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Paper 1

Geese are directing the plant and microbial communities of their Arctic forage habitat

Manuscript

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ABSTRACT

The presented study aims to add more field evidence of goose grazing impact on the structure of Arctic ecosystems, which is necessary to better understand the effect of rising goose numbers on complex ecosystem processes. The conducted research made use of long-term exclosures on Svalbard to study the influence of Barnacle Goose *Branta leucopsis* grazing on vascular plants, the moss layer and abiotic soil conditions. Molecular fingerprinting using PCR-DGGE was used to get also a first idea of the possible goose grazing effect on microbial communities.

Barnacle Goose grazing was found to significantly influence on the vegetation composition and to reduce species number, vegetation biomass and depth of the moss layer. Our results suggest also the effect to trickle down to the decomposer food web influencing the microbial community structure. Those differences are probably leading to changes in important ecosystem processes such as soil nutrient dynamics. The presented study adds thus to the growing body of evidence that geese are ecosystem engineers sculpturing Arctic ecosystem. Our results suggest, however, that the observed changes are reversible.

Key words: *Geese, Wetland, Moss, Vegetation, Soil, Microbial community, Arctic*

INTRODUCTION

Most species of Arctic breeding geese have experienced a dramatic increase in numbers during the last 50 years (Madsen et al. 1999a, Fox et al. 2005, Fox et al. 2010). Changes in climate, land use and the implementation of protective measures (e.g. reduced hunting pressure, improved refuge areas, feeding ...) dramatically improved the birds' ability to survive the winter (Madsen et al. 1999a, Fox et al. 2005, O'Connell et al. 2006). The enormous increase in numbers of Lesser Snow Geese *Chen caerulescens caerulescens* (Linnaeus, 1758) breeding in the Hudson Bay region in the Canadian Low Arctic is an example. Until the eighties positive ecosystem effects of grazing by Lesser Snow Geese were observed. Goose grazing and nutrient additions via faeces stimulated aboveground biomass production (Hik and Jefferies 1990) and the growth of graminoids (Cargill and Jefferies 1984, Hik and Jefferies 1990). However, increasing goose numbers resulted in overexploitation of the vegetation (Jefferies and Rockwell 2002). In combination with changed abiotic conditions (Iacobelli and Jefferies 1991, Jefferies and Rockwell 2002) this resulted in near irreversible soil degradation and widespread vegetation loss (Srivastava and Jefferies 1996, Handa et al. 2002, Jefferies et al. 2006b). We can thus distinguish three different ecosystem states: an ungrazed state with lower biomass production, a grazed state with higher biomass production and an overgrazed state without vegetation.

Also the populations of most European Arctic breeding geese have increased rapidly (Madsen 1991, Madsen et al. 1999a). On Svalbard for example, the population of Pink-footed Geese *Anser brachyrhynchus* (Baillon, 1834) more than doubled between 1965 and 2003 (Fox and Bergersen 2005) and the once endangered Svalbard Barnacle Goose *Branta leucopsis* (Bechstein, 1803) population even increased two orders of magnitude during the past 60 years (from 300 birds in 1948 to 30 000 birds in 2009; Pettifor et al. 1998, Tombre et al. 1998, Fox et al. 2010). The risk that European Arctic ecosystems could suffer a similar degradation due to goose grazing is of concern.

Previous research suggests that selective grazing by Barnacle Geese combined with increasing grazing pressure leads to changes in the vascular plant community (Drent et al. 1998, Loonen and Solheim 1998, Kuijper et al. 2006, Kuijper et al. 2009). Both Loonen and Solheim (1998) and Sjögersten et al. (2011) found a significant increase in vascular plant biomass due to exclusion of Barnacle Geese. Also the abundance of preferred forage plants like *Equisetum*

arvense Linnaeus and species diversity seemed to decrease by goose grazing. However, these studies were characterized by large variation and only limited sample size and none of the observations were statistically significant.

In addition to vascular plants, also the moss layer was found to be influenced by Barnacle Goose grazing. Mechanical disturbance, mainly by trampling and grazing of mosses can reduce the integrity and depth of the moss layer. As shown by multiple studies, this moss layer plays an important role in many Arctic ecosystems, e.g. maintaining moisture from snow melt, buffering soils from temperature extremes in summer and winter (Gornall et al. 2007) and affecting the competition for nutrients between graminoids and mosses (Gauthier et al. 1995, van der Wal and Brooker 2004).

Biotic and abiotic factors are both known to influence the soil borne microbial communities (Kuramae et al. 2011). Plants are known to influence microbial community structure and diversity, mainly in the rhizosphere (Kowalchuk et al. 2002, Berg and Smalla 2009). Specific plant species, plant diversity and plant community composition have all been shown to influence soil borne microbial communities and vice versa (Wardle et al. 2004). With respect to abiotic factors, soil characteristics as pH, moisture and temperature have been shown to be drivers of microbial community structure (Chen et al. 2003, Smith et al. 2010). It is thus clear that goose grazing can indirectly, through their impact on plant communities and soil conditions, affect microbial communities, the main players of important ecosystem processes as nutrient cycling in soil systems. However, as far as we know, the effect of (Barnacle) Goose grazing on the microbial community is almost not-documented. Actually, we are only aware of the studies of Zielke (2004) concerning the cyanobacterial community.

Speed et al. (2010a) found that resilience to disturbance by grubbing of Pink-footed Geese differed between plant communities. Those with higher moss cover and higher soil moisture, favoured by both Pink-footed Geese (Speed et al. 2009) and Barnacle Geese (Prop et al. 1984, Stahl and Loonen 1998), seemed most resilient. Barnacle Geese, however, feed in contrast to Pink-footed Geese almost exclusively on above ground plant material (Fox and Bergersen 2005). The response of biota (vegetation and microbial communities) and soil systems (pH, temperature and moisture) to grazing by the high densities of Barnacle Geese is therefore not necessarily similar to the response of grubbing by Pink-footed Geese. Zacheis et al. (2001) found indeed a difference in plant community response to below- and above-ground herbivory.

Therefore, our study aims to add more field evidence to the research of a recently established Barnacle Goose population in the Kongsfjorden area (Svalbard) to fill gaps of knowledge and to strengthen previous research about the effect of goose grazing on the structure of Arctic ecosystems. This is necessary to better understand the effect of rising goose numbers on complex ecosystem processes. Our study not only focussed on vascular plants, the moss layer and abiotic soil conditions. Advanced molecular techniques were used to get also a first idea of the possible goose grazing effect on microbial communities.

MATERIAL AND METHODS

Study site

The study was carried out in the Kongsfjorden area (78.55°N, 11.56°E) on Spitsbergen, Svalbard (figure B.2). 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 community. Grazing impact by other herbivores than Barnacle Geese is negligible. Just a few Pink-footed Geese were observed for a short time at beginning of the season and although 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 carried out in 2007-2009, four to six years after the set-up of the exclosures.

Field and laboratory techniques

Vegetation surveys existed of cover determinations for each species of vascular plants made by agreeing visual estimates between two recorders in 2008 and 2009. We used an adaptation of the Braun-Blanquet cover-abundance scale (Braun-Blanquet 1932, Braun-Blanquet 1964) as described in table 1.1.

Table 1.1. Scale used for vegetation surveys in this study, which is an adaptation of the Braun-Blanquet cover-abundance scale (Braun-Blanquet 1932, Braun-Blanquet 1964).

Obeservation	Value
1 specimen	0.01
< 1%	0.1
1-20%	Percentage, steps of 1%
20-100%	Percentage, steps of 5%

To determine biomass of different functional plant groups, we harvested four turfs of 9 cm^2 (end growing season, August 2007) or six cores of 9.68 cm^2 (start growing season, June 2008) or six turfs of 9 cm^2 (end growing season, August 2008) to a soil depth of 10 cm in each plot. At the start of the growing season a steel corer was used to take the biomass samples as soil was still frozen at time of sampling, at the end of the growing season a knife was used to avoid compaction. After harvesting samples were carefully sorted into mosses, vascular plants and roots. Moss tissue was split into photosynthetic active (green) and inactive (brown) fractions, vascular plants into functional groups (graminoids, dicotyledons and equisetales) and further into living shoots and litter. No attempt was made to make a distinction between the different functional groups and bio- and necromass for roots. Material from individual turfs was pooled to give one biomass value per plot. All samples were oven dried at 35°C until constant mass and weighed.

At the four sides of the turfs harvested for biomass determination in 2008 the distance between the top of the moss layer and the moss-soil interface (the point where moss shed old plant material) was measured with a ruler. A mean for each plot was made and used for further analysis.

At the start and the end of the growing season (2008) soil temperature at 10 cm depth was measured in each plot on four occasions spread equally over a day (24 hours) in order to calculate an average daily temperature and to get an idea of the daily fluctuations (amplitude). At the end of the growing season (2008) data loggers (DL6, ΔT, Cambridge, UK) were installed in each plot to measure the fluctuations in soil temperature at 2 cm depth from the moss-soil interface every 30 minutes over an entire year. Unfortunately only half of them survived the winter season.

Soil thaw depth (below moss surface) was measured by inserting a metal rod into the soil and recording depth at which it reached the frozen soil layer. Four measurements were taken per plot, averaged and adjusted by distracting the depth of the moss layer. Depth of the permafrost was measured two days after total snow thaw (start of the growing season) and on 15 August (end of the growing season). At the end of the growing season, in one couple of plots stones impeded a correct measurement.

To determine gravimetric moisture content and soil pH, small turfs were harvested in each plot at the start and the end of the growing season and separated in the moss layer and top 2 cm of the soil layer. One subsample of each soil sample and moss sample was weighed, dried at 105°C until constant weight and reweighed to calculate the moisture content. Other subsamples were used to determine both actual (pH-H_2O) and potential (pH-KCl) pH. We followed the protocol described by Houba et al. (1989). After fresh weight determination (4.00 ± 0.01 g), samples were shacked (1 hour) and incubated (± 23 hours) in 10 ml demineralised water and 1 M KCl (ratio 1:2.5 w/v) respectively. Water was squeezed out the moss layer from each plot to measure the pH of moss water. pH in solution was measured (Mettler-toledo GmbH SG2 (instrument) combined with Mettler-toledo Inlab 413 SG IP 67 (probe)).

Microbial community structure was analysed using PCR-DGGE analysis. Soil was collected from the Thiisbukta plots at the end (2007) and the start (2008) of the growing season. In each plot, four turfs of 1.5 cm by 3 cm and 11 cm deep were cut out using a steel knife. The vegetation layer was removed and the top 5 cm of the soil was pooled into sterile recipients.

Precautions were taken to prevent cross contamination. Samples were frozen to -80 °C within one hour after sampling and transported on dry ice. Upon thawing, after homogenization, the community DNA from three subsamples per soil sample was extracted and purified as described by Boon et al. (2000). The DNA was stored at -20 °C upon further analysis. 1 µL of the extracted DNA was amplified by PCR with the bacteria specific 16S rRNA forward primer 338f and the reverse primer 518r (Muyzer et al. 1993). The PCR product contains a GC-clamp of 40 bases, added to the forward primer. PCR products were subjected to DGGE as described previously (Boon et al. 2002). In brief, PCR samples were run for 17 hours at 38 V on 8 % (wt/vol) polyacrylamide gel with a denaturing gradient ranging from 45 - 60 % (where 100 % denaturant contains 7 M urea and 40 % formamide). After electrophoresis the gels were stained with SYBR Green I nucleic acid gel stain (1:10000 dilution; FMC BioProducts, Rockland, Maine) and photographed.

Data analysis

To test for differences in species composition between grazed and ungrazed plots we used a linear mixed model with treatment (grazed/exclosure), species and the interaction between them as fixed effects and replica as random effect. Species was indicated as repeated measurement.

We tested for differences in number of species, total plant cover, plant biomass, depth of the moss layer and abiotic conditions using a repeated two way ANOVA with treatment (grazed or exclosure) as fixed factor and replica as random factor (proc mixed). The analysis of the freeze-thaw cycles forms an exception using a coupled t-test after square root transformation to meet the prerequisite of normality. Analysis was carried out using proc mixed and proc univariate normal of the statistical software program SAS (SAS Institute Inc., Cary, NC, USA; Version 9.2, 2008).

DGGE fingerprint profiles were normalized and analysed using BioNumerics software (version 2.0, Applied Maths, Kortrijk, Belgium). The calculation of the similarity matrix was based on the Pearson correlation coefficient and the clustering algorithm of Ward was used to calculate dendrograms (Ward 1963).

RESULTS

Vegetation

In both years considerable differences in vegetation composition between exclosures and grazed plots were found (figure 1.1). In total ten different plant species were found in the exclosures, seven of them occurred also in the grazed plots were no additional species were found (figure 1.1). *Cerastium regelii* Ostenfeld, *Deschampsia alpine* (Linnaeus) Roem & Schultes and *Salix Polaris* Wahlenberg were not present in the grazed plots. The grazed treatment contained on average fewer species per plot than the exclosures (2.7 +/- 0.5, respectively 5.3 +/- 0.8; $F_{1,16} = 12.99$, $p = 0.0024$).

Both the mean cover of the vascular plants ($F_{1,70} = 12.59$, $p < 0.0007$; $F_{1,55} = 7.60$, $p = 0.0079$ for respectively 2008 and 2009) and the relative cover of the different plant species ($F_{13,70} = 6.86$, $p < 0.0001$; $F_{10,55} = 2.79$, $p = 0.0072$ for respectively 2008 and 2009) were significantly affected by the exclusion of geese. The cover of the different vascular plant species increased, even dramatically for some species like *Arctodupontia scleroclada*, or did not change after excluding geese, but for no species a decrease in cover could be found, resulting in an overall higher vascular plant cover in the exclosures compared to the grazed plots ($F_{1,16} = 10.71$, $p = 0.0048$). No impact of grazing on seedling abundance was found.

The higher cover of vascular plants in the exclosures compared to the grazed plots was translated in a significant higher biomass for all distinguished categories, namely dicotyledons, equisetales, graminoid shoots, graminoid litter and roots (table 1.2). Also the moss layer was affected by grazing: while there was no difference detected for the photosynthetic active part, biomass of the photosynthetic inactive part was significantly reduced by grazing (table 1.2). This was reflected in the depth of the moss layer (figure 1.2; $F_{1,16} = 41.92$, $p < 0.0001$).

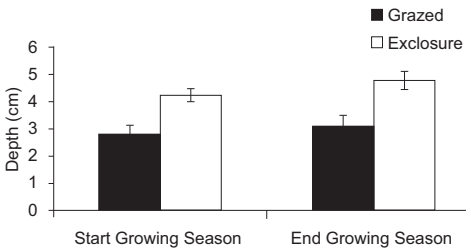

Figure 1.2. Depth of the moss layer (=the distance between the top of the moss layer and the moss soil interface at the start and the end of the growing season 2008. Data shown are mean values ± SE (error bars). The difference between grazed plots and exclosures is significant ($p < 0.0001$).

