UiT The Arctic University of Norway

Faculty of Biosciences, Fisheries, and Economics
Department of Arctic and Marine Biology

Short-term tundra plant-community nutrient responses to herbivory and warming

New insights from Near infrared-reflectance spectroscopy methodology

Matteo Petit Bon

A dissertation for the degree of Philosophiae Doctor (PhD) in Natural Sciences (Ecology) – June 2020

UNIS
The University Centre in Svalbard

Short-term tundra plant-community nutrient responses to herbivory and warming

New insights from Near infrared-reflectance spectroscopy methodology

Matteo Petit Bon

The University Centre in Svalbard (UNIS)
Department of Arctic Biology
Longyearbyen, Svalbard (Norway)

The Arctic University of Norway (UiT)
Faculty of Biosciences, Fisheries, and Economics
Department of Arctic and Marine Biology
Tromsø, Norway

A dissertation for the Degree of Philosophiae Doctor (PhD)

Author's address:

Matteo Petit Bon
Via Paracelso, 9
Reggio Emilia, 42122, Italy
Email: matteo.petitbon@gmail.com

Supervisors:

Professor Dr. **Ingibjörg Svala Jónsdóttir**

University of Iceland
Institute of Life and Environmental Sciences
Sturlugata 7, IS-101 Reykjavik, Iceland
(from 2017)

The University Centre in Svalbard (UNIS)
Department of Arctic Biology
PO Box 156, N-9171 Longyearbyen, Norway
(until 2018)

Professor Dr. **Kari Anne Bråthen**
The Arctic University of Norway (UiT)
Faculty of Biosciences, Fisheries, and Economics
Department of Arctic and Marine Biology
N-9037 Tromsø, Norway

Researcher Dr. **Virve Tuulia Ravolainen**
Norwegian Polar Institute (NPI)
Fram Centre, PO Box 6606 Langnes, N-9296 Tromsø, Norway

Cover picture: Bird-cliff at Midterhuken, high-Arctic Svalbard (August 2017). One of the most beautiful places I visited in my life.

All photographs in this thesis are taken by Matteo Petit Bon except those explicitly stated.

"Scientific progress is a cumulative process of uncertainty reduction that can only succeed if science itself remains the greatest skeptic of its explanatory claims"

Open Science Collaboration (2015)

Science, 349(6251): aac4716

Table of Contents

Preface VI

A word on the Instruction Components VII

Acknowledgements VIII

List of the Manuscripts X

Abbreviations XII

List of Tables and Figures XIV

SUMMARY 1

Chapter 1 – INTRODUCTION 3

- 1.1 – Herbivores in the tundra 4
- 1.2 – A rapidly warming Arctic 6
- 1.3 – What is missing? 8
- 1.4 – Aims 9

Chapter 2 – METHODS 14

- 2.1 – Study systems 14
  - 2.1.1 – Sub-Arctic/alpine Finnmark 14
  - 2.1.2 – High-Arctic Svalbard 15
- 2.2 – Study designs 18
  - 2.2.1 – Finnmark studies 18
  - 2.2.2 – Svalbard studies 19
- 2.3 – Sample collection 20
- 2.4 – Sample processing 22
- 2.5 – Data analyses 24

Chapter 3 – MAIN RESULTS and DISCUSSION 26

Chapter 4 – CONCLUSIONS 39

Chapter 5 – FUTURE PERSPECTIVES 41

REFERENCES 44

MANUSCRIPTS I–V

Preface

The multiple ways in which organisms interact with each other and with the surrounding environment drive all natural systems on Earth. Ecology is the quantitative study of the relationships between organisms and how such relationships affect – and are affected by – the physical environment. Climate is perhaps the most critical component: temperature and precipitation patterns ultimately control where organisms live, and how they affect one another.

Life in the Arctic is at its extremes. Being one of the most seasonal environments on Earth, the Arctic is characterized by cold, long winters and cool, short summers. Precipitation mainly comes in the form of snow, and is low. Such extreme conditions pose severe limits to all organisms living in the Arctic. Yet, adaptations are also at their edges and plants and animals cope well with short growing seasons dominated by 24-h light and with long, dark, snow-covered winters.

Perhaps for this reason, as an Ecologist, I have always been fascinated by extreme environments. At first, I got enchanted by snow-bed ecosystems on Italian Alps, where I was working for my Master Degree Thesis in Ecology and Nature Conservation. After that, I soon reached the Arctic to work on plant-herbivore interactions within the PhD program of The Arctic University of Norway (UiT) and The University Centre in Svalbard (UNIS). That PhD program led to the present thesis.

"Short-term tundra plant-community nutrient responses to herbivory and warming: New insights from Near infrared-reflectance spectroscopy methodology" is the outcome. I wish two different things for this thesis. First, I hope it can contribute for a teeny-tiny piece, already at this stage, in increasing our knowledge about plant-herbivore interactions in tundra ecosystems. Second, I hope that the research conducted throughout my PhD period and summarized here will lay the foundations for my future studies as an Arctic/alpine Ecologist.

Matteo Petit Bon

A word on the Instruction Components

The Research Committee at the BFE faculty made in case FU BFE 84-18 the following decision on January 15th 2019:

"The Research Committee gives final approval on the instruction component for PhD student in Natural Sciences Matteo Petit Bon, consisting of":

Emnekode	Tittel, institusjon	Stp.	Semester
AB-829	Arctic Winter ecology, UNIS	10 (2)	V-15
AB-826	Arctic Plant Ecology, UNIS	10 (2)	H-15
SVF-8600	Philosophy of science and ethics	6	V-16
Bio-8105	Ecological methodology: Study design and statistical analysis	10	V-16
Bio-8514	Advanced ecological statistics	10	H-17
Sum		**46 (30)**	

The approval of the Instruction Components was the very first step towards the end of my PhD pathway. It happened almost one and a half year before the delivery of the present thesis. I started the 'Arctic Winter Ecology' course 15 days after I landed on Svalbard for the first time on the 15th of February 2015, approximately five and a half years ago. After a few months, I took the 'Arctic Plant Ecology' course. Prof. Ingibjörg S. Jónsdóttir, my supervisor and main (former) instructor in both courses, laid down the foundations of what I know about high-Arctic environments. In 2016, it was the turn of the 'Ecological methodology: Study design and statistical analysis' course. Here, Prof. Nigel G. Yoccoz helped me to deepen my understanding of Linear Mixed-Effects Models, without which I would have never been able to finish my PhD. I took the 'Philosophy of science and ethics' course at the same time. It was mandatory for PhD students in Natural and Social sciences at the Arctic University of Norway (UiT); yet Prof. Peter Arbo enlightened me on how to talk about the role of science in the nowadays society. It could have been it! I had already 6 more credits than the 30 required to complete the Instruction Components at UiT. However, I will never regret my decision of taking the 'Advanced ecological statistics' course one year later, in Autumn 2017. Here, Prof. Raul Primicerio and Prof. Michael J. Greenacre introduced me to the vast world of Multivariate Data Analysis, statistical methods that I repeatedly used throughout my PhD.

Acknowledgements

Inga Svala, Kari Anne, and Virve; with your positive energy, creativity, experience, and knowledge, you taught me a lot. I would like to give you a special thank for two main reasons: first, thanks for giving me the opportunity to come study in the Arctic; second, thanks for letting me develop my own path. You not only made me grow as a Scientist, but also changed the Person I am and I will be forever grateful for this. Also, I here want to thank all the other people who have been involved in the studies that are part of this thesis. Without such a great Team, this work would have been impossible.

Filippo, it has now been 4.5 years since we first met at AMB. We did share our PhD paths, supporting each other through joys and struggles. But first and foremost I want to thank you for the countless laughs and the crazy moments. My PhD period would have never been the same without you.

Mathilde, my French n° 1, and Brage, together the NTNU Team; thanks for all the help and the support you indirectly gave me during my PhD. Starting from "he will drop in a couple of months" to all the great projects and ideas we had together: I am happy to confirm I did not drop, and I finally managed to submit my thesis. Now it is time to start applying for funding…

A special thanks to my parents: they did not pursue a PhD, but it was almost like they did. They have been supportive, comprehensive, and always ready to help, especially when 9 months ago I came back from Svalbard knowing that the struggle was not over, yet. Also, if it was not for how they raised me, I would not be the person I am, and likely I would not be here writing this section. You are the best.

I would like to thank the Alpine Ecology Group in Parma. Alessandro, Michele, and Marcello; you are my Italian scientific family. You are the ones who made me fall in love with Science some 7 years ago, and the ones who pushed me to pursue a PhD. Indirectly, you changed my life and I will be forever grateful for this too.

A special thanks to all my colleagues in the Department of Arctic Biology at the University Centre in Svalbard (UNIS) and in the Department of Arctic and Marine Biology at the Arctic University of Norway (UiT). It has been a privilege to work with you and share funny moments together. Also, I want to thank Inga Svala's research group for the fruitful discussions we had during the Lab meetings and the endless times I got motivating feedbacks on my work.

Jessica, there are no words to thank you for these last 9 months of life. Without your presence, this last period would have never been the same. Every moment I was down, you were there to cheer me up. Every time I was productive, you were ready to leave me alone and let me work. You have been the most important component in these last months of struggle with my PhD degree. Thanks for how you are.

I want to thank the cumpa RAGHASHISH. Guys, you are the best friends ever. Although we did see each other for only a few weeks per year during the last 5 years, I feel like we were always together. Cheers.

Also, thanks to Tommy, Gagga, Keso, Gianluca, Nicola, and Giada. You were always there when I needed it, even if it was just for a simple skype call when we were more than 3000 km apart (well, even 13523.93 km with some of you).

Venke I., Alex M., Larissa B., Arna O., Lena H., Kate LM., Ursula E., Bart P., Hanne H., Robin Z., Claudio G., Majsofie C., Caroline T., Giacomo R., Mikel M., Martin M., Lukas T., Carmen K., Niek H., Anton H., Calum B., Tor J., Xabi M., Sunil M., Henni B., Eli X., Sophia Z., Inge A., Svea Z., Freja F., Marta G., Solvei H., Jarad M., Magda W., Stuart T.. Thanks for (i) helping out with fieldwork/labwork, (ii) sharing nice moments with me, and/or (iii) making a (small) part of my life just unforgettable.

List of the Manuscripts

This thesis is based on the following five studies, which are referred to in the text by their respective Roman numerals. Manuscripts follow the main body of the thesis.

I. Petit Bon M., Böhner H., Kaino S., Moe T., Bråthen K.A.[⸸] (2020). One leaf for all: Chemical traits of single leaves measured at the leaf surface using Near infrared-reflectance spectroscopy (NIRS). *Methods in Ecology and Evolution*.
doi: https://doi.org/ 10.1111/2041-210X.13432.

II. Petit Bon M.[⸸], Inga K.G., Jónsdóttir I.S., Utsi T.A., Soininen E.M., Bråthen K.A. (2020). Interactions between winter and summer herbivory affect spatial and temporal plant nutrient dynamics in tundra grassland communities. *Oikos*.
doi: https://doi.org/10.1111/oik.07074.

III. Petit Bon M.[⸸], Inga K.G., Utsi T.A., Jónsdóttir I.S., Bråthen K.A. Silicon-based defence and nutrient levels in grasses under herbivory: Forage quality in tundra grasslands is altered through different mechanisms. *Manuscript draft* [‡].

IV. Petit Bon M.[⸸], Böhner H., Bråthen K.A., Ravolainen V.T., Jónsdóttir I.S. Variable chemical responses of three major ecosystem compartments to herbivory and warming in high-Arctic tundra. *Manuscript draft* [‡].

V. Petit Bon M.[⸸], Bråthen K.A., Ravolainen V.T., Böhner H., Jónsdóttir I.S. Nutrient contents and nutrient pools of high-Arctic plant communities: contrasting effects of herbivory and warming and plant functional type dependent responses. *Manuscript draft* [‡].

[⸸] Corresponding author

[‡] Manuscript drafts are found at different stages of preparation

Declaration of contributions (the order of names simply reflects their appearance in the co-author list of each manuscript [MS]*):

	MS I	**MS II**	**MS III**	**MS IV**	**MS V**
Concept and idea	MPB, SK, TM, KAB	MPB, KGI, TAU, KAB	MPB, KGI, TAU, KAB	MPB, KAB, VTR, ISJ	MPB, KAB, VTR, ISJ
Study design and methods	MPB, HB, SK, TM, KAB	MPB, KGI, TAU, KAB	MPB, KGI, TAU, KAB	MPB, HB, KAB, VTR, ISJ	MPB, KAB, HB, VTR, ISJ
Data gathering and interpretation	MPB, HB, SK, TM, KAB	MPB, KGI, ISJ, TAU, EMS, KAB	MPB, KGI, ISJ, TAU, KAB	MPB, HB, KAB, VTR, ISJ	MPB, KAB, HB, VTR, ISJ
Manuscript preparation	MPB, HB, SK, TM, KAB	MPB, KGI, ISJ, TAU, KAB	MPB, KGI, ISJ, TAU, KAB	MPB, HB, KAB, VTR, ISJ	MPB, KAB, HB, VTR, ISJ

* Specific contributions of each author are reported in the 'Author Contribution Statement' of each manuscript

Authors' initials (alphabetical order):

MPB = Matteo Petit Bon[1,2]

EMS = Eeva Marjatta Soininen[2]
HB = Hanna Böhner[1,2]
ISJ = Ingibjörg Svala Jónsdóttir[3]
KAB = Kari Anne Bråthen[2]
KGI = Katarina Gunnarsdotter Inga[2]
SK = Sissel Kaino[2]
TM = Torunn Moe[2]
TAU = Tove Aagnes Utsi[2]
VTR = Virve Tuulia Ravolainen[4]

Affiliations:

[1] The University Centre in Svalbard (UNIS)
Department of Arctic Biology
PO Box 156, N-9171 Longyearbyen, Norway

[2] The Arctic University of Norway (UiT)
Faculty of Biosciences, Fisheries, and Economics
Department of Arctic and Marine Biology
N-9037 Tromsø, Norway

[3] University of Iceland
Institute of Life and Environmental Sciences
Sturlugata 7, IS-101 Reykjavik, Iceland

[4] Norwegian Polar Institute (NPI)
Fram Centre, PO Box 6606 Langnes, N-9296 Tromsø, Norway

Cover picture MS I: The author while processing single, full leaves with Near infrared-reflectance spectroscopy (NIRS) methodology (photo credits: Filippo Marolla).

Cover picture MS II: The historical fence dividing Ifjordfjellet, Finnmark (Norway) in spring/fall migratory (right side) and summer (left side) pasture ranges for semi-domesticated reindeer.

Cover picture MS III: Reindeer grazing on tundra-grassland plant communities found in summer pasture ranges at Ifjordfjellet, Finnmark (Norway).

Cover picture MS IV: Pink-footed geese grubbing on recently exposed tundra-patches after snowmelt in Adventdalen, Svalbard.

Cover picture MS V: A rainbow colouring Adventdalen (Svalbard) and the mosaic of plant communities found therein.

Abbreviations

List of the abbreviations used throughout this thesis, presented in alphabetical order. Abbreviations are also introduced in the text at the first encounter, but reported here for completeness.

C	Carbon
CI	Confidence interval
CO_2	Carbon dioxide
C-to-N (C:N)	Carbon-to-nitrogen ratio
DA	Goose-disturbed/un-warmed plot
DW	Goose-disturbed/warmed plot
ITEX	International tundra Experiment
ME-habitats	Mesic habitats (mesic heath)
MO-habitats	Moist habitats (moss tundra)
N	Nitrogen
NIRS	Near infrared-reflectance spectroscopy
OTC	Open-top chamber
P	Phosphorus
PFT	Plant functional type
Ro−/Re−	Rodent-undisturbed/reindeer-ungrazed plot
Ro−/Re+	Rodent-undisturbed/reindeer-grazed plot
Ro+/Re−	Rodent-disturbed/reindeer-ungrazed plot
Ro+/Re+	Rodent-disturbed/reindeer-grazed plot
ROS	Rain-on-snow
Si	Silicon
Si-poor grasses	Silicon-poor grasses
Si-rich grasses	Silicon-rich grasses
Si-to-N (Si:N)	Silicon-to-nitrogen ratio
Si-to-P (Si:P)	Silicon-to-phosphorus ratio
UA	Goose-undisturbed/un-warmed plot

UiT	The Arctic University of Norway, Tromsø, Norway
UNIS	The University Centre in Svalbard, Longyearbyen, Svalbard (Norway)
UW	Goose-undisturbed/warmed plot
WE-habitats	Wet habitats (wetland)
%dw	Percentage dry weight

List of Tables and Figures

Table 1 (page 25). Overview of the research questions addressed by this thesis, main expectations, and methods utilized to test these predictions.

Table 2 (page 33). Ecosystem compartments differed in their chemical responses to treatments and responses also differed between habitats, but the between-year variation in ecosystem-compartment chemical composition was greater than responses to my experimental manipulations (main results from *Paper IV*).

Figure 1 (page 13). Schematic of the overarching question addressed by this thesis and of the more specific questions addressed by each study.

Figure 2 (page 14). Geographical position of the two study systems used in this work.

Figure 3 (page 18). Study and sampling design used to assess the extent to which small rodents and reindeer can affect tundra plant-community nutrient levels in the short-term.

Figure 4 (page 19). Study and sampling design used to assess the extent to which spring goose herbivory and summer warming can affect tundra plant-community nutrient levels in the short-term.

Figure 5 (page 26). Arctic-alpine NIRS calibration models used to predict chemical contents of tableted leaves can be applied on single, full leaves (main results from *Paper I*).

Figure 6 (page 28). Herbivores increased plant-community nitrogen and phosphorus levels in tundra-grasslands and effects were temporally consistent throughout the growing season (main results from *Paper II*).

Figure 7 (page 30). Both herbivores together decreased the quality of Si-rich grasses relative to that of Si-poor grasses (main results from *Paper III*).

Figure 8 (page 36). Spring goose disturbance and summer warming had generally opposing effects on plant-community and PFT nutrient contents and pools, and PFTs differed in their responses to treatments (main results from *Paper V*).

SUMMARY

Background. The study of tundra plant-community nutrient, mainly nitrogen and phosphorus, responses to herbivory and climate warming is critical for our understanding of ecosystem processes and trophic interactions in a rapidly changing Arctic. In the long-term, herbivores and climate warming have been shown to alter nutrient levels in tundra plant communities by changing the functional composition of the vegetation. Yet, the extent to which they affect tundra plant-community nutrient levels in the short-term by directly modifying the chemistry of plants has been poorly explored. Methodological constraints have often hampered detailed assessments of herbivore- and warming-induced changes in nutrient-related plant traits across plant communities.

Aims. In this thesis, I asked to what extent herbivores and summer warming can affect plant-community nitrogen and phosphorus levels in tundra ecosystems in the short-term (*II, III, IV, V*). I also considered grass-community silicon-based defence level (*III*) and vascular-plant, moss, and soil carbon content (*IV*) since silicon and carbon may influence the contribution of nitrogen and phosphorus to e.g. forage quality and nutrient cycling. My overall hypothesis was that herbivory and warming are critical short-term modifiers of tundra plant-community nutrient levels. To test this, I first developed a time- and cost-effective method that allowed me to account for the high variability in nutrient-related plant traits among plant individuals, and further scale up to the plant-community level (*I*). Then, I applied such methodology to investigate various aspects of short-term effects of herbivores (*II, III*) and herbivory and warming (*IV, V*) on tundra plant-community nutrient levels.

Locations. The combined effects of widespread tundra herbivores, namely small rodents and reindeer (caribou in North America), were assessed in a one-year field-experiment performed across sub-Arctic/alpine tundra-grasslands in norther Fennoscandia, Norway (*II, III*). The combined effects of goose herbivory and summer warming were addressed in a two-year field-experiment carried out across different habitats in a high-Arctic ecosystem in the archipelago of Svalbard (*IV, V*).

Results and Implications. Arctic-alpine Near infrared-reflectance spectroscopy calibration models used to predict chemical contents (nitrogen, phosphorus, silicon, and carbon) of plant samples constituted by merged and milled leaves were successfully extended to predict chemical contents of single leaves (*I*). Thus, I had the tool to properly investigate the immediate effects of herbivores and elevated summer temperatures on tundra plant-community nutrient levels. I found that plant communities in tundra-grasslands responded to small rodents and reindeer with an immediate increase in their nitrogen and phosphorus levels (*II*). Moreover, these positive effects of herbivores were temporally consistent throughout the summer and still evident at the onset of the winter period (*II*), suggesting that herbivores accelerate short-term nutrient cycling rates in these grasslands. Silicon-based defence relative to nitrogen and phosphorus levels (i.e. silicon-to-nutrient ratios) of grasses affected by small rodents and reindeer were never above those of grasses in their absence (*III*), demonstrating that herbivory immediately enhances the overall quality of the grass-community in tundra-grasslands. Yet, in the presence of both herbivores, the quality of inherently silicon-rich, less palatable grasses was decreased relative to that of silicon-poor, more palatable grasses (*III*), indicating that the apparent competition between these groups of grasses may be exacerbated by short-term herbivory. This pattern was dictated by grass silicon and nutrient responses, suggesting that both mechanisms are likely to contribute to the dominance of silicon-rich vegetation in tundra-grasslands subjected to high herbivory pressure. In the assessment of short-term carbon, nitrogen, and carbon-to-nitrogen ratio responses of vascular plants, mosses, and soil to goose herbivory and summer warming (*IV*), I found that these major ecosystem compartments show different chemical responses (vascular plants > soil > mosses), which also differ between habitats (mesic > moist > wet habitats). By differentially altering the chemical composition of vascular plants, mosses, and soil, herbivory and warming may have immediate effects on the functioning of tundra ecosystems. However, the degree of such effects varies among habitats, eventually affecting ecosystem processes across the tundra landscape at different rates. Overall, I found goose herbivory to increase plant-community nitrogen and phosphorus contents and decrease plant-community nutrient pools, whereas summer warming decreased plant-community nutrient contents but did not affect nutrient pools (*V*). These opposing responses are likely to have important implications for how these drivers alter nutrient available to herbivores in summer. Yet, changes in plant-community nutrient levels varied between habitats, and plant functional types showed differential responses, ultimately suggesting that herbivory and warming may also affect how herbivores utilize the tundra landscape throughout the growing season.

Conclusions. This thesis provides clear evidence that herbivores and climate warming are key, short-term modifiers of plant-community nutrient levels in tundra ecosystems, and that nutrient-level changes are happening at a much shorter time-scale than previously revealed. Considerable short-term changes in plant-community nutrient levels, as those detected in this work, are likely to have strong implications for the immediate functioning of tundra ecosystems and the trophic interactions established therein.

Keywords: Carbon (C), Community-weighted means, Fennoscandia, Forage quality, Forage quantity, Geese, Growth forms, Habitat-types, Herbivory Network (HN), High-Arctic tundra, International Tundra Experiment (ITEX), Near infrared-reflectance spectroscopy (NIRS), Nitrogen (N), Nutrient contents, Nutrient pools, Nutrient cycling, Phosphorus (P), Plant functional types (PFTs), Plant-herbivore interactions, *Rangifer* (reindeer/caribou), Silicon (Si), Small rodents, Sub-Arctic/alpine tundra, Svalbard, Tundra-grasslands.

Chapter 1

INTRODUCTION

The level of chemical constituents in plants influences processes and functions of terrestrial ecosystems worldwide.

Together with light, moisture, and temperature, plant nutrient contents (mainly nitrogen [N] and phosphorus [P]) exert a strong control over photosynthesis (Reich et al. 1997, Wright et al. 2004), i.e. the process by which carbon (C) and chemical energy enter ecosystems. N and P are required in large quantity, often much larger than their availability (Aerts and Chapin 1999), and it is therefore not surprising that nutrients are globally limiting in terrestrial ecosystems (Shaver and Chapin 1995, LeBauer and Treseder 2008, Vitousek et al. 2010). By controlling leaf photosynthetic rates, thus plant growth, plant nutrient levels ultimately regulate primary production across ecosystems (Elser et al. 2007).

Because the same nutrients that promote primary productivity also enhance the decomposability of plant litter (Hobbie 1992, Zhang et al. 2008), N and P contents in plants regulate the rates of nutrient cycling in terrestrial ecosystems. Moreover, litter decomposition contributes substantially to the C dioxide (CO_2) flux from soil to the atmosphere (Raich and Potter 1995). Hence, by affecting C assimilation in plants (i.e. primary production) and C loss through plant respiration and litter decomposition, plant nutrient levels regulate C cycling and C balance of ecosystems (De Deyn et al. 2008). Indeed, it is the tight link between C and nutrient cycling that ultimately links plant-soil C dynamics across terrestrial environments (Chapin et al. 2009).

Nutrient levels in plants also play an important role in mediating plant-herbivore interactions (White 2012). This is because N and P are essential elements for herbivore growth, but are often in short supply relative to demands by grazers (Sterner and Elser 2002). Indeed, across a wide range of ecosystems, nutrient content in plants has proved useful to predict herbivore habitat use (Albon and Langvatn 1992, Anderson et al. 2010, Iversen et al. 2014) and, consequently, herbivore fitness (Mysterud et al. 2001, Doiron et al. 2015). Yet, forage quality for herbivores not only depends on amount of essential nutrients in plants, but also on plant chemical and structural defences (Hanley 1997, Hartley and DeGabriel 2016, Moreira et al. 2018). For instance, several plant species, especially graminoids, have specialised in using silicon (Si) to increase their resistance to herbivory (Epstein 2009, Hartley and DeGabriel 2016). By enhancing the abrasiveness of plant material, Si acts as a feeding deterrent for herbivores (Massey et al. 2006). Furthermore, it has been shown that high Si-content in forage grasses can reduce herbivore fitness by preventing the absorption of essential nutrients such as N (Massey and Hartley 2006).

Understanding and articulating how biotic and abiotic drivers affect the chemical composition of plant communities is thus critical to predict changes in primary productivity, plant-mediated nutrient and C cycling pathways and rates, and herbivore forage quality. Perhaps nowhere is this more pertinent than in high latitude regions, such as the (sub-) Arctic, which are experiencing the fastest rate of environmental changes (Post et al. 2009, Post et al. 2019).

The tundra, the major biome at high-latitudes, is peculiar in that its biogeochemistry is largely controlled by the slow turnover rates of C and nutrients between soil and vegetation (Hobbie et al. 2002). The prevailing low soil temperatures slow down rates of organic matter decomposition (Cornelissen et al. 2007), which in turn severely restrict nutrient mineralization rates (Nadelhoffer et al. 1991). Slow N and P cycling rates constrain plant growth, and thus ecosystem C gain through primary productivity (Shaver and Chapin 1980, Rustad et al. 2001). Further, low plant biomass may limit the amount of nutrients available to herbivores utilizing the tundra landscape throughout the growing season. This is exacerbated by that the short Arctic summer constrains the time window when herbivores have access to forage plants. Under such limiting conditions, any factors that affect the chemical composition of plant communities may thus have prominent consequences for ecosystem processes and functions and trophic interactions in tundra environments.

1.1 – Herbivores in the tundra

Vertebrate herbivores are found almost everywhere across the circumpolar tundra (Barrio et al. 2016, Speed et al. 2019), and through their activities they substantially affect structure and function of plant communities (Jefferies et al. 1994, Mulder 1999). In the medium- and long-term, herbivore-induced changes in the functional composition of the vegetation can be regarded as one of the main mechanisms through which herbivores influence the chemical composition of tundra plant communities.

Herbivory can promote higher abundance of either palatable, nutrient-rich plant species (e.g. forbs and grasses) (Olofsson et al. 2001, Olofsson et al. 2004, Tuomi et al. 2018) or less palatable, nutrient-poor plant species (e.g. shrubs) (Pastor and Naiman 1992, Pastor et al. 1993, Grellmann 2002), thus increasing or decreasing the overall nourishing state of tundra plant communities. Since the nutrient-related plant traits that contribute to the palatability of foliage also govern the decomposability of plant litter (Grime et al. 1996, Cornelissen et al. 2004), herbivores sometimes accelerate and sometime retard nutrient-cycling rates in tundra ecosystems (Pastor et al. 2006, Stark 2007).

By affecting the functional composition of tundra plant communities, herbivores also manipulate the amount of nutrients carried by their own forage species. Indeed, intense herbivory can cause vegetation shifts towards either less (Srivastava and Jefferies 1996, Jefferies and Rockwell 2002, Jefferies et al. 2006) or more (Zimov et al. 1995, Olofsson et al. 2001, Olofsson and Oksanen 2002, Olofsson et al. 2004) productive vegetation states, ultimately influencing plant-community nutrient pools. Theoretical (Van der Wal 2006) and empirical (Freschet et al. 2014, Egelkraut et al. 2018b, Egelkraut et al. 2018a) evidence suggests that these herbivore-driven vegetation changes represent alternative stable states of tundra plant communities, which may persist over long time scales.

Such vegetation shifts are also accompanied by changes in forage quality. For instance, intense grazing by reindeer (caribou in North America) can promote the transition from heath- or shrub-dominated tundra (a less productive vegetation state) to graminoid-dominated tundra (a more productive vegetation state) (Olofsson et al. 2001). Graminoids, such as grasses, are generally more nutrient-rich compared to e.g. evergreen shrubs, and thus give a greater nutritional return to herbivores. Yet, tundra-grasslands that are heavily utilized by reindeer are often dominated by grasses that are high in Si-content (i.e. Si-rich grasses) (Bråthen et al. 2007), and hence are characterized by a lower nourishing state compared to grasslands dominated by grasses that are low in Si-content (i.e. Si-poor grasses) and/or forbs. Interestingly, studies that experimentally excluded herbivore activities found that herbivory only reduces the biomass of more palatable, Si-poor grasses (Bråthen and Oksanen 2001, Ravolainen et al. 2011), suggesting that a tundra vegetation state dominated by unpalatable, Si-rich grasses is maintained when the grazing pressure is high (Austrheim et al. 2007, Ravolainen et al. 2011).

A change in the functional composition of the vegetation is, however, *not* the only mechanism through which herbivores can alter the chemical composition of tundra plant communities. Indeed, herbivores may affect the chemical composition of plant communities by modifying the chemistry of plants, and these changes may be detected long time ahead compared to compositional changes.

For instance, herbivores may select leaves in early phenological stages and/or more nutritious plant parts (Bråthen and Oksanen 2001, Iversen et al. 2014), thus reducing overall nutrient levels in tundra plant communities. Such immediate reduction in the nourishing state of tundra plant communities could be further accentuated if short-term herbivory also induces the accumulation of chemical (i.e. secondary metabolites) and/or structural (e.g. Si) defences in plant leaves (Soininen et al. 2013a, Väisänen et al. 2013).

Conversely, herbivory could increase plant-community nutrient levels by returning readily available nutrients to soil through faeces and urine (Bazely and Jefferies 1985). This process shortcuts the slower litter-decomposition pathway and enhances soil microbial activity and plant nutrient availability (Stark et al. 2002, Van der Wal et al. 2004). Of all short-term grazing effects, this latter process is also commonly promoted as the main mechanism by which grazers can stimulate nutrient cycling in tundra ecosystems (Van der Wal et al. 2004) and positively manipulate the quality of their own food supply (Hik and Jefferies 1990).

Herbivores may also induce a rapid re-growth of highly-nutritious plant tissue, i.e. keep leaves in younger phenological stages (Chapin 1980, McNaughton 1983, Mysterud et al. 2011), which contribute to the maintenance of high plant-community nutrient levels. If the rapid re-growth of newly-formed plant tissue over-compensates the losses through herbivory (Wegener and Odasz-Albrigtsen 1997, Bråthen and Odasz-Albrigtsen 2000), herbivores could also promote an increase in the overall amount of nutrients carried by plant communities.

1.2 – A rapidly warming Arctic

Over the past 30 years, Arctic regions have warmed at a faster pace than the rest of the planet (Post et al. 2019). Such phenomenon, known as the ‘Arctic amplification’, has substantially altered the structure and function of tundra plant communities (Walker et al. 2006, Myers-

Smith et al. 2011, Elmendorf et al. 2012a, Elmendorf et al. 2012b, Myers-Smith et al. 2020). As for herbivory, temperature-driven changes in the functional composition of the vegetation can be regarded as one of the main mechanisms through which climate warming influences the chemical composition of tundra plant communities in the long-term.

For instance, one of the most widespread changes in vegetation composition that Arctic ecosystems are experiencing because of higher temperatures is the increase in shrub biomass, cover, and abundance (colloquially termed *shrubification*) (Myers-Smith et al. 2011, Myers-Smith et al. 2020). Shrub encroachment and associated changes in the amount of nutrients in the vegetation may have important consequences for nutrient cycling and C balance of tundra ecosystems (Cornelissen et al. 2007), and trophic interactions between herbivores and their food sources (Cornelissen et al. 2001, den Herder et al. 2008, Zhou et al. 2020).

A change in the functional composition of the vegetation is, however, *not* the only mechanism through which climate warming alters the chemical composition of tundra plant communities. As for herbivory, elevated temperatures may affect the chemical composition of plant communities by modifying the chemistry of plants, and these changes may happen much faster compared to structural changes.

For instance, higher temperatures may immediately increase soil nutrient mineralization rates, thus indirectly enhance nutrient availability for tundra plants (Rustad et al. 2001, Salazar et al. 2019) and ultimately their nutrient contents (Welker et al. 2005). Such warming-induced increase in soil process rates, thus nutrient availability for plants, could be further accentuated if herbivores concomitantly return nutrients to soil through animal-excreta (see above). Herbivore-grazed plant communities in a warmer Arctic may enjoy an even greater nutrient supply, allowing for greater plant-community nutrient contents and, eventually, nutrient pools. Higher plant-community nutrient levels throughout the summer may benefit herbivores insisting upon tundra ecosystems. Moreover, they could transpose to enhanced litter decomposition, thus faster nutrient cycling rates, if higher temperatures do not further affect nutrient resorption in senescence leaves (Aerts et al. 2007).

A multitude of experimental studies has shown that Arctic plants respond to higher summer temperatures by rapidly increasing their productivity (see reviews by Elmendorf et al. 2012a, Elmendorf et al. 2012b). Since a bigger plant individual will likely contain a higher amount of total nutrients, one may expect higher temperatures to increase plant-community nutrient pools in the short-term. This could be especially true if a warming-induced higher productivity is also accompanied by positive effects of warming on plant nutrient contents (see above). However, an increase in plant biomass due to higher temperatures does not necessarily correspond to higher plant nutrient pools (Doiron et al. 2014). Indeed, summer warming has often been associated to a decrease in tundra-plant nutrient contents (Tolvanen and Henry 2001, Aerts et al. 2009, Doiron et al. 2014). This is because the direct effects of higher temperatures (i.e. dilution of nutrients in the increased biomass and acceleration of plant phenology, thus senescence) can be stronger than their indirect effects (i.e. higher soil nutrient mineralization) (Dormann and Woodin 2002, Aerts et al. 2009), at least in the short-term (Michelsen et al. 2012).

1.3 – What is missing?

Whereas herbivore- and warming-driven changes in the functional composition of tundra plant communities have been the centre of investigation in several studies, much less is known about how herbivory and higher temperatures affect tundra plant-community nutrient levels in the short-term by directly modifying the chemistry of plants.

I see two main reasons for this. First, elemental contents in plants are costly measures to attain. Consequently, the majority of studies assessing the extent to which herbivory (e.g. Mysterud et al. 2011, Beard et al. 2019) or warming (e.g. Tolvanen and Henry 2001, Welker et al. 2005, Doiron et al. 2014) affect the chemistry of tundra-plants have often focused on a few plant species structuring the plant community. Second, most methods to measure foliar chemical contents require several leaves to be merged in order to obtain enough plant material for chemical analyses. Plant chemical contents obtained with such methods can only poorly assess intra-specific variation in plant chemistry, and might fail in accounting for intra-individual variability. Yet, we now know that the assumption that intra-specific plant-trait variability is negligible compared to inter-specific variability may not hold true (Albert et al. 2011), particularly in the Arctic (Bjorkman et al. 2018), and that levels of variation below that of species-specific differences are likely to influence plant-community responses to perturbations (Albert et al. 2010). Perhaps for this reason studies addressing the extent to which herbivore activities (Van der Wal et al. 2004, Barthelemy et al. 2015) or warming

(Dormann and Woodin 2002) affect the chemistry of plant functional types (PFTs) in tundra plant communities have often found variable and somewhat inconsistent responses.

Methodological constraints have often hampered detailed assessments of herbivore- and warming-induced changes in nutrient-related plant traits across plant communities. Yet, by causing immediate changes in the chemistry of plants, herbivores and elevated temperatures might affect nutrient levels in tundra plant communities, with likely important implications for ecosystem processes and functions and trophic interactions.

1.4 – Aims

In light of the knowledge gaps presented above, ***the overarching objective of this thesis was to assess the extent to which herbivory and climate warming can affect plant-community nutrient levels in tundra ecosystems in the short-term by directly modifying the chemistry of plants*** (Figure 1 – main question).

To achieve this, I first needed to develop a methodology that could allow me to include inter- and intra-specific variation in chemical contents of single leaves, and further scale up at the plant-community level. My research group at The Arctic University of Norway (UiT, Tromsø, Norway) had recently developed Arctic-alpine Near infrared-reflectance spectroscopy (NIRS) calibration models to predict chemical contents (nitrogen [N], phosphorus [P], carbon [C], and silicon [Si] – as % dry weight, hereafter %dw) of tableted-samples constituted by merged and milled leaves (Smis et al. 2014, Murguzur et al. 2019). NIRS is a high-throughput technology that had long been utilized for measuring plant constituents in agricultural contexts (Stuth et al. 2003), assessing the composition of plant and animal tissues (Foley et al. 1998, Chodak 2008), and quantifying soil chemical composition (Viscarra Rossel et al. 2016), but it has only been recently developed at UiT for plant ecological applications in Arctic and alpine environments. If Arctic-alpine NIRS calibration models were applicable to assess chemical contents of single, full leaves, NIRS methodology would have provided the opportunity to properly answer my overall question.

Thus, I first asked **to what extent Arctic-alpine NIRS calibration models used to assess N, P, C, and Si contents of merged, milled, and tableted leaves can be applied to predict chemical contents of single, full leaves** (Figure 1 – *Paper I*; Table 1a,b – *Paper I*).

My overarching question was then divided in two main aims, further subdivided into several specific questions. First, I investigated short-term plant-community nutrient-level responses to small rodents and reindeer in sub-Arctic/alpine tundra-grasslands throughout the summer season of 2015. Second, I assessed short-term plant-community nutrient-level responses to goose herbivory and climate warming in the high-Arctic tundra across different habitats in summers 2016 and 2017. Sub-Arctic/alpine and high-Arctic tundra highly differ in terms of both biotic and abiotic conditions (see below and Section '2.1 – Study systems'), allowing me to tackle my overarching question under different perspectives.

A. *Short-term plant-community nutrient-level responses to herbivores* – I asked **to what extent small rodents in winter and reindeer in summer can affect plant-community nutrient levels in the short-term** (Figure 1 pathway A – *Papers II, III*).

In *Paper II*, I asked: (1) To what extent can herbivores affect tundra plant-community N and P dynamics? (2) Is the impact of herbivores indicative of accelerating, neutral or retarding effects on N and P cycling rates? (Table 1a,b – *Paper II*)

In *Paper III,* I asked: (1) To what extent can herbivores affect Si-based defence levels in the community of tundra-grasses? (2) To what extent may such changes alter the quality of the grass-community in terms of Si-to-N (Si:N) and Si-to-P (Si:P) ratios? (Table 1a,b – *Paper III*)

Short-term tundra plant-community nutrient-level responses to herbivores were investigated across sub-Arctic/alpine tundra-grasslands in northern Fennoscandia, Norway (see Section '2.1.1 – Study systems: Sub-Arctic/alpine Finnmark'). For four main reasons these tundra-grasslands are a suitable system to test the effects of small rodents and reindeer on plant-community nutrient levels in the short-term. First, they are characterized by PFTs with high nutrient contents (i.e. forbs and grasses) (Aerts and Chapin 1999, Cornelissen et al. 2004), and thus are key hotspots for plant-herbivore interactions (Skarin et al. 2008, Soininen et al. 2013b). Second, they harbour both reindeer and small rodents, which are herbivores found across many regions of the Arctic biome (Ims and Fuglei 2005, Bernes et al. 2015, Uboni et al. 2016), yet that differ in their long- and short-term effects on plant community structure (Olofsson et al. 2009, Ravolainen et al. 2011, Olofsson et al. 2013) and ecosystem processes (Olofsson et al. 2004, Metcalfe and Olofsson 2015, Tuomi et al. 2018). This is partly because small rodents and reindeer differ in their spatial and temporal dynamics: small rodents can visibly disturb the vegetation both in summer and winter (Hambäck et al. 1998, Ims and

Fuglei 2005), whereas reindeer show large-scale spatial dynamics through their migratory behaviour and their impacts by grazing and trampling on vegetation are more likely to be seasonal (Bernes et al. 2015). Third, their long reindeer-grazing history (Hætta et al. 1994) and the ubiquitous presence of small rodents (Ims and Fuglei 2005) make these grasslands ideal to test whether short-term herbivory is promoting accelerating, neutral or retarding effects on nutrient cycling rates (*II*). Finally, the grass-community found in these grasslands is clearly differentiated in species that are high in Si-based defence levels (Si-rich grasses) and species that are low in Si-based defence levels (Si-poor grasses) (Bråthen et al. 2007, Ravolainen et al. 2011, Soininen et al. 2013a), creating the opportunity to test whether herbivory-driven changes in Si-content contributes to alterations in herbivore forage quality (*III*).

B. *Short-term plant-community nutrient-level responses to herbivory and warming* – I asked **to what extent spring goose herbivory and summer warming can affect tundra plant-community nutrient levels in the short-term** (Figure 1 pathway B – *Papers IV, V*).

In *Paper IV*, I asked: (1) To what extent do three major compartments of tundra ecosystems (i.e. vascular plants, mosses, and soil) differ in their C, N, and C-to-N (C:N) ratio responses to goose herbivory and warming? (2) To what extent do chemical responses vary between habitats? (3) To what extent do chemical responses vary between years? (Table 1a,b – *Paper IV*)

In *Paper V*, I asked: (1) To what extent do goose herbivory and warming affect plant-community N and P contents and N and P pools in different habitats? (2) Do PFTs show differential nutrient responses? (Table 1a,b – *Paper V*). Here, I limited my investigation to the second year of the experiment (2017).

Short-term tundra plant-community nutrient-level responses to goose herbivory and warming were investigated across different habitats on the archipelago of Svalbard, in the European high-Arctic (see Section '2.1.2 – Study systems: High-Arctic Svalbard'). For four main reasons the Svalbard tundra is a suitable system to test the effects of goose herbivory and higher temperatures on plant-community nutrient levels in the short-term. First, migratory goose populations have dramatically increased in size over the past few decades in many Arctic regions (Fox and Madsen 2017), suggesting a substantial increase in the potential for disturbance of the tundra (Jefferies and Rockwell 2002, Speed et al. 2009). For instance, the Svalbard population of pink-footed geese has increased from 15000 individuals in 1965 up to 90000 individuals in 2017 (Madsen et al. 2017), and a further expansion is predicted under a warmer climate scenario (Jensen et al. 2008, Wisz et al. 2008). Second, the Svalbard region has experienced one of the highest rates of warming in Arctic land areas and projections indicate a further increase up to 6-8 ˚C by 2100 (Førland et al. 2011). Thus, from an environmental management point of view, understanding the extent to which goose herbivory and warming are affecting plant-community nutrient levels in Svalbard may help predicting imminent changes in ecosystem functioning (Ravolainen et al. 2020). Third, geese disturb both vegetation and soil (Srivastava and Jefferies 1996, Jefferies and Rockwell 2002, Van der Wal et al. 2007), and thus may be particularly important drivers of the biogeochemistry of Arctic ecosystems (*IV*). Finally, geese utilise many habitats on Svalbard (Fox et al. 2006, Speed et al. 2009), which alternate within a few meters across the tundra landscape. Since tundra ecosystem responses to goose herbivory and elevated temperatures are likely to be contingent upon the characteristics of a system (Speed et al. 2010a, Elmendorf et al. 2012a), Svalbard offer a unique opportunity to assess whether plant-community nutrient-level responses to these perturbations vary across habitats (*IV, V*).

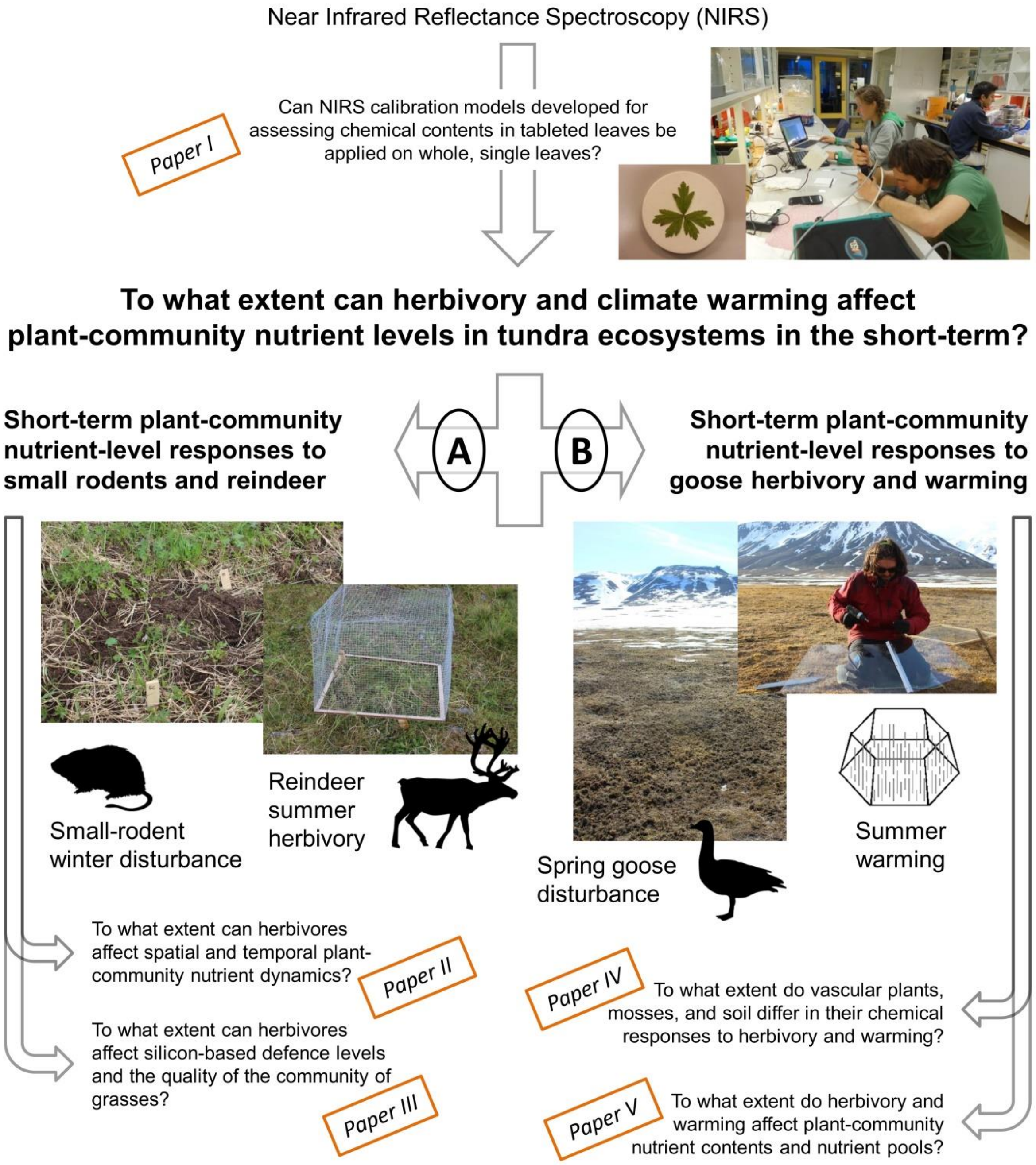


Figure 1. Schematic of the overarching question addressed by this thesis and of the more specific questions addressed by each study. *Silhouettes* presented in this figure for (A) small rodents and reindeer and (B) geese and warming will be consistent throughout the thesis. The *silhouette* utilized to represent 'warming' shows an open-top chamber [OTC] (Molau and Mølgaard 1996, Henry and Molau 1997), a well-established passive warming device that I employed to increase summer temperatures in the high-Arctic Svalbard tundra (see Section '2.2.2 – Study designs: Svalbard studies' for details). The description given for each *silhouette* is referred to consistently in this work (further explanations are given in 'Chapter 2 – Methods'). Figures from top to bottom and left to right: (1) The 'Alligators' team while processing whole, single leaves with NIRS in the former AMB Lab at UiT (photo credits: Kari Anne Bråthen); the inset shows a full leaf of *Trollius europaeus* ready to be scanned with NIRS; (2) Small-rodent winter-disturbed tundra-patch in a tundra-grassland in northern Fennoscandia, Norway; (3) Cages used to exclude reindeer in summer across tundra-grasslands in Fennoscandia; (4) Extensive natural spring goose grubbing as found in many pre-breeding sites in the Svalbard tundra; (5) Preparation of an OTC to be used in Svalbard to increase summer temperatures experienced by growing plants (photo credits: Anton Hochmuth).

Chapter 2

METHODS

2.1 – Study systems

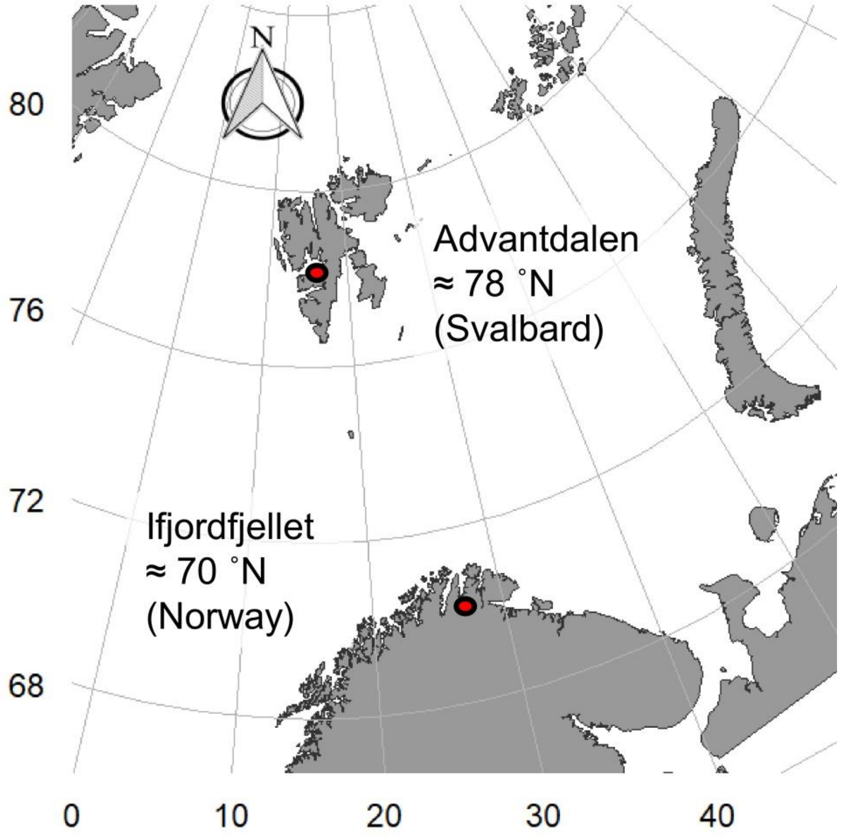


Figure 2. Geographical position of the two study systems used in this thesis. Plant-community nutrient-level responses to small rodents and reindeer (Figure 1; pathway A – *Papers II, III*) were assessed at Ifjordfjellet (70° 27′ N, 27° 08′ E), Finnmark, Northern Norway. Plant-community nutrient-level responses to goose herbivory and warming (Figure 1; pathway B – *Papers IV, V*) were assessed in Adventdalen (78° 10′ N, 16° 05′ E), Svalbard. To extend Arctic-alpine NIRS calibration models to the assessment of elemental contents of single, full leaves (Figure 1; *Paper I*), I utilized leaf samples from both biogeographic regions (see Section '2.3 – Sample collection').

2.1.1 – Sub-Arctic/alpine Finnmark

The extent to which small rodents in winter and reindeer in summer can affect tundra plant-community nutrient levels in the short-term was assessed within tundra-grasslands at Ifjordfjellet in the sub-Arctic/alpine Finnmark (Figure 2). The study was carried out in summer 2015.

The annual temperature of the study area in the warmest month (July) ranges from 8.2 to 13.6 ˚C (30-year period 1986–2015), with a mean of 8.6 ˚C in July 2015. Total annual precipitation for the same 30-year period ranges from 429 to 704 mm, with 564 mm fallen in 2015 (climate data were recorded at Ifjord camping, which is approximately 15 km from the study area; Norwegian Meteorological Institute, http://met.no).

The region is mainly characterized by dwarf-shrub heaths (Walker et al. 2005), whereas grasslands typically dominate river plains. A fence was built in 1950s and divided Ifjordfjellet in spring/fall migratory and summer pasture ranges for semi-domesticated reindeer *Rangifer tarandus tarandus* (Hætta et al. 1994). Historical differences in reindeer herbivory pressure on the two sides of the fence caused a divergence in plant-community composition of grassland communities. Grasslands found in the spring/fall migratory range are forb-dominated, whereas grasslands found in the summer range are grass-dominated. Species composition of the grass-community also largely differs between the two ranges. Whereas Si-poor grasses are the most abundant in migratory-range grasslands, Si-rich grasses dominate summer-range grasslands. Common plant species across these grasslands are: (i) forbs: *Alchemilla* spp., *Bistorta vivipara*, *Geranium sylvaticum*, *Rumex acetosa*, *Solidago virgaurea*, *Trollius* spp., and *Viola* spp.; (ii) Si-poor grasses: *Poa* spp., *Avenella flexuosa*, *Anthoxanthum* spp., *Phleum alpinum*, and *Festuca ovina*; (iii) Si-rich grasses: *Calamagrostis phragmitoides*, *Deschampsia cespitosa*, and *Nardus stricta*.

The semi-domesticated reindeer is the main large herbivorous mammal in the study area. Along with semi-domesticated reindeer, other wild large herbivores occasionally found here are moose *Alces alces*. The community of medium-sized vertebrate herbivores consists of ptarmigan *Lagopus lagopus* and *L. muta* and hare *Lepus timidus*. Three species of small rodents (tundra vole *Microtus oeconomus*, grey-sided vole *Myodes rufocanus*, and Norwegian lemming *Lemmus lemmus*) are active year-round; tundra vole is the species dominating the small-rodent guild in tundra-grasslands (Killengreen et al. 2007, Henden et al. 2011).

2.1.2 – High-Arctic Svalbard

The extent to which spring goose herbivory and summer warming can affect tundra plant-community nutrient levels in the short-term was assessed across different habitats in

Adventdalen, a wide, well-vegetated valley in Svalbard (Figure 2). The study was carried out during summers 2016 and 2017.

The annual temperature of the study area in the warmest month (July) ranges from 5.1 to 9.0 ˚C (30-year period 1988−2017), with a mean of 9.0 ˚C in 2016 and 6.9 ˚C in 2017. Total annual precipitation for the same 30-year period ranges from 176 to 239 mm, with 236 mm and 239 mm fallen in 2016 and 2017, respectively (climate data were recorded at Svalbard airport, which is approximately 10 km from the study area; Norwegian Meteorological Institute, http://met.no). It is worth noting the large differences in climatic conditions between Svalbard and Finnmark (cf. Section '2.1.1 – Study systems: Sub-Arctic/alpine Finnmark'), but also the large differences in July temperatures between the two experimental seasons in Svalbard.

