Geese impact on the nitrogen cycle and especially on the fate of litter nitrogen in Artic wetlands

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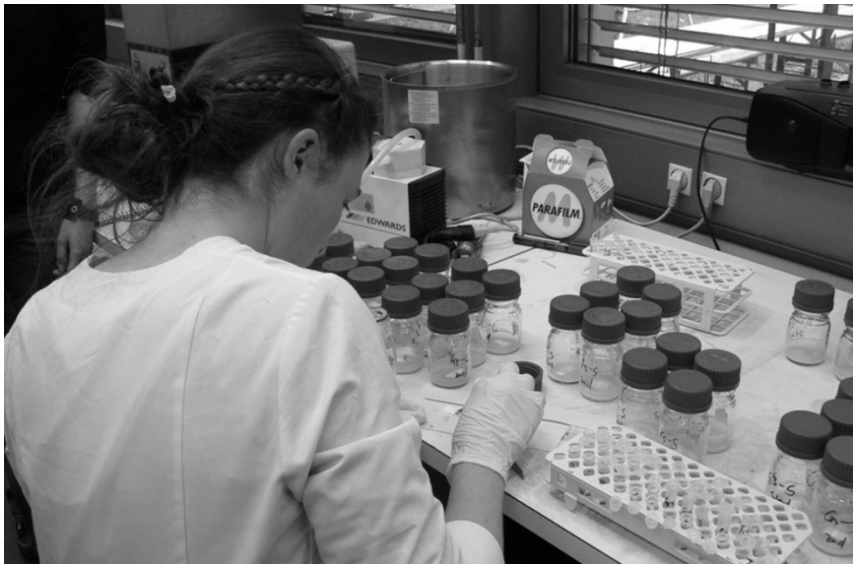
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Paper 3

Geese impact on the nitrogen cycle and especially on the fate of litter nitrogen in Artic wetlands

Manuscript

Lise Fivez, Ivan Janssens, Maarten Loonen, Pascal Boeckx, Patrick Meire

ABSTRACT

Due to land use changes and reduced hunting pressure in their wintering grounds, goose numbers increased dramatically over the past 50 years. To understand the consequences of these changes, studies on ecosystem processes of the breeding grounds in the Artic are indispensable. A key process affected by herbivores is decomposition, which in turn influences nutrient cycling and thus plant growth. Here, we investigated the influence of geese on the nitrogen cycle. In Spitsbergen (78° 55' N, 11° 56' E), we used paired long-term exclosures and control plots. Nitrogen incorporation from decomposing litter was studied by tracing the fate of ^{15}N originating from ^{15}N-labelled moss and grass litter. In this study we found indications of geese (grazing) impacting on almost all levels of nitrogen cycling. Geese change the start material for decomposition and nitrogen mineralisation by enhancing the nitrogen concentration and by redistribution of nitrogen among the different ecosystem compartments. Although goose grazing did not significantly alter nitrogen release from moss or grass litter, geese might indirectly have an impact on nitrogen release rates from plant litter by suppressing the production of grass litter, which was found to release nitrogen more readily than moss litter. Moreover, the fate of litter nitrogen varied through at least two mechanisms: i.e. the suppression of grass litter production and the reduction of the moss layer. Indeed, in this study a strong indication was found that nitrogen from grass litter is partly intercepted by the moss layer when it, after decomposition, migrates down to the rooting zone of vascular plants. In absence of geese the moss layer is thicker and more nitrogen from grass litter is intercepted. Already after one winter goose effects on release rates and redistribution from litter nitrogen were found. This means that geese even impact on the nitrogen cycle outside the growing season, when they overwinter further south, and underlines the need for more research over winter times.

Keywords: *N pools, decomposition, ^{15}N, nitrogen cycle, plant available nitrogen, herbivory, geese, Arctic*

INTRODUCTION

In Arctic ecosystems, most nutrients are fixed in the soil and undecomposed plant litter; only a low proportion is found in the living plant biomass (Jonasson et al. 1999a). The cold and wet soil environment and short summers, typically for the Arctic, slow down organic matter decomposition and nutrient mineralization. Consequently, despite the often very large nutrient pools (Jonasson 1983, Shaver et al. 1996), these ecosystems exhibit very low nutrient availability (Nadelhoffer et al. 1992) and ecosystem productivity is typically very low (Haag 1974, Ulrich and Gersper 1978, Chapin 1987). In terrestrial Arctic habitats nitrogen is often the most limiting factor for primary production (Nadelhoffer et al. 1992).

Changing the availability of nitrogen can impact microbial and plant communities, and ultimately affect herbivores, like grazing geese, if the quality and/or abundance of forage are altered (Bazely and Jefferies 1985). Geese might in turn also affect the nitrogen cycle in tundra systems (Cooch et al. 1991, Jano et al. 1998, Gornall et al. 2009). Herbivores are indeed found to impact on the nitrogen cycle in at least four different ways, namely by (i) redistributing the nitrogen among the different pools, (ii) influencing the decomposition process, (iii) altering the fate of nitrogen after decomposition and (iv) directing the form in which nitrogen becomes available.

First of all geese might change the distribution of nitrogen in the ecosystem (i). Indeed, they remove plant biomass and thus nitrogen, which is subsequently incorporated in goose biomass and faeces (figure 3.1). As geese are selective grazers (Black et al. 2007), biomass losses to foraging vary among plant species (paper 1, paper 2, Sjögersten et al. 2011). However, the distribution of nitrogen is not only a matter of (bio)mass but also of concentration. Because digestion efficiency in geese is poor, geese select for plants high in nitrogen (Mattocks 1971, Owen 1980, Prop and Vulink 1992, Alsos et al. 1998). Moreover geese are known to change the nitrogen content within plants species/functional groups (Cargill and Jefferies 1984, Phillips et al. 1999). Several mechanisms have been proposed to explain differences in nitrogen concentration of plant tissue between grazed and ungrazed areas (Bazely and Jefferies 1985, Sirotnak and Huntly 2000, Zacheis et al. 2002).

