values compared to plants (Fig. 3). Soil extractable N had lower δ^{15}N values at 0–5 cm depth in comparison to 5–10 cm (Tukey's HSD, $p = 0.004$) and 10–20 cm depth (Tukey's HSD, $p = 0.013$) (Fig. 3). Microbial N pool δ^{15}N did not differ with soil depth.

Initial ^{15}N recovery and enrichment

The total recovery of ^{15}N in plant N pools was 26.2% two weeks after the addition of the ^{15}N-labeled urea (Fig. 4). Aboveground plant biomass (Fig. 4a) was the largest sink of the added ^{15}N compared to belowground plant parts (total recovery of 0.4%, Fig. 4b). Mosses and *B. nana* had the highest ^{15}N recovery of the plant fractions (recovery of 5.6% and 4.2% respectively), followed by *E. hermaphroditum* with a recovery of 2.2% (Fig. 4a and Online Resource 5). Deciduous shrubs and *E. hermaphroditum* had higher ^{15}N recovery than graminoids and forbs while the other plant groups did not differ (Online Resource 5). Plant litter, with a recovery of 11.0%, had a higher recovery than of any of live plant species (Tukey's HSD, $p < 0.001$ for all). Lichens had the highest ^{15}N enrichment (0.6 ^{15}N excess in atom %) compared to the other plant groups (Tukey's HSD, $p < 0.001$ for all, Fig. 6a). Mosses were also ^{15}N enriched compared to graminoids (Tukey's HSD, $p < 0.001$) and forbs (Tukey's HSD, $p < 0.001$), while the other plant functional groups did not differ (Fig. 6a). Initial ^{15}N enrichment in roots, soil extractable N and microbial N was low (Fig. 6b).

Longer-term ^{15}N recovery and enrichment

One year after tracer addition total aboveground ^{15}N enrichment ($p < 0.001$) and ^{15}N recovery ($p < 0.001$, 50% increase) were higher than initial ^{15}N enrichment and recovery (Linear mixed effects model, Fig. 4a). This increase was driven by an increase in ^{15}N enrichment for