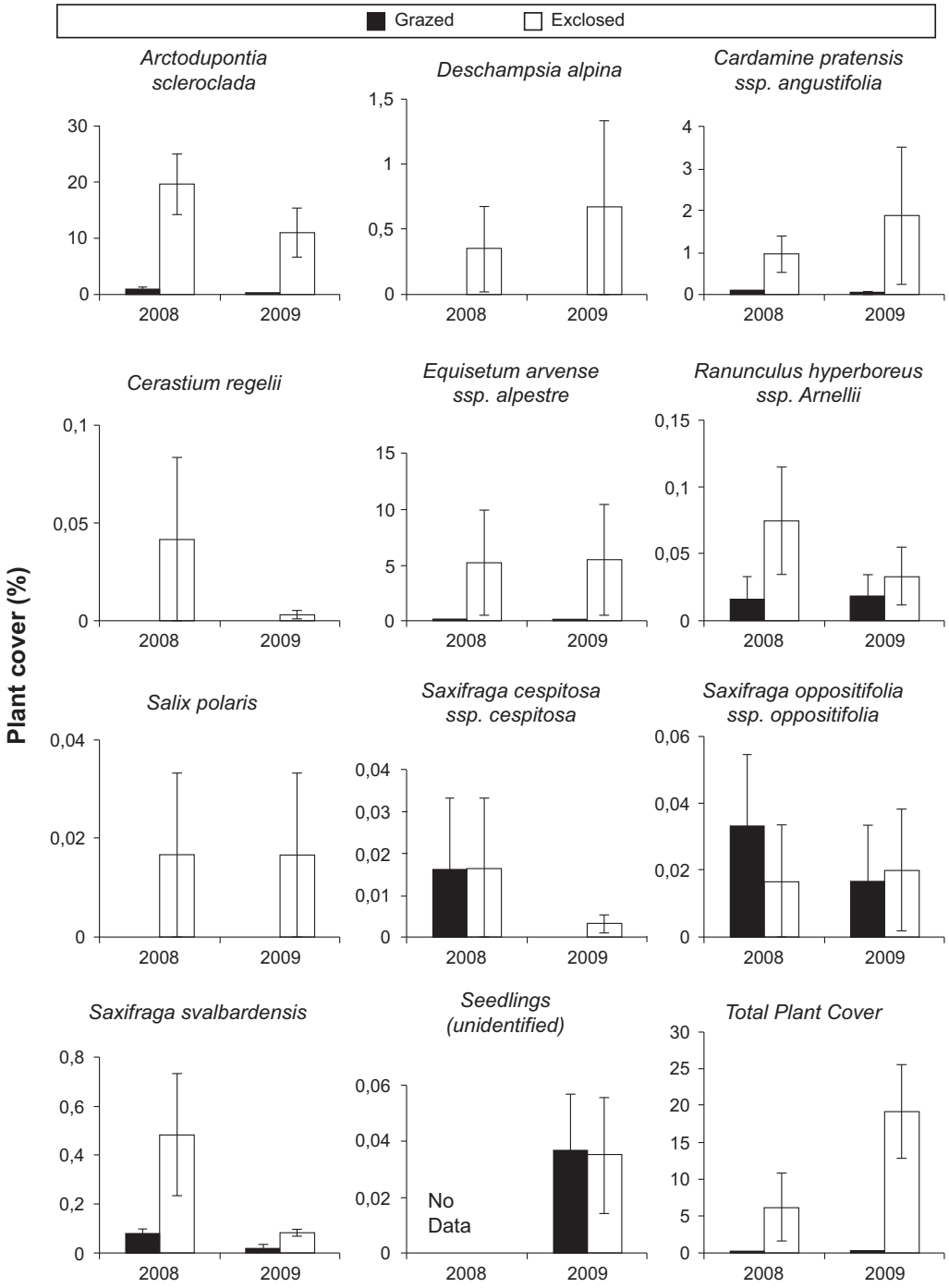

Figure 1.1. Results from the vegetation surveys at the peak (2009) and the end (2008) of the growing season. Vegetation surveys were made using an adaptation of the Braun-Blanquet cover-abundance scale (Braun-Blanquet 1932, Braun-Blanquet 1964) described in table 1.1 and only vascular plants were considered. Data shown are mean values ± SE (error bars) for grazed plots and exclosures.

Table 1.2. Biomass and necromass g/m² for different functional groups of plants on different key moments during the growing season. 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.

Plant (part)	End Growing Season (19/08/07)		Start Growing Season (21/06/08)		Peak Growing Season (08/08/08)		Interaction (Treatment x time)			Treatment		
	Grazed	Exclosure	Grazed	Exclosure	Grazed	Exclosure	*df*	*F*	*p*	*df*	*F*	*p*
Dycotyledons	0 ± 0	27 ± 15	3 ± 1	11 ± 3	2 ± 2	20 ± 7	2, 25	1.08	0.355	1, 27	11.24	**0.0024**
Equisetum sp.	1 ± 1	20 ± 6	3 ± 1	8 ± 2	7 ± 3	15 ± 6	2, 25	1.65	0.212	1, 27	12.44	**0.0015**
Graminoids Litter	9 ± 2	46 ± 16	14 ± 2	56 ± 15	13 ± 2	71 ± 22	2, 25	0.54	0.589	1, 27	28.18	**<0.0001**
Graminoids Shoots	10 ± 3	69 ± 19	8 ± 2	15 ± 4	10 ± 3	45 ± 17	2, 25	3.19	0.059	1, 25	15.68	**0.0005**
Moss Brown	1222 ± 106	1851 ± 227	1148 ± 176	1429 ± 129	959 ± 80	1271 ± 93	2, 25	0.89	0.423	1, 27	11.98	**0.0018**
Moss Green	414 ± 32	420 ± 56	257 ± 75	191 ± 59	338 ± 52	212 ± 45	2, 25	1.01	0.377	1, 27	2.64	0.1161
Roots	37 ± 11	251 ± 48	30 ± 8	94 ± 25	138 ± 44	484 ± 63	2, 25	6.82	0.004	1, 25	44.53	**<0.0001**

Table 1.3. Abiotic conditions (Soil temperature, pH and moistures) at the start and the end of the growing season. 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.

Soil parameter		Start Growing Season		End Growing Season		Treatment x time			Treatment		
		Grazed	Exclosure	Grazed	Exclosure	*df*	*F*	*p*	*df*	*F*	*p*
Soil Temperature	Average (°C)	3.75 ± 0.19	3.55 ± 0.19	2.92 ± 0.24	2.99 ± 0.69	1,10	0.19	0.670	1,11	0.05	0.834
	Amplitude (°C)	2.07 ± 0.24	1.52 ± 0.13	0.88 ± 0.17	1.07 ± 0.20	1,10	5.88	**0.036**	1,10	1.47	0.253
	Freeze-thaw Cycles (#)	na	na	5.7 ± 2.2	2.3 ± 1.9				n = 3	t=-3.78	0.063
	Depth of Permafrost (cm)	6.5 ± 1.5	5.5 ± 1.1	53 ± 11	42 ± 10	1,9	2.85	0.125	1,10	2.08	0.180
pH	Moss pH	7.17 ± 0.07	6.96 ± 0.14	7.35 ± 0.08	6.95 ± 0.19	1,7	0.01	0.939	1,8	8.39	**0.020**
	Actual Soil pH (H_2O)	6.95 ± 0.04	6.85 ± 0.02	7.35 ± 0.08	7.36 ± 0.07	1,10	0.94	0.355	1,11	1.16	0.305
	Total Soil pH (KCl)	6.60 ± 0.06	6.57 ± 0.06	6.91 ± 0.12	6.99 ± 0.15	1,10	0.29	0.599	1,11	0.00	0.954
Gravimetric moisture	Moss (% DW)	92.4 ± 1.0	92.0 ± 0.8	88.9 ± 1.7	88.3 ± 1.5	1,10	0.02	0.890	1,11	0.47	0.506
	Soil (% DW)	86.7 ± 6.6	87.4 ± 3.6	82.3 ± 4.6	85.5 ± 3.1	1,10	0.33	0.579	1,11	0.89	0.367

Soil characteristics

Almost none of the soil characteristics were significantly influenced by goose grazing. Both the average and the diurnal amplitude of the soil temperature, the depth of the permafrost layer, soil pH and gravimetric moisture of the soil and moss layer were similar in grazed plots and exclosures (table 1.3). The pH of the moss water forms the only truly significantly influenced exception (table 1.3). The number of freeze-thaw cycles was almost significantly reduced in the exclosures compared to the grazed plots (table 1.3).

Microbial Community

Clustering analysis after PCR-DGGE (figure 1.3) revealed differences in microbial community structure both in treatment as in time. Microbial fingerprints of the samples taken in the exclosures just after snow melt were distinct from all other fingerprints. Fingerprints from the exclosures at the end of the growing season showed higher similarities with those from the grazed plots than those from the same plots at the start of the growing season. Nevertheless also in this second cluster microbial communities from the exclosures seemed to differ from those from the grazed plots. Within the grazed plots seasonal differences in microbial community structure seemed to be less pronounced.

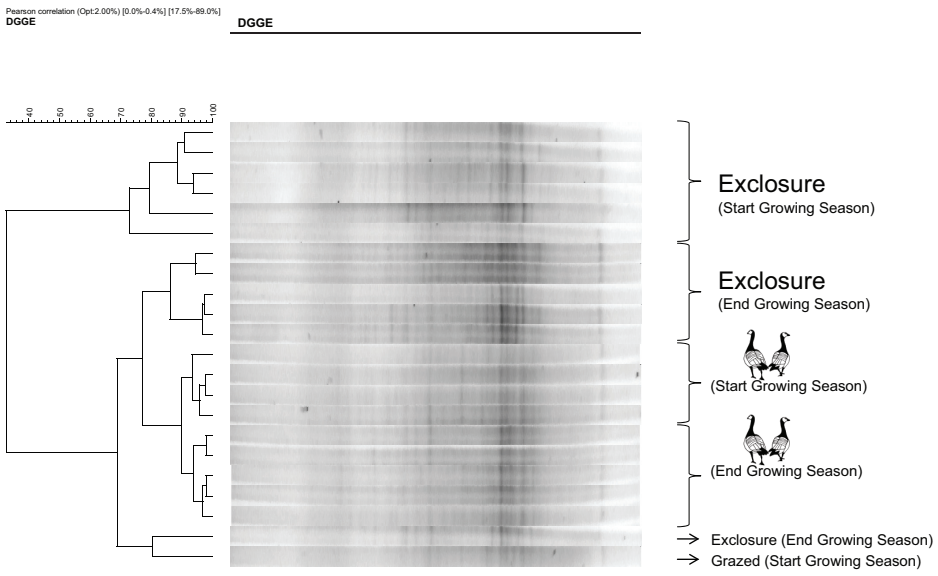

Figure 1.3. Clustering of the microbial communities present in grazed plots and exclosures based on their PCR-DGGE fingerprints. Samples were taken at the beginning and the end of the growing season.

DISCUSSION

Following Zimov et al. (1995) the vegetation in Arctic areas should have two alternative equilibriums, productive grassland with abundant large herbivores and low-productive moss tundra with few herbivores. The maintenance and promotion of a grazing lawn by herbivores was for example described for the increasing population of Black *Brant Branta bernicla nigricans* (Linnaeus, 1758) in Southwestern Alaska (Person et al. 2003). Like other terrestrial herbivores (Coppock et al. 1983, McNaughton 1984), geese indeed often create and maintain grazing lawns: vegetation swards dominated by a high density of grazing-tolerant plant species with high nutrient concentrations (Person et al. 2003). As the predominance of grazing-tolerant graminoids reduces soil moisture more and isolates the soil less than moss dominated vegetation, grazing and trampling should increase nutrient cycling and primary production by increasing soil temperature and by improving drainage.

However, if grazing pressure is too high and consumption rate of herbivores exceeds the growth rate of plants, grazing can lead to a rapid depletion of forage plants (Rowcliffe et al. 2001). Increasing grazing pressure will then lead to an increased rate of depletion (Vickery et al. 1995). Long-term effects of high grazing pressure may then result in vegetation changes. Overexploitation of vegetation occurred at the (sub-) Arctic breeding areas of Lesser Snow Geese in La Pérouse Bay, Canada. The intense grazing and grubbing of increasing numbers of geese led to the loss of vegetation (Jefferies and Rockwell 2002) and erosion of the surface organic layer (Kotanen and Jefferies 1997). These processes have led to the establishment of an alternative stable state (exposed unvegetated sediment) over large expanses of coastal marshes where geese stage or breed (Jefferies et al. 2006b). The lack of preferred high-quality food plants in these areas has forced geese to switch to alternative lower quality forage plants that were less tolerant to grazing (Zellmer et al. 1993, Gadallah and Jefferies 1995a). Increased grazing led to a rapid decrease of these species. Additionally, changed abiotic conditions prevented a recovery of the vegetation to its original state (Zellmer et al. 1993, Gadallah and Jefferies 1995b). However herbivore-driven state shifts are not necessarily so catastrophic, and may result in predictable and reversible vegetation state changes without dramatic reductions in ecosystem productivity (van der Wal 2006).

Our results also show depletion in preferred forage species as *Equisetum arvense* spp. *alpestre* and graminoids, corresponding with the depletion of high–quality food plants within

years and over years by grazing trials with captive geese in an unexploited area (Kuijper et al. 2009). As breeding geese demonstrate both fidelity to their breeding grounds and a faithfulness to brood rearing areas, they switch to alternative foods that are lower in quality when preferred resources are depleted (Cooch et al. 1993, Hughes et al. 1994, Gadallah and Jefferies 1995b, Lindberg and Sedinger 1998). This is also observed for Barnacle Geese on Svalbard, which show a high level of nest site fidelity (Tombre et al. 1998). While a graminoid-based diet is desirable for and preferred by non-breeders and family birds alike, Prop and Vulink (1992) showed that adult geese can cope with high moss contents in their diet through prolonged food retention.

Selective grazing of high quality plant species can directly affect the vegetation by reducing the plant standing crop and plant species composition. Exactly what we observed in this study and what was found by other authors (Zacheis et al. 2001). However the grazing effect is not only due to selective grazing, but also linked to the different grazing tolerance of plant species. Where many graminoid species have the capability to compensate and even over-compensate for light to moderate grazing, dicotyledonous species generally have less capability to compensate and are thus less tolerant to grazing. This might explain the disappearance of the two dicotyledonous species due to goose grazing, although, we have to remark that also a grass species, *Deschampsia alpine*, was not found in grazed plots.

Moreover the observed vegetation shift due to goose grazing could also be an indirect effect caused by an alteration in competitive interactions between plants. Selective grazing of one plant species may release other species from competition (Mulder and Ruess 1998, van der Wal et al. 2000a), or changed abiotic conditions may differentially affect competing plant species (Bazely and Jefferies 1985, van der Wal et al. 2001, van der Wal and Brooker 2004).

An important element of the structuring force of goose grazing is formed by the effect of the moss layer on abiotic conditions (Gornall et al. 2007, Gornall et al. 2009). Similar to other herbivores in the Arctic, goose grazing results in a considerable decrease in depth of the moss layer, as found in this study in agreement with, for instance, a study from van der Wal et al. (2001) at the same study site and a study of Miller et al. (1980) in coastal tundra at Barrow, Alaska. The observed reduction in depth of the moss layer by herbivory is probably both a result from trampling and grazing. Additionally, a reduction in shading by vascular plants may further decrease the moss layer as mosses grow maximally at less than full sunlight. High light intensity appears to limit growth because of photo-inhibition or photo-oxidative processes

and may be the most important limitation on moss production in tundra ecosystems (Clymo and Hayward 1982).