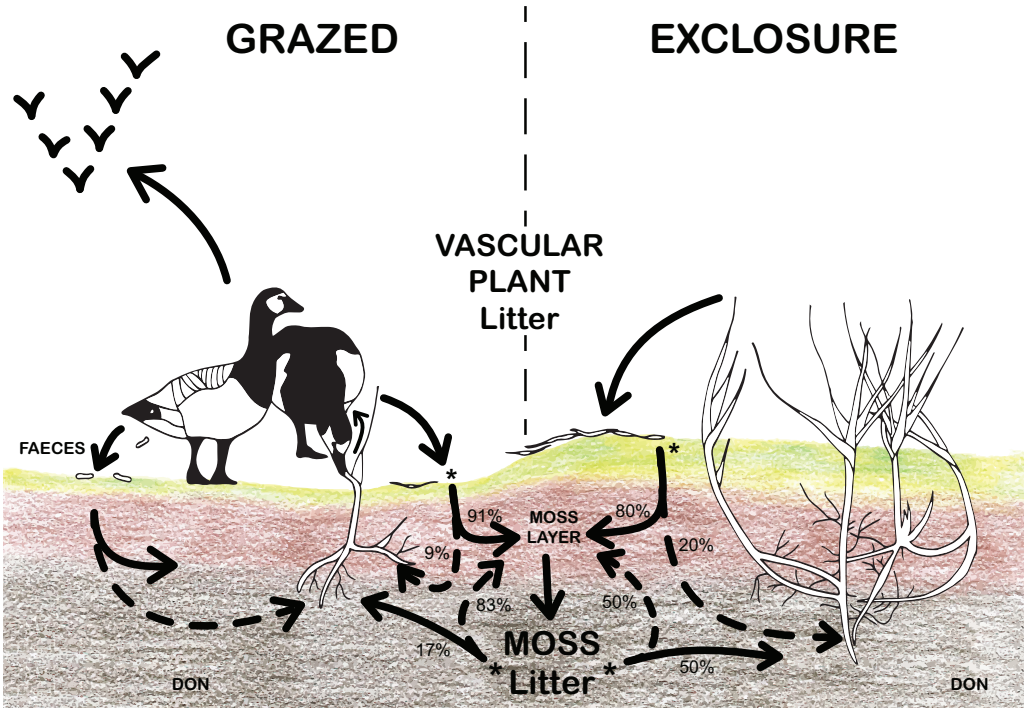


Figure 3.1. The influence of goose grazing on the nitrogen cycle in an Arctic wet tundra ecosystem. Arrows represent nitrogen fluxes. Different plausible ways of geese impacting on the tundra.

(i) Geese might change the **distribution of nitrogen** in the ecosystem. They remove N from plant biomass and incorporate it in their biomass and faeces.

(ii) Geese might impact on rates of **decomposition** and **nitrogen mineralization** (indicated by an *).

(iii) Geese might affect the **fate of nitrogen** after decomposition and mineralisation.

(iv) Geese might influence the availability of different **N forms** nitrate (NO_3^-) ammonium (NH_4^+) or dissolved organic nitrogen (DON).

Furthermore the redistribution of ^{15}N from labelled moss and grass litter after decomposition in moss (both photosynthetic active and non-active) and vascular plants (both aboveground and belowground) is given as measured in this study. The indicated percentages represent the mean relative recovery rate (n = 6).

One of those mechanisms is the goose impact on rates of decomposition and nitrogen mineralization, a second important mechanism through which these herbivores alter the nitrogen cycle (ii). Geese have been found to influence resource quality for decomposition (figure 3.1, paper 2). Indeed, goose grazing was found to impact severely on the vegetation composition in a range of Arctic habitats (Bazely and Jefferies 1986, Gauthier et al. 2004, Kuijper et al. 2009). Previous studies revealed that especially a shift in plant growth form composition can largely influence litter decomposition via a change in litter quality (Cornelissen et al. 2007). Moreover, geese are short-circuiting the litter production-decomposition cycle by returning faeces, which are swiftly decomposable and high in readily available nutrients (Bazely and Jefferies 1985, Hik and Jefferies 1990). Decomposition is also

affected by soil conditions and by microbial and invertebrate community structure (Swift et al. 1979). Geese impact on soil temperature (van der Wal et al. 2001), moisture and nutrient availability (Wilson and Jefferies 1996, Gornall et al. 2009), three environmental factors which are directly related to the rates of the decomposition process (Robinson et al. 1995, Hobbie 1996, Aerts et al. 2006). There is also ample evidence that herbivores, like geese, control the decomposer community. In unproductive ecosystems with low consumption rates, negative impacts on soil biota are most common (Bardgett et al. 1998, Bardgett and Wardle 2003). Research in the Nearctic has indeed revealed a rather negative impact on communities of soil invertebrates caused by goose grazing in wetlands (Sherfy and Kirkpatrick 2003). Moreover, geese were found to influence the microbial communities (paper 1). Finally, frequent trampling may accelerate decomposition by fragmenting the dead plant material and increase the rates of net nitrogen mineralization by incorporating litter into the soil (Zacheis et al. 2002, Sorensen et al. 2009). Geese thus have the capacity of impacting on the nitrogen availability for plants in soil.

A third mechanism through which geese affect the N-cycle encompasses the fate of nitrogen after decomposition and mineralisation (iii). Sjögersten et al. (2010) found indications that in a moss dominated system, mosses access more of the nitrogen released from faeces than the deeper rooting graminoids. The same might be true for nitrogen released from decomposing graminoid litter, which is found principally above the moss layer. In contrast nitrogen deriving from moss litter, shed at the moss-soil interface, might be primarily absorbed by graminoids (figure 3.1). The impact of geese on the ratio moss/graminoid litter in favour of moss litter (paper 2) and the decrease in depth of the moss layer due to grazing (paper 1, van der Wal et al. 2001) might thus limit the interception of nitrogen from decomposing litter by the moss layer.

Fourth and last, nitrogen occurs in many different forms and also the form in which nitrogen becomes available (nitrate, ammonium or dissolved organic nitrogen) and is taken up by plants might be influenced by herbivores (iv), as observed for cattle in grassland (Frank and Evans 1997).

Western Palearctic goose population numbers increased severely in the last 30 years (Madsen et al. 1996, O'Connell et al. 2006). Recent changes in climate, land use and the implementation of protective measures (e.g. reduced hunting pressure and improved refuge areas) were at the base as they have dramatically improved the birds' ability to survive the

winter (van Eerden et al. 1996, Fox et al. 2005, Gauthier et al. 2005, Kéry et al. 2006). Seen the potential of geese to alter ecosystem nitrogen turnover, this study aims to increase our understanding of the nitrogen cycle in Arctic coastal wetlands and specifically the impact of the high goose numbers. Long-term goose exclosures were erected in the Thiisbukta wetland (Kongsfjorden, Svalbard) frequented by a breeding colony of Barnacle Geese *Branta leucopsis* (Bechstein, 1803). An experiment with ^{15}N-labelled grass and moss litter, the two most abundant growth forms in the area, was set up within the exclosures and their control plots to test for following hypothesis:

- Nitrogen pool sizes are influenced by goose grazing, with especially a reduction in vascular plants;
- Grazing does change nitrogen release rates from plant litter and its fate;
- Goose grazing changes the plant available nitrogen content in the soil.

