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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 cm² (end growing season, August 2007) or six cores of 9.68 cm² (start growing season, June 2008) or six turfs of 9 cm² (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; $F_{1,16} = 41.92$, $p < 0.0001$).

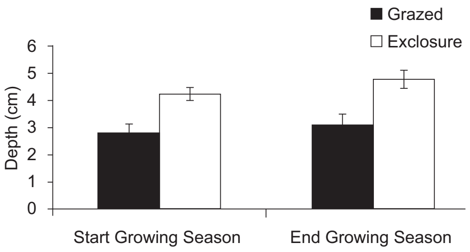

Figure 1.2. Depth of the moss layer (=the distance between the top of the moss layer and the moss soil interface at the start and the end of the growing season 2008. Data shown are mean values ± SE (error bars). The difference between grazed plots and exclosures is significant ($p < 0.0001$).

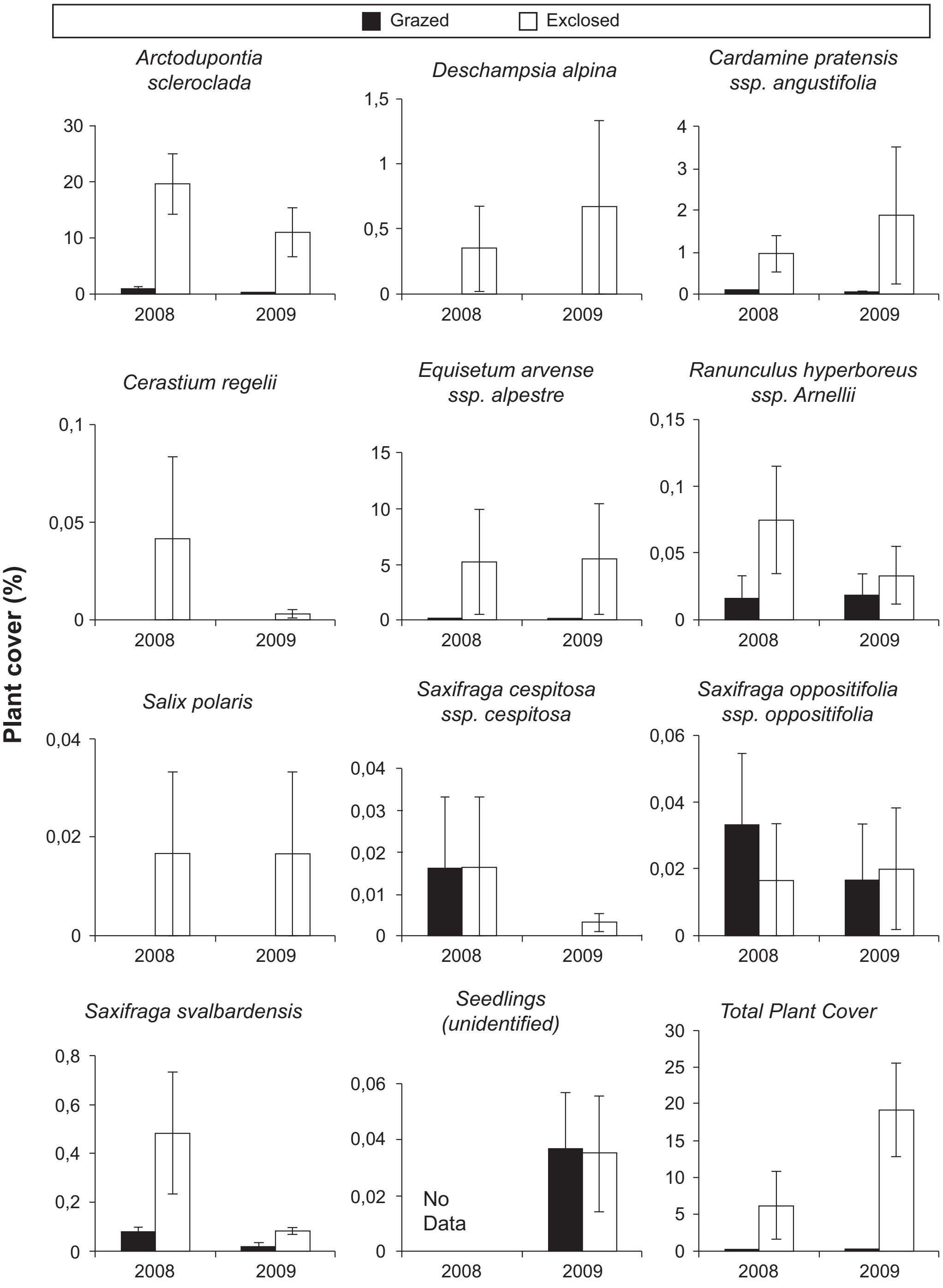

Figure 1.1. Results from the vegetation surveys at the peak (2009) and the end (2008) of the growing season. Vegetation surveys were made using an adaptation of the Braun-Blanquet cover-abundance scale (Braun-Blanquet 1932, Braun-Blanquet 1964) described in table 1.1 and only vascular plants were considered. 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 ≤ 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 ≤ 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.

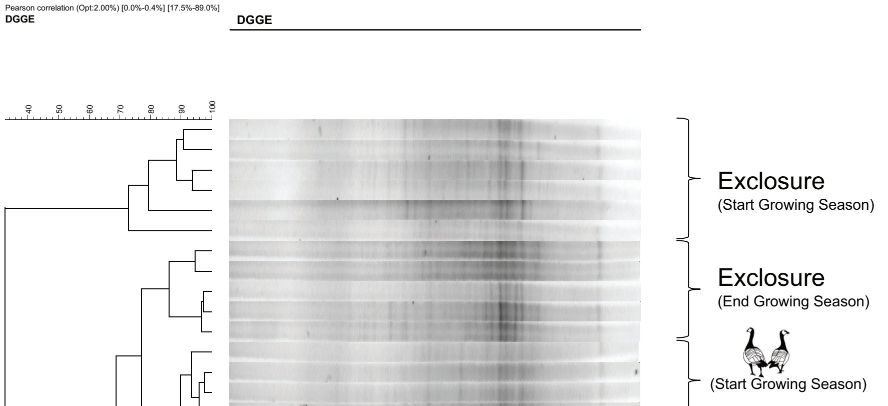

Figure 1.3. Clustering of the microbial communities present in grazed plots and exclosures based on their PCR-DGGE fingerprints. Samples were taken at the beginning and the end of the growing season.

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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