The moss layer is important in determining soil characteristics, such as soil temperature (Luthin and Guymon 1974, van der Wal and Brooker 2004) and moisture (Zimov et al. 1995, Gornall et al. 2007). Studies revealed both an increase in average temperature and temperature amplitude due to a reduction in the moss layer (Gornall et al. 2007). This is important as warmer temperatures have found to enhance plant metabolism and growth in the Arctic (Arft et al. 1999, Cooper et al. 2006). Furthermore deeper moss layers are found by Gornall et al. (2007) to delay the onset of soil thaw for several weeks. Such an effect may delay the onset of vascular plant growth early in summer, shortening their growing season by as much as 40%. This is likely to constrain vascular plant root growth even more (Brooker and Van der Wal 2003). As vascular plant growth is greatly reduced in chilled soil and moss growth is independent of soil temperature, a decrease in moss layer due to goose grazing means an increase in soil temperatures and thus more competition for resources creating a negative feedback on the depth of the moss layer. However, nevertheless the reduction in moss layer, we did not find a similar effect of goose exclusion on soil temperatures as the authors mentioned above. This could be due to the limited number of replicates (n = 6) combined with a strong variation between them.

At the other hand our data hints towards a possible increase in freeze-thaw cycles caused by goose grazing. This might be linked to the reduction of the insulating moss layer by goose grazing. Soil freeze–thaw cycles are important determinants of Northern ecosystems as they enhance litter decomposition, mineralization rates, nutrient leaching, and trace gas fluxes. Therefore freeze-thaw cycles have a considerable impact on the cycling of nutrients such as carbon and nitrogen. Furthermore freeze–thaw cycles can also destabilize soil aggregates, exposing substrates and stimulating microbial growth (Campbell et al. 2005).

The link between depth of the moss layer and soil moisture regime is far more complicated and contested (Gornall et al. 2007) and in this study the grazed plots with thin moss layers were comparable to the exclosures characterized by a thick moss layers with respect to gravimetric soil and moss moisture content.

Differences in substrate chemistry finally have important effects on dominant plant communities and ecosystem properties. Some of the most important effects are related to soil pH, which governs the availability of essential plant nutrients and creates distinctive plant

communities (Edlund 1982, Elvebakk 1982, Walker et al. 1998, Walker et al. 2005). Low pH restricts nitrification rates and increases concentrations of certain elements known to be toxic to many plants (e.g. aluminium). Soils in the circumneutral range (pH 5.5-7.2) are generally mineral rich, whereas the full suite of essential nutrients is often unavailable in acidic soils or in soils associated with calcareous bedrock (Walker et al. 2005). The pH values recorded in this study were situated around the upper limit of the circumneutral range. Soil pH , through its direct and indirect effect on plant and microbial communities, seems to function as an ultimate environmental driver that gives rise to and amplifies the interactions between above and belowground systems (Eskelinen et al. 2009). Goose grazing did not affect soil pH, but it did slightly, but significantly, elevate the pH of the moss water.

Shifts in soil conditions were at the base of the irreversible transitions in community assemblages observed at La Pérouse Bay (Jefferies and Rockwell 2002). The high consumption rate by geese led to loss of vegetation cover, exposure of surface sediments and development of hyper saline soils (Bazely and Jefferies 1997). In this study changes in soil conditions were minimal, probably because the moss layer was only reduced and still intact. Nonetheless, Kuijper et al. (2006) argued that goose grazing in these systems influences the potential for recovery after a disturbance event and thus in the long term plant species diversity and dynamics. This conclusion was based on the fact that geese have a strong effect on flower abundance and consequently on the seed bank in our study site. However, we observed a considerable potential for recovery. Already after 5 years of excluding geese three new species entered the exclosures. Moreover, in three older exclosures (13, 14 and 17 years old at the time of sampling), which were surveyed in 2008 four additional species were found, which were not present in grazed plots, namely *Bistorta vivipara* (Linneaus) S. F. Gray, *Cerastium alpinum* Linnaeus, *Cerastium Arcticum* Lange and *Cochlearia groenlandica* Linnaeus (L.F. & J.T., unpublished data). The similarity of the vegetation in the exclosures with the vegetation present at the study site before the goose colony established in the Kongsfjorden area (Reidar Elven, personal communication) shows that even after more than 30 years of goose grazing vascular plants have still the capacity to re-establish.

As preservation of seeds in the seed bank is hampered and clonal growth is not probable (no individuals of the returned species were observed close to the exclosures), the return of species means the existence of a nearby source. This could be seed or propagule dispersal from neighbouring populations. This process might even be facilitated by geese acting as

agents of dispersal (Bruun et al. 2008). Mostly, reproduction of vascular plants by means of seeds is seen as of marginally importance in the Arctic (Bell and Bliss 1980), though we observed seedlings in more than half of the plots.

Notwithstanding the effect seems to be reversible, we might conclude that geese do play an important role in structuring the vascular vegetation in the moss tundra wetland, just as they do in a range of other ecosystems (Hik et al. 1992, Mulder and Ruess 1998, Zacheis et al. 2001). Previous research revealed a strong link between the plant community composition and microbial community composition and differences in one compartment induce changes in others. The community structure of micro-organisms in soil is indeed mediated by among others plant biomass and plant litter biochemistry (Zak et al. 2003, Zak and Kling 2006, Eskelinen et al. 2009).

Both the observed changes in soil conditions (freeze-thaw cycles and pH) as in plant communities, which are probably at least partially linked, might thus explain the shift in microbial communities observed. The microbial community in turn affects the plant community among others by their crucial role in ecosystem processes as nutrient cycling (Wardle et al. 2004, Van der Heijden et al. 2008).

Nevertheless the important role of micro-organisms, evidence of (goose) grazing impact on microbial communities is still very scarce. The interesting result from the PCR-DGGE fingerprint analysis in this study emphasizes the need for more research effort in this direction for example by a more detailed study of the advantaged and disadvantaged groups. The huge improvement of molecular techniques over the last years might thereby be of incredible value. Pyrosequencing approaches could for example be used to further evaluate the effects of goose grazing on the microbial community structure in detail.

CONCLUSION

This study adds to the growing body of evidence that herbivores, like geese, are ecosystem engineers sculpturing Arctic ecosystems (Miller et al. 1980, van der Wal et al. 2001). In this study we found Barnacle Goose grazing to have a clear effect on species composition, vegetation biomass and depth of the moss layer. Our results suggest also the effect to trickle down to the decomposer food web influencing the microbial community structure. Those

differences are probably leading to changes in important ecosystem processes such as soil nutrient dynamics.

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Paper 2

Different mechanisms of goose influence both accelerate and retard the decomposition process in an Arctic wetland

Manuscript

Lise Fivez, Maarten Loonen, Patrick Meire

ABSTRACT

Background and aims Due to human induced changes in their wintering grounds, goose numbers increased dramatically over the past 50 years. To understand the consequences of these changes, studies on key ecosystem processes, like decomposition, on the breeding grounds in the generally severely nutrient limited Arctic are indispensable. This article reports on the influence of Barnacle Geese *Branta leucopsis* on the decomposition process and the release of nitrogen from litter on high-Arctic Svalbard (78° 55' N, 11° 56' E).

Methods The study made use of paired long-term exclosures and control plots. Litter and goose droppings were collected and subsequently analysed on chemical parameters to understand the influence of grazing via a change in dead organic matter quality within and between plant growth forms and faeces. Reciprocal transplantation of dead organic matter (graminoids, mosses, roots and faeces) between ungrazed (exclosures) and heavily grazed areas, using the litterbag technique, was used to study the goose grazing influence on litter decomposition and nitrogen release through a shift in environmental conditions. The possibly facilitating role of goose faeces was investigated by studying decomposition in separate subplots with faeces addition in some of the exclosures.

Results In the exclosures almost twice the necromass of grazed plots was present and the contribution of litter originating from graminoids and roots was respectively twice and four times as much in the exclosures compared to the grazed plots. This is important seen the fact that these growth forms were found to differ in litter quality. Together with the place of production and thus incubation, this resulted in a decrease in decomposition and nitrogen release rates in the following order: roots, graminoids and moss. Goose-induced changes in litter composition thus impeded decomposition. Environmental impact of geese, in contrast, was found to enhance decomposition, but not nitrogen release rates of the same litter type. Goose faeces, characterised by a distinct chemical quality, were found to decompose as slow as moss litter and release nitrogen as fast as graminoid litter.

Keywords: *litter quality, faeces, roots, nitrogen, decomposition, herbivory, goose, Arctic*

INTRODUCTION

Given their importance for ecosystem functioning and their possible influence on climate change, decomposition processes have been intensively studied with regard to global change. Mostly, these studies focused on the climate effects on decomposition (Aerts 1997, Cornelissen et al. 2007). However, global change encompasses much more. Examples of other well documented global changes are: alterations in the biogeochemistry of the global nitrogen cycle, on-going land use / land cover change (Vitousek et al. 1994) and an altered distribution and abundance of much of Earth's biota (Wardle and Bardgett 2004). The increased population numbers of the Western Palearctic Arctic breeding geese in the last 50 years (Madsen et al. 1996, O'Connell et al. 2006) are an example of the latter. Recent changes in climate, land use and the implementation of protective measures (e.g. reduced hunting pressure and improved refuge areas) 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), resulting in an increased grazing pressure both in the temperate wintering areas and in the higher latitude breeding sites of these migratory herbivores.

While the increase in goose numbers is primarily due to changes in the temperate wintering areas, the changes in decomposition processes due to grazing are likely to be of key importance in systems where low temperature and poor drainage result in low nutrient availability (Nadelhoffer et al. 1992). An example of such area is Svalbard's tundra, where the once endangered Svalbard Barnacle Geese *Branta leucopsis* (Bechstein, 1803) increased by two orders of magnitude over the past 60 years (from 300 birds in 1948 to almost 28 000 birds in 2006; Pettifor et al. 1998, O'Connell et al. 2006).

Grazing by geese and other herbivores strongly affects tundra systems (Cooch et al. 1991, Jano et al. 1998, Gornall et al. 2009). A major challenge in understanding herbivory effects on ecosystem functioning is to understand the linkages between above-ground and below-ground components in natural communities. In particular litter decomposition, a major factor for nutrient cycling and a strong determinant of the CO_2-fluxes from the soil to the atmosphere (Aerts 1997), is a key process that is likely influenced by (changes in) goose grazing. However despite the increased recognition of the importance of grazer effects on litter decomposition (Frank and Groffman 1998, Stark et al. 2000, Olofsson et al. 2001), our knowledge of how goose grazing influences decomposition is still limited.

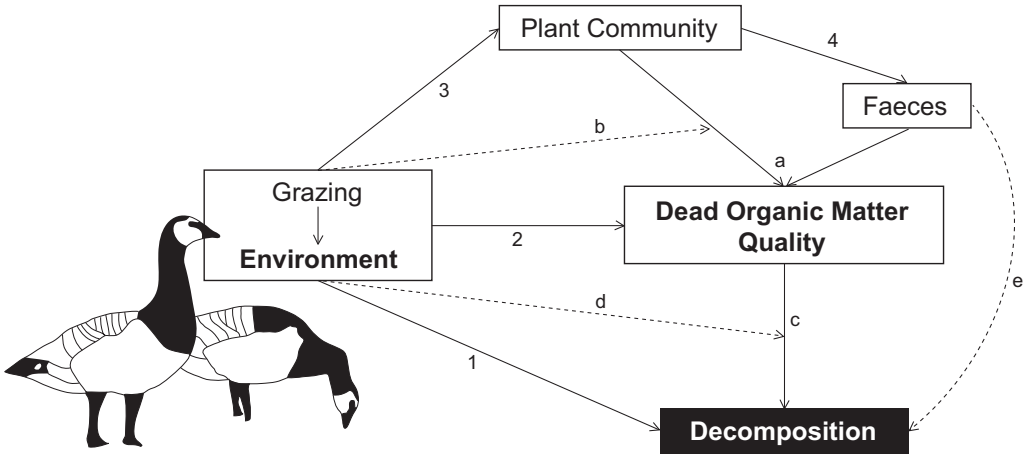

Figure 2.1. Simplified schematic representation of the ways in which goose grazing may affect decomposition i.e. through changes in the environmental conditions for decomposition (1) and more indirectly through changes in the quality of dead organic matter (2) which may be caused by shifts in community composition (3) and the production of faeces (4). Feedback mechanisms are not included. The first section of this paper focuses on dead organic matter quality. We investigated whether goose grazing affects the litter abundance of four common wetland growth forms (non-sphagnum mosses, monocotyledons, dicotyledons and equisetales) and roots (3), if growth forms and faeces differ in dead organic matter quality (a), whether goose grazing influences litter quality within growth forms (b). The second section focuses on the decomposability and nitrogen release rates. We investigated whether the dead organic matter produced in grazed plots differed in decomposability and nitrogen release rates compared to dead organic matter produced in exclosures (c), whether goose grazing influences decomposability and nitrogen release from identical dead organic matter (1) and if this alters the pattern observed for c (d). Finally we studied if faeces might stimulate decomposition and nitrogen release from organic matter (e). Answers to these questions are important if we are to understand potential effects of geese on the ecosystem level processes decomposition and nutrient availability. (Adapted from Dorrepaal et al. 2005)

Controls of litter decomposition include dead organic matter quality (figure 2.1, 2, Swift et al. 1979, Hobbie 1996, Lang et al. 2009) and environmental conditions (figure 2.1, 1, Vitousek et al. 1994, Aerts 1997, Berg and McClaugherty 2008), including the decomposer community (Swift et al. 1979, Ayres et al. 2009). Geese might interact with each of them (figure 2.1).

Selective grazing (Black et al. 2007) and the alteration of environmental conditions such as soil temperature following goose grazing (van der Wal et al. 2001), can alter vegetation composition (figure 2.1, 3). Indeed, goose grazing was found to be able to impact severely on the vegetation composition in a range of Arctic habitats (Bazely and Jefferies 1986, Gauthier et al. 2004) and also the Barnacle Goose population on Svalbard seems to induce a shift in both distribution of plant growth forms and species within growth forms (paper 1, Loonen and Solheim 1998, van der Wal et al. 2001, Stech 2008, 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 (figure 2.1, a, Cornelissen et al. 2007).

However, resource quality for decomposition is also influenced through grazing-induced changes in litter quality within species/growth forms (figure 2.1, 2, Kielland et al. 1997, Olofsson and Oksanen 2002), and responses might be species/growth form specific. Finally, (goose) herbivory transforms plant tissues into faeces (figure 2.1, 4), which tends to release nitrogen faster than plant litter (Bazely and Jefferies 1985), thus providing an important shortcut for nutrient cycling in the Arctic tundra where production and biomass accumulation are strongly nutrient limited (Shaver and Chapin 1986, Shaver and Chapin 1995, Jonasson et al. 1996). Moreover, the addition of nitrogen from faeces might stimulate the microbial activity (Bazely and Jefferies 1985) and thus enhance decomposition and nitrogen release from organic matter (figure 2.1, e).