Adventdalen contains a mosaic of different habitats due to fine-scale heterogeneities in topography and hydrological conditions. Habitats are characterized by diverse plant communities, which largely differ in their PFT composition. Three focal habitats distributed along a gradient of soil moisture, namely mesic (ME), moist (MO), and wet (WE) habitats, were selected for the present study. Habitats were selected based on the description of plant communities given by Rønning (1996), but also on descriptions provided by previous studies conducted in Adventdalen and encompassing different plant communities (e.g. Fox et al. 2006, Sjögersten et al. 2006, Speed et al. 2010a). ME-habitats were mainly characterized by rushes (e.g. *Luzula wahlenbergii*), which co-occurred with dwarf-shrubs (*Dryas octopetala* and *Salix polaris*) and grasses (e.g. *Poa arctica*), and mosses covered approx. 70-80% of the surface. MO-habitats were dominated by grasses (e.g. *Alopecurus magellanica*), deciduous dwarf-shrubs (*S. polaris*), and horsetails (*Equisetum arvense*), with mosses covering 100% of the surface. WE-habitats were largely dominated by grasses (e.g. *Dupontia fisheri*) and sedges (*Eriophorum scheuchzeri*) and were characterized by a lush moss-mat covering 100% of the surface. Forbs (e.g. *Bistorta vivipara*) occurred in all habitats, but at low densities.

In a first assessment of the distribution of pink-footed goose disturbance in Svalbard, geese were shown to prefer wetter habitats (Speed et al. 2009). Following the increase in population size, utilization of drier habitats has also increased (Pedersen et al. 2013a, Pedersen et al. 2013b), suggesting that most plant communities in Svalbard are exposed to spring goose disturbance. I decided to focus on mesic heath (ME), moss tundra (MO), and wetland (WE) vegetation since they all represent potential plant communities impacted by pink-footed geese (Fox et al. 2006, Speed et al. 2010a, Speed et al. 2010b).

The Svalbard trophic system is relatively simple (Hansen et al. 2013) and includes only three resident vertebrate herbivores, namely the wild Svalbard reindeer (*Rangifer tarandus platyrhynchus*), Svalbard rock ptarmigan (*Lagopus muta hyperborea*), and sibling vole (*Microtus levis*). The sibling vole is only found in a small bird cliff area (Yoccoz and Ims 1999) and is absent from my study area in Adventdalen. In summer, the Svalbard trophic system becomes more complex due to the arrival of many species of migratory birds. Two migratory goose species, namely the pink-footed goose (*Anser brachyrhynchus*) and barnacle goose (*Branta leucopsis*) heavily utilize Adventdalen as feeding ground during the pre-breeding period (mid May-mid June) (Fox et al. 2006). During this period, pink-footed geese feed almost exclusively on below-ground plant parts (i.e. roots and rhizomes of vascular plants) through grubbing. Conversely, barnacle geese mostly feed by grazing above-ground plant material, and mosses are an important part of their diet (Fox and Bergersen 2005).

In this thesis, I decided to focus on the effects of spring grubbing by pink-footed geese for two main reasons. First, several goose species from the genera *Anser* and *Chen* forage in spring through grubbing, which has been shown to cause severe disturbance to Arctic ecosystems in Svalbard (Van der Wal et al. 2007, Speed et al. 2009, Speed et al. 2010a) and elsewhere (Iacobelli and Jefferies 1991, Jefferies and Rockwell 2002, Jefferies et al. 2006). Since populations of these species are increasing across both the Nearctic and Palearctic (Fox and Madsen 2017), focusing on grubbing as a mechanism of disturbance for Arctic ecosystems may have management implications (Ravolainen et al. 2020) that extend beyond those for the fragile Svalbard tundra (cf. Section ‘1.4 – Aims’). Second, short-term effects of goose grubbing on several ecosystem attributes, such as vegetation structure (Speed et al. 2010a) and soil and vegetation C stocks (Van der Wal et al. 2007, Speed et al. 2010b), have been previously investigated. Yet, the extent to which goose grubbing affects plant-community nutrient levels has not been explored.

2.2 – Study designs

2.2.1 – Finnmark studies

Soon after snowmelt, I selected two grassland sites in forb-dominated grasslands and two grassland sites in grass-dominated grasslands (Figure 3a). Within each grassland site, twelve pairs of 60 × 60 cm plots were established (Figure 3b). Six plot-pairs were located in tundra-patches that showed evident signs of winter disturbance by small rodents (Ro+), whereas the other six plot-pairs were located in undisturbed tundra-patches (Ro−). Within each pair, plots were randomly assigned to be accessible to reindeer (Re+) or to be excluded to reindeer (Re−) (Figure 1c).

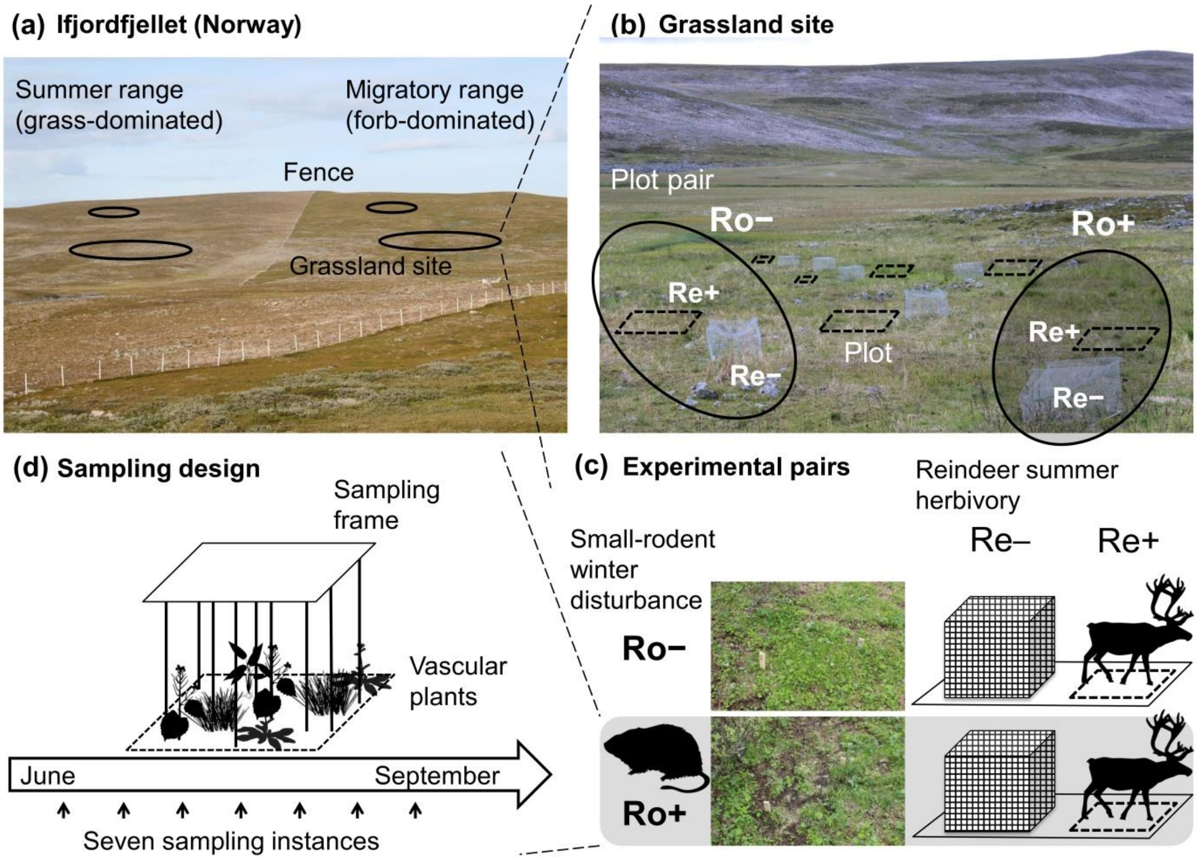


Figure 3. Study and sampling design used to assess the extent to which small rodents and reindeer can affect tundra plant-community nutrient levels in the short-term (Figure 1; pathway A – *Papers II, III*). (a-c) Hierarchical spatial structure of the study design. A full-factorial semi-randomized pair design was implemented in four tundra-grassland sites in which small-rodent winter disturbance was used as a quasi-experimental factor (Shadish et al. 2002) and reindeer summer herbivory as a fully experimental factor. In total, 96 plots were established. (d) Temporal structure of the sampling design. Leaf sampling was performed at seven instances from the start to the end of the growing season (between 28th of June and 10th of September, on average every 12 ± 1.3 days).

I used cages (70 × 70 cm area × 50 cm height) made of metal net to exclude reindeer herbivory in Re− plots throughout the summer. Since small rodents had free access to Re− plots, their summer effects are assumed to be equal in all plots. The coding for the four different treatment combinations, i.e. Ro−/Re−, Ro−/Re+, Ro+/Re−, and Ro+/Re+, is consistently utilized in *Papers II* and *III*. In this thesis, winter herbivory by small rodents is referred to as 'small-rodent winter disturbance', whereas summer herbivory by reindeer is referred to as 'reindeer summer herbivory'.

2.2.2 – Svalbard studies

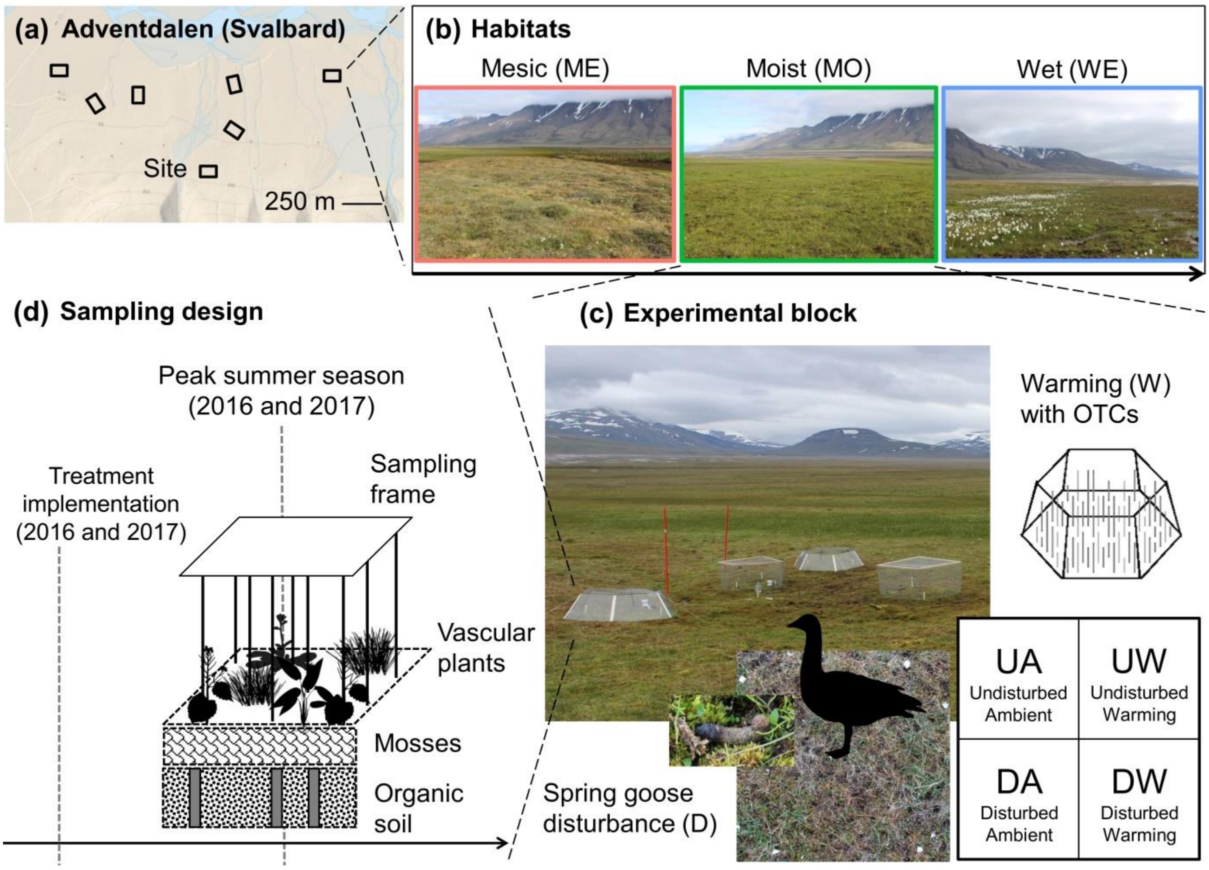


Figure 4. Study and sampling design used to assess the extent to which spring goose herbivory and summer warming can affect tundra plant-community nutrient levels in the short-term (Figure 1; pathway B – *Papers IV, V*). (a-c) Hierarchical spatial structure of the study design. A full-factorial randomized block design was implemented at seven sites, each encompassing the three focal habitats on a gradient of soil moisture, in which spring goose disturbance and summer warming were simulated over two growing seasons (2016 and 2017). In total, 84 plots were established. Due to a flooding, one of the selected experimental blocks for WE-habitats was discarded. (d) Temporal structure of the sampling design. Vascular-plant, moss, and soil sampling was performed at the peak of the growing season in both years (2016: between 19th and 29th of July; 2017: between 20th and 28th of July).

Seven replicate sites (Figure 4a), each encompassing the three focal habitats, namely ME-, MO-, and WE-habitats (Figure 4b), were chosen within Adventdalen in summer 2015. In spring 2016, I established an experimental block constituted by four 80 × 80 cm plots in all three habitats. Two treatments (i.e. spring goose grubbing and summer warming) with two levels each (i.e. control and treated) were randomly assigned to the plots in a full-factorial arrangement (Figure 4c), leading to four combinations: (1) UA: undisturbed/ambient [i.e. un-manipulated control plots]; (2) UW: undisturbed/warmed plots; (3) DA: disturbed/ambient plots; (4) DW: disturbed/warmed plots. The same treatments were applied to the same plots in both years. The coding for the four different treatment combinations, i.e. UA, UW, DA, and DW, is consistently utilized in *Papers IV* and *V*. In this thesis, spring grubbing by pink-footed geese is referred to as 'spring goose disturbance', whereas higher summer temperatures are referred to as 'summer warming'. All plots were caged off during summer to avoid natural herbivory as a possible confounding factor.

Spring goose disturbance was applied in spring and was simulated in a regular fashion to approx. 33% of the plot surface by using a sharpened steel tube (20 mm Ø) that was inserted to a depth of about 50 mm and twisted to remove plant and soil material, following Speed et al. (2010a). I then fertilized the same plots by adding 120 g of fresh goose faeces.

Summer warming was implemented throughout the summer by hexagonal open-top chambers (OTCs, 1.4 m Ø) following the International Tundra Experiment (ITEX) protocol (Molau and Mølgaard 1996, Henry and Molau 1997). Across years and habitats, OTCs increased mean (~0.7 ˚C) and maximum (~3.7 ˚C) July air temperature at 5 cm from the surface and mean (~0.8 ˚C) and maximum (~1.4 ˚C) July temperature measured at the moss surface (–2 cm) relative to un-manipulated control (UA) plots.

2.3 – **Sample collection**

To answer my overall research question, I performed highly resolute leaf sampling within my experiments. To study the effects of small-rodent winter disturbance and reindeer summer herbivory on plant-community nutrient dynamics (*II*) and quality of the grass-community (*III*) in tundra-grasslands, I sampled leaves at seven sampling occasions from the start to the end of the growing season (Figure 3d). To study chemical responses of major ecosystem compartments (i.e. vascular plants, mosses, and soil) to spring goose disturbance and summer warming (*IV*) and to what extent they affect plant-community nutrient contents and nutrient pools (*V*), sampling was conducted at the peak of the growing season in both years (Figure 4d). Here, in addition to leaf samples, I also collected moss shoots and samples of organic soil in order to compare their chemical responses to treatments with those of vascular plants (*IV*).

In Finnmark, leaf sampling was conducted randomly at each plot on each sampling occasion by using nine pins attached to a 42 × 46 cm metal frame (Figure 3d). The uppermost plant leaf touching each pin was collected, providing a total of 2831 independent leaf samples from 34 species/genera (mainly forbs and grasses) (*II*). This sampling design allowed me to encompass the whole range of leaf developmental stages, and thus quantify to what extent herbivores affect short-term plant-community nutrient dynamics in these grasslands (*II*). Since another aim of the sampling was to obtain at least one leaf sample for both Si-rich and Si-poor grasses at each plot (*III*), I randomly collected additional samples in the rare cases that the nine pins did not provide a leaf belonging to both groups of grasses. In *Paper III*, a total of 1182 independent leaf samples were used to assess to what extent herbivores affect Si-based defence levels and forage quality (i.e. Si:N and Si:P ratios) of the grass-community found in tundra-grasslands.

In Svalbard, leaf sampling was also conducted randomly at each plot by using a 50 × 50 cm frame based on the same principles of that utilised in Finnmark. Here, however, in order to obtain leaf samples from all the dominant species at each plot regardless of whether they were hit by a pin or not, I collected leaves from the second, third, a.s.o. closest species to a pin if the pin would have provided leaves from a species already sampled under previous pins. For each species identified with this process, I then randomly collected 3-5 leaves starting from the first pin at which the species was registered and continuing with the subsequent pins. Across habitats and years, I collected 2244 independent leaf samples from 14 species that encompassed over 99% of the vascular plant biomass within plots. These leaf samples were used to assess to what extent spring goose disturbance and summer warming affect plant-community nutrient contents (*IV, V*) and nutrient pools (*V*) and how chemical responses of the vascular-plant compartment compare with those of other two important ecosystem compartments, namely mosses and soil (*IV*) (see below and Section '2.4 – Sample processing').

I randomly collected 7-10 moss shoots close to where each of the nine pins used to sample vascular-plant leaves (see above) intercepted the moss layer (Figure 4d). I separated the green, photosynthetically-active part of the shoots from the brown, nearly-decomposed part, and only the former was retained. Given the random collection, I assume the number of moss shoots collected for a given moss species (not identified) to reflect its relative abundance within the plot. Finally, I collected three samples of organic soil from each plot (Figure 4d). By assessing the chemical composition of moss and soil samples (see Section '2.4 – Sample processing'), I was then able to compare short-term chemical responses of vascular plants, mosses, and soil to spring goose disturbance and summer warming (*IV*).

Several leaf samples collected in Finnmark and Svalbard (see above) were also used to extend Arctic-alpine NIRS calibration models developed for merged, milled, and tableted leaves to the assessment of elemental contents of single, full leaves (*I*). Moreover, I collected additional leaves in the mountainous areas surrounding the city of Tromsø in order to span a third biogeographic region, i.e. boreal-alpine. Overall, 1677 leaf samples from different species (high-Arctic Svalbard: 9; sub-Arctic/alpine Finnmark: 16; boreal/alpine Troms: 25) belonging to different PFTs and spanning different vegetation types and phenological stages were used in *Paper I*.

2.4 – **Sample processing**

I processed all leaf samples for their chemical contents (%dw) with NIRS using a FieldSpec 3 (ASD Inc., Boulder, Colorado, USA) in 350–2500 nm range and equipped with a 4 mm light-adapter for full-leaf scanning. Chemical contents for each leaf were initially predicted by applying Arctic-alpine NIRS calibration models developed for merged, milled, and tableted leaves (for leaf Si-content, see Smis et al. 2014, for leaf N-, P-, and C-content, see Murguzur et al. 2019). Depending on my specific questions (see Section '1.4 – Aims' and Table 1a), I retrieved the following chemical variables from each dataset:

- *Paper I* (extension of Arctic-alpine NIRS calibration models for the assessment of elemental contents in single, full leaves): leaf N, P, C, and Si contents;
- *Paper II* (plant-community nutrient dynamics): leaf N and P contents;
- *Paper III* (grass-community forage quality): leaf Si, N, and P contents;

- *Paper IV* (chemical responses of vascular plants – see below for mosses and soil): leaf C and N contents;
- *Paper V* (plant-community nutrient contents and nutrient pools): leaf N and P contents.

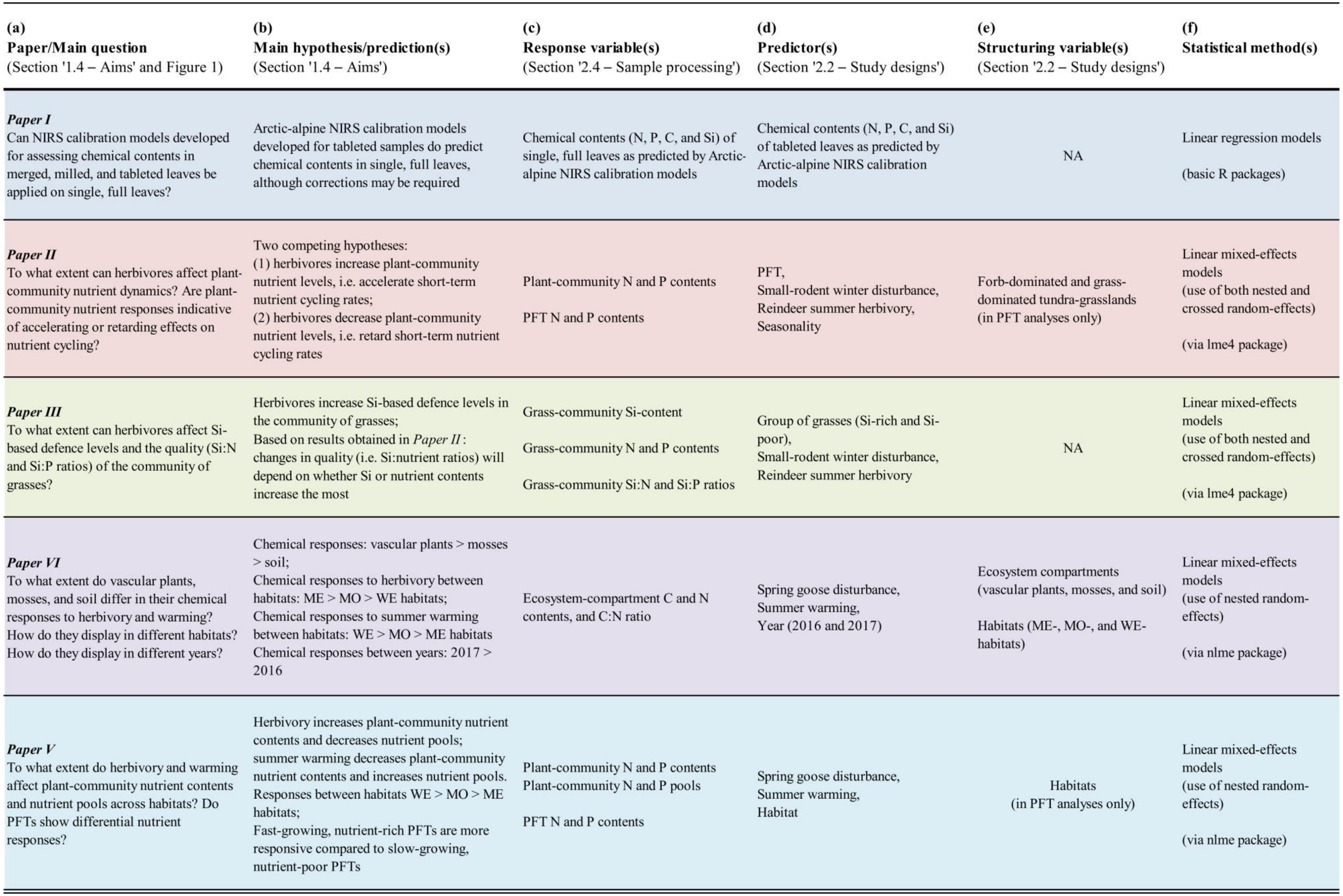
The 1677 leaf samples used to extend Arctic-alpine NIRS calibration models to the assessment of elemental contents of single, full leaves (*I*) were divided in 97 leaf-cohorts, based on biogeographical region, vegetation type, phenological stage, and species. Each leaf-cohort contained enough leaves for making a tablet (approx. 100 mg). I then merged all leaves within a cohort, pressed them into tablets, and processed them for their N, P, C, and Si contents (%dw) using NIRS. Finally, I used the predictions of the chemical contents of the tablets (for which the Arctic-alpine calibration models are developed) as blueprint to which the predicted chemical contents of single, full leaves was compared. I compared the average chemical contents per cohort as predicted from single, full leaves to the chemical contents of the cohort as predicted from its tablet, thus obtaining specific ‘correction factors’ for each element (see Chapter 3 ‘Main results and discussion’ for details). These ‘correction factors’ were applied to the initially predicted chemical values of each single leaf included in the datasets used for *Papers II-V* (see above) in order to obtain actual chemical contents of single, full leaves to be further used in the analyses.

The calculation of plant-community nutrient contents (*IV, V*) and nutrient pools (*V*) in Svalbard was based on the relative and absolute contribution of each species, respectively, to the live-leaf biomass within each plot. To achieve this, I determined relative and absolute live-leaf biomass in each plot by using the point intercept frequency method (PIM - Bråthen and Hagberg 2004). PFT-specific correlation coefficients to switch from number of contacts obtained with PIM for each vascular plant species in a plot and its actual live-leaf biomass (g m^{-2}) were achieved by fitting weighted linear-regression models between PIM and biomass data obtained on 17 plots external to the main experiment and *a priori* selected for destructive harvesting (*IV*), following Bråthen and Hagberg (2004). Finally, I calculated vascular-plant C and N community-weighted contents (and further obtained vascular-plant C:N ratio) (*IV*) and vascular-plant and PFT N and P community-weighted contents and pools (*V*) following Garnier et al. (2004). Hereafter, vascular-plant and PFT nutrient community-weighted contents and pools will be simply referred to as ‘vascular-plant and PFT nutrient contents and nutrient pools’.

Moss and soil samples collected in Svalbard were analysed for their C and N contents (%dw) using a CN analyser (Vario EL Cube, Elemental Analyzer GmbH, Hanau, Germany).

2.5 – **Data analyses**

All statistical analyses were conducted in the R environment version 3.6.1 (https://www.r-project.org). To avoid an overly-complicated description of the statistical analyses performed for each study, I here only report a summary of the response variables (Table 1c), predictors (Table 1d), structuring variables (i.e. variables not directly involved in data analyses, but e.g. used to guide modelling – Table 1e), and statistical methods (Table 1f) used to answer questions asked in *Papers I-V* (Table 1a) and test my specific predictions (Table 1b).



(a) **Paper/Main question** (Section '1.4 – Aims' and Figure 1)	(b) **Main hypothesis/prediction(s)** (Section '1.4 – Aims')	(c) **Response variable(s)** (Section '2.4 – Sample processing')	(d) **Predictor(s)** (Section '2.2 – Study designs')	(e) **Structuring variable(s)** (Section '2.2 – Study designs')	(f) **Statistical method(s)**
Paper I Can NIRS calibration models developed for assessing chemical contents in merged, milled, and tableted leaves be applied on single, full leaves?	Arctic-alpine NIRS calibration models developed for tableted samples do predict chemical contents in single, full leaves, although corrections may be required	Chemical contents (N, P, C, and Si) of single, full leaves as predicted by Arctic-alpine NIRS calibration models	Chemical contents (N, P, C, and Si) of tableted leaves as predicted by Arctic-alpine NIRS calibration models	NA	Linear regression models (basic R packages)
Paper II To what extent can herbivores affect plant-community nutrient dynamics? Are plant-community nutrient responses indicative of accelerating or retarding effects on nutrient cycling?	Two competing hypotheses: (1) herbivores increase plant-community nutrient levels, i.e. accelerate short-term nutrient cycling rates; (2) herbivores decrease plant-community nutrient levels, i.e. retard short-term nutrient cycling rates	Plant-community N and P contents PFT N and P contents	PFT, Small-rodent winter disturbance, Reindeer summer herbivory, Seasonality	Forb-dominated and grass-dominated tundra-grasslands (in PFT analyses only)	Linear mixed-effects models (use of both nested and crossed random-effects) (via lme4 package)
Paper III To what extent can herbivores affect Si-based defence levels and the quality (Si:N and Si:P ratios) of the community of grasses?	Herbivores increase Si-based defence levels in the community of grasses; Based on results obtained in *Paper II* : changes in quality (i.e. Si:nutrient ratios) will depend on whether Si or nutrient contents increase the most	Grass-community Si-content Grass-community N and P contents Grass-community Si:N and Si:P ratios	Group of grasses (Si-rich and Si-poor), Small-rodent winter disturbance, Reindeer summer herbivory	NA	Linear mixed-effects models (use of both nested and crossed random-effects) (via lme4 package)
Paper VI To what extent do vascular plants, mosses, and soil differ in their chemical responses to herbivory and warming? How do they display in different habitats? How do they display in different years?	Chemical responses: vascular plants > mosses > soil; Chemical responses to herbivory between habitats: ME > MO > WE habitats; Chemical responses to summer warming between habitats: WE > MO > ME habitats Chemical responses between years: 2017 > 2016	Ecosystem-compartment C and N contents, and C:N ratio	Spring goose disturbance, Summer warming, Year (2016 and 2017)	Ecosystem compartments (vascular plants, mosses, and soil) Habitats (ME-, MO-, and WE-habitats)	Linear mixed-effects models (use of nested random-effects) (via nlme package)
Paper V To what extent do herbivory and warming affect plant-community nutrient contents and nutrient pools across habitats? Do PFTs show differential nutrient responses?	Herbivory increases plant-community nutrient contents and decreases nutrient pools; summer warming decreases plant-community nutrient contents and increases nutrient pools. Responses between habitats WE > MO > ME habitats; Fast-growing, nutrient-rich PFTs are more responsive compared to slow-growing, nutrient-poor PFTs	Plant-community N and P contents Plant-community N and P pools PFT N and P contents	Spring goose disturbance, Summer warming, Habitat	Habitats (in PFT analyses only)	Linear mixed-effects models (use of nested random-effects) (via nlme package)

Table 1. Overview of the research questions addressed by this thesis, main expectations, and methods utilized to test these predictions. Summary of (a) main questions, (b) specific predictions, (c) response variables, (d) predictors, (e) structuring variables, and (f) statistical methods for *Papers I-V*.

Chapter 3

MAIN RESULTS and DISCUSSION

To what extent can Arctic-alpine NIRS calibration models used to predict chemical contents of merged, milled, and tableted leaves be applied on single, full leaves (Table 1a – *Paper I*)?

I found that the Arctic-alpine calibration models used to assess N (R^2=0.94), P (R^2=0.76), C (R^2=0.82), and Si (R^2=0.90) contents in tableted-samples (Smis et al. 2014, Murguzur et al. 2019) performed well in predicting chemical contents of single, full leaves (N: R^2=0.88; P: R^2=0.65; C: R^2=0.78; Si: R^2=0.67 [Si-model is based on Si-rich PFTs only, namely grasses, horsetails and sedges]) (Figure 5) (Petit Bon et al. 2020a). As expected (Table 1b – *Paper I*), regression lines deviated from an ideal relationship of 1:1 for all chemical variables (Figure 5) (Petit Bon et al. 2020a), indicating that 'correction factors' (i.e. intercept and slope of the relationship) must be used in order to achieve actual chemical contents of single, full leaves (see Section '2.4 – Sample processing' for details on how these 'correction factors' have been used to correct predicted chemical values of each single leaf included in the datasets used for *Papers II-V*).

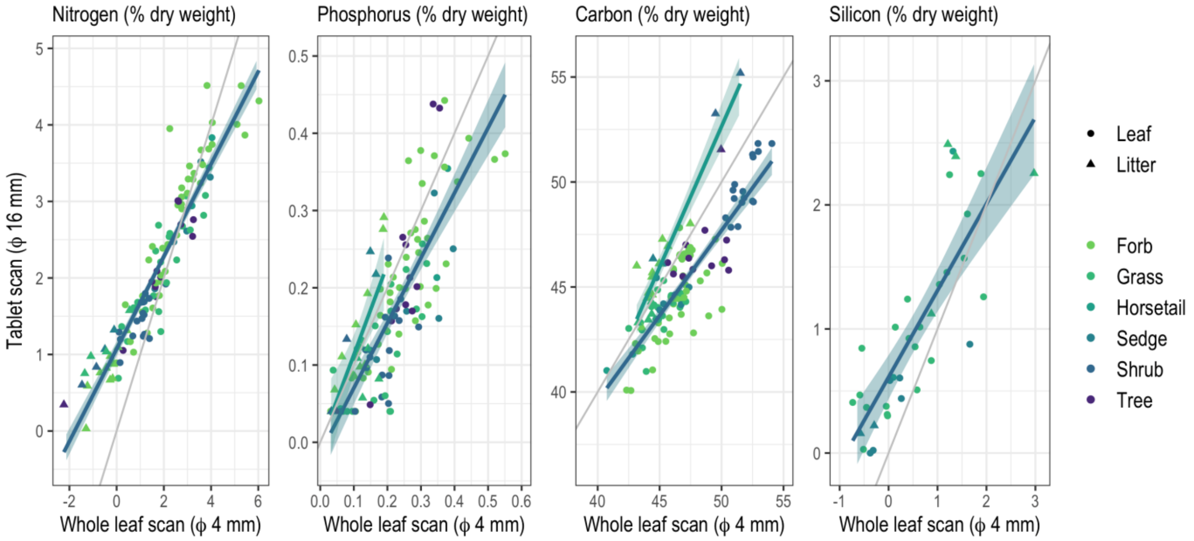


Figure 5. Arctic-alpine NIRS calibration models used to predict chemical contents of tableted leaves can be applied on single, full leaves (main results from *Paper I*). Relationships between Nitrogen (N), Phosphorus (P), Carbon (C), and Silicon (Si) contents of merged, milled, and tableted leaves (*y*-axis) and those of single, full leaves (*x*-axis). The grey line represents the ideal 1:1 relationship. When regression models performed better by separating between fresh leaves and litter, relationships are presented separately (see *Paper I* for more details).

Nutrient-related plant traits are among the plant functional traits (sensu Violle et al. 2007) that show the highest inter- and intra-specific variability (Siefert et al. 2015, Ely et al. 2019), and also the highest intra-specific phenological variation (Fajardo and Siefert 2016). Such strong variability in plant chemical traits characterized plant communities globally (Siefert et al. 2015), but is especially pronounced across the tundra biome (Bjorkman et al. 2018, Thomas et al. 2020).

The importance of accounting for both inter- and intra-specific variation in plant (chemical) traits in order to understand ecosystem processes and trophic interactions has been repeatedly emphasise (Albert et al. 2011, Bolnick et al. 2011). Yet, most methods require merging several leaves together in order to obtain enough plant material for chemical analyses, thus partially and almost totally hampering the assessment of inter- and intra-specific variability, respectively, in plant chemical composition. Moreover, the inclusion of such levels of variation can be even more difficult when working in Arctic and alpine environments, which host plant species with inherently small leaves.

The extension of the Arctic-alpine NIRS calibration models (Smis et al. 2014, Murguzur et al. 2019) provided here can be the tool through which both inter- and intra-specific variation in plant chemical traits can become levels of investigation when studying ecosystem processes and trophic interactions in tundra ecosystems. Furthermore, this novel methodology may help us uncover the still unexplored world of intra-individual variability in foliar chemical composition. Intra-individual trait variation (i.e. variation between e.g. leaves belonging to the same plant individual) can sometimes be larger compared to differences among individuals and populations, as it has been shown for leaf dry matter content of *Vaccinium myrtillus* across an alpine valley (Albert et al. 2010). The Arctic-alpine NIRS calibration models for single, full leaves could be used to test whether the same patterns hold true for a range of fundamental leaf chemical elements.

By developing NIRS methodology for single, full leaves (Petit Bon et al. 2020a), I was able to include inter- and intra-specific variation in leaf N, P, C, and Si contents when assessing short-term plant-community nutrient-level responses to herbivory and summer warming (*Papers II-V*).

Based on current theoretical and empirical evidence, I hypothesised that herbivores could have either positive or negative immediate effects on tundra-grassland plant-community nutrient levels, indicative of either accelerating or retarding short-term effects on nutrient dynamics and nutrient cycling rates (Table 1b – *Paper II*). On the one hand, by returning nutrients through faeces and urine (Bazely and Jefferies 1985) or by keeping leaves in young phenological stages (Chapin 1980), herbivores could enhance plant-community nutrient levels. On the other hand, by removing more nutrient-rich plant species or by selecting more nutritious plant parts (Pastor et al. 1993), herbivores could decrease plant-community nutrient levels.

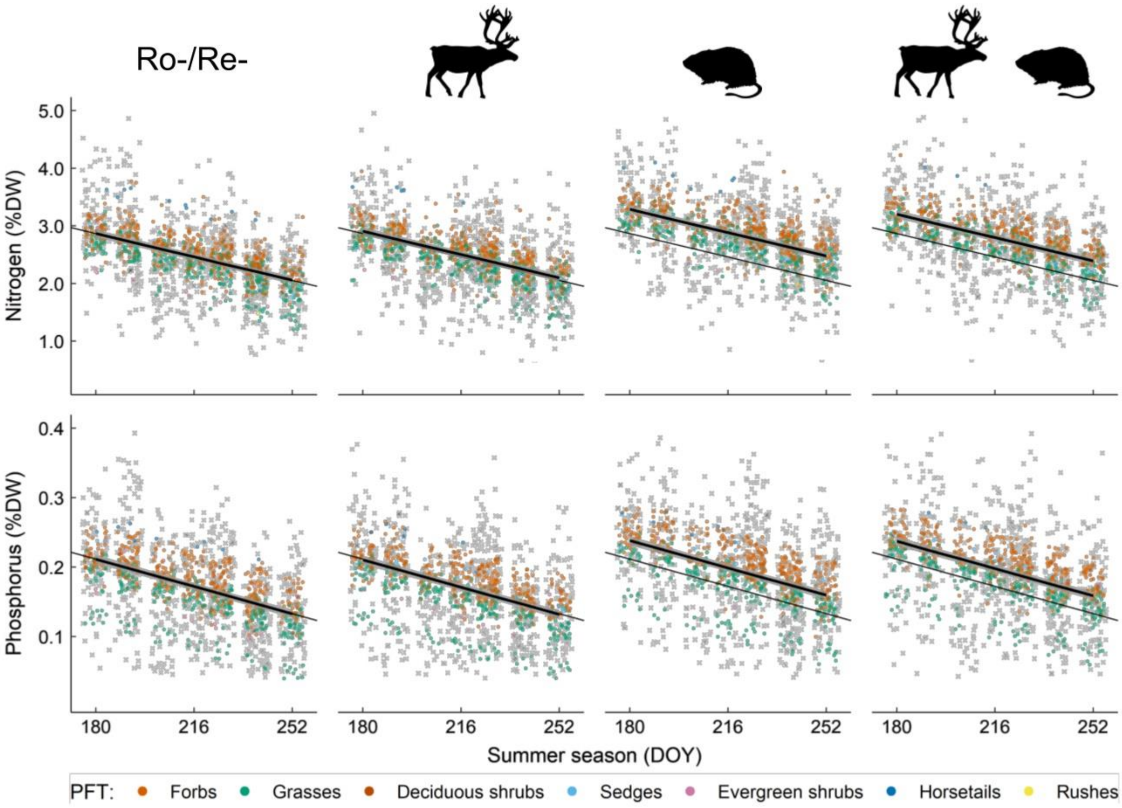


Figure 6. Herbivores increased plant-community nitrogen and phosphorus levels in tundra-grasslands and effects were temporally consistent throughout the growing season (main results from *Paper II*). Effects of small-rodent winter disturbance and reindeer summer herbivory on tundra-grassland plant-community nutrient levels throughout the growing season. Thick lines represent regression lines and bands are their 95% confidence interval (CI). The reference line (thin line) in absence of herbivores is maintained in each panel. Dots (raw and fitted values) were jittered within each of the seven sampling occasion to reduce overlapping.

I found that tundra-grassland communities responded to small-rodent winter disturbance with an overall increase in their N and P levels by up to 16%, whilst reindeer summer herbivory had more marginal effects (Figure 6) (Petit Bon et al. 2020b). These immediate, positive plant-community nutrient responses to herbivores paralleled those of dominant PFTs, namely forbs and grasses (Petit Bon et al. 2020b) (see also *Paper V*). Indeed, small-rodent winter disturbance and reindeer summer herbivory promoted generally higher N and P contents in both forb- and grass-dominated tundra-grassland communities (Petit Bon et al. 2020b).

Interestingly, these positive effects of herbivores were temporally consistent throughout the summer and higher nutrient levels were still detected in senescent leaves at the onset of the winter period (Figure 6) (Petit Bon et al. 2020b).

Herbivores thus acted as positive, short-term modifiers of tundra-grassland plant-community nutrient dynamics, potentially accelerating litter decay rate via change in litter quality (Quested et al. 2003) and enhancing soil process rates and nutrient turnover in tundra-grasslands. Overall, my findings from tundra-grasslands align with theoretical expectations of positive herbivore effects on nutrient cycling in relatively productive ecosystems, when dominant, nutrient-rich PFTs benefit from herbivory (Bardgett and Wardle 2003).

Results from Petit Bon et al. (2020b) also suggest that tundra herbivores, by enhancing plant-community N and P levels, may positively manipulate the immediate quality of their own food supply in tundra-grasslands. Yet, an increase in plant-community nutrient levels does not necessarily translate to higher forage quality, at least not for all food items (i.e. PFTs) available to herbivores.

Grasses, one of the dominant PFTs in my tundra-grasslands and food base for numerous herbivores, are known to employ Si-based defences to fight off herbivory (McNaughton and Tarrants 1983, Vicari and Bazely 1993, Hartley and DeGabriel 2016). Leaf Si-content reduces the palatability of grasses (Massey et al. 2006) and make them more difficult to be digested (Massey and Hartley 2009). Indeed, changes in Si-content in relation to levels of essential nutrients in forage grasses have been shown to be critical for the fitness of herbivores (Massey and Hartley 2006).

Whereas grasses have been shown to be capable of increasing their Si-based defence levels in response to herbivores (Massey et al. 2007, Huitu et al. 2014), evidence for Si changes following herbivory in grass species found in tundra ecosystems is inconsistent (Soininen et al. 2013a). I thus followed up from results obtained in Petit Bon et al. (2020b) to investigate how tundra herbivores affect the quality of their own forage grasses throughout the growing season (Paper *III*). I hypothesised that herbivores would generally increase Si-based defence levels of the community of grasses found in tundra-grasslands (Table 1b – *Paper III*). I expected that changes in the quality of the grass-community (measured as Si:N and Si:P ratios) will depend on the magnitude of positive changes in leaf Si and nutrient levels (Table 1b – *Paper III*).

Changes in Si-based defence levels were much weaker compared to those in nutrient levels, and were only observed in Si-rich, but not Si-poor, grasses (*III*). These findings clearly confirm that, also at the community-level, herbivores have little effects on Si-based defence levels of grasses found in tundra-grasslands (Soininen et al. 2013a). Si:nutrient ratios of grasses affected by herbivores were never above those of grasses in their absence (*III*), indicating that herbivory enhances the overall quality of the grass-community found in these grasslands in the short-term.

In direct opposition with my hypothesis, I found that reindeer summer herbivory alone decreased Si-content in Si-rich grasses by 7% (*III*). Young leaves generally have lower Si-content compared to older leaves (Rafi and Epstein 1999). Thus, my results suggest that reindeer may promote lower leaf Si-content in the community of grasses found in tundra-grasslands by keeping leaves in young phenological stages (Bañuelos and Obeso 2000). Such a reduction in Si-content also lowered leaf Si:N ratio of Si-rich grasses (*III*), indicating that higher forage quality can be achieved through an herbivore-driven reduction in Si-based defence relative to nutrient levels other than changes in C:N ratio, as has long been recognized (Mysterud et al. 2011, Mosbacher et al. 2019, Beard et al. 2019).

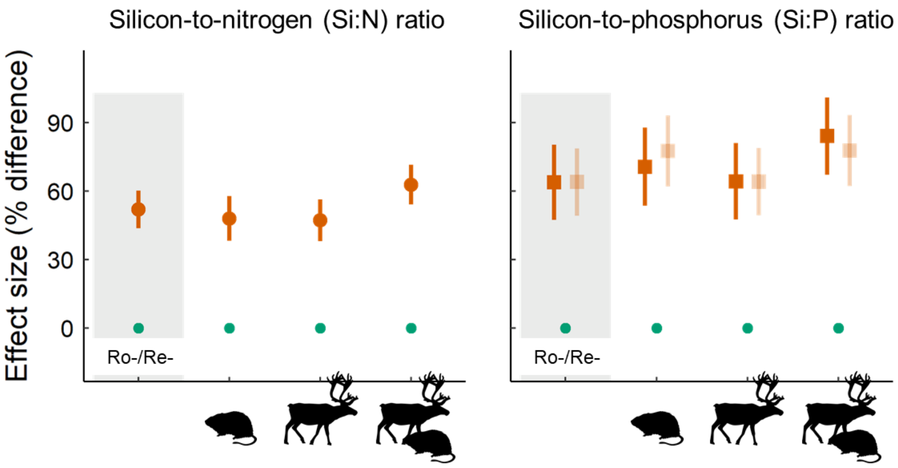


Figure 7. Both herbivores together decreased the quality of Si-rich grasses relative to that of Si-poor grasses (main results from *Paper III*). Contrast between Si-rich (orange) and Si-poor (green) tundra-grasses (effect sizes in % difference and their 95% CI), separately for the four herbivore-treatment combinations. Si-poor tundra-grasses is used as the reference level and is denoted with the dots at zero effect size. For Si:P ratio, effect sizes and their 95% CIs are presented for both full model (bold) and most parsimonious model (shaded) (see *Paper III* for more details).

In contrast with my prediction, I did not observe a net increase in grass Si-based defence levels following herbivory (*III*). Yet, the combined activities of small-rodent winter disturbance and reindeer summer herbivory both offset the decrease in Si-content of Si-rich grasses promoted by reindeer alone (see above) and reduced (for N) and cancelled out (for P) the positive effects of small rodents on their nutrient levels (*III*, see also *Paper II*). Ultimately, both herbivores together increased the quality (i.e. decreased Si:N and Si:P ratios) of Si-poor grasses, but not that of Si-rich grasses (*III*). More importantly, the quality of Si-rich grasses was decreased relative to that of Si-poor grasses, causing the two grass groups to have the largest difference in Si:nutrient ratios when affected by both herbivores (Figure 7). Here, the difference in Si:N and Si:P ratios between Si-rich and Si-poor grasses was increased by 11 and 20%, respectively, compared to their difference in absence of herbivores (Figure 7).

Hence, though herbivores did not cause a net Si accumulation in grass leaves and they generally enhanced the overall quality of the grass-community found in these tundra-grasslands, Si-rich grasses may still have a competitive advantage over Si-poor grasses by maintaining higher Si:nutrient ratios (i.e. lower nutritive quality) when the herbivory pressure is high (Moran and Hamilton 1980). This mechanism, which involved both grass Si and nutrient responses, causes apparent competition among these two groups of grasses (sensu Holt and Bonsall 2017). As such, it could also partly explain why, despite tundra-grasses have inconsistent responses to herbivores in terms of Si changes following herbivory (Soininen et al. 2013a, *Paper III*), Si-rich grasses are often favoured by heavy grazing, whereas Si-poor grasses lose their dominance when the grazing pressure is high (Bråthen and Oksanen 2001, Austrheim et al. 2007, Bråthen et al. 2007, Ravolainen et al. 2011).

Positive short-term effects of herbivores on plant-community nutrient contents in sub-Arctic/alpine tundra-grasslands (Petit Bon et al. 2020b, *Paper III*) were consistent with those of simulated spring goose disturbance on plant-community nutrient contents in the high-Arctic tundra (*Papers IV* and *V*). Here, however, I added two levels of complexity by investigating how summer warming plays out with spring goose disturbance in affecting plant-community nutrient levels and how the effects of these drivers display in different high-Arctic habitats. Within this framework, I investigated the extent to which short-term C, N, and C:N ratio responses of vascular plants compare with those from two other major compartments of ecosystems (i.e. mosses and soil) (*Paper IV*) and to what extent short-term responses in plant-community N and P contents are accompanied by responses in plant-community N and P pools (*Paper V*).

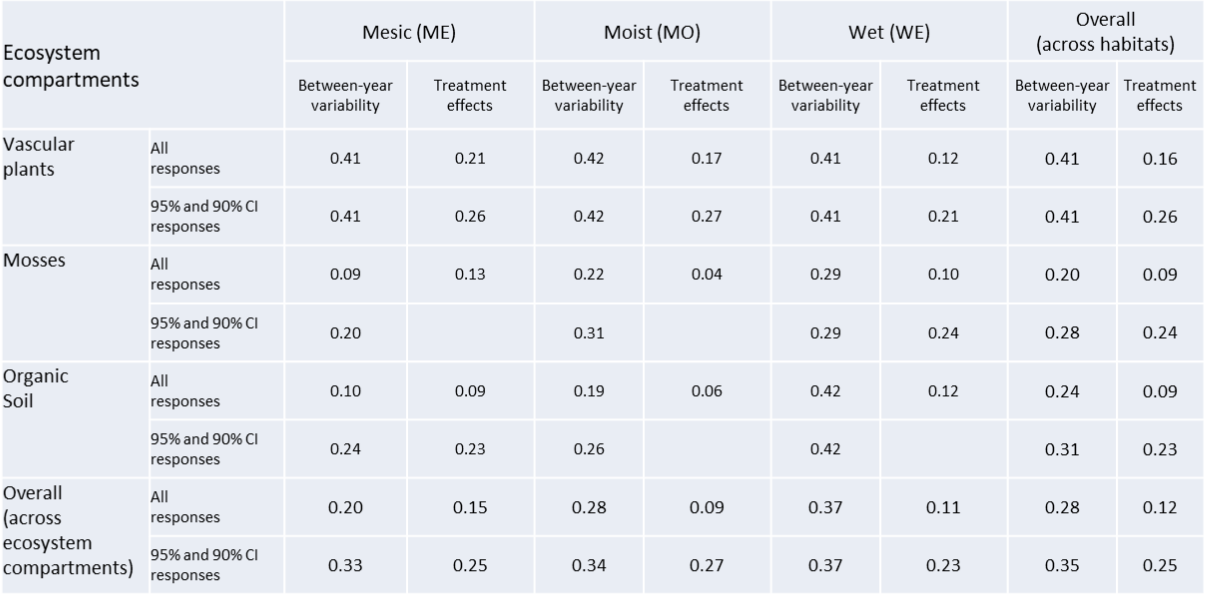
The importance of considering herbivory to understand tundra-ecosystem responses to warming and *vice versa* is widely acknowledged (e.g. Post and Pedersen 2008, Sjögersten et al. 2012, Ylänne et al. 2015). However, whether herbivores can affect plant-community nutrient-level responses to warming (or the other way around) has not been previously tested. It is of note that, in my Svalbard studies, I did not find any signs of interactions between my experimental treatments, across all response and structuring variables that I considered (Table 1b,e – *Papers IV* and *V*). Thus, a key, common finding of my studies from this high-Arctic ecosystem is that spring goose disturbance and summer warming, at least in the short-term, influence plant-community nutrient levels in an additive way. Therefore, in the following discussion, I do refer to the isolated effects of my experimental treatments when specifically addressing short-term effects of either spring goose disturbance or summer warming.

Herbivores that disturb both vegetation and soil, such as grubbing geese (Jefferies and Rockwell 2002), may be expected to be important drivers of C and N contents and C:N ratio of major tundra-ecosystem compartments (i.e. vascular plants, mosses, and soil). Indeed, by causing rapid vegetation loss and disruption of the moss-mat structure (Jefferies et al. 2006, Gornall et al. 2007, Gornall et al. 2009, Speed et al. 2010a), but also by leading to soil erosion (Srivastava and Jefferies 1996, Jefferies and Rockwell 2002), goose grubbing might cause differential chemical responses in vegetation and soil. However, the extent to which these major ecosystem compartments may differ in their capacity to immediately respond to goose disturbance has not been experimentally studied.

There is also ample evidence that these major compartments of tundra ecosystems may differ in their capacity to immediately respond to higher temperatures. For instance, vascular plants have been found to show generally fast chemical responses to elevated summer temperatures in diverse Arctic ecosystems (Tolvanen and Henry 2001, Welker et al. 2005, Doiron et al. 2014), whereas mosses and soil are known to be less responsive. Yet, most studies assessing C, N, and C:N ratio responses of tundra ecosystems to environmental changes have focused on either vegetation or soil compartment.

But do vascular plants, mosses, and soil differ in their chemical responses to perturbations? It is not a trivial question since differential sensitivity and magnitude of vegetation and soil C, N, and C:N ratio responses may have important implications for the role each ecosystem compartment has for the C balance and N cycling within the ecosystem (Hobbie et al. 2002). Uncertainty is added by that tundra ecosystem responses to perturbations are likely to be contingent upon the characteristics of a system (Shaver et al. 2000), and thus chemical responses of vascular plants, mosses, and soil might vary among tundra-habitats.

I hypothesised that C, N, and C:N ratio responses to spring goose disturbance and summer warming would vary among ecosystem compartments, habitats, and following one- or two-year of experimentally-imposed manipulations (Table 1b – *Paper IV*). The aim of the study was to compare short-term responsiveness and strength of the responses rather than their specific directions (i.e. positive or negative).



Ecosystem compartments		Mesic (ME)		Moist (MO)		Wet (WE)		Overall (across habitats)	
		Between-year variability	Treatment effects	Between-year variability	Treatment effects	Between-year variability	Treatment effects	Between-year variability	Treatment effects
Vascular plants	All responses	0.41	0.21	0.42	0.17	0.41	0.12	0.41	0.16
	95% and 90% CI responses	0.41	0.26	0.42	0.27	0.41	0.21	0.41	0.26
Mosses	All responses	0.09	0.13	0.22	0.04	0.29	0.10	0.20	0.09
	95% and 90% CI responses	0.20		0.31		0.29	0.24	0.28	0.24
Organic Soil	All responses	0.10	0.09	0.19	0.06	0.42	0.12	0.24	0.09
	95% and 90% CI responses	0.24	0.23	0.26		0.42		0.31	0.23
Overall (across ecosystem compartments)	All responses	0.20	0.15	0.28	0.09	0.37	0.11	0.28	0.12
	95% and 90% CI responses	0.33	0.25	0.34	0.27	0.37	0.23	0.35	0.25

Table 2. Ecosystem compartments differed in their chemical responses to treatments and responses also differed between habitats, but the between-year variation in ecosystem-compartment chemical composition was greater than responses to my experimental manipulations (main results from *Paper IV*). Average standardized effect sizes for between-year differences in chemical composition of ecosystem compartments and their chemical responses to treatments, sorted according to ecosystem compartments and habitats. Average standardized effect sizes for 'All responses' were calculated by averaging all effects addressed in the study, whereas average standardized effect sizes for '95% and 90% CI responses' were calculated by averaging only significant and close-to-significant effects. Empty cells for '95% and 90% CI responses' indicate that no significant or close-to-significant responses were detected (see *Paper IV* for more details).

The key result was that vascular plants showed higher chemical responsiveness (*IV*) and stronger chemical responses (as defined by their effect sizes) to herbivore disturbance and summer warming compared to mosses and soil, and that these responses were stronger in drier than wetter habitats (Table 2 – 'Treatment effects'). Such heterogeneity in chemical responses among ecosystem compartments indicates that disturbance and warming may have immediate effects on the functioning of tundra ecosystems through promoting e.g. decoupled vegetation-soil chemical responses (Hobbie et al. 2002, Bardgett et al. 2013). However, the degree of such effects will vary among habitats, eventually affecting ecosystem processes across the tundra landscape at different rates. Chemical responses were rather consistent between the two years (*IV*), suggesting that if an ecosystem compartment is prone to respond, this is likely to happen immediately and there is no need of repeated events of a disturbance.

