

SILICA ACCUMULATION IN GRASSES IN REPOSE TO A LARGE SCALE HERBIVORE EXCLOSURE EXPERIMENT



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ABSTRACT

Silica defenses in grasses have been recently proposed to be important for plant-herbivore interactions. High silica levels in grasses have been found to have a negative impact on herbivores performance and act as an herbivory deterrent. Moreover, accumulation of silica has been proposed to be inducible, i.e. highly grazed grasses accumulate silica in their leaves. In order to assess whether silica induction is an important mechanism of plant-herbivore interactions also in sub-arctic ecosystems, we conducted a large-scale herbivore exclosure experiment in northern Norway.

We measured silica concentrations in leaves of five common grass species. Two species showed differences in silica concentrations when herbivory was excluded and one species showed variation in the levels of silica between areas of different grazing pressure, otherwise, we found no clear results supporting silica accumulation in grasses as an important response to herbivory in sub-arctic areas.

Silica uptake in grasses is a complex process, regulated by several factors, where herbivory is one of them, but other abiotic factors (i.e. temperature, pH of the soil, etc.) may mask the effect of herbivory in grasses silica accumulation. A better understanding of the biotic and abiotic factors that affects silica induction is necessary to interpret correctly the role of silica in plant-herbivore interactions.

Keywords: Plant defenses, silica, tundra vegetation, exclosure, large scale.

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INTRODUCTION

Silicon (Si) is the second most abundant element in the earth's crust and in its form of silica (SiO_2) represents an important inorganic constituent in plants (Epstein 1999; Müller 2003). Although all plants contain silicon, the concentration of this element varies greatly among plant species (0,1-10% dry weight). Silicon content also varies vastly in different ecotypes and different parts of the plants. In higher plants, species of *Poaceae* (grasses) and *Cyperaceae* (sedges) families are considered Si-accumulating plants (Müller 2003). Grasses actively absorb silicon from the soil in the form of silicic acid (H_4SiO_4) and accumulate it as silica phytoliths in their tissues (Vicari and Bazely 1993; Massey and Hartley 2006b) or passively carry silica upwards in the transpiration stream and deposit it at sites of evaporation from plant surfaces (McNaughton, Tarrants et al. 1985). Anyhow, which silicon uptake mechanism occurs (passive or active), are dependent on both plant species and external silicon concentration (Liang, Hua et al. 2006).

Silica accumulation has been associated with a variety of functions in grasses, including protection against fungal attacks, resistance to water loss, stimulation of photosynthesis and structural support (Lux, Luxová et al. 2003; Müller 2003; Fauteux, Rémus-Borel et al. 2005; Hattori, Inanaga et al. 2005), in addition, silica has an important role in grass-herbivore interactions acting as an anti-herbivore mechanism (Vicari and Bazely 1993; Massey and Hartley 2006b).

Recently, there have been several investigations into the role of silica as a defense mechanism in grasses. Massey *et al* (2006a, 2006b, 2007, 2008, 2009) show in their studies in Great Britain, that accumulation of silica in grasses leads to an increase in leaf abrasiveness, act as feeding deterrent, reduce growth performance and change feeding preferences of folivorous insects, small herbivorous mammals (voles) and sheep. In addition, high concentrations of silica in grasses cause tooth wear in voles (Vicari and Bazely 1993) and deterioration of insect mandibles (Massey and Hartley 2009). Moreover, voles and insects have impact on silica accumulation, increasing silica concentration in grass leaves. Repeated feeding of mammals and insects on grasses produce a stronger response, i.e. frequently grazed grasses accumulate more silica in their leaves. Silica based defenses have been hypothesized, with empirical data, to play an important role in small rodent cycles (Massey, Smith et al. 2008). These findings propose silicon as an important element within plant-

herbivore interactions and we ask whether similar mechanisms may act in sub-arctic ecosystems.

In sub-arctic ecosystems, like in northern Norway, tundra heath vegetation dominates the landscape. However, in riparian sediment plains, moisture and nutrients are sufficient to sustain meadows and tall thickets of willows that represent hot-spots of plant-herbivore interactions (Bråthen, Ims et al. 2007). The meadows typically consist of a field layer of grasses, sedges, forbs and deciduous shrubs. Patches of dense willow thickets are interspersed within the meadows and they act as shelter and food source for herbivores. These habitats can support high diversity and productivity and may therefore be essential as food source for tundra herbivores (Bråthen, Ims et al. 2007; Ravolainen 2009).

The two focal groups of herbivores for our study in northern Norway were large ungulates (reindeer) and small rodents (lemmings and voles). In Finnmark, the main large herbivore is the semi-domesticated reindeer (*Rangifer tarandus*). Reindeer populations can reach very high densities in Finnmark, but their abundance and time of the year when they are present, varies because they are migratory animals. The focal small herbivores are represented in northern Norway by lemmings (*Lemmus lemmus*) and two species of voles (*Microtus oeconomus* and *Myodes rufocanus*). Populations of these small rodents, undergo cyclic high amplitude changes of density (Killengreen, Ims et al. 2007). Small rodents are disproportionally abundant during the peak years and they require a high rate of food intake (due to their high metabolic rate) (Ims, Yoccoz et al. 2007). Reindeer and small rodents grazing have been found to have a major impact on vegetation composition of riparian sediment plains (Ravolainen 2009).