Whereas goose grazing affects the decomposition process more indirectly by changing the resource quality, the goose-induced changes in the environment might also more directly impact decomposition and nutrient release rates (figure 2.1, 1). Indeed other authors observed an impact of geese on soil temperature (van der Wal et al. 2001) and moisture through a reduction in the insulating moss layer and nutrient availability (Wilson and Jefferies 1996, Gornall et al. 2009), three environmental factors which are directly related to the rate of the decomposition process (Robinson et al. 1995, Hobbie 1996, Aerts et al. 2006).

Moreover geese influence the microbial communities (paper 1), which are involved in the decomposition process. If the different soil communities are specialized in decomposing the litter produced above them, a so-called 'home-field advantage' might arise (Ayres et al. 2009); meaning that leaf litter decomposes more rapidly beneath the plant species it is derived from, than it does beneath different plant species (figure 2.1, d) (Bocock et al. 1960, Vivanco and Austin 2008). So found Olofsson and Oksanen (2002) for their study on reindeer grazing that shrub litter decomposed faster in the lightly grazed area where shrubs were common, and graminoid litter decomposed faster in the heavily grazed area where graminoids were common.

The objective of this study is to investigate following potential ways of Barnacle Goose impact on decomposition and nitrogen release rates:

- Goose-induced shifts in the quality of dead organic matter (figure 2.1, a, b)
- Goose-induced changes in the abiotic and biotic environment (figure 2.1, c, d)
- A facilitation effect of goose faeces (figure 2.1, e)

MATERIAL AND METHODS

Study site

The study was carried out in the Kongsfjorden area (78.55°N, 11.56°E) at Spitsbergen, Svalbard. 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 area in and around Ny-Ålesund, our study site, 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 beginning of the season and although 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 address the research questions we made use of paired grazed and ungrazed plots. For the ungrazed plots grazing was prevented by exclosures, which were made of chicken-wire 0.5 m high. Exclosures were erected on different times and in different habitats in the close neighbourhood of Ny Ålesund. The longest distance between two exclosures was 670 m. Exclosures were divided in three series. The first series, further named 'Solvatnet' was erected in 1998 and exists of five exclosures (0.8 m x 0.8 m) around the lake Solvatnet. The exclosures were characterised by dry (1), moist (2) and wet (2) vegetation. All were dominated by mosses. An area within 1 m distance from the exclosures with vegetation characterized by natural grazing was used as the reference paired grazed plot. The second series, further named 'Thiisbukta' exists of six exclosures (2 m x 2 m) erected in 2003 along a moisture gradient in the Thiisbukta wetland. At the same time an identical reference plot was defined for each exclosure in the close neighbourhood. The exclosures of both the Solvatnet and Thiisbukta series were protected with a cross of wires on top in order to prevent geese from landing in the exclosures. The 'old series' finally forms a third and more heterogeneous group which exists of older (two from 1991 and one from 1992, 1993 and 1994 each) exclosures of different sizes (between 0.5 m x 0.5 m and 1 m x 1 m) spread all over the village. As these exclosures lacked a proper reference plot, they were randomly assigned one in the close vicinity of the exclosure.

Relative abundance of different litter types

The relative abundance of different litter types was studied in grazed plots and exclosures of the Thiisbukta series at the end of the growing season in 2007. In each plot a pooled sample of four turfs (3 cm by 3 cm and 15 cm deep from the moss-soil surface, the limit for almost all roots) was taken. In the laboratory different litter types were carefully sorted out. Four categories were distinguished: moss litter (the brown photosynthetically inactive part of the moss layer which is still structurally intact), graminoid litter, dicotyledonous litter and roots (both bio- and necromass). A fifth category, *Equisetum* spec. litter, was expected but not found in the quantity samples. Litter was carefully cleaned, dried until constant weight at 35°C for at least 96 hours and weighted.

Quality analysis of litter

In autumn 2007 we also collected litter for quality analysis and the determination of decomposition and nitrogen release rates in both the exclosures and their control plots of all exclosure series. For the moss litter and roots, four turfs (5 cm by 5 cm and 15 cm deep) per plot were collected and sorted. Roots of all plants both dead and living were used. For the graminoids senescenced leaves, which were still attached to the plants were picked. No litter of dicotyledons or *Equisetum* spec. was collected as those litters were almost absent in the control plots and present only in very small amounts in the exclosures. Fresh goose faeces were collected around lake Solvatnet and in the Thiisbukta wetland and pooled in the end. All litter and faeces were air dried. Of each litter sample, a subsample was weighted, dried at 70°C until constant weight and reweighted to make the relation between air-dry and oven-dry mass and thus to calculate the initial oven-dry mass in each litterbag.

Table 2.1. Parameters and methods included in the Plant Quality Minimum Dataset from Palm and Rowland (1997) and used to characterize dead organic matter quality for decomposition +, -, * Indicates if the parameter is considered important for the process: (+) accelerating, (-) inhibiting or (*) important but depending on other factors (Melillo et al. 1989, Palm and Rowland 1997, Berg and McClaugherty 2008)

Parameters		**Methods**	**Short-term decomposition / Nutrient leaching**	**Long-term decomposition / SOM formation**
Carbon quality	'lignin'	ADF-H_2SO_4 (Van Soest 1963, Rowland and Roberts 1994)	-	-
	Soluble carbon	Aqueous methanol (50%) extraction followed by weight loss or simple sugars (Dubois et al. 1956, Allen 1989)	+	
	Soluble phenolics	Aqueous methanol (50%) extraction followed by Folin-Ciolcalteu assay (Constantinides and Fownes 1994a, b)	- (N-mineralization)	?
	α-Cellulose	ADF-residu (Van Soest 1963, Rowland and Roberts 1994)		*
Nutrient quality	Total nitrogen	CN element analysis (CN element analyser NC-2100, Carlo Erba Instruments, Italy)	+	+
	Total carbon	CN element analysis		
	Total Phosphorus	Kjeldahl (Anderson and Ingram 1993)	+	
	Ash-free dry weight	Ash for 3h 500°C	*	

Another subsample from the plant litters and goose faeces was analysed for the chemical parameters included in the minimum dataset for litter quality composed by Palm and Rowland (1997), namely 'lignin' (more correctly acid-insoluble-carbon as it may contain other recalcitrant carbon-fractions besides true lignin), soluble carbon, soluble phenolics, α-cellulose, total nitrogen, total carbon, total phosphorous and ash-free dry weight. Table 2.1 summarizes the protocols used for analysis of the different parameters and if the parameters are considered important for the short-term decomposition / nutrient release rate or the long-term decomposition / soil organic matter formation.

Before analysis litter was ground using a planetary ball mixer (Retsch, MM200, Germany). Especially for graminoid litter and to a lesser extent for roots not enough material could be collected in some grazed plots to analyse them for all parameters. In that case priority was given to carbon and nitrogen analysis followed by the other parameters in increasing order of mass needed for the analysis.

Decomposition and nitrogen release rates

The litterbag method (Bocock and Gilbert 1957) was utilized for estimating decomposition and nitrogen release rates. Litterbags (6 cm x 6 cm between stitching) were made of polyester gauze (0.3 mm mesh width) and filled with 0.2 g ± 0.002 g air-dried litter or goose faeces. Graminoid litter and roots from ungrazed plots, moss litter from grazed and ungrazed plots and goose faeces were incubated in separate litterbags in both the exclosures and their control plots.

For the six exclosures from the Thiisbukta series an extra set of litterbags was placed in separate subplots (20 cm x 25 cm) of each exclosure to investigate the possibly facilitating effect of goose faeces on the decomposition process. Therefore we added five fresh goose droppings from adult geese, corresponding circa 1.9 g of N m^{-2} (van der Wal and Loonen 1998) to these subplots at the end of the growing season in 2007 (start of incubation) and in 2008. This equals a realistic maximum of goose faeces at our study site (M.L., unpublished data). An overview of the amount of litterbags in every plot can be found in table 2.2.

Table 2.2. Overview of the number of litterbags incubated in the different exclosure types

Exclosure series	Treatment	Grass litter	Grass roots	Moss litter		Faeces
				Grazed	Exclosure	
Thiisbukta	Grazed	1	1	3	3	1
	Exclosure	1	1	3	3	-
	Exclosure subplotwith faeces	1	1	3	3	1
Lake Solvatnet	Grazed	1	1	2	2	1
	Exclosure	1	1	2	2	-
Old	Grazed	1	1	3	3	1
	Exclosure	1	1	3	3	-

Litterbags were incubated in the same plots as where their litter was originating from and the coupled plot at the end of the growing season (1-2 September 2007). Moss litter and roots were incubated in the decomposing moss layer, thus at the moss-soil interface. Grass litter and goose faeces were incubated above the moss layer and held in place with wooden skewers. This way we mimicked the position of the litter under natural circumstances.

Litterbags were collected after two years of incubation (30-31 August 2009). In the laboratory, extraneous litter, soil particles, organisms and roots were carefully removed with forceps. The remaining litter was dried at 70 °C until constant weight and the remaining mass was determined. All samples were ground and a 5-6 mg subsample was used for C/N analysis (CN element analyser NC-2100, Carlo Erba Instruments, Italy).

Data analysis

Statistical analyses were performed using SAS version 9.2 (SAS Institute Inc. 2008) and R version 2.10.1 (R Development Core Team 2009).

Relative abundance of different litter types was calculated as the percentage of the total litter mass in the plot concerned. We compared the total necromass and relative abundance of different litter types paired (corresponding grazed plots and exclosures) with Student's t or Signed Rank test depending on normality (SAS).

We tested for differences in litter quality using a mixed model ANOVA with two fixed and two random factors. Litter type (moss and roots and graminoids if enough data was available) and litter origin (grazed and ungrazed) and their interaction were treated as fixed factors. Exclosure series and replica, which is nested within exclosure series, were treated as random

factors (using the lme function in R). In this way, the problem of pseudoreplication (within-replica and within-exposure series correlations) are explicitly taken into account (Venables and Ripley 2002).

Differences in decomposition and nitrogen release rates between grazed and ungrazed plots were tested for using a four-way mixed ANOVA model. Dead organic matter type (moss, roots, graminoids and faeces) and incubation plot (grazed and ungrazed) were fixed factors, and as above, exclosure series and the nested replica effect were added as random effects to avoid pseudoreplication problems. Data were first analysed without the dead organic matter type 'faeces', because they were absent in most of the ungrazed plots and might induce spurious interactions due to the unbalanced design. However, after finding the interaction dead organic matter type – incubation plot to be non-significant we reintroduced the faeces category.

To test for the hypothesis that decomposition and nitrogen release rates differed depending on the origin of litter, we performed a similar four-way mixed ANOVA with moss litter origin (grazed and ungrazed) and incubation plot (grazed and ungrazed) as fixed factors and replica nested in exclosure series as random factors (lme function in R).

Finally we examined the influence of faeces on decomposition and nitrogen release rates using a mixed three-way ANOVA with litter type (moss, roots and graminoids) and incubation plot (only Thiisbukta series, grazed, ungrazed and ungrazed + faeces) as fixed factors and replica as random factor (lme function in R).

Effects were considered significant at $p \leq 0.05$. For the ANOVA, in case of significant effects, a posteriori comparison of means was performed with Tukey corrections for multiple comparisons.

RESULTS

Relative abundance litter

In the exclosures 1.7 times more necromass was present than in the grazed plots (2863 ± 372 g/m² respectively 1689 ± 149 g/m²; t=-2.53, n=6, p=0.05). Moss litter was by far the most abundant both in grazed plots and exclosures, followed by roots and graminoids (figure 2.2).

However, while the general pattern was the same for grazed plots and exclosures, the percentage of litter originating from graminoids and roots was respectively twice and four times as much in the exclosures compared to the grazed plots (figure 2.2).

All differences in relative abundance between grazed plots and exclosures were statistically significant (table 2.3). In none of the cores *Equisetum* spec. litter was found and only one sample, originating from one of the exclosures, contained a small amount of litter from dicotyledons.

Table 2.3. Results of pair wise comparisons of relative litter abundance between grazed plots and exclosures (n=6). Significant differences (p<0.05) are in bold.

Litter type	Test parameter	p
Graminoids	S = 10.5	0.0313
Moss	t = -5.15	0.0036
Roots	t= 5.46	0.0028

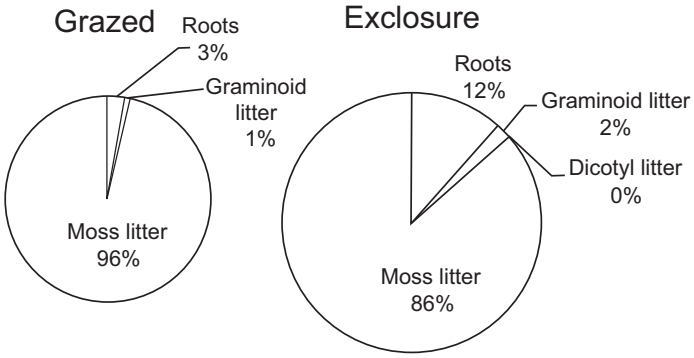

Figure 2.2 Mean relative abundance of different litter types in grazed plots and exclosures from the Thiisbukta series at the end of the growing season (n=6). Total necromass 2863±372 g/m² and 1689±149 g/m² for exclosures respectively grazed plots.

Litter Quality

We observed differences for all measured chemical parameters between litter types, e.g. moss, roots and graminoidsb (table 2.4 and table 2.5). Roots had the lowest concentration of structural compounds ('lignin' and α-cellulose) and the highest concentration of phosphorous, soluble carbohydrates, carbon and ash-free dry mass. For soluble phenolics content, roots showed an intermediate value. Nitrogen content did not differ between roots and graminoid litter or moss litter. Moss litter had the highest 'lignin' content (actually acid-insoluble carbon as mosses do not contain true lignin (Bland et al. 1968, Reddy 1984)) and lowest concentration of nitrogen, phosphorous, carbon, soluble phenolics, soluble carbohydrates and ash-free dry mass. The α-cellulose content of moss litter was intermediate between graminoid litter and roots. Graminoid litter had a higher concentration of α-cellulose, soluble phenolics and nitrogen, for all other parameters graminoid litter showed intermediate values.

Table 2.4. Initial chemical characteristics of litter types (means ± 1 SE). All values are expressed as % of dry weight. Different capitals indicate significant differences between treatments (grazed – exclosure) and different lower case letters indicate differences between litter types ($p \leq 0.05$).