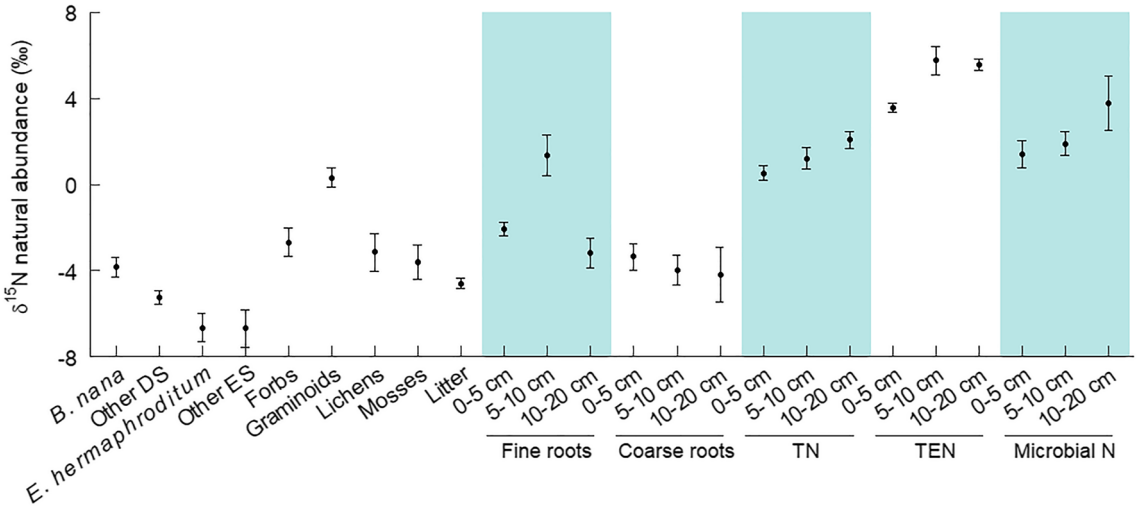

Fig. 3 δ^{15}N natural abundance (‰) for the nine plant groups (*B. nana*, Other DS: Other deciduous shrubs, *E. hermaphroditum*, Other ES: Other evergreen shrubs, Forbs, Graminoids, Lichens, Mosses and Litter), and for Fine and Coarse roots, TN: Total soil N, TEN: Total extractable N and Microbial N. δ^{15}N natural abundance was measured in 2017 (5 years after tracer addition) in each control plot. Three soil horizons were considered for the belowground ecosystem compartment. Mean values ± standard errors are presented. $n = 10$ in each ecosystem compartment except for *E. hermaphroditum*, graminoids, coarse roots at 5–10 cm ($n = 7$ for all), Other evergreen shrubs ($n = 4$) and TEN ($n = 8$)