To study the effect of herbivory in silica induction in grasses in northern Norway, we conducted a large-scale experiment in three different areas of Finnmark, northern Norway. Within three to four sections of each of these areas, we set up an enclosure experiment with three different treatments: control, exclusion of reindeer and exclusion of all mammal herbivores.

The following predictions were tested:

1. On the previous year to our study, high density peaks of small rodents populations were found in our study areas (Henden, Ims et al. 2011), together with stable seasonal high densities of reindeer. As a result of this potential herbivore grazing pressure, grasses from both exclosure treatments are expected to have less silica in their leaves than those from control plots.
2. As a result of spatial differences in grazing pressures as well as other environmental factors, between the three areas, silica levels in grasses will differ between different areas.
3. All plants have silicon in their tissues, but the constitutive levels of silicon differ between species. Therefore, the different grass species of our experiment should have different silica concentrations.

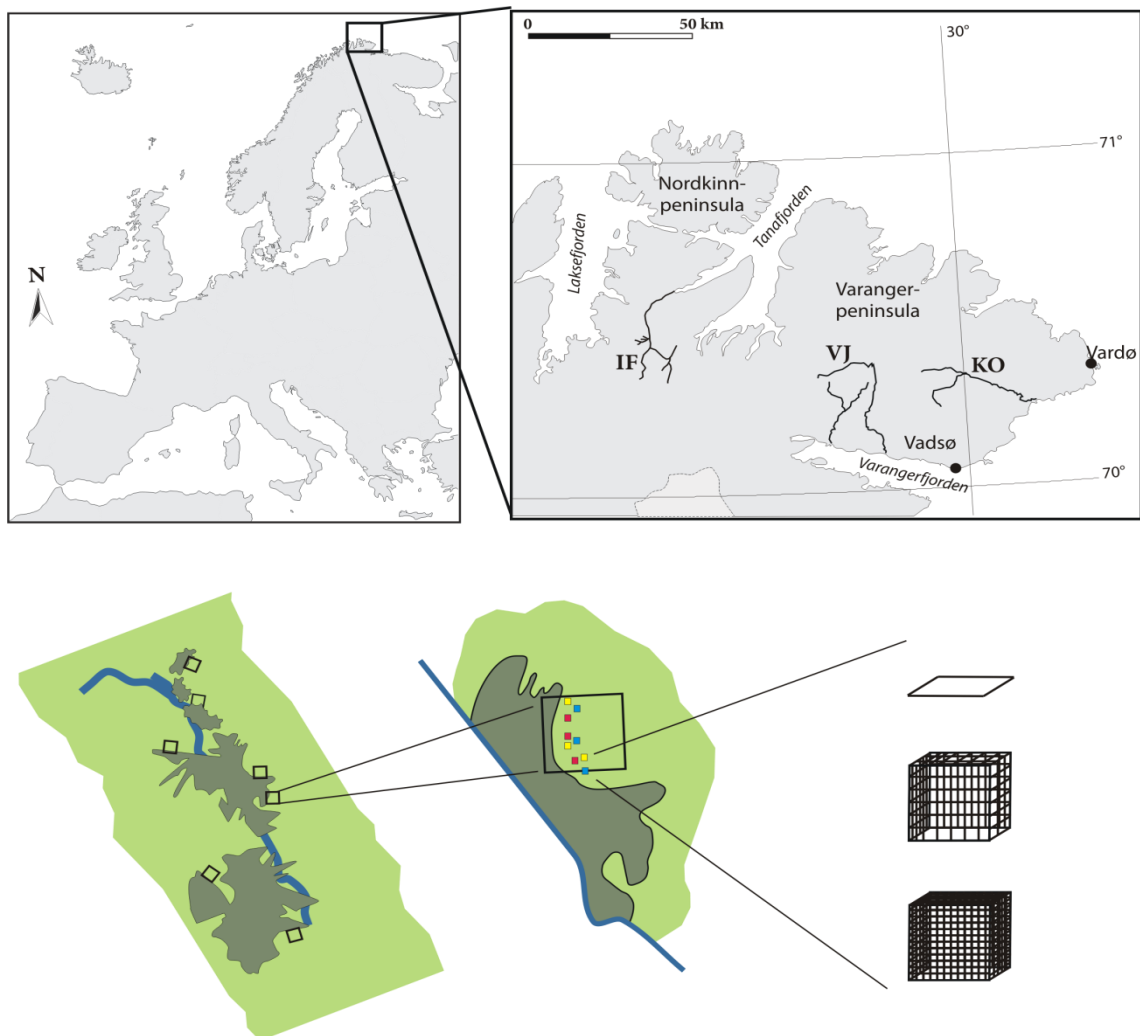
MATERIALS AND METHODS

Study Area

The study was conducted from 2006 to 2008 in two different regions of Finnmark (northern Norway): Varanger Peninsula and Laksefjordvidda. Two study areas were located on the Varanger Peninsula: Komagdalen ($70^{\circ}19' \text{ N}$, $30^{\circ}01' \text{ E}$) and Vestre Jakobselv ($70^{\circ}18' \text{ N}$, $29^{\circ}16' \text{ E}$) and one study area on Laksefjordvidda: Ifjordfjellet ($70^{\circ}25' \text{ N}$, $27^{\circ}20' \text{ E}$) (See Figure 1).