MATERIAL AND METHODS

Study site

The study was carried out in the Kongsfjorden area (78.55°N, 11.56°E) at Spitsbergen, Svalbard (figure B.1). The growing season is short with snowmelt around the beginning of June, followed by the thaw of the active layer covering the permafrost. The active layer gradually increases in depth until the end of August and the first new snow arrives around the start of September. Mean annual precipitation is 370 mm, which falls mostly outside the growing season, and mean annual temperature is -4.4 °C (data from www.eKlima.no, delivered by the Norwegian Meteorological Institute). In 1980, a first couple of breeding Barnacle Geese was observed in the area (Tombre et al. 1998). Over the subsequent years the new established population grew until a high of 900 adults in 1999 to fall back and stabilize between 450 and 800 adults (Kuijper et al. 2009). Barnacle Geese breed mainly on the islands in the fjord (Tombre et al. 1998). After hatching, during chick rearing and moulting, the Thiisbukta wetland in Ny-Ålesund, our studysite, is intensively used as forage habitat by families and non-breeders alike (Loonen et al. 1998). The depth of the soil organic layer is variable and exists mainly of poorly decomposed moss litter. The vegetation of this wetland is characterized by a continuous mat of mosses (*Calliergon* spec. as the most abundant) (Kuijper

et al. 2009). *Arctodupontia scleroclada* (Ruprecht) Tzvelev dominates the vascular plant composition. Grazing impact by other herbivores than Barnacle Geese is negligible. Just a few Pink-footed Geese *Anser brachyrhynchus* (Baillon, 1834) were observed for a short time at the beginning of the season and although Svalbard reindeer *Rangifer tarandus platyrhynchus* (Linnaeus, 1758) are observed throughout the season, grazing pressure by them is considered to be low (Kuijper et al. 2009).

Experimental design

To test our hypothesis we made use of six paired grazed and ungrazed plots (2 m x 2 m) in the Thiisbukta wetland. For the ungrazed plots, grazing was prevented by exclosures erected in 2003. The exclosures were made of chicken wire (0.5 m high) and protected with a cross of wires on top in order to prevent geese from landing in the exclosures, which proved effective. At the same time an identical reference plot was defined for each exclosure in the close neighbourhood. Our study was started in 2007, four years after the setup of the exclosures.

Production and incubation of labelled litter

We performed an incubation experiment with ^{15}N labelled litter of grasses and mosses. Mosses were labelled by spraying a plot of 1.5 m^2 with almost the same species composition as the experimental site three times a week from 4 July until 23 August 2007, with 1 L 3 mM of >98 atom% $^{15}NH_4^+$ $^{15}NO_3^-$. The labelling plot was fenced to prevent herbivores to remove the labelled mosses. At the end of the growing period the central part (0.75 m^2) was harvested. The photosynthetically active (green) part was subsequently removed and the resulting photosynthetically inactive (brown) moss was homogenized and used as a proxy for fresh moss litter.

1200 Young grass shoots of *Arctodupontia scleroclada*, the most common and abundant grass species in the Thiisbukta wetland were grown up in a greenhouse on a substrate of sand with ten percent of turf. Plants were harvested on 4 July 2007 in the neighbourhood of the experimental plots and only a small part of the roots was kept to make sure plants used the added (labelled) nutrients and didn't rely too much on their reserves. A labelled nutrient solution, a dilution of Murashige & Skoog nutrient solution (Murashige and Skoog 1962), made with premixed salts (Sigma-Aldrich) was added weekly from 4 July until 23 August 2007.

The ^{15}N labelled (>98%) $^{15}NH_4^+$ $^{15}NO_3^-$ was added as extra nitrogen. In total 10 % of the nitrogen in the nutrient solution consisted of $^{15}NH_4^+$ $^{15}NO_3^-$. Over the whole growing season nitrogen addition was 20 kg ha^{-1} (approximately four times the local atmospheric deposition or the typical nitrogen stock in vascular plants). Moisture was regulated by adding tap water. At the end of the growing period all grass was harvested. The root system was subsequently removed and the resulting grass litter was homogenized.

Labelling resulted in 1.30 and 5.02 atom% ^{15}N in excess present in moss and grass litter, respectively. ^{15}N-labelled litter from grasses (5.72 g DW m^{-2}) and mosses (328 g DW m^{-2}) was placed in two separate subplots (0.5 m x 0.5 m) in both the grazed plots and exclosures on 26 August 2007. This means that the concerned litter pool was on average increased by circa 25%, adding enough labelled litter without influencing litter abundance too much. Grass litter was incubated inside the green part of the moss layer, where grass litter is typically deposited also preventing it from being blown away. Moss litter was incubated at the place of moss litter production, namely at the moss-soil interface.

Sampling and chemical analysis

On 19 August 2007, 21 June 2008 and 8 August 2008, respectively before addition of labelled litter and after a winter and one year of incubation, samples were taken from the different ecosystem parts to determine the total mass, carbon (C), phosphorous (P) and nitrogen (N), natural abundance ^{15}N and ^{15}N enrichment in each compartment. In each plot we harvested four turfs of 9 cm^2 (end growing season 2007), six cores of 9.68 cm^2 (three in each subplot, start growing season 2008) or six turfs of 9 cm^2 (three in each subplot, end growing season 2008) to a soil depth (= depth under the moss-soil interface) of 10 cm. We used a knife at the end of the growing season to avoid compaction and a steel corer at the beginning of the growing season when the soil was still frozen at the time of sampling. After harvesting, samples were carefully sorted into mosses, vascular plants and roots. Moss tissue was split into photosynthetic active and inactive fractions, vascular plants into functional groups (graminoids, dicotyledons and equisetales) and further into living shoots and litter. For roots no attempt was made to make a distinction between the different functional groups or bio- and necromass, so total root mass was measured. Material from individual turfs was pooled

to give one value per plot. All samples were oven dried until constant mass at 35°C (> 96 h) and weighed and transported to the laboratory for total C, ^{15}N and N determination.

The organic soil was weighed (wet). After homogenisation four sub samples were taken. One sample was used to determine the ratio between wet and oven dry weight. Two other samples (10 g oven dry equivalent) were used to determine microbial N. The soil left was dried at 35°C and transported to the lab for total C, ^{15}N and N determination.

Microbial biomass N in the soil was measured using the chloroform fumigation direct extraction (CFDE) protocol (Brookes et al. 1985). Extraction and fumigation were started within 24 hours after sampling.

Samples for total C, total N and ^{15}N determination were ground with a planetary ball mill (Retsch, MM200, Germany) and analysed in duplicate using an elemental analyser (EA) interfaced to an isotope ratio mass spectrometer (IRMS) (20–20, SerCon, UK). Machine error (n=10) of this EA-IRMS system is 0.2‰ for $\delta^{15}N$.

Concentrations of total N, P of green moss and graminoid samples of 2007 were determined following an acid digestion (Walinga et al. 1989). Concentrations were determined on a colorimetric segmented flow analyser (Skalar, FAS, SA 20/40, Skalar Analytical B.V., Breda, the Netherlands) for N and P.

Plant available N was determined both during growing and winter season using PRS^{TM}-probes (Western Ag Innovations Inc., Saskatoon, SK, Canada). Four anion and cation PRS™-probes per plot were placed vertically in the soil to measure the nitrogen supply rates. The PRS™-probes were buried among plant roots, which provided a net nutrient supply rate (i.e., measuring the difference between total soil nutrient supply and plant uptake), therefore, yielding a measure of nutrient surplus rather than net mineralization over the burial period. However if we would exclude root competition we would still have competition from mosses.

