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Grazing decreases N partitioning among coexisting plant species

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Summary

1. Herbivores play a key role in shaping ecosystem structure and functions by influencing plant and microbial community composition and nutrient cycling.

2. This study investigated the long-term effects of herbivores on plant resource acquisition. We explored differences in the natural $\delta^{15}N$ signatures in plant, microbial and soil N pools, and examined mycorrhizal colonization in two tundra sites that have been either lightly or

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heavily grazed by reindeer for more than 50 years. The study examined changes in nutrient acquisition in five common tundra plants with contrasting traits and mycorrhiza status; the mycorrhizal dwarf shrubs, *Betula nana*, *Vaccinium myrtillus* and *Empetrum hermaphroditum*; a mycorrhizal grass, *Deschampsia flexuosa*, and a non-mycorrhizal sedge, *Carex bigelowii*.

3. There were large variations in $\delta^{15}N$ among coexisting plant species in the lightly grazed sites. This variation was dramatically reduced in the heavily grazed sites. At an individual species level, $\delta^{15}N$ was higher in *E. hermaphroditum* and lower in *C. bigelowii* in the heavily grazed sites. Mycorrhizal colonization in *B. nana* and *E. hermaphroditum* roots were also lower in the heavily grazed sites. The $\delta^{15}N$ signatures of the total soil N pool and of the microbial N pools were higher in the heavily grazed sites.

4. Since the strong $\delta^{15}N$ differentiation among plant species has been interpreted as a result of plants with different mycorrhizal types using different sources of soil nitrogen, we suggest that the lower variation in $\delta^{15}N$ in heavily grazed sites indicates a lower niche differentiation in nitrogen uptake among plants. Reduced mycorrhiza-mediated nitrogen uptake by some of the species, a shift towards a more mineral nutrition due to higher nutrient turnover, and uptake of labile nitrogen from dung and urine in the heavily grazed sites could all contribute to the changes in plant $\delta^{15}N$.

5. We conclude that herbivores have the potential to influence plant nutrient uptake and provide the first data suggesting that herbivores decrease nutrient partitioning on the basis of chemical N forms among plant species. Reduced niche complementarity among species is potentially important for estimates of the effects of herbivory on plant nutrient availability and species coexistence.

Key words: Above- belowground linkages, Arctic tundra, Microbial N biomass, Mycorrhizal colonization, Nutrient cycling, Plant-herbivore interactions, Plant nutrient uptake, Ungulate Grazing

Introduction

In arctic tundra, nitrogen (N) is a critical element limiting plant growth and a key determinant of plant species composition and the structure of tundra ecosystems (Nadelhoffer *et al.* 1992; Kielland 1994; Nadelhoffer *et al.* 1996). Dissolved organic nitrogen (DON) dominates the cycling of N in tundra where most of the soil N is found in complex organic compounds (Neff, Chapin & Vitousek 2003; Read & Perez-Moreno 2003), and nitrate and ammonium concentrations are low (Kielland 1994). A large proportion of the ecosystem N pools is immobilized in microbial biomass, which results in strong plant-microbe competition for nutrients (Jonasson *et al.* 1996; Michelsen *et al.* 1999). However, in nutrient limited environments plants can commonly bypass the N mineralisation by directly using organic N forms as a large part of their nutrition (Kielland 1994; Schimel & Chapin 1996; McKane *et al.* 2002).

Although non-mycorrhizal plant species can take up low molecular weight organic N from the soil solution (Chapin, Moilanen & Kielland 1993; Kielland 1994), symbiosis between mycorrhizal fungi and plant fine roots appears to be the major pathway for the acquisition of organic N under low nutrient availability. This is the case especially in ericoid mycorrhizal and ectomycorrhizal fungi which possess strong hydrolytic capacities to depolymerise complex organic N compounds (Read & Perez-Moreno 2003; Schimel & Bennett 2004). However, recent studies reveal that also arbuscular mycorrhizae could be more active in

organic N uptake than previously assumed (Whiteside *et al.* 2012), in addition to their well-recognized role in plant phosphorus nutrition.

Plant coexistence in nutrient-poor environments could be achieved by nutrient partitioning in space and time based on the chemical form of N taken up directly by plants or via their mycorrhizal symbionts (McKane *et al.* 2002). The large variety of inorganic and organic N forms in soils (Kielland 1994), the fluctuations of inorganic N and DON pools through the growing season (Stark & Väisänen 2014), and differences in uptake affinities for amino acids, ammonium and nitrate among tundra plant species (Chapin, Moilanen & Kielland 1993; Kielland 1994), suggest that partitioning on the basis of chemical N form could be an important mechanism facilitating species coexistence and maintaining plant diversity in the arctic.

Large herbivores exert a strong control over ecosystem functioning by influencing plant community composition, soil processes and nutrient cycling in natural ecosystems. Herbivores can increase nutrient cycling and mineral nutrient availability by favouring nutrient-rich and fast-growing plant species which produce easily decomposable litter (Bardgett & Wardle 2003) and through the conversion of plant biomass into nutrient-rich urine and dung that spatially redistribute nutrients within the ecosystems (Hobbs 1996) and stimulate microbial N mineralization (van der Wal *et al.* 2004). Enhanced soil nutrient availability increases plant nutrient content, which improves the quality of litter and creates a positive feedback that further enhances soil N cycling (Wardle *et al.* 2004).

In high latitudes, reindeer (*Rangifer tarandus*) may drive such positive feedbacks by causing a shift in the tundra vegetation from a moss-rich and dwarf shrub-rich heath into a grass dominated meadow (Olofsson *et al.* 2001) which results in more decomposable litter (Olofsson & Oksanen 2002), and increased soil nutrient availability (Olofsson, Stark & Oksanen 2004; Stark & Väisänen 2014). This positive feedback may be further intensified in cold systems through a reduction of the moss layer, which increases soil temperatures that in turn stimulate microbial activities and enhance soil nutrient availability for plants (Olofsson, Stark & Oksanen 2004; van der Wal & Brooker 2004; Gornall *et al.* 2007).

Herbivores also commonly affect plant symbiotic interactions. They may influence root mycorrhizal colonization and thus how plants acquire nutrients from the soil solution (1) directly by reducing carbon allocation to the roots due to the loss of photosynthetic tissues resulting from defoliation (Smith & Read 1997; Gehring & Whitham 2002) and (2) by changing soil nutrient availability as a consequence of tramping and urine and dung deposition (Smith & Read 1997; Tuomi, Kytoviita & Hardling 2001). The response of mycorrhizal root colonization to grazing ranges from negative to positive depending on the host plant species, ecosystem type and timing and intensity of herbivory (Eom, Wilson & Hartnett 2001; Barto & Rillig 2010; Murray, Frank & Gehring 2010; Ruotsalainen & Eskelinen 2011), and the response to intense and long-term grazing is poorly understood.