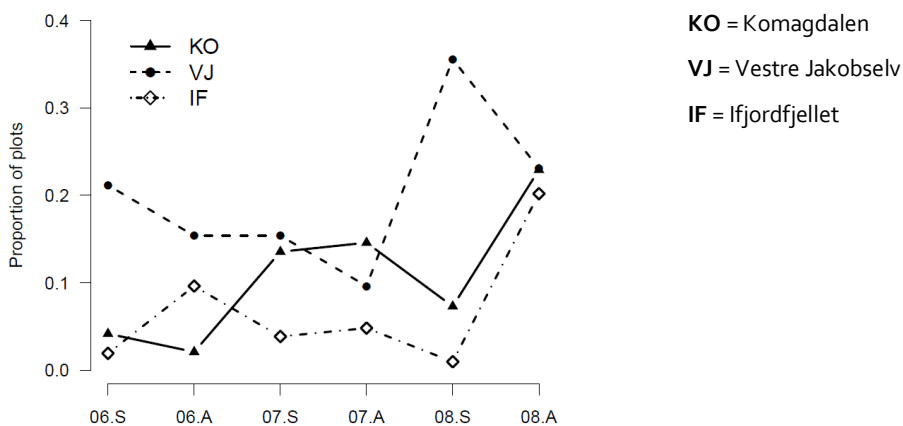
Figure 1. Location of the study area. Top figure represents a map of the study areas. IF=Ifjordfjellet; VJ=Vestre Jakobselv; KO=Komagdalen.

The bottom figure is a schematic illustration of the study and sampling area. The left illustration is a representation of the riparian plains. The middle is a representation of the sampling grids. In grey are represented the willow thickets and in green the meadow vegetation. The right shows the three different treatments: control, reindeer enclosure and all mammal herbivores enclosure.



The study region is bioclimatically classified as a low arctic shrub-tundra (Walker, Raynolds et al. 2005). Mean temperatures in July has a range from 7,9 °C to 11,1 °C and annual precipitation from 365 mm to 460 mm (Norwegian-Meteorological-Institute). Our study areas are situated above the tree line and are mostly represented by *Empetrum hermaphroditum* dominated heath. However, in riparian plains we can find meadow vegetation with patches of willow thicket formations. The thickets mainly consist of *Salix phylicifolia*, *Salix glauca* and *Salix lanata* and can be over two meters high. The meadows associated with the thickets are characterized by a great variety of growth forms like mosses, forbs, grasses (e.g. *Avenella flexuosa*, *Deschampsia cespitosa*), sedges (*Carex spp*), and herbaceous dicotyledons (e.g. *Viola biflora*, *Bistorta vivipara*) (Ravolainen 2009). These rich areas represent hot-spots habitats within arctic ecosystems.

Figure 2. Reindeer abundance from 2006 to 2008. S=Summer, A=Autumn. Measures represent average presence of feces per plot. Based on eight plots of 0,5 x 0,5 meters regularly spaced along the perimeter of each grid (EcoFinn project data).



The dominating large herbivore is the semi-domestic reindeer (*Rangifer tarandus*). Varanger Peninsula is used as summer pasture for an average of 11500 reindeer (on average 3,2 reindeer/Km²). Ifjordfjellet is a spring and autumn transition area, and support less reindeer densities than Varanger Peninsula (Figure 2) (Anonymous 2009). As small mammal herbivores, we find Norwegian lemmings (*Lemmus lemmus*), Grey-sided Voles (*Myodes rufocanus*) and Root Voles (*Microtus oeconomus*) (Killengreen, Ims et al. 2007). They are present year-round in the area and they have high density cycles (Ims and Fuglei 2005), in northern Norway these cycles normally have a 5-year period (Yoccoz and Ims 2004). The year previous to the collection of grasses, there was a population peak in every area but of different magnitude and different species (Figure 3).

Different areas had different herbivores densities (Figures 2 and 3). Sites at Varanger Peninsula had a higher Reindeer pressure than Ifjordfjellet (Figure 2). Both, the amplitude of small rodents' population peak and which species were the most abundant were different between the study areas. Komagdalen (at Varanger Peninsula) was the site with highest peak of rodents whereas Vestre Jakobselv was the site with the lowest (Figure 3).