After removal, the PRS™-probes were washed with deionized water, bulked per plot (anion and cation PRS™-probes that make up one sample were analysed together), and then eluted for one hour using 0.5 M HCl. The eluate was analysed for levels of ammonium (NH_4^+) and nitrate (NO_3^-) using automated colorimetric flow injection analysis system (Technicon autoanalyzer, Bran and Lubbe, Inc., Buffalo, NY). Nutrient supply rates generated with the PRS™-probes were reported as the amount of nutrient adsorbed per amount of adsorbing surface area per time of burial in soil.

Data analysis

Recovery rate of ^{15}N (RR, %) was calculated for plant material and soil by accounting for the natural abundance of ^{15}N.

$$RR(\%) = \frac{N\ \left(mol\,m^{-2}\right) x \left[{}^{15}N\ \left(At\%\right) - {}^{15}N\ \left(At\%\right)\ background\right]}{{}^{15}N\ added\ in\ excess\ \ (mol\,m^{-2})}$$

Relative recovery rates of ^{15}N (RRR %) for the mosses and vascular plants were calculated by summing the recovery rates of the concerned plant group and dividing by the total ^{15}N recovery in plants.

$$RRR\,Moss(\%) = \frac{RR\,Moss\,Green + RR\,Moss\,Brown}{RR\,Moss\,Green + RR\,Moss\,Brown + RR\,Roots + RR\,Graminoids\,Biomass}$$

$$RRR\,Vascular\ plants(\%) = \frac{RR\,Roots + RR\,Graminoids\,Biomass}{RR\,Moss\,Green + RR\,Moss\,Brown + RR\,Roots + RR\,Graminoids\,Biomass}$$

RR Graminoid litter is not taken up in the equation because in the case of labelled grass litter incubation, ^{15}N was added to this compartment.

We compared nitrogen limitation, total necromass and relative abundance of different litter types paired (corresponding grazed plots and exclosures) with a Student's t or Signed Rank test depending on normality. We tested for differences in nitrogen pool size, nitrogen content, ^{15}N recovery rate and plant available nitrogen using a repeated two way ANOVA with treatment (grazed or exclosure) as fixed factor and replica as random factor (proc mixed). To test if there was already a difference in ^{15}N recovery rate after only one winter of incubation or a difference in ^{15}N natural abundance values we used a coupled t-test (proc univariate normal). Effects were considered significant at $p \leq 0.05$ and data were transformed if necessary to meet the model criteria. Statistical analyses were performed using SAS version 9.2 (SAS Institute Inc. 2008).

RESULTS

Nitrogen pools and concentration (table 3.1, table 3.2)

A higher concentration of nitrogen was present in plant material of grazed plots compared to exclosures. The difference was significant for graminoids (shoots and litter) and mosses (photosynthetically active and inactive). For roots and soil no significant difference was found, although the nitrogen concentration in soil was almost significantly higher in the grazed plots (p=0.0507).

Relative to phosphorous, nitrogen concentrations can provide an indication whether or not nitrogen was a growth-limiting factor. The nitrogen to phosphorous ratios (N:P) were between 5.4 and 16.7 for graminoid shoots and 9.2 and 6.2 for photosynthetically active moss (figure 3.2). No significant difference was found between grazed plots and exclosures (n = 6, S = -4.5, p = 0.438 and n = 2, S = 1.5, p = 0.5 for mosses respectively graminoids).

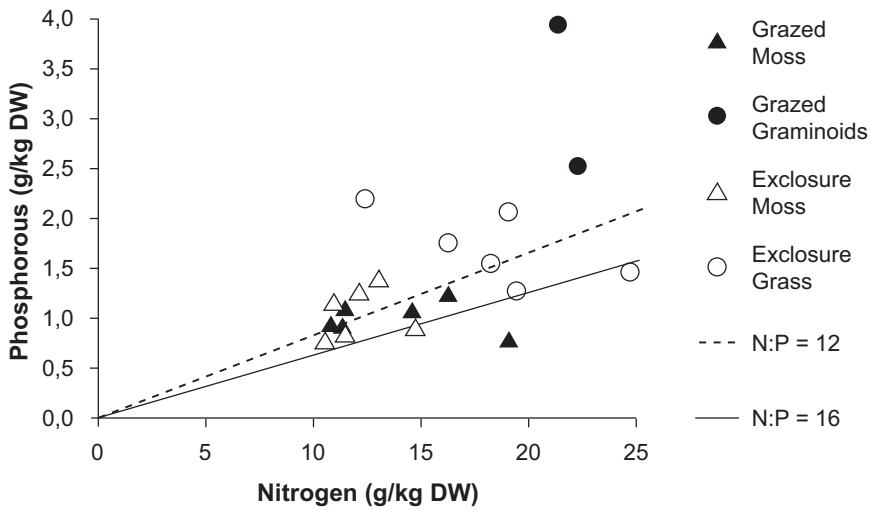


Figure 3.2 Foliar N:P ratios for moss (triangles) and graminoids (rounds) growing in grazed plots (black) and exclosures (open). The solid line represents an N:P ratio of 16, all samples beneath this line suggest phosphorous limitation, The dashed line represent an N:P ratio of 12, all samples above this line suggest nitrogen limitation, between both lines probably both N and P limitation occurs (Koerselman and Meuleman 1996, Aerts and Chapin 2000).

Table 3.1. Nitrogen concentration (%) in different components of the ecosystem. Data shown are mean values ± SE for grazed plots and exclosures. Statistical comparison between grazed and ungrazed plots (=treatment) is given and differences ($p \leq 0.05$) are indicated in bold.

	End Growing Season (19/08/'07)						Start Growing Season (21/06/'08)						Peak Growing Season (08/08/'08)						Treatment x time			Treatment		
	Grazed			Exclosure			Grazed			Exclosure			Grazed			Exclosure			*df*	*F*	*p*	*df*	*F*	*p*
Soil	0.6	±	0.1	0.6	±	0.1	0.73	±	0.19	0.64	±	0.20	0.95	±	0.15	0.53	±	0.14	2, 25	1.74	0.196	1, 27	4.18	**0.0507**
Dicotyls		na		2.4	±	0.5		na			na			na			na							
Equisetum sp.	2.2			1.9	±	0.3		na			na			na			na							
Graminoids Litter	1.5	±	0.3	1.3	±	0.1	1.79	±	0.11	1.53	±	0.14	1.65	±	0.13	1.35	±	0.16	2, 21	15.25	0.779	1, 23	16.44	**0.0005**
Graminoids Shoots	3.0	±	0.4	2.1	±	0.2	3.92	±	0.30	3.23	±	0.29	2.77	±	0.08	2.53	±	0.27	2, 20	1.15	0.336	1, 22	24.1	**<0.0001**
Moss Brown	1.2	±	0.1	1.1	±	0.1	1.38	±	0.07	1.12	±	0.05	1.36	±	0.09	1.18	±	0.06	2, 25	0.85	0.439	1, 27	16.75	**0.0003**
Moss Green	1.4	±	0.1	1.4	±	0.1	1.89	±	0.09	1.22	±	0.05	2.04	±	0.20	1.25	±	0.08	2, 24	9.09	**0.001**	1, 24	35.16	**<0.0001**
Roots	1.6	±	0.3	1.3	±	0.2	1.75	±	0.06	1.89	±	0.24	1.60	±	0.13	1.57	±	0.25	2, 25	1.52	0.239	1, 27	0.45	0.5074

Table 3.2. Nitrogen pools (g m^{-2}) in different components of the ecosystem. Data shown are mean values ± SE for grazed plots and exclosures. Statistical comparison between grazed and ungrazed plots (=treatment) is given and differences ($p \leq 0.05$) are indicated in bold.