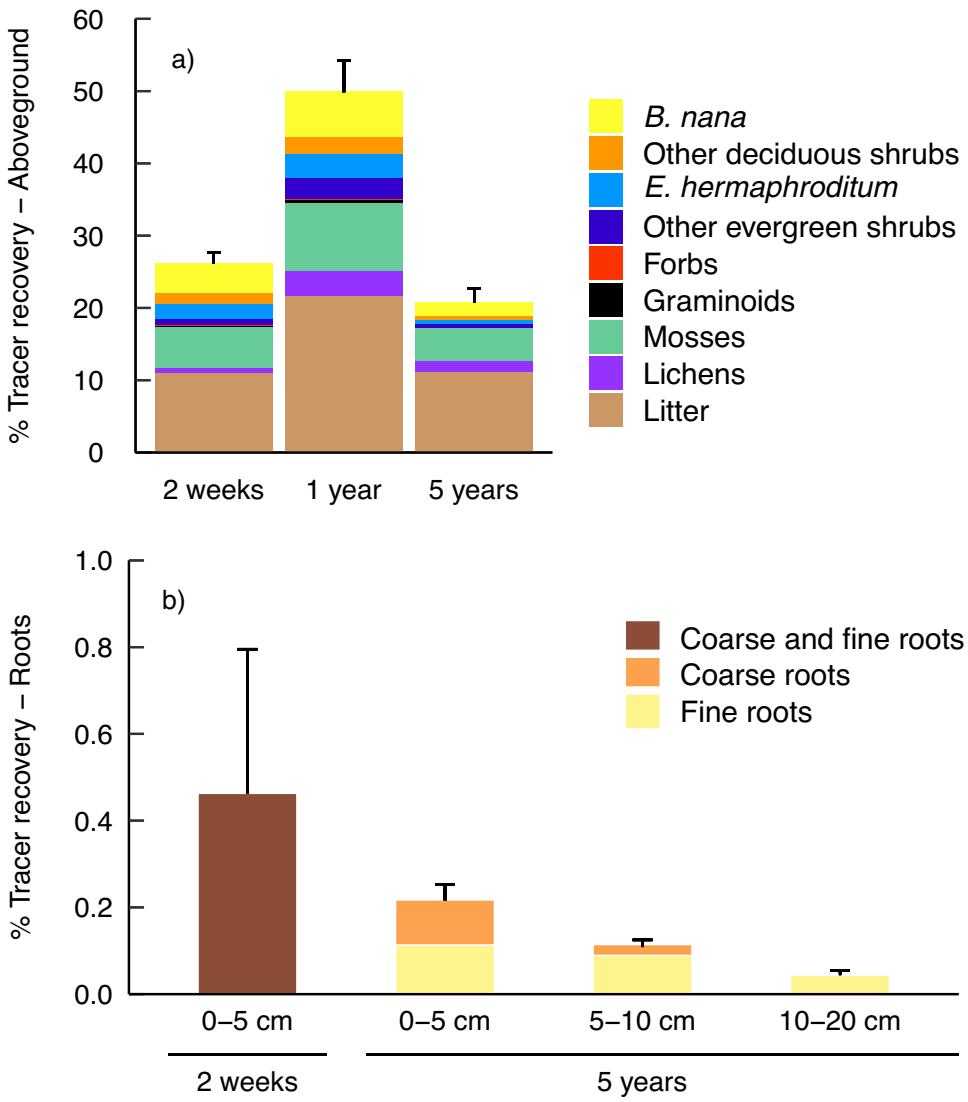

Fig. 4 ^{15}N recovery (%) for aboveground ecosystem pools 2 weeks, 1 year and 5 years after tracer addition (**a**). Bars represent means for each ecosystem pool and the error bars account for the standard error of the total recovery for each sampling year. ^{15}N recovery (%) for Roots 2 weeks and 5 years after tracer addition (**b**). Mean values ± standard errors are presented. For aboveground recovery, $n = 10$ in each compartment at 2 weeks and 5 years and $n = 6$ at 1 year

deciduous shrubs ($p = 0.012$), graminoids ($p = 0.021$), lichen ($p = 0.026$) and in the litter layer ($p = 0.001$) (Linear mixed effects model). Five years after tracer addition total plant and soil extractable and microbial ^{15}N recovery was 24% lower than after 1 year (Linear mixed effects model, $p < 0.05$), with aboveground plants (incl. litter) still being the largest sink of the added ^{15}N-urea with a recovery of 20.7% (Figs. 4 and 5). Total aboveground ^{15}N recovery and ^{15}N recovery of the three largest ^{15}N sinks, *B. nana*, mosses and litter after 5 years did not differ from the initial recovery after two weeks (Online Resource 5) while ^{15}N recovery in deciduous shrubs, *E. hermaphroditum*, and forbs decreased after 5 years (Online Resource 5). Total ^{15}N enrichment in aboveground plants was 42% lower after 5 years compared to the initial ^{15}N enrichment after 2 weeks (Linear mixed effects model, $p < 0.001$, Fig. 5a). A large decrease in ^{15}N enrichment was observed for all vascular plant groups (Linear mixed effects model, $p < 0.001$, 78% decrease) but not for mosses and lichens (29% decrease, Fig. 6a) which still had the highest ^{15}N enrichment (Linear mixed effects model, $p < 0.001$), with no difference between the other groups.

Plant roots had a higher ^{15}N enrichment after 5 years compared to the initial enrichment (+0.09% enrichment, Linear mixed effects model, $p = 0.001$, Fig. 6b), but were still a weak sink of the ^{15}N-urea with a recovery of 0.38% (Fig. 4b). Root ^{15}N enrichment was higher at 0–5 cm compared to 10–20 cm depth (Tukey's HSD, $p < 0.001$). Both tracer ^{15}N recovery and ^{15}N enrichment were still low in extractable N and microbial N pools after 5 years compared to aboveground N pools (Figs. 4, 5, 6). ^{15}N enrichment in extractable N was higher after 5 years compared to the initial enrichment (Linear mixed effects model, $p = 0.0023$) but still low for all soil depths, while ^{15}N enrichment in microbial N did not differ (Fig. 6b). Microbial N ^{15}N enrichment was on average 72% higher at 5–10