Ecosystem processes in the tundra are highly dependent on stochastic, year-to-year variation in temperature conditions (Jonasson et al. 2001), which is especially pronounced in high-Arctic environments, and Svalbard does not make an exception (Førland et al. 2011, Pelt et al. 2016). The two years encompassed by my study markedly differed in their climatic conditions, and effects of OTCs on summer temperatures were weaker compared to such natural between-year variability (cf. Sections '2.1.2 – Study systems: high-Arctic Svalbard' and '2.2.2 – Study designs: Svalbard studies'). Consequently, strong differences in vascular-plant, moss, and soil C and N contents and C:N ratio between 2016 and 2017 may have been expected. Indeed, the chemical composition of the three ecosystem compartments largely differed between the two years (*IV*). More interestingly, the between-year variation in ecosystem-compartment chemical composition was much higher compared to short-term chemical responses induced by my experimental treatments (Table 2 – 'Between-year variability'). Thus, despite I found mosses and soil to be relatively unresponsive to simulated summer warming (Table 2, *IV*), differences in their chemical composition between the two years suggest that these ecosystem compartments can indeed respond to variations in temperature conditions, although they likely need to be stronger than those elicited by OTCs in my experiment. Furthermore, such strong variability in ecosystem-compartment C and N contents and C:N ratio between an extreme year (2016) and a more average year (2017) clearly indicate that stronger chemical responses should be expected as the intensity of perturbations increases.

Results from *Paper IV* pointed to that the vascular-plant compartment of my focal high-Arctic ecosystem in Svalbard was the most responsive to spring goose disturbance and summer warming in terms of chemical composition (C and N contents and C:N ratio) (Table 2). As the short Arctic summer constrains the time window when herbivores have access to forage plants, changes in nutrient contents of vascular plants, an index of forage quality, may have major feedbacks to herbivores fitness (Doiron et al. 2015).

Yet, above-ground plant biomass in high-Arctic ecosystems is generally very low (Van der Wal and Stien 2014) and thus the amount of nutrients in plants, i.e. available plant nutrient pools, may be as limiting for herbivore fitness as plant nutrient contents. However, plant biomass has often been used as an index of forage quantity for Arctic herbivores (Van der Wal et al. 2000), whereas nutrient pools in plant communities have received little attention.

I thus followed up from results obtained in *Paper IV* to investigate the extent to which spring goose disturbance and summer warming affect tundra plant-community N and P contents and N and P pools following two summers of experimental manipulations (*Paper V*). I hypothesised that goose disturbance will generally increase plant-community nutrient contents and decrease plant-community nutrient pools, whereas warming will have opposite effects (Table 1b – *Paper V*).

Complexity is added by that nutrients for herbivores are distributed spatially in terms of PFTs, which often shift their dominance relations according to environmental factors (Wookey et al. 2009). Indeed, PFTs differ in both primary productivity, thus in their nutrient biomass, and in nutrient contents (Cornelissen et al. 2004, Arndal et al. 2009). If goose disturbance and climate warming can cause stronger nutrient responses in nutrient-rich, herbivore-preferred PFTs as opposed to nutrient-poor, less-palatable PFTs, changes in nutrient available to herbivores are likely to occur. I thus also assessed whether PFTs showed differential short-term nutrient responses to my treatments, predicting that nutrient-rich, fast-responsive PFTs (e.g. forbs and grasses) will show stronger responses compared to nutrient-poor, slow-responsive PFTs (e.g. dwarf-shrubs and rushes) (Table 1b – *Paper V*).

I found that spring goose disturbance and summer warming had opposing effects on plant-community nutrient levels, although the magnitude of nutrient responses differed between habitats. Disturbance increased plant-community nutrient contents in mesic (+14%) and wet (+8%), but not moist, habitats, whilst it decreased plant-community nutrient pools in all habitats (moist: –49% > mesic and wet: –32%) (Figure 8a,b – upper panel). Warming decreased plant-community nutrient contents in moist (–12%) and mesic (–10%), but not wet, habitats, whilst it did not affect plant-community nutrient pools (Figure 8a,b – upper panel).

By affecting plant-community nutrient contents, goose disturbance enhanced and warming decreased the overall nourishing state of these high-Arctic plant communities, thus altering forage quality. My results at the community-level are generally in line with those from previous studies addressing the effects of goose herbivory (Cargill and Jefferies 1984, Hik and Jefferies 1990, Beard et al. 2019) and summer warming (Tolvanen and Henry 2001, Aerts et al. 2009, Doiron et al. 2014) on nutrient contents of single tundra-plant species.

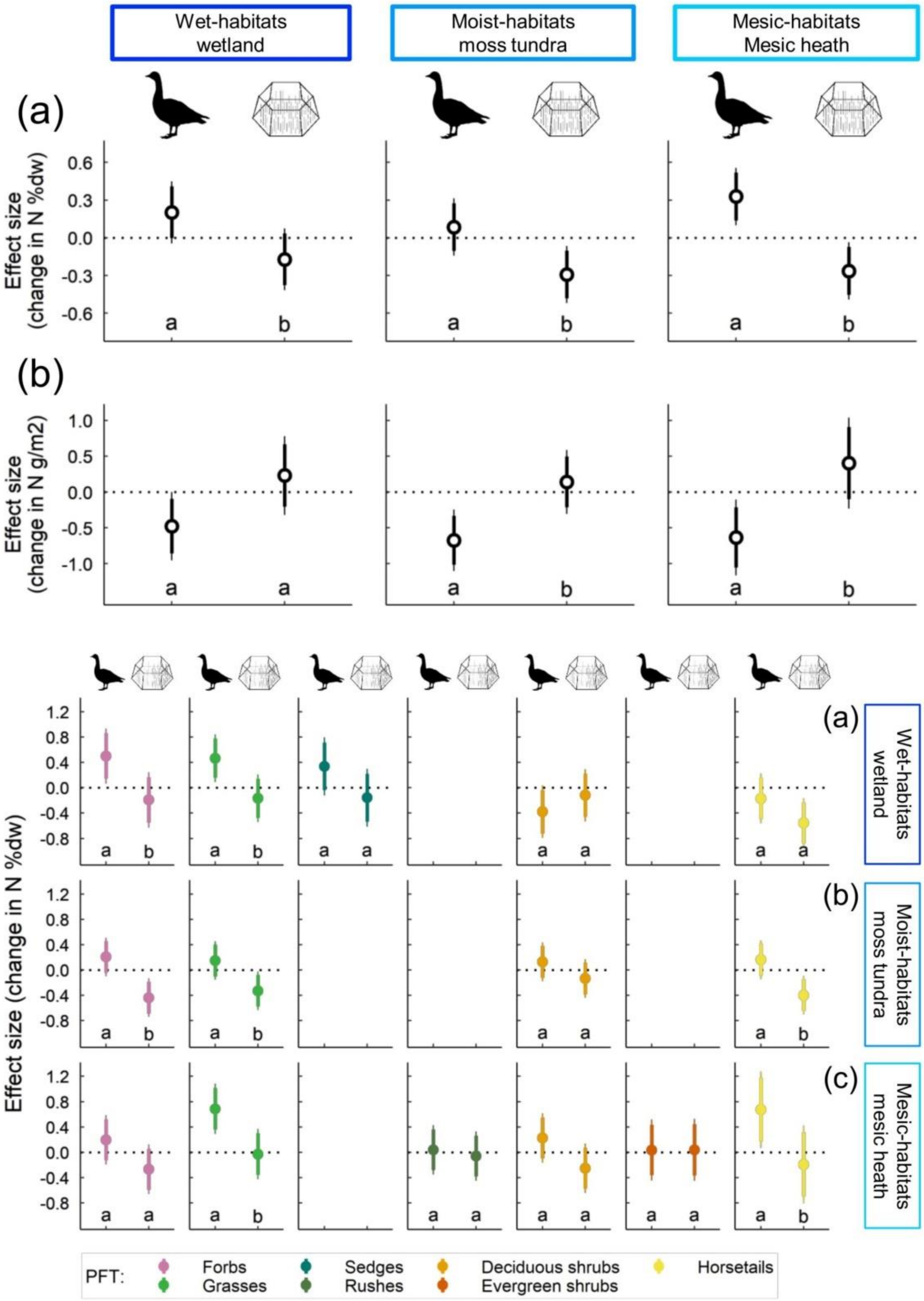


Figure 8. Spring goose disturbance and summer warming had generally opposing effects on plant-community and PFT nutrient contents and pools, and PFTs differed in their responses to treatments (main results from *Paper V*). Effects of spring goose disturbance and summer warming on (upper panel) [a] plant-community N content and [b] plant-community N pool and on (lower panel) PFT N content [a-c]. Treatment-specific effect sizes, their 90% confidence interval [CI] (thick line), and their 95% CI (thin line) are given for the main effects of spring goose disturbance and summer warming as contrasts to un-manipulated control plots, i.e. the reference level denoted with the line at zero effect size. Different letters at the base of each panel indicate that the difference between spring goose disturbance alone and summer warming alone was statistically significant. Results for P are not shown here (see *Paper V* for more details).

I here add to this by showing that immediate changes in plant-community nutrient pools should also be expected: goose disturbance strongly decreased N and P pools in all habitats, suggesting that the increase in plant nutrient contents promoted by geese is unlikely to balance out the loss in total nutrients within the vegetation. Contrary to my general expectation, the increase in plant biomass following higher summer temperatures did not offset the negative effects of warming on plant nutrient contents, thus leaving plant-community nutrient pools seemingly unaltered (Doiron et al. 2014). Habitat-specific nutrient responses of plant communities, as the ones detected here, suggest that goose disturbance and warming may also affect how herbivores utilize the tundra landscape throughout the growing season.

Nutrient-rich PFTs were the most affected by spring goose disturbance and summer warming. Disturbance increased nutrient contents of forbs and grasses (wet habitats) and nutrient contents of grasses and horsetails (mesic habitats) by about 21% (Figure 8a,c – lower panel). Warming decreased nutrient contents of forbs, grasses, and horsetails (moist habitats) and horsetails (wet habitats) by about 13% (Figure 8b,c – lower panel). Nutrient contents of nutrient-poor rushes and evergreen dwarf-shrubs were not affected by treatments.

Species belonging to nutrient-rich PFTs, such as forbs and grasses, generally show faster responses to herbivory (Chapin 1980, Grellmann 2002, Van der Wal et al. 2004) and warming (Chapin et al. 1995, Aerts et al. 2009) compared to species belonging to nutrient-poor PFTs, such as shrubs. My results clearly show that, at the community-level, PFTs manifest differential responses to herbivory and elevated temperatures, and that as predicted they were stronger in nutrient-rich PFTs. Horsetails are usually not included in these comparisons; yet, the fact their responses aligned with those of grasses and forbs is not surprising since they are also highly nutritious PFT (Thomas and Prevett 1982). These findings imply that consequences of plant-community nutrient-level changes may be exacerbated by that both spring goose disturbance and summer warming mainly alter nutrient levels in herbivore forage species.

I simulated spring goose disturbance by mimicking goose grubbing in a regular fashion and by subsequently adding fresh goose faeces. Despite habitats on Svalbard vary in the natural level of goose disturbance they experience (Speed et al. 2009), my experimental set-up allowed me to compare chemical responses of vascular plants, mosses, and soil (*IV*) and changes in plant-community nutrient contents and nutrient pools (*V*) among habitats (cf.

Speed et al. 2010a). My manipulation can be regarded as a fairly realistic disturbance, as opposed to more extreme ones applied by others, such as the complete removal of the moss layer (e.g. Gornall et al. 2007, Gornall et al. 2009, Speed et al. 2010a).

The increase in summer temperatures caused by OTCs was minor compared to future temperature changes expected for Arctic regions in general (Post et al. 2019) and Svalbard in particular (Førland et al. 2011). Furthermore, such temperature increase was lower compared to that observed in several other studies from Arctic/alpine ecosystems that also employed OTCs to increase summer temperatures (cf. Marion et al. 1997, Welker et al. 2005, Carbognani et al. 2018 and Section '2.2.2 – Study designs: Svalbard studies').

In sum, I expect my findings to be rather conservative and I find unlikely that my manipulations induced unrealistic vegetation and soil chemical responses in this high-Arctic ecosystem. Hence, my results indicate that short-term spring goose disturbance and summer warming can influence the immediate C and N contents and C:N ratio of major compartments of tundra ecosystems (*IV*), as well as tundra plant-community N and P levels (*V*). Together with findings obtained in sub-Arctic/alpine tundra-grasslands in norther Fennoscandia (*II*, *III*), this work clearly demonstrates that herbivory and warming are critical short-term modifiers of tundra plant-community nutrient levels.

Chapter 4

CONCLUSIONS

Nutrient levels in plants affect primary productivity of plant communities and their overall nutrient pools, carbon balance and nutrient cycling of ecosystems, and forage quality for herbivores. Insights into the drivers of tundra plant-community nutrient levels are of particular concern given the fast changes Arctic ecosystems are experiencing. However, actual elemental contents in plants are costly measures to attain and seldom reported in ecological studies with sufficient resolution to be useful explanatory variables of underlying ecological processes.

To date, it has been widely acknowledged that herbivores and warming can alter the structure of tundra plant communities in the long-term, thus affecting their chemical composition and eventually ecosystem processes and functions. Yet, the extent to which they also alter the chemistry of tundra plant communities in the short-term had remained relatively unexplored.

By developing NIRS methodology for single, full leaves (*I*), I was able to provide estimates of nitrogen, phosphorus, silicon, and carbon contents at the level of the functional unit of plants (i.e. the leaf), ultimately scaling up to the plant-community level (*II*-*V*). This thesis provides novel evidence that herbivores and climate warming are key, short-term modifiers of the chemical composition of plant communities in tundra ecosystems, and that changes in plant chemistry are happening at a much shorter time-scale than previously revealed. Considerable short-term changes in the chemistry of tundra plant communities following herbivory and summer warming, as those detected in this work, likely have strong implications for the immediate functioning of tundra ecosystems and the trophic interactions established therein.

By showing that herbivores and elevated temperatures can promote immediate changes in tundra-plant community nutrient levels, and that these changes are stronger than previously thought, this thesis may help re-thinking upon the relative importance of bottom-up and top-down mechanisms in regulating natural communities. Although the assessment of whether bottom-up and/or top-down forces shape tundra ecosystems was outside the scope of this thesis, the fact that plant communities quickly responded to perturbations in terms of their chemical composition implies that plants can take an active role in driving population changes and, eventually, community composition at different trophic levels. For instance, by showing short-term nutrient responses to herbivores and warming, plant communities are likely to affect the immediate dynamics of herbivores themselves, such as foraging patterns, habitat utilization, and ultimately herbivore fitness and performances. Thus, I argue that considering plants as an active, and not only passive, player in ecosystems would help us better understand how food webs are regulated.

Chapter 5

FUTURE PERSPECTIVES

The natural step forward of this thesis is to assess the extent to which immediate changes in tundra plant-community nutrient levels following herbivory and warming can transpose to changes in ecosystem processes in the short-term, such as early-stage decomposition rates and instantaneous C dynamics. In this context, NIRS methodology would provide an ideal framework to link changes in the chemical composition of tundra plant communities and further changes at the ecosystem level.

For instance, by using my experimental setup in Svalbard (see Section '2.2.2 – Study designs: Svalbard studies'), I conducted a one-year litter decomposition experiment in 2016 by employing the standard litter-bag method. Early-stage decomposition was assessed for three plant species belonging to three different PFTs, namely *Bistorta vivipara* (forb), *Alopecurus magellanica* (grass), and *Salix polaris* (deciduous dwarf-shrub). Litter decomposition rates are known to vary between PFTs, generally in the order forbs ≥ grasses > deciduous dwarf-shrubs. This experiment will enable me to identify the relative role of PFTs, herbivore disturbance, summer warming, and habitats (and eventually their interactions) in driving litter decomposition in this high-Arctic ecosystem. What about NIRS? I used NIRS methodology to estimate pre-incubation N and P contents of plant material used within the experiment, separately for each litter bag. These additional measurements will allow me to test whether and to what extent the actual N and P contents of plant litter is a predictor of litter decomposition rates. Moreover, by considering the specific nutrient contents of plant material within each litter bag, I will be able to establish whether intra-PFT variation in litter chemistry can explain a significant part of the variability observed in litter mass loss. Finally, upon the extension of Arctic-alpine NIRS calibration models for predicting chemical contents of plant litter *after* early-stage decomposition, it might be possible to assess nutrient leaching by e.g. comparing pre- and post-incubation nutrient levels of the plant material utilized within the experiment.

Within my experimental setup in Svalbard, I also measured instantaneous CO_2 fluxes between ecosystem and atmosphere throughout two growing seasons. Ecosystem CO_2 fluxes were measured with a custom-built chamber attached to an Infra-red gas analyser, and measurements were taken within the same spots at each plot (i.e. subplots) that were also used to estimate plant live-leaf biomass (see Section '2.4 – Sample processing'). Both net ecosystem exchange and ecosystem respiration were measured, and gross ecosystem photosynthesis was obtained by subtracting ecosystem respiration fluxes from net ecosystem exchange fluxes. What about NIRS? I used NIRS methodology to estimate community-weighted N and P contents and pools, both at the level of the entire plant-community and at the level of specific PFTs (*V*). The combination of CO_2 flux data and community-weighted chemistry data could be used to assess the extent to which nutrient-related plant traits influence instantaneous C exchange rates between ecosystem and atmosphere, and whether established relationships vary under herbivore disturbance, summer warming, and across different habitats.

Higher temperatures throughout the growing season are only one side of the many ways global climate changes may affect short-term plant-community nutrient levels across tundra ecosystems. For instance, the rate of winter warming that Svalbard has experienced in the last decades is almost double compared to that of summer warming. One of the most prominent consequences of warming outside the growing season is that the long Arctic winter is interrupted by more and more frequent warm spells, during which temperatures rise well above 0 °C. During such warm periods, which can last from a few hours to several days, precipitations fall in the form of rain instead of snow. For this reason, these phenomena are identified with the self-explanatory term of 'rain-on-snow' (ROS) events. The rain percolates through the snowpack and often reaches the frozen soil before forming a thick layer of ground-ice. Whereas ROS effects on herbivore population dynamics have been subject of investigation, little is known about their potential consequences on high-Arctic vegetation. ROS events are likely to affect short-term plant-community nutrient dynamics by influencing the time the vegetation becomes snow-free in spring, thus the phenological development of plants, but also by changing patterns of C and nutrient allocation within plants. In Svalbard, a field-experiment aiming to assess the extent to which experimentally-formed ground-ice can affect tundra vegetation has been carried out since I started my PhD-project in 2015. NIRS methodology would offer a great opportunity to investigate the understudied aspect of winter warming effects on plant-community nutrient dynamics.

Finally, although my thesis focused on the extent to which herbivores and summer warming can cause immediate changes in tundra plant-community nutrient levels, this does not imply that plant chemical responses to the same drivers cannot happen over longer time scales. In summer 2018, I conducted a sampling campaign within long-term exclosures in Svalbard in order to assess the extent to which long-term exclusion of herbivores can affect plant nutrient contents. Here, the combination of plant-community nutrient data and plant-community compositional data could also lead to a better understanding of how long-term herbivory-driven changes in plant nutrient levels relate to changes in the functional composition of the vegetation brought about by herbivores across high-Arctic plant communities.

This is what I hope to continue with…

REFERENCES

Aerts, R. and Chapin, F. S. 1999. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. – Adv. Ecol. Res. 30: 1-67

Aerts, R. et al. 2007. Climate change has only a minor impact on nutrient resorption parameters in a high-latitude peatland. – Oecologia 151: 132-139.

Aerts, R. et al. 2009. Seasonal climate manipulations result in species-specific changes in leaf nutrient levels and isotopic composition in a sub-arctic bog. – Funct. Ecol. 23: 680-688.

Albert, C. H. et al. 2011. When and how should intraspecific variability be considered in trait-based plant ecology? – Perspect. Plant Ecol. Evol. Syst. 13: 217-225.

Albert, C. H. et al. 2010. Intraspecific functional variability: extent, structure and sources of variation. – J. Ecol. 98: 604-613.

Albon, S. D. and Langvatn, R. 1992. Plant phenology and the benefits of migration in a temperate ungulate. – Oikos 65: 502-513.

Anderson, T. M. et al. 2010. Landscape-scale analyses suggest both nutrient and antipredator advantages to Serengeti herbivore hotspots. – Ecology 91: 1519-1529.

Arndal, M. F. et al. 2009. Seasonal variation in gross ecosystem production, plant biomass, and carbon and nitrogen pools in five high Arctic vegetation types. – Arct. Antarct. Alp. Res. 41: 164-173.

Austrheim, G. et al. 2007. Interactions between sheep, rodents, graminoids, and bryophytes in an oceanic alpine ecosystem of low productivity. – Ecoscience 14: 178-187.

Bañuelos, M. J. and Obeso, J. R. 2000. Effect of grazing history, experimental defoliation, and genotype on patterns of silicification in *Agrostis tenuis* Sibth. – Ecoscience 7: 45-50.

Bardgett, R. D. and Wardle, D. A. 2003. Herbivore-mediated linkages between aboveground and belowground communities. – Ecology 84: 2258-2268.

Bardgett, R. D. et al. 2013. Hierarchical responses of plant–soil interactions to climate change: consequences for the global carbon cycle. – J. Ecol. 101: 334-343.

Barrio, I. C. et al. 2016. Biotic interactions mediate patterns of herbivore diversity in the Arctic. – Glob. Ecol. Biogeog. 25: 1108-1118.

Barthelemy, H. et al. 2015. Strong responses of subarctic plant communities to long-term reindeer feces manipulation. – Ecosystems 18: 740-751.

Bazely, D. R. and Jefferies, R. L. 1985. Goose faeces: a source of nitrogen for plant growth in a grazed salt marsh. – J. Appl. Ecol. 22: 693-703.

Beard, K. H. et al. 2019. Migratory goose arrival time plays a larger role in influencing forage quality than advancing springs in an Arctic coastal wetland. – PloS One 14: e0213037.

Bernes, C. et al. 2015. What are the impacts of reindeer/caribou (*Rangifer tarandus* L.) on arctic and alpine vegetation? A systematic review. – Environ. Evid. 4: 4.

Bjorkman, A. D. et al. 2018. Plant functional trait change across a warming tundra biome. – Nature 562: 57-62.

Bolnick, D. I. et al. 2011. Why intraspecific trait variation matters in community ecology. – Trends Ecol. Evol. 26: 183-192.

Bråthen, K. A. and Odasz-Albrigtsen, A. M. 2000. Tolerance of the arctic graminoid Luzula arcuata ssp. confusa to simulated grazing in two nitrogen environments. – Can. J. Bot. 78: 1108-1113.

Bråthen, K. A. and Oksanen, J. 2001. Reindeer reduce biomass of preferred plant species. – J. Veg. Sci. 12: 473-480.

Bråthen, K. A. and Hagberg, O. 2004. More efficient estimation of plant biomass. – J. Veg. Sci. 15: 653-660.

Bråthen, K. A. et al. 2007. Induced shift in ecosystem productivity? Extensive scale effects of abundant large herbivores. – Ecosystems 10: 773-789.

Carbognani, M. et al. 2018. Different temperature perception in high-elevation plants: new insight into phenological development and implications for climate change in the alpine tundra. – Oikos 127: 1014-1023.

Cargill, S. M. and Jefferies, R. L. 1984. The effects of grazing by lesser snow geese on the vegetation of a sub-Arctic salt marsh. – J. Appl. Ecol. 21: 669-686.

Chapin, F. S. 1980. Nutrient allocation and responses to defoliation in tundra plants. – Arct. Alp. Res. 12: 553-563.

Chapin, F. S. et al. 1995. Responses of arctic tundra to experimental and observed changes in climate. – Ecology 76: 694-711.

Chapin, F. S. et al. 2009. The changing global carbon cycle: linking plant–soil carbon dynamics to global consequences. – J. Ecol. 97: 840-850.

Chodak, M. 2008. Application of near infrared spectroscopy for analysis of soils, litter and plant materials. – Pol. J. Environ. Stud. 17: 631-642.

Cornelissen, J. H. C. et al. 2004. Leaf digestibility and litter decomposability are related in a wide range of subarctic plant species and types. – Funct. Ecol. 18: 779-786.

Cornelissen, J. H. C. et al. 2001. Global change and arctic ecosystems: is lichen decline a function of increases in vascular plant biomass? – J. Ecol. 89: 984-994.

Cornelissen, J. H. C. et al. 2007. Global negative vegetation feedback to climate warming responses of leaf litter decomposition rates in cold biomes. – Ecol. Lett. 10: 619-627.

De Deyn, G. B. et al. 2008. Plant functional traits and soil carbon sequestration in contrasting biomes. – Ecol. Lett. 11: 516-531.

den Herder, M. et al. 2008. Reindeer herbivory reduces willow growth and grouse forage in a forest-tundra ecotone. – Basic Appl. Ecol. 9: 324-331.

Doiron, M. et al. 2014. Effects of experimental warming on nitrogen concentration and biomass of forage plants for an arctic herbivore. – J. Ecol. 102: 508-517.

Doiron, M. et al. 2015. Trophic mismatch and its effects on the growth of young in an Arctic herbivore. – Glob. Change Biol. 21: 4364-4376.

Dormann, C. F. and Woodin, S. J. 2002. Climate change in the Arctic: using plant functional types in a meta-analysis of field experiments. – Funct. Ecol. 16: 4-17.

Egelkraut, D. et al. 2018a. The role of plant–soil feedbacks in stabilizing a reindeer-induced vegetation shift in subarctic tundra. – Funct. Ecol. 32: 1959-1971.

Egelkraut, D. et al. 2018b. Multiple feedbacks contribute to a centennial legacy of reindeer on tundra vegetation. – Ecosystems 21: 1545-1563.

Elmendorf, S. C. et al. 2012a. Global assessment of experimental climate warming on tundra vegetation: heterogeneity over space and time. – Ecol. Lett. 15: 164-175.

Elmendorf, S. C. et al. 2012b. Plot-scale evidence of tundra vegetation change and links to recent summer warming. – Nat. Clim. Change 2: 453-457.

Elser, J. J. et al. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. – Ecol. Lett. 10: 1135-1142.

Ely, K. S. et al. 2019. Spectroscopy can predict key leaf traits associated with source–sink balance and carbon–nitrogen status. – J. Exp. Bot. 70: 1789-1799.

Epstein, E. 2009. Silicon: its manifold roles in plants. – Ann. Appl. Biol. 155: 155-160.

Fajardo, A. and Siefert, A. 2016. Phenological variation of leaf functional traits within species. – Oecologia 180: 951-959.

Foley, W. J. et al. 1998. Ecological applications of near infrared reflectance spectroscopy – a tool for rapid, cost-effective prediction of the composition of plant and animal tissues and aspects of animal performance. – Oecologia 116: 293-305.

Førland, E. J. et al. 2011. Temperature and precipitation development at Svalbard 1900-2100. – Adv. Meteorol. 2011: 1-14.

Fox, A. et al. 2006. Diet and habitat use of Svalbard Pink-footed Geese *Anser brachyrhynchus* during arrival and pre-breeding periods in Adventdalen. – Ardea 94: 691-699.

Fox, A. D. and Bergersen, E. 2005. Lack of competition between barnacle geese *Branta leucopsis* and pink-footed geese *Anser brachyrhynchus* during the pre-breeding period in Svalbard. – J. Avian Biol. 36: 173-178.

Fox, A. D. and Madsen, J. 2017. Threatened species to super-abundance: The unexpected international implications of successful goose conservation. – Ambio 46: 179-187.

Freschet, G. T. et al. 2014. Aboveground and belowground legacies of native Sami land use on boreal forest in northern Sweden 100 years after abandonment. – Ecology 95: 963-977.

Garnier, E. et al. 2004. Plant functional markers capture ecosystem properties during secondary succession. – Ecology 85: 2630-2637.

Gornall, J. et al. 2009. Herbivore impacts to the moss layer determine tundra ecosystem response to grazing and warming. – Oecologia 161: 747-758.

Gornall, J. L. et al. 2007. Arctic mosses govern below-ground environment and ecosystem processes. – Oecologia 153: 931-941.

Grellmann, D. 2002. Plant responses to fertilization and exclusion of grazers on an arctic tundra heath. – Oikos 98: 190-204.

Grime, J. P. et al. 1996. Evidence of a causal connection between anti-herbivore defence and the decomposition rate of leaves. – Oikos 77: 489-494.

Hætta, J. I. et al. 1994. Reindriften i Finnmark: Lovgivning og Distriktsinndeling – Reindriftsadministrasjonen, Alta (in Norwegian).

Hambäck, P. A. et al. 1998. Winter herbivory by voles during a population peak: the relative importance of local factors and landscape pattern. – J. Anim. Ecol. 67: 544-553.

Hanley, T. A. 1997. A nutritional view of understanding and complexity in the problem of diet selection by deer (Cervidae). – Oikos 79: 209-218.

Hansen, B. B. et al. 2013. Climate events synchronize the dynamics of a resident vertebrate community in the high Arctic. – Science 339: 313-315.

Hartley, S. E. and DeGabriel, J. L. 2016. The ecology of herbivore-induced silicon defences in grasses. – Funct. Ecol. 30: 1311-1322.

Henden, J.-A. et al. 2011. Population dynamics of tundra voles in relation to configuration of willow thickets in southern arctic tundra. – Polar Biol. 34: 533-540.

Henry, G. H. R. and Molau, U. 1997. Tundra plants and climate change: the International Tundra Experiment (ITEX). – Glob. Change Biol. 3: 1-9.

Hik, D. S. and Jefferies, R. L. 1990. Increases in the net above-ground primary production of a salt-marsh forage grass: a test of the predictions of the herbivore-optimization model. – J. Ecol. 78: 180-195.

Hobbie, S. E. 1992. Effects of plant species on nutrient cycling. – Trends Ecol. Evol. 7: 336-339.

Hobbie, S. E. et al. 2002. A synthesis: The role of nutrients as constraints on carbon balances in boreal and arctic regions. – Plant Soil 242: 163-170.

Holt, R. D. and Bonsall, M. B. 2017. Apparent competition. – Annu. Rev. Ecol. Evol. Syst. 48: 447-471.

Huitu, O. et al. 2014. Silicon, endophytes and secondary metabolites as grass defenses against mammalian herbivores. – Front. Plant Sci. 5: 478.

Iacobelli, A. and Jefferies, R. L. 1991. Inverse salinity gradients in coastal marshes and the death of stands of Salix: the effects of grubbing by geese. – J. Ecol. 1: 61-73.

Ims, R. A. and Fuglei, E. 2005. Trophic interaction cycles in tundra ecosystems and the impact of climate change. – BioScience 55: 311-322.

Iversen, M. et al. 2014. Phenology and cover of plant growth forms predict herbivore habitat selection in a high latitude ecosystem. – PLoS one 9: e100780.

Jefferies, R. et al. 1994. Vertebrate herbivores and northern plant communities: reciprocal influences and responses. – Oikos 71: 193-206.

Jefferies, R. L. and Rockwell, R. F. 2002. Foraging geese, vegetation loss and soil degradation in an Arctic salt marsh. – Appl. Veg. Sci. 5: 7-16.

Jefferies, R. L. et al. 2006. A biotic agent promotes large-scale catastrophic change in the coastal marshes of Hudson Bay. – J. Ecol. 94: 234-242.

Jensen, R. A. et al. 2008. Prediction of the distribution of Arctic-nesting pink-footed geese under a warmer climate scenario. – Glob. Change Biol. 14: 1-10.

Jonasson, S. et al. 2001. Biogeochemistry in the Arctic: patterns, processes, and controls. – In: Ernst-Detlef, S. et al. (eds), Global biogeochemical cycles in the climate system. Elsevier, pp. 139-150.

Killengreen, S. T. et al. 2007. Structural characteristics of a low Arctic tundra ecosystem and the retreat of the Arctic fox. – Biol. Conserv. 135: 459-472.

LeBauer, D. S. and Treseder, K. K. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. – Ecology 89: 371-379.

Madsen, J. et al. 2017. Svalbard Pink-footed Goose. – Population Status Report 2016-2017.

Marion, G. M. et al. 1997. Open-top designs for manipulating field temperature in high-latitude ecosystems. – Glob. Change Biol. 3: 20-32.

Massey, F. P. and Hartley, S. E. 2006. Experimental demonstration of the antiherbivore effects of silica in grasses: impacts on foliage digestibility and vole growth rates. – Proc. R. Soc. B 273: 2299-2304.

Massey, F. P. and Hartley, S. E. 2009. Physical defences wear you down: progressive and irreversible impacts of silica on insect herbivores. – J. Anim. Ecol. 78: 281-291.

Massey, F. P. et al. 2006. Silica in grasses as a defence against insect herbivores: contrasting effects on folivores and a phloem feeder. – J. Anim. Ecol. 75: 595-603.

Massey, F. P. et al. 2007. Herbivore specific induction of silica-based plant defences. – Oecologia 152: 677-683.

McNaughton, S. and Tarrants, J. 1983. Grass leaf silicification: natural selection for an inducible defense against herbivores. – Proc. Natl. Acad. Sci. 80: 790-791.

Metcalfe, D. B. and Olofsson, J. 2015. Distinct impacts of different mammalian herbivore assemblages on arctic tundra CO2 exchange during the peak of the growing season. – Oikos 124: 1632-1638.

Michelsen, A. et al. 2012. Two decades of experimental manipulations of heaths and forest understory in the subarctic. – Ambio 41: 218-230.

Molau, U. and Mølgaard, P. 1996. International tundra experiment (ITEX) manual. – Danish Polar Center, Copenhagen, Denmark.

Moran, N. and Hamilton, W. 1980. Low nutritive quality as defense against herbivores. – J. Theor. Biol. 86: 247-254.

Moreira, X. et al. 2018. Latitudinal variation in plant chemical defences drives latitudinal patterns of leaf herbivory. – Ecography 41: 1124-1134.

Mosbacher, J. B. et al. 2019. Muskoxen modify plant abundance, phenology, and nitrogen dynamics in a High Arctic fen. – Ecosystems 22: 1095-1107.

Mulder, C. P. H. 1999. Vertebrate herbivores and plants in the Arctic and subarctic: effects on individuals, populations, communities and ecosystems. – Perspect. Plant Ecol. Evol. Syst. 2: 29-55.

Murguzur, F. J. A. et al. 2019. Towards a global arctic-alpine model for Near-infrared reflectance spectroscopy (NIRS) predictions of foliar nitrogen, phosphorus and carbon content. – Sci. Rep. 9: 8259.

Myers-Smith, I. H. et al. 2011. Shrub expansion in tundra ecosystems: dynamics, impacts and research priorities. – Environ. Res. Lett. 6: 045509.

Myers-Smith, I. H. et al. 2020. Complexity revealed in the greening of the Arctic. – Nat. Clim. Change 10: 106-117.

Mysterud, A. et al. 2001. Plant phenology, migration and geographical variation in body weight of a large herbivore: the effect of a variable topography. – J. Anim. Ecol. 70: 915-923.

Mysterud, A. et al. 2011. Plant quality, seasonality and sheep grazing in an alpine ecosystem. – Basic Appl. Ecol. 12: 195-206.

Nadelhoffer, K. J. et al. 1991. Effects of temperature and substrate quality on element mineralization in six Arctic soils. – Ecology 72: 242-253.

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

Olofsson, J. et al. 2004. Reindeer influence on ecosystem processes in the tundra. – Oikos 105: 386-396.

Olofsson, J. et al. 2013. Complex biotic interactions drive long-term vegetation dynamics in a subarctic ecosystem. – Proc. R. Soc. B 368: 20120486.

Olofsson, J. et al. 2001. Effects of summer grazing by reindeer on composition of vegetation, productivity and nitrogen cycling. – Ecography 24: 13-24.

Olofsson, J. et al. 2009. Herbivores inhibit climate-driven shrub expansion on the tundra. – Glob. Change Biol. 15: 2681-2693.

Open Science Collaboration. 2015. Estimating the reproducibility of psychological science. – Science 349: aac4716.

Pastor, J. and Naiman, R. J. 1992. Selective foraging and ecosystem processes in boreal forests. – Am. Nat. 139: 690-705.

Pastor, J. et al. 2006. The roles of large herbivores in ecosystem nutrient cycles. – In: Danell, K., Bergström, R., Duncan, P. and Pastor, J. (ed.), Large herbivore ecology: ecosystem dynamics and conservation. Cambridge Univ. Press, pp. 289-325.

Pastor, J. et al. 1993. Moose browsing and soil fertility in the boreal forests of Isle Royale National Park. – Ecology 74: 467-480.

Pedersen, Å. et al. 2013a. Prevalence of pink-footed goose grubbing in the arctic tundra increases with population expansion. – Polar Biol. 36: 1569-1575.

Pedersen, Å. Ø. et al. 2013b. Spatial patterns of goose grubbing suggest elevated grubbing in dry habitats linked to early snowmelt. – Polar Res. 32: 1-9.

Pelt, W. et al. 2016. Multidecadal climate and seasonal snow conditions in Svalbard. – J. Geophys. Res. Earth Surf. 121: 2100-2117.

Petit Bon, M. et al. 2020a. One leaf for all: Chemical traits of single leaves measured at the leaf surface using Near infrared-reflectance spectroscopy (NIRS). – Methods Ecol. Evol., doi: https://doi.org/10.1111/2041-210X.13432.

Petit Bon, M. et al. 2020b. Interactions between winter and summer herbivory affect spatial and temporal plant nutrient dynamics in tundra grassland communities. – Oikos, doi: https://doi.org/10.1111/oik.07074.

Post, E. and Pedersen, C. 2008. Opposing plant community responses to warming with and without herbivores. – Proc. Natl. Acad. Sci. 105: 12353-12358.

Post, E. et al. 2019. The polar regions in a 2° C warmer world. – Sci. Adv. 5: eaaw9883.

Post, E. et al. 2009. Ecological dynamics across the Arctic associated with recent climate change. – Science 325: 1355-1358.

Quested, H. M. et al. 2003. Decomposition of sub-arctic plants with differing nitrogen economies: a functional role for hemiparasite. – Ecology 84: 3209-3221.

Rafi, M. M. and Epstein, E. 1999. Silicon absorption by wheat (*Triticum aestivum* L.). – Plant Soil 211: 223-230.

Raich, J. W. and Potter, C. S. 1995. Global patterns of carbon dioxide emissions from soils. – Global Biogeochem. Cycles 9: 23-36.

Ravolainen, V. et al. 2020. High Arctic ecosystem states: Conceptual models of vegetation change to guide long-term monitoring and research. – Ambio 49: 666-677.

Ravolainen, V. T. et al. 2011. Rapid, landscape scale responses in riparian tundra vegetation to exclusion of small and large mammalian herbivores. – Basic Appl. Ecol. 12: 643-653.

Reich, P. B. et al. 1997. From tropics to tundra: global convergence in plant functioning. – Proc. Natl. Acad. Sci. 94: 13730-13734.

Rønning, O. I. 1996. The Flora of Svalbard. – Norwegian Polar Institute.

Rustad, L. et al. 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. – Oecologia 126: 543-562.

Salazar, A. et al. 2019. Faster nitrogen cycling and more fungal and root biomass in cold ecosystems under experimental warming: a meta-analysis. – Ecology 101: e02938.

Shadish, W. R. et al. 2002. Experimental and quasi-experimental designs for generalized causal inference. – Cengage learning.

Shaver, G. R. and Chapin, F. S. 1980. Response to fertilization by various plant growth forms in an Alaskan tundra: nutrient accumulation and growth. – Ecology 61: 662-675.

Shaver, G. R. and Chapin, F. S. 1995. Long-term responses to factorial, NPK fertilizer treatment by Alaskan wet and moist tundra sedge species. – Ecography 18: 259-275.

Shaver, G. R. et al. 2000. Global warming and terrestrial ecosystems: A conceptual framework for analysis. – BioScience 50: 871-882.

Siefert, A. et al. 2015. A global meta-analysis of the relative extent of intraspecific trait variation in plant communities. – Ecol. Lett. 18: 1406-1419.

Sjögersten, S. et al. 2006. Small-scale hydrological variation determines landscape CO2 fluxes in the high Arctic. – Biogeochemistry 80: 205-216.

Sjögersten, S. et al. 2012. Impacts of grazing and climate warming on C pools and decomposition rates in Arctic environments. – Ecosystems 15: 349-362.

Skarin, A. et al. 2008. Summer habitat preferences of GPS-collared reindeer *Rangifer tarandus tarandus*. – Wildlife Bio. 14: 1-15.

Smis, A. et al. 2014. Determination of plant silicon content with near infrared reflectance spectroscopy. – Front. Plant Sci. 5: 496.

Soininen, E. M. et al. 2013a. More than herbivory: levels of silica-based defences in grasses vary with plant species, genotype and location. – Oikos 122: 30-41.

Soininen, E. M. et al. 2013b. Arctic small rodents have diverse diets and flexible food selection. – PLoS one 8: e68128.

Speed, J. D. et al. 2019. Trophic interactions and abiotic factors drive functional and phylogenetic structure of vertebrate herbivore communities across the Arctic tundra biome. – Ecography 42: 1152-1163.

Speed, J. D. M. et al. 2010a. Plant community properties predict vegetation resilience to herbivore disturbance in the Arctic. – J. Ecol. 98: 1002-1013.

Speed, J. M. et al. 2010b. Extrapolating herbivore-induced carbon loss across an arctic landscape. – Polar Biol. 33: 789-797.

Speed, J. M. et al. 2009. Predicting habitat utilization and extent of ecosystem disturbance by an increasing herbivore population. – Ecosystems 12: 349-359.

Srivastava, D. S. and Jefferies, R. 1996. A positive feedback: herbivory, plant growth, salinity, and the desertification of an Arctic salt-marsh. – J. Ecol. 1: 31-42.

Stark, S. 2007. Nutrient cycling in the tundra. – In: Marschner, P. and Rengel, Z. (ed.), Nutrient cycling in terrestrial ecosystems. Springer, pp. 309-331.

Sterner, R. W. and Elser, J. J. 2002. Ecological stoichiometry: the biology of elements from molecules to the biosphere. – Princeton Univ. Press.

Stuth, J. et al. 2003. Direct and indirect means of predicting forage quality through near infrared reflectance spectroscopy. – Field Crops Res. 84: 45-56.

Thomas, H. J. et al. 2020. Global plant trait relationships extend to the climatic extremes of the tundra biome. – Nat. Comm. 11: 1-12.

Thomas, V. and Prevett, J. 1982. The role of horsetails (Equisetaceae) in the nutrition of northern-breeding geese. – Oecologia 53: 359-363.

Tolvanen, A. and Henry, G. H. 2001. Responses of carbon and nitrogen concentrations in high arctic plants to experimental warming. – Can. J. Bot. 79: 711-718.

Tuomi, M. et al. 2018. Herbivore effects on ecosystem process rates in a low-productive system. – Ecosystems 22: 1-17.

Uboni, A. et al. 2016. Long-term trends and role of climate in the population dynamics of Eurasian reindeer. – PloS one 11: e0158359.

Väisänen, M. et al. 2013. Phenolic responses of mountain crowberry (*Empetrum nigrum* ssp. *hermaphroditum*) to global climate change are compound specific and depend on grazing by reindeer (*Rangifer tarandus*). – J. Chem. Ecol. 39: 1390-1399.

Van der Wal, R. 2006. Do herbivores cause habitat degradation or vegetation state transition? Evidence from the tundra. – Oikos 114: 177-186.

Van der Wal, R. and Stien, A. 2014. High-arctic plants like it hot: a long-term investigation of between-year variability in plant biomass. – Ecology 95: 3414-3427.

Van der Wal, R. et al. 2004. Vertebrate herbivores and ecosystem control: cascading effects of faeces on tundra ecosystems. – Ecography 27: 242-252.

Van der Wal, R. et al. 2000. Trading forage quality for quantity? Plant phenology and patch choice by Svalbard reindeer. – Oecologia 123: 108-115.

Van der Wal, R. et al. 2007. Spring feeding by pink-footed geese reduces carbon stocks and sink strength in tundra ecosystems. – Glob. Change Biol. 13: 539-545.

Vicari, M. and Bazely, D. R. 1993. Do grasses fight back? The case for antiherbivore defences. – Trends Ecol. Evol. 8: 137-141.

Violle, C. et al. 2007. Let the concept of trait be functional! – Oikos 116: 882-892.

Viscarra Rossel, R. A. et al. 2016. A global spectral library to characterize the world's soil. –Earth-Sci. Rev. 155: 198-230.

Vitousek, P. M. et al. 2010. Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen–phosphorus interactions. – Ecol. Appl. 20: 5-15.

Walker, D. A. et al. 2005. The Circumpolar Arctic vegetation map. – J. Veg. Sci. 16: 267-282.

Walker, M. D. et al. 2006. Plant community responses to experimental warming across the tundra biome. – Proc. Natl. Acad. Sci. 103: 1342-1346.

Wegener, C. and Odasz-Albrigtsen, A. M. 1997. Effects of laboratory simulated grazing on biomass of the perennial Arctic grass *Dupontia fisheri* from Svalbard: evidence of overcompensation. – Oikos 79: 496-502.

Welker, J. M. et al. 2005. Leaf mineral nutrition of Arctic plants in response to warming and deeper snow in northern Alaska. – Oikos 109: 167-177.

White, T. C. 2012. The inadequate environment: nitrogen and the abundance of animals. – Springer.

Wisz, M. S. et al. 2008. Where might the western Svalbard tundra be vulnerable to pink-footed goose (*Anser brachyrhynchus*) population expansion? Clues from species distribution models. – Divers. Distrib. 14: 26-37.

Wookey, P. A. et al. 2009. Ecosystem feedbacks and cascade processes: understanding their role in the responses of Arctic and alpine ecosystems to environmental change. – Glob. Change Biol. 15: 1153-1172.

Wright, I. J. et al. 2004. The worldwide leaf economics spectrum. – Nature 428: 821-827.

Ylänne, H. et al. 2015. Vegetation shift from deciduous to evergreen dwarf shrubs in response to selective herbivory offsets carbon losses: evidence from 19 years of warming and simulated herbivory in the subarctic tundra. – Glob. Change Biol. 21: 3696-3711.

Yoccoz, N. G. and Ims, R. A. 1999. Demography of small mammals in cold regions: the importance of environmental variability. – Ecol. Bull. 47: 137-144.

Zhang, D. et al. 2008. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. – J. Plant Ecol. 1: 85-93.

Zhou, J. et al. 2020. Enhanced shrub growth in the Arctic increases habitat connectivity for browsing herbivores. – Glob. Change Biol., doi: https://doi.org/10.1111/gcb.15104.

Zimov, S. A. et al. 1995. Steppe-tundra transition: A herbivore-driven biome shift at the end of the Pleistocene. – Am. Nat. 146: 765-794.

Paper I

One leaf for all: Chemical traits of single leaves measured at the leaf surface using Near infrared-reflectance spectroscopy (NIRS)

One leaf for all: Chemical traits of single leaves measured at the leaf surface using Near infrared-reflectance spectroscopy (NIRS)

Matteo Petit Bon[1,2], Hanna Böhner[1], Sissel Kaino[1], Torunn Moe[1], Kari Anne Bråthen[1]*

[1] Department of Arctic and Marine Biology, UiT - The Arctic University of Norway, N-9037 Tromsø, Norway

[2] Department of Arctic Biology, University Centre in Svalbard (UNIS), PO Box 156, N-9171 Longyearbyen, Norway

* Corresponding author: kari.brathen@uit.no, tel +47 776 46629

Key words (max eight)

leaf chemical traits, functional groups, phenological stages, Nitrogen, Phosphorus, Carbon, Silicon, arctic-alpine

A running headline (< 45 characters**)**

Chemical traits of single leaves

Word count

6777 (excluding title page and abstract)

Tweetable abstract

How to measure several chemical traits from a single, whole, small- or large-sized leaf

Abstract

1. The leaf is an essential unit for measures of plant ecological traits. Yet, measures of plant chemical traits are often achieved by merging several leaves, masking potential foliar variation within and among plant individuals. This is also the case with cost-effective measures derived using Near-infrared reflectance spectroscopy (NIRS). The calibration models developed for converting NIRS spectral information to chemical traits are typically based on spectra from merged and milled leaves. In this study we ask if such calibration models can be applied to spectra derived from whole leaves, providing measures of chemical traits of single leaves.

2. We sampled cohorts of single leaves from different biogeographic regions, growth forms, species and phenological stages in order to include variation in leaf and chemical traits. For each cohort we first sampled NIRS-spectra from each whole, single leaf, including leaf sizes down to Ø 4 mm (the minimum area of our NIRS application). Next, we merged, milled and tableted the leaves and sampled spectra from the cohort as a tablet. We applied arctic-alpine calibration models to all spectra and derived chemical traits. Finally, we evaluated the performance of the models in predicting chemical traits of whole, single leaves by comparing the traits derived at the level of leaves to that of the tablets.

3. We found that the arctic-alpine calibration models can successfully be applied to single, whole leaves for measures of Nitrogen (R^2=0.88, RMSE=0.824), Phosphorus (R^2=0.65, RMSE=0.081), and Carbon (R^2=0.78, RMSE=2.199) content. For Silicon content we found the method acceptable when applied to Silicon-rich growth forms (R^2=0.67, RMSE=0.677). We found a considerable variation in chemical trait values among leaves within the cohorts.

4. This time- and cost-efficient NIRS-application provides non-destructive measures of a set of chemical traits in single, whole leaves, including leaves of small sizes. The application can facilitate research into the scales of variability of chemical traits and include intraindividual variation. Potential trade-offs among chemical traits and other traits within the leaf unit can be identified and be related to ecological processes. In sum this NIRS-application can facilitate further ecological understanding of the role of leaf chemical traits.

Introduction

The essential role of chemical constituents in plants and ecosystem functioning is repeatedly emphasized (White 1993; Elser *et al.* 1996; Aerts & Chapin 2000; Güsewell 2004; Elser *et al.* 2007; LeBauer & Treseder 2008; Cebrian *et al.* 2009; Elser *et al.* 2010; Fay *et al.* 2015). Foliar chemical constituents show interspecific variability at both spatial and temporal scales within and across ecosystems, and are closely related to plant performance and ecological interactions (Güsewell 2004). Furthermore, foliar chemical content is among the plant traits with the highest intraspecific variability (Albert *et al.* 2010; Siefert *et al.* 2015; Fajardo & Siefert 2016), that may also include intraindividual variability (Ely *et al.* 2019). For instance, trait measures at the leaf level have been found to explain a considerable part of trait variability among tropical trees (Messier, McGill & Lechowicz 2010), large leaved food plants (Ely *et al.* 2019) and alpine plants (Albert *et al.* 2010). Albert *et al.* (2010) found intraindividual trait variation to be the largest for leaf dry matter content (LDMC) (ratio of dry to fresh leaf mass) in the dwarf shrub *Vaccinium myrtillus.* LDMC variability of 8 %, 37 % and 55 % was explained by differences among populations, individuals and leaves respectively. However, most methods to measure foliar chemical content require more than a single leaf, especially when working with small arctic and alpine plant species, causing knowledge about intraspecific variability in chemical traits to be at a rudimentary stage. Yet, the single leaf is a plant unit involved in ecological interactions. Thus, chemical trait information at the leaf level is likely to prove useful both to eco-physiological understanding of trade-offs within plants, and to how chemical traits, their variability and stoichiometry, affect ecosystem process rates (Bolnick *et al.* 2003; Albert *et al.* 2011; Bolnick *et al.* 2011). In this study, we address time- and cost-efficient methodology for measuring several chemical traits in single leaves, a scale of potential relevance to many ecological questions.

Near infra-red reflectance spectroscopy (NIRS) has recently been found to provide cost-efficient and accurate measures of leaf chemical traits independent of species, phenology, ecological context and region (Serbin *et al.;* Petisco *et al.* 2005; Serbin *et al.* 2014; Smis *et al.* 2014; Couture *et al.* 2016; Murguzur *et al.* 2019). Such cost-efficient measures open avenues for incorporating leaf chemical traits in large-scale ecological studies. This is strengthened by the fact that a single measure, one spectrum, of a sample is enough for predicting several chemical traits. Furthermore, the sampling of a spectrum is non-destructive, causing analyzed plant material to be available for further studies of, for example, the content of other constituents or of ecological processes such as decomposition rates. However, typical NIRS-applications are based on calibration models of NIRS derived spectra from dried and milled leaves versus their analytically derived chemical content. In order to have enough milled leaf material it is often necessary to merge several leaves, especially from species with small leaves, masking the potential chemical trait variability among leaves. And although cost-efficient, the process-time for milling is still a constraint (Couture *et al.* 2016). Here we ask if arctic-alpine calibration models for NIRS-based measures of chemical traits (Smis *et al.* 2014; Murguzur *et al.* 2019), models that are based on NIRS-spectra from dried, milled and tableted leaves, can be applied to NIRS-spectra of single, dried, whole leaves and still provide accurate measures of chemical content.

The precision of NIRS calibration models for measures of chemical constituents is dependent on the precision and bias of the analytical techniques from which the chemical constituents are retrieved and the NIRS-spectra are fitted (Chodak 2008). NIRS calibration models can thus only be as precise as the chemical analysis methods upon which they are based (Coates 2002). Any analytical technique imprecision can reduce the fit between the actual constituent values and the NIRS-spectra, whereas still be within the acceptable range that apply to standard method performance for analytical methods (AOAC International 2016), Because precision requirements are lower for small contents (Horwitz & Albert 2006), the fit can be poorer for nutrients with small content. Furthermore, any bias, i.e. a systematic shift in measured quantity above or below the true content, will reduce the fit with spectra derived from NIRS. It is therefore a great challenge to assess the actual accuracy of NIRS-based measures. Still, NIRS calibration models such as the arctic-alpine NIRS calibration models, are not inferior to chemical analysis in terms of accuracy and perform well for a range of chemical constituents (Smis *et al.* 2014; Murguzur *et al.* 2019). These models are developed for measures of foliar Nitrogen, Phosphorus, Carbon (Murguzur *et al.* 2019), and Silicon content (Smis *et al.* 2014) (Table 1). We chose to apply these models in this particular study because they provide accurate measures of chemical traits for a range of growth forms and species, at a range of phenological stages in both arctic and alpine environments (Table 1). Hence, they can potentially provide robust measures of chemical content of single leaves from any species in these environments. Furthermore, there is potential to build on these arctic-alpine NIRS calibration models to become global (Murguzur *et al.* 2019), making them useful for measures of chemical content of single leaves from a range of other environments.

Table 1. Performance of *arctic-alpine calibration models* for foliar chemical content (data from Smis *et al.* 2014; Murguzur *et al.* 2019). The calibration models are based on samples from nine (five for Silicon) functional groups, three levels of phenology, a range of habitats and three biogeographic regions (one region for Silicon). Model parameters are presented for external validation of the calibration models along with information about the samples upon which the calibrations were based. Bias is the mean error between predicted values and chemically measured values.