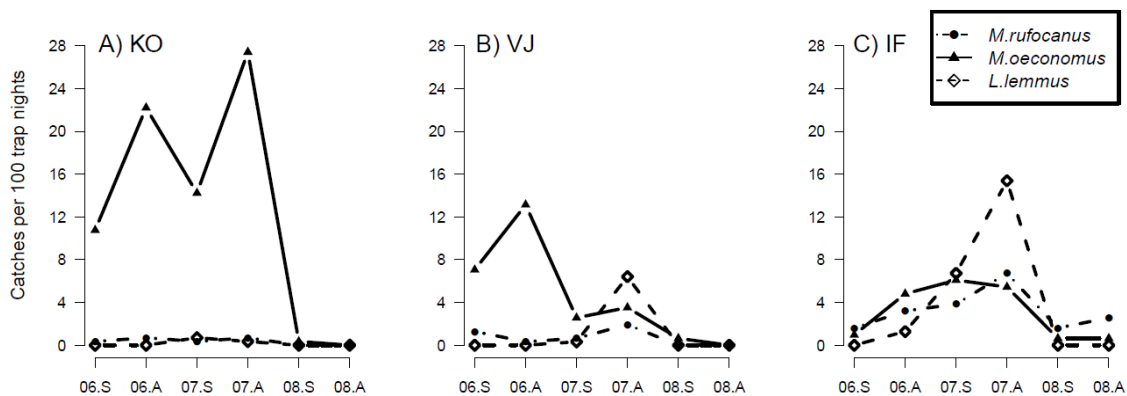


Figure 3. Small rodents' abundance from 2006 to 2008. S=summer, A=autumn. Values expressed in %. Three rodent traps at each corner of the grid. Set for 48 hours (EcoFinn project data).

Areas: **KO** = Komagdalen

VJ = Vestre Jakobselv

IF = Ifjordfjellet

Study design

In each study areas, three to four sections were selected. At Ifjordfjellet (mean soil pH = 4,4) we chose four sections along the mountain pass and in a range of altitude between 260-360 meters above sea level. At Varanger peninsula, the two study areas; Vestre Jakobselv and Komagdalen, were located between 110-290 meters over the sea level. At Vestre Jakobselv (mean soil pH = 4,7) we selected three sections, two side valleys of Jakobselv river and one more in the upper part of the river (Jakobselvkroken). At Komagelvdalen (mean soil pH = 4,6) another three sections were selected along the river valley, starting 15 km from the coast. Sampling grids associated to thicket formations were established in every section (Table 1). The minimum distance between grids was 164 meters and the average distance was 652 meters. The sampling grids (n=36 in total, between 1 and 5 per section) consist of 15x15 meter square placed in riparian herbaceous vegetation, with one side aligned with the edge of a thicket (Figure 1).

Table.1 Hierarchical structure and number of study units (sampling grids) of the study design. See map in figure 1 for the situation of the region and area levels.

Region	Area	Section	Sampling Grids
Laksefjordvidda	Ifjordfjellet (IF)	Aestorjohka	4
		Gurojohka	1
		Sulojavri	2
		Stuorrajohka	4
Varanger Peninsula	Komagdalen (KO)	Upper Komag river	5
		Middle Komag river	5
		Lower Komag river	2
Vestre Jakobselv (VJ)	Vestre Jakobselv (VJ)	Lavdnevarjohka	4
		Bearalveaijohka	5
		Jakobselvkroken	4

Within each grid, three blocks of three experimental plots (in total nine plots per grid) were established. Within the blocks, each plot was randomly assigned to one treatment. The different treatments were: (1) exclusion of all vertebrate herbivores by placing small-meshed cages (iron mesh size of approximately 1x1 cm), (2) exclusion of large herbivores by placing large-meshed cages (mesh size of approximately 3x3 cm) and (3) non-enclosed control plots that were potentially grazed by any kind of herbivore (see Figure 1)

The exclosures were set up in June 2006, so ensure no grazing from the selected herbivores during two years prior to the sampling (2008). The cages were approximately 50 cm sideways and approximately 60 cm high, thus reaching above the vegetation. They were placed between 0,5 and 6 meters from the thicket edge (Figure 1). All exclosures were dug 5-10 cm into the ground (below the root layer) and similar cut was dug in the soil of the control plots.

Study Species

Seven of the most common grass species of the region were selected for the study. The selected species were *Avenella flexuosa*, *Anthoxanthum nipponicum*, *Deschampsia cespitosa*, *Nardus stricta*, *Festuca* spp, *Calamagrostis phragmitoides* and *Phleum alpinum*. These grasses are common in northern Norway, and represent in average 6% of the total standing crop and 75% of the grasses present in the study sites (Ravolainen unpublished data). These species have been found to be part of the diet of large herbivores (see review in (Bråthen and Oksanen 2001)) and small rodents. (Soininen, Valentini et al. 2009). All species present at a given treatment plot were sampled, however, because of an uneven presence of species the final sample size of the selected species was also uneven (see Table 2).

Table 2. Number of samples analyzed from the three different areas and treatments. Due to small sample size, *Festuca* spp. and *Nardus stricta* were excluded from the analysis. Samples from *Deschampsia cespitosa* and *Phleum alpinum* from Ifjordfjellet were also excluded from the analysis. **Treatments:** C=control, R=reindeer enclosure and S=all mammal enclosure.