Despite the crucial ecological importance of reindeer for soil nutrient cycling and plant-soil interactions in tundra, evidence for long-term effect of herbivores on how plants access different N sources is lacking. Natural N isotope ratios ($^{15}N/^{14}N$) in ecosystem pools provide an effective tool for analysing ecosystem nutrient dynamics, as changes in plant and soil ^{15}N natural abundance integrate the net effect of mechanisms in plant nutrient uptake and N

cycling (Nadelhoffer & Fry 1994; Högberg 1997; Robinson 2001). For example, enhanced N cycling is often associated with increased losses of the ^{15}N-depleted products of N mineralization from the soil system (gaseous N forms or leaching of inorganic N), which leave the remaining inorganic N pool enriched in ^{15}N (Amundson *et al.* 2003). If herbivores enhance soil N cycling, they are thus also expected to increase the δ^{15}N of the system.

Foliar δ^{15}N in plants does not only depend on soil δ^{15}N, but is also associated with the presence and the type of mycorrhizal association. The lowest δ^{15}N signatures are reported for plants associated with ericoid mycorrhizae, followed by ectomycorrhizae and arbuscular mycorrhizae with the highest δ^{15}N signatures found in non-mycorrhizal plants (Craine *et al.* 2009). The ^{15}N-depleted signatures observed in mycorrhizal plants are the result of the ^{15}N fractionation associated to N assimilation from the rhizosphere into the fungal hyphae (Macko *et al.* 1986; Jin *et al.* 2005), internal transfer reactions (Hobbie & Hobbie 2006), and from the proportion of ^{15}N retained in fungal symbionts which is expected to decrease the plant δ^{15}N under strong nutrient limitation (Hobbie & Colpaert 2003). Independent of the relative importance of the processes involved, herbivores would be expected to reduce the δ^{15}N differentiation among plants if herbivory reduces the importance of mycorrhizae in plant nutrient acquisition and reduces the resource partitioning among plant species based on chemical N form.

Reindeer grazed tundra is a perfect study system to address these questions since the differentiation in foliar δ^{15}N is pronounced in nutrient-poor environment (Michelsen *et al.* 1996; Nadelhoffer *et al.* 1996; Michelsen *et al.* 1998) and the effect of herbivores on nutrient cycling is strong (Olofsson, Stark & Oksanen 2004; van der Wal *et al.* 2004). In our study site, intense defoliation, trampling and dung and urine deposition have transformed the initial

heath vegetation into a graminoid-dominated vegetation where the abundance of mosses and dwarf shrubs is severely reduced (Olofsson, Stark & Oksanen 2004). Concentrations of mineral nutrients are also drastically higher (Stark & Väisänen 2014) as a result of dung and urine deposition and higher plant litter quality (Olofsson & Oksanen 2002).

In this study, we investigated the long-term effects of reindeer on plant N uptake by exploring differences in $\delta^{15}N$ signatures in plant, microbe and soil N pools. We tested the following hypotheses: (1) Plant species have a pronounced differentiation in foliar $\delta^{15}N$ in the lightly grazed tundra (2) Reindeer reduce the differentiation in foliar $\delta^{15}N$ among plant species (3) Reindeer reduce mycorrhizal colonization of fine roots (4) Reindeer increase the $\delta^{15}N$ signatures of soil and microbial biomass N.

Material and Methods

Study site

The research site is located above the treeline (600-700m a.s.l) on the northern slope of Raisduoddar Fjell (69°31´N, 21°19´E) in the suboceanic northern Norway. Reindeer husbandry forms the dominant land-use in the area. At the study site, the annual precipitation is 935 mm and the annual mean temperature is -0.6 ° C (2006-2015, Norwegian Water Resources and Energy Directorate, www.senorge.no). The study was conducted across a 1-1.5 m high reindeer fence established in the 1960s to reduce the risk that reindeer enter migration areas during summer. The fence runs for several kilometres across the tundra and separates the landscape into two grazing areas; the heavily grazed summer range, and the adjacent lightly grazed spring and the autumn migration range (Olofsson, Stark & Oksanen 2004). The two sides of the fence differ only in terms of grazing intensity since there is no difference in the seasonal timing of grazing that takes place during second half of August.

At the lightly grazed sites, a dwarf shrub heath vegetation dominates with typical species such as *Empetrum hermaphroditum, Vaccinium vitis-idaea, Vaccinium uliginosum, Vaccinium myrtillus* and *Betula nana* (Olofsson *et al.* 2001) which are usually associated with high ectomycorrhizal and ericoid mycorrhizal colonization rates (Michelsen *et al.* 1998; Wang & Qiu 2006). Mosses and lichens are abundant with as the most common moss species *Pleurozium schreberi* and *Dicranum* sp. and as the most common lichen species *Nephroma arctica*, and *Peltigera* sp. Graminoids and herbs are present, but rare. At the heavily grazed sites, intense reindeer grazing transformed the original dwarf shrub vegetation into a graminoid dominated vegetation with strong increase in grass and sedge abundance, in particular *Carex bigelowii, Festuca ovina, Deschampsia flexuosa, Poa alpina* and *Phleum alpinum* with only some scattered dwarf shrubs (Olofsson *et al.* 2001). These graminoid species are either colonized by arbuscular mycorrhizae or are non-mycorrhizal (Michelsen *et al.* 1998; Wang & Qiu 2006).

Sampling

Foliar $\delta^{15}N$ and mycorrhizal colonization were measured in five common plant species which occur in the two grazing regimes. Study species included three dwarf shrubs, *B. nana, V. myrtillus* and *E. hermaphroditum*, a grass, *D. flexuosa* and a sedge, *C. bigelowii*. These were the only plant species that were common enough to get adequate samples from both sides of the fence. We selected 10 locations along the reindeer fence, apart from each other of about 30-40 m. In each of the 10 locations, a site was selected at the heavily and at the lightly grazed areas. All sites were within a distance of 5 m from the fence. Sites with similar physical abiotic conditions (i.e topography, sunlight and soil moisture) were selected and the

main differences in vegetation between the sides of the fence should thus be only controlled by a top down effect of reindeer.

Foliar tissues were collected from 5 to 10 individuals of each plant species at the 10 locations in late July 2011. Total plant biomass was also harvested in subplots (25 cm x 25 cm) randomly selected at each sites. Plant species were sorted at the species level with the exception of grass and sedge which were pooled into the graminoid functional group. All the plant samples were oven-dried (60° C, 48 h) and weighed. Root samples were harvested from 10 individuals within three of the 10 locations by tracing the fine roots from the main root down into the soil in late July 2012. Root samples were kept moist in plastic bags, brought to the laboratory, thoroughly washed with water and stored in plastic vials in 70 % ethanol. Fresh reindeer dung was collected at close location of the reindeer herd at the heavily grazed sites.