	End Growing Season (19/08/07)						Start Growing Season (21/06/08)						Peak Growing Season 08/08/08)						Treatment x time			Treatment		
	Grazed			Exclosure			Grazed			Exclosure			Grazed			Exclosure			*df*	*F*	*p*	*df*	*F*	*p*
Soil	168	±	27	160	±	25	186	±	64	145	±	39	253	±	57	150	±	17	2, 25	0.72	0.495	1, 27	2.5	0.1256
Dycotyls	0.000	±	0.000	0.586	±	0.282		na			na			na			na					1, 5	S^a =5	0.125
Equisetum sp.	0.026	±	0.026	0.413	±	0.199		na			na			na			na					1, 5	S^a=7.5	0.0625
Graminoids Litter	0.132	±	0.020	0.622	±	0.241	0.246	±	0.036	0.846	±	0.250	0.215	±	0.036	0.925	±	0.275	2, 25	0.27	0.766	1, 27	25.24	**<0.0001**
Graminoids Shoots	0.261	±	0.072	1.429	±	0.385	0.299	±	0.084	0.498	±	0.183	0.280	±	0.072	1.067	±	0.347	2, 23	4.08	**0.031**	1, 23	18.85	**0.0002**
Microbial	0.229	±	0.159	0.140	±	0.056	0.084	±	0.019	0.108	±	0.017	0.123	±	0.035	0.108	±	0.031	2, 25	0.30	0.747	1, 27	0.05	08222
Moss Brown	15.13	±	1.88	20.49	±	2.67	15.36	±	1.90	15.85	±	1.30	12.77	±	0.83	14.82	±	0.80	2, 25	1.08	0.356	1, 27	3.6	0.0684
Moss Green	5.819	±	0.743	5.714	±	0.599	4.963	±	1.583	2.297	±	0.689	7.134	±	1.640	2.754	±	0.687	2, 25	3.01	0.068	1, 27	9.66	**0.0044**
Roots	0.460	±	0.120	3.333	±	1.158	0.538	±	0.146	1.925	±	0.760	1.934	±	0.431	7.266	±	1.103	2, 25	4.71	**0.018**	1, 25	36.31	**<0.0001**
Root/Shoot	2.022	±	0.452	1.442	±	0.379	6.391	±	0.508	8.855	±	1.384	1.878	±	0.225	4.555	±	1.551	2,23	1.84	0.181	1,25	3.23	0.084

In contrast to nitrogen concentrations, the nitrogen pools in the vegetation are larger in the exclosures compared to the grazed plots. Graminoid litter and shoots, photosynthetically active moss and roots encompassed significantly more nitrogen in the exclosures than in the grazed plots. No differences between grazed and ungrazed plots were found for the nitrogen pool sizes of photosynthetically inactive moss, equisetum and dicotyls (both litter and biomass). Also the microbial and soil nitrogen pool is similar for both grazed and ungrazed plots. For the nitrogen distribution (root to shoot ratio) the difference between grazed and ungrazed plots was only significant at the 0.1 level (p=0.084).

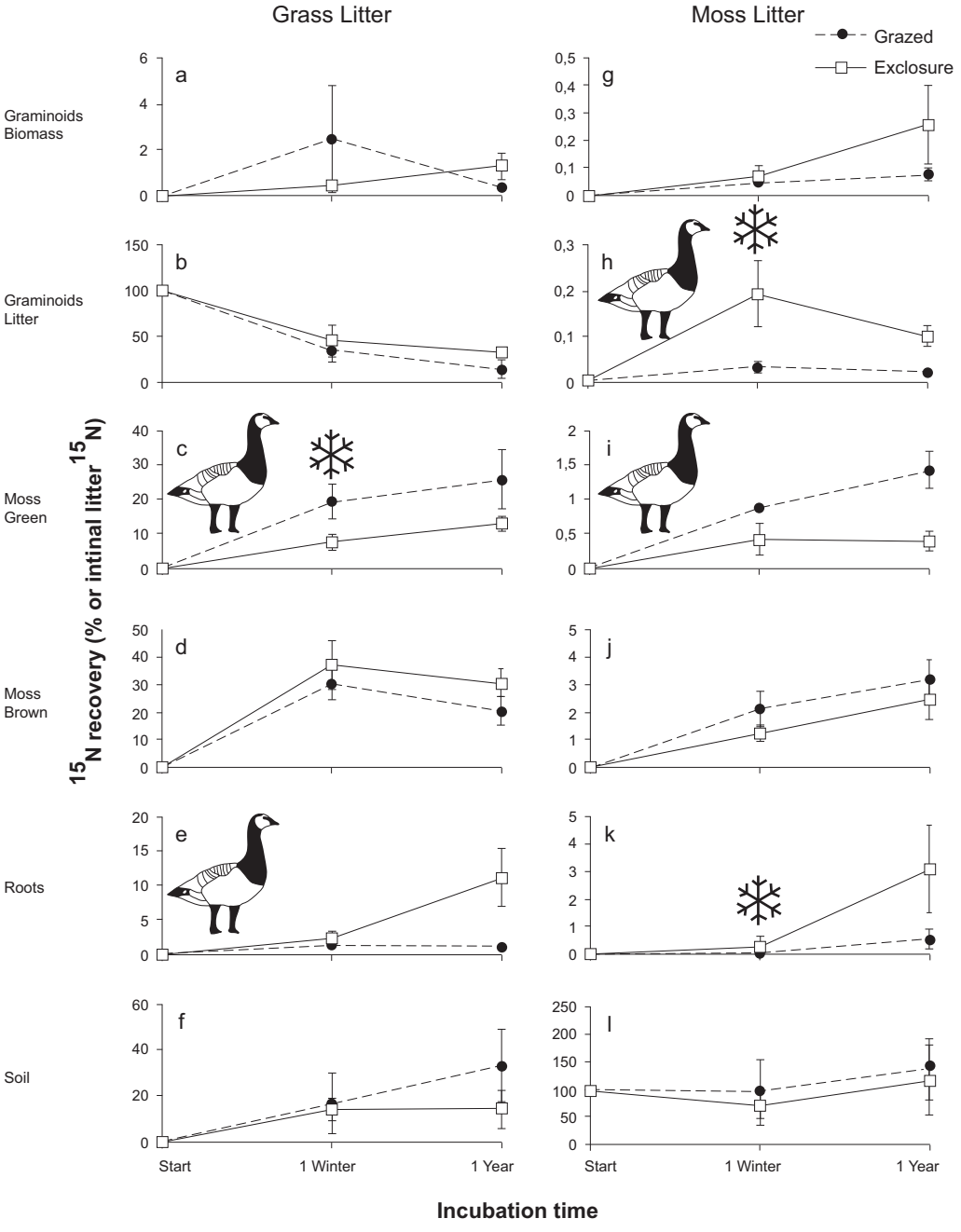
N-dynamics (figure 3.3, table 3.3)

After the first winter, substantial amounts of nitrogen (>50%) were already released from grass litter and redistributed among different ecosystem components (figure 3.3.B). The nitrogen release and redistribution from grass litter continued during the growing season. In contrast, moss litter released almost no nitrogen, not even after one year of incubation (figure 3.3.L). No difference in nitrogen release from litter types has been found between grazed plots and exclosures (figure 3.3.B and 3.3.L).