	No of species	A Content (% dry weight)		B Model parameters				
Chemical trait	(no of samples)	Mean	Range	Intercept	Slope	Bias	R^2	RMSE
Nitrogen	97 (552)	2.33	0.34 - 6.01	0.09	0.99	-0.08	0.94	0.20
Phosphorus	79 (291)	0.21	0.04 - 0.64	0.05	0.77	0.01	0.76	0.05
Carbon	96 (424)	46.05	32.56 - 56.22	2.29	0.95	0.14	0.88	1
Silicon	29 (442)	0.47	0 - 9.99	-0.001	0.95	-0.05	0.90	0.24

For our testing, we sampled cohorts of leaves from a range of plant individuals from three biogeographic regions, from different vegetation types, growth forms, species and phenological stages. With this wide range of leaf-cohorts we aimed to maximize the range of leaf types, and the range of foliar chemical content of our samples, according to guidelines for how to develop optimally performing methods (AOAC International 2016). Within each leaf-cohort, we dried the leaves, sampled spectra and predicted the chemical content of each single, whole leaf. We then calculated the average content per cohort and compared this average to the prediction achieved from the same cohort in the form of a tablet (all the leaves of the cohort merged, milled and pressed into a tablet). We also assessed to what extent the single leaves within a cohort showed variation in their chemical traits, including stoichiometric relations. We hypothesized that the arctic-alpine NIRS calibration models (Table 1) performed well for the prediction of chemical trait values of single, whole leaves. We also hypothesized that the chemical trait values differed among the single leaves within the leaf-cohorts.

Methods

Leaf sampling

The sampling was conducted on Svalbard, in Finnmark and in Troms (Norway), representing the biogeographic regions of the high-Arctic, the sub-Arctic alpine and the Boreal-alpine respectively. The sampling in Svalbard was conducted in Adventdalen (78° 10' N, 16° 05' E), a wide, formerly glaciated valley on the island of Spitsbergen, during the summer of 2016. We sampled leaves in dry heaths, mesic heaths, and wetlands, which represent the majority of habitat-types found across the archipelago (Elvebakk 2005). Both Finnmark and Troms belong to the Norwegian part of Fennoscandia. The sampling in Finnmark was conducted in the low alpine zone at 300–400 m a.s.l. at Ifjordfjellet (70° 27' N, 27° 08' E), during the summer season of 2015. The region is mainly characterized by dwarf-shrub heaths (Walker et al. 2005), whereas we sampled leaves mainly from tundra grasslands that typically dominate river plains and that host a wide variety of growth forms. The sampling in Troms was conducted in the low alpine zone at 400-500 m a.s.l. in the mountainous areas surrounding the city of Tromsø (69° 40' N, 18° 55' E) during the summer of 2017. Additional sampling of senescent leaves and litter, hereafter denoted as leaf litter, was conducted in the fall in 2017 in the boreal forest of Troms at approx. 50-100 m a.s.l..

We collected a total of 1677 fresh leaves for a total of 97 leaf-cohorts (set of single leaves merged into tablets), and we collected leaf litter for a total of 20 litter-cohorts (without separating between single leaves) (Table 2). Within each biogeographic region the cohorts were collected from different vegetation types, growth forms, species and from different phenological stages.

Table 2. Overview of leaf-cohorts (set of single leaves merged into tablets) sorted according to biogeographic region and growth form, and further split into phenological stage as indicated by sampling in early (June/July), mid (July/August) and late (August/September) summer, and including an overview of litter-cohorts (sampled in fall). Numbers in parenthesis refer to number of unique species within each growth form. More information about the cohorts is provided in the data overview (https://opendata.uit.no).

	Leaf-cohorts												Litter	
	Biogeographic region													
	Svalbard (high-Arctic)				Finnmark (sub-Arctic alpine)				Troms (Boreal-alpine)					Overall
	Overall	Summer			Overall	Summer			Overall	Summer			Fall	
		Early	Mid	Late		Early	Mid	Late		Early	Mid	Late		
Forbs	5 (1)	3		2	18 (7)	6	7	5	28 (14)		17		11	51
Grasses	6 (3)	3		3	12 (4)	4	6	2	8 (3)		4		4	26
Sedges	4 (2)	2		2	2 (1)	1	1		2 (1)				2	8
Horsetails	1	1			1	1								2
Shrubs	3 (2)	1		2	2 (2)			2	13 (3)		11		2	18
Trees					1		1		11 (4)		10		1	12
Overall	19				36				62					117

In addition to assessing if the arctic-alpine calibration models can be applied to spectra derived from whole leaves, we also assessed the number of spectra needed for predicting accurate chemical content in whole, single leaves as a guide to future sampling. For this purpose, we sampled fresh leaves from the Varanger Peninsula in Finnmark (70° N, 30° E) during the summer season of 2018. We sampled 22 single leaves of different leaf sizes from a total of 18 species, representing forbs, grasses and shrubs (Table S1), and sorted them in size classes of small leaves (Ø < 1 cm), medium leaves (Ø between 1 and 3 cm) and large leaves (Ø > 3 cm).

Sample processing

All leaves were sampled individually and immediately put in teabags, pressed dry between filter papers for at least 72 h and then dried at 60°C for at least 24 h. In a few cases when we did not have immediate access to plant press and oven-facilities, sampled leaves were stored as dry as possible, pressed at the latest during the evening of the sampling and finally dried in an oven within 5 days.

Per cohort we sampled leaves for a total of approx. 100 mg, which is a leaf mass large enough for making a tablet. The final number of leaves per leaf-cohort was on average 17.29, but varied dependent on both the leaf size of the species and the biogeographic region (Table 3). First, we sampled NIRS-spectra from whole leaves.

From the leaf-cohorts spectra were sampled separately from each single leaf, whereas from the litter-cohorts, leaves were stacked and NIRS-spectra were sampled from the leaves collectively. After sampling of spectra from whole leaves, all leaves within a cohort were merged and milled into fine powder using a ball mill (Mixer Mill, MM301; Retsch GmbH & Co. Haan, Germany) and pressed into tablets (Ø 16 mm, 1 mm thick) using a hydraulic press with 4 tons of pressure. Finally, we sampled spectra from each tablet.

Because water shows strong absorption patterns in the near infra-red region (Givens, De Boever & Deaville 1997) both the whole leaves and the tablets were oven-dried for 2 h at 60ºC to remove any potential water films, after which they were stored in a desiccator at room temperature (approx. 20ºC) until the sampling of spectra.

Spectral measurements

All spectra were recorded with a portable NIRS spectrometer (FieldSpec 3, Asd Inc., Boulder, Colorado). Spectra of whole leaves were recorded using a custom-made adaptor that can be attached to the ASD Contact probe (Asd Inc., Boulder, Colorado) and allows for measurements of an area as small as Ø 4 mm (Figure 1). The adaptor was made using Delrin, a non-absorptive material similar to that of the original plant adaptor (advice communicated by Asd Inc., Boulder, Colorado). Spectra of tablets were recorded using a similar setup but with an adaptor for an area of Ø 16 mm, exactly matching the size of the tablets (Smis *et al.* 2014).

Spectra were recorded with monochromatic radiation in the wavelength range of 350-2500 nm with NIR, SWIR1 and SWIR2 sensors. The spectra were interpolated to 1 nm intervals based on recordings every 1.4 nm in the 350-1050 nm region and every 2 nm from 1050 to 2500 nm. The arctic-alpine calibration models are based on a subset of the wavelength range, and do not include wavelength regions for which the different sensors overlap (i.e. 350-380 nm, 760-840 nm, 1700-1800 nm and 2450-2500 nm) and the visible part of the spectrum (380-720 nm). The same subset should be applied when using the arctic-alpine calibration models for prediction, and hence we prepared the spectra accordingly.

The number of sampled spectra per single, whole leaf was dependent on the leaf area, causing a range in spectra sampled (Table 3). Overall, we sampled from 1 to 12 spectra with an average number of 3.53 replicate spectra per leaf and an average number of 61.03 replicate spectra per leaf-cohort, and 14.1 replicate spectra per litter-cohort. For the tablets the average was 3 and 4 replicate spectra for leaves and leaf litter respectively. For the assessment of the number of spectra needed for predicting accurate content of chemical constituents in single leaves, we sampled 10 replicate spectra from small-sized leaves, an average of 14.5 replicate spectra per medium-sized leaves and an average of 32.5 replicate spectra per large-sized leaves. Each spectrum was recorded as absorbance (log 1/ R, where R = reflectance) and the chemical content predicted using the arctic-alpine models for Nitrogen, Phosphorus, Carbon (Murguzur *et al.* 2019) and Silicon content (Smis *et al.* 2014).

Table 3. Overview of number of leaves per leaf-cohort and number of spectra sampled per leaf sorted according to biogeographic region.

	Biogeographic region					
	Svalbard (high-Arctic)		Finnmark (sub-Arctic alpine)		Troms (Boreal-alpine)	
	Range	Mean	Range	Mean	Range	Mean
Number of leaves per cohort	20 - 140	48	1 - 22	8.92	3 - 126	10.57
Number of replicates of spectra per leaf	3 - 9	4.96	1 - 12	2.47	1 - 11	1.34

Assessment of method performance

We used the predictions of the chemical content of the tablets (for which the arctic-alpine calibration models are developed) as blueprint to which the predicted chemical content of whole leaves was compared. For the whole, leaves we first calculated the average predicted chemical content per single leaf, after which we calculated the average content per leaf-cohort. For the leaf litter we calculated the average predicted chemical content per litter-cohort directly. We compared the chemical content per cohort as predicted from whole leaves to the chemical content of the cohort as predicted from its tablet. We used linear regression models to assess prediction fit (intercept and slope) and prediction accuracy (root-mean-square error of prediction (RMSEP) and coefficient of determination (R2)). All cohorts were included in the linear regression models for the predictions of Nitrogen, Phosphorus and Carbon content. For the prediction of Silicon content only the silicon-rich growth forms were included (horsetails and graminoids), for which also the arctic-alpine model of Silicon performs best (Smis *et al.*

2014). Negative predictions of Phosphorus content from tablets of two of the cohorts were adjusted to a Phosphorus content of 0.04 % dry weight, the minimum content included in the arctic-alpine calibration model of Phosphorus and measured with chemical analysis (Murguzur *et al.* 2019). The regression analysis was also conducted for leaves and leaf litter separately. The final model was based on all cohorts only if this model was equal or better in accuracy to that of the leaf model, otherwise two separate models (i.e. one for leaf-cohorts and one for litter-cohorts) are presented.

For the assessment of chemical content variation among single leaves within leaf-cohorts, we first corrected predicted values using correction factors achieved from the regression analyses described above. For the chemical constituents where the fit between predicted content from the whole leaves vs the tablets was not 1:1, we applied the intercept and slope as correction factors to adjust the predicted content per leaf. After correction the predicted Phosphorus and Silicon content was negative for a few leaves. These leaves were given a minimum value of content equal to 0.01 % Phosphorus and 0.1 % Silicon, similar to the lowest values included in the arctic-alpine calibration models (Table 1).

We assessed intra-cohort variation using a subset of the samples. For intra-cohort variation in chemical content we used leaf-cohorts of *Bistorta vivipara*, the only species represented with cohorts from all the three biogeographic regions as well as several phenological stages per region. For intra-cohort variation in stoichiometric ratios we used graminoid cohorts sampled from Svalbard in the late season, representing a range of genera for which the predicted chemical content was based on at least four sampled spectra and for which we could include Silicon content. We assessed whether stoichiometric ratios would be more accurately predicted using calibration models based on stoichiometric ratios directly. We made a calibration model for the ratio between Nitrogen and Carbon (Figure S1A). A comparison between the stoichiometric ratios derived from the arctic-alpine calibration models and the new stoichiometric calibration model indicated they were equally precise (Figure S1B), and we proceeded with the arctic-alpine calibration models.

To estimate the number of NIRS-spectra necessary to accurately predict the chemical content of single, whole leaves we sampled a minimum of 10 and a maximum of 42 spectra per leaf. First, we predicted the chemical content from each single spectrum using the arctic-alpine calibration models. Next, we averaged these predictions between an increasing number of replicates (average of predictions from the two first replicate spectra, the three first replicate spectra and so on up to the maximum number of replicate spectra for each leaf). Finally, we compared these averages by calculating their differences. We repeated this procedure 10 times, from each of 10 randomizations of the order in which the spectra were taken. We plotted the differences in predictions as a function of the number of replicate spectra. Based on a graphical presentation of the differences we assessed at what number of replicate spectra the difference in predictions levelled off, with differences approaching zero considered the number of spectra required for accurate predictions of chemical content in single, whole leaves.

All statistical analyses were run in the R environment version 3.4.4 (http://www.r-project.org) using ggplot2 for all graphical presentations.

Results

The range of chemical content derived from sampled NIRS-spectra of milled and tableted leaves with the Ø 16 mm plant adaptor (Figure 1) was considerable (Table 4A), providing a range in chemical contents for which to pursue the comparison between predictions from whole leaves and tablets.

We found the arctic-alpine calibration models performed well in predicting content of chemical traits of whole leaves. Predictions of chemical content of a cohort when based on spectra sampled from leaves (using a Ø 4 mm plant adaptor) correlated well with that of predictions based on sampled spectra from the same leaves as milled and tableted (the standardized way of preparing leaf material for measurement of chemical content using NIRS) (Figure 2, Table 4B). For Nitrogen and Silicon, we found both leaf- and litter-cohorts were fitting in a common model (Figure 2). For Phosphorus and Carbon, we found the slope of the litter-cohorts was steeper than that of the leaf-cohorts (Figure 2). Overall, the predicted content from whole leaves differed in range to that of the predicted content from tablets (Figure 2), and the intercept and slope of the regressions deviated from an ideal relationship of 1:1 for all the chemical traits (Table 4). Hence, in order to achieve actual chemical content predictions from the Ø 4 mm sampled spectra of whole leaves, the initial predictions from the arctic-alpine calibration models must be corrected.

Table 4. Chemical content of milled and tableted leaf- and litter-cohorts as predicted by the arctic-alpine models (Smis *et al.* 2014; Murguzur *et al.* 2019) and applied in this study (A). Model parameters for the regression analysis between chemical content predicted from single leaves and tablets (B). For Phosphorus and Carbon the model parameters improved when separating leaf- and litter-cohorts, whereas for Nitrogen and Silicon the best model included both cohorts. *The Silicon model only includes graminoids and horsetails as these are growth forms with higher Silicon content.

		A Content (% dry weight)		B Model parameters			
Chemical trait		Mean	Range	Intercept	Slope	R^2	RMSE
Nitrogen		2.100	0.032 - 4.515	1.073	0.604	0.88	0.824
Phosphorus	Leaf-cohorts	0.184	0.040 - 0.443	-0.014	0.842	0.65	0.081
	Litter-cohorts	0.127	0.040 - 0.291	-0.003	1.165	0.56	0.053
Carbon	Leaf-cohorts	45.35	40.07 - 51.84	7.165	0.811	0.78	2.199
	Litter-cohorts	46.48	43.07 - 55.19	-14.403	1.341	0.91	1.654
Silicon*		0.991	0 - 2.489	0.612	0.699	0.67	0.677

The predicted chemical content of single leaves within *Bistorta* leaf-cohorts showed a considerable variation (Figure 3). The range in chemical content among leaves within a cohort was particularly large for the cohorts from Svalbard (Figure 3, Figure S2), and with a larger range in chemical content in early as opposed to late season. In general, the range in predicted chemical content among leaves within cohorts was equal to or larger than the range in predicted content among seasons and biogeographic regions, as indicated by the predicted content of tablets.

The single, whole leaf predictions were attained on the basis of several spectra sampled per leaf but for a few leaves from the *Bistorta* leaf-cohorts of the Finnmark and Troms regions that were based on one spectrum only (Figure 3). The predicted content of tablets was both larger, similar and smaller than that of the average of predicted chemical content of the single leaves (Figure 3; for all cohorts see Figure 2). In particular there was a large discrepancy between leaves and tablets for the predicted content of Carbon in the *Bistorta* leaf-cohorts from the Troms region, the region with most leaves with only one replicate spectrum.

The predicted stoichiometric ratios of single leaves within the graminoid cohorts from Svalbard showed a considerable variation (Figure 4). In general, the three cohorts of grasses showed most variation among leaves, and especially the grass *Calamagrostis,* where the variation in the ratio among chemical constituents ranged from seven-fold to 40-fold. The sedge *Eriophorum* showed the least variation among leaves but for the Silicon / Nitrogen ratio, where it showed the largest variation. For most cohorts the average of predicted stoichiometric ratios of the leaves overlapped or were close to that of the tablets.

The precision in predicted chemical content per leaf was dependent on the number of sampled spectra. There was a sharp increase in precision already at 4-5 sampled spectra per leaf, as indicated by a sharp decrease in difference in predictions between 2-3 and 4-5 sampled spectra (Figure 5). When comparing the difference in predicted chemical content to that of the average chemical content of the leaves (insets), the maximum prediction inaccuracy was up to 12.5 % when using only two replicate spectra and dropped to approx. 3 % when using 5 spectra. This supports that a few spectra only provide an accurate prediction of foliar chemical content of single leaves.

Discussion

This study shows that the foliar content of a range of key chemical elements can be measured, using NIRS, from a whole, single leaf and from leaf sizes as small as Ø 4 mm. The NIRS-application is both time- and cost-efficient, and is non-destructive. In a time when marked changes to the environment are happening, and especially in arctic and alpine regions where predicted changes to biogeochemistry are considerable (Jonasson, Chapin &

Shaver 2001), we believe our efficient method to achieve chemical traits is a welcomed contribution (Halbritter *et al.* 2019). This is further supported in terms of reduced sampling impact on vegetation when for instance working in experimental or long-term monitoring plots. Also, the quick measure of a range of foliar chemical traits at the level of single leaves opens avenues for research. For instance, chemical traits can be related to that of other traits along with their trade-offs at the level of single leaves, and compared to trade-offs at the level of individuals and populations. Inter- and intra-individual variability in foliar chemical traits can become levels of investigation when studying ecosystem processes such as herbivory and decomposition. In sum, ecological questions for which chemical traits in single leaves are relevant, can easily be addressed through our NIRS-application.

The arctic-alpine calibration models are based on spectral data of milled plant material pressed into tablets. The purpose of milling and tableting leaf material is to create a homogeneous surface and reduce random light scattering (Smis et al. 2014). Reduced precision is found when predicting from fresh leaves as opposed to dried and milled leaves, yet the loss in precision does not make predictions from fresh leaves inferior (Couture et al. 2016). Furthermore, any gain in information acquired from having time to process more samples (when avoiding tedious processing of leaf samples) may compensate for less accurate predictions (Couture et al. 2016). In our study any reduced accuracy in the prediction of chemical trait values from the dried and pressed leaves compared to that of the tablets could not be estimated directly as most single leaves were too small for tableting (and too small for providing wet chemistry measures of the chemical content). However, we found that the accuracy of the measure of chemical content in a single leaf increased with the number of spectra sampled, suggesting the uneven surface of a leaf (such as that of veins and other structures) does not interfere with predictions as long as several spectra are sampled.

The arctic-alpine calibration model of Silicon performs best for Silicon-rich growth forms (Smis *et al.* 2014). Perhaps for this reason we found the model was only applicable to single leaves of Silicon-rich growth forms. Furthermore, species of growth forms with low Silicon content such as forbs, shrubs and trees made up two thirds of all samples (Table 2), hence their inclusion would have caused a bias in the regression analysis towards small content. Also, the predicted Silicon content of single leaves of these Silicon-poor species was sometimes spurious. This indicates aspects of the leaf surface, in turn affecting the spectral signature, interfered with the

Silicon-model and that foliar Silicon content of Silicon-poor growth forms are best measured in a homogeneous surface such as that of milled material (Smis et al. 2014).

The ability to address chemical traits of single leaves provides the opportunity to assess intraspecific chemical trait variability at several scales, including the within individual variability (Albert *et al.* 2011; Bolnick *et al.* 2011). Indeed, the Nitrogen content related trade-off, or dilemma, of leaves being palatable and efficient in production as opposed to investing in defenses (Díaz *et al.* 2016), may play out differently among single leaves within a plant individual. For instance, plant herbivore interactions between trees and large ungulates can promote changes at both the modular and genetic level (Danell *et al.* 2003). In response to herbivory by moose the deciduous tree *Betula pendula* allocates more Nitrogen to leaves on shoots browsed by the herbivore than to leaves on lesser-browsed shoots (Danell, Huss-Danell & Bergstrom 1985). There are also several other ways by which intraspecific trait variation - that is variation both within and among individuals of the same species - could alter community structure or dynamics (Bolnick *et al.* 2011). For instance, ecological interactions may depend non-linearly on the variations in a trait, or trait variation may determine the number of ecological interactions taking place (Bolnick *et al.* 2011), hence knowledge of the intraindividual trait variation may increase our predictive ability of ecological interactions. In turn, variation in chemical or stoichiometric traits among leaves is likely to drive differences in biodiversity among individual plants. The arctic-alpine calibration models, and potentially also other NIRS-based calibration models, provide an opportunity to address such within-individual variation for a range of chemical traits. Furthermore, NIRS-based spectral information at the leaf level also hold the potential for being scaled up to larger scales. Measures at the leaf level within individual plots can be scaled up to canopy, community and landscape levels, and even larger scales, where for each level confounding factors that blur understanding can be addressed. Such scaling also provide efficient measures of biodiversity (Cavender-Bares *et al.* 2017).

There are several aspects of leaves for which a focus on their chemical content may be worth-while. Leaves are functional units for photosynthesis. Leaves are modular units constantly produced and discarded from plant individuals. Leaves are the units often selected for by herbivores. All these functional roles of leaves suggest their chemical content varies, and that measuring their chemical traits at the scale of the functional leaf unit opens avenues to what questions we can ask in ecology. The arctic-alpine calibration models for NIRS-based prediction of foliar Nitrogen, Phosphorus, Carbon (Murguzur *et al.* 2019) and Silicon content (Smis *et al.* 2014) can be applied to achieve chemical traits from single, whole leaves, and as such may be the method to open these avenues. Merging the arctic-alpine calibration models with existing calibration models from other regions may further open these avenues towards a global level.

Acknowledgement

We are grateful to Francisco Javier Ancin Murguzur for discussions on NIRS applications and for designing the adaptors, and to Adriaan Smis, Kelsey Lorberau, Mikel Moriano and Jake Robinson for assistance in processing samples. This study received financial support to KAB from the High North Research Centre for Climate and the Environment (The FRAM Centre, www.framsenteret.no) and is an integral study to the Climate-ecological Observatory of the Arctic Tundra, COAT (www.coat.no). There is no conflict of interest to declare for this study.

Author Contributions Statement

MPB, KAB, TM and SK conceived and developed the study, MPB drafted the study design and study protocol, HB did the modelling, KAB drafted the paper with help of MPB and HB. All authors gave final approval for publication.

Competing Financial Interests statement

There are no competing financial interests associated to this study.

Data availability

Data are accessible at UiT Open Research Data (https://opendata.uit.no, doi.org/10.18710/MHX1Z7).

References

Aerts, R. & Chapin, F.S. (2000) The mineral nutrition of wild plants revisited: A re-evaluation of processes and patterns. *Advances in Ecological Research, Vol 30*, pp. 1-67.

Albert, C.H., Grassein, F., Schurr, F.M., Vieilledent, G. & Violle, C. (2011) When and how should intraspecific variability be considered in trait-based plant ecology? *Perspectives in Plant Ecology, Evolution and Systematics,* **13,** 217-225.

Albert, C.H., Thuiller, W., Yoccoz, N.G., Soudant, A., Boucher, F., Saccone, P. & Lavorel, S. (2010) Intraspecific functional variability: extent, structure and sources of variation. *Journal of Ecology,* **98,** 604-613.

AOAC International (2016) Official Methods of Analysis. *Appendix F: Guidelines for Standard Method Performance Requirements*. www.eoma.aoac.org/app_f.pdf.

Bolnick, D.I., Amarasekare, P., Araújo, M.S., Bürger, R., Levine, J.M., Novak, M., Rudolf, V.H.W., Schreiber, S.J., Urban, M.C. & Vasseur, D.A. (2011) Why intraspecific trait variation matters in community ecology. *Trends in Ecology & Evolution,* **26,** 183-192.

Bolnick, D.I., Svanbäck, R., Fordyce, J.A., Yang, L.H., Davis, J.M., Hulsey, C.D. & Forister, M.L. (2003) The ecology of individuals: Incidence and implications of individual specialization. *American Naturalist,* **161,** 1-28.

Cavender-Bares, J., Gamon, J.A., Hobbie, S.E., Madritch, M.D., Meireles, J.E., Schweiger, A.K. & Townsend, P.A. (2017) Harnessing plant spectra to integrate the biodiversity sciences across biological and spatial scales. *American Journal of Botany,* **104,** 966-969.

Cebrian, J., Shurin, J.B., Borer, E.T., Cardinale, B.J., Ngai, J.T., Smith, M.D. & Fagan, W.F. (2009) Producer Nutritional Quality Controls Ecosystem Trophic Structure. *Plos One,* **4,** e4929.

Chodak, M. (2008) Application of Near Infrared Spectroscopy for Analysis of Soils, Litter and Plant Materials. *Polish Journal of Environmental Studies,* **17**.

Coates, D.B. (2002) Is near infrared spectroscopy only as good as the laboratory reference values? An empirical approach. *Spectroscopy Europe,* **14,** 24-26.

Couture, J.J., Singh, A., Rubert-Nason, K.F., Serbin, S.P., Lindroth, R.L. & Townsend, P.A. (2016) Spectroscopic determination of ecologically relevant plant secondary metabolites. *Methods in Ecology and Evolution,* **7,** 1402-1412.

Danell, K., Bergström, R., Edenius, L. & Ericsson, G. (2003) Ungulates as drivers of tree population dynamics at module and genet levels. *Forest Ecology and Management,* **181,** 67-76.

Danell, K., Huss-Danell, K. & Bergstrom, R. (1985) Interactions between Browsing Moose and Two Species of Birch in Sweden. *Ecology,* **66,** 1867-1878.

Díaz, S., Kattge, J., Cornelissen, J.H.C., Wright, I.J., Lavorel, S., Dray, S., Reu, B., Kleyer, M., Wirth, C., Colin Prentice, I., Garnier, E., Bönisch, G., Westoby, M., Poorter, H., Reich, P.B., Moles, A.T., Dickie, J., Gillison, A.N., Zanne, A.E., Chave, J., Joseph Wright, S., Sheremet'ev, S.N., Jactel, H., Baraloto, C., Cerabolini, B., Pierce, S., Shipley, B., Kirkup, D., Casanoves, F., Joswig, J.S., Günther, A., Falczuk, V., Rüger, N., Mahecha, M.D. & Gorné, L.D. (2016) The global spectrum of plant form and function. *Nature,* **529,** 167-171.

Elser, J.J., Bracken, M.E.S., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B. & Smith, J.E. (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters,* **10,** 1135-1142.

Elser, J.J., Dobberfuhl, D.R., MacKay, N.A. & Schampel, J.H. (1996) Organism size, life history, and N: P stoichiometry toward a unified view of cellular and ecosystem processes. *Bioscience,* **46,** 674-684.

Elser, J.J., Fagan, W.F., Kerkhoff, A.J., Swenson, N.G. & Enquist, B.J. (2010) Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. *New Phytologist,* **186,** 593-608.

Elvebakk, A. (2005) A vegetation map of Svalbard on the scale 1: 3.5 mill. *Phytocoenologia,* **35,** 951-967.

Ely, K.S., Burnett, A.C., Lieberman-Cribbin, W., Serbin, S. & Rogers, A. (2019) Spectroscopy can predict key leaf traits associated with source–sink balance and carbon–nitrogen status. *Journal of Experimental Botany,* **70,** erz061.

Fajardo, A. & Siefert, A. (2016) Phenological variation of leaf functional traits within species. *Oecologia,* **180,** 951-959.

Fay, P.A., Prober, S.M., Harpole, W.S., Knops, J.M.H., Bakker, J.D., Borer, E.T., Lind, E.M., MacDougall, A.S., Seabloom, E.W., Wragg, P.D., Adler, P.B., Blumenthal, D.M., Buckley, Y., Chu, C.J., Cleland, E.E., Collins, S.L., Davies, K.F., Du, G.Z., Feng, X.H., Firn, J., Gruner, D.S., Hagenah, N., Hautier, Y., Heckman, R.W., Jin, V.L., Kirkman, K.P., Klein, J., Ladwig, L.M., Li, Q., McCulley, R.L., Melbourne, B.A., Mitchell, C.E., Moore, J.L., Morgan, J.W., Risch, A.C., Schutz, M., Stevens, C.J., Wedin, D.A. & Yang, L.H. (2015) Grassland productivity limited by multiple nutrients. *Nature Plants,* **1**.

Givens, D.I., De Boever, J. & Deaville, E. (1997) The principles, practices and some future applications of near infrared spectroscopy for predicting the nutritive value of foods for animals and humans. *Nutrition research reviews,* **10,** 83-114.

Güsewell, S. (2004) N : P ratios in terrestrial plants: variation and functional significance. *New Phytologist,* **164,** 243-266.

Halbritter, A.H., De Boeck, H.J., Eycott, A.E., Reinsch, S., Robinson, D.A., Vicca, S., Berauer, B., Christiansen, C.T., Estiarte, M., Grünzweig, J.M., Gya, R., Hansen, K., Jentsch, A., Lee, H., Linder, S., Marshall, J., Peñuelas, J., Kappel Schmidt, I., Stuart-Haëntjens, E., Wilfahrt, P., Group, t.C.W. & Vandvik, V. (2019) The handbook for standardized field and laboratory measurements in terrestrial climate change experiments and observational studies (ClimEx). *Methods in Ecology and Evolution,* **00,** 1-16.

Horwitz, W. & Albert, R. (2006) The Horwitz ratio (HorRat): a useful index of method performance with respect to precision. *Journal of AOAC International,* **89,** 1095-1109.

Jonasson, S., Chapin, F.S. & Shaver, G.R. (2001) 1.10 - Biogeochemistry in the Arctic: Patterns, Processes, and Controls. *Global Biogeochemical Cycles in the Climate System* (eds E.-D. Schulze, M. Heimann, S. Harrison, E. Holland, J. Lloyd, I.C. Prentice & D. Schimel), pp. 139-150. Academic Press, San Diego.

LeBauer, D.S. & Treseder, K.K. (2008) Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology,* **89,** 371-379.

Messier, J., McGill, B.J. & Lechowicz, M.J. (2010) How do traits vary across ecological scales? A case for trait-based ecology. *Ecology Letters,* **13,** 838-848.

Murguzur, F.J.A., Bison, M., Smis, A., Böhner, H., Struyf, E., Meire, P. & Bråthen, K.A. (2019) Towards a global arctic-alpine model for Near-infrared reflectance spectroscopy (NIRS) predictions of foliar nitrogen, phosphorus and carbon content. *Scientific Reports,* **9,** 8259.

Petisco, C., García-Criado, B., Vázquez de Aldana, B.R., Zabalgogeazcoa, I., Mediavilla, S. & García-Ciudad, A. (2005) Use of near-infrared reflectance spectroscopy in predicting nitrogen, phosphorus and calcium contents in heterogeneous woody plant species. *Analytical and Bioanalytical Chemistry,* **382,** 458-465.

Serbin, S.P., Singh, A., McNeil, B.E., Kingdon, C.C. & Townsend, P.A. (2014) Spectroscopic determination of leaf morphological and biochemical traits for northern temperate and boreal tree species. *Ecological Applications,* **24,** 1651-1669.

Serbin, S.P., Wu, J., Ely, K.S., Kruger, E.L., Townsend, P.A., Meng, R., Wolfe, B.T., Chlus, A., Wang, Z. & Rogers, A. From the Arctic to the tropics: multibiome prediction of leaf mass per area using leaf reflectance. *New Phytologist,* **0**.

Siefert, A., Violle, C., Chalmandrier, L., Albert, C.H., Taudiere, A., Fajardo, A., Aarssen, L.W., Baraloto, C., Carlucci, M.B., Cianciaruso, M.V., de L. Dantas, V., de Bello, F., Duarte, L.D.S., Fonseca, C.R., Freschet, G.T., Gaucherand, S., Gross, N., Hikosaka, K., Jackson, B., Jung, V., Kamiyama, C., Katabuchi, M., Kembel, S.W., Kichenin, E., Kraft, N.J.B., Lagerström, A., Bagousse-Pinguet, Y.L., Li, Y., Mason, N., Messier, J., Nakashizuka, T., Overton, J.M., Peltzer, D.A., Pérez-Ramos, I.M., Pillar, V.D., Prentice, H.C., Richardson, S., Sasaki, T., Schamp, B.S., Schöb, C., Shipley, B., Sundqvist, M., Sykes, M.T., Vandewalle, M. & Wardle, D.A. (2015) A global meta-analysis of the relative extent of intraspecific trait variation in plant communities. *Ecology Letters,* **18,** 1406-1419.

Smis, A., Murguzur, F.J.A., Struyf, E., Soininen, E.M., Jusdado, J.G.H., Meire, P. & Bråthen, K.A. (2014) Determination of plant silicon content with near infrared reflectance spectroscopy. *Frontiers in Plant Science,* **5,** 496.

Walker, D.A., Raynolds, M.K., Daniëls, F.J.A., Einarsson, E., Elvebakk, A., Gould, W.A., Katenin, A.E., Kholod, S.S., Markon, C.J., Melnikov, E.S., Moskalenko, N.G., Talbot, S.S., Yurtsev, B.A. & Franklin, J. (2005) The Circumpolar Arctic vegetation map. *Journal of Vegetation Science,* **16,** 267-282.

White, T. (1993) *The inadequate environment: nitrogen and the abundance of animals*. Springer-Verlag, Berlin.

FIGURE LEGENDS

Figure 1. **A**. The plant probe along with custom-made adaptors with a Ø 16 mm area and a Ø 4 mm area for measuring a NIRS-spectrum of a tablet or a single, whole leaf. **B**. The Ø 4 mm adaptor attached to the plant probe ready for scanning a leaf placed on a white pad (a Spectralon) of Ø 10 cm. **C**. Leaves of *Bistorta vivipara*, *Geranium sylvaticum* and *Vaccinimum myrtillus* prepared for scanning. **D**. Narrow grass leaves prepared for scanning.

Figure 2. Relationships between Nitrogen, Phosphorus, Carbon and Silicon content of whole leaves and tablets, separate for leaf-cohorts (Leaf) and litter-cohorts (Litter) when their separation improved the linear regression models. The correlation for Silicon content is based on silicon-rich growth forms only. The grey line shows the ideal 1:1 relationship.

Figure 3. Violin plots of foliar content of Nitrogen, Phosphorus and Carbon (% dry weight) of leaf-cohorts of *Bistorta vivipara*. Each violin represents one leaf-cohort and each dot within each violin represents the chemical content of a unique, single leaf with the size of each dot representing the number of replicate spectra as basis for the predicted chemical content. Cohorts are sorted according to the biogeographic region (Svalbard, Finnmark and Troms) and the season (early, mid and late) they were sampled. The chemical content of the cohort tablet (T) and the cohort average across all leaves (S) are projected onto its respective violin. The leaves sampled from Svalbard were inherently smaller in size than in the two other regions causing the cohorts to have many more leaves (and hence the dense appearance in the plot).

Figure 4. Violin plots of stoichiometric ratios between Nitrogen, Phosphorus, Carbon and Silicon content (% dry weight) of leaf-cohorts of graminoid species sampled from Svalbard in the late season. Each violin represents one leaf-cohort and each dot within each violin represents the stoichiometric ratio of a single leaf, with the size of the dot representing the number of replicate spectra as basis for the predicted stoichiometric ratio. The stoichiometric ratios of the cohort tablets (T) and the average across the cohort leaves (S) are projected onto its respective violin. The graminoid genera included are *Alopecurus (Alo), Calamagrostis (Cal), Duponita (Dup), Eriophorum (Eri)* and *Luzula (Luz).*

Figure 5. Differences in predicted foliar Nitrogen, Phosphorus, Carbon and Silicon content (% dry weight) as a function of the number of sampled NIRS-spectra per leaf, displayed separately for large-, medium- or small-sized leaves. Each dot represents the average difference in prediction, obtained from randomizing the order of the spectra sampled from a leaf and calculating the differences 10 times (with differences between one and two spectra presented as two sampled spectra, differences between two and three spectra as three sampled spectra a.s.o.). The density-curves demonstrate the overall pattern across all leaves within a leaf-size group. For a comparison to the scale of the chemical content of the leaves, insets show the average chemical content per leaf-size group. Examples of species representing the different leaf-size groups are the grass *Phleum alpinum* and the forbs *Trollius europaeus* and *Soildago virgaurea* for large-sized leaves, the grasses *Anthoxanthum nipponicum* and *Calamagrostis phragmitoides* and the forbs *Bistorta vivipara* and *Rumex acetosa* for medium-sized leaves, and finally the dwarf shrubs *Vaccinium myrtillus* and *Betula nana* for small-sized leaves. In total the leaves of 18 species were included (Table S1).

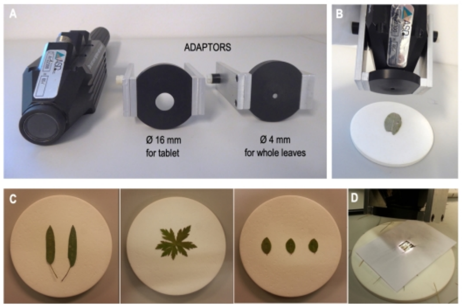


Figure 1

338x190mm (224 x 224 DPI)

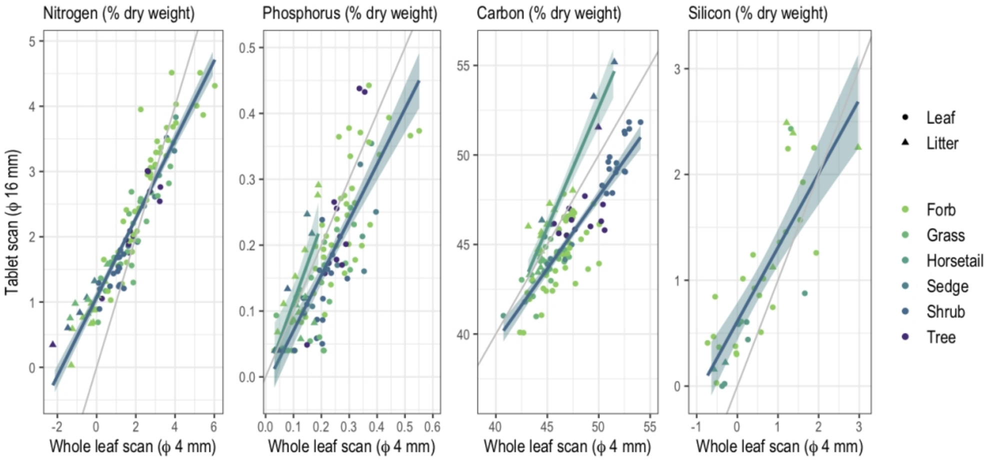


Figure 2

238x116mm (150 x 150 DPI)

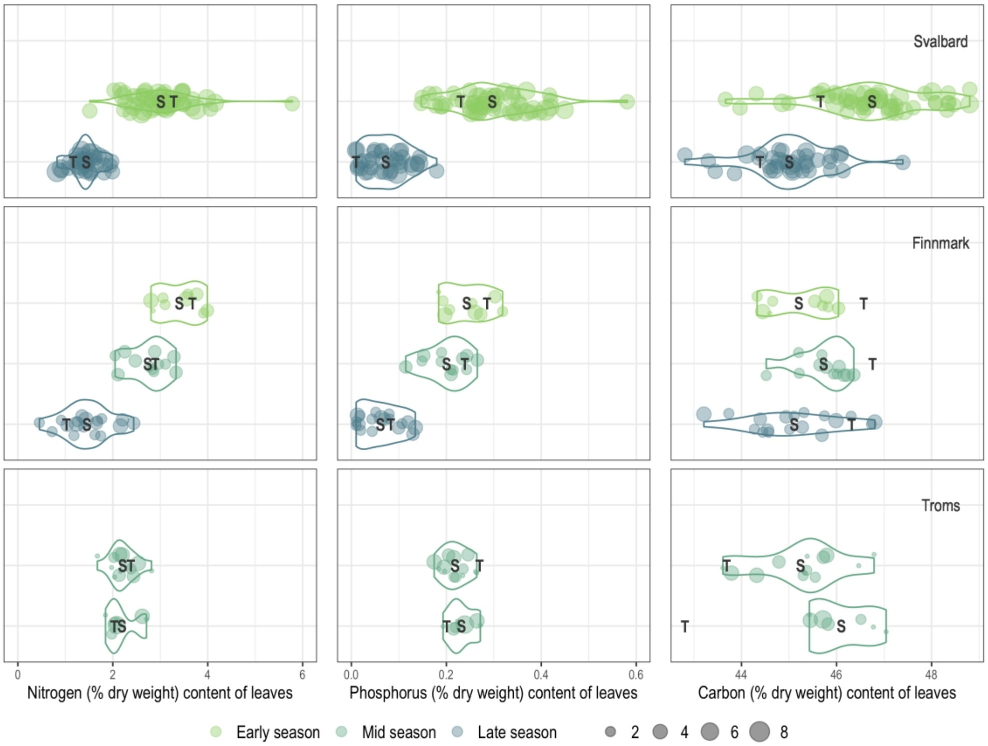


Figure 3

239x181mm (300 x 300 DPI)

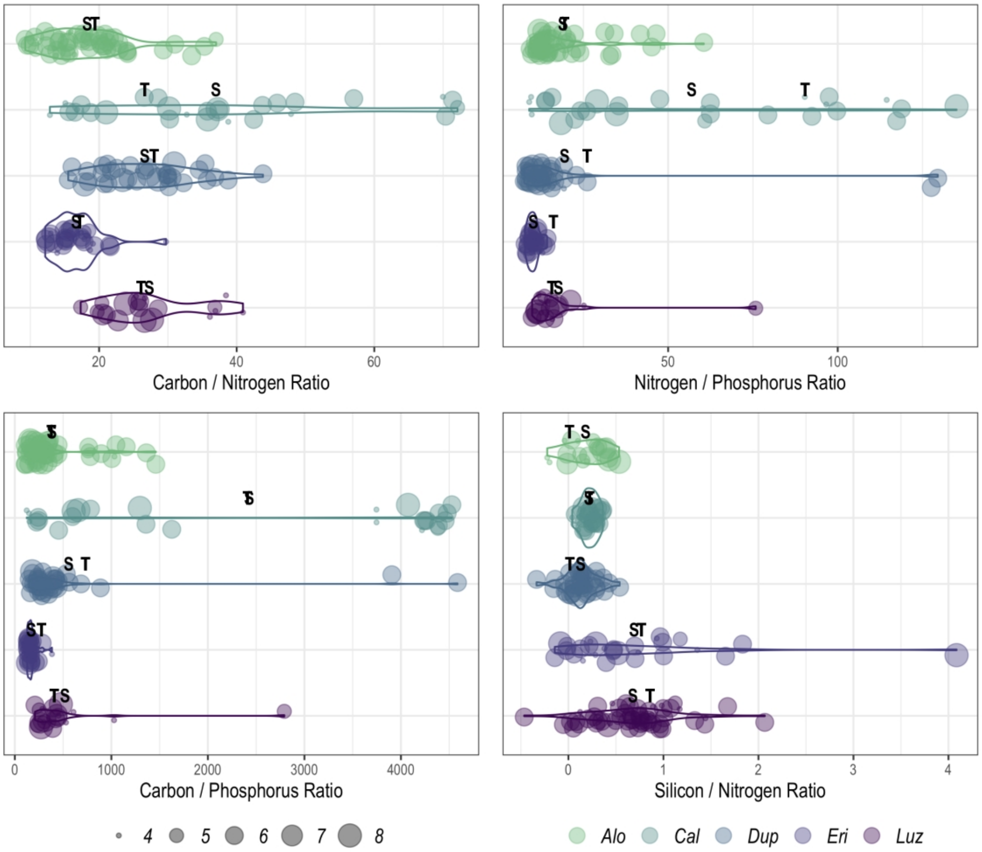


Figure 4

205x177mm (300 x 300 DPI)

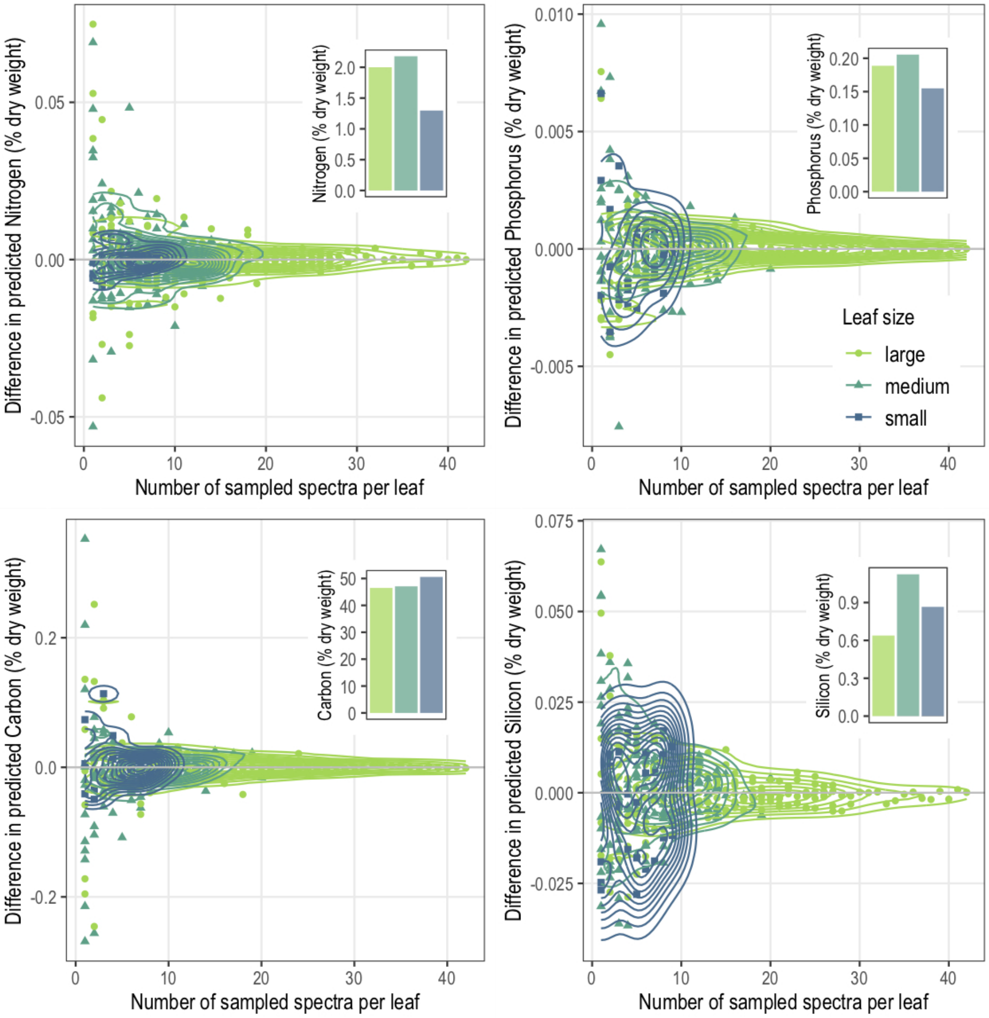


Figure 5

191x196mm (150 x 150 DPI)

SUPPLEMENTARY INFORMATION

One leaf for all: Chemical traits of single leaves measured at the leaf surface using Near infrared-reflectance spectroscopy (NIRS)

Matteo Petit Bon[1,2], Hanna Böhner[1], Sissel Kaino[1], Torunn Moe[1], Kari Anne Bråthen[1]*

[1] Department of Arctic and Marine Biology, UiT - The Arctic University of Norway, N-9037 Tromsø, Norway

[2] Department of Arctic Biology, University Centre in Svalbard (UNIS), PO Box 156, N-9171 Longyearbyen, Norway

* Corresponding author: kari.brathen@uit.no, tel +47 776 46629

Table S1. Overview of samples used to assess the number of spectra needed for predicting accurate chemical content in single leaves (Figure 4). Samples are sorted according to source species and the leaf-size category applied.

Species	Large leaf (Ø > 3 cm)	Medium leaf (Ø >1 U < 3 cm)	Small leaf (Ø < 1 cm)
Anthoxanthum nipponicum		1	
Betula nana		1	1
Bistorta vivipara		2	
Calamagrostis phragmitoides		1	
Comarum palustre	1		
Chamaepericlymenum suecicum			1
Deschampsia cespitosa		1	
Geranium sylvaticum	1		
Phleum alpinum	1		
Poa spp		1	
Rumex acetosa	1	1	
Solidago virgaurea	1	1	
Stellaria nemorum		1	
Trientalis europaea		1	
Trollius europaeus	1		
Vaccinium myrtillus		1	
Vaccinium vitis-idea			1
Viola spp		1	
Sum of leaves	6	13	3

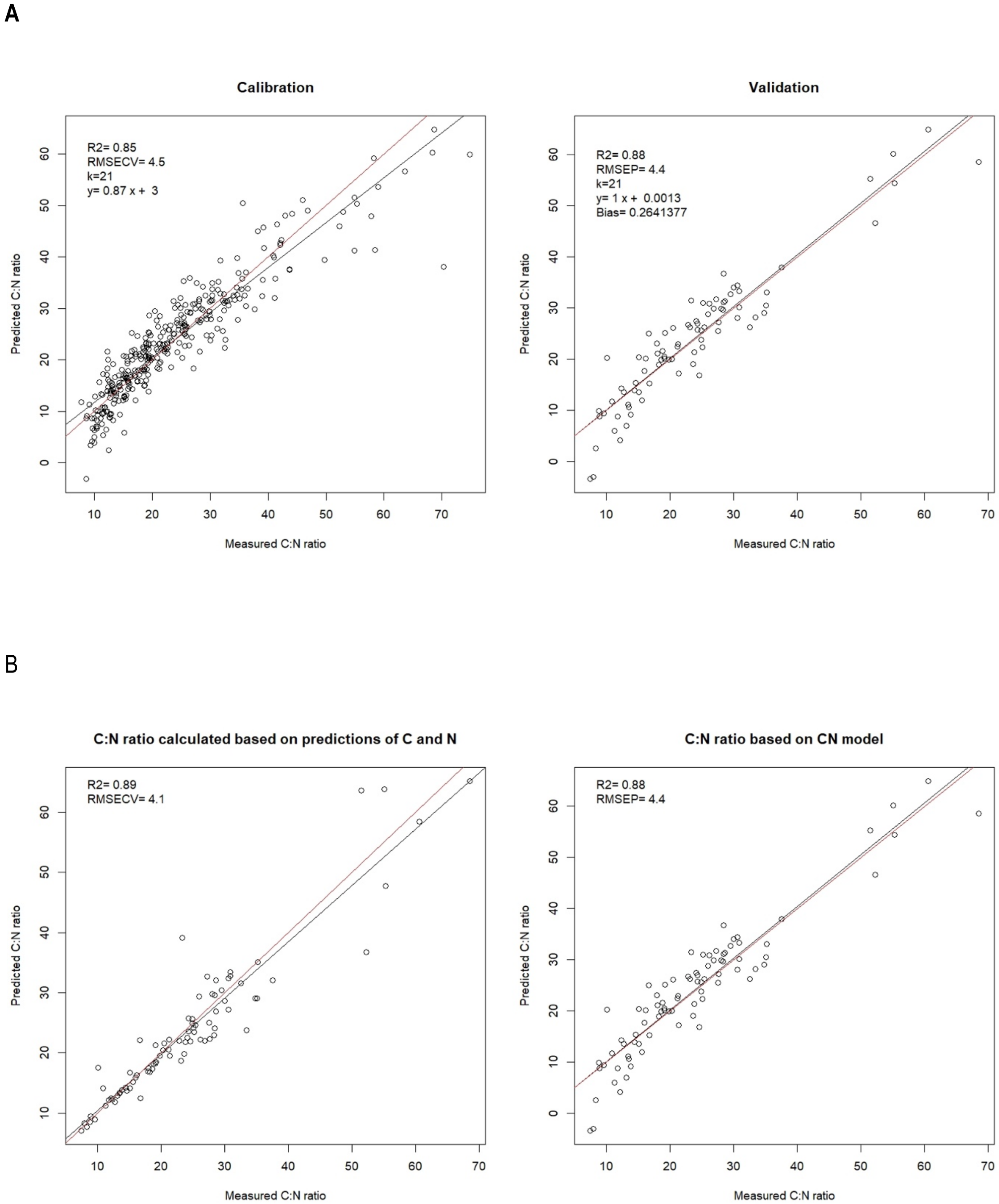
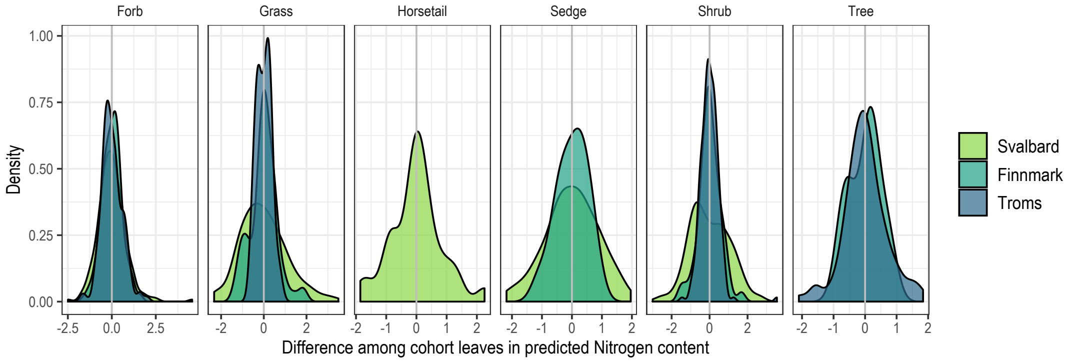
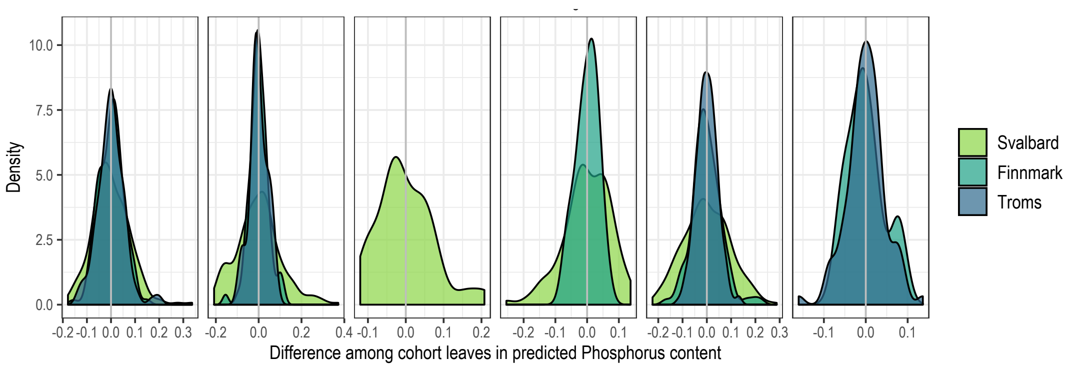
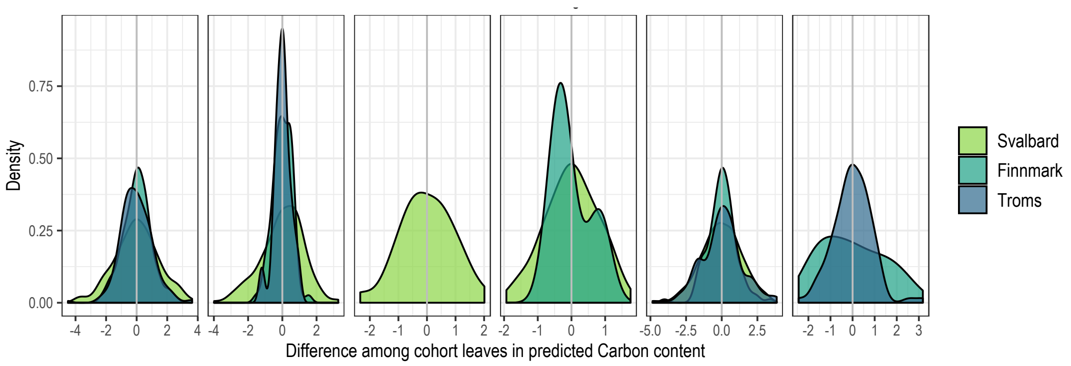


Figure S1. Calibration model of the stoichiometric ratio of Carbon to Nitrogen (CN model) and its validation (A). The predicted stoichiometric ratio of Carbon to Nitrogen based on the arctic-alpine calibration models (Murguzur et al. 2019) and the CN model, each compared to the ratio based on chemically measured Carbon and Nitrogen (B).