A first set of three soil cores (total estimated volume of 500 cm^3) were taken for determining total soil N and $\delta^{15}N$ at three sites along the fence. Another set of three soil samples were collected at each plant sampling site in 2011 for determining extractable and microbial N and $\delta^{15}N$. Only the thin humus layer was sampled in each sampling sites and the mineral layer was discarded. Soil samples were kept frozen until analysis. Soil temperatures were recorded at mid-day at 10 cm depth at each sampling location with a rugged thermometer coupled to a HI-765BL probe, Hanna Instrument (range -50° C to 150° C, resolution 0.1° C). The sampling was timed to correspond with periods of peak plant biomass (end of July- beginning of August).

Mycorrhizal colonization analysis

Root samples for mycorrhizal colonization analysis were cleared, stained and mounted on slides. Mycorrhizal type was determined for each plant species and colonization rate was assessed as the proportion of arbuscules, fungal mantle or fungal coils intercepted in 1 cm long root segments (<1 mm diameter) by using a modification of the line intersection procedure (McGonigle *et al.* 1990). A detailed description of the root sample preparation and mycorrhizal colonization analysis is presented in Appendix S1 in Supporting information.

Chemical analysis

Leaf and dung samples were dried at 60° C and the first set of soil samples at 105° C (48 h). Samples were finely grinded and subsamples were encapsulated into preweighed tin capsules for estimation of total N and $\delta^{15}N$. The remaining larger set of soil samples, for estimation of extractable and microbial N and $\delta^{15}N$, was thawed at room temperature and sieved (mesh 2 mm). Soil water content was measured gravimetrically (12 h, 105° C) and organic matter content was determined by loss on ignition (12 h, 475° C).

Soil microbial N was determined as the chloroform labile fraction using the chloroform-fumigation-extraction technique described by Brookes *et al.* (1985). A subsample of 2 g of soil was 0.5 M K_2SO_4 extracted without fumigation for determination of total extractable N (N_e) and another subsample of 2 g of soil was extracted after fumigation for determination of microbial N (N_{mic}). All the extracts were filtered through Whatman No 1 filter paper. Total extractable N in the non-fumigated extracts was determined by oxidizing the entire extractable N to NO_3^- (Williams *et al.* 1995) which was then measured by automated flow analyser (N, FIA Perstorp). Microbial N content was calculated by subtracting total

extractable N from the N present in the fumigated extracts (N_f). To determine extractable $\delta^{15}N$ ($\delta^{15}N_e$) and microbial $\delta^{15}N$ ($\delta^{15}N_{mic}$), fumigated and non-fumigated extracts were analyzed for $^{15}N/^{14}N$ isotopic ratio following a modification of the acid trap diffusion technique on a subsample of 10 ml of digested extracts (Holmes *et al.* 1998). Plant and soil samples, and diffusion disks were analysed for $\delta^{15}N$ and elemental N content 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 University of California, Davis. Dung samples were analysed for $\delta^{15}N$ and elemental N content on a Roboprep, a Dumas-type continuous-Flow CHN analyser, coupled to a tracermass isotope ratio mass spectrometer (Europa Scientific Ltd, UK), at the University of Copenhagen. A detailed description of the chloroform-fumigation-extraction method, acid trap diffusion technique and isotopic calculation is presented in Appendix S2.

Statistics

Differences in plant biomass between the lightly and heavily grazed sites were tested using Wilcoxon rank sum test with continuity correction. The deciduous shrub *B. nana* was only present in the sampled biomass at the lightly grazed sites in 2011. Thus, the effects of grazing intensity on *B. nana* abundance were not tested. Differences in soil properties, total extractable and microbial N pool, $\delta^{15}N_{soil}$, $\delta^{15}N_{mic}$ and mycorrhizal colonization of fine roots between the lightly and heavily grazed sites were compared using Student`s t-test. Differences in foliar $\delta^{15}N$ and N concentrations among species and within-species differences in the response to grazing intensity were tested using two-way analysis of variance (ANOVA) with plant species and grazing intensity as categorical variables. Akaike´s information criteria and residual plots were used to assess the fit of the model. A Tukey`s studentized range (honestly significant difference) test was used to examine posteriori differences among

species means. The data fulfill the assumption of normality and heteroscedasticity with the exception of plant biomass data. All statistical analyses were performed using the R statistical package (R development Core Team 2016).

Results

Plant species abundance

The long term heavy reindeer pressure on the tundra vegetation strongly changed the abundance of the studied plant species (Fig. 1). The dwarf shrubs *E. hermaphroditum* and *B. nana* almost totally disappeared in the heavily grazed sites (*E. hermaphroditum*: $P<0.001$), while the abundance of the dwarf shrub *V. myrtillus* was strongly reduced ($P=0.004$). By contrast, there was a dramatic increase in the aboveground biomass of the graminoids under intense reindeer grazing ($P<0.001$).

Plant and dung ^{15}N natural abundance

In the lightly grazed tundra, foliar δ^{15}N signatures differed dramatically among plants with δ^{15}N values ranging from about -7.67 ‰ to 5.01 ‰ (Table 1, Fig. 2). Plant species identity explained a large part of the variation in δ^{15}N. The lowest signatures were recorded in *E. hermaphroditum*, followed by *V. myrtillus*, *B. nana*, *D. flexuosa* and *C. bigelowii* (Fig. 2). Plant species ^{15}N abundance was closely correlated with the presence and the type of mycorrhizal association in the plant root with the lowest values in the two shrubs with ericoid mycorrhiza (*E. hermaphroditum* and *V. myrtillus*), followed by the shrub with ectomycorrhizae (*B. nana*). The grass with arbuscular mycorrhiza (*D. flexuosa*) had intermediate and the non-mycorrhizal sedge (*C. bigelowii*) highest δ^{15}N signatures (Fig. 2).

Reindeer had no effect on the average $\delta^{15}N$ values in all plants if species identity is not considered (heavily grazed: -2.25; lightly grazed: -2.29, P=0.936). However, the range in $\delta^{15}N$ signature among plant species was lower in the heavily grazed sites (8.61 ‰) than in the lightly grazed sites (12.68 ‰), resulting in a significantly smaller variance in $\delta^{15}N$ in the heavily grazed sites (heavily grazed: 2.46 ‰; lightly grazed: 6.21 ‰, P=0.001). This decrease in the range of $\delta^{15}N$ values among plant species was caused by an increase in the ^{15}N values in the species with the lowest $\delta^{15}N$ signature (*E. hermaphroditum*) with an enrichment by 1.39 ‰ (P=0.031) and a decrease in the ^{15}N values in the species with the highest $\delta^{15}N$ signature (*C. bigelowii*) with a depletion by 2.14 ‰ (P=0.015) in the heavily grazed sites (Table 1, Fig. 2). The $\delta^{15}N$ signatures tended to be higher at the heavily grazed sites in the two other shrubs species with ericoid mycorrhizae and ectomycorrhizae, *V. myrtillus* and *B. nana*, albeit not statistically significant. $\delta^{15}N$ natural abundance in the arbuscular mycorrhizal grass *D. flexuosa* was not influenced by reindeer (P=0.940, Fig. 2).