Species		<i>Avenella flexuosa</i>	<i>Anthoxantum nipponicum</i>	<i>Calamagrostis Phragmitoides</i>	<i>Deschampsia cespitosa</i>	<i>Festuca</i> Spp.	<i>Nardus stricta</i>	<i>Phleum alpinum</i>
Ifjordfjellet								
Treatment	C	19	12	7	0	2	2	1
	R	11	7	2	0	0	0	0
	S	14	5	1	0	1	4	0
Vestre Jakobselv								
Treatment	C	24	30	23	8	2	2	17
	R	18	16	13	3	3	0	3
	S	19	12	6	1	1	0	9
Komagelva								
Treatment	C	33	18	10	35	1	5	21
	R	10	9	4	16	0	1	2
	S	18	7	1	18	1	1	12

Samples analysis

Every sample collected in the field was folded in a paper bag and labeled with the species name, location, plot number and date. The samples were dried at room temperature and stored. Before the analysis, the specimens were re-dried at 60°C during two days. Dry leaves of each sample were cut and weighted. We selected green leaves, excluding leaves with a dead tip longer than 5mm. When the sample size was less than 0,2 grams, we pooled material of different samples, always with samples of the same grid, same treatment and same species, (see Table 3) until we reached the minimum weight of 0,2 grams. Samples were sorted and grinded using a ball mill (Mixer Mill, MM301; Retsch GmbH & Co. Haan, Germany) for 30 seconds, 30 rotations per second, until we had a homogeneous powder. Thereafter, the grass powder was pressed using a hydraulic press with 11 tons of pressure to produce flat pellets of 13mm of diameter. To analyze the concentration of silica in our samples we used the X-Ray Fluorescence (XRF) method, using a Nilton XL3t portable XRF analyzer (Thermo Fisher Scientific, Inc.) at the University of Sussex. The samples were irradiated during 30 seconds under helium atmosphere to avoid signal loss by air absorption (Reidinger, Hartley et al *in prep*). Every 20 analyses, one control sample (sample of known Si concentration) was tested to verify that the calibration was correct. In addition, random samples of all species (n=42) were re-analyzed to test consistency in the method (mean variation < 0,02%).

Table 3. Number of samples pooled together for silica analysis.

Samples pooled	Total	C	R	S
<i>Avenella flexuosa</i>	40	22	9	9
<i>Anthoxantum nipponicum</i>	51	25	14	12
<i>Festuca spp.</i>	6	2	1	3
<i>Phleum alpinum</i>	37	19	3	15
<i>Deschampsia cespitosa</i>	11	6	3	2
<i>Calamagrostis phragmitoides</i>	7	4	2	1
<i>Nardus stricta</i>	2	2	0	0

Treatment :

C = control

R = reindeer exclusion

S = all mammal herbivores exclusion

Statistical analysis

Two analyses were conducted: one to analyze the effect of treatment and area in silica levels within species and other to analyze differences in silica between species. Data from *Nardus stricta* and *Festuca spp.* were excluded from the analyses, due to their low sample size.

The effect of treatments (control and exclusions) and spatial variation (three different areas) were analyzed with a linear mixed effects model fit by REML (function `lme` in `nlme` package of R, (Pinheiro, Bates et al. 2009)). We used both treatments and area as fixed effects in an additive model and grid was included as a random effect in the model. We used an additive model instead of an interaction model due to low sample size.

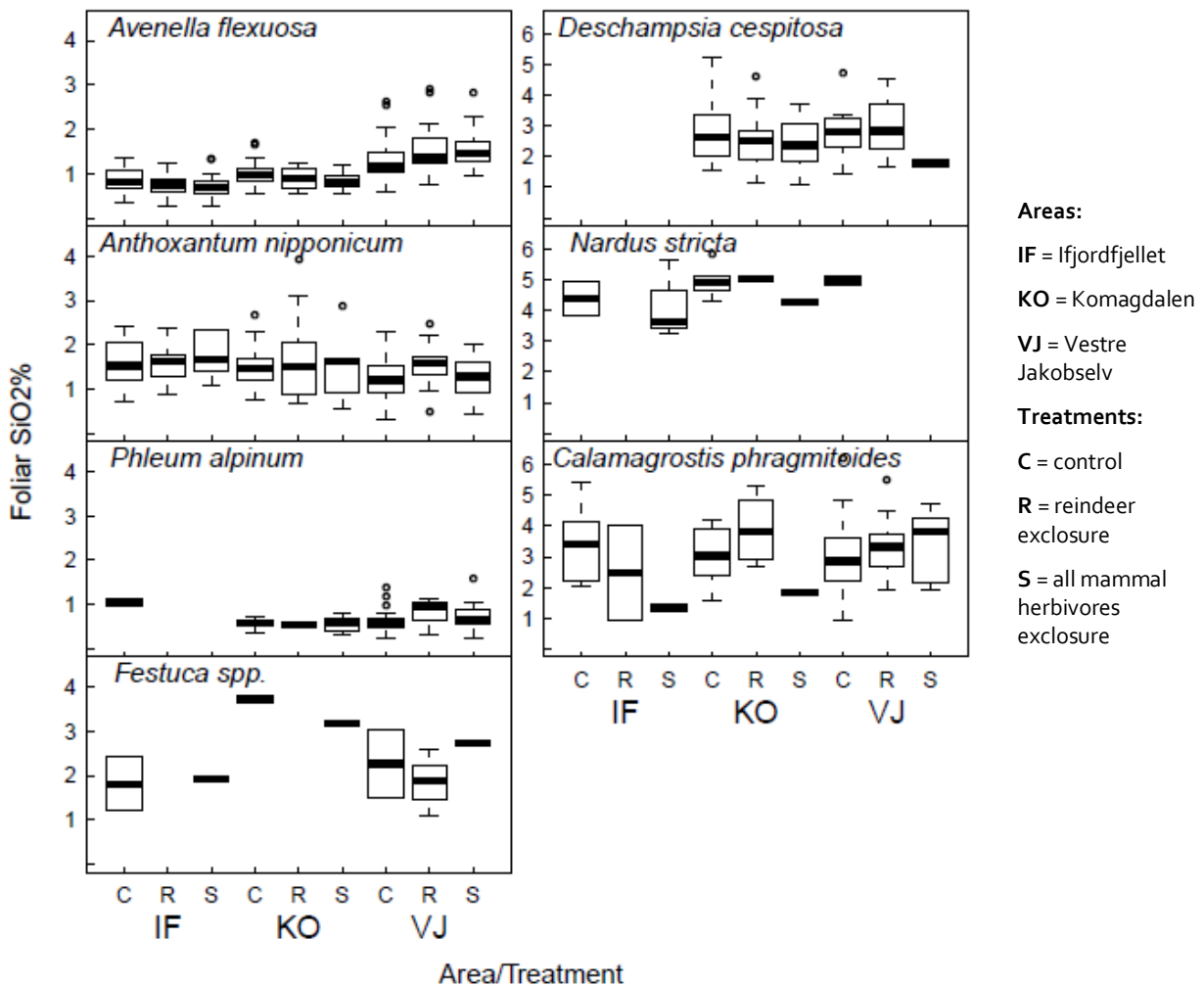
To analyze differences in silica content between species, a linear mixed effects model was run (Referenced above). Species was used as fixed effect and again grid as random effect. Only data from the control plots were analyzed to exclude treatments effect.