Litter type	'lignin'	Soluble Carbohydrates	Soluble Phenolics	α-Cellulose	Total Nitrogen	Total Carbon	Total Phosphorus	Ash-free dry weight
Grazed	A	A	A	A	A	A	A	A
Roots	5.3 ± 0.9b	34.6 ± 4.9a	0.65 ± 0.05a	16.5 ± 0.6b	1.40 ± 0.14ab	43.0 ± 0.9a	0.206 ± 0.026a	0.839 ± 0.131a
Graminoids	na	na	na	na	1.48 ± 0.19a	42.7 ± 0.9a	0.169 ± 0.071b	na
Moss	13.0 ± 0.8a	2.9 ± 0.4b	0.22 ± 0.017b	22.2 ± 1.3a	1.19 ± 0.07b	34.6 ± 1.4b	0.119 ± 0.007b	0.778 ± 0.030b
Faeces	5.9 ± (*)	2.3 ± (*)	1.16 ± (*)	19.2 ± (*)	2.00 ± (*)	39.8 ± (*)	0.404 ± (*)	0.832 ± (*)
Exclosure	B	A	A	B	B	A	B	A
Roots	3.4 ± 0.5b	35.4 ± 3.1a	0.65 ± 0.03b	16.0 ± 0.9c	1.20 ± 0.08ab	44.5 ± 0.2a	0.146 ± 0.018a	0.968 ± 0.002a
Graminoids	4.2 ± 0.3b	6.1 ± 0.8b	1.07 ± 0.05a	25.1 ± 0.7a	1.35 ± 0.05a	44.1 ± 0.2a	0.127 ± 0.010b	0.923 ± 0.010a
Moss	12.6 ± 0.9a	2.7 ± 0.3b	0.24 ± 0.03c	20.7 ± 1.1b	1.10 ± 0.05b	33.6 ± 1.5b	0.112 ± 0.006b	0.777 ± 0.025b

(*) Pooled samples

Besides those differences in litter quality between litter types, differences between litter produced in grazed plots and exclosures were found (table 2.4 and table 2.5). Litter produced in exclosures exhibited lower amounts of 'lignin', α-cellulose, nitrogen and phosphorous than in ungrazed plots (between 3 – 35 % reduction, with for all parameters but α-cellulose lowest differences for moss litter).

Goose droppings showed intermediate values for structural compounds, higher values for nitrogen, phosphorous and soluble phenolics and lower values for soluble carbohydrates, carbon (except moss from exclosures) and ash-free dry mass (table 2.4).

Differences in 'lignin', carbon, nitrogen and phosphorous content resulted in differences in the most commonly used ratios, C:N, C:P, 'lignin':N and 'lignin':P (table 2.5, figure 2.3) between litter types (all parameters) and between litter produced in grazed plots and exclosures (only C:N and 'lignin':N). Faeces had the lowest values for all quality parameters, suggesting they decompose relatively easy.

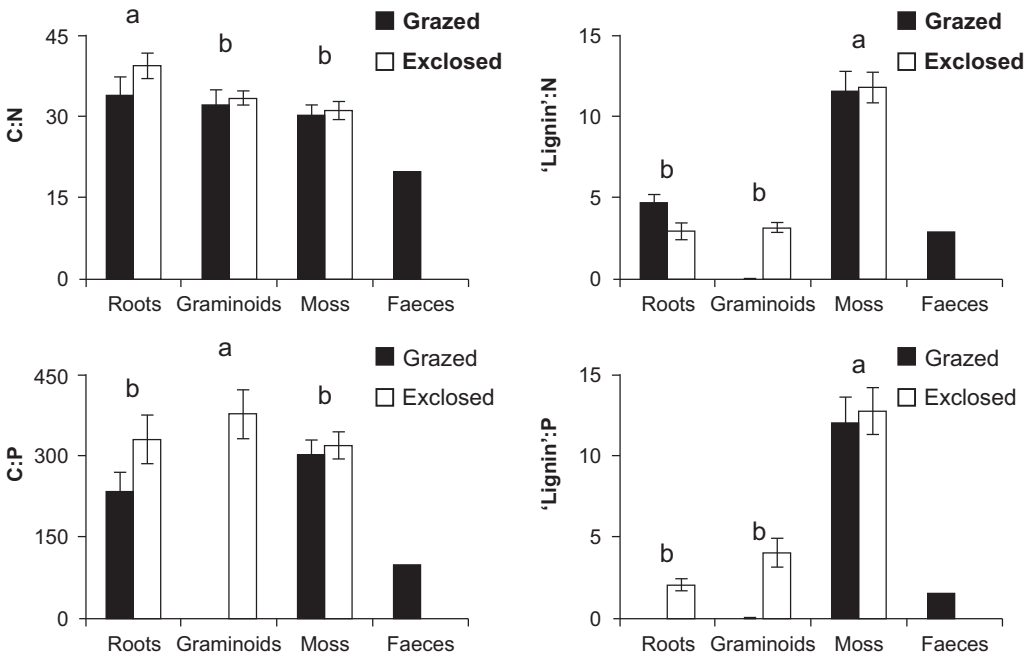

Figure 2.3. Selection of most commonly used ratios of chemical parameters to describe dead organic matter quality (C:N, 'lignin':N, C:P, 'lignin':P) for different litter types produced in grazed plots and exclosures. Error bars indicate ± 1 SE. Different letters indicate differences between litter types, differences between treatments are indicated by a putting them in bold ($p \leq 0.05$).

Table 2.5. Results from the four-way ANOVA's for the different litter quality parameters. Fixed variables were litter type (moss, roots and shoots, or only moss and roots if indicated by *) and treatment (grazed, exclosure), random variables were replica nested in exclosure series. Significant differences ($p \leq 0.05$) are in bold.

Litter quality parameter	Litter type			Treatment			Litter type x Treatment		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
'Lignin'	2, 45	108.32	**<0.0001**	1, 33	23.91	**<0.0001***	1, 32	0.09	0.7697*
Soluble Carbohydrates	2, 58	114.24	**<0.0001**	1, 42	1.11	0.2986*	1, 41	0.05	0.8321*
Soluble Phenolics	2, 58	210.94	**<0.0001**	1, 42	0.65	0.4249*	1, 41	0.14	0.7092*
α-Cellulose	2, 45	20.59	**<0.0001**	1, 33	5.69	**0.0230***	1, 32	0.12	0.7358*
Total Nitrogen	2, 67	7.08	**0.0016**	1, 67	4.28	**0.0424**	2, 65	0.35	0.706
Total Carbon	2, 67	82.74	**<0.0001**	1, 67	3.64	0.0607	2, 65	1.08	0.3447
Total Phosphorus	2, 35	13.90	**<0.0001**	1,35	5.05	**0.031**	2, 33	2.21	0.1258
Ash-free mass	2, 21	18.08	**<0.0001**	1, 18	0.00	0.9558*	1, 17	1.01	0.3282*
C:N	2, 67	5.98	**0.0041**	1, 67	4.45	**0.0386**	2, 65	0.87	0.4255
'lignin':N	2, 45	69.91	**<0.0001**	1, 33	11.05	**0.0022***	1, 32	0.08	0.7842*
C:P	2, 34	8.56	**0.001**	1, 34	1.51	0.2269	1, 32	2.09	0.1397
'lignin':P	2, 25	30.64	**<0.0001**	1, 17	1.68	0.212*	1, 16	0.01	0.9379*

Decomposition rates

After two years on average between 17% (moss grazed, exclosure) and 54% (roots, grazed) of the litter was broken down (figure 2.4.A).

Decomposition rates were significantly increased by excluding geese ($F_{1,104}$=4.456, p=0.0372) and by dead organic matter type ($F_{3,123}$=30.53, p<0.0001). Whereas all dead organic matter types – graminoids, roots, moss, faeces – differed from each other in decomposition rates except moss litter and faeces (figure 2.4.A), no interaction effect of goose grazing and dead organic matter type on decomposition rates could be observed ($F_{1,102}$=0.1606, p=0.8519). Goose grazing impact on decomposition was thus not significant different between dead organic matter types.

No support was found for the facilitation of litter decomposition in the plot of origin. More detailed analysis of the decomposition rates of moss litter revealed that the breakdown of moss litter was not significantly influenced by the origin of the litter ($F_{1,46}$= 1.689, p=0.2002, figure 2.4.A). Also no evidence was found for a change in decomposition rates when goose faeces were added to the exclosures ($F_{2,61}$=2.126, p=0.1281, table 2.6).

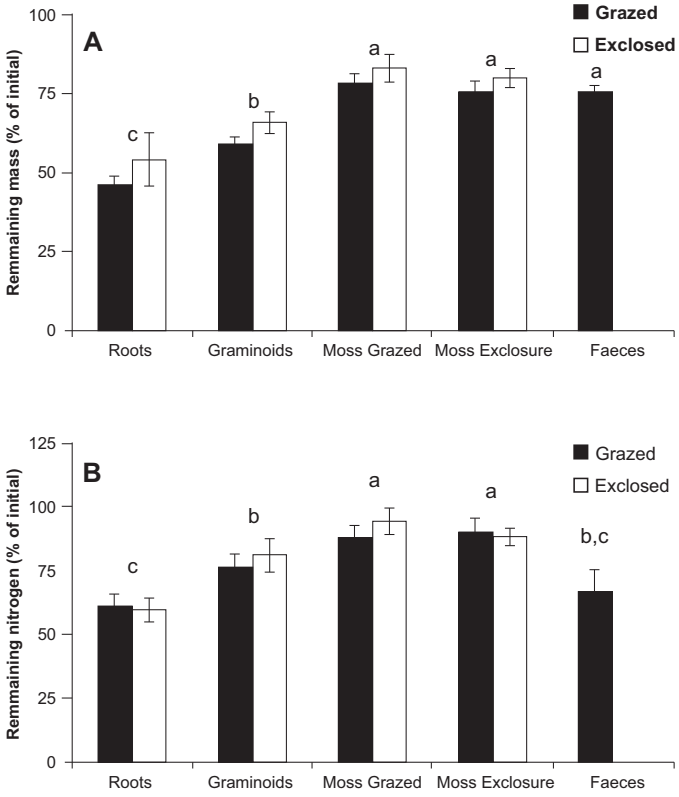

Figure 2.4. Remaining litter mass (A) and nitrogen (B) for different litter types in grazed plots and exclosures. Error bars indicate ± 1 SE. Different letters indicate significant differences between litter types, difference between treatments are indicated by putting them in bold ($p \leq 0.05$).

Table 2.6. Decomposition and nitrogen release from litter in the Thiisbukta plots. Values are given as means ± 1 SE.

Plot type	Litter type	Remaining Mass (% of initial)	Remaining Nitrogen (% of initial)
Grazed	Roots	48.3 ± 4.0	60.5 ± 8.0
	Graminoids	61.3 ± 1.9	88.5 ± 8.8
	Moss Grazed	87.7 ± 4.7	99.6 ± 7.0
	Moss Exclosure	84.8 ± 5.5	97.0 ± 12.0
	Faeces	76.0 ± 7.8	86.7 ± 16.8
Exclosure + Faeces	Roots	52.4 ± 6.8	63.4 ± 12.0
	Graminoids	70.2 ± 8.7	101.2 ± 13.3
	Moss Grazed	94.3 ± 3.4	101.0 ± 7.1
	Moss Exclosure	96.7 ± 2.8	100.5 ± 2.1
	Faeces	79.1 ± 9.6	89.5 ± 13.0
Exclosure	Roots	67.0 ± 20.9	58.3 ± 9.4
	Graminoids	70.3 ± 6.3	93.4 ± 11.2
	Moss Grazed	100.7 ± 5.5	112.6 ± 10.0
	Moss Exclosure	89.0 ± 3.5	96.3 ± 6.5

Nitrogen release

Although at first glance the patterns for nitrogen release might seem rather similar to the patterns for mass loss, they differ on quite essential parts. After two years between 6% (grazed moss, exclosure) and 40% (roots, exclosure) of the nitrogen was released from the litter (figure 2.4.B). Rates of nitrogen release were only significantly influenced by dead organic matter type ($F_{3,123}$=21.37, p<0.0001) and not by goose grazing ($F_{1,104}$=0.21969, p=0.6403) or the interaction between both ($F_{1,102}$=0.2229, p=0.8006). Dead organic matter types which differed significantly in nitrogen release rates were moss and faeces, moss and roots and graminoids and roots. Just as for mass loss no support was found for the facilitation of nitrogen release in the plot of origin; rates of nitrogen release from moss litter were not significantly influenced by the origin of the litter ($F_{1,46}$= 0.2291, p=0.6344, figure 2.4.B). Finally, also similar to mass loss no evidence could be found for a change in nitrogen release rates when goose faeces were added to the exclosures ($F_{2,61}$=0.36969, p=0.6925, table 2.6).

DISCUSSION

The goal of this study was to examine the different ways in which goose grazing might affect decomposition processes in the high Arctic. First we focused on the dead organic matter: plant litter and in the case of goose grazing also faeces. We both examined litter quantity and resource quality.

Litter production itself could not be studied but the necromass (graminoids, dicotyledons, *Equisetum* spec. and moss excluding the peaty soil) and total root mass was considered as a proxy for litter production. As decomposition rates varied between litter types the relative production of litter will not be the same as the relative abundance of litter. The use of the total root mass further biases the picture. However, assuming that goose grazing has no (or only a minor) effect on root mortality, the differences in relative abundance of litter between grazed and ungrazed plots will be translated in analogue differences in litter production as the difference in decomposition rates between different litter types was not influenced by goose grazing.

Also while discussing the effect of goose grazing on resource quality we have to be careful. It was indeed not possible for us to separate roots trustfully into biomass and necromass, let

alone that we would be able to distinguish between recently senescenced and old root litter. Visual separation of roots has lots of limitations and especially distinguishing live from dead fine roots is subjective and requires tedious microscopic examination of nearly every fine root segment (Clemensson-Lindell and Persson 1995, Ruess et al. 2006); a reason why a series of chemical methods were developed (Clemensson-Lindel 1994, Ruf and Brunner 2003). It goes without saying that we couldn't adopt these methods as we intended to use the root litter for chemical analysis and to study decomposition. As a result we decided to use total root mass, but we are aware of the problems this might cause for comparing litter quality between roots and other litter types.

Keeping these objections in mind, we found that grazing by geese diminishes the amount of litter present. It might sound evidently because plant parts are removed by geese before they can senescence, however the reduction of litter by herbivory is not a general rule. Ford and Grace (1998) found for example 85% more necromass in grazed plots compared to exclosures, which was explained by the destructive feeding habit of their study species: wild boar and nutria. However, other studies on geese found no difference (Zacheis et al. 2002, Sjögersten et al. 2011) or in line with our research a decreased litter production (Bazely and Jefferies 1986, Zacheis et al. 2001). Also the study conducted by Sjögersten et al. (2011) on the same exclosures earlier in the season found only a tendency of decreased graminoid litter presence and no overall effect as we found in this study. This might be due to seasonal differences, as she collected litter at the peak of the growing season before senescense and we collected litter at the end of the season after senescence.

On top of the overall impact of Barnacle Goose grazing on litter quantity we found a shift in relative abundance of litter types between growth forms. The relative abundance of graminoid litter, dicotyledonous litter and roots was reduced in grazed plots while relatively more moss litter was present. This is reflecting the food selection of Barnacle Geese, which prefer grasses and sedges followed by forbs above mosses (Prop and Vulink 1992, Alsos et al. 1998).