Plant and dung N concentration

Foliar N concentrations differed among species (Table 1, Fig. 3). The deciduous dwarf shrub *B. nana* and the sedge *C. bigelowii* had the highest foliar N concentrations, the evergreen dwarf shrub *E. hermaphroditum* and the grass *D. flexuosa* the lowest N concentration, while the dwarf shrub *V. myrtillus* was intermediate (Fig. 3). Foliar N concentrations were also higher (P=0.005) in all plant species in the heavily grazed sites, with no interactions between species and grazing regimes (Table 1). The largest effect of reindeer grazing was observed in *B. nana*, with 12 % higher N concentrations in the heavily grazed sites (Fig. 3, P=0.002). The N concentration was positively correlated with the $\delta^{15}N$ values in all plants (R=0.72, P<0.001). When each species was analysed separately, positive correlations were detected in *E. hermaphroditum* (R=0.67, P<0.001) and *V. myrtillus* (R=0.72, P<0.001), while no

significant correlations were detected in the other species. Dung had higher N concentration (2.32 ± 0.44 %, Fig. 3) than most of the sampled tundra plant species.

Mycorrhizal colonization of fine roots

The two ericaceous shrub species, *E. hermaphroditum* and *V. myrtillus* were colonized by ericoid mycorrhiza and the dwarf shrub *B. nana* formed ectomycorrhizal symbiosis. The grass species *D. flexuosa* was colonized by arbuscular mycorrhizae, while the sedge *C. bigelowii* was non-mycorrhizal at all sites in the two grazing systems. Dual colonisations by different types of mycorrhizal fungi did not occur in any of our sampled plant species. Mycorrhizal colonization of fine roots of *B. nana* differed strongly between the two grazing regimes with a decrease by about 50 % ectomycorrhizal colonization rate in the heavily grazes sites (P<0.001) (Fig. 4a). Mycorrhizal colonization of fine roots of *E. hermaphroditum* was also significantly higher at the lightly grazed sites (P=0.014, Fig. 4b), while no significant differences were observed in *V. myrtillus* and *D. flexuosa* between the two reindeer grazing systems (Figs 4c and 4d).

Soil properties

Reindeer influenced soil properties and nutrient availability (Table 2, Figs 5 and 6). Soil temperature and moisture contents were higher (P<0.001 and P=0.025, respectively) and organic matter content (P=0.033) was lower in the heavily grazed sites (Table 2). Extractable N pool per unit of SOM was two times higher in the heavily grazed sites than in the lightly grazed sites (P=0.006, Fig. 5a). The microbial soil N pool did not differ statistically significantly between the two grazing regimes (P=0.373, Fig. 5b). The $\delta^{15}N$ signature in the soil extractable N pool (-10 ‰) was lower and the $\delta^{15}N$ signature in the microbial N pool (+17 ‰) was higher than corresponding values for any of the plant species (Fig. 6). $\delta^{15}N$

values of the total soil N pool and microbial N pool were higher in the heavily grazed sites (P=0.049 and P=0.040 respectively, Fig. 6).

Discussion

We tested the hypothesis that reindeer grazing reduces foliar $\delta^{15}N$ differentiation among coexisting plant species. We found strong support for this hypothesis. Large differences in the $\delta^{15}N$ signatures were observed among plant species in the lightly grazed sites, while the differences were smaller among plant species in the heavily grazed sites. These observations contribute to a growing body of evidence showing that $\delta^{15}N$ in arctic plants varies consistently among species and functional types (Michelsen *et al.* 1996; Nadelhoffer *et al.* 1996; Michelsen *et al.* 1998; Craine *et al.* 2009), and reveal for the first time that the interspecific variation in $\delta^{15}N$ signatures is diminished by herbivores. Lower variation in plant $\delta^{15}N$ signatures in the heavily grazed sites, without corresponding differences in the average $\delta^{15}N$, demonstrates a high potential of reindeer to influence plant nutrient uptake and to decrease partitioning of N forms among coexisting arctic plant species. We suggest that lower variation in $\delta^{15}N$ signatures among coexisting plant species in the heavily grazed sites derives from several interacting mechanisms, including nitrogen sources, plant mycorrhizal type and the importance of mycorrhizal-mediated nutrient uptake, and ^{15}N discriminating soil processes.

At high mineral nutrient availability, conditions that predominate in the heavily grazed sites, all plants could access the more easily available N sources. 50 years of reindeer grazing has indeed increased soil nitrogen availability, evident both from a higher extractable N in the soil and higher N concentrations in all plant species investigated in this study, and from a higher mineral nutrient availability in the soil throughout the growing season (Stark &

Väisänen 2014). In line with previous work (Olofsson, Stark & Oksanen 2004), soil temperatures and soil moisture were higher at the heavily grazed sites. Since graminoid litter decomposes faster than ericoid litter, the change in vegetation and in soil microclimates contributes to an increased nutrient turnover together with nutrients from dung and urine (Olofsson & Oksanen 2002). Intense but infrequent herbivory often induces a large reduction in root biomass as a result from a higher allocation of nutrients and carbohydrate in the aboveground plant parts and from an increase in root mortality (Guitian & Bardgett 2000). Since plants in the heavily grazed sites were also more defoliated, the more similar plant $\delta^{15}N$ signatures could thus result from a reduced capacity of defoliated plants to access complex organic N sources.

Difference in the mycorrhiza-mediated N uptake between the lightly and the heavily grazed sites is also a possible mechanism that could contribute to the reduced variation in plant $\delta^{15}N$ signatures. In the lightly grazed sites, the foliar $\delta^{15}N$ signatures correspond to our predictions based on their mycorrhizal type (Michelsen *et al.* 1996; Nadelhoffer *et al.* 1996; Michelsen *et al.* 1998; Craine *et al.* 2009): shrubs with ericoid mycorrhizae (*Empetrum hermaphroditum* and *Vaccinium myrtillus*) had lower $\delta^{15}N$, followed by the shrub with ectomycorrhizae (*Betula nana*), the graminoid with arbuscular mycorrhizas (*Deschampsia flexuosa*) was intermediate and the non-mycorrhizal graminoid (*Carex bigelowii*) had the highest $\delta^{15}N$. In the lightly grazed tundra, litter from mycorrhizal dwarf shrubs is probably the main source of amino acids which dominate the soil N pools of arctic soils and are more ^{15}N-depleted than other N pools such as hydrolysable ammonium (Yano *et al.* 2010). ^{15}N-depleted amino acids constitute a large part of the dwarf shrub nutrition (Chalot & Brun 1998), and have been suggested to cause the depleted ^{15}N signal of ericoid mycorrhizal and ectomycorrhizal plants (Michelsen *et al.* 1996). Lower $\delta^{15}N$ in ericoid mycorrhizal relative to ectomycorrhizal plants

could result from higher saprotrophic capacities of ericoid mycorrhizae that enable these fungi to access and degrade a larger variety of organic compounds, including polyphenols (Bending & Read 1996).