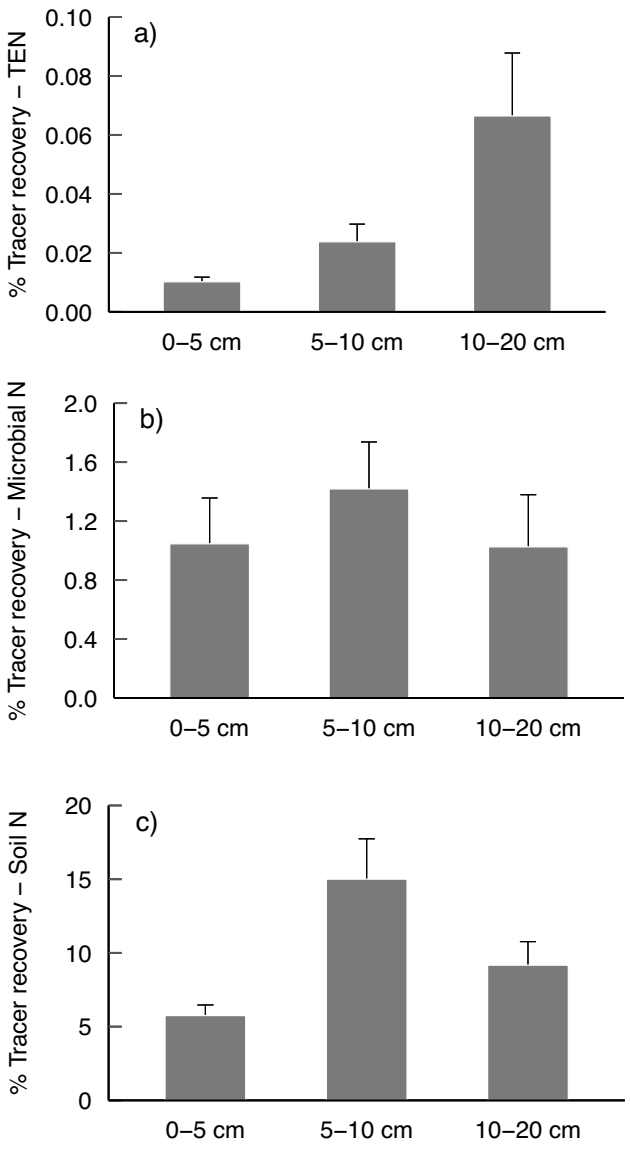

Fig. 5 ^{15}N recovery (%) for belowground ecosystem soil pools 5 years after tracer addition; TEN: Total Extractable N (**a**), Microbial N (**b**) and total soil N (**c**). Bars represent mean recovery for each belowground compartment with standard error. $n = 10$ for all soil depths

cm soil layer than at 0–5 cm (Tukey's HSD, $p < 0.001$) and 10–20 cm (Tukey's HSD, $p = 0.001$) layers.

Recovery in the extractable N pool increased with depth (Tukey's HSD, $p = 0.009$) while the microbial ^{15}N recovery did not. Soil total ^{15}N recovery (0–20 cm depth) was 30% of added ^{15}N after 5 years, with higher recovery at 5–10 cm compared to 0–5 cm depth (Tukey's HSD, $p < 0.0112$, Fig. 5c).

Discussion

In this study, we applied the main N component of urine as trace amount of ^{15}N labelled urea over the plant canopy. We traced the ^{15}N-urea pathway from plant canopy to belowground ecosystem compartments over a 5-year period in a mesic tundra heath with low level of herbivory. In agreement with our first hypothesis, we found that most of the ^{15}N-urea was rapidly assimilated into the plant canopy, with high ^{15}N enrichment in all aboveground plant parts 2 weeks after tracer addition. This is in line with previous studies reporting rapid foliar absorption rates of ^{15}N urea by deciduous shrubs at the same site (Blok et al. 2016), by tundra plants in northern Norway (Barthelemy et al. 2018) and by plants from other biomes (Dong et al. 2002; Uscola et al. 2014; Ruan and Gerendas 2015). All vascular and non-vascular plants, independently of differences in traits, were efficient in absorbing applied ^{15}N-urea and they did so in proportion to their abundance in the vegetation. Our results support that urine can be an easy and rapid source of N for Arctic plant species in nutrient-poor tundra ecosystems, in a much faster way than nutrients released during the slow process of plant litter decomposition. Interestingly, short-term ^{15}N enrichment in plants was of the same magnitude as ^{15}N enrichment in tundra plants adapted to high level of herbivory when normalized per biomass (Barthelemy et al. 2018), indicating that plants with historically low level of herbivory might be as efficient as highly grazed plants in the uptake of N compounds derived from urine. While this study does not investigate the exact molecular and physiological mechanisms behind tundra plant urea uptake, it suggests that, in partial contrast to our first hypothesis, foliar and root absorption of N-urea is not affected by the level of herbivory pressure on the ecosystem.

In accordance with our second hypothesis, mosses and lichens were the largest sink of the ^{15}N from the applied ^{15}N urea compared to the other plant functional groups. Cryptogams are known to efficiently incorporate large amount of mineral and organic nutrients with their highly absorptive surfaces (Weber and Vancleve 1981; Crittenden 1989; Jonsdottir et al. 1995; Eckstein and Karlsson 1999; Krab et al. 2008). Interestingly, while vascular plant ^{15}N enrichment was strongly reduced 5 years after tracer addition, ^{15}N enrichment for mosses and lichens was almost identical to the initial ^{15}N enrichment measured at 2 weeks. Mosses are long-living, decompose slowly and have a high internal nutrient turnover (Eckstein and Karlsson 1999; Eckstein 2000; Lang et al. 2009).