Although a shift in relative litter abundance of different species / growth forms by herbivory is often insinuated based on the results of vegetation surveys or biomass production (Olofsson and Oksanen 2002), we found almost no other study which actually quantified the possible shift. An exception is he study of Persson et al. (2005) who found a significant difference in litter production of different plants caused by herbivory. Shifts in vegetation composition

suggest that herbivores might both favour and deplete their preferred food plants depending on among others habitat characteristics (Semmartin et al. 2004) and grazing intensity (Jefferies et al. 2003).

Actually, as digestion efficiency in geese is poor, geese select for plants high in nutrients and low in structural components as 'lignin' (Mattocks 1971, Owen 1980, Prop and Vulink 1992, Alsos et al. 1998), two main characteristic of high quality litter (Berg and McClaugherty 2008). So as Barnacle Geese prefer grasses above mosses we expected grasses to produce better decomposable litter. Indeed we found grass litter having higher nutrient (nitrogen and phosphorous) and lower 'lignin' concentration than mosses and this not only counts for aboveground graminoid litter, but also for the often neglected roots. In general, main nutrients, as nitrogen and phosphorous, primarily enhance decomposition during the early stage, whereas 'lignin' exerts a dominant negative control over the later stages (Berg and McClaugherty 2008). Therefore, we might conclude that the change in litter composition towards a lower percentage of graminoid litter and roots caused by goose grazing is probably unfavourable both for short-term and for long-term decomposition.

The other dead organic matter quality parameters are all seen as important determinants of the decomposition process, but their role is less clear. For instance mass loss of dead organic matter is, especially in the long term, positively associated with α-cellulose, but is also negatively associated with 'lignin' concentration (Hobbie 1996, Palm and Rowland 1997). Melillo et al. (1989) combined both lignin and holocellulose (α-cellulose plus hemicellulose) in their lignocellulose index (lignin to lignin and cellulose ratio) for estimating plant quality and long-term decomposition trends. Graminoid litter has the highest α-cellulose content followed by moss and then roots. Based on α-cellulose alone we might thus expect moss to have a better quality for long-term decomposition than roots. However, the much higher lignin-like content and low nitrogen values of moss litter might mitigate the small decomposition advantage the higher α-cellulose content provides. The concentration of soluble phenolics on the other hand is considerably higher in the graminoid litter and roots than in the moss litter suggesting reduced rates of nitrogen release but not necessarily carbon mineralization (Palm and Rowland 1997).

Concerning the commonly used ratios to express litter quality, differences of 'lignin':N and 'lignin':P between the litter types were most obvious and indicate mosses as having the worst

quality for decomposition (highest values). In contrast C:N and C:P values indicate a slightly worse quality for roots respectively graminoid litter.

In summary, we might conclude that the shift in relative litter abundance of different growth forms caused by goose grazing indeed results in a shift in litter quality since, in agreement with other studies (e.g. Hobbie 1996, Dorrepaal et al. 2005), we found a difference in litter quality between growth forms. As different litter quality parameters point in different directions, it is less clear which growth form has the most favourable overall litter quality for decomposition. However, we might prudently think about graminoid litter and roots, both with a lower relative abundance due to goose grazing, having a better organic matter quality for decomposition than moss. If so the shift in growth form composition of litter by goose grazing deteriorates litter quality.

As should have become clear above, a difference exists in organic matter quality between roots and graminoid aboveground litter. Though roots were not sorted in growth forms, we might look at them as the below ground part of graminoids as dicotyledonous litter was only found in very small amounts in two exclosures. Given the known impact of herbivore exclusion on the root biomass, both in positive and negative direction (Milchunas and Lauenroth 1993), this difference in organic matter quality between roots and shoots shows, unintentionally, an indication for a barely documented way in which goose grazing might impact on the organic matter quality: namely by altering the resource allocation pattern. However, data on root litter separated from living roots is necessary to understand if and how this potential mechanism plays.

On top of this we found goose grazing impact on the litter quality of the same plant organ within growth forms, 'lignin', α-cellulose, nitrogen and phosphorous content are higher in litter from grazed plots compared to exclosures. The goose-induced increment in 'lignin' concentrations might impede decomposition. 'Lignin' is one of the major determinants of litter quality for decomposition (Meentemeyer 1978). In spite of this, the effect of (vertebrate) herbivory on the 'lignin' content of litter seems to be hardly documented, especially for moss litter and for roots. Nevertheless, at least one other study by Semmartin et al. (2008) also studied the grazing effect on 'lignin' in roots. Contrary to our results they did not find a difference in 'lignin' content but found that plants from a grassland grazed by cattle produced litter with lower 'lignin':N ratios than those from the ungrazed site, which likely

contributed to accelerate the decomposition of their litter (Aber and Melillo 1991, Vivanco and Austin 2006).

Litter nutrient (nitrogen and phosphorous) content on the other hand is enhanced in grazed plots, ameliorating litter quality for decomposition. This pattern reflects the often found elevated nutrient contents of grazed plants (Cargill and Jefferies 1984, Phillips et al. 1999). Several mechanisms have been proposed to explain differences in nitrogen concentrations of plant tissue between grazed and ungrazed areas (Bazely and Jefferies 1985, Sirotnak and Huntly 2000, Zacheis et al. 2002). In Ydenberg and Prins (1981) the enhanced nitrogen concentrations were explained by the consequent sustained regeneration of young protein-rich plant tissues as a result of repeated grazing by Barnacle Geese. However, this may not explain the elevated levels of nutrients in grazed litter. The enhanced nitrogen availability through incorporation of litter (Zacheis et al. 2002) and faeces (Sorensen et al. 2009) into the soil by trampling, in contrary, might be (a part of) the explanation. The conversion of plant material into faeces might be another. The addition of faeces and urine or uric acid alone, without grazing, has indeed been shown to result in increased plant nitrogen concentration in some grazing systems (Bazely and Jefferies 1985, Hik and Jefferies 1990).

In fact, grazing short-circuits the decomposition process by the production of faeces (Bryant et al. 1983). This influences the time of conversion of living plant tissue to dead organic matter and the form of dead organic matter: faeces rather than dead leaves (Maclean 1974). Like other authors (Floate 1970, Bazely and Jefferies 1985), we found much higher concentrations of total nitrogen and phosphorous in faeces compared to plant litter resulting in lower - thus more favourable - values for all commonly used ratios to express litter quality for decomposition (C:N, C:P, 'lignin':N, 'lignin':P).

Moreover, faeces seem to provide a readily accessible form of nitrogen (Floate 1970, Bazely and Jefferies 1985). Furthermore, Floate (1970) found that for plant litter large amounts of phosphorous were immobilized, while for faeces only by very low temperatures phosphorous was immobilized. We did not measure the remaining phosphorous, but the rather high loss of nitrogen of goose faeces we found (compared with the main plant material they consisted of, namely mosses, L.F. Personal Observation), supports the theory that goose grazing enhances the plant availability of nitrogen through the production of faeces (Bazely and Jefferies 1985).

Analogous to the fast nitrogen release rates we might expect rather high decomposition rates for goose faeces even more as all commonly used ratios to predict decomposability (i.e. C:N,

C:P, 'lignin':N and 'lignin':P) were favourable. Yet, goose faeces were together with mosses the slowest to decompose. Actually, mosses confirmed their status of being slow to decompose and to release nitrogen (Hobbie 1996, Cornelissen et al. 2007). Unfortunately we were not able to collect enough dicotyledonous litter, but litter decomposition research in cold biomes by Cornelissen et al. (2007) consistently found forb litter to decompose even faster than graminoid litter.

The reduced relative abundance of roots, dicotyledons and graminoids through goose grazing thus impedes both mass loss and nitrogen release from litter while the latest is on the other hand accelerated by the transformation of plant biomass in faeces.

In this study no indication was found for a difference in decomposition or nitrogen release rate between litter from the same type produced in a grazed plot or an exclosure. However, as we were not able to collect enough root or graminoid litter in the grazed plots this part of the study was only performed with moss litter which chemistry was least influenced by goose grazing. In other words, the importance of the effect of goose grazing on litter quality within species for decomposition might be more important than our results suggest.

Even a small difference in litter quality caused by goose grazing and the linked decomposability in Arctic ecosystems can play a pivotal role in determining the amount and quality of organic matter that accumulates in soil because small differences in decomposability at the surface can produce large differences in the proportion of litter that is transferred to depths, where decomposition is lowered by cold and wet conditions (Jones and Gore 1978, Heal et al. 1981).

Measured differences in decomposition rates were not only the result of the differences in litter quality. Indeed also the place of litter production and thus decomposition was mimicked in our experiment. Therefore, root and moss litter experienced the same colder and wetter decomposition conditions prevailing at the moss-soil interface, compared to the conditions at the surface where grass litter decomposes.

The differences in mass loss rates between grazed and ungrazed plots are then again a result of the impact of goose grazing on the conditions for decomposition. The decomposition process is indeed very sensitive for environmental conditions like soil temperature, moisture, nutrient availability and the decomposer community (Swift et al. 1979, Hobbie 1996, Ayres et al. 2009, Liu et al. 2010) and on all of them herbivory was found to have an impact.

Barnacle Geese reduced thickness of the moss layer (paper 1) by trampling and consumption. As moss is a good isolator a reduction in the moss layer results in higher soil temperatures (van der Wal and Brooker 2004, Gornall et al. 2007), which in turn is found to enhance the decomposition process (Hobbie 1996). On top of this a deep moss layer retains water within its structure and reduces evaporation from the soil surface, resulting in wetter soils. A considerable amount of the plots was inundated in spring and the other plots were at least soaked by melt water. Exceptionally high (or low) soil moisture contents may limit decomposition rates (Flanagan and Veum 1974); a reduction in soil moisture content in grazed plots will thus enhance decomposition rates.

The possible increase in soil temperature and decrease in soil moisture by the reduction in moss layer depth can only explain the accelerated litter decomposition of moss litter and roots as those litter types were incubated at the moss-soil interface. We thus should think of other mechanisms to explain the increased decomposition rate of graminoid litter incubated above the moss layer by goose grazing. It might be the production of faeces, wherein nitrogen is present in a soluble form (Bazely and Jefferies 1985). However we did not find an indication of facilitated litter decomposition by the addition of faeces. Also the reduction and incorporation in the soil by trampling, which was reported to increase nitrogen mineralization by Zacheis et al. (2002), is excluded as explaining mechanism since we used litterbags. Changed decomposition rates might be explained by the observed shift in microbial community structure (paper 1), but we do not have any evidence for this. Sure is that we didn't find any indication which points towards a "home field advantage", decomposition facilitation of the litter produced in the same plot, as observed in the reindeer study of Olofsson and Oksanen (2002).

CONCLUSION

In this study we found Barnacle Geese influencing decomposition rates in several ways. They altered the plant species composition, changed the plant chemistry within functional groups and produced faeces. The result is a change in dead organic matter entering the decomposition process.

This resulted on one hand in a decrease of the decomposition process and nitrogen release rates caused by the suppression of graminoid litter production by goose grazing. The goose-induced changes in the decomposition environment, on the other hand, positively impacted on the decomposition. The production of goose faeces finally was, surprisingly, not enhancing the process.

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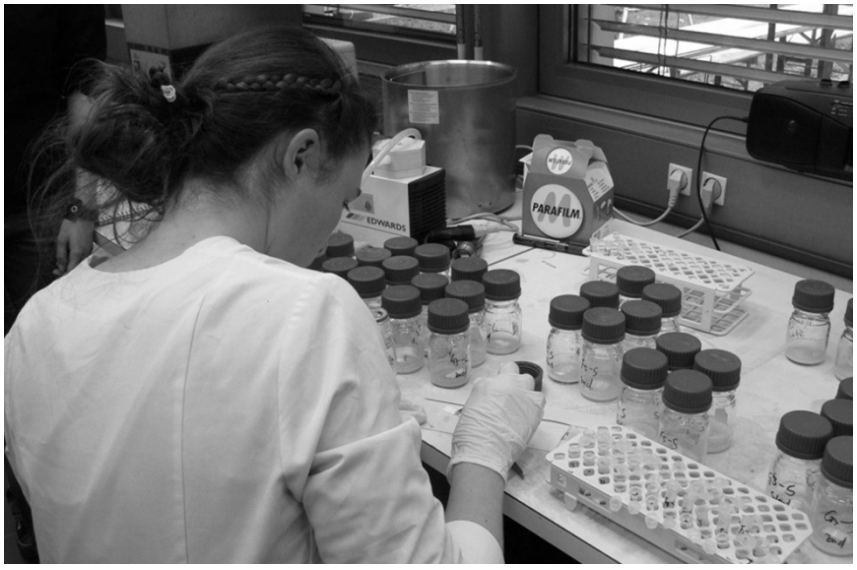
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Paper 3

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).

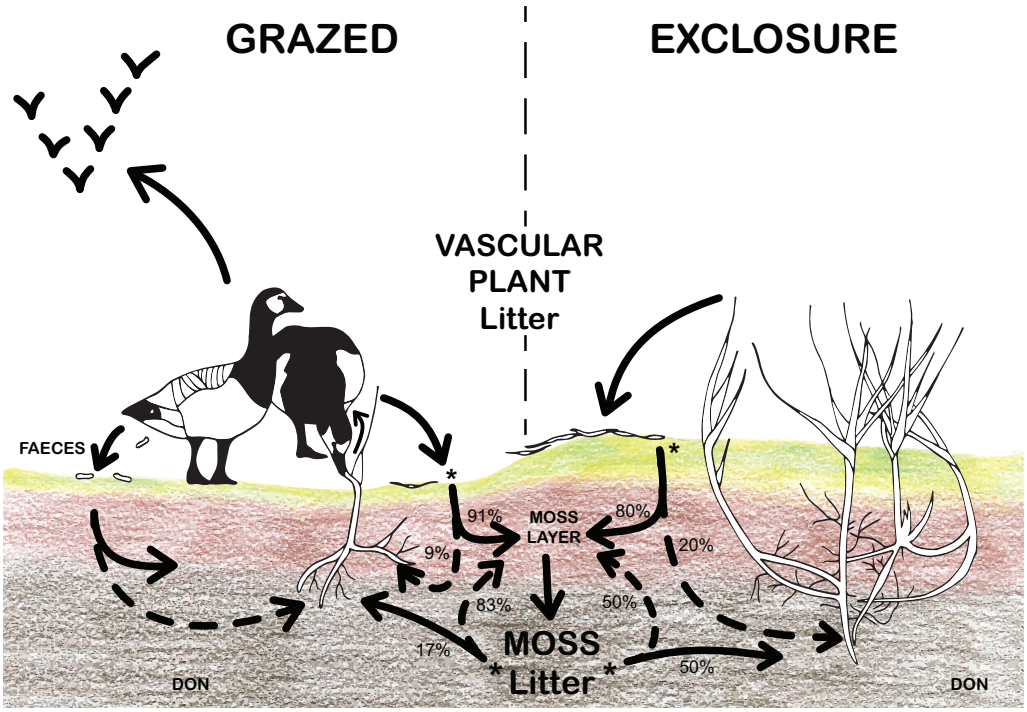


Figure 3.1. The influence of goose grazing on the nitrogen cycle in an Arctic wet tundra ecosystem. Arrows represent nitrogen fluxes. Different plausible ways of geese impacting on the tundra.