The models were run with $\log+1$ data, to meet assumption of normality. Model results of p -values $< 0,05$ were considered significant. Results are presented as effect size, i.e. the modeled contrast between levels of fixed effects. All analyses were conducted in the R-environment (R Development Core R_Development_Core_Team 2010).

RESULTS

Leaf silica content of our grass species did not show consistent variation between the different treatments (all mammal herbivores enclosure, reindeer enclosure and control) (see Table 4 and Figure 4). However, we found significant differences in *A. nipponicum*, where leaves from reindeer enclosure treatment had higher silica than leaves sampled in the control plots (back-transformed effect size = 0,079%, p-value = 0,032) (Table 4). Also, leaves of *D. cespitosa* contained higher silica in the control plots than in the all mammal herbivores enclosures (back-transformed effect size = -0,122%, p-value = 0,046) (Table 4).

Figure 4. Average foliar silica content in different grass species from different areas and treatments. Note that the “y” axis has different graduation in the left and right panels. Foliar silica values are expressed in percentage SiO₂ of dry weight. *Nardus stricta* and *Festuca* spp. have not been included in the statistical analysis, but are included here.



A significant difference in grass silica content between the study areas was found for leaves of *Avenella flexuosa* which had higher silica concentrations in Vestre Jakobselv than Komagelva (back-transformed effect size = 0,227%, p-value = 0,0007) (Table 4) and also had higher silica levels in Vestre Jakobselv than Ifjordfjellet (back-transformed effect size = 0,353%, p-value = 0,000)(Table 4). Also we found a tendency (p-value = 0,087) for *Avenella flexuosa*, to have higher silica in Komagelva than Ifjordfjellet (see Table 4 and Figure 4).

Table 4. Model estimates of silica content of all species (additive linear mixed-effect model). The intercept corresponds to the region "KO" and the treatment "Control". All values expressed in Log(x+1). Significant values (p<0,05) are indicated in bold.

	Contrast	<i>Avenella flexuosa</i>			<i>Anthoxantum Nipponicum</i>			<i>Phleum alpinum</i>		
		Estimate	DF	p-value	Estimate	DF	p-value	Estimate	DF	p-value
Fixed Effects										
<i>Intercept</i>		0,648	132	0,000	0,928	86	0,000	0,438	41	0,000
<i>Treatment</i>	<i>CvsR</i>	0,002	132	0,921	0,076	86	0,032	0,084	41	0,099
<i>Treatment</i>	<i>CvsS</i>	-0,015	132	0,521	0,032	86	0,402	-0,004	41	0,890
<i>Treatment</i>	<i>RvsS</i>	-0,018	132	0,530	-0,044	86	0,307	-0,089	41	0,099
<i>Area</i>	<i>KOvsIF</i>	-0,096	29	0,087	-0,01	25	0,923			
<i>Area</i>	<i>KOvsVJ</i>	0,205	29	0,0007	-0,151	25	0,141	0,096	19	0,102
<i>Area</i>	<i>IFvsVJ</i>	0,302	29	0,000	-0,141	25	0,148			
Random Effects										
<i>Grid</i>		0,109			0,194			0,112		
<i>Residual</i>		0,129			0,152			0,098		