Mosses and lichens can constitute a large part of the diet for some herbivores, for example in early spring for pink-footed geese (*Anser brachyrhynchus*) (Fox et al.

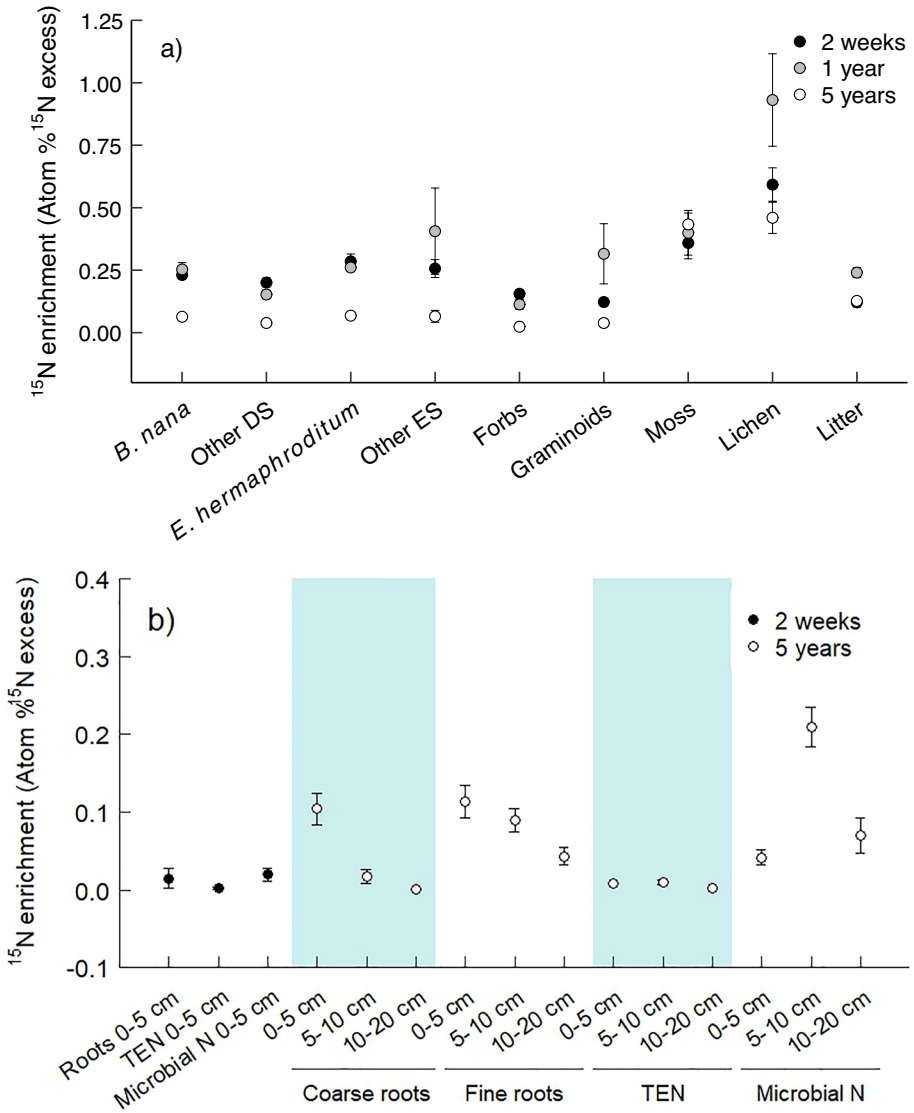

Fig. 6 Aboveground ^{15}N enrichment (Atom % ^{15}N excess) 2 weeks, 1 year and 5 years after tracer addition (**a**). The nine plant groups are *B. nana*, Other DS: Other deciduous shrubs, *E. hermaphroditum*, Other ES: Other evergreen shrubs, Forbs, Graminoids, Lichens, Mosses and Litter. Belowground ^{15}N enrichment (Atom % ^{15}N excess) 2 weeks and 5 years after tracer addition (**b**). The belowground N pools are Roots, TEN: Total extractable N and Microbial N. Three soil horizons were considered in 2017 (5 years after tracer addition). Mean values ± standard errors are presented. In cases where the standard errors are not visible, they are smaller than the data point. Aboveground ^{15}N enrichment, $n = 10$ in each compartment for at 2 weeks and 5 years (variation between 4–10 depending on the compartment) and $n = 6$ at 1 year (variation between 2–6 depending on the compartment). Belowground ^{15}N enrichment, $n = 10$ in each compartment except for TEN (n = 9) and microbial N ($n = 8$)

2006) and in winter for Svalbard reindeer (*Rangifer tarandus platyrhynchus*) (Zhao et al. 2019) and Norwegian lemming (*Lemmus lemmus*) (Saetnan et al. 2009). Other herbivores, including reindeer and muskox (*Ovibos moschatus*) which are widely present in the Arctic, forage selectively on forbs, graminoids and deciduous shrubs during the growing season (Nieminen and Heiskari 1989; Kristensen et al. 2011). This indicates that most N compounds derived from urine can be incorporated and sequestered into non-forage plant species for some important herbivore populations in the Arctic. In tundra ecosystems under moderate or high herbivore pressure, the N-urea captured by cryptogams is likely to be released to other ecosystem compartments after a few years, via herbivores' foraging behavior or trampling. For instance, geese grubbing activities (Speed et al. 2010), reindeer and muskox trampling (Gornall et al. 2009; Mosbacher et al. 2019) or rodents peaks (Rydgren et al. 2007) are all well known to strongly reduce the cryptogam layer, potentially enabling more nutrients to enter the soil system. However, in Arctic systems with historically low levels of herbivory, mosses and lichens can constitute a long-term sink of N by immobilizing N compounds for years and making them unavailable to vascular plants and soil microbes.