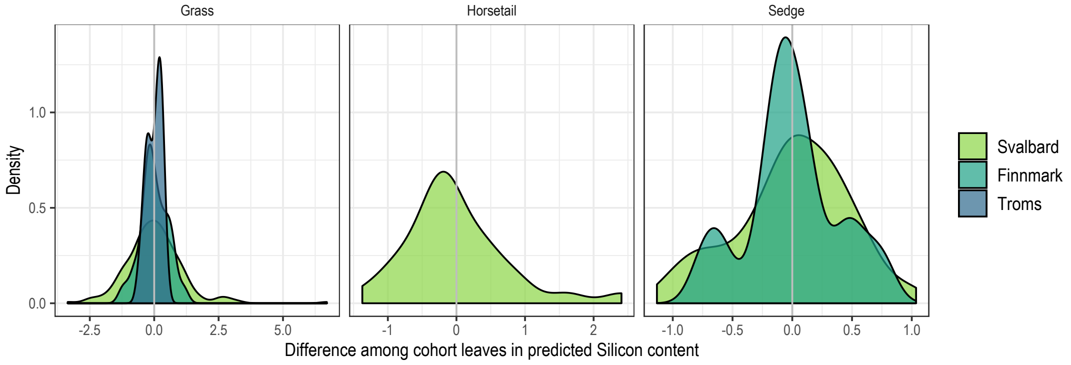


Figure S2. Density plots of the standardized predicted chemical content among leaves within cohorts (standardized towards the average content across leaves within the cohorts), sorted according to growth form and biogeographic region.

Murguzur, F.J.A., Bison, M., Smis, A., Böhner, H., Struyf, E., Meire, P. & Bråthen, K.A. (2019) Towards a global arctic-alpine model for Near-infrared reflectance spectroscopy (NIRS) predictions of foliar nitrogen, phosphorus and carbon content. *Scientific Reports,* **9,** 8259.

Paper II

Interactions between winter and summer herbivory affect spatial and temporal plant nutrient dynamics in tundra grassland communities

OIKOS

Research

Interactions between winter and summer herbivory affect spatial and temporal plant nutrient dynamics in tundra grassland communities

Matteo Petit Bon, Katarina Gunnarsdotter Inga, Ingibjörg Svala Jónsdóttir, Tove Aagnes Utsi, Eeva Marjatta Soininen and Kari Anne Bråthen

M. Petit Bon (https://orcid.org/0000-0001-9829-8324) ✉ (matteo.petitbon@gmail.com), Dept of Arctic Biology, Univ. Centre in Svalbard (UNIS), PO Box 156, NO-9171 Longyearbyen, Norway. – MPB, K. Gunnarsdotter Inga, T. A. Utsi (https://orcid.org/0000-0003-0776-2369), E. M. Soininen (https://orcid.org/0000-0003-4280-8350), K. A. Bråthen (https://orcid.org/0000-0003-0942-1074), Dept of Arctic and Marine Biology, Faculty of Biosciences, Fisheries, and Economics, Arctic Univ. of Norway (UiT), Tromsø, Norway. – I. S. Jónsdóttir (https://orcid.org/0000-0003-3804-7077), Inst. of Life and Environmental Sciences, Univ. of Iceland, Reykjavik, Iceland.

Oikos
00: 1–14, 2020
doi: 10.1111/oik.07074

Subject Editor: Eric Seablom
Editor-in-Chief: Dries Bonte
Accepted 18 April 2020

In the long-term, herbivores can alter nutrient dynamics in terrestrial ecosystems by changing the functional composition of plant communities. Here, we ask to what extent herbivores can affect plant-community nutrient dynamics in the short-term. We provide theoretical expectations for immediate effects of herbivores on tundra-grassland plant-community nutrient levels throughout a single growing season and empirically evaluate these predictions. We established an experiment within two forb-dominated and two grass-dominated tundra-grassland communities. We selected tundra-patches disturbed by small rodents during the previous winter, and neighbouring undisturbed tundra-patches. Within each tundra-patch, we set up a reindeer-open and a reindeer-exclusion plot. Throughout the summer, we randomly collected over 2800 leaf samples from 34 vascular plant species/genera and analysed their nitrogen and phosphorus contents. Plant-community nutrient levels were consistently higher in tundra-patches affected by small rodents, both across tundra-grassland types and throughout the growing season. Forbs and grasses growing in small-rodent disturbed tundra-patches had 11% and 25% higher nutrient content, respectively, compared to undisturbed tundra-patches. Reindeer affected only grasses growing in grass-dominated tundra-grasslands and the outcome was dependent on small-rodent winter disturbance. Reindeer increased grass nitrogen content in undisturbed tundra-patches (+7%) and weakened the positive effects of small rodents in disturbed tundra-patches (from 25% to 15% higher nutrient content [both nitrogen and phosphorus]). By enhancing plant nutrient levels throughout a single growing season, herbivores were key, immediate modifiers of plant-community nutrient dynamics in tundra-grasslands. Higher nutrient contents still detected in senescent leaves at the end of the summer in herbivore-affected tundra suggest that herbivory is accelerating short-term tundra-grassland nutrient cycling rates. Our findings from tundra-grassland communities align with theoretical expectations of positive herbivore effects on nutrient cycling in relatively productive ecosystems.

NORDIC SOCIETY OIKOS

www.oikosjournal.org

Keywords: near infrared reflectance spectroscopy (NIRS), nitrogen (N), phosphorus (P), plant functional types (PFTs), *Rangifer* (reindeer/caribou), small rodents

Introduction

Mammalian herbivores have long been recognized as fundamental drivers of the nutrient cycling in terrestrial ecosystems worldwide (reviewed by Pastor et al. 2006, Harrison and Bardgett 2008, Sitters and Olde Venterink 2015). In the long-term, herbivores modify the rate of nutrient cycling through several pathways, such as altering plant species composition and thus the quantity and quality of resources returned to the soil, affecting soil physical/chemical properties and/or influencing the movement of nutrients between habitats (McNaughton et al. 1997, Olofsson and Oksanen 2002, Schrama et al. 2013, Stark et al. 2015). The plant nutrient-related traits that contribute to the palatability of foliage can also govern the decomposability of plant litter (Grime et al. 1996, Cornelissen et al. 2004). Perhaps for this reason long-term herbivore-driven changes in ecosystem nutrient cycling of cold, nutrient-limited environments, such as (sub-)Arctic and alpine tundra, can be contingent on herbivore-induced changes in the functional composition of plant communities (reviewed by Stark 2007). Here, long-term herbivory can promote higher abundance of either palatable, nutrient-rich plant species (e.g. forbs and grasses) or less palatable, nutrient-poor plant species (e.g. shrubs), thus either accelerating (Olofsson et al. 2004a, Tuomi et al. 2018) or retarding (Pastor et al. 1993, Grellmann 2002) nutrient-cycling rates. However, it remains an open question whether herbivores can also cause immediate changes in tundra plant-community nutrient levels, indicative of accelerating, neutral or retarding effects on nutrient cycling rates.

There is a set of mechanisms through which herbivores may provoke immediate changes in tundra plant-community nutrient levels. Herbivores may select leaves in early phenological stages and/or more nutritious plants and plant parts (Bråthen and Oksanen 2001, Iversen et al. 2014), thus reducing the overall nutrient status of plant communities. Conversely, herbivores can increase plant-community nutrient levels by returning readily available nutrients to soil through faeces and urine (Bazely and Jefferies 1985). The latter process shortcuts the slower litter-decomposition pathway and enhances soil microbial activity and plant nutrient availability (Stark et al. 2002, Van der Wal et al. 2004). Further, herbivory may induce rapid re-growth of highly-nutritious plant tissue, i.e. keep leaves in younger phenological stages (Chapin 1980, McNaughton 1983, Mysterud et al. 2011). Consequently, one may expect herbivores to either accelerate or retard short-term nutrient-cycling rates in tundra ecosystems depending on the relative strength of these opposing mechanisms, which operate simultaneously in all ecosystems (Bardgett and Wardle 2003).

Phenological development of plants causes plant nutrient levels to change over a growing season. Because newly emergent leaves in early summer have higher nutrient contents than older, senescing leaves in late summer (Aerts and Chapin 1999), food quality for herbivores declines throughout the growing season (Albon and Langvatn 1992, Mysterud et al. 2011). However, a large variety of plant species, belonging to several plant functional types (PFTs), co-exist in plant communities (sensu Chapin et al. 1996). Nutrient levels vary among PFTs from nutrient-rich forbs, through grasses to the less nutritious shrubs (Cornelissen et al. 2004). Moreover, PFTs at high latitudes are characterized by diverse patterns in phenological development (Iversen et al. 2009), which dictate tundra plant-community nutrient status at a given time. Thus, plant-community nutrient levels vary according to plant-species composition and time of the year, which in turn are likely to mediate the interactions between tundra herbivores and their food sources (Ims and Fuglei 2005, Iversen et al. 2014). In addition, within- and between-season herbivore attraction to a given plant community, i.e. tundra-patch, is likely to be either intensified or diminished if herbivores can increase or decrease, respectively, nutrient contents of dominant plant species (Hik and Jefferies 1990). Therefore, through immediate impacts on plant-community nutrient levels, herbivores may drive spatial and temporal nutrient dynamics of tundra plant communities and manipulate their own food supply, potentially influencing habitat selection of other herbivores.

Tundra ecosystems harbour a range of different-sized mammalian herbivores, which differ in their temporal and spatial displacement (Ims and Fuglei 2005, Ims et al. 2007). Small rodents, such as voles and lemmings, strongly disturb tundra vegetation during their population density peaks, both in summer and winter (Olofsson et al. 2004b, Ims and Fuglei 2005). However, observations conducted during their population heights reveal extreme heterogeneity in small-rodent activity, with some tundra-patches experiencing a much higher disturbance level compared to nearby ones (Hambäck et al. 1998). In contrast, large vertebrates, such as *Rangifer* (reindeer/caribou), may impact the vegetation on a larger spatial and temporal scale through a migratory behaviour (Bernes et al. 2015). The conspicuous mobility of reindeer enable them to locate and utilise highly nutritious vegetation-patches distributed in space and time (Iversen et al. 2014). Studies on how different-sized mammalian herbivores affect the functional composition of tundra plant communities indicate that herbivory may have mixed effects on ecosystem nutrient cycling. Either alone or in combination, small and large herbivores have been shown to benefit sometimes species that accelerate (Olofsson et al. 2001, Van der Wal 2006, Tuomi et al. 2018) and sometimes species that retard (Bråthen and Oksanen 2001, Grellmann 2002, Bråthen et al. 2007, Ravolainen et al. 2011) tundra-ecosystem nutrient dynamics in the long-term. Yet, how do tundra-herbivores modify plant-community nutrient dynamics in the short-term? Here, we assess to what extent herbivore interactions affect tundra plant-community nutrient levels during the short duration of an alpine/sub-Arctic summer.

Tundra-grasslands are suitable systems to test short-term effects of herbivores on plant-community nutrient

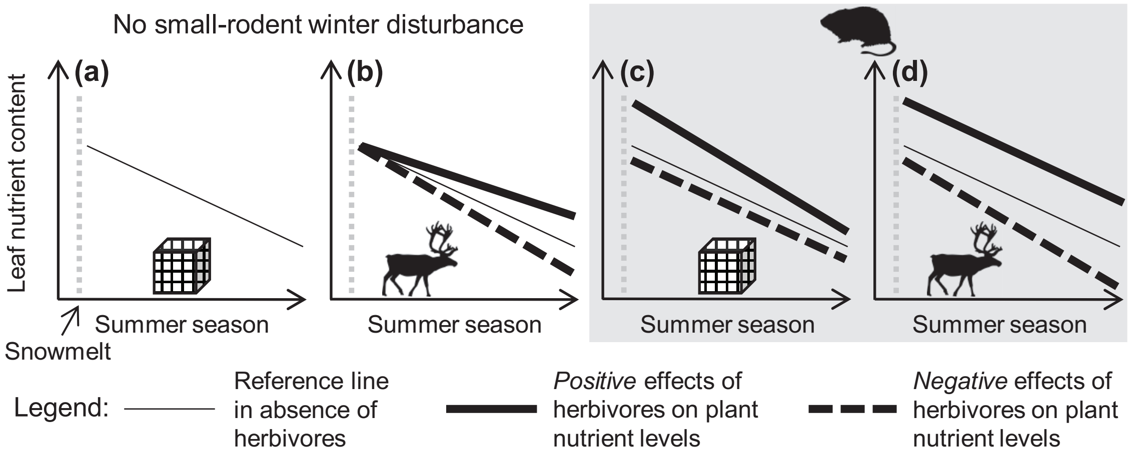


Figure 1. Schematic presentation of the two competing hypotheses addressed in this study. (a) Reference line in absence of herbivores, with plant nutrient levels decreasing along the summer. (b–d) Positive (thick solid lines) or negative (dashed lines) effects of (b) reindeer summer herbivory alone, (c) small-rodent winter disturbance alone and (d) both herbivores together on plant nutrient levels (see main text for details). The reference line in absence of herbivores (thin solid line) is maintained in each panel for an easier comparison with our competing hypotheses.

levels. First, they are characterized by plant species with high nutrient contents (i.e. forbs and grasses), and thus are key hotspots for plant–herbivore interactions (Skarin et al. 2008, Soininen et al. 2013b). Second, tundra-grasslands are characterized by both a long reindeer-grazing history (Hætta et al. 1994) and the ubiquitous presence of small rodents (Ims and Fuglei 2005). We performed an herbivore-interaction experiment during summer in two forb-dominated and two grass-dominated tundra-grasslands in northern Fennoscandia. We excluded reindeer summer herbivory both within and outside tundra-patches that had been disturbed by small rodents during the previous winter. Higher plant-community nutrient levels in the presence of herbivores would suggest that herbivory is accelerating short-term nutrient cycling rates in these tundra-grasslands. Conversely, lower plant-community nutrient levels in the presence of herbivores would indicate that herbivory is retarding short-term tundra-grassland nutrient cycling rates. Given the strong inter- and intra-specific variability of nutrient-related plant traits (Siefert et al. 2015), we sampled all the main species found within tundra-grasslands in our experiment. Moreover, to account for the high intra-specific phenological variation of nutrient-related plant traits (Fajardo and Siefert 2016), we encompassed the whole range of plant-leaf developmental stages by repeatedly sampling throughout the whole growing season.

Given the seasonal component in plant nutrient contents (Aerts and Chapin 1999), we expected that, in absence of herbivores, plant-community nutrient levels will be highest at the beginning of the season and that they will decrease as the summer progresses (Fig. 1a). Based on current theoretical and empirical evidence, we formulated two competing hypotheses for immediate effects of herbivores on tundra-grassland plant-community nutrient levels. By returning nutrients through faeces and urine (Bazely and Jefferies 1985) or by keeping leaves in young phenological stages (Chapin 1980), small-rodent winter disturbance or reindeer summer herbivory alone will enhance plant-community nutrient levels (Fig. 1b–c – thick solid lines). Alternatively, by removing more nutrient-rich plant species or by selecting more nutritious plant parts (Pastor et al. 1993), either herbivore alone will cause negative plant-community nutrient responses (Fig. 1b–c – dashed lines). Both herbivores together will cause either positive (Fig. 1d – thick solid line) or negative (Fig. 1d – dashed line) plant-community nutrient responses depending on magnitude and direction of their main effects. We expected plant-community nutrient responses to herbivores to be paralleled by responses of the dominant PFTs, i.e. forbs and grasses. The effects of reindeer on plant nutrient levels will grow stronger as the season proceeds, reflecting repeated events of herbivory. Effects of small-rodent winter disturbance, if positive, will instead be mainly visible at the beginning of the summer due to a sudden release of nutrients in the system soon after snowmelt, after which plant-community nutrient dynamics will converge towards those of plant communities not affected by herbivores.

Methods

Study area

The study was conducted in the low alpine zone at 300–400 m a.s.l. at Ifjordfjellet (70°27′N, 27°08′E), Finnmark, northern Norway (Fig. 2a) during the summer season of 2015. The annual temperature of the study area in the warmest month (July) ranges from 8.2 to 13.6°C (period 1986–2015), with a mean of 8.6°C in July 2015. Total annual precipitation for the same period ranges from 429 to 704 mm, with 564 mm fallen in 2015 (Norwegian Meteorological Institute, <http://met.no>). The bedrock consists of sedimentary rocks, mainly sandstone and mudstone (Geological Survey of Norway, <http://ngu.no>). Date of snowmelt varies from early to

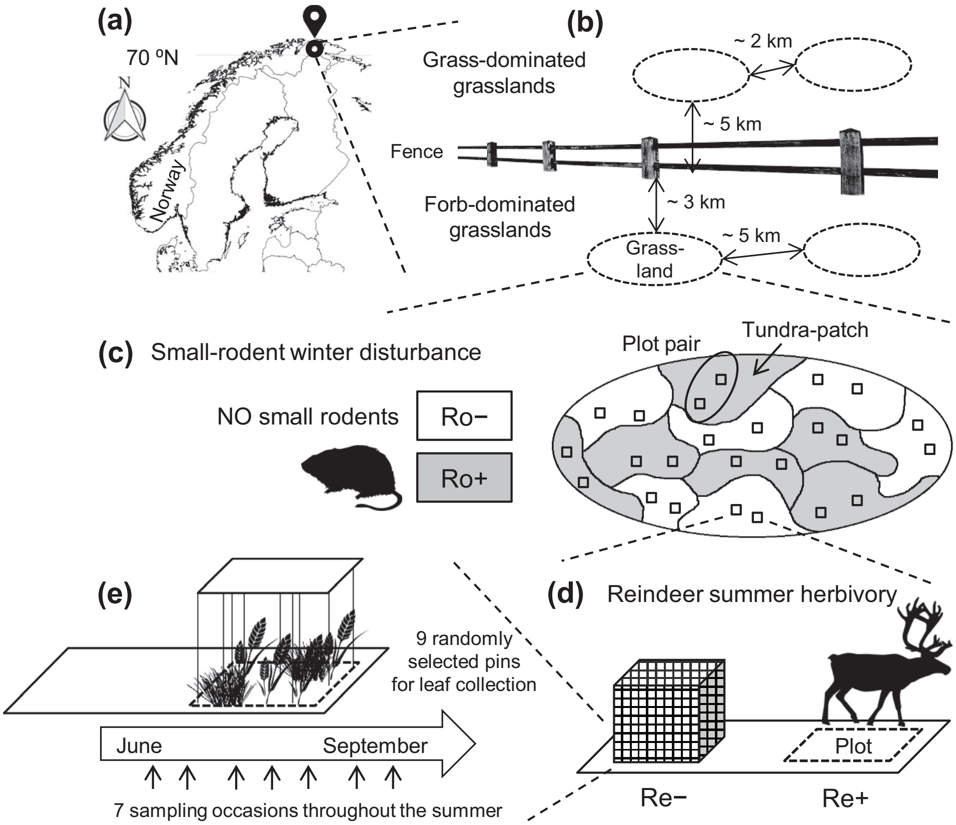


Figure 2. Study and sampling design. (a) Study location, (b–d) hierarchical spatial structure of the study design (four grassland sites [two sites in each of the two grassland types], for a total of 48 plot pairs) and (e) temporal structure of the sampling design adopted for plant leaf collection.

late June. In 2015, our study area was snow-free by the end of June.

The region is mainly characterized by dwarf-shrub heaths (Walker et al. 2005), whereas grasslands typically dominate river plains. A fence was built in 1950s and divided Ifjordfjellet in spring/fall migratory and summer pasture ranges for semi-domesticated reindeer *Rangifer tarandus tarandus* (Hætta et al. 1994). Historical differences in reindeer herbivory pressure on the two sides of the fence caused a divergence in plant-community composition of grassland communities. Grasslands found in the spring/fall migratory range are forb-dominated, whereas grasslands found in the summer range are grass-dominated. Reindeer faeces counts in permanent plots during the last five years showed that reindeer also utilize forb-dominated grasslands in summer. Yet, a higher reindeer grazing pressure in grass-dominated grasslands was evident throughout the summer season of 2015 (Supplementary material Appendix 1 Fig. A1a). Common plant species across these grasslands are forbs, such as *Alchemilla* spp., *Bistorta vivipara*, *Geranium sylvaticum*, *Rumex acetosa*, *Solidago virgaurea*, *Trollius* spp. and *Viola* spp. along with grasses, such as *Poa* spp., *Calamagrostis phragmitoides*, *Deschampsia cespitosa* and *Nardus stricta*. Plant names follow the Pan-Arctic Flora (<http://nhm2.uio.no/paf>).

The semi-domesticated reindeer is the main large herbivorous mammal in the study area, which is encompassed by the Lágesduotter reindeer herding district, where a density of 4.8 reindeer km^{-2} was estimated in summer 2015 (<https://landbruksdirektoratet.no>). Along with semi-domesticated reindeer, other wild large herbivores occasionally found in these areas are moose *Alces alces*. The community of medium-sized vertebrate herbivores consists of ptarmigan *Lagopus lagopus* and *L. muta* and hare *Lepus timidus*. Three species of small rodents (tundra vole *Microtus oeconomus*, grey-sided vole *Myodes rufocanus* and Norwegian lemming *Lemmus lemmus*) are active year-round; tundra vole is the species dominating the small-rodent guild in tundra-grasslands (Killengreen et al. 2007).

Study design

We implemented a full-factorial semi-randomized pair design in which small-rodent winter disturbance was used as a quasi-experimental factor (Shadish et al. 2002) and reindeer summer herbivory as a fully experimental factor. Immediately after snowmelt, we selected two relatively homogeneous grassland sites of about 1 km^2 within each of the two grassland-types (Fig. 2b). Twelve pairs of plots were laid within each grassland site. Six plot-pairs were located in tundra-patches disturbed by small rodents during the previous winter (disturbed tundra-patches: Ro+), whereas the other six plot-pairs were arranged in tundra-patches with no evident signs of small-rodent disturbance (undisturbed tundra-patches: Ro−) (Fig. 2c). Finally, plots within a pair were randomly assigned to be either reindeer-open (Re+) or reindeer-exclusion (Re−) plots (Fig. 2d). In total, 96 plots were established. The area of each plot was 60 × 60 cm. To exclude reindeer, we used cages made of metal net (70 × 70 cm area × 50 cm height, mesh-size 1.3 × 1.3 cm).

Distance between plots within a pair was maximum 3 m, whilst distance between neighbouring plot-pairs was at least 3 m. Suitability of tundra-patches was evaluated following two criteria: 1) plots within a pair were characterized by similar plant species composition and micro-topographical features, and 2) for Ro+ plot-pairs at least 80% of the plot surface should show signs of winter disturbance by small rodents, whereas for Ro− plot-pairs there should be no visible signs of small-rodent disturbance. Small-rodent winter activity had visibly altered the vegetation in disturbed tundra-patches, where most aboveground plant parts were cut down and found in the form of a litter layer often mixed with soil particles and rodent waste products (Supplementary material Appendix 1 Fig. A2a–b). The extreme heterogeneity in small-rodent winter disturbance observed within our relatively homogeneous grassland sites suggests a rather random fine-scale spatial variation in winter herbivory (Hambäck et al. 1998). Gaps between cages and the ground allowed small rodents to have free access into Re− plots. Small-rodent faeces counts in the permanent plots indicated that the number of small rodents within the study area in early summer 2015 was low, but it increased throughout the growing season at both grassland-types (Supplementary material Appendix 1 Fig. A1b). Small-rodent effects throughout the summer are assumed to be equal in all plots.

Sampling design, sample analyses and data processing

Leaf sampling was performed within the 96 plots at seven instances (hereafter sampling occasions) from the start to the end of the growing season; on average every 12 ± 1.3 days, between 29 June and 9 September (Supplementary material Appendix 1 Table A1). This sampling design allowed us to encompass the whole range of leaf developmental stages, i.e. from newly emergent leaves soon after snowmelt to old senescent leaves before the first snowfall. Leaf sampling was conducted randomly by using nine pins attached to a metal frame (42 × 46 cm area × 40 cm height) consisting of 50 regularly-distributed pin placements (Fig. 2e, Supplementary material Appendix 1 Fig. A2c). We used a sampling frame smaller than the plot size to minimize possible edge effects. The position of the pins was changed at each sampling occasion, but not the position of the metal frame, and pin placements used in the previous two sampling occasions were excluded from the random selection in order to minimize the possibility of sampling repeatedly the same ramets. The uppermost plant leaf touching each pin was collected. Additional leaves touching the pin were collected whenever the first leaf was not considered to be large enough for nutrient content analyses (a minimum leaf area of 4 mm in diameter). Since grasses intercept pins more easily than other PFTs (Bråthen and Hagberg 2004) and the aim of the sampling was to obtain a complete spectrum of the plant community composition in our plots, a correction for the Poaceae family was introduced. When Poaceae species were hit by more than two pins, the second uppermost plant leaf was sampled for the consecutive pins as long as it belonged to a species (or genera when the species were not further identified) of a different PFT. The final number of species sampled within a plot at each sampling occasion ranged between a minimum of three and a maximum of nine; on average 4.3 ± 1.5 species. We assume the number of samples collected for a given species or PFT to reflect its relative abundance within our grasslands.

We collected in total 2831 plant leaf samples from 34 species/genera belonging to seven broadly-classified PFTs, i.e. (number of species in brackets) forbs (21), grasses (5), sedges (1), deciduous (2) and evergreen (3) shrubs, rushes (1) and horsetails (1). As a consequence of the random sampling and/or the development of our tundra-grassland communities throughout the summer, the frequency of occurrence of different plant species/genera varied between sampling occasions (Supplementary material Appendix 1 Table A2). During fieldwork, each sample was placed in a tea-filter bag and pressed within 1–10 h after collection by using a plant press. In the lab, all leaf samples were oven-dried flat at 60°C for 48 h within four days of collection and subsequently stored in their original tea-filter bags in a dry and dark place at room temperature.

All samples were analysed for nitrogen (N) and phosphorus (P) contents (% of dry weight – %DW) using near infrared reflectance spectroscopy (NIRS) with a FieldSpec 3 in 350–2500 nm range and equipped with a 4 mm light-adapter for full-leaf scanning. Prior to analysis, plant samples were cleaned from dust particles. Because traces of water can cause distortion in the light absorbance and undermine predictions of leaf elemental contents, samples were oven-dried again at 60°C for 2 h following Smis et al. (2014). Subsequently, samples were cooled down in a desiccator until scanning by NIRS. For species with leaves narrower than 4 mm, leaves were cut and stacked together in order to cover the minimum area needed for analyses. For each plant sample, between 4 and 32 measurements (i.e. scans) were taken (on average 7 ± 3.1 scans), leading to a total of 19 834 spectra. The high variability in the number of scans per sample reflects the variability of both number and size of the leaves constituting that sample. Each sample spectrum was converted to N- and P-content by applying the prediction models based on milled and tableted plant samples (Murguzur et al. 2019) and with correction factors for full leaves (Petit Bon et al. 2020). For each sample, we finally used the median of the replicate scans for data analyses. In total, 63 (2.23% of the data) predicted median values for leaf P-content fell outside the calibration range of our prediction model (range for leaf P-content: 0.04–0.70 %DW; Murguzur et al. 2019). To avoid possible statistical artefacts, these data were excluded from the analyses.

In sampling occasion four (3–5 of August, peak of the growing season), point intercept frequency method (PIM – Bråthen and Hagberg 2004) was performed to assess differences in plant-community composition between forb- and grass-dominated tundra-grasslands. Within each plot, all intercepts between the nine randomly selected pins for leaf sampling and each vascular plant species were counted. Point intercepts for each species were finally converted into above-ground plant biomass ($g\,m^{-2}$) following Bråthen and Hagberg (2004) and by using the correlation coefficients in Ravolainen et al. (2010) and Tuomi et al. (2018). A pin density of nine pins per $0.25\,m^{-2}$ area (our sampling frame covered $0.19\,m^{-2}$ area) is within the recommendations for efficient estimation of plant biomass (Bråthen and Hagberg 2004) and has been utilized in previous studies quantifying tundra-grassland plant-community responses to herbivory (Ravolainen et al. 2011). Soil environmental variables were also measured to characterize forb- and grass-dominated tundra-grasslands. We measured soil moisture within plots throughout the summer. In sampling occasion five (13–15 August), we collected samples of organic soil within plots and assessed their pH and N- and P-content. Plant-species composition, PFT above-ground biomass and soil environmental characteristics for forb- and grass-dominated grasslands are presented in Supplementary material Appendix 1 Table A3, Fig. A3.

Statistical analysis

Prior to model fitting, data exploration was conducted following standardized protocols (Zuur et al. 2010). In particular, we explored possibly introduced non-random trends in missing observations for leaf P-content. No patterns were identified that could affect the estimates of the models and hence modify our biological conclusions.

Plant communities

The effect of herbivores on plant-community nutrient levels was evaluated by using linear mixed-effects models (LMMs). We fitted a separate LMM for the two response variables of interest (plant leaf nitrogen [N] and phosphorus [P] contents – %DW), in which the initial full fixed-effects structure included the three-way interaction between 'small-rodent winter disturbance' (two-level factor: undisturbed [Ro−] and disturbed [Ro+] tundra-patches), 'reindeer summer herbivory' (two-level factor: reindeer-exclusion [Re−] and reindeer-open [Re+] plots), and 'seasonality' (continuous variable: seven sampling occasions throughout the summer). Given the hierarchical spatial structure of the study design and the fact that the same plant species could be sampled across all plots, both nested and crossed random-effects had to be specified (Baayen et al. 2008). In both LMMs, the study design was entered as nested random factors, with 'plots' nested within 'tundra-patch', and subsequently nested within 'grassland site. 'Plots' accounted for both the hierarchical nested design of our study and the repeated measures over the summer. 'Plant species' was entered as a crossed random-effect with the hierarchical nested design. We fitted full random-intercept LMMs and avoided random-slope LMMs to prevent over-parameterization and convergence problems, as suggested by Bates et al. (2015a).

Plant functional types (PFTs)

Plant-community composition significantly differed between forb- and grass-dominated tundra-grasslands (Supplementary material Appendix 1 Fig. A3 and Supplementary material Appendix 3), and thus the effects of herbivores on PFT nutrient levels were evaluated separately for the two grassland-types. We analysed the data obtained for forbs and grasses (over 91% of the collected plant leaf samples). Results for sedges and deciduous shrubs (6.7% of the samples), for which sample sizes did not allow full model fitting, are presented in Supplementary material Appendix 1 Table A4, A5, Fig. A4, A5. Evergreen shrubs, rushes and horsetails (2% of the samples) were not analysed statistically because of too small sample sizes for model fitting (summary statistics for these PFTs are given in Supplementary material Appendix 1 Table A6). We fitted a separate LMM for forb- and grass-dominated grasslands and for the two response variables of interest (leaf N and P – %DW). We avoided a direct statistical comparison between forb- and grass-dominated grasslands given only two grassland sites within each grassland-type. We first created full LMMs with a basic fixed-effects structure including the four-way interaction between 'PFT' (two-level factor: forbs and grasses), 'small-rodent winter disturbance', 'reindeer summer herbivory' and 'seasonality'. 'Site' (two-level factor) was technically considered as random-effect, but incorporated as additive fixed-effect in the models since too few levels were available to properly determine its variance component (Bolker et al. 2009). In all full LMMs, which were fitted with random-intercepts only (see above), the study design was entered as nested random factors, with 'plots' nested within 'tundra-patch'. 'Plant species' was entered as a crossed random-effect with the hierarchical nested design.

For each LMM, we first selected the better random-effects structure by removing from the models those random terms for which the variance was estimated as zero. We then selected the better fixed-effects structure using likelihood ratio test (model parameters estimated using maximum likelihood – ML). We here only present the results from the most parsimonious LMMs (fitted by restricted ML), for which statistically significant effects were defined by 95% confidence intervals not encompassing 0. We proceeded to model validation by assessing homogeneity of variances in the residuals for the fixed-effects retained in each model and checking for approximate linearity between observed and fitted values. We estimated the significance of the fixed-effects using parametric bootstrapping with 10 000 replicates.

Additional details on statistical analyses are provided in Supplementary material Appendix 3. All statistical analyses were conducted in the R environment ver. 3.6.1 (<www.r-project.org>) with the packages 'lme4' (Bates et al. 2015b), 'emmeans' (Lenth et al. 2018) and 'ggplot2' (Wickham 2016).

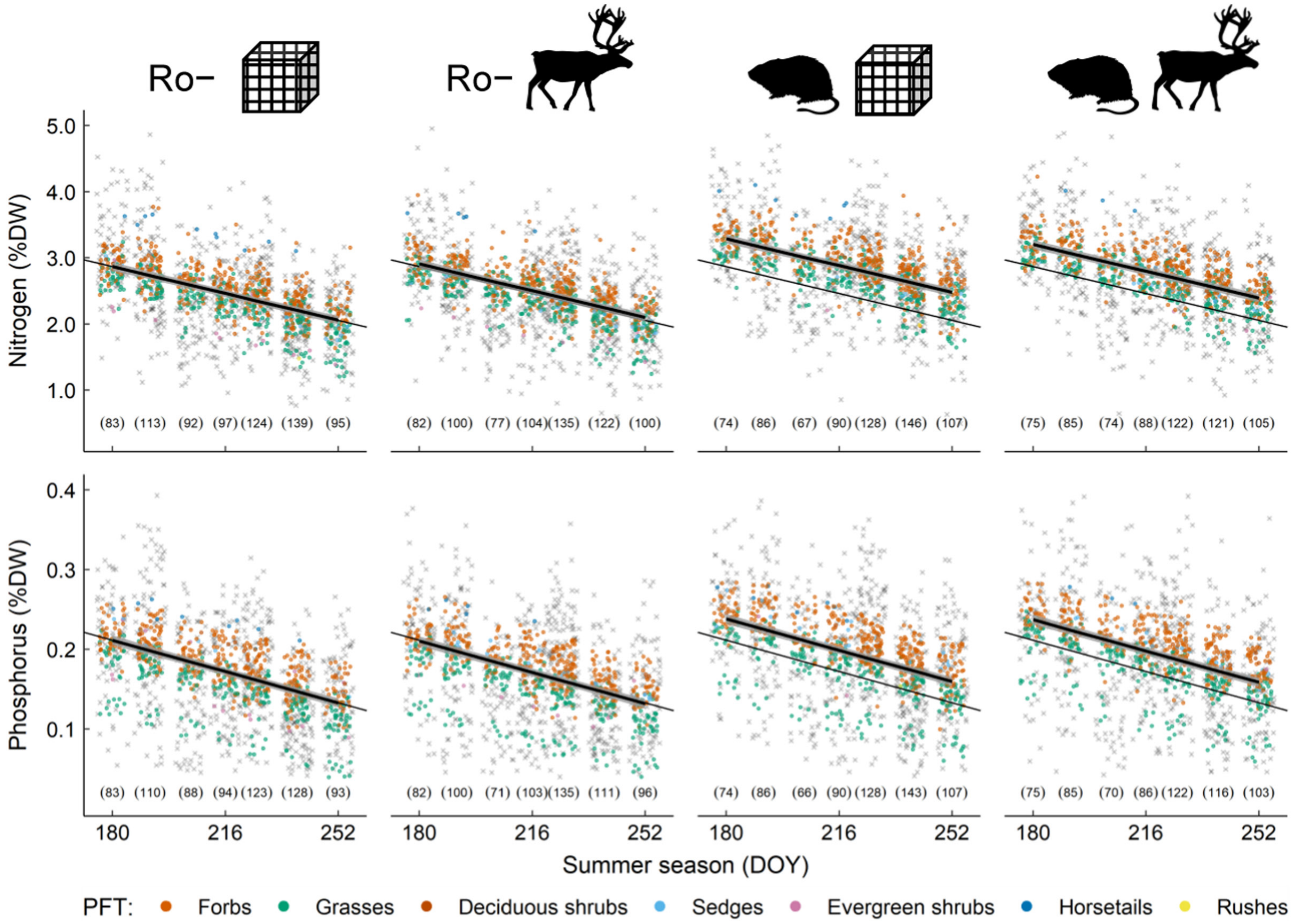


Figure 3. Effects of herbivores on overall tundra-grassland plant-community nutrient levels. Effects of small-rodent winter disturbance, reindeer summer herbivory and seasonality on leaf nitrogen [N] content (upper panel) and leaf phosphorus [P] content (lower panel) (% of dry weight – %DW) in plant communities of tundra-grasslands. Thick lines and bands represent regression lines for the fitted values and their 95% confidence intervals (CIs). Fitted values were acquired from the prediction models on plant-community N- and P-content. The reference line (thin line) in absence of herbivores [Ro−/Re−] is maintained in each panel to facilitate the visualization of the effects of herbivores on plant-community nutrient levels. Coloured dots represent fitted values for each plant leaf sample, whereas grey dots represent raw values. All dots were spaced apart within each of the seven sampling occasions to reduce overlapping. Numbers in parentheses represent the number of plant leaf samples collected at each sampling occasion in each herbivore–treatment combination. Parameter estimates and their CI are provided in Table 1.

Results

Plant-community and PFT leaf nutrient levels in absence of herbivores

Plant-community nutrient levels peaked in early summer soon after snowmelt and significantly decreased by the end of the growing season (N-content: −28%; P-content: −37%) (Fig. 3 [Ro−/Re−], Table 1). Forbs and grasses differed in their nutrient contents and dynamics throughout the summer, although a seasonal decrease in nutrient levels characterized both PFTs (Fig. 4, 5 [Ro−/Re−], Table 2, 3). Soon after snowmelt, forbs in forb-dominated grasslands had on average 22% higher N- and P-content compared to grasses (Fig. 4 [Ro−/Re−], Table 2). In grass-dominated grasslands, the difference was similar for N-content, but forbs had 52% higher P-content than grasses (Fig. 5 [Ro−/Re−], Table 3). Although nutrient levels were consistently higher in forbs, the relative difference in PFT nutrient contents decreased throughout the growing season (significant two-way 'PFT × seasonality' interactions – Table 2, 3). At the end of the summer, forbs and grasses in forb-dominated grasslands did not significantly differ in their nutrient levels (Fig. 4 [Ro−/Re−]), whereas in grass-dominated grasslands, forbs still had higher nutrient contents (14% and 25% higher N and P, respectively) compared to grasses (Fig. 5 [Ro−/Re−]). Nutrient levels of sedges and deciduous shrubs also significantly decreased throughout the summer (Supplementary material Appendix 1 Table A4, A5, Fig. A4, A5).

Plant-community leaf-nutrient levels in presence of herbivores

Small-rodent winter disturbance alone significantly increased plant-community N- and P-content of about 16% and this

Table 1. Parameter estimates for linear mixed-effects models for the effects of herbivores on overall tundra-grassland plant-community nutrient levels. Parameter estimates of fixed-effects (Estimates) and their 95% confidence interval (CI – lower and upper bounds) for the most parsimonious models in which predictors are 'small-rodent winter disturbance', 'reindeer summer herbivory' and 'seasonality' and responses are (a) leaf nitrogen [N] content and (b) leaf phosphorus [P] content (% of dry weight – %DW) in plant communities of tundra-grasslands. Intercept is calculated for undisturbed tundra-patches (Ro−), reindeer-exclusion plots (Re−) and first sampling occasion (DOY 180, 29 June). Estimates with bold indicate that their 95% CI does not include 0. Random-effects retained in the final models are presented as standard deviations. Empty cells indicate that a predictor was not statistically significant, thus it was removed from the model. Observations refer to the number of plant leaf samples used in each model. Marginal R^2 represents the variance explained by the model when only fixed-effects are considered, whereas the conditional R^2 represents the variance explained by the model when both fixed- and random-effects are considered.

	(a) Nitrogen (%DW)		(b) Phosphorus (%DW)	
Fixed-effects (Predictors)	Estimates	CI (95%)	Estimates	CI (95%)
(Intercept) [Ro−/Re−/DOY = 180]	**2.86*** **	**2.65; 3.07**	**0.212*** **	**0.193; 0.231**
Small-rodent winter disturbance [Ro+]	**0.42*** **	**0.34; 0.50**	**0.0267*** **	**0.020; 0.033**
Reindeer summer herbivory [Re+]	0.04	−0.01; 0.10	−0.001	−0.005; 0.003
Seasonality [+1 DOY]	**−0.011*** **	**−0.012; −0.010**	**−0.0011*** **	**−0.0012; −0.0010**
Herbivore interaction [Ro+ × Re+]	**−0.13** **	**−0.21; −0.04**		
Random-effects	SD		SD	
Grassland site	0.12		0.012	
Grassland site: Tundra-patch	0.09		0.009	
Plant species	0.41		0.029	
Residual	0.55		0.056	
Observations	2831		2768	
Marginal R^2/Conditional R^2	0.162/0.483		0.157/0.373	

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

effect was consistent throughout the summer (Fig. 3 [Ro+/Re−], Table 1). Reindeer summer herbivory alone did not significantly affect plant-community nutrient levels. However, reindeer significantly weakened the positive effects of small rodents on plant-community N-content (significant two-way 'Ro+ × Re+' interaction – Table 1a). Indeed, both herbivores together significantly increased plant-community N-content of about 13%, i.e. less than small rodents alone (Fig. 3 [cf. Ro+/Re+ and Ro+/Re−]). Such counteractive effect of reindeer on positive plant-nutrient responses induced by small rodents only characterized grass-dominated grasslands, but not forb-dominated grasslands, when the two grassland-types were analysed separately (see below and cf. Fig. 4, 5).

PFT leaf-nutrient levels in presence of herbivores

Small-rodent winter disturbance had clear effects on PFT nutrient contents. Small rodents alone significantly increased nutrient levels in forbs (about 11% increase in N- and P-content) and even more in grasses (about 25% increase in N- and P-content) (significant two-way 'PFT × Ro+' interactions – Table 2, 3). This diminished partially (in grass-dominated grasslands) and completely (in forb-dominated grasslands) the nutrient-level gap between the two PFTs observed in absence of herbivores (Fig. 4, 5 [Ro+/Re−]). Forbs and grasses in small-rodent disturbed tundra-patches of forb-dominated grasslands did not significantly differ in their nutrient levels at any time during the summer (Fig. 4 [Ro+/Re−]). Furthermore, nutrient levels in plants affected by small rodents alone remained consistently higher compared to those in absence of herbivores throughout the entire summer season (Fig. 4, 5 [Ro+/Re−], Table 2, 3). Grasses in small-rodent disturbed tundra-patches at the end of the summer had on average only a non-significant 5% lower N- and P-content compared to those not affected by herbivores in early summer soon after snowmelt (Fig. 4, 5 [Ro+/Re−]). Similar nutrient responses also characterized forbs and grasses affected by both herbivores in forb-dominated grasslands (Fig. 4 [cf. Ro+/Re+ and Ro+/Re−], Table 2). N-content in sedges and N- and P-content in deciduous shrubs were also significantly increased by small rodents alone (Supplementary material Appendix 1 Table A4, A5, Fig. A4, A5).

Reindeer summer herbivory had weaker and more variable effects than small-rodent winter disturbance. Reindeer did not significantly affect nutrient levels in forbs, but consistently affected those of grasses throughout the entire summer season. However, such effects were only detected in grass-dominated grasslands and were found to be dependent on small-rodent winter disturbance (significant three-way 'PFT × Ro+ × Re+' interaction – Table 3). In undisturbed tundra-patches, reindeer significantly increased grass N-content by 7% (Fig. 5 [Ro−/Re+], Table 3a). In contrast, in disturbed tundra-patches, reindeer significantly weakened the positive effects of small rodents by reducing grass N- and P-content of about 10% compared to small-rodent disturbed/reindeer-exclusion plots (Fig. 5 [cf. Ro+/Re+ and Ro+/Re−], Table 3). Yet, grasses affected by both herbivores still had a significant 15% higher N- and P-content than grasses not affected by herbivores (Fig. 5 [Ro+/Re+]). A similar counteractive effect of reindeer summer herbivory when acting in small-rodent disturbed tundra-patches was also observed for leaf N-content in deciduous shrubs, whereas reindeer alone did not affect nutrient levels in either deciduous shrubs or sedges (Supplementary material Appendix 1 Table A4, A5, Fig. A4, A5).

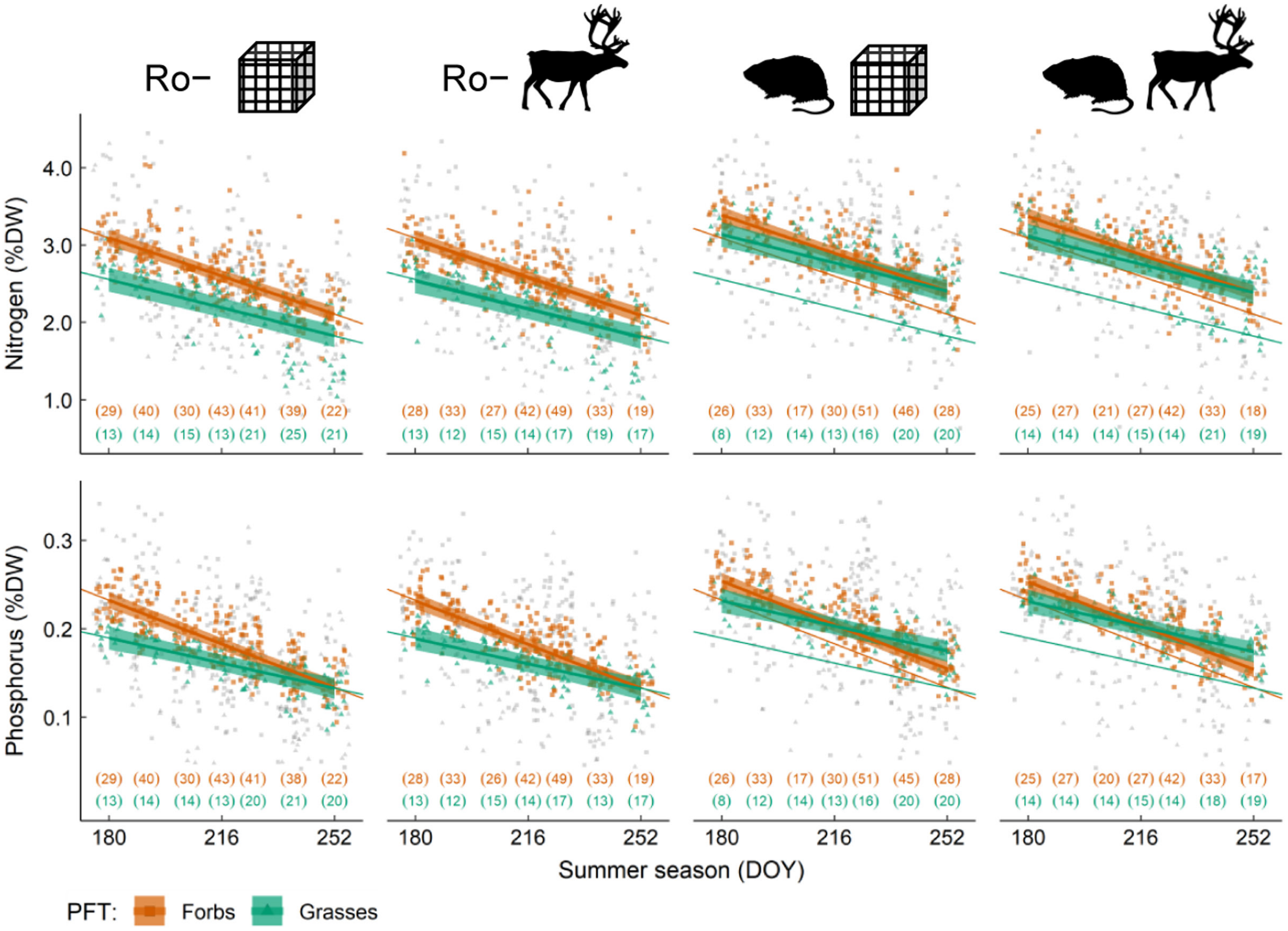


Figure 4. Effects of herbivores on plant functional type (PFT) nutrient levels in forb-dominated tundra-grasslands. Effects of small-rodent winter disturbance, reindeer summer herbivory and seasonality on leaf nitrogen [N] content (upper panel) and leaf phosphorus [P] content (lower panel) (% of dry weight – %DW) in forbs and grasses. Fitted values were acquired from the prediction models on PFT N- and P-content in forb-dominated grasslands. The reference lines (thin lines) are given separately for forbs and grasses. Description of graph content is provided in Fig. 3. Parameter estimates and their CI are provided in Table 2.

Discussion

Based on current theoretical/empirical evidence, we hypothesized that mammalian herbivores could have either positive or negative short-term effects on tundra-grassland plant-community nutrient levels. The key result of this study is that tundra-grassland communities responded to herbivores with an increase in their N and P levels. Contrary to our predictions, we found that these positive effects of herbivores were temporally consistent throughout the summer and higher nutrient levels were still detected in senescent leaves at the onset of the winter period. This demonstrates that herbivores act as positive, short-term modifiers of tundra-grassland plant-community nutrient dynamics, potentially accelerating short-term nutrient cycling rates by enhancing plant nutrient levels. As expected, these immediate, positive plant-community nutrient responses to herbivores paralleled those of the dominant, nutrient-rich PFTs, i.e. forbs and grasses. Overall, our findings from tundra-grasslands align with theoretical expectations of positive herbivore effects on nutrient cycling in relatively productive ecosystems.

In our study, tundra-plant N- and P-content were assessed within one growing season and significant community-level nutrient responses to herbivores were readily detected. By using near infrared reflectance spectroscopy (NIRS) methodology, we were able to assess plant nutrient contents at the bite-size level of the herbivores, ultimately providing precise estimates of herbivore-induced nutrient-level changes at the plant-community level. We are not aware of other studies from tundra ecosystems that report such fine-scale estimates for plant nutrient responses to herbivores at the level of the whole plant community. However, previous field-based studies focusing on the most abundant plant species and/or PFTs within tundra-grasslands have typically found little or no effects of herbivore activities on plant nutrient contents (Van der Wal et al. 2004, Mysterud et al. 2011, Barthelemy et al. 2015). These studies used methods that required merging several leaves together in order to obtain enough plant

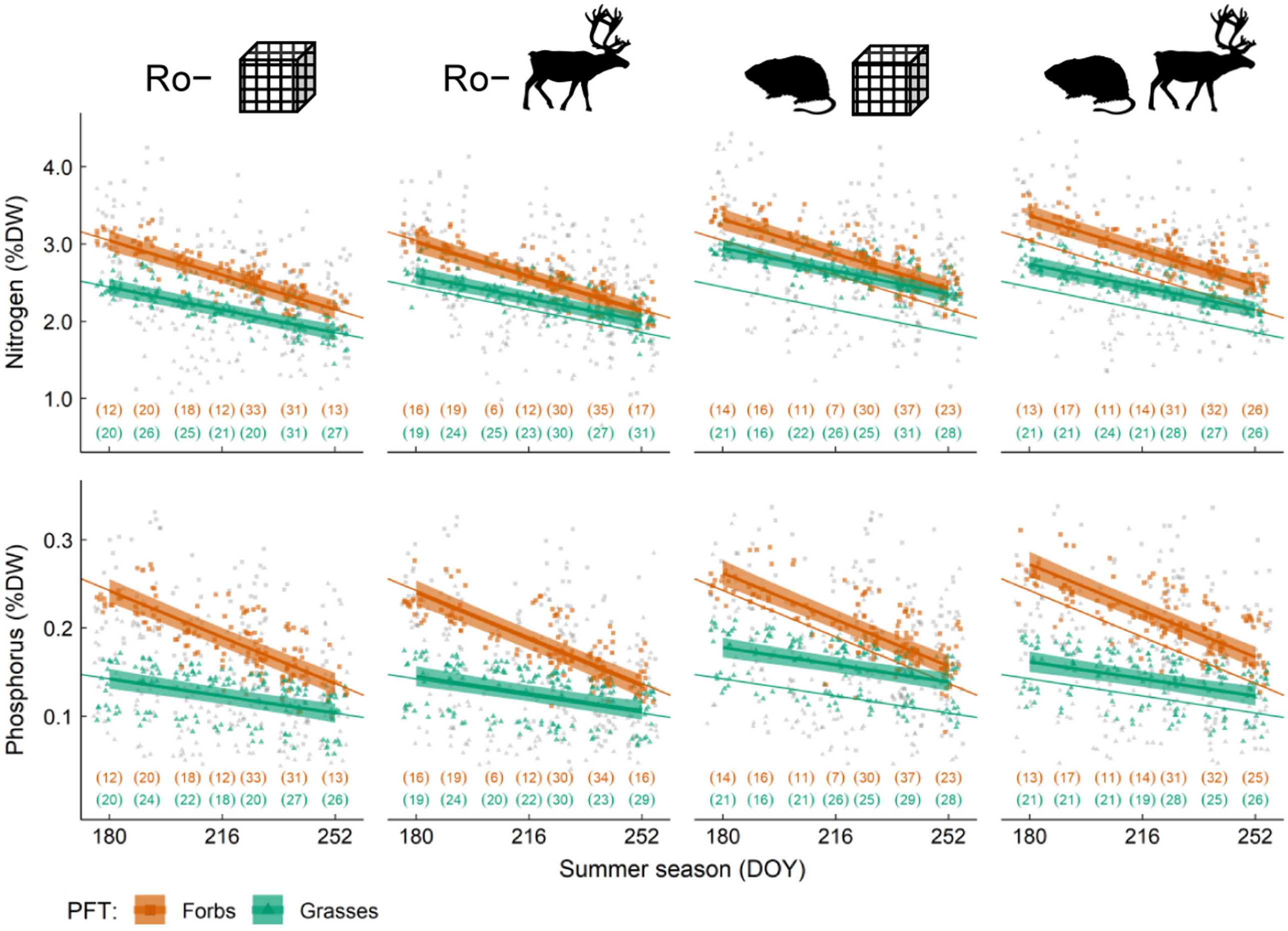


Figure 5. Effects of herbivores on plant functional type (PFT) nutrient levels in grass-dominated tundra-grasslands. Effects of small-rodent winter disturbance, reindeer summer herbivory and seasonality on leaf nitrogen [N] content (upper panel) and leaf phosphorus [P] content (lower panel) (% of dry weight – %DW) in forbs and grasses. Fitted values were acquired from the prediction models on PFT N- and P-content in grass-dominated grasslands. The reference lines (thin lines) are given separately for forbs and grasses. Description of graph content is provided in Fig. 3. Parameter estimates and their CI are provided in Table 3.

material for analyses. Given the high variability in plant nutrient-related traits within and among plant individuals (Siefert et al. 2015), pooling leaves together may thus have hampered the detection of plant nutrient responses to herbivores. NIRS methodology allowed us to quantify nutrient contents of single leaves (Petit Bon et al. 2020) for all the main species found in our grasslands and to properly incorporate such level of detail into our analyses. For the first time, we show that tundra-grassland nutrient dynamics are considerably modified by mammalian herbivores and that this can occur at a much shorter time-scale than previously revealed.