(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).

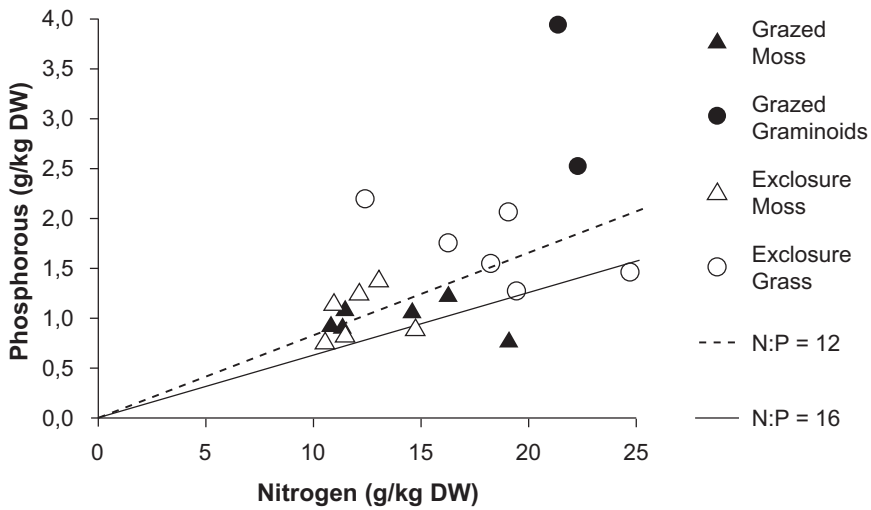


Figure 3.2 Foliar N:P ratios for moss (triangles) and graminoids (rounds) growing in grazed plots (black) and exclosures (open). The solid line represents an N:P ratio of 16, all samples beneath this line suggest phosphorous limitation, The dashed line represent an N:P ratio of 12, all samples above 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).

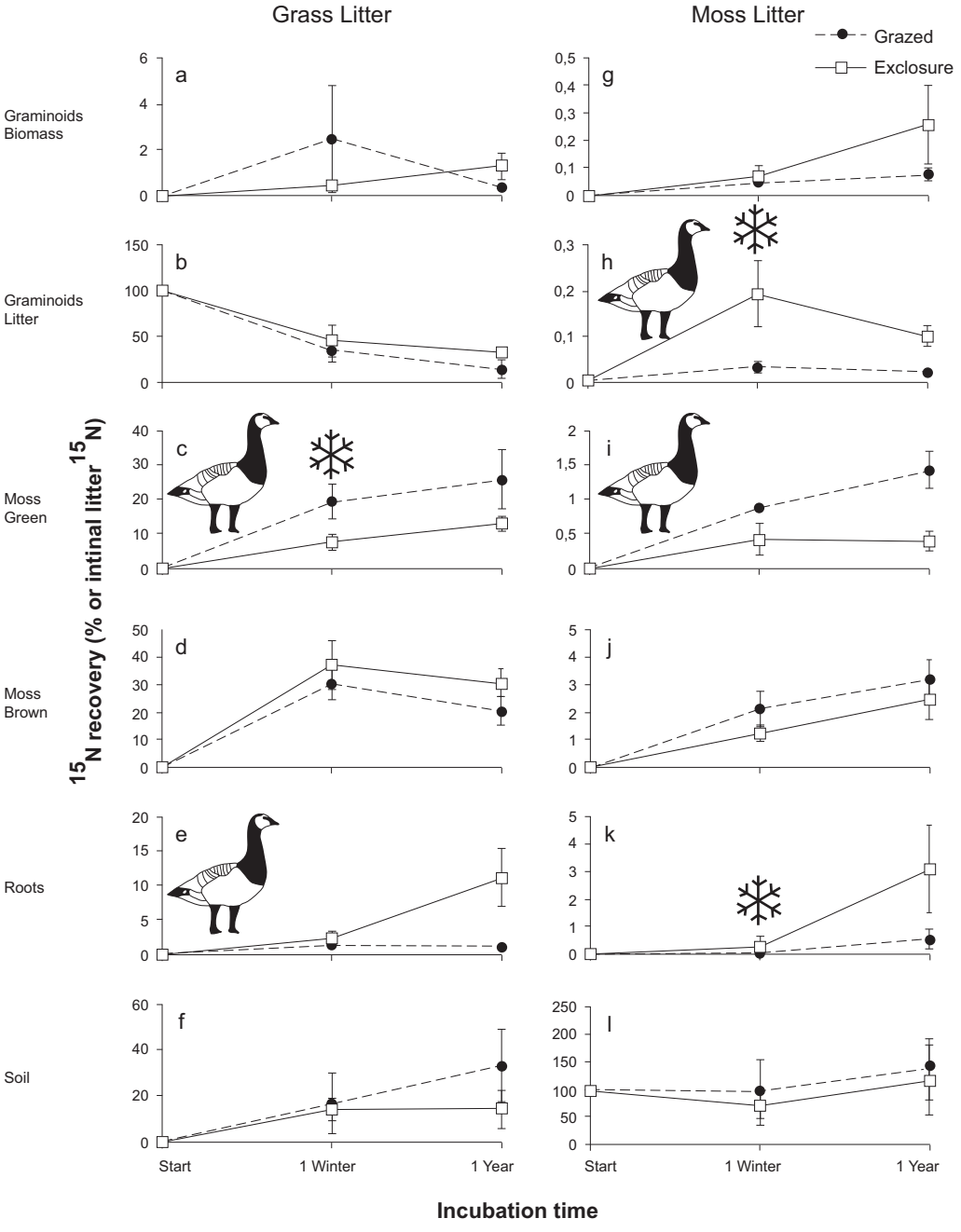
N-dynamics (figure 3.3, table 3.3)

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).

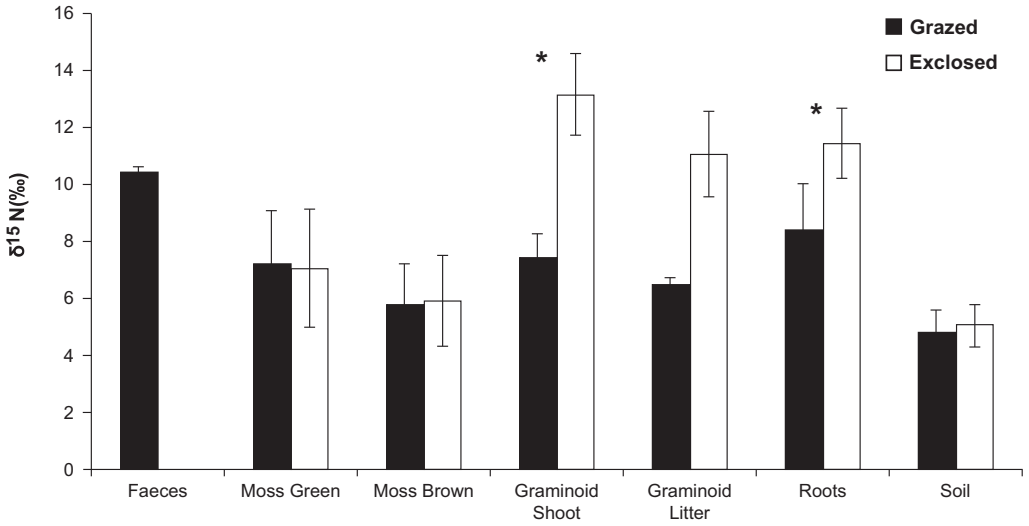


Figure 3.4. Impact of the grazing treatment on background $\delta^{15}N$ values for different ecosystem compartments. Means ± 1 SE are shown (n=6). Significant differences indicated by an asterix ($p \leq 0.05$).

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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Paper 4

Influence of goose grazing on plant availability of nutrients

Manuscript

Lise Fivez, Johannes Teuchies, Maarten Loonen, Patrick Meire

ABSTRACT

Nutrient availability is a primary limiting factor of biotic functioning in Arctic environments. We hypothesized that geese, whose numbers have increased dramatically, impact on the plant availability of nutrients. The moss layer was thought to play a key role herein. To test for these hypotheses we measured plant availability of macro- and micronutrients over the winter and growing season and moss depth in a goose exclosure experiment. Our results show that important nutrients were significantly influenced by goose grazing. For some elements this could be partially explained by the grazing impact on the moss layer. During the winter season nutrient availability was remarkably high and was influenced by geese, urging the need for more ecological research during this period.

Keywords: *Nutrient availability, nitrogen, goose, Arctic, moss, PRS^{TM}-probes, winter processes*

ARTICLE

Nutrient availability is a primary limiting factor of biotic functioning in Arctic environments. Geese, whose numbers increased dramatically over the past 50 years due to human induced changes, were found to impact cycling and availability of nitrogen (paper 3, Ruess et al. 1989). Possible mechanisms of goose grazing impact are: (i) the impact on resource quality for decomposition by changes in vegetation composition (Bazely and Jefferies 1986, Gauthier et al. 2004, Kuijper et al. 2009) and the production of faeces, which are easily decomposable and high in readily available nutrients, (ii) the fragmentation of the dead plant material and the incorporation of litter into the soil due to trampling, accelerating decomposition and net nitrogen mineralization (Zacheis et al. 2002, Sorensen et al. 2009) and (iii) the reduction of the moss layer which is of crucial importance for ecosystem functioning (van der Wal et al. 2001, Gornall et al. 2009).

Whereas we do not want to neglect the importance of the first two mechanisms, the impact on the moss layer is likely to play a key role in the goose grazing effect on nitrogen availability for plants. The depth of the isolating moss layer indeed governs soil temperature, soil moisture and the number of freeze-thaw cycles, all of crucial importance for decomposition and mineralization processes (see also paper 1 and 3, Campbell et al. 2005, Gornall et al. 2007). A reduction in the moss layer might result in warmer and wetter soils containing more plant available nitrogen as found by Gornall et al. (2007). Moreover, ion exchange capacity of mosses is typically high and mosses are able to effectively take up nutrients through their entire surface because they lack a cuticle (Brown and Bates 1990). As a consequence mosses may swiftly take up soluble nitrogen preventing further access for vascular plants (paper 3, Kotanen 2002, Sjögersten et al. 2010).

Although the same mechanisms might hold for other nutrients, virtually nothing is known about the effect that geese might have on the plant availability. This study aims to fill this knowledge gap by addressing following questions: (1) Does goose grazing affect the availability of macro- and micronutrients for plants? (2) Does the moss layer play a key role in this process? This study aims to answer these questions by studying the plant availability of both macro- and micronutrients in the wet moss tundra-dominated brood-rearing area of the Kongsfjorden Barnacle goose population *Branta leucopsis* (Bechstein, 1803), Spitsbergen (78° 55' N, 11° 56' E). Nutrient availability was determined in three series of long–term goose

exclosures and their control plots (figure 4.1.A). More detailed information about the field site and experimental setup can be found in paper 2.

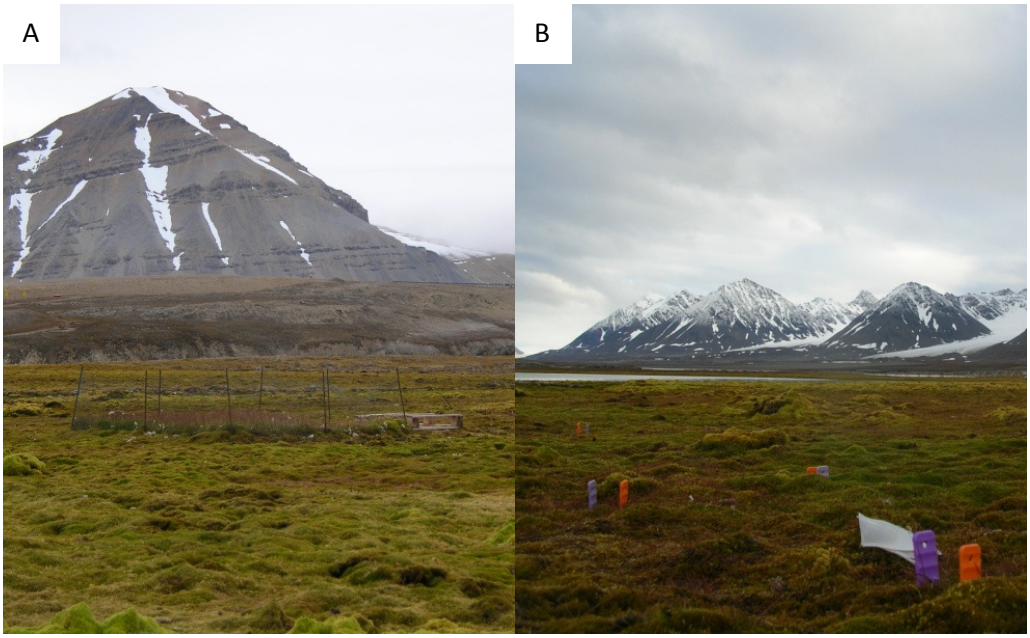


Figure 4.1. Experimental setup. a) Exclosure, mark the difference between the vegetation in and surrounding the exclosure. b) PRS^{TM}-probes in the field.

Nutrient supply rates for plants were assessed both during the winter (geese absent, 17/08/08-25/06/09) and the summer season (geese present, 25/06/08-17/08/08), using Plant Root Simulator probes (PRS^{TM}-probes, Western Ag Innovations Inc., Saskatoon, SK, Canada, figure 4.1.B). To account for soil heterogeneity within each replicate four pairs (i.e. cation- and anion-exchange) of PRS™-probes were spread throughout each experimental unit and combined for analysis. PRS™-probes were inserted vertically, downwards from the top of the rooting zone, at the start of the winter season when the soil started freezing. We removed buried PRS™-probes in spring just after snowmelt when the soil was still frozen and then re-inserted fresh PRS™-probes in the same soil slot until the end of the summer season. Such long-term burials allow accounting for temporal factors affecting nutrient supply, including ion diffusion from greater distances and the slow release of nutrients from mineralization and dissolution. After removal, the PRS™-probes were washed with deionized water, bulked according to treatment plot and transported on ice. In the lab probes were 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). Inductively-coupled argon plasma optical emissions spectrophotometry (ICP-OES, Optima 7300 DV, PerkinElmer) was used to measure levels of phosphorous (P), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), boron (B).