The lower differentiation of foliar $\delta^{15}N$ signatures and the ^{15}N enrichment of *E. hermaphroditum* in the heavily grazed sites indicate that reindeer reduce the importance of the specific mechanisms of the nutrition of ericoid mycorrhizal and ectomycorrhizal plants. With increasing N availability, dwarf shrubs could decrease their uptake of $\delta^{15}N$-depleted amino acids and other forms of organic N via the mycorrhizae. Since the depleted ^{15}N observed in mycorrhizal plants is also affected by the ^{15}N fractionation associated to N assimilation, transfer and retention within fungal hyphae (Macko *et al.* 1986; Hobbie & Colpaert 2003; Jin *et al.* 2005; Hobbie & Hobbie 2006), both N sources used and the role of mycorrhiza in N uptake could contribute to the observed changes in foliar $\delta^{15}N$ signatures.

In accordance with our hypothesis, the mycorrhizal colonization in fine roots of *B. nana* and *E. hermaphroditum* was lower in the heavily grazed sites. The increased nutrient availability in the soil, trampling and defoliation could all underlie the reduction in mycorrhizal colonization. Defoliation reduces allocation of C to roots by reducing photosynthetic capacity of plants and could thus reduce mycorrhizal colonization of fine roots, although a recent meta-analysis concluded that this occurs only in a limited subset of plant species (Barto & Rillig 2010). In line with that, reindeer had no effect on mycorrhizal colonization rate in *V. myrtillus* and *D. flexuosa* in our study.

As isotopic fractionation associated with mycorrhizal symbiosis does not occur in the non-mycorrhizal sedge, any changes in *C. bigelowii* $\delta^{15}N$ signatures are due to differences in direct plant N uptake. Thus, the decrease in foliar $\delta^{15}N$ values indicates that *C. bigelowii* utilizes N sources with lower $\delta^{15}N$ signatures in the heavily grazed sites. In this line, in addition to a probable higher uptake of inorganic N, *C. bigelowii* could readily use labile N compounds leached from reindeer urine and dung since both dung and heavily grazed *C. bigelowii* individuals have a $\delta^{15}N$ signatures of about -1 which is within the range of the $\delta^{15}N$ signatures documented for reindeer urine (Finstad & Kielland 2011). Ammonia from urine and dung volatilizes very rapidly and up to 40 % of the inorganic N from animal wastes can be lost via NH_3^- volatilization (Bussink & Oenema 1998). The absorption of ^{15}N-depleted NH_3^- volatilized from ungulate urine have been shown to decrease the foliar $\delta^{15}N$ of graminoids in temperate grazed grasslands (Frank, Evans & Tracy 2004). Although ammonia volatilization rates in this system are unknown, this mechanism could also contribute to the decrease in $\delta^{15}N$ signatures of *C. bigelowii* in the heavily grazed sites. Although *Carex.* sp $\delta^{15}N$ varies substantially among sites depending on local climatic conditions and soil properties (Welker, Jonsdottir & Fahnestock 2003), the high $\delta^{15}N$ signature for *C. bigelowii* at the lightly grazed sites is in line with previous observations in nutrient-poor arctic tundra for the same species and for other for non-mycorrhizal plants with typical values ranging between 0 to ±4 ‰ (Michelsen *et al.* 1996; Michelsen *et al.* 1998; Welker, Jonsdottir & Fahnestock 2003). This high $\delta^{15}N$ signature is also not restricted to *C. bigelowii* at the lightly grazed sites but can be extended to the entire graminoid community (Barthelemy, unpublished data). High $\delta^{15}N$ in non-mycorrhizal tundra plants has been attributed to the strong microbial immobilization and concurrent microbial ^{15}N discrimination which result in a ^{15}N enrichment of the remaining soil ammonium and of the plants which predominantly take up this inorganic N (Michelsen *et al.* 1996).

At the ecosystem level, niche partitioning in plant nutrient uptake is jointly governed by nutrient acquisition by the plant species and changes in the relative abundances of species in the plant community. By strongly reducing the abundances of both the ericoid mycorrhizal species *E. hermaphroditum* and *V. myrtillus*, and the ectomycorrhizal species *B. nana* and by increasing the abundance of arbuscular or non-mycorrhizal graminoids, intense reindeer grazing induces a shift in mycorrhizal plant community composition and substantially increases the proportion of non-mycorrhizal plants in the plant community. In the heavily grazed sites, graminoids replace dwarf shrubs although they are intensively defoliated, since they tolerate herbivory better through a higher capacity for compensatory growth under higher nutrient availability and are less sensitive to trampling than woody species (Chapin, McKendrick & Johnson 1986). A recent study with experimental reindeer dung addition also indicated that increased N concentration alone does not induce the vegetation shift towards graminoids, but disturbance by trampling is needed to break down the dominance of dwarf shrubs (Barthelemy, Stark & Olofsson 2015).

We hypothesized that reindeer grazing increases ^{15}N enrichment in soil and microbial biomass. In this line, soil $\delta\,^{15}N$ signatures were higher in the heavily than lightly grazed sites supporting previous findings of higher nutrient turnover (Olofsson, Stark & Oksanen 2004; Stark & Väisänen 2014). However, 50 years of heavy reindeer grazing have not changed the total nitrogen pool (Olofsson, Stark & Oksanen 2004), indicating a neutral long-term balance between nutrient inputs and outputs during this time. The higher microbial $\delta^{15}N$ in the heavily grazed sites in turn is likely directly related to the correspondingly higher $\delta^{15}N$ in the soil N pools. Similar effects of grazing have previously been reported across a cattle manure gradient (Dijkstra *et al.* 2006b). Dung and urine may increase $\delta\,^{15}N$ in the soil by providing

nutrient-rich highly decomposable resources with relatively high $\delta^{15}N$ (Olofsson & Strengbom 2000; Lindwall *et al.* 2013).

Soil extractable N pool was strongly ^{15}N-depleted and this findings is in line with previous studies in arctic soils (Yano *et al.* 2010). By contrast, microbial $\delta^{15}N$ showed substantial ^{15}N enrichment in comparison to soil and plant N pools, which agrees with earlier studies demonstrating that microbial biomass across ecosystems is consistently enriched in ^{15}N relative to the surrounding soil (Dijkstra *et al.* 2006a; Dijkstra *et al.* 2006b; Portl *et al.* 2007; Dijkstra *et al.* 2008; Coyle *et al.* 2009). This study is the first to analyse $\delta^{15}N$ in the microbial biomass in an arctic heathland. It is conceived that this enrichment derives from repeated processing of humified soil organic matter in which microbial biomass constitutes a major component (Dijkstra *et al.* 2006a). Similar to ^{15}N enrichment when crossing trophic level, each round of the internal microbial N cycling would enrich the ^{15}N in the remaining microbial biomass. As internal N cycling within the microbial biomass constitutes a particularly important component of element cycles in arctic soils (Schimel & Mikan 2005), this mechanism could yield to high microbial $\delta^{15}N$ across the arctic.