Effects of small-rodent winter disturbance were greater than effects of reindeer summer herbivory. Small-rodent activity in winter may have increased nutrient availability for soil and plant compartments in spring by providing animal-excreta, which are rich in labile nutrients (Bazely and Jefferies 1985). Soon after snowmelt, higher nutrient availability in disturbed tundra-patches may have stimulated soil microbial activity (Van der Wal et al. 2004), nutrient mineralization (Olofsson et al. 2004a) and ultimately plant nutrient acquisition (Hik and Jefferies 1990, Olofsson et al. 2004a). Yet, contrary to our expectation that a positive effect of small-rodent winter disturbance would dampen as the summer proceeded, plant-community nutrient levels in disturbed tundra-patches were consistently higher than in undisturbed ones across the entire growing season. Tundra-grasslands have a prominent accumulation of snow during winter and a large amount of water is released into the system in spring during snowmelt. Large inputs of water early in the season, combined with high soil moisture levels throughout the summer, may have promoted both a gradual release of nutrients from small-rodent waste products (van der Wal et al. 2004) and the maintenance of high microbial activity throughout the growing season (Illeris et al. 2003). These findings show that, within tundra-grasslands, intense and localised small-rodent activities occurring outside the growing season can induce immediate, positive plant-community nutrient responses lasting an entire summer season. This, in turn, could accelerate litter decay rate via change in litter quality (Quested et al. 2003), eventually enhancing soil process rates and nutrient turnover in tundra-grasslands.

Table 2. Parameter estimates for linear mixed-effects models for the effects of herbivores on plant functional type (PFT) nutrient levels in forb-dominated tundra-grasslands. Parameter estimates of fixed-effects (Estimates) and their 95% confidence interval (CI – lower and upper bounds) for the most parsimonious models in which predictors are 'PFT', 'small-rodent winter disturbance', 'reindeer summer herbivory' and 'seasonality' and responses are (a) leaf nitrogen [N] content and (b) leaf phosphorus [P] content (% of dry weight – %DW). Intercepts are calculated for forbs, undisturbed tundra-patches (Ro−), reindeer-exclusion plots (Re−) and first sampling occasion (DOY 180, 29 June), separately for the two forb-dominated grassland sites (conventionally named site A and B) since site (technically considered as random-effect) was retained in the final model (see 'Statistical analysis' for details). Estimates with bold indicate that their 95% CI does not include 0. Random-effects retained in the final models are presented as SD. Empty cells indicate that a predictor was not statistically significant, thus it was removed from the model. Observations refer to the number of plant leaf samples used in each model. Marginal R^2 represents the variance explained by the model when only fixed-effects are considered, whereas the conditional R^2 represents the variance explained by the model when both fixed- and random-effects are considered.

	(a) Nitrogen (%DW)		(b) Phosphorus (%DW)	
Fixed-effects (Predictors)	Estimates	CI (95%)	Estimates	CI (95%)
(Intercept site A) [Forbs/Ro−/Re−/DOY = 180]	**2.99*****	**2.75; 3.23**	**0.223*****	**0.208; 0.239**
(Intercept site B) [Forbs/Ro−/Re−/DOY = 180]	**3.24*****	**2.99; 3.48**	**0.242*****	**0.226; 0.257**
Plant functional type (PFT) [grasses]	**−0.60***	**−1.10; −0.10**	**−0.047*****	**−0.073; −0.020**
Small-rodent winter disturbance [Ro+]	**0.30*****	**0.18; 0.42**	**0.021*****	**0.009; 0.032**
Reindeer summer herbivory [Re+]	−0.02	−0.08; 0.04	−0.001	−0.007; 0.005
Seasonality [+1 DOY]	**−0.014*****	**−0.015; −0.012**	**−0.0014*****	**−0.0015; −0.0012**
PFT and Small rodents interaction [Grasses × Ro+]	**0.28*****	**0.15; 0.41**	**0.022*****	**0.009; 0.035**
PFT and Seasonality interaction [Grasses × DOY]	**0.004***	**0.001; 0.006**	**0.0006*****	**0.0003; 0.0009**
Random-effects	SD		SD	
Grassland site: Tundra-patch	0.11		0.011	
PFT: Plant species	0.44		0.018	
Residual	0.55		0.055	
Observations	1342		1321	
Marginal R^2/Conditional R^2	0.236/0.543		0.229/0.329	

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Table 3. Parameter estimates for linear mixed-effects models for the effects of herbivores on plant functional type (PFT) nutrient levels in grass-dominated tundra-grasslands. Parameter estimates of fixed-effects (Estimates) and their 95% confidence interval (CI – lower and upper bounds) for the most parsimonious models in which predictors are 'PFT', 'small-rodent winter disturbance', 'reindeer summer herbivory' and 'seasonality' and responses are (a) leaf nitrogen [N] content and (b) leaf phosphorus [P] content (% of dry weight – %DW). Intercept is calculated for forbs, undisturbed tundra-patches (Ro−), reindeer-exclusion plots (Re−) and first sampling occasion (DOY 180, 29 June). Estimates with bold indicate that their 95% CI does not include 0. Random-effects retained in the final models are presented as standard deviations. Empty cells indicate that a predictor was not statistically significant, thus it was removed from the model. Observations refer to the number of plant leaf samples used in each model. Marginal R^2 represents the variance explained by the model when only fixed-effects are considered, whereas the conditional R^2 represents the variance explained by the model when both fixed- and random-effects are considered.

	(a) Nitrogen (%DW)		(b) Phosphorus (%DW)	
Fixed-effects (Predictors)	Estimates	CI (95%)	Estimates	CI (95%)
(Intercept) [Forbs/Ro−/Re−/DOY = 180]	**3.01*****	**2.83; 3.20**	**0.241*****	**0.220; 0.262**
Plant functional type (PFT) [Grasses]	**−0.58*****	**−0.88; −0.29**	**−0.099*****	**−0.135; −0.062**
Small-rodent winter disturbance [Ro+]	**0.29*****	**0.14; 0.43**	**0.021****	**0.006; 0.036**
Reindeer summer herbivory [Re+]	−0.02	−0.14; 0.11	−0.002	−0.015; 0.010
Seasonality [+1 DOY]	**−0.013*****	**−0.015; −0.010**	**−0.0015*****	**−0.0017; −0.0013**
PFT and Small rodents interaction [Grasses × Ro+]	**0.21***	**0.04; 0.39**		
PFT and Reindeer interaction [Grasses × Re+]	**0.16***	**0.0001; 0.33**		
PFT and Seasonality interaction [Grasses × DOY]	**0.004****	**0.002; 0.007**	**0.0010*****	**0.0007; 0.0012**
PFT, Small rodents and Reindeer interaction [Grasses × Ro+ × Re+]	**−0.41*****	**−0.65; −0.17**	**−0.031***	**−0.055; −0.006**
Random-effects	SD		SD	
Grassland site: Tundra-patch	0.09		0.009	
PFT: Plant species	0.22		0.029	
Residual	0.53		0.053	
Observations	1242		1204	
Marginal R^2/Conditional R^2	0.236/0.362		0.294/0.470	

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Grasses responded more strongly than forbs to small-rodent winter disturbance in both grassland-types. Notably, in forb-dominated tundra-grasslands, small-rodent winter disturbance increased N and P-content of grasses to the level of forbs. In grass-dominated grasslands, grasses, but not forbs, responded to reindeer summer herbivory, although here nutrient levels of grasses never reached those of forbs. Forbs and grasses are fast-growing and productive PFTs (Aerts and Chapin 1999) and the fact that both would show short-term nutrient responses to herbivores could be expected. However, graminoids can have higher nutrient absorption rates compared to forbs (Hong et al. 2018) and they have been found to respond more rapidly than other PFTs in nutrient amendment and mammalian excreta-addition experiments from the tundra (Grellmann 2002, Barthelemy et al. 2015). Thus, grasses may have been able to absorb a relatively higher proportion of nutrients released from herbivore waste products compared to forbs. Moreover, graminoids, but not forbs, have basal meristems in their leaves causing leaf rejuvenation following herbivory (McNaughton 1983). This mechanism is likely to affect nutrient dynamics within grass individuals, and could have contributed to the higher responsiveness of grasses compared to forbs. Yet, despite grasses had a relatively higher increase in N- and P-content compared to forbs in response to herbivory, nutrient levels in grasses never exceeded those of forbs. Overall, the nourishing state of our tundra-grassland communities was consistently increased by short-term herbivore activities.

Reindeer summer herbivory affected only grasses growing in grass-dominated tundra-grasslands. We see two main reasons for this. First, reindeer grazing pressure throughout the summer in forb-dominated grasslands may have not been high enough to cause immediate nutrient responses of grasses. Second, different species composition of the community of grasses in the two grassland-types could have resulted in these different responses. Grass-dominated grasslands were dominated by grass species that are high in silica content (i.e. silica-rich grasses), whereas silica-poor grasses were the most abundant in forb-dominated grasslands. Reindeer select for palatable, silica-poor grass species, thus reducing their abundance in tundra plant communities (Bråthen and Oksanen 2001, Bråthen et al. 2007). On the other hand, plant communities dominated by unpalatable, silica-rich grasses are maintained by reindeer summer herbivory (Bråthen et al. 2007, Ravolainen et al. 2011), suggesting that high silica levels may confer a competitive advantage to these grass species in the presence of herbivores (Soininen et al. 2013a). Here, we show that grass communities with dominance of silica-rich grass species can increase their nutrient levels in response to reindeer summer herbivory. This could be another potential mechanism under which these communities thrive.

As part of our theoretical/empirical framework, we hypothesised that the impact of both herbivores together on plant-community nutrient levels would reflect the magnitude and direction of the effects of either herbivore alone. In contrast to this prediction, reindeer herbivory in grass-dominated tundra-grasslands partly counteracted the positive effects of small rodents on grass nutrient levels, but it increased grass N-content in undisturbed tundra-patches. Although grass nutrient levels were still largely higher in the presence of both herbivores than in their absence, these results suggest that diverse plant–reindeer interactions can arise in tundra-patches that differ in their initial nutrient status. The mechanisms behind these opposing grass nutrient responses could not be identified directly in our study, and manipulative experiments able to disentangle under which conditions either negative or positive effects of reindeer prevail would be needed. However, one possible explanation might lie in that the net effect of herbivores on plant-community nutrient levels is often determined by the subtle balance between selective grazing and nutrient return to forage species through animal-excreta (Pastor et al. 2006, Harrison and Bardgett 2008). Ruminants are highly selective towards plant individuals with high nutrient contents (White 1983), and reindeer show preferences for nutrient-rich vegetation-patches across the tundra landscape (Iversen et al. 2014). Higher plant-community nutrient levels promoted by small rodents may have intensified reindeer grazing in disturbed tundra-patches relatively to undisturbed ones, causing the removal of highly-nutritious grass parts. Here, more intense and selective grazing may have outbalanced the immediate return of nutrients to the system through faeces and urine (Pastor et al. 1993), thus lowering grass nutrient contents. In undisturbed tundra-patches, conversely, a lower grazing intensity may have promoted a larger nutrient investment in leaf re-growth (Chapin 1980, McNaughton 1983), thus enhancing grass nutrient contents. As disturbed and undisturbed tundra-patches alternate within a few meters, and reindeer actively move across their feeding landscape (Iversen et al. 2014), a reindeer-mediated transfer of nutrients was likely to be maintained across tundra-patches throughout the growing season, as has already been suggested (Stark et al. 2015). Irrespective of the underlying mechanisms, our results clearly show that, within grass-dominated tundra-grasslands, interactions between winter and summer herbivory can lead to the formation of a mosaic of tundra-patches that differ in their nutrient-level states and that persist till the end of the summer season. In turn, such patchiness in landscape nutrient distribution could have important consequences for the spatial heterogeneity of ecosystem process rates within tundra-grasslands.

Conclusions

Nutrient limitation of terrestrial ecosystems is globally distributed (Elser et al. 2007, LeBauer and Treseder 2008). Hence, any factors that modify nutrient cycling rates may have significant effects on processes and functions of terrestrial environments. Insights into the drivers of tundra nutrient cycling are of particular concern given the fast changes high-latitude ecosystems are experiencing, both in their biotic and abiotic components. However, N- and P-content in plants are costly measures to attain and they are seldom reported in ecological studies with sufficient resolution to be useful explanatory variables of underlying ecological processes. By using NIRS, which allowed us to process a large amount of samples, thus working at the plant-community level, this study demonstrated a breakthrough in the assessment of short-term effects of plant–herbivore interactions on tundra-grassland nutrient dynamics.

Overall, we show that herbivores cause immediate, positive changes in tundra-grassland plant-community N and P levels by enhancing leaf nutrient contents of the dominant, nutrient-rich PFTs, i.e. forbs and grasses. Considering the long-term grazing history characterizing our tundra-grassland communities, these PFTs are also the ones that have been promoted here by several decades of herbivory. Thus, our short-term findings indicate that forbs and grasses are currently having positive nutrient-level responses to herbivore activities in these tundra-grasslands, which align with theoretical expectations of positive herbivore effects on nutrient cycling in relatively productive ecosystems, when dominant, nutrient-rich PFTs benefit from herbivory (Bardgett and Wardle 2003).

Data accessibility

Data and R script are available viaDataverseNO UiT Open Research Data Repository <https://doi.org/10.18710/XCEXJ1>.

Acknowledgements – We are grateful to Freja Fagerholm, Victor Toscani, Torunn Moe and Francisco Murguzur for help with field assistance and Hanna Böhner for providing continuous updates on NIRS calibration models. We are also grateful to Michele Carbognani and Nigel Yoccoz for precious advice during the preliminary data analyses and Alessandro Petraglia for useful discussions during the preparation of this manuscript. Finally, we thank the leader of the reindeer herding district for being positive to the study. This work is part of the Tall Shrub Tundra module of the Climate ecological Observatory in the Arctic Tundra (COAT, <http://coat.no>).

Conflicts of interest – There are no conflicts of interest to declare.

Author contributions – KAB conceived the idea with contributions from MPB, KGI and TAU, and all four collected the data. MPB analysed the data and wrote the manuscript with contributions from KAB, ISJ, TAU and KGI. EMS provided and interpreted data concerning small-rodent and reindeer dynamics at our study area. ISJ and KAB financed the study through their research grants. All authors read the manuscript and approved the final version for publication.

References

Aerts, R. and Chapin, F. S. 1999. The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. – Adv. Ecol. Res. 30: 1–67.

Albon, S. D. and Langvatn, R. 1992. Plant phenology and the benefits of migration in a temperate ungulate. – Oikos 65: 502–513.

Baayen, R. H. et al. 2008. Mixed-effects modeling with crossed random effects for subjects and items. – J. Mem. Lang. 59: 390–412.

Bardgett, R. D. and Wardle, D. A. 2003. Herbivore-mediated linkages between aboveground and belowground communities. – Ecology 84: 2258–2268.

Barthelemy, H. et al. 2015. Strong responses of subarctic plant communities to long-term reindeer feces manipulation. – Ecosystems 18: 740–751.

Bates, D. et al. 2015a. Parsimonious mixed models. – arXiv:1506.04967v2 [stat.ME].

Bates, D. et al. 2015b. Fitting linear mixed-effects models using lme4. – J. Stat. Softw. 67: 1–48.

Bazely, D. R. and Jefferies, R. L. 1985. Goose faeces: a source of nitrogen for plant growth in a grazed salt marsh. – J. Appl. Ecol. 22: 693–703.

Bernes, C. et al. 2015. What are the impacts of reindeer/caribou (*Rangifer tarandus* L.) on arctic and alpine vegetation? A systematic review. – Environ. Evid. 4: 4.

Bolker, B. M. et al. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. – Trends Ecol. Evol. 24: 127–135.

Bråthen, K. A. and Oksanen, J. 2001. Reindeer reduce biomass of preferred plant species. – J. Veg. Sci. 12: 473–480.

Bråthen, K. A. and Hagberg, O. 2004. More efficient estimation of plant biomass. – J. Veg. Sci. 15: 653–660.

Bråthen, K. A. et al. 2007. Induced shift in ecosystem productivity? Extensive scale effects of abundant large herbivores. – Ecosystems 10: 773–789.

Chapin, F. S. 1980. Nutrient allocation and responses to defoliation in tundra plants. – Arct. Alp. Res. 12: 553–563.

Chapin, F. S. et al. 1996. Plant functional types as predictors of transient responses of arctic vegetation to global change. – J. Veg. Sci. 7: 347–358.

Cornelissen, J. H. C. et al. 2004. Leaf digestibility and litter decomposability are related in a wide range of subarctic plant species and types. – Funct. Ecol. 18: 779–786.

Elser, J. J. et al. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. – Ecol. Lett. 10: 1135–1142.

Fajardo, A. and Siefert, A. 2016. Phenological variation of leaf functional traits within species. – Oecologia 180: 951–959.

Grellmann, D. 2002. Plant responses to fertilization and exclusion of grazers on an arctic tundra heath. – Oikos 98: 190–204.

Grime, J. P. et al. 1996. Evidence of a causal connection between anti-herbivore defence and the decomposition rate of leaves. – Oikos 77: 489–494.

Hætta, J. I. et al. 1994. Reindriften i Finnmark: lovgivning og distriktsinndeling. – Reindriftsadministrasjonen, Alta, in Norwegian.

Hambäck, P. A. et al. 1998. Winter herbivory by voles during a population peak: the relative importance of local factors and landscape pattern. – J. Anim. Ecol. 67: 544–553.

Harrison, K. A. and Bardgett, R. D. 2008. Impacts of grazing and browsing by large herbivores on soils and soil biological properties. – In: Gordon, I. J. and Prins, H. H. T. (eds), The ecology of browsing and grazing. Springer, pp. 201–216.

Hik, D. S. and Jefferies, R. L. 1990. Increases in the net aboveground primary production of a salt-marsh forage grass: a test of the predictions of the herbivore-optimization model. – J. Ecol. 78: 180–195.

Hong, J. et al. 2018. Which root traits determine nitrogen uptake by alpine plant species on the Tibetan Plateau? – Plant Soil 424: 63–72.

Illeris, L. et al. 2003. Soil plus root respiration and microbial biomass following water, nitrogen and phosphorus application at a high arctic semi desert. – Biogeochemistry 65: 15–29.

Ims, R. A. and Fuglei, E. 2005. Trophic interaction cycles in tundra ecosystems and the impact of climate change. – BioScience 55: 311–322.

Ims, R. A. et al. 2007. Can reindeer overabundance cause a trophic cascade? – Ecosystems 10: 607–622.

Iversen, M. et al. 2009. Predictors of plant phenology in a diverse high-latitude alpine landscape: growth forms and topography. – J. Veg. Sci. 20: 903–915.

Iversen, M. et al. 2014. Phenology and cover of plant growth forms predict herbivore habitat selection in a high latitude ecosystem. – PLoS One 9: e100780.

Killengreen, S. T. et al. 2007. Structural characteristics of a low Arctic tundra ecosystem and the retreat of the Arctic fox. – Biol. Conserv. 135: 459–472.

LeBauer, D. S. and Treseder, K. K. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. – Ecology 89: 371–379.

Lenth, R. et al. 2018. emmeans: estimated marginal means, aka least-squares means. – R package ver. 1.4.5. <https://cran.r-project.org/web/packages/emmeans/index.html>.

McNaughton, S. 1983. Compensatory plant growth as a response to herbivory. – Oikos 40: 329–336.

McNaughton, S. J. et al. 1997. Promotion of the cycling of diet-enhancing nutrients by African grazers. – Science 278: 1798–1800.

Murguzur, F. J. A. et al. 2019. Towards a global arctic-alpine model for near-infrared reflectance spectroscopy (NIRS) predictions of foliar nitrogen, phosphorus and carbon content. – Sci. Rep. 9: 8259.

Mysterud, A. et al. 2011. Plant quality, seasonality and sheep grazing in an alpine ecosystem. – Basic Appl. Ecol. 12: 195–206.

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

Olofsson, J. et al. 2001. Effects of summer grazing by reindeer on composition of vegetation, productivity and nitrogen cycling. – Ecography 24: 13–24.

Olofsson, J. et al. 2004a. Reindeer influence on ecosystem processes in the tundra. – Oikos 105: 386–396.

Olofsson, J. et al. 2004b. Importance of large and small mammalian herbivores for the plant community structure in the forest tundra ecotone. – Oikos 106: 324–334.

Pastor, J. et al. 1993. Moose browsing and soil fertility in the boreal forests of Isle Royale National Park. – Ecology 74: 467–480.

Pastor, J. et al. 2006. The roles of large herbivores in ecosystem nutrient cycles. – In: Danell, K. et al. (eds), Large herbivore ecology: ecosystem dynamics and conservation. Cambridge Univ. Press, pp. 289–325.

Petit Bon, M. et al. 2020. One leaf for all: chemical traits of single leaves measured at the leaf surface using near infrared-reflectance spectroscopy (NIRS). – bioRxiv doi: 10.1101/2020.03.26.010462

Supplementary material (available online as Appendix oik-07074 at <www.oikosjournal.org/appendix/oik-07074>). Appendix 1.

Quested, H. M. et al. 2003. Decomposition of sub-arctic plants with differing nitrogen economies: a functional role for hemiparasite. – Ecology 84: 3209–3221.

Ravolainen, V. T. et al. 2010. Additive partitioning of diversity reveals no scale-dependent impacts of large ungulates on the structure of tundra plant communities. – Ecosystems 13: 157–170.

Ravolainen, V. T. et al. 2011. Rapid, landscape scale responses in riparian tundra vegetation to exclusion of small and large mammalian herbivores. – Basic Appl. Ecol. 12: 643–653.

Schrama, M. et al. 2013. An integrated perspective to explain nitrogen mineralization in grazed ecosystems. – Perspect. Plant Ecol. Evol. Syst. 15: 32–44.

Shadish, W. R. et al. 2002. Experimental and quasi-experimental designs for generalized causal inference. – Cengage Learning.

Siefert, A. et al. 2015. A global meta-analysis of the relative extent of intraspecific trait variation in plant communities. – Ecol. Lett. 18: 1406–1419.

Sitters, J. and Olde Venterink, H. O. 2015. The need for a novel integrative theory on feedbacks between herbivores, plants and soil nutrient cycling. – Plant Soil 396: 421–426.

Skarin, A. et al. 2008. Summer habitat preferences of GPS-collared reindeer *Rangifer tarandus tarandus*. – Wildl. Biol. 14: 1–15.

Smis, A. et al. 2014. Determination of plant silicon content with near infrared reflectance spectroscopy. – Front. Plant Sci. 5: 496.

Soininen, E. M. et al. 2013a. More than herbivory: levels of silica-based defences in grasses vary with plant species, genotype and location. – Oikos 122: 30–41.

Soininen, E. M. et al. 2013b. Arctic small rodents have diverse diets and flexible food selection. – PLoS One 8: e68128.

Stark, S. 2007. Nutrient cycling in the tundra. – In: Marschner, P. and Rengel, Z. (eds), Nutrient cycling in terrestrial ecosystems. Springer, pp. 309–331.

Stark, S. et al. 2002. Reindeer grazing and soil microbial processes in two suboceanic and two subcontinental tundra heaths. – Oikos 97: 69–78.

Stark, S. et al. 2015. When do grazers accelerate or decelerate soil carbon and nitrogen cycling in tundra? A test of theory on grazing effects in fertile and infertile habitats. – Oikos 124: 593–602.

Tuomi, M. et al. 2018. Herbivore effects on ecosystem process rates in a low-productive system. – Ecosystems 22: 1–17.

Van der Wal, R. 2006. Do herbivores cause habitat degradation or vegetation state transition? Evidence from the tundra. – Oikos 114: 177–186.

Van der Wal, R. et al. 2004. Vertebrate herbivores and ecosystem control: cascading effects of faeces on tundra ecosystems. – Ecography 27: 242–252.

Walker, D. A. et al. 2005. The circumpolar arctic vegetation map. – J. Veg. Sci. 16: 267–282.

White, R. G. 1983. Foraging patterns and their multiplier effects on productivity of northern ungulates. – Oikos 40: 377–384.

Wickham, H. 2016. ggplot2: elegant graphics for data analysis. – Springer.

Zuur, A. F. et al. 2010. A protocol for data exploration to avoid common statistical problems. – Methods Ecol. Evol. 1: 3–14.

Supplementary Material

Below are the supplementary methods, tables, and figures supporting the study "**Interactions between winter and summer herbivory affect spatial and temporal plant nutrient dynamics in tundra grassland communities**".

Matteo Petit Bon[1,2]*, Katarina Gunnarsdotter Inga[2], Ingibjörg Svala Jónsdóttir[3], Tove Aagnes Utsi[2], Eeva Marjatta Soininen[2], Kari Anne Bråthen[2]

[1] Department of Arctic Biology, University Centre in Svalbard (UNIS), PO Box 156, N-9171 Longyearbyen, Norway

[2] Department of Arctic and Marine Biology, Faculty of Biosciences, Fisheries, and Economics, Arctic University of Norway (UiT), N-9037 Tromsø, Norway

[3] Institute of Life and Environmental Sciences, University of Iceland, Sturlugata 7, IS-101 Reykjavik, Iceland

[*] Corresponding author. E-mail: matteo.petitbon@unis.no; Fax: (+47) 79 02 33 01; Phone: (+47) 966 59 147; ORCID-ID: https://orcid.org/0000-0001-9829-8324

KGI: katarina.inga@biegga.com
ISJ: isj@hi.is (ORCID-ID: https://orcid.org/0000-0003-3804-7077)
TAU: tove.a.utsi@uit.no (ORCID-ID: https://orcid.org/0000-0003-0776-2369)
EMS: eeva.soininen@uit.no (ORCID-ID: https://orcid.org/0000-0003-4280-8350)
KAB: kari.brathen@uit.no (ORCID-ID: https://orcid.org/0000-0003-0942-1074)

Appendix 1: Supporting tables and figures

Supporting Tables

Sampling occasion	Sample collection (fieldwork)	Day of the year (DOY)
1	28 - 30 June	179 - 181
2	10 - 12 July	191 - 193
3	23 - 25 July	204 - 206
4	3 - 5 August	215 - 217
5	13 - 15 August	225 - 227
6	26 - 28 August	238 - 240
7	8 - 10 September	251 - 253

Table A1. Overview of the sampling schedule for plant leaf collection. In total, seven sampling occasions have been performed throughout the summer season of 2015 in all 96 plots, spanning from the end of June soon after snowmelt (28th–30th of June, days of the year [DOY]: 179–181) to mid September before the first snowfall (8th–10th of September, DOY: 251–253). Day of the year (DOY, i.e. seasonality) has been used as continuous predictor of plant leaf nitrogen and phosphorus contents in data analysis (see main text for details).

Plant Functional Type (PFT)	**Species**	**Forb-dominated grasslands**							**Grass-dominated grasslands**						
		1	**2**	**3**	**4**	**5**	**6**	**7**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Forbs	*Alchemilla* spp.	14	12	17	12	17	16	15	0	1	0	1	3	3	3
	Astragalus spp.	2	2	0	2	1	4	3	0	0	0	0	0	0	1
	Bistorta vivipara	19	17	7	15	18	12	4	32	33	25	17	45	34	15
	Cirsium spp.	0	0	2	0	0	1	0	0	0	0	0	0	0	0
	Geranium sylvaticum	13	18	17	18	16	13	7	1	0	0	0	0	0	0
	Gnaphalium supinum	1	0	0	0	1	2	0	0	0	0	0	0	1	0
	Hieracium spp.	0	0	0	0	1	1	0	0	0	0	0	0	8	0
	Leontodon spp.	2	0	0	0	0	0	0	3	0	0	0	0	0	0
	Omalotheca norvegica	0	2	0	2	2	2	2	1	0	0	0	0	1	1
	Potentilla spp.	0	0	0	0	0	0	0	0	0	0	0	1	2	0
	Pyrola spp.	1	2	2	2	2	4	1	0	0	0	2	2	0	1
	Ranunculus spp.	0	1	1	4	3	1	2	7	7	6	4	9	8	14
	Rumex acetosa	7	11	5	15	17	16	10	2	11	3	4	14	15	7
	Saussurea spp.	4	2	0	6	8	3	3	0	0	0	0	0	0	1
	Sibbaldia spp.	1	4	0	5	8	5	4	0	2	0	0	3	2	2
	Solidago virgaurea	4	24	17	26	33	24	19	0	4	2	1	11	14	6
	Taraxacum spp.	0	4	1	2	2	2	3	0	2	0	6	15	11	12
	Trientalis europaea	1	3	0	1	5	5	1	1	2	1	0	3	6	2
	Trollius spp.	13	11	10	17	15	20	8	4	2	5	3	4	5	4
	Vicia spp.	0	0	2	1	4	0	0	0	0	0	0	0	0	0
	Viola spp.	26	20	14	14	30	20	5	4	8	4	7	14	25	10
	TOTAL FORBS	**108**	**133**	**95**	**142**	**183**	**151**	**87**	**55**	**72**	**46**	**45**	**124**	**135**	**79**
Grasses	*Avenella flexuosa*	3	0	6	0	18	34	33	1	0	4	1	5	7	12
	Calamagrostis phragmitoides	7	11	6	12	15	12	14	14	19	25	25	37	30	25
	Deschampsia cespitosa	4	0	10	2	1	1	2	6	8	6	6	8	3	3
	Nardus stricta	0	1	0	0	0	1	0	34	23	25	31	34	34	37
	Poa spp.	34	40	36	41	34	37	28	26	37	36	28	19	42	35
	TOTAL GRASSES	**48**	**52**	**58**	**55**	**68**	**85**	**77**	**81**	**87**	**96**	**91**	**103**	**116**	**112**
Sedges	*Carex* spp.	3	6	2	7	2	2	6	5	7	4	8	10	12	16
Deciduous shrubs	*Salix herbacea*	0	2	2	8	2	2	9	2	10	0	0	10	1	7
	Salix spp.	2	2	2	11	0	9	5	3	0	0	1	0	7	3
Evergreen shrubs	*Empetrum nigrum*	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	Vaccinium myrtillus	1	2	1	0	1	1	2	0	0	0	0	0	0	0
	Vaccinium vitis-idaea	2	2	1	2	3	2	3	1	0	0	2	1	2	0
Rushes	*Luzula* spp.	0	0	0	0	0	0	0	0	0	0	0	0	2	0
Horsetails	*Equisetum* spp.	0	6	2	6	1	1	0	3	3	1	1	1	0	0

Table A2. Overview of the plant leaf samples collected from the main species/genera within the experiment. In total, 2831 plant leaf samples were collected from 34 species/genera and utilized in the present study to assess the effects of small-rodent winter disturbance, reindeer summer herbivory, and seasonality on plant leaf nitrogen and phosphorus contents within tundra-grasslands. Number of plant leaf samples are given for each species/genera and further summarized within each plant functional type (PFT). Plant leaf samples for each species/genera are grouped by grassland-types (i.e. forb-dominated and grass-dominated tundra-grasslands) and sampling occasion (from one to seven, Supplementary material Appendix 1 Table A1).

	Forb-dominated grasslands			Grass-dominated grasslands		
(a) **Plant Functional Type (PFT)**	mean	±SD	n	mean	±SD	n
Forbs (g/m^2)	81.9	62.3	48	19.1	14.1	48
Grasses (g/m^2)	100.6	68.6	48	108.8	48.5	48
Sedges (g/m^2)	4.0	10.2	48	4.5	7.7	48
Deciduous shrubs (g/m^2)	8.9	14.6	48	1.2	3.4	48
(b) **Soil features**	mean	±SD	n	mean	±SD	n
Moisture (%vol)	44.06	13.03	48	66.20	11.13	48
Nitrogen (%DW)	9.49	3.37	35	13.14	4.30	36
Phosphorus (%DW)	1.37	0.56	35	1.81	0.67	36
pH	4.29	0.27	35	4.25	0.26	36

Table A3. Plant functional type (PFT) above-ground biomass and soil environmental characteristics within tundra-grasslands. Summary statistics for (a) PFT above-ground biomass (g m^{-2}) and (b) soil environmental characteristics in forb-dominated and grass-dominated tundra-grasslands. Mean, standard deviation [SD], and number of sampled plots [n] are shown. (a) Above-ground biomass for forbs, grasses, sedges, and deciduous shrubs is presented (overall, 98% of the above-ground biomass across our tundra-grasslands). Above-ground biomass for evergreen shrubs, rushes, and horsetails (overall, 2% of the above-ground biomass across our tundra-grasslands) is not shown. (b) Soil moisture data were collected in three random spots within each plot at each sampling occasion (except occasion one, 28th–30th of June) using a SM150 soil moisture sensor (Delta-T Devices, Cambridge, UK). Measurements were firstly registered as millivolt units (mV) and later converted to soil moisture (m^3H_2O/m^3Soil or %vol) using the polynomial conversion for a generalized organic soil (SM150 manual). First, we took the average of the three soil moisture measurements in each plot to obtain a soil moisture average per plot at each sampling occasion. We then averaged plot-mean soil moisture across sampling occasions in order to obtain a single soil moisture value per plot that was representative for the whole growing season (i.e. seasonal-average soil moisture, which is represented here). Given the high correlation between seasonal-average soil moisture and average soil moisture at each sampling occasion (Supplementary material Appendix 1 Fig. A6), seasonal-average soil moisture is used in Supplementary material Appendix 1 Fig. A3 because more likely biologically relevant in determining a differentiation between tundra-grassland plant communities in the long-term. In sampling occasion five (13th–15th of August), three samples of organic soil were collected from all plots using a soil sample cylinder (25 mm Ø and 30-50 mm deep). However, due to logistic constraints, only half of the reindeer-exclusion plots were sampled. Within maximum 16 hours after collection, soil samples were transported to the laboratory and stored in a refrigerator at 3−4 ˚C until further analyses. Immediately after collection and during the transport to the laboratory, soil samples were placed in a Styrofoam box with ice to maintain low temperatures. The three samples of organic soil belonging to each plot were pooled together prior to analyses. Half of the material was used to assess soil pH, whereas the remaining soil sample was oven-dried at 60 ˚C for 48 h. Following the same procedure applied to plant leaf samples (see main text for details), we first created Near Infrared Reflectance Spectroscopy (NIRS) prediction models for organic soil (Supplementary material Appendix 1 Fig. A7) and finally used NIRS to analyse our samples for their nitrogen (N) and phosphorus (P) contents (% of dry weight – %DW) (for a similar approach, see Viscarra Rossel et al. 2016). Soil pH, soil N-content, and soil P-content are used in Supplementary material Appendix 1 Fig. A3.

	(a) Nitrogen (%DW)		(b) Phosphorus (%DW)	
Fixed effects (*Predictors*)	*Estimates*	*CI (95%)*	*Estimates*	*CI (95%)*
(*Intercept*) [Ro– / Re– / DOY = 180]	**3.15** ***	**2.80** ; **3.50**	**0.262** ***	**0.228** ; **0.295**
Small-rodent winter disturbance [Ro+]	**0.39** *	**0.03** ; **0.73**	0.005	–0.021 ; 0.031
Reindeer summer herbivory [Re+]	–0.14	–0.38 ; 0.10	–0.012	–0.039 ; 0.014
Seasonality [+1 DOY]	**–0.017** ***	**–0.022** ; **–0.013**	**–0.0016** ***	**–0.0021** ; **–0.0010**
Random Effects	*St.dev.*		*St.err.*	
Grassland site : Tundra-patch	0.36			
Residual	0.48		0.061	
Observations	90		90	
Marginal R^2 / Conditional R^2	0.381 / 0.600		0.290 / 0.265	

* *p<0.05* ** *p<0.01* *** *p<0.001*

Table A4. Parameter estimates for linear (mixed-effects) models for the effects of herbivores on leaf nutrient contents of sedges found in tundra-grasslands. Parameter estimates (*Estimates*) and their 95% confidence interval (*CI* – lower and upper bounds) for the most parsimonious models in which predictors are 'small-rodent winter disturbance' (two-level factor: undisturbed [Ro–] and disturbed [Ro+]), 'reindeer summer herbivory' (two-level factor: reindeer-exclusion [Re–] and reindeer-open [Re+]), and 'seasonality' (continuous variable: seven sampling occasions throughout the summer; day of the year [DOY]) and responses are (a) leaf nitrogen [N] content and (b) leaf phosphorus [P] content (% of dry weight – %DW) in sedges found across forb-dominated and grass-dominated tundra-grasslands (see main text for details). Intercept is calculated for undisturbed tundra-patches (Ro–), reindeer-exclusion plots (Re–), and first sampling occasion (DOY 180, 29th of June). Estimates with bold indicate that their 95% CI does not include 0. Random effects retained in the final models are presented as standard deviations. For leaf P-content, a linear model was fitted since no random effects were retained in the most parsimonious model, thus 'residual' represents the standard error of the residuals. Observations refer to the number of plant leaf samples used in each model (3.2% of the collected plant leaf samples). Marginal

R^2 represents the variance explained by the model when only fixed-effects are considered, whereas the conditional R^2 represents the variance explained by the model when both fixed- and random-effects are considered. For leaf P-content, which is described by a linear model without random-effects, the first value refers to the un-adjusted R^2, whereas the second value refers to the adjusted R^2. Specifications of the fitted models are presented in Supplementary material Appendix 2 and results are displayed in Supplementary material Appendix 1 Fig. A4.

	(a) Nitrogen (%DW)		**(b) Phosphorus (%DW)**	
Fixed Effects (*Predictors*)	*Estimates*	*CI (95%)*	*Estimates*	*CI (95%)*
(*Intercept*) [Ro– / Re– / DOY = 180]	**2.60** ***	**2.32** ; **2.89**	**0.242** ***	**0.214** ; **0.272**
Small-rodent winter disturbance [Ro+]	**0.91** ***	**0.57** ; **1.25**	**0.042** **	**0.018** ; **0.067**
Reindeer summer herbivory [Re+]	0.21	–0.05 ; 0.47	–0.004	–0.029 ; 0.021
Seasonality [+1 DOY]	**–0.009** ***	**–0.013** ; **–0.005**	**–0.0017** ***	**–0.0022** ; **–0.0012**
Herbivore interaction [Ro+ × Re+]	**–0.55** **	**–0.93** ; **–0.17**		
Random Effects	*St.dev*		*St.err*	
Grassland site : Tundra-patch	0.28			
Residual	0.44		0.061	
Observations	100		99	
Marginal R^2 / Conditional R^2	0.358 / 0.548		0.328 / 0.307	

* *p<0.05* ** *p<0.01* *** *p<0.001*

Table A5. Parameter estimates for linear (mixed-effects) models for the effects of herbivores on leaf nutrient contents of deciduous shrubs found in tundra-grasslands. Parameter estimates (*Estimates*) and their 95% confidence interval (*CI* – lower and upper bounds) for the most parsimonious models in which predictors are small-rodent winter disturbance (two-level factor: undisturbed [Ro−] and disturbed [Ro+]), reindeer summer herbivory (two-level factor: reindeer-exclusion [Re−] and reindeer-open [Re+]), and seasonality (continuous variable: seven sampling occasions throughout the summer; day of the year [DOY]) and responses are (a) leaf nitrogen [N] content and (b) leaf phosphorus [P] content (% of dry weight – %DW) in deciduous shrubs found across forb-dominated and grass-dominated tundra-grasslands (see main text for details). Intercept is calculated for undisturbed tundra-patches (Ro−), reindeer-exclusion plots (Re−), and first sampling occasion (DOY 180, 29th of June). Estimates with bold indicate that their 95% CI does not include 0. Random effects retained in the final models are presented as standard deviations. Empty cells indicate that a predictor was not statistically significant, thus it was removed from the model. For leaf P-content, a linear model was fitted since no random effects were retained in the most parsimonious model, thus ‘residual’ represents the standard error of the residuals. Observations refer to the number of plant leaf samples used in each model (3.5% of the collected plant leaf samples). Marginal R^2 represents the variance explained by the model when only fixed-effects are considered, whereas the conditional R^2 represents the variance explained by the model when both fixed- and random-effects are considered. For leaf P-content, which is described by a linear model without random effects, the first value refers to the un-adjusted R^2, whereas the second value refers to the adjusted R^2. Specifications of the fitted models are presented in Supplementary material Appendix 2 and results are displayed in Supplementary material Appendix 1 Fig. A5.

Plant Functional Type (PFT)	**(A) Nitrogen %DW (±SD) [*n*]**			
	Ro−/Re−	Ro−/Re+	Ro+/Re−	Ro+/Re+
Evergreen shrubs	2.16 (0.79) [16]	1.69 (0.61) [9]	0.85;2.69	2.52 (0.14) [3]
Rushes	1.26		1.20	
Horsetails	3.56 (0.62) [10]	3.22 (0.37) [5]	3.96 (0.50) [7]	4.11 (0.34) [3]
	(B) Phosphorus %DW (±SD) [*n*]			
	Ro−/Re−	Ro−/Re+	Ro+/Re−	Ro+/Re+
Evergreen shrubs	0.140 (0.066) [15]	0.150 (0.083) [8]	0.088;0.270	0.209 (0.053) [3]
Rushes			0.128	
Horsetails	0.233 (0.087) [10]	0.252 (0.055) [5]	0.270 (0.086) [7]	0.299 (0.104) [3]

Table A6. Summary statistics for leaf nutrient contents of evergreen shrubs, rushes, and horsetails found in tundra-grasslands under different herbivore-treatment combinations. Summary statistics for (a) leaf nitrogen [N] content and (b) leaf phosphorus [P] content (% of dry weight, %DW) in evergreen shrubs, rushes, and horsetails (2% of the collected plant leaf samples), for which the sample sizes did not allow us to perform statistical analyses for the effects of herbivores on leaf nutrient contents at the plant functional type (PFT) level. For both (a) leaf N-content and (b) leaf P-content, the mean, standard deviation [SD], and number of samples [*n*] for each PFT are presented according to herbivore-treatment combinations: (i) Ro−/Re−: small-rodent undisturbed/reindeer-exclusion plots, (ii) Ro−/Re+: small-rodent undisturbed/reindeer-open plots, (iii) Ro+/Re−: small-rodent disturbed/reindeer-exclusion plots, and (iv) Ro+/Re+: small-rodent disturbed/reindeer-open plots (see main text for details). When the number of samples for a given herbivore-treatment combination was $n < 3$, the actual nutrient values for each sample are presented and the calculation of mean and SD has not been performed. Empty cells indicate that no samples for a given herbivore-treatment combination were available.

Supporting Figures

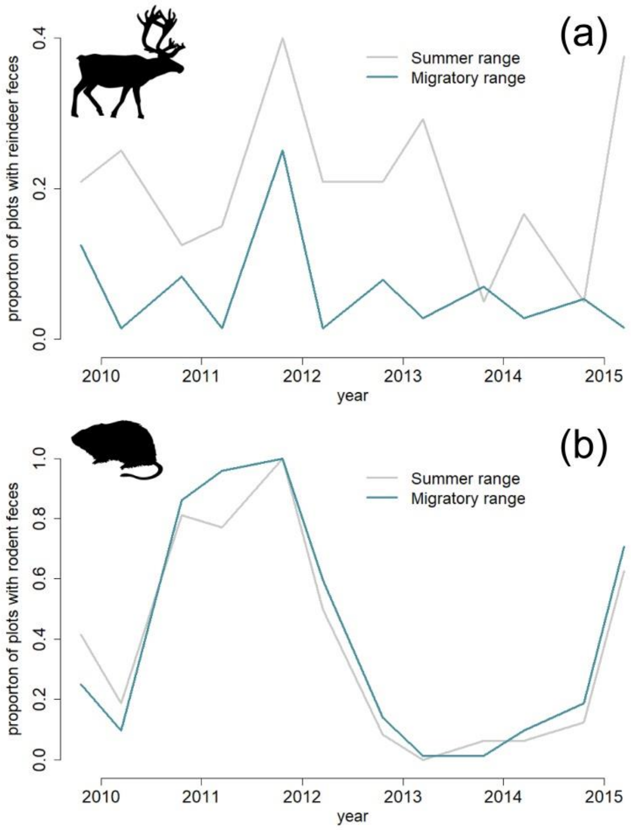


Figure A1. Time-series of faeces counts for small rodents and reindeer in the study area. Time-series (2010−2015) of faeces counts for (a) reindeer and (b) small rodents at permanent plots within tundra-grassland sites found in the study area. Nine grassland sites are monitored in the migratory range (i.e. in forb-dominated grasslands) and seven grassland sites are monitored in the summer range (i.e. in grass-dominated grasslands). At each grassland site, 8 permanent plots (50 × 50 cm) are cleaned from faeces twice a year; early July (1st–3rd July – spring/summer count) and early September (1st–3rd September – autumn count). The method was modified in 2013; since then small-rodent faeces were not removed at each count, and the presence of small rodents was recorded based on fresh faeces and/or other fresh signs of activity. Data are presented as the mean proportion of plots with herbivore presence, separately for summer (i.e. grass-dominated grasslands) and migratory (i.e. forb-dominated grasslands) ranges.

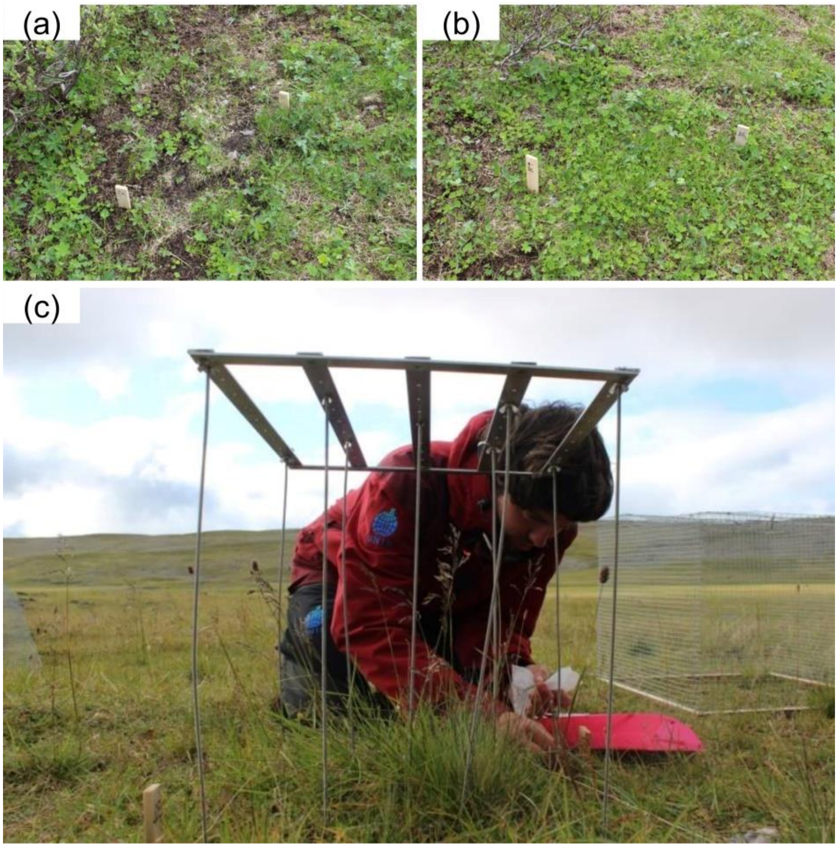


Figure A2. Contrast between (a) small-rodent disturbed (Ro+) and (b) small-rodent undisturbed (Ro−) tundra-patches, as selected for the present study, in one of the forb-dominated tundra-grasslands. Woody sticks marked one of the diagonal of each plot (photos: 10th–12th of July 2015). (c) A metal frame with nine randomly-distributed pins is being utilized for conducting leaf sampling in an experimental plot in one of the grass-dominated tundra-grasslands (photos: 3rd–5th of August).

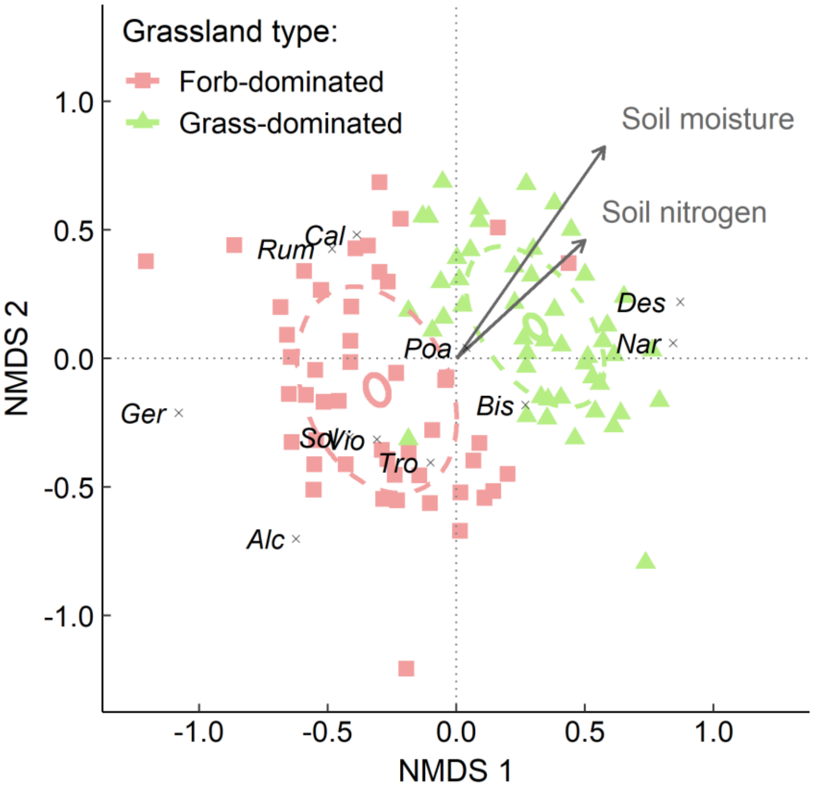


Figure A3. Plant species composition in tundra-grasslands. Two-dimensional NMDS ordination (based on Bray-Curtis dissimilarity index) of plot plant species/genera above-ground biomass (*log-transformed* [g m^{-2}]+1) within forb-dominated and grass-dominated tundra-grasslands (n = 96, stress = 0.25, Non-metric fit r^2 = 0.94, Linear fit r^2 = 0.68). Ellipses represent the 95% confidence interval for the mean (i.e. grassland-type centroids – solid lines) and for the standard deviation of the mean (dashed lines): Grassland-type: R^2 = 0.18, F-statistics = 20.1, $P < 0.001$ (via vegan package: ordiellipse-function and adonis-function run with 10000 restricted permutations to account for the hierarchical spatial structure of the study design; see main text for details). Fit of the environmental parameters (i.e. soil environmental variables, Supplementary material Appendix 1 Table A3) when *a posteriori* regressed on the two axes of the biplot: Soil moisture: r^2 = 0.45, P = 0.01; Soil nitrogen-content: r^2 = 0.21, P = 0.04; Soil pH and soil phosphorus content did not significantly correlate with the NMDS ordination (P > 0.1) and thus are not displayed (envfit-function via vegan package run with 10000 restricted permutations to account for the hierarchical spatial structure of the study design; see main text for details). The ordination shows only the main species/genera characterizing our tundra-grassland communities (species names abbreviated as follow: *Alc*: *Alchemilla* spp.; *Bis*: *Bistorta vivipara*; *Cal*: *Calamagrostis phragmitoides*; *Des*: *Deschampsia cespitosa*; *Ger*: *Geranium sylvaticum*; *Nar*: *Nardus stricta*; *Poa*: *Poa* spp.; *Rum*: *Rumex acetosa*; *Sol*: *Solidago virgaurea*; *Tro*: *Trollius* spp.; *Vio: Viola* spp. – see Supplementary material Appendix 1 Table A2). Specifications on the implementation of the NMDS ordination and details on statistical analyses of plant species composition and soil environmental variables are described in Supplementary material Appendix 2.

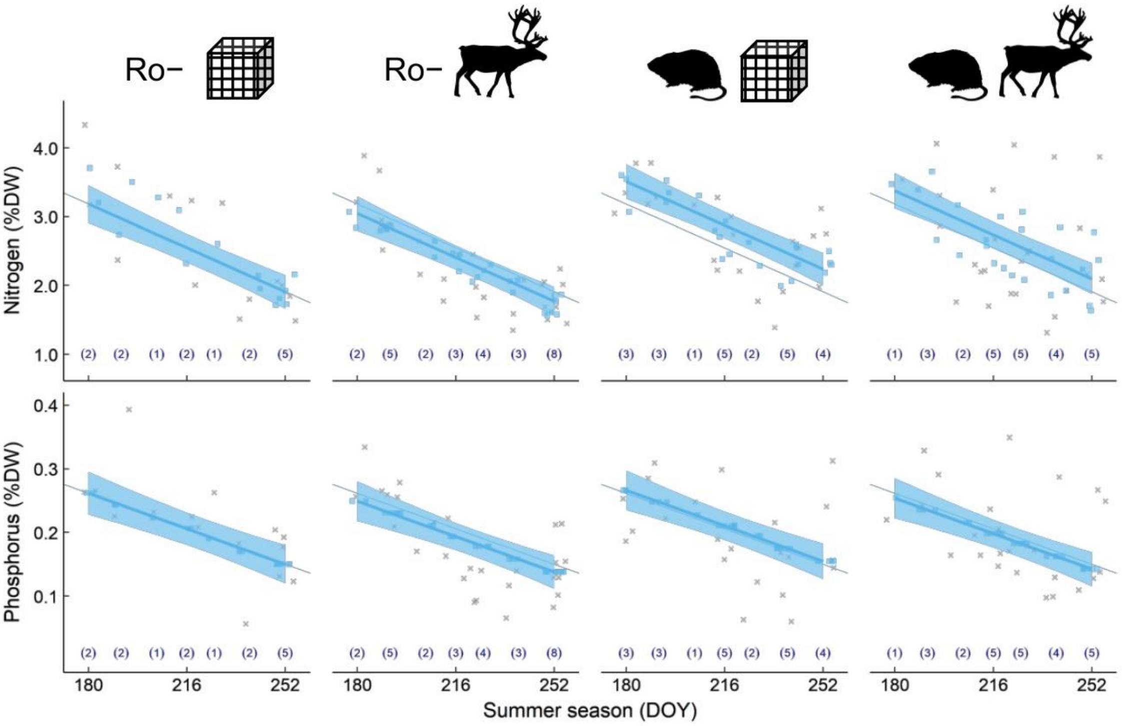


Figure A4. Effects of herbivores on leaf nutrient levels of sedges found in tundra-grasslands. Effects of small-rodent winter disturbance, reindeer summer herbivory, and seasonality on leaf nitrogen [N] content (upper panel) and leaf phosphorus [P] content (lower panel) (% of dry weight − %DW) in sedges found across forb-dominated and grass-dominated tundra-grasslands (see main text for details). Thick lines and bands represent regression lines for the fitted values and their 95% confidence intervals (CIs). Fitted values were acquired from the prediction models on sedge N- and P-content. The reference line (thin line) in absence of herbivores [Ro−/Re−] is maintained in each panel to facilitate the visualization of the effects of herbivores on sedge nutrient levels. Coloured dots represent fitted values for each plant leaf sample, whereas grey dots represent raw values. All dots were spaced apart within each of the seven sampling occasions to reduce overlapping. Numbers in parentheses represent the number of plant leaf samples collected at each sampling occasion (Supplementary material Appendix 1 Table A1) in each herbivore-treatment combination. Specifications of the fitted models are presented in Supplementary material Appendix 2. Parameter estimates and their CI are provided in Supplementary material Appendix 1 Table A4.