The litter layer was also an important sink of the ^{15}N from the applied ^{15}N urea. Initially, plant litter from recently senesced ^{15}N enriched plant materials would account only for a small fraction of the ^{15}N enrichment in the litter since the first sampling occurred at the peak of the growing season, 2 weeks after tracer addition. This initial high ^{15}N enrichment and recovery in the litter fraction is more likely the result of high microbial immobilization associated with the decomposition of plant litter. Free-living saprotrophic fungi are important colonizers of the litter layer and ascomycetes and basidiomycetes dominated the surface litter fungal communities at the site (Christiansen et al. 2017). Fungal

communities control the early stage of plant litter decomposition by the release of hydrolytic exoenzymes in the litter layer (Read and Perez-Moreno 2003; Schimel and Bennett 2004) and their activities in arctic ecosystems are often limited by N availability rather than carbon availability (Liu et al. 2018). A large increase in ^{15}N enrichment and in ^{15}N recovery (twice as high as the initial recovery at 2 weeks) was observed in the litter layer 1 year after tracer addition. This is probably due to the deposition of ^{15}N enriched foliar material and leaching from plants during plant senescence at the end of the first growing season. After 5 years, ^{15}N enrichment and recovery in litter decreased compared to the levels observed at 1 year but were still as high as the initial levels measured at 2 weeks. Fungal enzymatic activities and litter decomposition rates are strongly controlled by ground surface litter microclimate, in particular temperature and moisture regimes. Litter mass and carbon loss from decomposing plant litter is much lower in our mesic heath tundra sites compared to an adjacent sedge vegetation with higher moisture availability (Christiansen et al. 2017). Our findings suggest that in dry tundra ecosystems with slow litter decomposition, a large fraction of N compounds from urine incorporated in plant biomass can be sequestered in the litter layer for many years before entering the soil compartment.