To conclude, this study reveals that intense grazing can reduce $\delta^{15}N$ signatures among coexisting plant species. Since variation in foliar $\delta^{15}N$ is considered to result from variation in uptake mechanisms and in the chemical form of N taken up by plants, our results indicate that herbivores do not only influence the soil nutrient availability, but also how plants acquire nutrients. Consequently, soil nitrogen concentration in different grazing regimes does not fully depict the nutrient availability for plants. Although further experimental manipulations are needed to identify the exact mechanisms and their relative importance, we suggest that the combined effect of reduced mycorrhiza-mediated nitrogen uptake, a shift towards direct

uptake of mineral forms of N, and the uptake of N compounds deriving from dung and urine was at the origin of the observed shift in plant $\delta^{15}N$ in heavily grazed areas.

Author contribution

HB and JO conceived the ideas and designed methodology; HB performed the sample collection; HB and SS conducted soil analysis, and HB and MMK conducted mycorrhizal analysis; HB conducted statistical analysis and led writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data accessibility

Data associated with this article are archived at the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.78084 (Barthelemy *et al.* 2017)

References

Amundson, R., Austin, A.T., Schuur, E.A.G., Yoo, K., Matzek, V., Kendall, C., Uebersax, A., Brenner, D. & Baisden, W.T. (2003) Global patterns of the isotopic composition of soil and plant nitrogen. *Global Biogeochemical Cycles,* **17,** 1031-1041.

Bardgett, R.D. & Wardle, D.A. (2003) Herbivore-mediated linkages between aboveground and belowground communities. *Ecology,* **84,** 2258-2268.

Barthelemy, H., Stark, S., Kytöviita, M.M., & Olofsson, J. (2017) Data from: Grazing decreases N partitioning among coexisting plant species. *Dryad Digital Repository,* http://dx.doi.org/10.5061/dryad.78084

Barthelemy, H., Stark, S. & Olofsson, J. (2015) Strong Responses of Subarctic Plant Communities to Long-Term Reindeer Feces Manipulation. *Ecosystems,* **18,** 740-751.

Barto, E.K. & Rillig, M.C. (2010) Does herbivory really suppress mycorrhiza? A meta-analysis. *Journal of Ecology,* **98,** 745-753.

Bending, G.D. & Read, D.J. (1996) Nitrogen mobilization from protein-polyphenol complex by ericoid and ectomycorrhizal fungi. *Soil Biology & Biochemistry,* **28,** 1603-1612.

Brookes, P.C., Landman, A., Pruden, G. & Jenkinson, D.S. (1985) Chloroform fumigation and the release of soil nitrogen- A rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biology & Biochemistry,* **17,** 837-842.

Bussink, D.W. & Oenema, O. (1998) Ammonia volatilization from dairy farming systems in temperate areas: a review. *Nutrient Cycling in Agroecosystems,* **51,** 19-33.

Chalot, M. & Brun, A. (1998) Physiology of organic nitrogen acquisition by ectomycorrhizal fungi and ectomycorrhizas. *Fems Microbiology Reviews,* **22,** 21-44.

Chapin, F.S., McKendrick, J.D. & Johnson, D.A. (1986) Seasonal-changes in carbon fractions in Alaskan tundra plants of differing growth form - Implications for herbivory. *Journal of Ecology,* **74,** 707-731.

Chapin, F.S., Moilanen, L. & Kielland, K. (1993) Preferential use of organic nitrogen for growth by a nonmycorrhizal arctic sedge. *Nature,* **361,** 150-153.

Coyle, J.S., Dijkstra, P., Doucett, R.R., Schwartz, E., Hart, S.C. & Hungate, B.A. (2009) Relationships between C and N availability, substrate age, and natural abundance C-

13 and N-15 signatures of soil microbial biomass in a semiarid climate. *Soil Biology & Biochemistry,* **41,** 1605-1611.

Craine, J.M., Elmore, A.J., Aidar, M.P., Bustamante, M., Dawson, T.E., Hobbie, E.A., Kahmen, A., Mack, M.C., McLauchlan, K.K., Michelsen, A., Nardoto, G.B., Pardo, L.H., Penuelas, J., Reich, P.B., Schuur, E.A., Stock, W.D., Templer, P.H., Virginia, R.A., Welker, J.M. & Wright, I.J. (2009) Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytol,* **183,** 980-992.

Dijkstra, P., Ishizu, A., Doucett, R., Hart, S.C., Schwartz, E., Menyailo, O.V. & Hungate, B.A. (2006a) C-13 and N-15 natural abundance of the soil microbial biomass. *Soil Biology & Biochemistry,* **38,** 3257-3266.

Dijkstra, P., LaViolette, C.M., Coyle, J.S., Doucett, R.R., Schwartz, E., Hart, S.C. & Hungate, B.A. (2008) N-15 enrichment as an integrator of the effects of C and N on microbial metabolism and ecosystem function. *Ecology Letters,* **11,** 389-397.

Dijkstra, P., Menyailo, O.V., Doucett, R.R., Hart, S.C., Schwartz, E. & Hungate, B.A. (2006b) C and N availability affects the N-15 natural abundance of the soil microbial biomass across a cattle manure gradient. *European Journal of Soil Science,* **57,** 468-475.

Eom, A.H., Wilson, G.W.T. & Hartnett, D.C. (2001) Effects of ungulate grazers on arbuscular mycorrhizal symbiosis and fungal community structure in tallgrass prairie. *Mycologia,* **93,** 233-242.

Finstad, G.L. & Kielland, K. (2011) Landscape Variation in the Diet and Productivity of Reindeer in Alaska Based on Stable Isotope Analyses. *Arctic Antarctic and Alpine Research,* **43,** 543-554.

Frank, D.A., Evans, R.D. & Tracy, B.F. (2004) The role of ammonia volatilization in controlling the natural N-15 abundance of a grazed grassland. *Biogeochemistry,* **68,** 169-178.

Gehring, C.A. & Whitham, T.G. (2002) Mycorrhiza-Herbivore Interactions: Population and Community Consequences. *Mycorrhizal Ecology, Ecological Studies* (eds M. van der Heijden & I. Sanders), pp. 295-320. Springer, Heidelberg.

Gornall, J.L., Jónsdóttir, I.S., Woodin, S.J. & Van der Wal, R. (2007) Arctic mosses govern below-ground environment and ecosystem processes. *Oecologia,* **153,** 931-941.