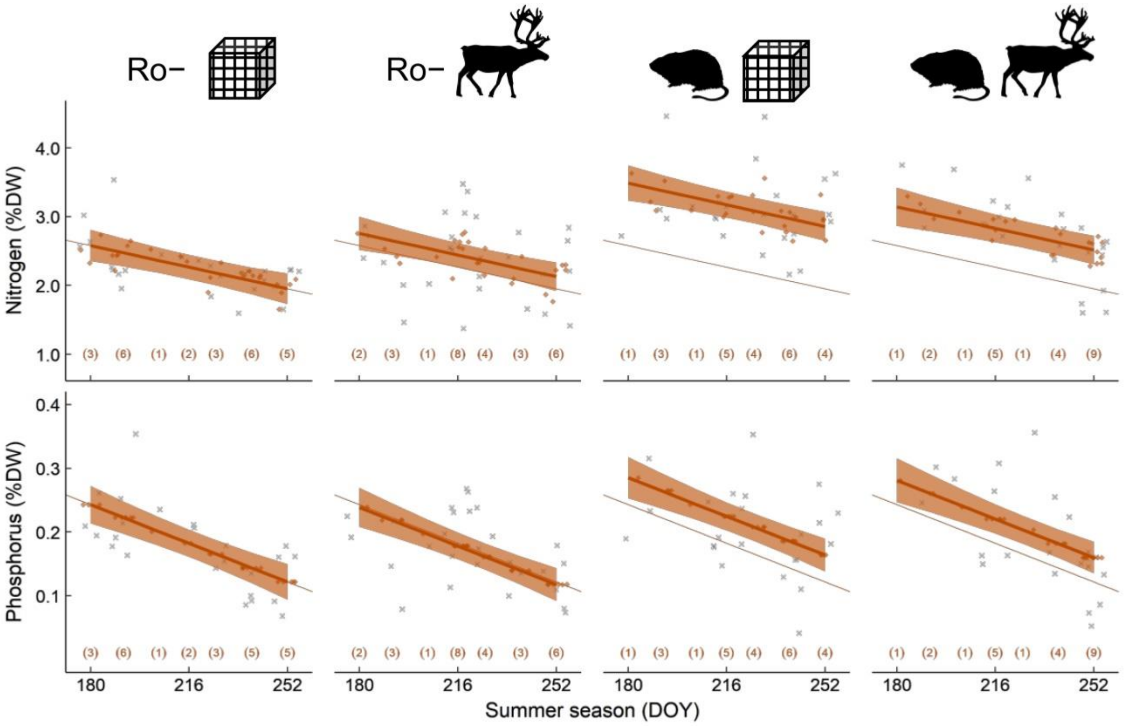


Figure A5. Effects of herbivores on leaf nutrient levels of deciduous shrubs found in tundra-grasslands. Effects of small-rodent winter disturbance, reindeer summer herbivory, and seasonality on leaf nitrogen [N] content (upper panel) and leaf phosphorus [P] content (lower panel) (% of dry weight − %DW) in deciduous shrubs found across forb-dominated and grass-dominated tundra-grasslands (see main text for details). Thick lines and bands represent regression lines for the fitted values and their 95% confidence intervals (CIs). Fitted values were acquired from the prediction models on deciduous-shrub N- and P-content. The reference line (thin line) in absence of herbivores [Ro−/Re−] is maintained in each panel to facilitate the visualization of the effects of herbivores on deciduous-shrub nutrient levels. Coloured dots represent fitted values for each plant leaf sample, whereas grey dots represent raw values. All dots were spaced apart within each of the seven sampling occasions to reduce overlapping. Numbers in parentheses represent the number of plant leaf samples collected at each sampling occasion (Supplementary material Appendix 1 Table A1) in each herbivore-treatment combination. Specifications of the fitted models are presented in

Supplementary material Appendix 2. Parameter estimates and their CI are provided in Supplementary material Appendix 1 Table A5.

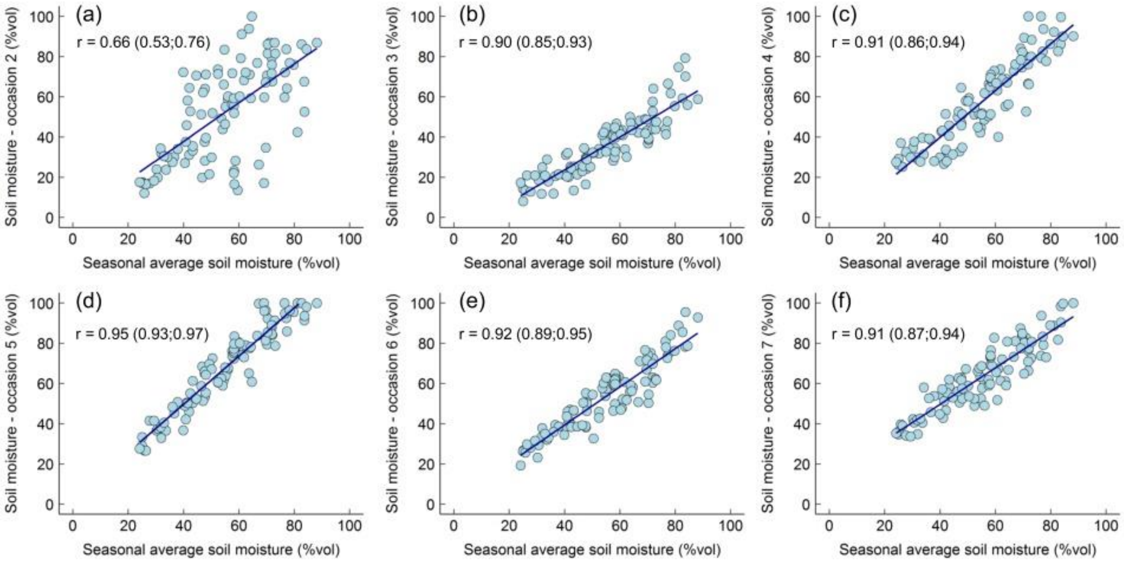


Figure A6. Correlations between seasonal-average soil moisture and average soil moisture at each sampling occasion. Correlations between plot seasonal-average soil moisture (*x* axis) and plot average soil moisture at each sampling occasion (*y* axis) (panels [a–f] correspond to sampling occasions 2–7, respectively; notice that we did not measure soil moisture in sampling occasion one, Supplementary material Appendix 1 Table A3). Pearson correlation coefficients (r) and their 95% confidence interval for each relationship are given in each panel of the figure. Soil moisture data were collected at each sampling occasion following the schedule for plant leaf sampling (Supplementary material Appendix 1 Table A1).

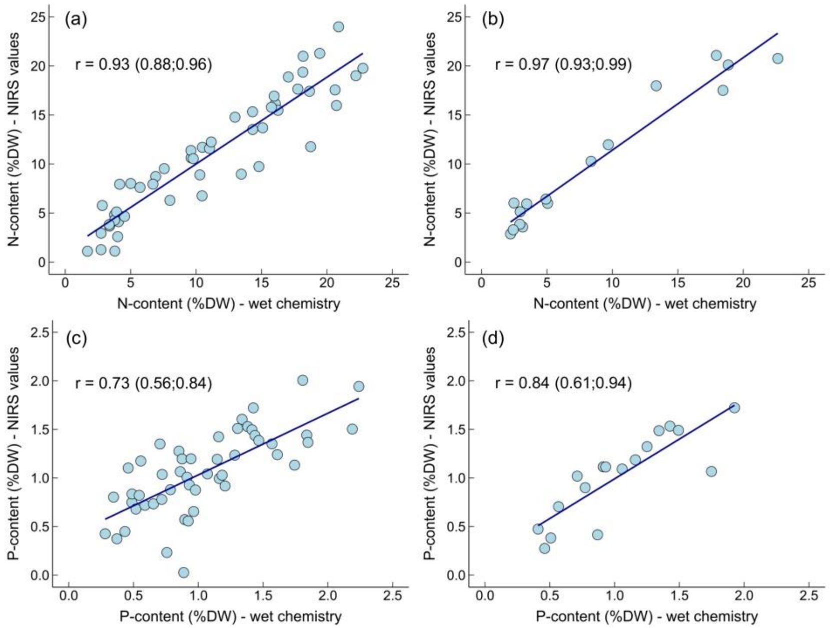


Figure A7. Correlations between soil nutrient levels obtained using wet chemistry and using Near Infrared Reflectance Spectroscopy (NIRS) methodology. Correlations between nutrient values obtained with wet chemistry and nutrient values obtained with NIRS in the (a,c) calibration sample sets and (b,d) validation sample sets for nitrogen [N] content [upper panels] and phosphorus [P] content [lower panels] in the organic soil. Pearson correlation coefficients (r) and their 95% confidence interval for each relationship are given in each panel of the figure. Calibration models derived from (a,c) the calibration sample sets and further validated using (b,d) the validation sample sets have been used to predict N- and P-content in the organic soil samples used in this study to characterize the abiotic component of our forb-dominated and grass-dominated tundra-grasslands (Supplementary material Appendix 1 Table A3, Fig. A3).

Appendix 2: Details on statistical analyses

All statistical analyses were conducted in the R environment version 3.6.1 (https://www.r-project.org).

Plant-community composition in forb- and grass-dominated grasslands

A nonmetric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity index (Legendre and Legendre 2012) was implemented using the metaMDS-function via vegan package (Oksanen et al. 2018) to explore the overall plant community composition of our tundra-grasslands. The NMDS was based on the *log-transformed+1* above-ground biomass values (g m^{-2}) of the species/genera at each plot. A visual screening of the plant communities clearly showed that species composition varied widely between the two grassland-types (Supplementary material Appendix 1 Fig. A3). All forb species (except *Bistorta vivipara*) displayed higher biomass in forb-dominated tundra-grasslands, whereas silica-rich grasses such as *Nardus stricta* and *Deschampsia cespitosa* were the main species prevailing in grass-dominated tundra-grasslands. *Poa* spp. biomass was approximately the same in all our tundra-grassland communities.

We statistically tested differences in species composition between forb- and grass-dominated tundra-grassland communities by implementing Permutational Multivariate Analysis of Variance (PERMANOVA) (adonis-function via vegan package) based on the Bray-Curtis distance matrix of species composition (see above). We run PERMANOVA with 10000 restricted permutations (defined *a-priori* using the how-function via permute package; Simpson et al. 2019) to account for the hierarchical spatial structure of the study design. In particular, ‘Plots’ were allowed to permute only within the ‘Tundra-patch’ they belonged to (see main text for details). Results are reported in Supplementary material Appendix 1 Fig. A3.

Finally, the statistical significance of soil environmental characteristics (Supplementary material Appendix 1 Table A3) when *a posteriori* fitted onto the NMDS ordination was evaluated by using 10000 restricted permutations to account for the hierarchical spatial structure of the study design (envfit-function via vegan package). In particular, 'tundra-patches' nested within 'grassland sites' were allowed to permute only within the 'grassland-type' (i.e. forb- and grass-dominated tundra-grasslands) they belonged to (see main text for details). The interpretation of significant soil environmental characteristics (vectors) is that they significantly correlate with the Bray-Curtis distance matrix of species composition (see above). As such, the direction of the vectors in the NMDS ordination space indicates towards which direction these vectors change most rapidly and the direction to which they have maximal correlations with the ordination configuration. The projections of points (i.e. experimental plots) onto vectors have maximum correlation with corresponding soil environmental characteristics. Results are reported in Supplementary material Appendix 1 Fig. A3.

Linear mixed-effects models (LMMs) presented in the main text

Given the fervent debate around the simplification of the random-effects structure (Barr et al. 2013, Bates et al. 2015a), we also performed all the statistical analyses maintaining the maximal random-effects structure (i.e. all the random intercepts) in each linear mixed-effects model (LMM). Since the selected random-effects structure did not significantly influence the final estimates for the fixed-effects, we decided to present the LMMs with the simplest random-effects structure (Bates et al. 2015a).

The selection of the better random-effects structure for each LMM was accomplished by using a combination of two alternative computationally-intensive methods:

1) Parametric bootstrapping with 10000 replicates with the confint-function (base R package) applied to lmer-objects (i.e. LMMs fitted by lmer-function via lme4 package – see main text for details).
2) Simulations (i.e. permutations) with 10000 replicates with the simulate- and refit-functions (base R package), as suggested by Gałecki and Burzykowski (2013).

Although the ratio of the total sample size to the number of fixed-effects levels being tested and the number of random-effects levels were large enough for likelihood ratio (LR) tests (Bolker et al. 2009), we repeated the selection of the better fixed-effects structure using two alternative methods:

1) Computationally-efficient analysis of variance (ANOVA) with the anova-function (base R package).
2) Computationally-intensive parametric bootstrapping with 10000 replicates with the PBmodcomp-function via pbkrtest package (Halekoh and Højsgaard 2014).

The two methods led to identical results (i.e. the final most parsimonious LMMs were the same independently of the methodology used to select their better fixed-effects structure), further confirming the robustness of our LMMs.

Fixed- and random-effects (i.e. model structure) retained in the most parsimonious LMMs are presented below (as specified by using the lmer-function via lme4 package in R):

Model for plant-community leaf nitrogen content across tundra-grasslands:

Fixed-effects structure: (‘Small-rodent winter disturbance’ + ‘Reindeer summer herbivory’ + ‘Seasonality’ + ‘Small-rodent winter disturbance × Reindeer summer herbivory’)

Random-effects structure: (‘Grassland site’ + ‘Grassland site : Tundra-patch’ + ‘Plant species’)

Model for plant-community leaf phosphorus content across tundra-grasslands:

Fixed-effects structure: (‘Small-rodent winter disturbance’ + ‘Reindeer summer herbivory’ + ‘Seasonality’)

Random-effects structure: (‘Grassland site’ + ‘Grassland site : Tundra-patch’ + ‘Plant species’)

Model for plant functional type (PFT) leaf nitrogen content in forb-dominated grasslands:

Fixed-effects structure: (‘Grassland site’ + ‘PFT’ + ‘Small-rodent winter disturbance’ + ‘Reindeer summer herbivory’ + ‘Seasonality’ + ‘PFT × Small-rodent winter disturbance’ + ‘PFT × Seasonality’)

Random-effects structure: (‘Grassland site : Tundra-patch’ + ‘PFT : Plant species’)

Model for plant functional type (PFT) leaf phosphorus content in forb-dominated grasslands:

Fixed-effects structure: (‘Grassland site’ + ‘PFT’ + ‘Small-rodent winter disturbance’ + ‘Reindeer summer herbivory’ + ‘Seasonality’ + ‘PFT × Small-rodent winter disturbance’ + ‘PFT × Seasonality’)

Random-effects structure: (‘Grassland site : Tundra-patch’ + ‘PFT : Plant species’)

Model for plant functional type (PFT) leaf nitrogen content in grass-dominated grasslands:

Fixed-effects structure: (‘PFT’ + ‘Small-rodent winter disturbance’ + ‘Reindeer summer herbivory’ + ‘Seasonality’ + ‘PFT × Small-rodent winter disturbance’ + ‘PFT × Reindeer summer herbivory’ + ‘PFT × Seasonality’ + ‘PFT × Small-rodent winter disturbance × Reindeer summer herbivory’)

Random-effects structure: (‘Grassland site : Tundra-patch’ + ‘PFT : Plant species’)

Model for plant functional type (PFT) leaf phosphorus content in grass-dominated grasslands:

Fixed-effects structure: (‘PFT’ + ‘Small-rodent winter disturbance’ + ‘Reindeer summer herbivory’ + ‘Seasonality’ + ‘PFT × Seasonality’ + ‘PFT × Small-rodent winter disturbance × Reindeer summer herbivory’)

Random-effects structure: (‘Grassland site : Tundra-patch’ + ‘PFT : Plant species’)

<u>Linear models (LMs) and linear mixed-effects models (LMMs)for the analyses of sedges and deciduous shrubs (Supplementary material Appendix 1 Tables A4-A5 and Figs. A4-A5)</u>

We proceeded by analysing the data obtained for sedges (n = 90) and deciduous shrubs (n = 100) (6.7% of the collected plant samples) by using LMs fitted by lm-function via base R package and LMMs fitted by lmer-function via lme4 package (Bates et al. 2015b). The influence of herbivores on sedges and deciduous shrub leaf nutrient levels was evaluated across tundra-grasslands (i.e. encompassing both forb- and grass-dominated tundra-grasslands). Differences in model structure between these models and the models presented in the main text for forbs and grasses were due to too low sample sizes for sedges and deciduous shrubs in order to fit separate models for forb- and grass-dominated tundra-grasslands.

We fitted a separate model for the two plant functional types (PFTs) and for the two response variables of interest (plant nitrogen [N] and phosphorus [P] contents %DW), for a total of 4 models. We first created full LMMs with a basic fixed-effects structure including the three-way interaction between 'small-rodent winter disturbance' (two-level factor: undisturbed [Ro−] and disturbed [Ro+]), 'reindeer summer herbivory' (two-level factor: reindeer-exclusion [Re−] and reindeer-open [Re+]), and 'seasonality' (continuous variable: seven sampling occasions throughout the summer). In all full LMMs, the hierarchical spatial structure of the study design was entered as nested random factors, with 'plots' nested within 'tundra-patches', and subsequently nested within 'grassland sites'. 'Plots' accounted for both the nested design of our study and the repeated measures over the summer. We only fitted full random-intercept LMMs and avoid random-slope LMMs to prevent over-parameterization and convergence problems, as suggested by Bates et al. (2015a).

Selection of the better random- and fixed-effects structure for each model (i.e. most parsimonious models) was performed following the methodology applied for the main analyses presented in the main text (see also above). As for the analyses presented in the main text, we also performed all the analyses maintaining the maximal random-effects structure in each model. Since the selected random-effects structure did not significantly influence the final estimates for the fixed-effects, we decided to present the models with the simplest random-effects structure. When the variance of all random factors in a model was estimated as 0, we fitted simple linear models.

Fixed- and random-effects (i.e. model structure) retained in the most parsimonious LMs/LMMs are presented below (as specified by using the lm-function via basic R and the lmer-function via lme4 package):

Model for leaf nitrogen content in sedges:

Fixed-effects structure: ('Small-rodent winter disturbance' + 'Reindeer summer herbivory' + 'Seasonality')

Random-effects structure: ('Grassland site : Tundra-patch')

Model for leaf phosphorus content in sedges:

Fixed-effects structure: ('Small-rodent winter disturbance' + 'Reindeer summer herbivory' + 'Seasonality')

Random-effects: No random-effects were retained

Model for leaf nitrogen content in deciduous shrubs:

Fixed-effects structure: ('Small-rodent winter disturbance' + 'Reindeer summer herbivory' + 'Seasonality' + 'Small-rodent winter disturbance × Reindeer summer herbivory')

Random-effects structure: ('Grassland site : Tundra-patch')

Model for leaf phosphorus content in deciduous shrubs:

Fixed-effects structure: (‘Small-rodent winter disturbance’ + ‘Reindeer summer herbivory’ + ‘Seasonality’)

Random-effects structure: No random-effects were retained

Model validation process and definition of statistically significant effects was performed following the methodology applied for the main analyses presented in the main text. All graphs presented in Supplementary material Appendix 1 were made using the ggplot2 package (Wickham 2016).

References for Supplementary material

Barr, D. J. et al. 2013. Random effects structure for confirmatory hypothesis testing: keep it maximal. – J. Mem. Lang. 68: 255-278.

Bates, D. et al. 2015a. Parsimonious mixed models. – arXiv:1506.04967v2 [stat.ME].

Bates, D. et al. 2015b. Fitting linear mixed-effects models using lme4. – J. Stat. Softw. 67: 1-48.

Bolker, B. M. et al. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. – Trends Ecol. Evol. 24: 127-135.

Gałecki, A. and Burzykowski, T. 2013. Linear mixed-effects models using R: a step-by-step approach. – Springer.

Halekoh, U. and Højsgaard, S. 2014. A Kenward-Roger approximation and parametric bootstrap methods for tests in linear mixed models: the R package pbkrtest. – J. Stat. Softw. 59: 1-32.

Legendre, P. and Legendre, L. 2012. Numerical ecology. – Elsevier.

Oksanen, J. et al. 2018. vegan: community ecology package. – R package ver. 2.5-1.

Simpson, G. L. et al. 2019. Package permute. – R package ver. 0-9-5.

Viscarra Rossel, R. A. et al. 2016. A global spectral library to characterize the world's soil. – Earth-Sci. Rev. 155: 198-230.

Wickham, H. 2016. ggplot2: elegant graphics for data analysis. – Springer.

Paper III

Silicon-based defence and nutrient levels in grasses under herbivory: Forage quality in tundra grasslands is altered through different mechanisms

Silicon-based defence and nutrient levels in grasses under herbivory: Forage quality in tundra grasslands is altered through different mechanisms

Matteo Petit Bon[1,2*†], Katarina Gunnarsdotter Inga[2*], Tove Aagnes Utsi[2], Ingibjörg Svala Jónsdóttir[3], and Kari Anne Bråthen[2]

[*] Shared first-authorship

[1] Department of Arctic Biology, The University Centre in Svalbard (UNIS), PO Box 156, N-9171 Longyearbyen, Norway

[2] Department of Arctic and Marine Biology, Faculty of Biosciences, Fisheries, and Economics, The Arctic University of Norway, N-9037 Tromsø, Norway

[3] Institute of Life and Environmental Sciences, University of Iceland, Sturlugata 7, IS-101 Reykjavik, Iceland

[†] Corresponding author. E-mail: matteo.petitbon@unis.no; Fax: (+47) 79 02 33 01; Phone: (+47) 966 59 147; ORCID-ID: https://orcid.org/0000-0001-9829-8324

KGI: katarina.inga@biegga.com

TAU: tove.a.utsi@uit.no (ORCID-ID: https://orcid.org/0000-0003-0776-2369)

ISJ: isj@hi.is (ORCID-ID: https://orcid.org/0000-0003-3804-7077)

KAB: kari.brathen@uit.no (ORCID-ID: https://orcid.org/0000-0003-0942-1074)

Authors’ contributions and authors’ statement

KAB conceived the idea with contributions from MPB, KGI, and TAU, and all four collected the data. MPB analysed the data. MPB led the writing with contributions from all authors. ISJ and KAB financed the study through their research grants. There are no conflicts of interest to declare.

Keywords: Near infrared reflectance spectroscopy (NIRS), Nitrogen (N), Phosphorus (P), Plant-herbivore interactions, *Rangifer* (reindeer/caribou), Silicon(Si)-to-N and Si-to-P ratios, Si-poor and Si-rich grasses, Small rodents, sub-Arctic/alpine ecosystems

Abstract

Herbivore-induced changes in silicon (Si)-based defence and nutrient levels of grasses are central mechanisms through which grazers alter the quality of their own food supply across grassland ecosystems. In tundra-grassland communities, herbivory has been shown to increase nutrient contents in forage grasses; yet, it is an open question whether herbivores can affect grass Si-content. We asked if and to what extent herbivores affect leaf Si-based defence levels and leaf Si-to-nutrient (nitrogen and phosphorus) ratios in the community of grasses found in tundra-grasslands.

We performed an herbivore-interaction field-experiment combining tundra-patches that had been disturbed or not by small rodents during winter with reindeer exclosures in summer, spanning four tundra-grassland communities. We collected over 1150 leaf-samples of Si-rich and Si-poor grass species throughout an entire summer season and analysed their elemental contents.

Small-rodent winter disturbance alone did not affect grass Si-content, but increased the quality of the grass-community (i.e. lowered Si-to-nutrient ratios) by enhancing nutrient levels of both Si-rich (+21%) and Si-poor (+30%) grasses. Reindeer summer herbivory alone increased the quality of Si-rich grasses by decreasing their Si-content (−7%). The two herbivores together offset both the increase in nutrient levels promoted by small rodents and the decrease in Si-content promoted by reindeer in Si-rich grasses. Thus, they increased the difference in Si-to-nitrogen and Si-to-phosphorus ratios between Si-rich and Si-poor grasses by 11 and 20%, respectively.

Results from this study provide the first community-level field-based evidence that herbivory-driven changes in both grass Si- and nutrient-contents contribute to alter herbivore forage quality in tundra-grasslands. Si-to-nutrient ratios of grasses affected by herbivores were never above those of grasses in their absence, indicating that herbivory enhances the overall quality of the grass-community. Yet, despite grasses became generally more palatable also when affected by both herbivores, the quality of Si-rich grasses was decreased relative to that of Si-poor grasses, thus causing apparent competition among these grass groups. The ability of maintaining a lower nutritive quality may confer a competitive advantage to Si-rich grasses over Si-poor grasses against herbivores utilizing tundra-grasslands throughout the summer.

Introduction

Grasslands, including managed rangelands and pastures, cover approximately 40% of the Earth’s land surface (Gibson 2009) and they have long been recognized as important hotspots for plant-herbivore interactions (Olff and Ritchie 1998, Bardgett and Wardle 2003). Several studies from a wide range of grassland ecosystems have documented the substantial, often positive, effects that mammalian herbivores exert on plant nutrient levels (Frank and Evans 1997, McNaughton et al. 1997, Petit Bon et al. 2020b). Herbivores potentially increase plant nutrient contents either directly by inducing re-growth of highly nutritious plant tissue or indirectly by enhancing plant nutrient availability (mainly nitrogen [N] and phosphorus [P]) (Bardgett and Wardle 2003). These mechanisms are also commonly promoted as the key processes by which herbivores positively manipulate the quality of their own food supply (McNaughton et al. 1997, Drent and Van der Wal 1999, Hamilton and Frank 2001). Yet, it is the structural and chemical defences in plants that ultimately determine the availability of nutrients for herbivores (Hanley 1997, Massey and Hartley 2006), and their levels in plants are also modified by grazers (e.g. Huitu et al. 2014).

Grasses (Poaceae), which dominate grassland ecosystems worldwide, are known to employ silicon-based defences to fight off herbivory (McNaughton and Tarrants 1983, Vicari and Bazely 1993). Silicon (Si), deposited as phytoliths or silica bodies in leaves of grasses (Ma and Yamaji 2006), increases the abrasiveness of leaf material, thus acting as a feeding deterrent for herbivores (Massey and Hartley 2006). As such, high leaf Si-content reduces the palatability of grasses (Gali-Muhtasib et al. 1992, Massey et al. 2006, Cotterill et al. 2007) and alters herbivore feeding preferences for different grass species (Massey et al. 2006, Massey et al. 2007a, Massey et al. 2009). Moreover, it has been demonstrated that Si-rich diets can reduce the digestive efficiency of herbivores and, consequently, their fitness (Harbers et al. 1981, Massey and Hartley 2009). Experimentally enhanced grass Si-content up to 400-600% negatively affected the growth rate of a small rodent (*Microtus agrestis*) commonly found across European grasslands by reducing of about 10-40% the proportion of N that voles could absorb from grasses (Massey and Hartley 2006). These findings indicate that changes in Si-content relative to levels of essential nutrients in forage grasses are likely to be critical for herbivores of grassland ecosystems. This could be particularly relevant in high-latitude regions, such as the tundra, where herbivores have a short time window to access forage plants and hence any change in plant quality can have important consequences for their fitness. We have recently shown that, within a single summer, mammalian herbivores modify plant N and P levels in tundra-grassland communities (Petit Bon et al. 2020b). Here, we ask to what extent herbivores can modify the quality of the community of grasses found in tundra-grasslands by altering grass Si, N, and P levels.

McNaughton and Tarrants (1983) first proposed Si accumulation in grass leaves as an ‘inducible defence’ against mammalian herbivory in African Savannah grasslands. More recently, greenhouse and field experiments have demonstrated that similar mechanisms also hold for commonly occurring European grass species (Massey et al. 2007b, Garbuzov et al. 2011, Huitu et al. 2014, Ruffino et al. 2018). However, some grass species do not show Si accumulation following herbivory (Massey et al. 2007b), whereas in other species certain threshold levels of damage have been shown to be a pre-requisite for Si accumulation (Garbuzov et al. 2011). Yet, for grasses in cold ecosystems with a short growing season, the evidence for Si changes following herbivory is inconsistent (Soininen et al. 2013). Instead, variations in grass Si-content in tundra-grasslands have been mainly ascribed to plant species, genotype, and location (Soininen et al. 2013). This is surprising since tundra-grass Si levels are comparable to those found in grasses dominating grasslands further south and where grasses are capable of Si changes following herbivory (cf. McNaughton and Tarrants 1983, Massey et al. 2007b, Soininen et al. 2013, Smis et al. 2014). Moreover, in tundra-grassland communities, unpalatable, Si-rich grasses have been shown to be maintained by the grazing activity of large ungulates (Bråthen and Oksanen 2001, Austrheim et al. 2007, Ravolainen et al. 2011), whilst palatable, Si-poor grasses often lose their dominance when the grazing pressure is high (Bråthen and Oksanen 2001, Bråthen et al. 2007). These patterns suggest that, also in tundra-grasslands, grasses can benefit from having high Si-content in the presence of herbivores. However, whether tundra-grasses also have the capacity of accumulating foliar Si in response to herbivory remain unclear.

Tundra-grasslands are habitat to a range of herbivores with different spatial and temporal dynamics (Jefferies et al. 1994). Small rodents, such as voles and lemmings, have major impacts on the functioning of most Arctic ecosystems and are well-renowned for their population density cycles (Ims and Fuglei 2005). During peak abundances, which can occur both in summer and winter, small rodents visibly disturb the vegetation (Hambäck et al. 1998, Ims and Fuglei 2005, Olofsson et al. 2012). In contrast, *Rangifer* (reindeer/caribou) show large-scale spatial dynamics through their migratory behaviour and their impacts by grazing and trampling on vegetation are more likely to be seasonal (Bernes et al. 2015). Tundra-grasslands are therefore affected by multiple herbivores that often co-occur in space, but less often in time (Ims and Fuglei 2005, Ravolainen et al. 2011). The extent to which these herbivores modify tundra grass-community defence and nutrient contents will ultimately determine changes in the quality of their pastures.

A full-factorial field-experiment was undertaken within four sub-Arctic/alpine tundra-grasslands in northern Norway, in which natural disturbance by small rodents that had occurred over the winter was combined with the exclusion of reindeer throughout the following summer. We studied both absolute changes in Si-based defence and changes relative to nutrient levels following herbivory within the community of Si-poor and Si-rich tundra-grasses throughout a short Arctic summer. We asked (i) to what extent herbivore interactions modify leaf Si-based defence levels of both Si-poor and Si-rich tundra-grasses and (ii) the extent to which such changes alter grass quality in terms of leaf Si-to-N (Si:N) and Si-to-P (Si:P) ratios. We hypothesised that herbivores will have complementary impacts and will promote an increase in Si-based defence levels of tundra-grasses. However, we also expected herbivores to enhance grass N and P levels, thus making the expectations for altered grass Si:N and Si:P ratios dependent on the magnitude of changes in leaf Si and nutrient levels. Predictions were studied for both Si-rich and Si-poor tundra-grasses.

Material and methods

Study area

The study took place in the low alpine zone at 300–400 m a.s.l. at Ifjordfjellet (70˚ 27′ N, 27˚ 08′ E), Finnmark, Northern Norway (Fig. 1a) during summer 2015. The region is generally snow-covered until mid-late June and primarily characterized by dwarf-shrub tundra heaths (Walker et al. 2005). Rivers descending from the inland towards the coast have created large riparian sediment plains mainly dominated by grasslands. Ifjordfjellet is bisected by a fence that was built in 1950s to separate spring/fall migratory and summer pasture ranges for semi-domesticated reindeer (*Rangifer tarandus tarandus*) (Hætta et al. 1994). However, the two ranges reflect more an historical division than a current one; reindeer faeces counts in permanent plots during the period 2011-2015 show that reindeer also utilize migratory ranges in summer, although to a lesser extent than summer pasture ranges (Petit Bon et al. 2020b). Our study area encompassed both migratory and summer ranges within the Lágesduotter reindeer herding district, where a density of 4.8 reindeer km^{-2} was estimated in summer 2015 (https://landbruksdirektoratet.no).

Due to historical differences in reindeer herbivory pressure, grasslands found on the two sides of the fence differ in their plant-community composition. The migratory range is characterized by forb-rich grasslands, whilst the summer range is characterized by grass-dominated grasslands (Petit Bon et al. 2020b). Moreover, grass species composition also differs between the two ranges. Whereas silicon-poor grass species (Si-poor grasses) were most abundant in migratory-range grasslands (e.g. *Poa* spp., *Avenella flexuosa*, *Anthoxanthum* spp., *Phleum alpinum*, and *Festuca ovina*), silicon-rich grass species (Si-rich grasses) were most abundant in summer-range grasslands, of which *Nardus stricta*, *Calamagrostis phragmitoides*, and *Deschampsia cespitosa* were the dominant ones. Plant names follow the Pan-Arctic Flora (http://nhm2.uio.no/paf).

At Ifjordfjellet, as in most of the Arctic tundra biome, migratory reindeer (caribou in North America) is the main large herbivorous mammal. Moose (*Alces alces*) is another wild large herbivore occasionally found within the study area. Small rodents (i.e. voles and lemmings) dominate among the small herbivores and are active year-round. Tundra vole (*Microtus oeconomus*) is the most common species within tundra grasslands (Killengreen et al. 2007).

Study design

Soon after snowmelt, we selected two grassland sites within each range (Fig. 1b). Within each site, twelve pairs of 60 × 60 cm plots were established. Six plot-pairs were located in tundra-patches that showed evident signs of winter disturbance by small rodents (disturbed tundra-patches: Ro+), whereas the other six plot-pairs were located in undisturbed patches (undisturbed tundra-patches: Ro−). Within each pair, plots were randomly assigned to be accessible to reindeer (open-grazed plots: Re+) or to be excluded to reindeer (reindeer-exclusion plots: Re−) (Fig. 1c). We used cages (70 × 70 cm area × 50 cm height) made of metal net (mesh-size 1.3 × 1.3 cm) to exclude reindeer herbivory in Re− plots throughout the summer. Gaps between the bottom part of the cage and the ground allowed small rodents to have free access into the cages. The number of small rodents within the study area was very low in early summer 2015, but it increased towards the end of the growing season (Petit Bon et al. 2020b). Small-rodent effects throughout the summer are assumed to be generally minimal and equal in all plots.

The selection of suitable tundra-patches for plot establishment followed three main criteria: (i) paired plots were max. 3 m apart and distance to other plot-pairs was min. 3 m, (ii) plot-pairs within tundra-patches were similar in both plant species composition and micro-topographical features, and (iii) at least 80% of the Ro+ plot-pair surface should show signs of small-rodent winter disturbance, whereas there should be no visible signs in Ro− plot-pairs (Fig. 1c). The scatter of small-rodent disturbed and undisturbed tundra-patches observed within our relatively homogeneous grassland sites points to a rather random fine-scaled spatial variation in winter herbivory (cf. Hambäck et al. 1998).

Sampling design and sample processing

Grass species were *a priori* assigned to either Si-rich grasses or Si-poor grasses following foliar Si-content estimates provided by Soininen et al. (2013) and Smis et al. (2014) for grass samples collected in the same region. *Nardus stricta*, *Calamagrostis phragmitoides*, and *Deschampsia cespitosa* were assigned to the group of Si-rich grasses, whilst the other grass species (see above) were assigned to the group of Si-poor grasses.

Leaf sampling was performed within each plot at seven sampling instances throughout the summer (Fig. 1d), in order to span all grass-leaf developmental stages available to herbivores throughout the growing season. To guide leaf sampling, we used nine pins attached to nine randomly-selected pin placements on a metal frame (42 × 46 cm area × 40 cm height) consisting of 50 regularly-distributed pin placements. Pin placements were changed at each sampling occasion, and those used in the previous two rounds were excluded in order to reduce the possibility of sampling repeatedly the same ramets. We collected the uppermost plant-leaf touching each pin if it belonged to a grass species. Additional leaves within a plot were collected whenever the first sampled leaf was not considered to be large enough for elemental content analyses (a minimum leaf area of 4 mm Ø, see below). In this case, we proceeded as follows: (i) first, we collected additional leaves touching the same pin if they were available, (ii) second, we collected additional leaves touching other pins if the original pin did not provide enough plant-leaf tissue for analyses, and (iii) third, we searched the required plant-leaf tissue within the entire plot when also the remaining pins did not touch or provide enough leaves to complete the sample.

In order to obtain balanced estimates of Si, N, and P levels for Si-rich and Si-poor grasses, we aimed at collecting at least one leaf sample for both groups of grasses within each plot at each sampling occasion. In total, we collected 1182 leaf samples (Si-rich grasses: 576, Si-poor grasses: 606) evenly distributed between herbivore-treatment combinations and sampling instances (see Figure S1 in Supporting Information). Yet, grass species differed in their abundance across our tundra-grassland communities. We assume the number of samples collected for a given species to reflect its abundance within our tundra-grasslands. During fieldwork, each sample was placed in a separate tea-filter bag. All samples were pressed with a plant-press within max. 10 hours after collection and subsequently oven-dried flat at 60 ˚C for 48 h within 4 days of collection. Samples were cleaned from soil/dust particles and stored in their original tea-filter bags in a dry and dark place at room temperature. Prior to analyses, all samples were oven-dried again at 60 ˚C for 2 h and subsequently cooled down in a desiccator.

All leaf samples were analysed for their elemental content (% of dry weight, hereafter %DW) using Near Infrared Reflectance Spectroscopy (NIRS) with a FieldSpec 3 (ASD Inc., Boulder, Colorado, USA) in 350–2500 nm range and equipped with a 4 mm light-adapter for full-leaf scanning (Petit Bon et al. 2020a). Narrow leaves were cut and stacked together in order to cover the minimum area needed for analyses. We took between 4 and 32 NIRS-measurements for each leaf sample (on average 6.3 ± 3.2), depending on number and size of the leaves constituting that sample. Each NIRS-measurement (in total 7498) was converted to Si-, N-, and P-content (%DW) by applying the prediction models based on milled and tableted plant samples (for Si-content, see Smis et al. 2014, for N- and P-content, see Murguzur et al. 2019) and adjusted for full-leaf scanning (Petit Bon et al. 2020a). We finally used the median of the replicate NIRS-measurements of each sample for data analyses.

Soil moisture data were collected in three random spots within each plot at each sampling instance (except occasion one, 28th-30th of June) using a SM150 soil moisture sensor (Delta-T Devices, Cambridge, UK). We then took the average of the three readings to obtain mean soil moisture at each plot.

Statistical analysis

Prior to model fitting, we explored the data following standardized protocols (Zuur et al. 2010). In total, 24 (2% of the data) and 51 (4.3% of the data) predicted median values for leaf Si- and P-content, respectively, fell outside the calibration range of our prediction models (range for leaf Si-content: 0.0008-3.73 %DW, Smis et al. 2014, range for leaf P-content: 0.04-0.70 %DW, Murguzur et al. 2019). We excluded these data from the analyses to avoid introducing possible statistical artefacts. However, since non-random patterns in missing values have been shown to strongly bias statistical inference in ecology (Soininen et al. 2017), we explored possibly introduced trends in missing observations. We did not discover non-random missing-value patterns that could affect model estimates and, consequently, our biological conclusions.

Data were analysed using linear mixed-effects models fitted by lmer-function via lme4 package (Bates et al. 2015a) in the R environment version 3.6.1 (https://www.r-project.org).

‘Grass silicon level’ (two-level factor: Si-poor and Si-rich grasses), ‘small-rodent winter disturbance’ (two-level factor: undisturbed [Ro−] and disturbed [Ro+] tundra-patches), ‘reindeer summer herbivory’ (two-level factor: reindeer-exclusion [Re−] and reindeer-open [Re+] plots), and their interactions were used as predictors in full models with leaf Si-, N-, P-content (%DW), Si:N, and Si:P ratios as response variables (five models in total). We took into account the hierarchical spatial structure of the study design and the correlation among plot-level observations by specifying ‘plot’ nested in ‘tundra-patch’ nested within ‘grassland-site’ as nested random-effects. However, ‘plot’ variance was always estimated as zero, thus this term was removed from the models, as suggested by Bates et al. (2015b). ‘Sampling instance’ was entered as crossed random-effect to account for the fact that leaf sampling was repeated seven times throughout the summer across all plots within the experiment (for details on the use of nested and/or crossed random-effects, see Baayen et al. 2008, Bolker et al. 2009). This model structure allowed us to quantify if and to what extent herbivores affect leaf Si-, N-, and P-content and leaf Si:N and Si:P ratios in the community of grasses found in tundra-grasslands across the whole summer. To avoid over-parameterization and convergence problems, we only fitted full models including random intercepts, following Bates et al. (2015b). We $log_e(y+1)$-transformed leaf P-content, Si:N, and Si:P ratios prior to analyses in order to achieve homogeneity in the residual variance. Other transformations, such as the $log_e(y)$ and square-root(y), yielded comparable results.

For the comparison of effect sizes between models, we used a common model structure including the three-way ‘grass silicon level × small-rodent winter disturbance × reindeer summer herbivory’ interaction (i.e. set of full models – see above). This three-way interaction, however, was statistically significant only in the full models fitted for leaf Si-content and leaf Si:N ratio as response variables. Therefore, for the other three response variables (i.e. leaf N-, P-content, and Si:P ratio), we determined the model that produced the least unexplained variation given the constraint that all its predictors were statistically significant (i.e. set of most parsimonious models). Model simplification was performed by using likelihood ratio test (models fitted using maximum likelihood – ML) until the most parsimonious models were obtained, following Bolker et al. (2009). Both sets of models produced similar effect sizes, but differed in how well they explained the model variance. We present results from the set of full models, and include results from the most parsimonious models in figures to enable the reader to compare between the two sets of models.

We originally tested ‘soil moisture’ (continuous predictor) as a covariate in the fixed-effects structure of both sets of models to determine whether it improved model fit. Soil moisture was not a significant predictor of any of the response variables, except in the full and most parsimonious models fitted for leaf N-content. Nevertheless, the inclusion of ‘soil moisture’ in these models neither statistically changed the other parameter estimates nor their biological interpretation. Thus, we excluded soil moisture from the models reported in *Results*.

Statistically significant effects (models fitted using restricted ML) were defined as having their 95% confidence interval not overlapping zero. Each model was validated by assessing normality and homogeneity of variances in the residuals for the fixed-effects and checking for approximate linearity between observed and fitted values. In the display of the results, we focus on main and interactive effects of different herbivore-treatment combinations on the five response variables, separately for Si-rich and Si-poor grasses. Linear contrasts between factor levels were calculated with the ‘emmeans’ package (Lenth 2018) and all graphs were made using the ‘ggplot2’ package (Wickham 2016). Model summaries for the set of full and reduced models are provided in Tables S1-S5.

Results

Leaf Si, N, and P levels and Si:N and Si:P ratios of grasses in absence of herbivores

Our subdivision of the grass species structuring our tundra-grassland communities in Si-poor and Si-rich grasses was *a posteriori* confirmed by their leaf Si-content, with Si-rich grasses having 65% higher Si-content than Si-poor grasses (Fig. 2a). The two grass groups also differed in their leaf N-content, but not P-content, with Si-rich grasses having 11% higher N-content than Si-poor grasses (Fig. 2b,c). Si-rich grasses also had 52% higher Si:N ratio and 64% higher Si:P ratio compared to Si-poor grasses (Fig. 2d,e).

Effects of herbivores on leaf Si, N, and P levels of grasses

Herbivores significantly affected leaf Si-content of Si-rich grasses, but not of Si-poor grasses (Fig. 3a,b). Reindeer summer herbivory alone decreased Si-content of Si-rich grasses by 7%, whereas small-rodent winter disturbance alone did not affect grass Si-content. Si-content of Si-rich grasses impacted by both herbivores was 9% higher than that of Si-rich grasses affected by reindeer alone.

Si-rich grasses had higher leaf Si-content compared to Si-poor grasses under all herbivore combinations (Fig. 3c), but this difference varied significantly from 56% higher when affected by small rodents alone to 67% higher when affected by both herbivores together.

Herbivores significantly affected leaf nutrient levels in both Si-rich and Si-poor grasses, and effect sizes were larger compared to changes in Si-content (Fig. 3, cf. N and P *vs* Si). Small-rodent winter disturbance alone increased N-content of both grass-groups by 22-26% (Fig. 3d,e) and P-content by 19% in Si-rich grasses and by 34% in Si-poor grasses (Fig. 3g,h; significant two-way 'grass silicon level × small-rodent winter disturbance' interaction – Table S3). Reindeer summer herbivory alone did not affect grass nutrient levels (Fig. 3, N and P), but significantly weakened the positive effects of small rodents on nutrient levels of Si-rich grasses (significant two-way 'small-rodent winter disturbance × reindeer summer herbivory' interactions – Tables S2, S3). Indeed, both herbivores together increased N-content of Si-rich grasses by 12%, i.e. less than small rodents alone, and did not affect their P-content (Fig. 3d,g).

Herbivores significantly altered the difference in leaf nutrient levels between Si-rich and Si-poor grasses observed in absence of herbivores (Fig. 3f,i). Under either herbivore alone, N-content in Si-rich grasses was still higher than that of Si-poor grasses (9-13% higher), but this difference was cancelled out in the presence of both herbivores (Fig. 3f). P-content in Si-rich grasses still did not differ from that of Si-poor grasses under reindeer alone, but was 11% lower under small rodents alone and 15% lower in the presence of both herbivores (Fig. 3i).

Effects of herbivores on leaf Si:N and Si:P ratios of grasses

Through their differential effects on leaf elemental contents of Si-rich and Si-poor grasses, herbivores significantly, and differently, affected leaf Si:N and Si:P ratios of the two groups of grasses (Fig. 4). Small-rodent winter disturbance alone decreased Si:N and Si:P ratios by 14-20% in both grass-groups (Fig. 4a,b,e), whereas reindeer summer herbivory alone only decreased Si:N ratio of Si-rich grasses by 11% (Fig. 4a). Both herbivores together did not affect Si:nutrient ratios of Si-rich grasses (Fig. 4a,d; significant two-way 'small-rodent winter disturbance × reindeer summer herbivory' interaction – Tables S4), but in Si-poor grasses they decreased Si:N ratio by 20% and Si:P ratio by 26% (Fig. 4b,e).

Si-rich grasses had higher leaf Si:nutrient ratios compared to Si-poor grasses under all herbivore combinations (Fig. 4c,f), but this difference was significantly greater under the impact of both herbivores. Here, herbivores increased the difference in Si:N and Si:P ratios between Si-rich and Si-poor grasses by 11 and 20%, respectively, compared to their difference in absence of herbivores.

Discussion

In this study, we assessed the extent to which small rodents and reindeer, two keystone Arctic herbivores, alter the quality of the community of grasses found in tundra-grasslands by modifying grass Si, N, and P levels. Contrary to our hypotheses, herbivores did not cause any net increase in grass Si-based defence levels and reindeer summer herbivory alone even decreased Si-content in Si-rich grasses. Herbivory also increased grass nutrient contents, but effects were stronger in Si-poor than Si-rich grasses. Si:nutrient ratios of grasses affected by herbivores were never above those of grasses in their absence, indicating that herbivory enhances the overall quality of the grass-community found in these tundra-grasslands. Yet, despite grasses became generally more palatable also when affected by both herbivores, the quality of Si-rich grasses was decreased relative to that of Si-poor grasses, possibly exacerbating the apparent competition among these grass groups. The ability of maintaining a lower nutritive quality may confer a competitive advantage to Si-rich grasses over Si-poor grasses against herbivores utilizing tundra-grasslands throughout the summer.

The magnitude of changes in grass Si-content following herbivory was relatively small compared to that observed for changes in nutrient levels. At the landscape-scale, spatial and temporal heterogeneity in factors other than herbivory is known to affect Si availability in the environment and Si uptake by grasses, eventually causing higher variation in leaf Si responses to herbivores (Soininen et al. 2013, Hartley and DeGabriel 2016, Ruffino et al. 2018). These factors may include environmental characteristics such as soil water availability (Quigley and Anderson 2014), but also genotypic and phenotypic plasticity within species (Soininen et al. 2013, Hartley et al. 2015). Indeed, inconsistent Si responses of five tundra-grass species to herbivores have been ascribed to the intra-specific variations in leaf Si-content, which is highly pronounced even within a few meters across tundra-grasslands (Soininen et al. 2013). However, soil moisture was not a significant predictor of grass Si-content in our study. Moreover, the high spatial and temporal resolution of our sampling was likely to capture intra-specific variations in leaf Si-content found within and across tundra-grasslands and throughout the summer season. Hence, our results clearly confirm that, also at the community-level, herbivores have little effects on Si-based defence levels of grasses found in tundra-grasslands (Soininen et al. 2013).

We found reindeer summer herbivory alone to decrease Si-content of Si-rich grasses, which directly opposed our expectation of higher grass Si-based defence levels in the presence of herbivores. Grass Si accumulation is influenced by leaf transpiration rates, which cause younger leaves to have generally lower Si-content compared to older leaves (Rafi and Epstein 1999). Moreover, since Si cannot be remobilized after its deposition in leaves (Richmond and Sussman 2003), grass Si-content often increases with both plant and leaf age (Brizuela et al. 1986, Cid et al. 1989, Kindomihou et al. 2006, Reynolds et al. 2012). In our study, reindeer herbivory may have promoted lower leaf Si-content in Si-rich grasses throughout the summer by keeping leaves in young phenological stages, as has previously been suggested (Bañuelos and Obeso 2000). It is of note that such decrease in Si-content was also responsible for lower leaf Si:N ratio of Si-rich grasses when grazed upon by reindeer. The capacity of tundra herbivores to enhance forage quality by reducing leaf carbon-to-nutrient ratios has long been recognized (Mysterud et al. 2011, Mosbacher et al. 2019, Beard et al. 2019), whereas their ability to increase grass-community forage quality by also reducing Si-based defence relative to nutrient levels is first confirmed in this study.

Small-rodent winter disturbance alone increased nutrient levels in both Si-rich and Si-poor tundra-grasses, ultimately increasing their quality. The fact that grasses growing in small-rodent disturbed tundra-patches would have higher nutrient levels than those found in absence of herbivores was expected. By using the same experimental set-up employed in this study, we have recently demonstrated that small rodents in winter increase tundra-grassland plant-community nutrient dynamics during the summer (Petit Bon et al. 2020b). We here add to this by showing that small-rodent activities occurring outside the growing season strongly increase the summer quality of the whole grass-community found in these tundra-grasslands, and that this happens through changes in grass nutrient, and not Si, levels. The effect of providing a grass-community with lower Si:N ratios, thus higher nutrient-reward potential, may have marked impacts on herbivores utilizing tundra-grasslands throughout the summer. This could be particularly relevant for reindeer; ruminants are selective herbivores (Hanley 1997) and high-quality forage would give higher return of essential nutrients for each bite and lower rumination time, potentially ameliorating their performance (Belovsky and Jordan 1978, White 1983).

We did not observe a net increase in grass Si-based defence levels following herbivory. Yet, Si-rich grasses impacted by both herbivores had higher Si-content than those under reindeer herbivory alone, but not higher Si-content than those found either in the presence of small rodents alone or in absence of herbivores. Since reindeer counteracted the positive effects of small rodents on nutrient levels of Si-rich grasses, we are confident that this interactive effect between herbivores on grass Si-content was not an artefact of that reindeer avoided disturbed tundra-patches in summer. A plausible explanation could be that the higher quality of the grass-community affected by small rodents attracted reindeer in disturbed tundra-patches (Petit Bon et al. 2020b). Here, more intense grazing may have impeded leaf rejuvenation of Si-rich grasses, thus causing their Si-content to remain unchanged throughout the summer season. These findings indicate that small rodents in winter can mediate reindeer effects on Si-content of Si-rich grasses in summer, eventually re-establishing higher Si-content in the grass-community when plants are affected by both herbivores. Thus, although herbivores did not cause a net Si accumulation in grass leaves in our tundra-grasslands, Si-rich grasses may still have a competitive advantage over Si-poor grasses by maintaining high Si-based defence levels when the herbivory pressure is high (Soininen et al. 2013).

There is yet another mechanism, which involved both grass Si and nutrient responses, that could confer a competitive advantage to Si-rich grasses over Si-poor grasses when the herbivory pressure is high. Indeed, the combined activity of both herbivores in our study not only offset the decrease in Si-content of Si-rich grasses promoted by reindeer alone (see above), but also reduced (for N) and cancelled out (for P) the positive effects of small rodents on their nutrient levels. Ultimately, the combined activity of both herbivores increased the quality (i.e. decreased Si:nutrient ratios) of Si-poor grasses, but not that of Si-rich grasses. Even more importantly, the quality of Si-rich grasses was decreased relative to that of Si-poor grasses, causing the two grass groups to have the largest difference in Si:nutrient ratios when affected by both herbivores. Such lower nutritive quality of Si-rich grasses compared to Si-poor grasses may act as an effective defence against herbivory (Moran and Hamilton 1980), thus influencing feeding selection by herbivores. The establishment of these negative indirect interactions, i.e. apparent competition (Holt and Bonsall 2017), between Si-rich and Si-poor grasses may partly explain why vegetation states dominated by Si-rich grasses are often promoted by tundra herbivores (Bråthen and Oksanen 2001, Austrheim et al. 2007, Ravolainen et al. 2011).

Conclusions

The quality of plants is crucial for herbivore performance (White 1983). Yet, plant quality can be difficult to quantify satisfactorily, as it depends on both plant defence and nutrient levels. By using Near infrared reflectance spectroscopy (NIRS) methodology, we were able to provide estimates of Si-based defence relative to nutrient levels for single tundra-grass leaves (Petit Bon et al. 2020a), i.e. at the bite-size level of the herbivores, and further scale up to the grass-community level. Our study provides the first field-based quantitative assessment of the role of herbivores for the quality of the whole grass-community found in tundra-grasslands.

High latitude regions are experiencing the fastest rate of environmental changes (Post et al. 2019), and there is extensive evidence that climate warming is affecting plant-communities in ways that may reduce forage quality for herbivores (Doiron et al. 2014, Zamin et al. 2017). By increasing nutrient relative to Si-based defence levels in forage grasses, tundra herbivores could thus be a key factor partially counteracting this decline in grass nutritive quality. Importantly, however, herbivores did not affect homogeneously the grass-community, but rather amplified differences in grass quality between tundra-patches and between Si-rich and Si-poor tundra-grasses. This mosaic in grass quality is likely to differ depending on the spatial and temporal dynamics of herbivores, and is in turn likely to affect the dynamics of herbivores themselves, causing tundra-grasslands to be a continuously changing foodscape.

Acknowledgements

We are grateful to Freja Fagerholm, Victor Toscani, Torunn Moe, and Francisco Murguzur for help with field assistance. We thank the leader of the reindeer herding district for being positive to the study. This work is part of the Tall Shrub Tundra module of the Climate ecological Observatory in the Arctic Tundra (COAT, http://coat.no).

References

Austrheim, G. et al. 2007. Interactions between sheep, rodents, graminoids, and bryophytes in an oceanic alpine ecosystem of low productivity. – Ecoscience 14: 178-187.

Baayen, R. H. et al. 2008. Mixed-effects modeling with crossed random effects for subjects and items. – J. Mem. Lang. 59: 390-412.

Bañuelos, M. J. and Obeso, J. R. 2000. Effect of grazing history, experimental defoliation, and genotype on patterns of silicification in *Agrostis tenuis* Sibth. – Ecoscience 7: 45-50.

Bardgett, R. D. and Wardle, D. A. 2003. Herbivore-mediated linkages between aboveground and belowground communities. – Ecology 84: 2258-2268.