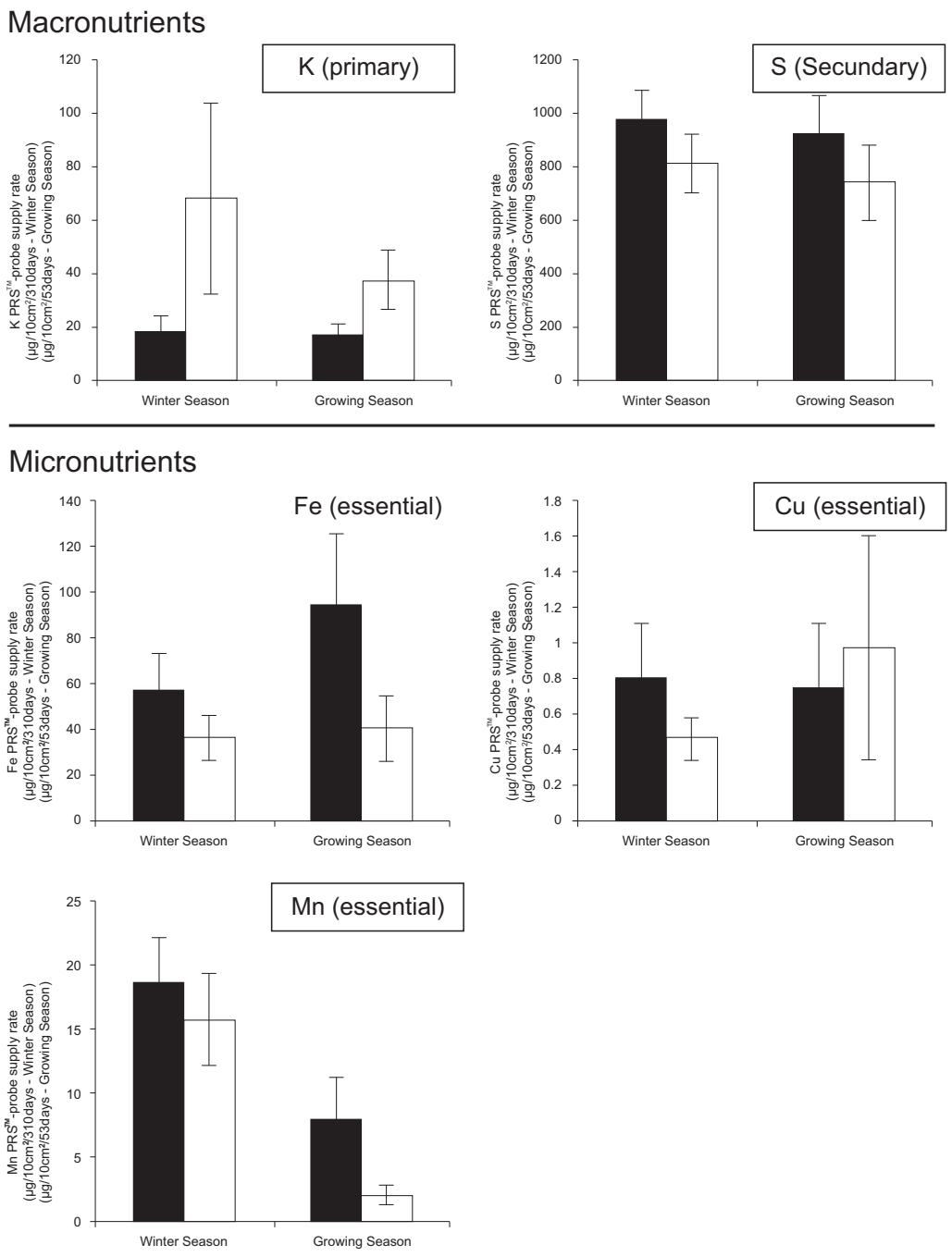
Difference in nutrient availability for plants between grazed and ungrazed plots was statistically tested with a three-way ANOVA accounting for repeated measures. Goose exclosure was set as fixed variable, series and replica nested in series as random variables.

To investigate the role of the moss layer, turfs of 9 cm^2 to a soil depth of 10 cm were cut in each plot. At the four sides of the turfs the distance between the top of the moss layer and the moss-soil interface (the point where moss shed old plant material) was measured with a ruler. A mean for each plot was made and used for further analysis. Results from different time periods were analysed separately using an ANCOVA with moss depth as continuous variable and treatment (grazed – ungrazed) as categorical fixed variable. Series and replica nested within series were fit into the model as random variables. Effects were considered significant at $p \leq 0.05$. All statistical analyses were performed using SAS version 9.2 (SAS Institute Inc. 2008) and data were transformed if necessary to meet the requirement of normality.

In accordance to other studies, grazing significantly reduced the depth of the isolating moss layer with 1.65 cm (exclosure: 4.19 ± 0.46 cm, grazed: 2.54 ± 0.30 cm, $n = 15$, $t = -5.90$, $p < 0.0001$). However, this did not result in the expected increase in nitrogen availability. Actually, in contrast with the study of Gornall et al. (2007), we did not find a correlation between moss depth and nitrogen availability (table 4.1). Also, plant availability of phosphorous, magnesium, zinc and boron was not affected by either goose exclosure or moss depth (table 4.1).

However, our results show a significant effect of goose grazing on potassium, which is as a primary macronutrient needed in large quantities by plants, sulphur, a secondary macronutrient, and iron, manganese and cupper, all essential micronutrients (table 4.1, figure 4.2). The change in nutrient availability might be the result of a change in plant composition (paper 1, Thiisbukta plots), the production of faeces and the reduction of the moss layer. Analogue with previous research on nitrogen (Ruess et al. 1989, Gornall et al. 2007) higher nutrient availability was observed in the grazed plots for all the nutrients except for cupper during the growing season (no difference) and potassium.

Nutrients		Season	PRS™-probe supply rate (µg/10cm²/53days – Growing Season) (µg/10cm²/310days – Winter Season)						Results statistical analysis											
									Grazing effect (ANOVA)						Contribution moss layer (ANCOVA)					
									Season * Geese			Geese			Geese excl. moss			Moss		
			Grazed			Exclosed			*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
Primary macro-nutrients	Total N	Growing	49.3	±	24.5	44.2	±	22.6	1, 43.2	0.14	0.713	1, 44.2	0.91	0.345	1, 19.8	0.48	0.496	1, 23.3	0.05	0.830
		Winter	92.7	±	36.8	102.4	±	51.1							1, 17.4	1.24	0.280	1, 19.4	0.85	0.367
	NO_3-N	Growing	44.7	±	24.7	39.6	±	22.9	1, 43.2	0.00	0.978	1, 44.2	1.18	0.283	1, 19.9	0.44	0.513	1, 22.4	0.03	0.876
		Winter	89.9	±	36.6	95.0	±	51.2							1, 18.4	0.12	0.735	1, 20.8	0.00	0.963
	NH_4-N	Growing	4.76	±	0.72	4.93	±	0.72	1, 42.7	0.32	0.572	1, 43.7	0.57	0.454	1, 22.9	1.08	0.317	1, 20.1	1.59	0.222
		Winter	3.33	±	0.39	8.67	±	5.47							1, 22.6	4.20	0.052	1, 22.9	2.68	0.115
	P	Growing	2.06	±	0.47	1.63	±	0.35	1, 42.6	1.47	0.233	1, 43.6	0.04	0.849	1, 21.2	0.01	0.917	1, 16.6	1.12	0.305
		Winter	1.90	±	0.44	2.24	±	0.40							1, 18	0.80	0.383	1, 9.71	0.04	0.855
	K	Growing	16.69	±	3.93	37.29	±	11.17	1, 43	0.13	0.719	1, 44	6.47	**0.015**	1, 23.6	23.40	0.140	1, 23	0.11	0.740
		Winter	17.76	±	5.96	67.80	±	35.63							1, 26	0.00	0.954	1, 26	1.55	0.224
Secondary macro-nutrients	Ca	Growing	2045	±	168	1766	±	204	1, 42.4	1.71	0.198	1, 43.4	2.42	0.127	1, 19.2	0.83	0.375	1, 17.7	0.01	0.920
		Winter	2055	±	116	2025	±	156							1, 14.4	3.44	0.084	1, 17.1	6.81	**0.018**
	Mg	Growing	311.8	±	21.5	292.3	±	29.3	1, 42.6	1.14	0.292	1, 43.6	0.07	0.792	1, 20.7	1.28	0.271	1, 15.4	0.94	0.348
		Winter	327.4	±	18.3	340.3	±	20.6							1, 21.8	0.17	0.680	1, 25.5	0.04	0.838
	S	Growing	922	±	142	740	±	142	1, 42.4	0.02	0.892	1, 43.4	6.65	**0.013**	1, 9.54	0.41	0.537	1, 14.3	4.66	**0.048**
		Winter	977	±	106	812	±	110							1, 12.5	1.86	0.197	1, 14.4	11.71	**0.004**
Micro-nutrients	Fe	Growing	94.75	±	30.88	40.14	±	14.34	1, 42.8	0.40	0.534	1, 43.8	6.58	**0.014**	1, 21.4	0.00	0.980	1, 27.7	3.54	0.070
		Winter	57.24	±	15.87	36.13	±	9.84							1, 19.6	0.39	0.538	1, 24.4	3.90	0.060
	Mn	Growing	8.01	±	3.26	2.09	±	0.79	1, 42.6	8.46	**0.006**	1, 42.6	7.38	**0.009**	1, 22.1	4.01	0.058	1, 25.4	0.00	0.944
		Winter	18.67	±	3.54	15.78	±	3.58							1, 12.5	1.40	0.258	1, 24.4	1.58	0.220
	Cu	Growing	0.75	±	0.36	0.98	±	0.63	1, 43.2	2.66	0.11	1, 44.2	4.55	0.039	1, 23.5	0.34	0.563	1, 25.7	1.42	0.245
		Winter	0.81	±	0.30	0.46	±	0.12							1, 16.4	1.12	0.305	1, 12.2	0.88	0.366
	Zn	Growing	3.01	±	1.29	3.31	±	1.69	1,42.2	0.02	0.899	1, 43.3	1.34	0.253	1, 20.3	2.72	0.114	1, 24.9	2.06	0.164
		Winter	2.40	±	0.69	2.41	±	0.46							1, 17.1	0.02	0.894	1, 24.6	0.77	0.387
	B	Growing	1.88	±	0.16	1.76	±	0.12	1, 41.9	0.00	0.981	1, 42.9	0.96	0.333	1, 23.2	1.45	0.240	1, 27.9	0.40	0.534
		Winter	1.97	±	0.13	1.86	±	0.11							1, 22.6	0.12	0.732	1, 20.1	0.02	0.885



↑ Figure 4.2. Plant availability of some macro- and micronutrients expressed as mean PRS™-probe supply rate, error bars represent standard errors. Plant availability of the shown elements differed significantly ($p < 0.05$) between grazed plots (black bars) and exclosures (white bars).

← Table 4.1. Plant availability of macro- and micronutrients, measured as PRS™-probe supply rate, in grazed plots and exclosures. Mean values ± SE and the results of the statistical tests are given. Significant ($p \leq 0.05$) results are indicated in bold.

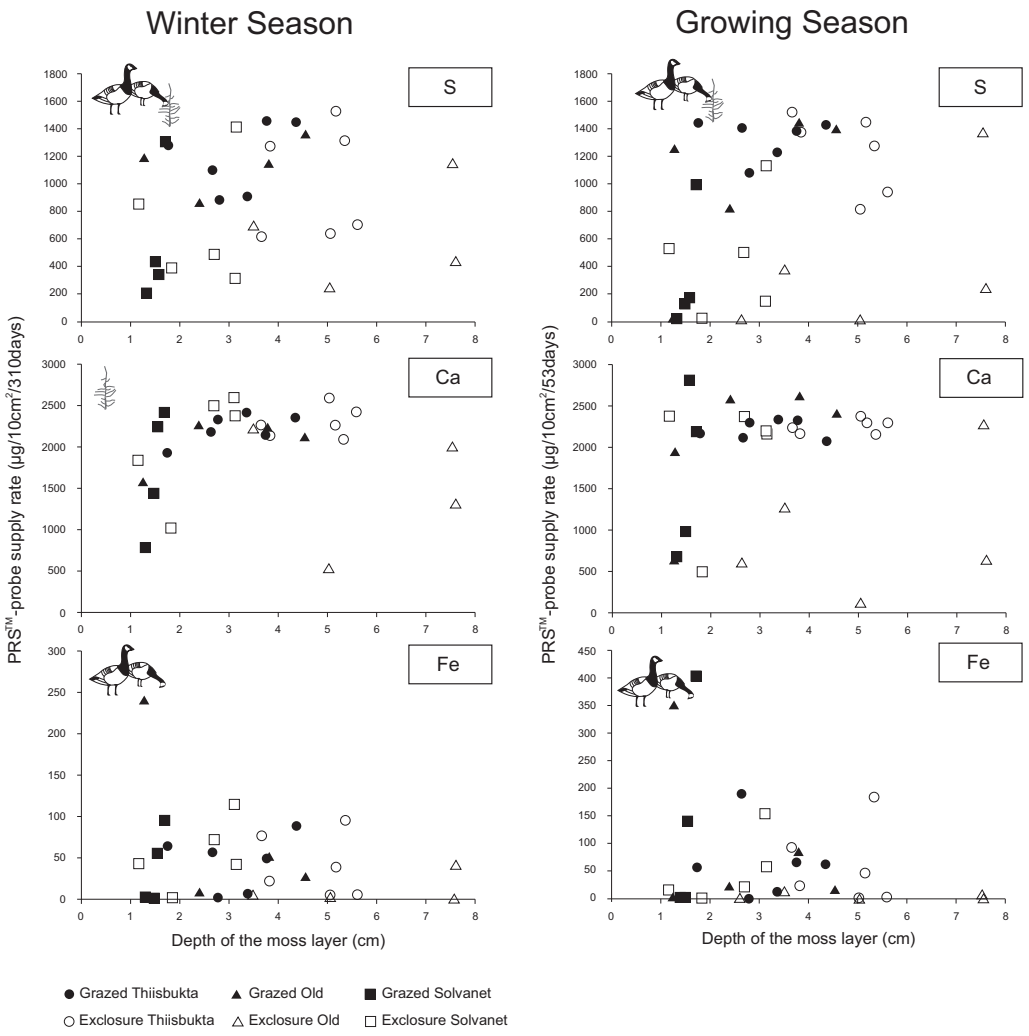


Figure 4.3. Plant availability of some macro- and micronutrients in function of the depth of the moss layer in grazed plots and exclosures. Significant differences ($p \leq 0.05$) between grazed and ungrazed plots in plant availability are indicated by , a significant correlation between moss depth and plant availability by .

Separating the effect of the reduced moss layer and 'other grazing mechanisms' revealed that - except for manganese during the growing season - the nutrient availability is not significantly influenced by 'other grazing mechanisms' only (table 4.1). The grazing impact on nutrient availability is thus at least partly mediated by the moss layer. For sulphur and calcium (only winter season) we even found a significant positive correlation between the plant availability and the depth of the moss layer, for iron this correlation was negative and only marginally significant (table 4.1, figure 4.3). This suggests that other mechanisms than the

insulation capacity of the moss layer and the absorption of nutrients by mosses (Gauthier et al. 1995, Kotanen 2002) might underlay the moss effect on the availability of sulphur and calcium. Indeed, based on these mechanisms we expected an increased nutrient availability due to the moss layer reduction by grazing as observed for iron in this study and nitrogen in the study of Gornall et al. (2007).

Until now most ecological research concerning nutrient availability is limited to nitrogen and sometimes also phosphorous. However, as the overall effect of goose grazing on plant availability of nutrients and the underlying mechanisms seems to differ considerably between elements, we urge to broaden the scope of this research to other essential plant nutrients. In addition we want to emphasize the need to continue ecological studies during winter when geese are absent as nutrient availability outside the growing season is relatively high and for some elements influenced by grazing.

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