Guitian, R. & Bardgett, R.D. (2000) Plant and soil microbial responses to defoliation in temperate semi-natural grassland. *Plant and Soil,* **220,** 271-277.

Hobbie, E.A. & Colpaert, J.V. (2003) Nitrogen availability and colonization by mycorrhizal fungi correlate with nitrogen isotope patterns in plants. *New Phytologist,* **157,** 115-126.

Hobbie, J.E. & Hobbie, E.A. (2006) N-15 in symbiotic fungi and plants estimates nitrogen and carbon flux rates in Arctic tundra. *Ecology,* **87,** 816-822.

Hobbs, N.T. (1996) Modification of ecosystems by ungulates. *Journal of Wildlife Management,* **60,** 695-713.

Holmes, R.M., McClelland, J.W., Sigman, D.M., Fry, B. & Peterson, B.J. (1998) Measuring N-15-NH4+ in marine, estuarine and fresh waters: An adaptation of the ammonia diffusion method for samples with low ammonium concentrations. *Marine Chemistry,* **60,** 235-243.

Högberg, P. (1997) Tansley review No 95 - N-15 natural abundance in soil-plant systems. *New Phytologist,* **137,** 179-203.

Jin, H., Pfeffer, P.E., Douds, D.D., Piotrowski, E., Lammers, P.J. & Shachar-Hill, Y. (2005) The uptake, metabolism, transport and transfer of nitrogen in an arbuscular mycorrhizal symbiosis. *New Phytologist,* **168,** 687-696.

Jonasson, S., Michelsen, A., Schmidt, I.K., Nielsen, E.V. & Callaghan, T.V. (1996) Microbial biomass C, N and P in two arctic soils and responses to addition of NPK fertilizer and sugar: Implications for plant nutrient uptake. *Oecologia,* **106,** 507-515.

Kielland, K. (1994) Amino-acid-absorption by arctic plants- Implications for plant nutrition and nitrogen cycling. *Ecology,* **75,** 2373-2383.

Lindwall, F., Vowels, T., Ekblad, A. & Bjork, R.G. (2013) Reindeer grazing has contrasting effect on species traits in Vaccinium vitis-idaea L. and Bistorta vivipara (L.) Gray. *Acta Oecologica-International Journal of Ecology,* **53,** 33-37.

Macko, S.A., Estep, M.L.F., Engel, M.H. & Hare, P.E. (1986) Kinetic fractionation of stable nitrogen isotopes during amino-acid transamination. *Geochimica Et Cosmochimica Acta,* **50,** 2143-2146.

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L. & Swan, J.A. (1990) A new method which gives an objective-measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. *New Phytologist,* **115,** 495-501.

McKane, R.B., Johnson, L.C., Shaver, G.R., Nadelhoffer, K.J., Rastetter, E.B., Fry, B., Giblin, A.E., Kielland, K., Kwiatkowski, B.L., Laundre, J.A. & Murray, G. (2002) Resource-based niches provide a basis for plant species diversity and dominance in arctic tundra. *Nature,* **415,** 68-71.

Michelsen, A., Graglia, E., Schmidt, I.K., Jonasson, S., Sleep, D. & Quarmby, C. (1999) Differential responses of grass and a dwarf shrub to long-term changes in soil microbial biomass C, N and P following factorial addition of NPK fertilizer, fungicide and labile carbon to a heath. *New Phytologist,* **143,** 523-538.

Michelsen, A., Quarmby, C., Sleep, D. & Jonasson, S. (1998) Vascular plant N-15 natural abundance in heath and forest tundra ecosystems is closely correlated with presence and type of mycorrhizal fungi in roots. *Oecologia,* **115,** 406-418.

Michelsen, A., Schmidt, I.K., Jonasson, S., Quarmby, C. & Sleep, D. (1996) Leaf N-15 abundance of subarctic plants provides field evidence that ericoid, ectomycorrhizal and non- and arbuscular mycorrhizal species access different sources of soil nitrogen. *Oecologia,* **105,** 53-63.

Murray, T.R., Frank, D.A. & Gehring, C.A. (2010) Ungulate and topographic control of arbuscular mycorrhizal fungal spore community composition in a temperate grassland. *Ecology,* **91,** 815-827.

Nadelhoffer, K. & Fry, B. (1994) Nitrogen isotope studies in forest ecosystems. *Stable isotopes in ecology and environmental science* (eds K. Lajtha & R.H. Michener), pp. 22-44. Blackwell Scientific Publications, Boston.

Nadelhoffer, K., Shaver, G., Fry, B., Giblin, A., Johnson, L. & McKane, R. (1996) N-15 natural abundances and N use by tundra plants. *Oecologia,* **107,** 386-394.

Nadelhoffer, K.J., Giblin, A.E., Shaver, G.R. & Linkins, A.E. (1992) Microbial processes and plant nutrient availability in arctic soils. *Arctic Ecosystems in a Changing Climate: An Ecophysiological Perspective* (eds F.S. Chapin III, R.L. Jefferies, J.F. Reynolds, G.R. Shaver & J. Svoboda), pp. 281-300. Academic Press, San Diego.

Neff, J.C., Chapin, F.S. & Vitousek, P.M. (2003) Breaks in the cycle: dissolved organic nitrogen in terrestrial ecosystems. *Frontiers in Ecology and the Environment,* **1,** 205-211.

Olofsson, J., Kitti, H., Rautiainen, P., Stark, S. & Oksanen, L. (2001) Effects of summer grazing by reindeer on composition of vegetation, productivity and nitrogen cycling. *Ecography,* **24,** 13-24.

Olofsson, J. & Oksanen, L. (2002) Role of litter decomposition for the increased primary production in areas heavily grazed by reindeer: a litterbag experiment. *Oikos,* **96,** 507-515.

Olofsson, J., Stark, S. & Oksanen, L. (2004) Reindeer influence on ecosystem processes in the tundra. *Oikos,* **105,** 386-396.

Olofsson, J. & Strengbom, J. (2000) Response of galling invertebrates on Salix lanata to reindeer herbivory. *Oikos,* **91,** 493-498.

Portl, K., Zechmeister-Boltenstern, S., Wanek, W., Ambus, P. & Berger, T.W. (2007) Natural N-15 abundance of soil N pools and N2O reflect the nitrogen dynamics of forest soils. *Plant and Soil,* **295,** 79-94.

R development Core Team (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Read, D.J. & Perez-Moreno, J. (2003) Mycorrhizas and nutrient cycling in ecosystems - a journey towards relevance? *New Phytologist,* **157,** 475-492.

Robinson, D. (2001) delta N-15 as an integrator of the nitrogen cycle. *Trends in Ecology & Evolution,* **16,** 153-162.

Ruotsalainen, A.L. & Eskelinen, A. (2011) Root fungal symbionts interact with mammalian herbivory, soil nutrient availability and specific habitat conditions. *Oecologia,* **166,** 807-817.