	Contrast	<i>Deschampsia cespitosa</i>			<i>Calamagrostis phragmitoides</i>		
		Estimate	DF	p-value	Estimate	DF	p-value
Fixed Effects							
<i>Intercept</i>		1,2990	64	0,000	1,388	40	0,000
<i>Treatment</i>	<i>CvsR</i>	-0,058	64	0,364	0,051	40	0,473
<i>Treatment</i>	<i>CvsS</i>	-0,130	64	0,046	-0,003	40	0,976
<i>Treatment</i>	<i>RvsS</i>	-0,072	64	0,337	-0,055	40	0,615
<i>Area</i>	<i>KOvsIF</i>				-0,062	22	0,664
<i>Area</i>	<i>KOvsVJ</i>	0,220	13	0,808	0,006	22	0,956
<i>Area</i>	<i>IFvsVJ</i>				0,068	22	0,604
Random Effects							
<i>Grid</i>		0,079			0,178		
<i>Residual</i>		0,227			0,243		

Treatment:

C = control

R = reindeer exclusion

S = all mammal exclusion

Area:

KO = Komagdalen

VJ = Vestre Jakobselv

IF = Ifjordfjellet

All species had significantly different silica content from each other except *Deschampsia cespitosa* (back transformed effect size = 2,629%) and *Calamagrostis phragmitoides* (back-transformed effect size = 2,92%) (see Table 5). These two species were found to have the highest silica content (Table 5), after which in descending order of silica content were *Anthoxantum nipponicum* (back-transformed effect size = 1,363%), *Avenella flexuosa* (back-transformed effect size = 0,96%) and *Pleum alpinum* (back-transformed effect size = 0,576%) (Table 5).

Table 5. Model estimates of silica content of all species (linear mixed-effects model). All values correspond to samples from the treatment "control". The values are expressed in log(x+1). Random Effect Values: Grid = 0,116; Residual = 0,183.

	<i>Avenella flexuosa</i>			<i>Anthoxantum nipponicum</i>			<i>Phleum alpinum</i>		
	Estimate	DF	p-value	Estimate	DF	p-value	Estimate	DF	p-value
<i>Intercept</i>	0,673	230	0	0,86	230	0	0,455	230	0
<i>A.flexuosa</i>	-	-	-	-0,187	230	0	0,218	230	0
<i>A.nipponicum</i>	0,187	230	0	-	-	-	0,405	230	0
<i>P.alpinum</i>	-0,218	230	0	-0,405	230	0	-	-	-
<i>D.cespitosa</i>	0,616	230	0	0,429	230	0	0,834	230	0
<i>C.phragmitoides</i>	0,692	230	0	0,506	230	0	0,911	230	0

	<i>Deschampsia cespitosa</i>			<i>Calamagrostis phragmitoides</i>		
	Estimate	DF	p-value	Estimate	DF	p-value
<i>Intercept</i>	1,2890	230	0	1,366	230	0
<i>A.flexuosa</i>	-0,616	230	0	-0,692	230	0
<i>A.nipponicum</i>	-0,429	230	0	-0,506	230	0
<i>P.alpinum</i>	-0,834	230	0	-0,911	230	0
<i>D.cespitosa</i>	-	-	-	-0,077	230	0,078
<i>C.phragmitoides</i>	0,077	230	0,078	-	-	-

DISCUSSION

We conducted a large scale enclosure experiment to test silica accumulation in sub-arctic tundra grasses as a defense mechanism against herbivory. We predicted that (1) grasses would respond to herbivore exclusion with decreased silica content, that (2) silica levels would differ both spatially between individuals of a species and (3) between species. The experiment was set up in areas of continuous grazing by reindeer and where small rodent population fluctuations are occurring. Collection of grass leaves was following a peak year of small rodent grazing.

We found no consistent variation in silica levels of grass leaves of the different study species when herbivory was excluded. Two of the five study species responded to herbivore exclusion, whereas the other three did not. Of these two, *D.cespitosa* responded with a decrease of silica content to exclusion of all mammal herbivores (supporting prediction 1), whereas reindeer exclusion alone had no effect. The other species, *A.nipponicum*, responded with an increase of silica content to reindeer exclusion (contradicting prediction 1). In addition, we only found one species (*A.flexuosa*) that had different silica levels between the study areas, thus prediction 2 was supported by only one species. Silica concentrations found in *A.flexuosa* were correlated with the abundance of reindeer in the different areas, that is, where reindeer were most abundant (Vestre Jakobselv), we found the highest leaf silica levels, whereas in the area with the lowest densities of reindeer (Ifjordfjellet) leaves of *A.flexuosa* had the lowest levels of silica. However, as long as these silica levels was not matched by reduced silica levels in response to reindeer exclusion, silica levels of *A.flexuosa* grass leaves from different areas, may not be explained by reindeer grazing pressure (or grazing in general) but more likely by other ecological (e.g. temperature) or genetic (different genotypes within species) differences. Finally, we found that different grass species have different levels of silica content (supporting prediction 3) under natural conditions (i.e. controls).