Initial ^{15}N enrichment was relatively low in soil extractable N and microbial biomass, despite that the microbial N pool is of similar magnitude as that of plants (Fig. 2b). When applied from above the vegetation, ^{15}N is rapidly sequestered by plant canopy and the litter layer at the soil surface. The very small ^{15}N enrichment measured in roots 2 weeks after tracer addition also supports this idea. Our results contrast with the much higher ^{15}N recovery (40–80%) in soil and large microbial immobilization observed for studies where ^{15}N compounds have been injected directly in tundra soil (Jonasson et al. 1996; Grogan et al. 2004; Nordin et al. 2004; Clemmensen et al. 2008). Our findings also indicate that urine would have a fast effect on plants rather than a slow indirect effect via stimulation of microbial activity and organic matter decomposition, leading to increased soil nutrient availability from which plants will benefit (van der Wal et al. 2004). Both the ^{15}N enrichment and ^{15}N recovery in the plant community increased 1 year after tracer addition. Most likely, some ^{15}N compounds derived from the added urea have moved from aboveground plants to the litter and belowground compartments over the first year following tracer addition (i.e. due to plant senescence and root exudation). Plants subsequently could have acquired some of this ^{15}N in the soil, causing the observed increase in ^{15}N recovery in deciduous shrubs, graminoids and litter after 1 year.

We expected in our third hypothesis that in the short term (1st year) aboveground plant biomass should capture and retain most of the incoming N from the urea (either as intact urea molecule or in mineralized forms), while in the long-term N compounds derived from urea should eventually reach the soil matrix via plant turnover. We found mixed support for this hypothesis. Five years after tracer addition, belowground ^{15}N enrichment in extractable and microbial fractions was very low and ^{15}N recovery in microbial pool N minimal, with c. 1% ^{15}N recovered in 0–5, 5–10 and 10–20 cm soil depth. On the other hand, other fractions of the ^{15}N gradually released from microbes over the five years were absorbed to non-labile particles in the soil matrix, as demonstrated by the higher (5–15%) total soil ^{15}N recovery after 5 years in the same soil depths. We did not find any support for our hypothesis that ^{15}N enrichment should differ between plants with different traits and morphology as ^{15}N enrichment decreased equally for all vascular plant species. Evergreen shrubs with a slower turnover than fast growing graminoids and forbs did not have a higher capacity to retain the ^{15}N compounds from the applied ^{15}N urea. This indicates a high reallocation of nutrients to woody stems or belowground biomass before plant senescence: a physiological mechanism which can be typical for nutrient limited Arctic ecosystems (Aerts and Chapin 2000).

The large difference in ^{15}N natural abundance between co-occurring plant species reflects varying uptake of N between mycorrhizal and non-mycorrhizal plants and a strong niche partitioning in plant nutrient uptake based on chemical N form and mycorrhizal fungal associates (Michelsen et al. 1996, 1998; Barthelemy et al. 2017). All shrubs, evergreen or deciduous, were associated with either ericoid or ectomycorrhizal fungi, whereas graminoids were mainly non-mycorrhizal. Still, only small differences in ^{15}N enrichment by urea addition were observed between vascular plant species, indicating that mycorrhizal symbioses did not provide any competitive advantage to acquire ^{15}N compounds compared to non-mycorrhizal plants. It is likely that, even if plants absorbed intact urea through their foliage, ^{15}N-urea would, when reaching the soil, be rapidly hydrolyzed into ^{15}N-ammonium by urease, an extracellular enzyme (Fujii et al. 2018). Then, the ^{15}N form in the soil would have been ^{15}N-ammonium, a mineral form of N easily taken up by both mycorrhizal and non-mycorrhizal plants.