However, the fate of the nitrogen released during decomposition did differ between grazed and ungrazed plots. Looking at the nitrogen fluxes after one year of incubation, we found green moss to capture significantly higher amounts of nitrogen in grazed plots compared to exclosures for grass litter (figure 3.3.C). For moss litter this pattern was almost significant (p = 0.06; figure 3.3.I). In contrast, in graminoid litter (only relevant for moss incubation as for grass litter incubation this was the labelled pool) and roots, higher nitrogen recovery rates were found in the exclosures compared to the grazed plots (figure 3.3.H, 3.3.E and 3.3.K).

Moreover we noticed that already after one winter of labelled litter incubation, differences in ^{15}N uptake by certain compartments occurred between grazed plots and exclosures. For grass litter incubation the green moss compartment recovered less ^{15}N in the exclosures compared to the grazed plots (figure 3.3.H). For moss litter both the graminoid litter and roots compartments recovered more ^{15}N in the exclosures compared to the grazed plots (figure 3.3.C and 3.3.K). For the compartments graminoids biomass, photosynthetically inactive (brown) moss and soil, no significant difference in ^{15}N recovery was found between grazed

plots and exclosures, neither for grass litter nor for moss litter (figure 3.3.A, 3.3.G, 3.3.D, 3.3.J and 3.3.F).



← Figure 3.3. Average recovery rates of ^{15}N (= the percentage of ^{15}N which was originally present in the labelled litter) originating from grass respectively moss litter for different ecosystem components (n=6) after a winter season and one year of incubation in grazed plots and exclosures. Error bars represent the standard error. The left part (panels A-F) represents the subplots with grass litter incubation and the right part (panels G-L) those with moss litter incubation. Please note that the scale of the y-axis is varying between graphs. The labelled compartment is indicated by putting the graph in bold. For grass litter this is obvious namely the graminoid litter compartment. Moss litter at the other hand was incubated at the moss soil interface and as such became part of the soil compartment. The compartments indicated by a goose had significantly different recovery rates for the grazed plots compared to the exclosures. Significant differences in recovery rates after only one winter of incubation are indicated by an ice crystal ($p \leq 0.05$).

The relative recovery of ^{15}N in the vascular and moss biomass is shown in figure 4.1. The relative recovery of ^{15}N in the moss layer is the same (moss litter incubation in the exclosure) or much higher than the relative recovery of ^{15}N in the vascular plants (moss litter in the grazed plot, grass litter in both the grazed plot and exclosure). Both for the grazed plots as for the exclosures the relative ^{15}N recovery rate in vascular plants is higher for nitrogen derived from decomposing moss litter than from decomposing grass litter. The relative difference between ^{15}N recovery rate in vascular plants for nitrogen derived from decomposing moss litter and from decomposing grass litter is higher in the exclosures (2.50 x) than in the grazed plots (1.89 times).

Nitrogen availability (table 3.4)

The availability of total nitrogen, nitrate and ammonium is not significantly influenced by goose grazing. The method used does not allow comparing nitrogen availability between incubation periods if they differ in length, which was the case in this study. However, the fact that the cumulative nitrate availability is more or less twice as high over wintertime than summertime (+74% and +133% for grazed plots respectively exclosures) and the cumulative ammonium availability in wintertime is only +10% to +56% summertime availability (for respectively grazed plots and exclosures), suggests a higher nitrate to ammonium ratio over the wintertime compared to the growing season.

Table 3.3. Comparison of ^{15}N recovery rates for different ecosystem compartments between grazed and ungrazed plots (=Treatment). ^{15}N was originating from ^{15}N labelled grass and moss litter which was incubated in the graminoid litter compartment respectively the soil (indicated in italic). Significant differences ($p \leq 0.05$) are indicated in bold.

^{15}N Origin	Ecosystem compartment	Winter -Treatment			Year - Treatment x Time			Year - Treatment		
		n	*t*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Grass Litter	Graminoids Biomass	6	S=0.5	1.000	1,12.4	0.34	0.571	1,14.4	0.14	0.714
	Graminoids Litter	*6*	*0.584*	*0.585*	*1,15*	*0.30*	*0.591*	*1,16*	*1.91*	*0.186*
	Moss Green	6	-3.021	**0.029**	1,15	0.01	0.920	1,16	7.78	**0.013**
	Moss Brown	6	0.673	0.531	1,20	0.07	0.791	1,21	1.74	0.206
	Roots	6	1.218	0.277	1,14.4	5.79	**0.030**	1,15.3	9.07	**0.009**
	Soil	6	S=1.5	0.844	1,13.1	0.22	0.644	1,14.1	0.44	0.517
Moss Litter	Graminoids Biomass	6	S=1.5	0.813	1,20	1.24	0.279	1,21	2.98	0.099
	Graminoids Litter	6	S=10.5	**0.031**	1,11.3	0.97	0.346	1,17	9.64	**0.006**
	Moss Green	6	S=-0.5	1.000	1,13.6	0.41	0.533	1,14.6	4.14	0.060
	Moss Brown	6	-1.772	0.137	1,15	0.01	0.922	1,16	2.62	0.125
	Roots	6	2.661	**0.045**	1,15	0.01	0.934	1,16	13.2	**0.002**
	Soil	*6*	*-0.190*	*0.859*	*1,19*	*0.07*	*0.795*	*1,20*	*0.40*	*0.535*

Table 3.4. Plant available nitrogen (PRS™-probe supply rate µg/10cm²/310days – Winter Season respectively µg/10cm²/53days – Growing Season). Data shown are mean values ± SE for grazed plots and exclosures. Statistical comparison between grazed and ungrazed plots (=treatment) is given.

Nitrogen fraction	Winter season		Growing season		Treatment x time			Treatment		
	Grazed	Exclosure	Grazed	Exclosure	*df*	*F*	*p*	*df*	*F*	*p*
Ammonium-N	4.17 ± 0.82	4.8 ± 2.5	3.80 ± 0.56	3.07 ± 0.49	1,10	0.09	0.770	1,11	2.73	0.127
Nitrate-N	176 ± 81	222 ± 114	101 ± 62	95 ± 57	1,10	1.73	0.217	1,11	0.93	0.356
Total N	180 ± 81	226 ± 114	105 ± 62	98 ± 56	1,10	1.89	0.199	1,11	0.93	0.355

Background $\delta^{15}N$ (figure 3.4)

Roots, graminoid shoots and graminoid litter from exclosures were most enriched in ^{15}N, followed by goose faeces; roots, graminoid shoots and graminoid litter from grazed plots; green moss; brown moss and soil in that order. Differences in $\delta^{15}N$ between grazed and ungrazed plots were only significant for roots (n=6, t=2.62, p= 0.047) and the graminoid shoots (n=4, t=24.07, p=0.0002).