Schimel, J.P. & Bennett, J. (2004) Nitrogen mineralization: Challenges of a changing paradigm. *Ecology,* **85,** 591-602.

Schimel, J.P. & Chapin, F.S. (1996) Tundra plant uptake of amino acid and NH4+ nitrogen in situ: Plants compete well for amino acid N. *Ecology,* **77,** 2142-2147.

Schimel, J.P. & Mikan, C. (2005) Changing microbial substrate use in Arctic tundra soils through a freeze-thaw cycle. *Soil Biology & Biochemistry,* **37,** 1411-1418.

Smith, S.E. & Read, D.J. (1997) *Mycorrhizal symbiosis Second ed*. Academic Press, San Diego CA USA.

Stark, S. & Väisänen, M. (2014) Insensitivity of soil microbial activity to temporal variation in soil N in subarctic tundra: Evidence from responses to large migratory grazers. *Ecosystems,* **17,** 906-917.

Tuomi, J., Kytoviita, M.M. & Hardling, R. (2001) Cost efficiency of nutrient acquisition and the advantage of mycorrhizal symbiosis for the host plant. *Oikos,* **92,** 62-70.

van der Wal, R., Bardgett, R.D., Harrison, K.A. & Stien, A. (2004) Vertebrate herbivores and ecosystem control: cascading effects of faeces on tundra ecosystems. *Ecography,* **27,** 242-252.

van der Wal, R. & Brooker, R.W. (2004) Mosses mediate grazer impacts on grass abundance in arctic ecosystems. *Functional Ecology,* **18,** 77-86.

Wang, B. & Qiu, Y.L. (2006) Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza*, **16,** 299-363.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setala, H., van der Putten, W.H. & Wall, D.H. (2004) Ecological linkages between aboveground and belowground biota. *Science,* **304,** 1629-1633.

Welker, J.M., Jonsdottir, I.S. & Fahnestock, J.T. (2003) Leaf isotopic (delta C-13 and delta N-15) and nitrogen contents of Carex plants along the Eurasian Coastal Arctic: results from the Northeast Passage expedition. *Polar Biology*, **27,** 29-37.

Whiteside, M.D., Digman, M.A., Gratton, E. & Treseder, K.K. (2012) Organic nitrogen uptake by arbuscular mycorrhizal fungi in a boreal forest. *Soil Biology & Biochemistry,* **55,** 7-13.

Williams, B.L., Shand, C.A., Hill, M., O´Hara, C., Smith, S. & Young, M.E. (1995) A procedure for the simultaneous oxidation of total soluble N and P in extracts of fresh

and fumigated soils and litters. *Communications in Soil Science and Plant Analysis*, **26,** 91-106.

Yano, Y., Shaver, G.R., Giblin, A.E. & Rastetter, E.B. (2010) Depleted N-15 in hydrolysable-N of arctic soils and its implication for mycorrhizal fungi-plant interaction. *Biogeochemistry*, **97,** 183-194.

Supporting information

Additional supporting information may be found in the online version of this article:

Appendix S1. Supplementary methods on mycorrhiza colonization analysis

Appendix S2. Supplementary methods on soil analysis

Figure legends

Figure 1 Aboveground biomass (g m^{-2}) of *Empetrum hermaphroditum, Vaccinium myrtillus*, *Betula nana*, and graminoids at the lightly and heavily grazed sites. The deciduous shrub *B. nana* was only present in the sampled biomass at the lightly grazed sites in 2011. Mean values ± standard errors are presented. Asterisks denote significance at $^{**}P < 0.01$ and $^{***}P < 0.001$.

Figure 2 $\delta^{15}N$ natural abundance (‰) in dung collected at the heavily grazed reindeer area and foliar $\delta^{15}N$ natural abundance in *Empetrum hermaphroditum*, *Vaccinium myrtillus*, *Betula nana*, *Deschampsia flexuosa* and *Carex bigelowii* in the lightly and heavily grazing sites with ERI, ericoid mycorrhizal fungi; ECM, ectomycorrhizal fungi; AM, arbuscular mycorrhizal

fungi; NON, non-mycorrhizal fungi. Mean values ± standard errors are presented. Asterisks (*) denote significance at $P < 0.05$.

Figure 3 N concentration (%) in dung collected at the heavily grazed reindeer area and in *Empetrum hermaphroditum*, *Vaccinium myrtillus*, *Betula nana*, *Deschampsia flexuosa* and *Carex bigelowii* in the lightly and heavily grazing sites. Mean values ± standard errors are presented. Asterisks (*) denote significance at $P < 0.05$.

Figure 4 Percentage of fine roots colonized by ectomycorrhizal fungi (ECM) in *Betula nana* (a), by ericoid mycorrhizal fungi (ERI) in *Empetrum hermaphroditum* (b) and *Vaccinium myrtillus* (c) and by arbuscular mycorrhizal fungi (AM) in *Deschampsia flexuosa* (d). Mean values ± standard errors are presented. Asterisks denote significance at $*P < 0.05$ and $***P < 0.001$.

Figure 5 (a) Soil total extractable N and (b) soil microbial N (μg g^{-1} SOM) at the lightly and heavily reindeer grazed sites. SOM, Soil organic matter. Mean values ± standard errors are presented. Asterisks (*) denote significance at $P < 0.05$.

Figure 6 $\delta^{15}N$ natural abundance in soil total N, soil extractable N and soil microbial biomass at the lightly and heavily reindeer grazed sites. Mean values ± standard errors are presented. Asterisks (*) denote significance at $P < 0.05$.

Table 1 Analyses of variance of the within plant species differences and grazing intensity effects on foliar $\delta^{15}N$ and N concentrations. The sampled plant species were *Empetrum hermaphroditum*, *Vaccinium myrtillus*, *Betula nana*, *Deschampsia flexuosa* and *Carex bigelowii*. The grazing intensity corresponds to the lightly and heavily reindeer grazing sites. Degrees of freedom (Df) and *F*-values are presented and the asterisks denote significance at $*P < 0.05$, $**P < 0.01$, $***P < 0.001$

	Df	$\delta^{15}N$	N
Plant species	4,87	28.11***	74.98***
Grazing intensity	1,87	0.90	13.01***
Plant species x Grazing intensity	4,87	4.49**	0.51

Table 2 Soil temperature, moisture and organic matter content (SOM) at the lightly and heavily grazed reindeer sites, mean ± SE are expressed. For each soil variables means with different letters are significantly different (Student´s t-test, α=0.05)

	Soil temperature (ºC)	Moisture (%)	SOM (%)
Lightly grazed	7.46 ± 0.20 a	38.07 ± 3.04 a	64. 73 ± 6.36 a
Heavily grazed	8.76 ± 0.15 b	47.54 ± 2.37 b	46.88 ± 4.24 b