Silica accumulation in response to herbivory

In grasses, silica accumulation has been proposed to play a key role within grass-herbivore interactions (e.g. (McNaughton and Tarrant 1983; Vicari and Bazely 1993; Massey and Hartley 2006b; Massey, Smith et al. 2008)). In recent years, several studies have been conducted in the subject, leading to propose silica defenses as one of the most important inducible defense mechanisms in grasses, and it has even been suggested to shape cyclic small rodent population dynamics (Massey and Hartley 2006b; Massey, Smith et al. 2008). Grasses with high silica content, have a deterrent effect on mammal herbivores and insects (Vicari and Bazely 1993; Massey, Ennos et al. 2006a; Massey and Hartley 2006b; Massey and Hartley 2009). The effects of silica based defenses and their induction in grasses in response to herbivory, have been found to be important for plant-herbivore interactions in different areas of Africa, Europe and America (McNaughton and Tarrant 1983; Brizuela, Detling et al. 1986; Cid, Detling et al. 1989; Massey, Roland Ennos et al. 2007; Massey, Smith et al. 2008), but we propose that this was not the case in Finnmark. Our results do not suggest that silica accumulation is a general response to grazing of sub-arctic grasses, either when herbivory was excluded or when grazing pressure was different. One reason may be that grasses in our study area are common, but do not represent the majority of vegetation, hence selection pressure for induction may be not sufficient, in addition, among our study species, only *D.cespitosa* and *C.phragmitoides* are silica rich grasses. Alternatively, these contrasting results may be due to different ecological conditions for silica induction in grasses in sub-arctic tundra (i.e. temperature, soil pH, length of growing season, etc.) compared to grasses from southern areas on which similar studies were conducted.

The physiological mechanism of silica uptake in grasses is a complex process. Previous studies suggest that in silica accumulator species, like grasses (Epstein 1999; Müller 2003), external factors affect the uptake of silicon from the soil. Physical and chemical factors like low temperatures or acid soils have been found to inhibit the uptake of silicon from the soil in grasses (Liang, Hua et al. 2006). The low temperatures and low soil pH (4-5) (Ancin-Murguzur unpublished data) from our study areas may be an explanation of the lack of silica accumulation in response to herbivory found in our experiment, i.e. previous studies that showed silica induction in grasses (Massey and Hartley 2006b; Massey and Hartley 2009), were conducted under greenhouse condition where temperature and soil pH were more favorable for silicon uptake.

Anyhow, parallel studies in Finnmark from the same enclosure experiment (Kollstrøm 2009; Ravolainen 2009) showed a clear effect of the herbivore exclusion on the vegetation of the area. When mammal herbivores were excluded from grazing, significant changes in plant species community composition occurred (Ravolainen 2009).

Specific silica concentrations

We found different levels of silica between all studied species (supporting prediction 3) except for *D. cespitosa* and *C. phragmitoides* which both had higher silica levels than the other species. These two species may have higher constitutive defenses (higher concentration of silica) as a strategy against grazing. Accordingly, biomass of species with high silica levels, namely *D. cespitosa* decreased in the enclosure treatments while biomass of non-silica rich grasses increased (Ravolainen 2009). These results may suggest that in our study area, silica rich grasses had a competitive advantage in presence of herbivores.

Silica defenses in arctic tundra

Silica defenses have been proposed to be an inducible defense mechanism (e.g.(Massey, Roland Ennos et al. 2007)), and the deterrent effect of silica in different herbivore grazers have been empirically demonstrated (e.g.(Massey and Hartley 2006b)). However, as we showed, constitutive level of silica varies between species, indicating level of deterrence varies between species. We showed that *D. cespitosa* (silica rich species) had less silica concentration when mammal herbivores were excluded. Similar responses were expected to happen in *C. phragmitoides*, that also is a silica rich species, but we have not found any significant response to the enclosure treatments for this species. This lack of response may however be due to low sample size and hence low statistical power, as trends were in the same direction as that for *D. cespitosa*. Induction of silica may thus be a successful strategy for those species with high constitutive levels of silica but less likely to those with low constitutive levels of silica. Alternatively, the snow free season in Finnmark is too short for grasses to be grazed several times by herbivores, thus may not lead to silica induction in grasses. Nevertheless, we propose that silica as an inducible defense mechanism may be only effective against herbivory when the constitutive levels of silica are high, otherwise, different

defense mechanisms (i.e. endophytes) or strategies (tolerance instead of defense) may be more plausible.

CONCLUSIONS

We have not found clear evidences that support silica accumulation as a response to herbivory in grasses from Finnmark. Only one species (*D.cespitosa*) presented lower silica levels when all mammal herbivores where excluded. In addition, one species (*A.flexuosa*) showed differences on silica concentrations in grasses from different areas, but did not vary when herbivory was excluded.

Silica in grasses is likely to play an important role in plant-herbivore interactions, i.e. silica rich grasses are more abundant when grazing pressure is high. Anyhow, in our areas, silica is neither induced by herbivore grazing but for one species, nor reaches high levels in the leaves of common sub-arctic grasses, which indicates that silica accumulation in grasses do not shape rodent cycles in sub-arctic ecosystems.

The particular ecological conditions of Finnmark (short growing season, low temperatures, etc.) are likely to affect strongly the silicon uptake mechanisms, possibly causing that no general pattern of silica accumulations in response to herbivory have been found.

In order to correctly interpret the role of silica in natural systems, a better understanding of the factors causing silica induction, are needed. The process of silica uptake and the different biotic and physicochemical conditions that affect these processes are still not clear, and further studies would be desirable.

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