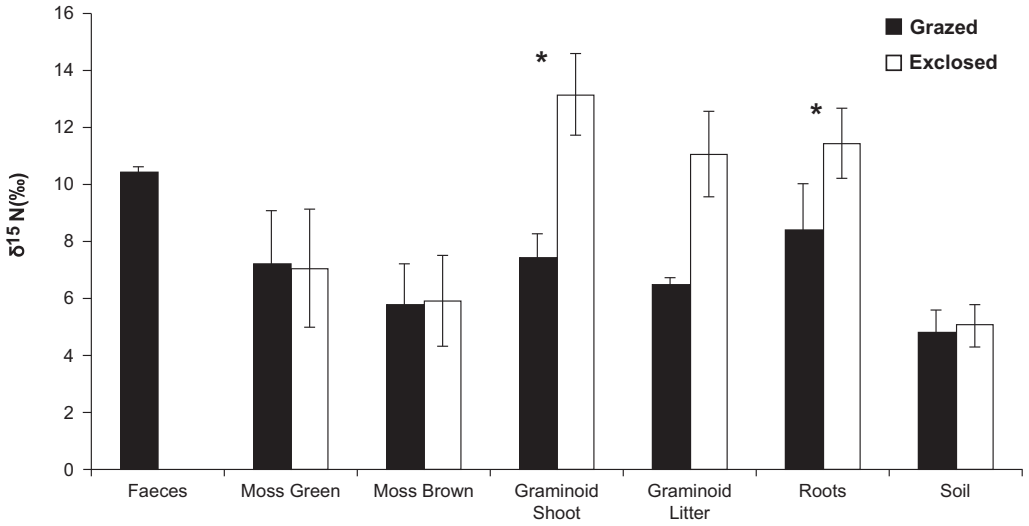


Figure 3.4. Impact of the grazing treatment on background $\delta^{15}N$ values for different ecosystem compartments. Means ± 1 SE are shown (n=6). Significant differences indicated by an asterix ($p \leq 0.05$).

DISCUSSION

Foliar nitrogen to phosphorous ratios indicate that the majority of vascular plants in our study plots are nitrogen limited (N:P ratios between 5 and 12) (Koerselman and Meuleman 1996, Aerts and Chapin 2000). This stresses further the importance of well understanding the ecosystem-processes that drive the nitrogen cycle at this tundra site.

Goose grazing and nitrogen pools and concentrations

Goose grazing removes plant biomass and thus plant nitrogen from the different plant pools. The work presented in paper 1 and a study by Sjögersten et al. (2011) revealed for the same study site a decrease in biomass of all plant (tissues) caused by goose grazing, which was in this study significant for all categories except for green moss. The nitrogen pools, however, are not only determined by biomass stocks, but also by the nitrogen concentrations. Overall the measured nitrogen concentrations in the vascular plants (graminoids, dicotyledons) were high compared to other Arctic studies in a similar habitat (Shaver and Chapin 1991, Shaver et al. 2001), those of bryophytes were comparable (Shaver and Chapin 1991).

Both for vascular plants and bryophytes nitrogen concentrations increased due to goose grazing. Ydenberg and Prins (1981) explained elevated nitrogen concentrations in grazed plots by the subsequent sustained regeneration of young, protein-rich plant tissues as a result of repeated grazing by Barnacle Geese. Other proposed mechanisms are linked to herbivores changing rates of decomposition and nitrogen mineralization and are extensively discussed below. For geese the elevated plant nitrogen concentrations imply a higher nutritional value, which is important since their digestion efficiency is poor (Mattocks 1971, Owen 1980, Prop and Vulink 1992, Alsos et al. 1998).

Even though nitrogen concentration in plants was increased by goose grazing, this did not compensate for the biomass loss and thus nitrogen loss caused by grazing; i.e. nitrogen pool sizes of bryophytes and graminoids decreased. This nitrogen was not found back in any other nitrogen pool, but is incorporated in goose mass and faeces.

On the other hand Zielke et al. (2004) found, at a nearby grazed site, that the same goose colony enhanced the cyanobacterial nitrogen fixation activity. This is explained as the combined effect of two opposite mechanisms. At the one hand geese facilitate the release of nitrogen from dead material by producing faeces, which are readily decomposable and high in labile nutrients (Bazely and Jefferies 1985, Hik and Jefferies 1990), and by increasing nitrogen mineralization through trampling (Zacheis et al. 2002). At the other hand grazing resulted in a reduction in plant biomass and thus less nitrogen containing litter entered the decomposition process.

In case that in our study site the net resultant of these processes is also an increase in nitrogen fixation, this mitigates at least partially the nitrogen losses from the marsh by goose grazing.

Nitrogen release from litter

As described above, nitrogen fluxes between the different pools were measured starting from the decomposition of labelled litter. Inherently to the used methodology artefacts could arise due to "mixed" sampling of different pools. However, both sampling and sorting was executed extremely carefully and our data does not suggest a significant contamination problem. In what follows we will first describe the nitrogen release from litter, which is logically the fraction of the originally labelled litter which is not recovered in the labelled pool, but distributed among the other ecosystem compartments.

Contrary to our expectations, no difference in nitrogen recovery and thus release rates from litter between grazed plots and exclosures was observed. This confirms the results of the work presented in paper 2. In contrast to the here presented research, the mentioned study used litterbags which hampered the effect of trampling by geese causing litter fragmentation and soil incorporation; a mechanism indicated by Zacheis et al. (2002) to have a primary role in the nitrogen dynamics of Arctic salt marshes in Cook Inlet, Alaska, grazed by Lesser Snow Geese *Chen caerulescens caerulescens* (Linnaeus, 1758) and Canada Geese *Branta Canadensis* (Linnaeus, 1758). The presented work thus also excludes this mechanism to have significant effect on nitrogen release rates in our study site.

While we did not observe a direct effect of goose grazing on nitrogen release rates from moss or graminoid litter, the difference between both reveals an indirect effect. Even after one year moss litter did not release any significant amount of nitrogen in contrast to graminoid litter which lost already after one winter of incubation about 50% of its nitrogen. This is probably due to the poor litter quality of mosses. Moss litter is high in lignin and low in nutrient concentrations (paper 2) and is therefore not only hard to decompose (Dorrepaal et al. 2005, Eskelinen et al. 2009), but it also immobilizes more nutrients per unit mass loss than litter with high nutrient and low lignin concentrations like graminoids (Aber and Melillo 1982, Melillo et al. 1982). In general, Barnacle Geese, whose digestion efficiency is poor, select for plants high in nutrients and low in structural components like lignin (Mattocks 1971, Owen

1980, Prop and Vulink 1992, Alsos et al. 1998) and thus cause a shift in litter composition towards less decomposable plants such as mosses.