Our fourth hypothesis suggested that ^{15}N loss would be small in this tundra heath system with a closed N cycling. Our results largely confirmed that our study system was indeed N-limited. Soil nutrient availability (both NH_4^+ and NO_3^-) and plant N pools were significantly lower in our site compared to other tundra heaths in Greenland (Michelsen et al. 1998) and in Scandinavia (Barthelemy et al. 2017). The wide range in plant ^{15}N natural abundance and low ^{15}N natural abundance in the soil matrix is often found in nutrient limited Arctic ecosystems (Michelsen et al. 1996, 1998; Hobbie and Hobbie 2006; Barthelemy et al. 2018). Yet, a considerable fraction of N from urea was lost from the ecosystem with a 5-year retention of approx. 20% in plants and

approx. 30% in soil to 20 cm depth. We suggest that a rapid volatilization shortly after the application of the ^{15}N-urea may contribute to initial loss of ^{15}N from our tundra ecosystem. While volatilization generally is considered low in Arctic ecosystems, it could occur for the ^{15}N-urea deposited over the plant canopy or the fraction in the soil compartment. When urea is applied to vegetation, like via natural herbivore urination, a large proportion is immobilized in the litter layer, where a higher temperature and pH may promote urea hydrolysis and the conversion of ammonium into volatile ammonia (Ernst and Massey 1960). Soil and plant surfaces can reach 28 °C at the site and in 2014 surfaces were on average 7.7 ± 0.9 °C warmer than air temperature at 1.5 m height during summer (Lindwall et al. 2016). Hence, it is likely that a proportion of the urea added over the vegetation has been lost into volatile ammonia. Precipitation events following the application of urea are also critical for ammonia volatilization rates (Engel et al. 2011; Holcomb et al. 2011). Yet, meteorological data retrieved from the Greenland Ecosystem Monitoring Database reported no rainfall between the ^{15}N urea application and the first sampling 2 weeks later, which likely prevented most of the urea from moving deeper into the soil matrix, below our sampling profile. In the longer term, ^{15}N ecosystem losses may result from the downward movement of ^{15}N compounds along the soil profile and the following ^{15}N leaching. This is supported by the highest ^{15}N recovery for soil extractable N found at the deepest soil horizon at 10–20 cm depth after 5 years. Microbial denitrification has been often reported to explain N loss through the conversion of nitrate into atmospheric N (Groffman and Tiedje 1989; Aulakh et al. 1992), but denitrification is moderate in the dry heath tundra (Xu et al. 2023) with low soil moisture, cold temperatures, low nitrate concentration, and aerobic conditions prevailing along the soil profile and in the litter layer.

Our ^{15}N tracer study in a tundra vegetation with low herbivore density demonstrates that urea can be rapidly captured by the plant canopy, in particular by mosses and lichens, and that plants under low herbivore pressure are as efficient as highly grazed plants in taking advantage of this incoming N source from urine. Whereas vascular plant ^{15}N enrichment strongly declined over the long-term, mosses and lichens retained the same amount of labelled N as initially even after five years, demonstrating that in the absence of herbivores, cryptogams may constitute a very long-term sink of N. By contrast, although short-term microbial N uptake contributed to ecosystem retention of added urea-N, long-term ^{15}N recovery in this pool was small, indicating that herbivores can increase the quality of their forage plants more rapidly via urine deposition than via changes in microbial activities and coupled increase in soil nutrient availability. However, N-urea losses from the system right after urine excretion by N volatilization and leaching may be large despite a strong nutrient-limitation of the ecosystem. Our findings suggest that herbivores colonizing new territories (i.e. herbivore reintroduction or range expansion due to climate change (Olofsson and Post 2018) could rapidly increase plant litter quality by the deposition of nutrient rich and labile urine. Herbivores will benefit immediately from this improved forage quality, in contrast to other mechanisms also enhancing N cycling but operating at longer time scale such as slow vegetation shift to more palatable plants due to selective grazing.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00300-023-03209-6.

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Author contributions The project was conceived and designed by HB, AM and JO. HB, LAN and AM conducted the fieldwork campaigns. HB and LAN prepared all samples and HB and LAN conducted the laboratory analyses with the assistance of AM, SS and MV. LAN and AM performed preliminary data analyses and HB conducted the full data analyses and drafted the manuscript. All authors provided feedbacks on the manuscript through several rounds of revision.

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Data availability All data that support the findings of this study is openly available. Sample data are available in Dryad with the name: Short- and long-term plant and microbial uptake of ^{15}N-labelled urea in a mesic tundra heath, West Greenland, with DOI https://doi.org/10.5061/dryad.m0cfxpp92.

Declarations

Conflict of interest We have no conflicts of interest to disclose.

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