The negative impact of geese on litter composition is, however, at least partially compensated by the transformation of ingested plants into faeces, which are readily decomposable and high in labile nutrients (paper 2, Bazely and Jefferies 1985, Hik and Jefferies 1990).

Fate of nitrogen after mineralization

A higher recovery of nitrogen from litter in the roots and graminoid litter (only relevant for moss litter) from the exclosures compared to the grazed plots was found. This is probably a result of the higher mass of these compartments in the exclosures compared to the grazed plots. Indeed, a more than three and four fold increment of roots respectively graminoid litter was found in the exclosures compared to the grazed plots (paper 1). The higher amount of label in the green moss from the grazed plots might be a result from the reduced competition for nitrogen with vascular plants. Vascular plant biomass is indeed strongly reduced by goose grazing (paper 1). Moreover, already after one winter a difference in nitrogen uptake from litter existed between grazed plots and exclosures. This means that the influence of geese is not limited to the period they are present and underlines the need for more research over winter times.

In order to better understand the path of nitrogen through the ecosystem we had a more detailed look at the ^{15}N recovery in the vegetation (Relative Recovery Rates represented in figure 3.1). In the grazed plots, a larger fraction of nitrogen originating both from grass and moss litter ended up in the moss layer compared to the vascular plants. This might surprise us, as unlike higher plants, mosses lack developed root and vascular systems, which is thought to limit their access to soil nutrients. Nonetheless they do take up nitrogen from soil (Ayres et al. 2006) and as they lack a cuticle they have the ability to effectively acquire nutrients through their entire surface (Brown and Bates 1990). In addition, the biomass of mosses compared to vascular plants is much higher. The high percentage of nitrogen deriving from litter decomposition taken up by mosses is thus at least partially a result of their dominance in the studied ecosystem.

The fraction of the released nitrogen taken up by vascular plants is almost (grazed plots) or more than twice as much (exclosures) for the nitrogen originating from the moss litter

compared to the nitrogen originating from the grass litter (figure 3.1). This might be explained by the absorption of nutrients by mosses as suggested by a number of studies (Gauthier et al. 1995, Kotanen 2002, Sjögersten et al. 2010), which prevents further access of nutrients by vascular plants. As mosses acquire nutrients through their entire surface (Brown and Bates 1990), they can take up soluble nutrients released by decomposing grass litter before they reach the vascular plant roots in the lower parts of the vegetation layer. Moss litter at the other hand is shed and decomposed at the moss-soil interface, where also a considerable part of vascular plant roots.

Previous research already suggested the possibility that mosses have greater access to nitrogen from faeces than grasses (Lee et al. 2009, Sjögersten et al. 2010). Indeed, Lee et al. (2009) found greater ranges in $\delta^{15}N$ in mosses than in grasses in habitats close to seabird colonies, where faeces with high $\delta^{15}N$ ratios are deposited on the vegetation. This clearly suggested that mosses have greater access to nitrogen from faeces than grasses. In our study we found evidence that the same is true for nitrogen released from decomposing grass litter. The suppressed production of grass litter by goose grazing (paper 2) thus reduces the direct flux of nitrogen from decomposing grass litter to the mosses. On the other hand, geese produce faeces whose nitrogen (after decomposition) seems to follow the same route as the suppressed grass litter, thus (partly) offsetting the effect of declined litter production.

If we compare the results for the grazed plots to the results for the exclosures with respect to the fate of nitrogen from litter, two observations are definitely worth remarking. First, relatively more nitrogen is taken up by the vascular plants in the exclosures (figure 3.1). This could be explained by the fact that vascular plants benefit more from the removal of grazing than mosses as these plants are preferred by geese.

Secondly the fraction of nitrogen taken up by vascular plants is more than twice as much for the nitrogen originating from the moss litter (figure 3.1). In other words the difference between the fate of nitrogen from grass litter and from moss litter is more pronounced in the exclosures, probably because of the thicker moss layer (paper 1) creating a longer distance over which mosses can intercept nitrogen from grass litter before it reaches the vascular plant roots. This adds another element to the importance of the moss layer for ecosystem functioning and the impact of herbivory on this moss layer which was extensively described by Gornall et al. (2009) and van der Wal et al. (2001).

Nitrogen availability for plants

Indications exist that geese elevate the soil nitrogen concentration. As discussed above this is probably at least partially a combined result of goose faeces production and the reduction of the moss layer depth and might be also linked to a possible increase in cyanobacterial nitrogen fixation activity (Bazely and Jefferies 1985, Zielke et al. 2004).

So goose grazing might provide extra available nitrogen in these nutrient limited ecosystems. However, in this study no difference in plant availability of nitrogen was found. High microbial immobilization of this surplus of nitrogen might explain why the seemingly higher nitrogen concentration in grazed soils is not translated in a higher plant availability of both nitrate and ammonium. Harmsen and van Schreven (1955) and Campbell (1978) report that the generally accepted values for equilibrium between net rates of immobilization and mineralization of nitrogen are carbon to nitrogen ratios of 20-25:1 and a soil nitrogen content of 1.5-2.0%. Although there is a large range of variability in the critical percentages of nitrogen and in carbon to nitrogen ratios at which net immobilization gives way to net mineralization (Haynes 1986), high carbon to nitrogen ratios (20-40%, L.F., unpublished data) and the low nitrogen values in the soil (0.2-1%, L.F., unpublished data) taken together indicate that net immobilization might predominate in the sediments.

Nitrogen sources used by plants

$\delta^{15}N$ signatures of graminoids and roots are considerably different between grazed plots and exclosures and high compared to soil. This might look surprising, but $\delta^{15}N$ of either bulk soil or soil organic matter cannot be used as an indicator of the nitrogen source to plants. Most nitrogen in soils is bound in highly recalcitrant organic matter and thus unavailable to plants, the dissolved labile nitrogen pool is small, transient, and may have a significantly different isotopic composition than bulk soil (Bergersen et al. 1990). The increase in $\delta^{15}N$ values of grasses and roots after goose exclusion might point toward a different nitrogen source used by them.

CONCLUSION

In this study we found indications of geese (grazing) impacting on almost all levels of nitrogen cycling. Geese change the start material for decomposition and nitrogen mineralisation by enhancing the nitrogen concentration, thereby improving their own forage quality, by redistribution of nitrogen among the different ecosystem compartments and by the production of faeces.

Goose grazing does affect the rates of nitrogen release by suppressing the production of grass litter, which was found to release nitrogen more easyly than moss litter. Goose grazing affects the fate of nitrogen from litter by at least two mechanisms: i.e. the suppression of the grass litter production and the reduction of the moss layer depth. We found indeed a strong indication that nitrogen from grass litter is partly intercepted by the moss layer when it, after decomposition, migrates down to the rooting zone of vascular plants. In absence of geese the moss layer is thicker and more nitrogen from grass litter is intercepted.

Finally, we found even after only one winter of decomposition a difference between grazed plots and exclosures in the uptake from litter nitrogen. This means that geese even impact on the nitrogen cycle outside the growing season when they overwinter further south and it underlines the need for more research over winter times.

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