Bates, D. et al. 2015a. Fitting linear mixed-effects models using lme4. – J. Stat. Softw. 67: 1-48.

Bates, D. et al. 2015b. Parsimonious mixed models. – arXiv:1506.04967v2 [stat.ME].

Beard, K. H. et al. 2019. Migratory goose arrival time plays a larger role in influencing forage quality than advancing springs in an Arctic coastal wetland. - PloS one 14: e0213037.

Belovsky, G. E. and Jordan, P. A. 1978. The time-energy budget of a moose. – Theor. Popul. Biol. 14: 76-104.

Bernes, C. et al. 2015. What are the impacts of reindeer/caribou (*Rangifer tarandus* L.) on arctic and alpine vegetation? A systematic review. – Environ. Evid. 4: 4.

Bolker, B. M. et al. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. – Trends Ecol. Evol. 24: 127-135.

Bråthen, K. A. and Oksanen, J. 2001. Reindeer reduce biomass of preferred plant species. – J. Veg. Sci. 12: 473-480.

Bråthen, K. A. et al. 2007. Induced shift in ecosystem productivity? Extensive scale effects of abundant large herbivores. – Ecosystems 10: 773-789.

Brizuela, M. A. et al. 1986. Silicon concentration of grasses growing in sites with different grazing histories. – Ecology 67: 1098-1101.

Cid, M. et al. 1989. Patterns in grass silicification: response to grazing history and defoliation. – Oecologia 80: 268-271.

Cotterill, J. V. et al. 2007. Boosting silica levels in wheat leaves reduces grazing by rabbits. – Pest Manag. Sci. 63: 247-253.

Doiron, M. et al. 2014. Effects of experimental warming on nitrogen concentration and biomass of forage plants for an arctic herbivore. – J. Ecol. 102: 508-517.

Drent, R. and Van der Wal, R. 1999. Cyclic grazing in vertebrates and the manipulation of the food resource. In: Olff, H. et al. (eds), Between plants and predators. Blackwell, pp. 271-299.

Frank, D. A. and Evans, R. D. 1997. Effects of native grazers on grassland N cycling in Yellowstone National Park. – Ecology 78: 2238-2248.

Gali-Muhtasib, H. et al. 1992. The effect of silica in grasses on the feeding behavior of the prairie vole, Microtus ochrogaster. – Ecology 73: 1724-1729.

Garbuzov, M. et al. 2011. Interactive effects of plant-available soil silicon and herbivory on competition between two grass species. – Ann. Bot. 108: 1355-1363.

Gibson, D. J. 2009. Grasses and grassland ecology. – Oxford University Press.

Hætta, J. I. et al. 1994. Reindriften i Finnmark: lovgivning og distriktsinndeling. – Reindriftsadministrasjonen, Alta, in Norwegian.

Hambäck, P. A. et al. 1998. Winter herbivory by voles during a population peak: the relative importance of local factors and landscape pattern. – J. Anim. Ecol. 67: 544-553.

Hamilton, E. W. and Frank, D. A. 2001. Can plants stimulate soil microbes and their own nutrient supply? Evidence from a grazing tolerant grass. – Ecology 82: 2397-2402.

Hanley, T. A. 1997. A nutritional view of understanding and complexity in the problem of diet selection by deer (Cervidae). – Oikos 79: 209-218.

Harbers, L. et al. 1981. The role of plant epidermal silica as a structural inhibitor of rumen microbial digestion in steers. – Nutrition Reports International.

Hartley, S. E. and DeGabriel, J. L. 2016. The ecology of herbivore-induced silicon defences in grasses. – Funct. Ecol. 30: 1311-1322.

Hartley, S. E. et al. 2015. Defending the leaf surface: intra-and inter-specific differences in silicon deposition in grasses in response to damage and silicon supply. – Front. Plant Sci. 6: 35.

Holt, R. D. and Bonsall, M. B. 2017. Apparent competition. – Annu. Rev. Ecol. Evol. Syst. 48: 447-471.

Huitu, O. et al. 2014. Silicon, endophytes and secondary metabolites as grass defenses against mammalian herbivores. – Front. Plant Sci. 5: 478.

Ims, R. A. and Fuglei, E. 2005. Trophic interaction cycles in tundra ecosystems and the impact of climate change. – BioScience 55: 311-322.

Jefferies, R. et al. 1994. Vertebrate herbivores and northern plant communities: reciprocal influences and responses. – Oikos 71: 193-206.

Killengreen, S. T. et al. 2007. Structural characteristics of a low Arctic tundra ecosystem and the retreat of the Arctic fox. – Biol. Conserv. 135: 459-472.

Kindomihou, V. et al. 2006. Effect of defoliation on silica accumulation in five tropical fodder grass species in Benin. – Belg. J. Bot. 139: 87-102.

Lenth, R. 2018. emmeans: estimated marginal means, aka least-squares means. – R package ver. 1.4.5, https://cran.rproject.org/web/packages/emmeans/index.html.

Ma, J. F. and Yamaji, N. 2006. Silicon uptake and accumulation in higher plants. – Trends Plant Sci. 11: 392-397.

Massey, F. P. and Hartley, S. E. 2006. Experimental demonstration of the antiherbivore effects of silica in grasses: impacts on foliage digestibility and vole growth rates. – Proc. R. Soc. B 273: 2299-2304.

Massey, F. P. and Hartley, S. E. 2009. Physical defences wear you down: progressive and irreversible impacts of silica on insect herbivores. – J. Anim. Ecol. 78: 281-291.

Massey, F. P. et al. 2006. Silica in grasses as a defence against insect herbivores: contrasting effects on folivores and a phloem feeder. – J. Anim. Ecol. 75: 595-603.

Massey, F. P. et al. 2007a. Grasses and the resource availability hypothesis: the importance of silica-based defences. – J. Ecol. 95: 414-424.

Massey, F. P. et al. 2007b. Herbivore specific induction of silica-based plant defences. – Oecologia 152: 677-683.

Massey, F. P. et al. 2009. Impacts of silica-based defences in grasses on the feeding preferences of sheep. – Basic Appl. Ecol. 10: 622-630.

McNaughton, S. and Tarrants, J. 1983. Grass leaf silicification: natural selection for an inducible defense against herbivores. – Proc. Natl. Acad. Sci. 80: 790-791.

McNaughton, S. J. et al. 1997. Promotion of the cycling of diet-enhancing nutrients by African grazers. – Science 278: 1798-1800.

Moran, N. and Hamilton, W. 1980. Low nutritive quality as defense against herbivores. – J. Theor. Biol. 86: 247-254.

Mosbacher, J. B. et al. 2019. Muskoxen modify plant abundance, phenology, and nitrogen dynamics in a High Arctic fen. – Ecosystems 22: 1095-1107.

Murguzur, F. J. A. et al. 2019. Towards a global arctic-alpine model for Near-infrared reflectance spectroscopy (NIRS) predictions of foliar nitrogen, phosphorus and carbon content. – Sci. Rep. 9: 8259.

Mysterud, A. et al. 2011. Plant quality, seasonality and sheep grazing in an alpine ecosystem. – Basic Appl. Ecol. 12: 195-206.

Olff, H. and Ritchie, M. E. 1998. Effects of herbivores on grassland plant diversity. – Trends Ecol. Evol, 13: 261-265.

Olofsson, J. et al. 2012. Vole and lemming activity observed from space. – Nat. Clim. Chang. 2: 880-883.

Petit Bon, M. et al. 2020a. One leaf for all: Chemical traits of single leaves measured at the leaf surface using Near infrared-reflectance spectroscopy (NIRS). – Methods Ecol. Evol., doi: https://doi.org/10.1111/2041-210X.13432.

Petit Bon, M. et al. 2020b. Interactions between winter and summer herbivory affect spatial and temporal plant nutrient dynamics in tundra grassland communities. – Oikos, doi: https://doi.org/10.1111/oik.07074.

Post, E. et al. 2019. The polar regions in a 2° C warmer world. – Sci. Adv. 5: eaaw9883.

Quigley, K. M. and Anderson, T. 2014. Leaf silica concentration in Serengeti grasses increases with watering but not clipping: insights from a common garden study and literature review. – Front. Plant Sci. 5: 568.

Rafi, M. M. and Epstein, E. 1999. Silicon absorption by wheat (*Triticum aestivum* L.). – Plant Soil 211: 223-230.

Ravolainen, V. T. et al. 2011. Rapid, landscape scale responses in riparian tundra vegetation to exclusion of small and large mammalian herbivores. – Basic Appl. Ecol. 12: 643-653.

Reynolds, J. J. et al. 2012. Delayed induced silica defences in grasses and their potential for destabilising herbivore population dynamics. – Oecologia 170: 445-456.

Richmond, K. E. and Sussman, M. 2003. Got silicon? The non-essential beneficial plant nutrient. – Curr. Opin. Plant Biol. 6: 268-272.

Ruffino, L. et al. 2018. Population-level manipulations of field vole densities induce subsequent changes in plant quality but no impacts on vole demography. – Ecol. Evol. 8: 7752-7762.

Shadish, W. R. et al. 2002. Experimental and quasi-experimental designs for generalized causal inference. – Cengage learning.

Smis, A. et al. 2014. Determination of plant silicon content with near infrared reflectance spectroscopy. – Front. Plant Sci. 5: 496.

Soininen, E. M. et al. 2017. Importance of study design and robust analyses in ecology – what is the evidence for silica–vole interactions? – Funct. Ecol. 31: 1847-1852.

Soininen, E. M. et al. 2013. More than herbivory: levels of silica-based defences in grasses vary with plant species, genotype and location. – Oikos 122: 30-41.

Vicari, M. and Bazely, D. R. 1993. Do grasses fight back? The case for antiherbivore defences. – Trends Ecol. Evol. 8: 137-141.

Walker, D. A. et al. 2005. The Circumpolar Arctic vegetation map. – J. Veg. Sci. 16: 267-282.

White, R. G. 1983. Foraging patterns and their multiplier effects on productivity of northern ungulates. – Oikos 40: 377-384.

Wickham, H. 2016. ggplot2: elegant graphics for data analysis. – Springer.

Zamin, T. J. et al. 2017. Experimental warming alters migratory caribou forage quality. – Ecol. Appl. 27: 2061-2073.

Zuur, A. F. et al. 2010. A protocol for data exploration to avoid common statistical problems. – Methods Ecol. Evol. 1: 3-14.

Figures

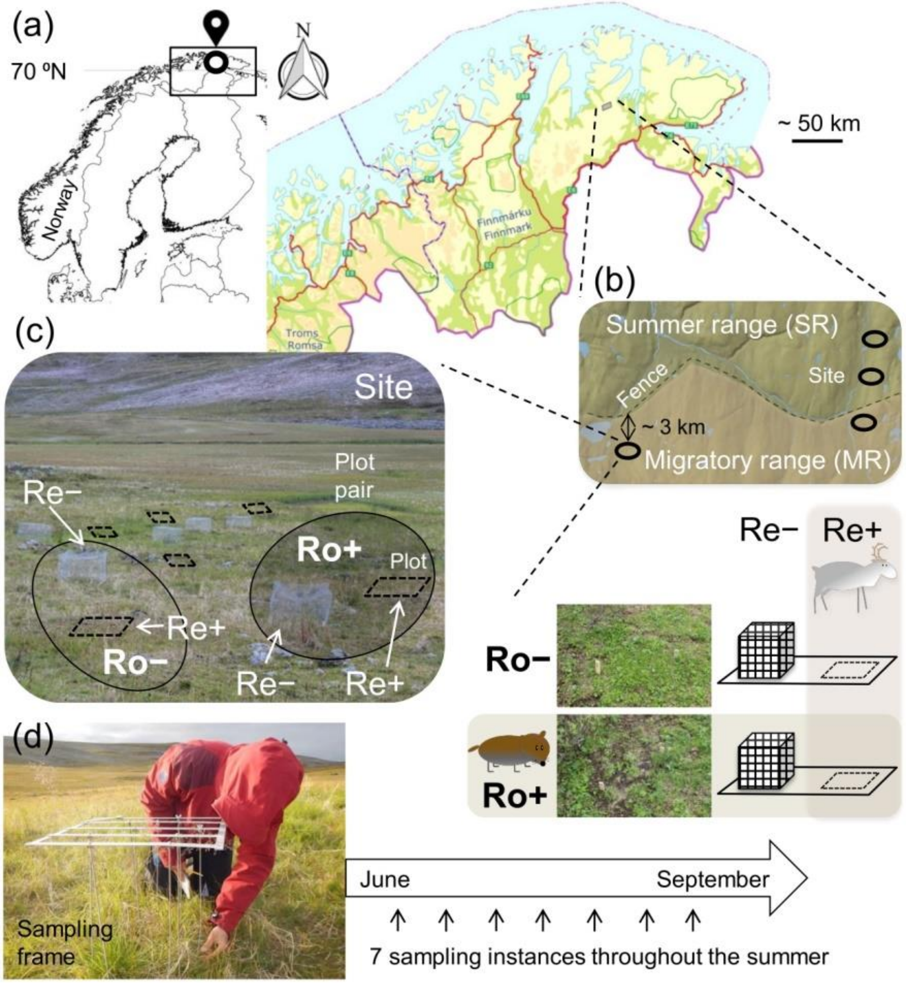


Figure 1. Study and sampling design. (a) Study location. (b-c) Hierarchical spatial structure of the study design. A full-factorial semi-randomized pair design was implemented in four tundra-grassland sites in which small-rodent winter disturbance was used as a quasi-experimental factor (Shadish et al. 2002) and reindeer summer herbivory as a fully experimental factor. In total, 96 plots were established (across all grassland sites, each treatment was replicated 24 times). (d) Temporal structure of the sampling design. Leaf sampling was performed at seven instances from the start to the end of the growing season (between 28th of June and 10th of September, on average every 12 ± 1.3 days). Treatment coding and animal *silhouettes* presented in this figure will be consistent throughout the manuscript.

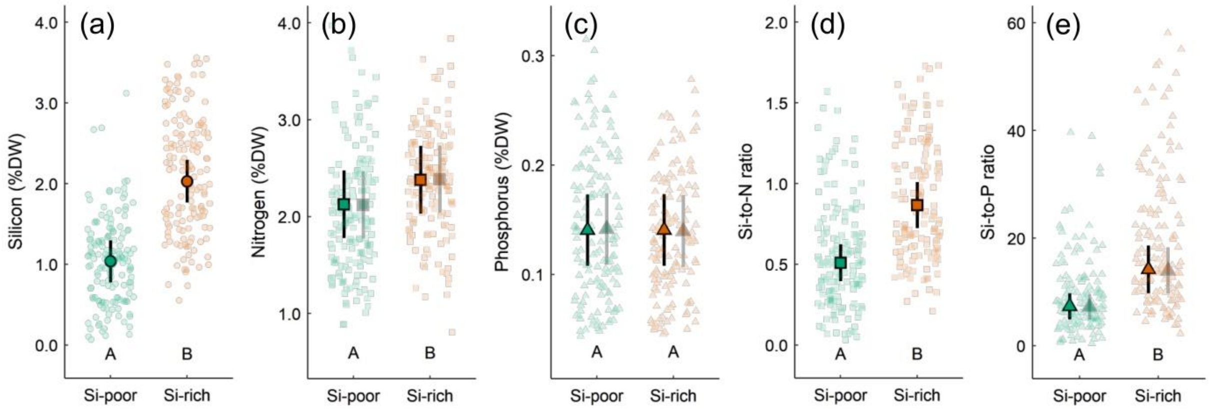


Figure 2. Baseline leaf elemental content, i.e. leaf elemental content in absence of herbivores, in Si-poor and Si-rich tundra-grasses. Model predictions and their 95% confidence intervals [CIs] are given for leaf (a) Silicon [Si], (b) Nitrogen [N], and (c) Phosphorus [P] contents [% dry weight – %DW] and (d) Si:N and (e) Si:P ratios of Si-poor and Si-rich tundra-grasses in absence of herbivores (Ro−/Re− plots) (model predictions for leaf P-content and Si:N and Si:P ratios were back-transformed on the response scale prior to presentation). Different letters at the base of each panel indicate significant differences between Si-poor and Si-rich grasses. For N, P, and Si:P ratio, model predictions and their 95% CIs are presented for both full models (bold) and most parsimonious models (shaded). Dots in the background show the raw data, which were spaced within each grass-group to reduce overlapping. Colour coding for Si-poor and Si-rich grasses and symbol (shape) coding for the five response variables will be consistent throughout the manuscript.

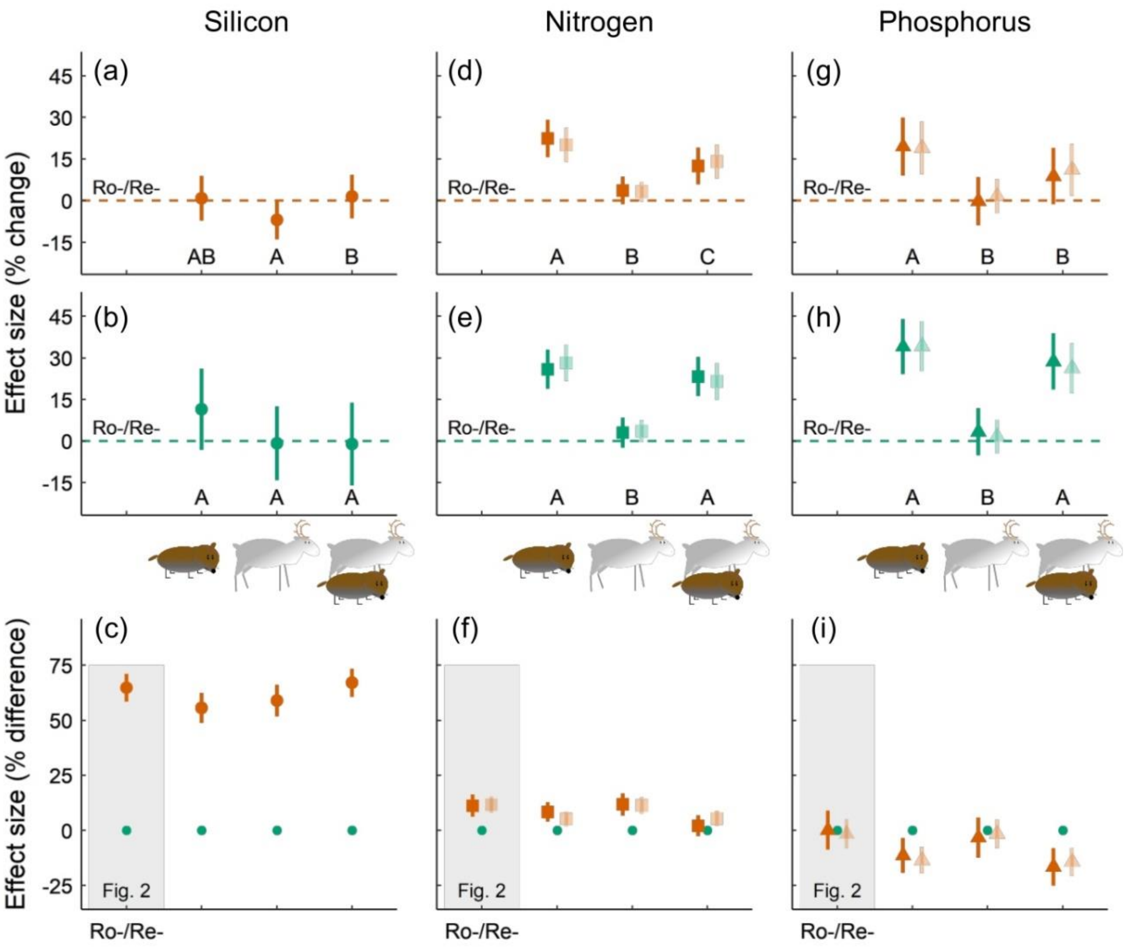


Figure 3. Effects of herbivores on leaf silicon, nitrogen, and phosphorus levels in tundra-grass communities. (Upper panels) Effect sizes [as % change] and their 95% confidence intervals [CIs] are given for the effects of small-rodent winter disturbance alone, reindeer summer herbivory alone, and small-rodent winter disturbance + reindeer summer herbivory as contrasts to plots not affected by herbivores, separately for Si-rich [orange] and Si-poor [green] tundra-grasses (i.e. a positive effect size indicates higher leaf elemental contents in the presence of herbivores than in their absence). Plots not affected by herbivores [Ro−/Re− plots] is used as the reference level and is denoted with the dashed lines at 0 effect size (refer to *Figure 2* for actual values of leaf elemental contents in Ro−/Re− plots). Different letters at the base of each panel indicate that the difference in leaf elemental contents between two herbivore-treatment combinations was statistically significant. (Lower panels) Contrast between Si-rich and Si-poor tundra-grasses (effect sizes in % difference and their 95% CI),

separately for the four herbivore-treatment combinations (see *Figure 2* for actual values of leaf elemental contents in Ro−/Re− plots). Si-poor tundra-grasses under the four herbivore-treatment combinations is used as the reference level and is denoted with the dots at 0 effect size. Effect sizes (and their CIs) were acquired from the prediction models on tundra-grass community silicon, nitrogen, and phosphorus levels. For nitrogen and phosphorus contents, effect sizes and their 95% CIs are presented for both full models (bold) and most parsimonious models (shaded).

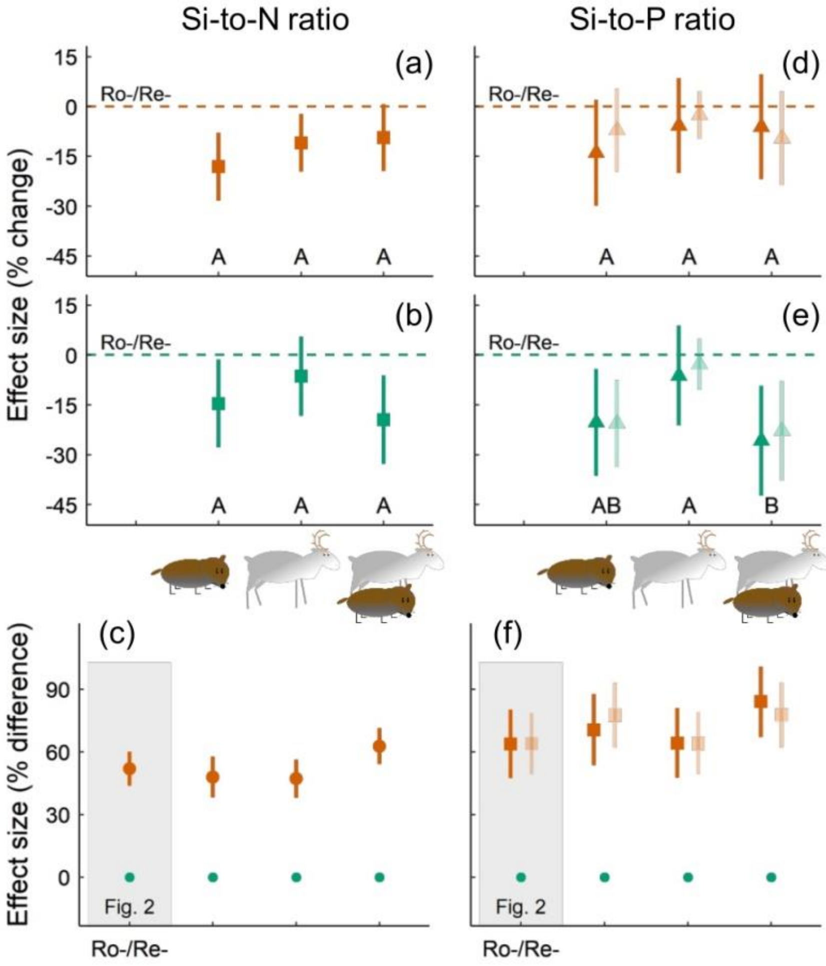


Figure 4. Effects of herbivores on leaf silicon-to-nitrogen and silicon-to-phosphorus ratios in tundra-grass communities. (Upper panels) Effect sizes [as % change] and their 95% confidence intervals [CIs] are given for the effects of herbivores as contrasts to plots not affected by herbivores, separately for Si-rich [orange] and Si-poor [green] tundra-grasses. Plots not affected by herbivores [Ro−/Re− plots] is used as the reference level and is denoted with the dashed lines at 0 effect size. Different letters at the base of each panel indicate that the difference in leaf elemental ratios between two herbivore-treatment combinations was statistically significant. (Lower panels) Contrast between Si-rich and Si-poor tundra-grasses (effect sizes in % difference and their 95% CI), separately for the four herbivore-treatment combinations. Si-poor tundra-grasses under the four herbivore-treatment combinations is used as the reference level and is denoted with the dots at 0 effect size. Effect sizes (and their

CIs) were acquired from the prediction models on tundra-grass community Si:N and Si:P ratios. For Si:P ratio, effect sizes and their 95% CIs are presented for both full model (bold) and most parsimonious model (shaded). Full description of graph content is provided in *Figure 3*.

Supplementary Material

Below are the supplementary methods, tables and figures supporting the study "**Silicon-based defence and nutrient levels in grasses under herbivory: Forage quality in tundra grasslands is altered through different mechanisms**".

Matteo Petit Bon[1,2*†], Katarina Gunnarsdotter Inga[2*], Tove Aagnes Utsi[2], Ingibjörg Svala Jónsdóttir[3], and Kari Anne Bråthen[2]

[*] Shared first-authorship

[1] Department of Arctic Biology, The University Centre in Svalbard (UNIS), PO Box 156, N-9171 Longyearbyen, Norway

[2] Department of Arctic and Marine Biology, Faculty of Biosciences, Fisheries, and Economics, The Arctic University of Norway, N-9037 Tromsø, Norway

[3] Institute of Life and Environmental Sciences, University of Iceland, Sturlugata 7, IS-101 Reykjavik, Iceland

[†] Corresponding author. E-mail: matteo.petitbon@unis.no; Fax: (+47) 79 02 33 01; Phone: (+47) 966 59 147; ORCID-ID: https://orcid.org/0000-0001-9829-8324

KGI: katarina.inga@biegga.com

TAU: tove.a.utsi@uit.no (ORCID-ID: https://orcid.org/0000-0003-0776-2369)

ISJ: isj@hi.is (ORCID-ID: https://orcid.org/0000-0003-3804-7077)

KAB: kari.brathen@uit.no (ORCID-ID: https://orcid.org/0000-0003-0942-1074)

Supporting Tables

Table S1. Parameter estimates for the linear mixed-effects model for the effects of herbivores on leaf silicon content in tundra-grass communities. Parameter estimates of fixed-effects (*Estimates*) and their 95% confidence interval (*CI* – lower and upper bounds) for the model on leaf silicon content (% of dry weight – %DW) in tundra-grass communities, in which predictors are ‘grass-groups’, ‘small-rodent winter disturbance’, and ‘reindeer summer herbivory’. Intercept is calculated for Si-poor grasses, undisturbed tundra-patches (Ro−), and reindeer-exclusion plots (Re−). Estimates with bold indicate that their 95% CI does not include 0. Random-effects retained in the model are presented as standard deviations. Observations refer to the number of grass leaf-samples used in the model. Marginal R^2 represents the variance explained by the model when only fixed-effects are considered, whereas the conditional R^2 represents the variance explained by the model when both fixed- and random-effects are considered.

	Silicon (%DW)	
Fixed Effects (*Predictors*)	*Estimates*	*CI (95%)*
(*Intercept*) [Si-poor grasses / Ro– / Re–]	**1.04** ***	**0.78 ; 1.30**
Grass-group [Si-rich grasses]	**0.99** ***	**0.85 ; 1.14**
Small-rodent winter disturbance [Ro+]	0.12	–0.03 ; 0.27
Reindeer summer herbivory [Re+]	–0.01	–0.15 ; 0.13
Grass-group and Small-rodent interaction [Si-rich grasses × Ro+]	–0.10	–0.31 ; 0.10
Grass-group and Reindeer interaction [Si-rich grasses × Re+]	–0.13	–0.33 ; 0.06
Small-rodent and Reindeer interaction [Ro+ × Re+]	–0.12	–0.32 ; 0.08
Grass-group, Small-rodent, and Reindeer interaction [Si-rich grasses × Ro+ × Re+]	*0.28* †	*–0.01 ; 0.56*
Random Effects	*St.dev.*	
Grassland site	0.18	
Grassland site / Tundra-patch	0.12	
Sampling instance	0.22	
Residual	0.61	
Observations	1158	
Marginal R^2 - Conditional R^2	0.327 - 0.462	

†*p=0.05* * *p<0.05* ** *p<0.01* *** *p<0.001*

Table S2. Parameter estimates for linear mixed-effects models for the effects of herbivores on leaf nitrogen content in tundra-grass communities. Parameter estimates of fixed-effects (*Estimates*) and their 95% confidence interval (*CI* – lower and upper bounds) for (a) the full model and (b) the most parsimonious model on leaf nitrogen content (% of dry weight – %DW) in tundra-grass communities, in which predictors are ‘grass-groups’, ‘small-rodent winter disturbance’, and ‘reindeer summer herbivory’. Intercept is calculated for Si-poor grasses, undisturbed tundra-patches (Ro−), and reindeer-exclusion plots (Re−). Estimates with bold indicate that their 95% CI does not include 0. Empty cells in (b) indicate that a predictor was not statistically significant, thus it was removed from the model. Random-effects retained in the models are presented as standard deviations. Observations refer to the number of grass leaf-samples used in the models. Marginal R^2 represents the variance explained by the models when only fixed-effects are considered, whereas the conditional R^2 represents the variance explained by the models when both fixed- and random-effects are considered.

	(a) Nitrogen (%DW) [*full model*]		**(b) Nitrogen (%DW)** [*reduced model*]	
Fixed Effects (*Predictors*)	*Estimates*	*CI (95%)*	*Estimates*	*CI (95%)*
(*Intercept*) [Si-poor grasses / Ro– / Re–]	**2.13** ***	**1.78 ; 2.48**	**2.12** ***	**1.77 ; 2.47**
Grass-group [Si-rich grasses]	**0.25** ***	**0.13 ; 0.37**	**0.26** ***	**0.17 ; 0.35**
Small-rodent winter disturbance [Ro+]	**0.55** ***	**0.40 ; 0.70**	**0.60** ***	**0.46 ; 0.74**
Reindeer summer herbivory [Re+]	0.07	–0.05 ; 0.18	*0.08* †	*–0.01 ; 0.16*
Grass-group and Small-rodent interaction [Si-rich grasses × Ro+]	–0.02	–0.19 ; 0.16	*–0.12* †	*–0.25 ; 0.01*
Grass-group and Reindeer interaction [Si-rich grasses × Re+]	0.02	–0.14 ; 0.19		
Small-rodent and Reindeer interaction [Ro+ × Re+]	–0.12	–0.29 ; 0.04	**–0.22** ***	**–0.34 ; –0.10**
Grass-group, Small-rodent, and Reindeer interaction [Si-rich grasses × Ro+ × Re+]	–0.20	–0.44 ; 0.04		
Random Effects	*St.dev.*		*St.dev.*	
Grassland site	0.16		0.16	
Grassland site / Tundra-patch	0.17		0.17	
Sampling instance	0.39		0.39	
Residual	0.52		0.52	
Observations	1182		1182	
Marginal R^2 / Conditional R^2	0.114 - 0.505		0.113 - 0.504	

†*p=0.07* * *p<0.05* ** *p<0.01* *** *p<0.001*

Table S3. Parameter estimates for linear mixed-effects models for the effects of herbivores on leaf phosphorus content in tundra-grass communities. Parameter estimates of fixed-effects (*Estimates*) and their 95% confidence interval (*CI* – lower and upper bounds) for (a) the full model and (b) the most parsimonious model on leaf phosphorus content (log[% of dry weight + 1]) in tundra-grass communities, in which predictors are 'grass-groups', 'small-rodent winter disturbance', and 'reindeer summer herbivory'. Intercept is calculated for Si-poor grasses, undisturbed tundra-patches (Ro−), and reindeer-exclusion plots (Re−). Estimates with bold indicate that their 95% CI does not include 0. Empty cells in (b) indicate that a predictor was not statistically significant, thus it was removed from the model. Random-effects retained in the models are presented as standard deviations. Observations refer to the number of grass leaf-samples used in the models. Marginal R^2 represents the variance explained by the models when only fixed-effects are considered, whereas the conditional R^2 represents the variance explained by the models when both fixed- and random-effects are considered.

	(a) Phosphorus log(%DW+1) [*full model*]		**(b) Phosphorus log(%DW+1)** [*reduced model*]	
Fixed Effects (*Predictors*)	*Estimates*	*CI (95%)*	*Estimates*	*CI (95%)*
(*Intercept*) [Si-poor grasses / Ro– / Re–]	**0.131** ***	**0.103 ; 0.160**	**0.132** ***	**0.104 ; 0.161**
Grass-group [Si-rich grasses]	0.0001	–0.011 ; 0.011	–0.002	–0.010 ; 0.006
Small-rodent winter disturbance [Ro+]	**0.041** ***	**0.029 ; 0.053**	**0.042** ***	**0.031 ; 0.052**
Reindeer summer herbivory [Re+]	0.004	–0.006 ; 0.015	0.002	–0.006 ; 0.010
Grass-group and Small-rodent interaction [Si-rich grasses × Ro+]	**–0.017** *	**–0.033 ; –0.002**	**–0.019** **	**–0.030 ; –0.007**
Grass-group and Reindeer interaction [Si-rich grasses × Re+]	–0.004	–0.019 ; 0.011		
Small-rodent and Reindeer interaction [Ro+ × Re+]	–0.010	–0.025 ; 0.004	**–0.011** *	**–0.022 ; –0.001**
Grass-group, Small-rodent, and Reindeer interaction [Si-rich grasses × Ro+ × Re+]	–0.002	–0.024 ; 0.019		
Random Effects		*St.dev.*		*St.dev.*
Grassland site		0.020		0.020
Grassland site / Tundra-patch		0.010		0.010
Sampling instance		0.025		0.025
Residual		0.045		0.045
Observations	1131		1131	
Marginal R^2 - Conditional R^2	0.072 - 0.405		0.071 - 0.405	

** p<0.05 ** p<0.01 *** p<0.001*

Table S4. Parameter estimates for the linear mixed-effects model for the effects of herbivores on leaf silicon-to-nitrogen ratio in tundra-grass communities. Parameter estimates of fixed-effects (*Estimates*) and their 95% confidence interval (*CI* – lower and upper bounds) for the model on leaf silicon-to-nitrogen ratio (log[silicon-to-nitrogen ratio +1]) in tundra-grass communities, in which predictors are ‘grass-groups’, ‘small-rodent winter disturbance’, and ‘reindeer summer herbivory’. Intercept is calculated for Si-poor grasses, undisturbed tundra-patches (Ro−), and reindeer-exclusion plots (Re−). Estimates with bold indicate that their 95% CI does not include 0. Random-effects retained in the model are presented as standard deviations. Observations refer to the number of grass leaf-samples used in the model. Marginal R^2 represents the variance explained by the model when only fixed-effects are considered, whereas the conditional R^2 represents the variance explained by the model when both fixed- and random-effects are considered.

	Silicon-to-nitrogen log(Si:N+1)	
Fixed Effects (*Predictors*)	*Estimates*	*CI (95%)*
(*Intercept*) [Si-poor grasses / Ro– / Re–]	**0.412** ***	**0.336 ; 0.487**
Grass-group [Si-rich grasses]	**0.212** ***	**0.170 ; 0.254**
Small-rodent winter disturbance [Ro+]	**–0.051** *	**–0.096 ; –0.005**
Reindeer summer herbivory [Re+]	–0.022	–0.062 ; 0.019
Grass-group and Small-rodent interaction [Si-rich grasses × Ro+]	–0.038	–0.098 ; 0.023
Grass-group and Reindeer interaction [Si-rich grasses × Re+]	–0.031	–0.088 ; 0.027
Small-rodent and Reindeer interaction [Ro+ × Re+]	0.004	–0.054 ; 0.062
Grass-group, Small-rodent, and Reindeer interaction [Si-rich grasses × Ro+ × Re+]	**0.092** *	**0.009 ; 0.175**
Random Effects		*St.dev.*
Grassland-site		0.043
Grassland-site / Tundra-patch		0.038
Sampling instance		0.073
Residual		0.179
Observations	1165	
Marginal R^2 - Conditional R^2	0.214 - 0.380	

* *p<0.05* ** *p<0.01* *** *p<0.001*

Table S5. Parameter estimates for linear mixed-effects models for the effects of herbivores on leaf silicon-to-phosphorus ratio in tundra-grass communities. Parameter estimates of fixed-effects (*Estimates*) and their 95% confidence interval (*CI* – lower and upper bounds) for (a) the full model and (b) the most parsimonious model on leaf silicon-to-phosphorus ratio (log[silicon-to-phosphorus ratio +1]) in tundra-grass communities, in which predictors are 'grass-groups', 'small-rodent winter disturbance', and 'reindeer summer herbivory'. Intercept is calculated for Si-poor grasses, undisturbed tundra-patches (Ro−), and reindeer-exclusion plots (Re−). Estimates with bold indicate that their 95% CI does not include 0. Empty cells in (b) indicate that a predictor was not statistically significant, thus it was removed from the model. Random-effects retained in the models are presented as standard deviations. Observations refer to the number of grass leaf-samples used in the models. Marginal R^2 represents the variance explained by the models when only fixed-effects are considered, whereas the conditional R^2 represents the variance explained by the models when both fixed- and random-effects are considered.

	(a) Silicon-to-phosphorus log(Si:P+1) [*full model*]		**(b) Silicon-to-phosphorus log(Si:P+1)** [*reduced model*]	
Fixed Effects (*Predictors*)	*Estimates*	*CI (95%)*	*Estimates*	*CI (95%)*
(*Intercept*) [Si-poor grasses / Ro– / Re–]	**2.12** ***	**1.83 ; 2.41**	**2.10** ***	**1.82 ; 2.39**
Grass-group [Si-rich grasses]	**0.60** ***	**0.46 ; 0.74**	**0.60** ***	**0.50 ; 0.70**
Small-rodent winter disturbance [Ro+]	**–0.20** **	**–0.34 ; –0.05**	**–0.20** ***	**–0.31 ; –0.09**
Reindeer summer herbivory [Re+]	–0.06	–0.19 ; 0.08	–0.02	–0.09 ; 0.04
Grass-group and Small-rodent interaction [Si-rich grasses × Ro+]	0.06	–0.14 ; 0.25	*0.13* †	*–0.01 ; 0.27*
Grass-group and Reindeer interaction [Si-rich grasses × Re+]	0.001	–0.19 ; 0.19		
Small-rodent and Reindeer interaction [Ro+ × Re+]	–0.004	–0.19 ; 0.19		
Grass-group, Small-rodent, and Reindeer interaction [Si-rich grasses × Ro+ × Re+]	0.14	–0.13 ; 0.41		
Random Effects	*St.dev.*		*St.dev.*	
Grassland site	0.22		0.22	
Grassland site / Tundra-patch	0.10		0.10	
Sampling instance	0.22		0.22	
Residual	0.58		0.58	
Observations	1115		1115	
Marginal R^2 - Conditional R^2	0.212 - 0.405		0.211 - 0.404	

†*p=0.07* * *p<0.05* ** *p<0.01* *** *p<0.001*

Supporting Figures

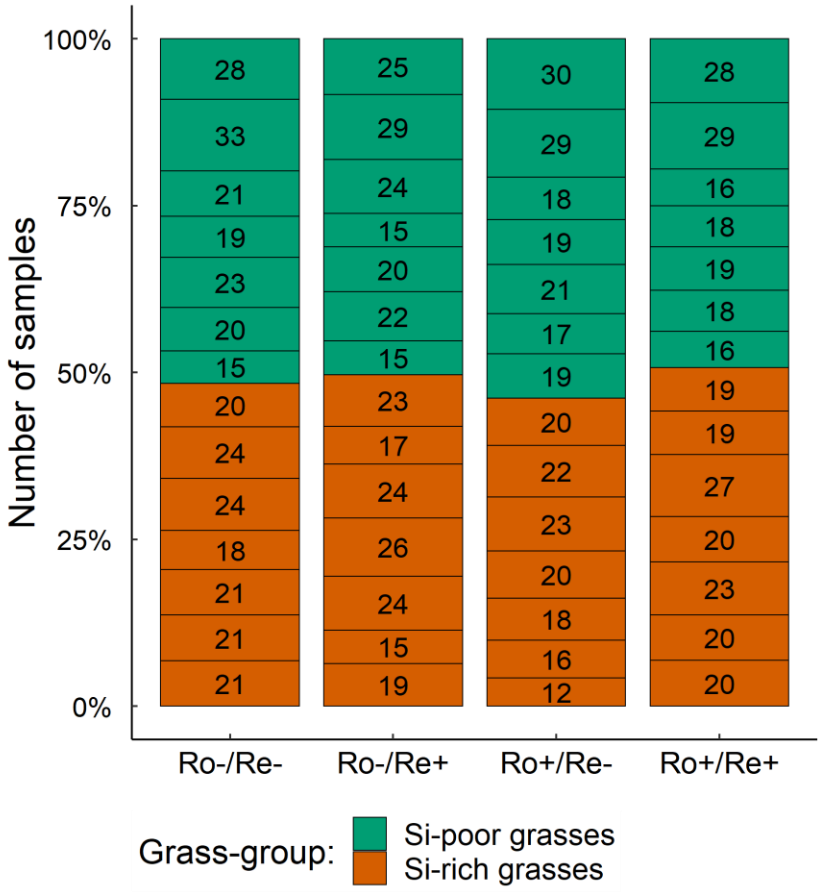


Figure S1. Overview of the number of grass leaf-samples collected, processed, and analysed for the present study. The number of leaf-samples for Si-poor and Si-rich grasses is given as percentage of the total number of grass leaf-samples collected within each herbivore-treatment combination, separately for each sampling instance (from one to seven; see main text for details). Within each herbivore-treatment combination and grass-group, sampling instance 'one' is displayed at the bottom, whereas sampling instance 'seven' is displayed at the top.

Paper IV

Variable chemical responses of three major ecosystem compartments to herbivory and warming in high-Arctic tundra

Variable chemical responses of three major ecosystem compartments to herbivory and warming in high-Arctic tundra

Matteo Petit Bon[1,2,*], Hanna Böhner[1,2], Kari Anne Bråthen[2], Virve Tuulia Ravolainen[3], Ingibjörg Svala Jónsdóttir[4]

[1] Department of Arctic Biology, University Centre in Svalbard (UNIS), PO Box 156, N-9171 Longyearbyen, Norway

[2] Department of Arctic and Marine Biology, Faculty of Biosciences, Fisheries, and Economics, Arctic University of Norway (UiT), N-9037 Tromsø, Norway

[3] Norwegian Polar Institute (NPI), Fram Centre, PO Box 6606 Langnes, N-9296 Tromsø, Norway

[4] Institute of Life and Environmental Sciences, University of Iceland, Sturlugata 7, IS-101 Reykjavik, Iceland

[*] Corresponding author. E-mail: matteo.petitbon@unis.no; Fax: (+47) 79 02 33 01; Phone: (+47) 966 59 147; ORCID-ID: https://orcid.org/0000-0001-9829-8324

HB: hanna.bohner@uit.no
KAB: kari.brathen@uit.no (ORCID-ID: https://orcid.org/0000-0003-0942-1074)
VTR: virve.ravolainen@npolar.no (ORCID-ID: https://orcid.org/0000-0001-8411-7238)
ISJ: isj@hi.is (ORCID-ID: https://orcid.org/0000-0003-3804-7077)

Authors' contributions and authors' statement

MPB, ISJ, KAB, and VTR conceived the idea. MPB and HB collected the data. MPB analysed the data and led the writing of the manuscript with inputs from all authors. There are no conflicts of interest to declare.

Keywords: Carbon (C), Community-weighted means, C-to-N ratio, Goose grubbing, Habitat-types, ITEX (OTC), Mosses, Near infrared reflectance spectroscopy (NIRS), Nitrogen (N), Pink-footed geese, Soil, Svalbard, Vascular plants

Abstract

Most studies assessing chemical responses of tundra ecosystems to environmental changes have focused on either vegetation or soil compartments. Yet, the extent to which they differ in their short-term chemical responses to perturbations may have immediate implications for processes and functions of tundra ecosystems. Here, we aimed at comparing short-term carbon (C), nitrogen (N), and C:N ratio responses of three major ecosystem compartments (i.e. vascular plants, mosses, and soil) to herbivore disturbance and summer warming in the Arctic.

Spring grubbing and faeces deposition by pink-footed geese (*Anser brachyrhynchus*) and summer warming were simulated in a two-year full-factorial experiment within three habitats along a soil moisture gradient in the high-Arctic archipelago of Svalbard.

Ecosystem compartments differed in their chemical responses to treatments (vascular plants > soil > mosses), and responses also differed between habitats (mesic > moist > wet). Chemical responses were generally consistent across the two consecutive summers, despite large differences in temperature conditions and greater between-year variation in the chemical composition of ecosystem compartments compared to their responses to treatments. By differentially altering the chemical composition of vascular plants, mosses, and soil, herbivore disturbance and summer warming may have immediate effects on the functioning of tundra ecosystems. However, the degree of such effects will vary among habitats, eventually affecting ecosystem processes across the tundra landscape at different rates. Consistent short-term chemical responses of vascular plants, mosses, and soil found across years that widely differed in temperature conditions indicate that herbivores and warming are predictable modifiers of biogeochemical processes in Arctic ecosystems. Yet, larger between-year differences in ecosystem-compartment C and N contents and C:N ratio compared to treatment effects suggest that stronger responses should be expected as the intensity of perturbations increases.

Introduction

The biogeochemistry of high-latitude ecosystems is largely controlled by the slow turnover rates of carbon (C) and nutrients between soil and vegetation (Hobbie et al. 2002). The prevailing low soil temperatures slow down rates of organic matter decomposition (Cornelissen et al. 2007), which in turn severely limit nutrient mineralization rates (Nadelhoffer et al. 1991). Slow nutrient cycling rates (particularly of nitrogen [N]) constrain plant growth, and thus ecosystem C gain through primary productivity (Shaver and Chapin 1980, Rustad et al. 2001). Under such limiting conditions, any factors that modify C and N contents of soil and vegetation can have large effects on processes and functions of cold ecosystems.

Studying the extent to which C and N contents of soil and vegetation can immediately respond to perturbations may help us understand the capacity of cold ecosystems to resist disturbance and maintain their functioning (Hobbie et al. 2002). For instance, alterations in the C-content of soil and vegetation may affect C exchange rates and C budget of ecosystems. Similarly, changes in their N-content may alter ecosystem N cycling. But do soil and vegetation differ in their chemical responses to perturbations? C and N contents and the stoichiometry (i.e. carbon-to-nitrogen [C:N] ratio) of soil and vegetation differ widely (Shaver et al. 1992), even within vegetation compartments (i.e. vascular plants vs mosses). As well, these ecosystem compartments may differ in their capacity to immediately respond to perturbations. Differential sensitivity and magnitude of soil and vegetation C and N responses may have important implications for the role each ecosystem compartment has for the C balance and N cycling within the ecosystem (Shaver et al. 1992, Hobbie et al. 2002). Here, we report on a field experiment aimed at revealing the extent to which three major compartments of tundra ecosystems, i.e. vascular plants, mosses, and soil, differ in their short-term C, N, and C:N ratio responses to warming and herbivory in the Arctic.

With an increase in average annual temperature by 0.75 °C over the past decade, Arctic regions are warming faster than any other areas on Earth (Post et al. 2019). Several studies have investigated the effects of experimental warming on C and N contents of either vegetation or soil in Arctic ecosystems. While over longer time scales (i.e. 10-20 years), vascular plants (Sorensen et al. 2008), mosses (Sorensen et al. 2012), and soil (Alatalo et al. 2017) appear to at least partially restore their chemical composition that could have initially changed in response to elevated temperatures, short-term chemical responses may still differ between these tundra-ecosystem compartments. Vascular plants showed C and N responses to experimentally-imposed higher summer temperatures already after 2-6 years of manipulations in diverse low-Arctic (Welker et al. 2005, Aerts et al. 2009) and high-Arctic

(Tolvanen and Henry 2001, Doiron et al. 2014) ecosystems. Mosses and soil are often less responsive, although changes in their chemical composition following short-term (2-4 year) experimental warming have been documented (Biasi et al. 2008, Deane-Coe et al. 2015). It is likely that vascular plants, mosses, and soil differ in their short-term C and N responses to higher summer temperatures, yet a direct comparison from tundra ecosystems is lacking.

In a rapidly warming Arctic, it is important to consider the capacity of tundra ecosystems to resist disturbances such as herbivory. Herbivores are a key component of Arctic ecosystems (Barrio et al. 2016, Speed et al. 2019) and strongly influence their functioning (Mulder 1999). Herbivores disturbing both vegetation and soil, such as grubbing geese (Jefferies and Rockwell 2002), could be particularly important drivers of the biogeochemistry of tundra ecosystems. During the pre-breeding period in spring, migratory geese of the genera *Anser* and *Chen* forage on below-ground plant parts, i.e. roots and rhizomes, through grubbing (Fox et al. 2006). Grubbing is a disturbance that occurs at the landscape-scale (Figure 1A), although each affected tundra-patch can be small in extent (Figure 1B) (Ravolainen et al. 2020). By causing rapid vegetation loss and disruption of the moss-mat structure, but also by leading to soil erosion, goose grubbing has the potential to cause severe disturbance across the tundra landscape (Jefferies and Rockwell 2002, Speed et al. 2010a). The dramatic increase of migratory goose populations in both the Nearctic and Palearctic (Fox and Madsen 2017) suggests that short-term impacts of geese on C and N contents of vegetation and soil may be considerable. Yet, chemical responses of tundra ecosystems to goose grubbing have not been investigated. The archipelago of Svalbard, in the European high-Arctic, has seen a noticeable rise in both the number of pink-footed geese (Anser brachyrhynchus – from 15000 individuals in 1965 up to 90000 individuals at present; Madsen et al. 2017) and average annual temperatures (1.25 ˚C/decade for the period 1989-2011; Førland et al. 2011) during the last decades. It therefore represents a suitable system for testing the extent to which vascular plants, mosses, and soil differ in their short-term C and N responses to goose disturbance, and explore whether interactive effects with warmer summers are at stake.

Arctic ecosystems contain a mosaic of different habitats due to fine-scale heterogeneities in topography and hydrological conditions (Walker 2000). Arctic habitats are characterized by diverse plant communities, which differ in their C and N contents (Welker et al. 2005). Peculiarly, tundra ecosystems are often dominated by mosses, whose N-content is lower than that of vascular plants (Aerts et al. 2009, Turetsky et al. 2012), but are effective in competing with them for available nutrients deposited from e.g. animal-excreta (Gornall et al. 2009, Sjögersten et al. 2010). Moreover, the moss layer insulates the soil and affects the below-ground environment, thus playing a critical role in mediating biogeochemical processes in the tundra (Gornall et al. 2007, Turetsky et al. 2012). Further, Arctic habitats are associated to diverse soil types, which also differ in their C and N contents (Giblin et al. 1991, Bardgett et al. 2007). Evidence suggests that tundra-ecosystem responses to perturbations are contingent upon the characteristics of a system (Shaver et al. 2000). For instance, Speed et al. (2010a) found that Svalbard plant communities in wetter habitats are more resilient to goose grubbing than those in drier habitats. Conversely, in a meta-analysis of experimental warming studies, Elmendorf et al. (2012) found higher temperatures to cause larger changes in tundra plant-community composition in moister compared to drier habitats. Though virtually no studies have compared C and N responses of vascular plants, mosses, and soil to herbivory and warming across soil moisture gradients, one may expect them to vary between tundra-habitats.

The current study aimed at comparing short-term (one- and two-year) chemical responses of vascular plants, mosses, and soil to spring goose disturbance and summer warming in high-Arctic tundra. We asked three specific questions. (Q1) To what extent do these ecosystem compartments differ in their C, N, and C:N ratio responses to spring goose disturbance and summer warming? (Q2) To what extent do chemical responses vary between habitats? (Q3) To what extent do chemical responses vary between years? Furthermore, we explored the possibility of that goose disturbance in spring acts in concert with higher summer temperatures in determining chemical responses of the three ecosystem compartments. To achieve this, a two-year field experiment was carried out in Svalbard, in which spring goose disturbance and summer warming were simulated in a full-factorial arrangement across three habitats along a gradient of soil moisture. Chemical responses were evaluated at the peak of the growing season in both years. We predicted that chemical response would be greatest for vascular plants, followed by mosses, and lowest for soil. We hypothesised chemical responses to goose disturbance to be highest in drier habitats and lowest in wetter habitats, whereas responses to warming were expected to be strongest in wetter habitats and weakest in drier habitats. We expected stronger chemical responses following two years of manipulations.

Material and methods

Study area

The study was carried out in Adventdalen, a wide, well-vegetated valley in Svalbard, Norway (78˚ 10′ N, 16˚ 05′ E) (Figure 2A), during summers 2016 and 2017. The average annual temperature for the period 1988-2017 was −4.2 ˚C, with a mean of −0.1 ˚C in 2016 and −2.2 ˚C in 2017. Average annual precipitation for the same 30-year period was 199 mm, with 236 mm and 239 mm fallen in 2016 and 2017, respectively. The two experimental seasons largely differed in spring and summer temperatures. Spring 2016 was unusually warm (May mean temperature: 1.4 ˚C; average for the period 1988-2017: −2.4 ˚C), whereas spring 2017 was unusually cold (May mean temperature: −3.9 ˚C). Also, mean summer (June to August) temperature was 6.6 ˚C in 2016 and 5.9 ˚C in 2017 (average for the period 1988-2017: 5.4 ˚C) (climate data were recorded at Svalbard airport, which is approximately 10 km from the study area; http://met.no).

Adventdalen becomes snow-free relatively early and is thus heavily utilized by pink-footed geese (*Anser brachyrhynchus*) as feeding ground during the pre-breeding period in spring (Fox et al. 2006). Snowmelt generally commences in mid May, but the date at which the tundra becomes snow-free differs from year to year and depends on climatic conditions (cf. Pelt et al. 2016). The onset of the growing season was particularly early in 2016 and delayed in 2017. The start of the growing season also varies at the landscape-scale due to different snow accumulation patterns in winter; yet, above-ground plant biomass generally reaches an average peak in late July/early August in most habitats (Van der Wal and Stien 2014). Additional details on environmental characteristics of the study area and its trophic system are given in the Supplementary material Table S1 and Appendix 1A.

Study design and experimental treatments

In order to capture different moisture regimes among the experimental units of this study, we selected 7 sites within a 10-km^2 area on the southern side of Adventdalen (Figure 2B), each encompassing mesic (ME), moist (MO), and wet (WE) habitats (Figure 2C). These habitats represent potential pink-footed goose habitats on Svalbard (Speed et al. 2010a, Speed et al. 2010b). Distance between sites was greater than the distance between habitats within site. ME-habitats mainly developed on flat terrains that dry out as the summer progresses and have relatively thin snow-cover in winter. MO-habitats were found on gently sloping grounds and are characterized by considerable seepage of water in the soil throughout the season and by having a moderate snow-cover in winter. WE-habitats were generally confined in depressions, which are featured by the presence of standing water in spring, close-to-saturated soil throughout the summer, and an appreciable snow-cover in winter.

Habitats were chosen based on descriptions of Rønning (1996) and differed in the relative abundance of dominant plant species. ME-habitats were mainly characterized by the rush *Luzula wahlenbergii*, which co-occurred with dwarf shrubs, grasses, and forbs (common species were *Dryas octopetala*, *Salix polaris*, *Alopecurus magellanica*, *Poa arctica*, and *Bistorta vivipara*). The moss compartment was mainly constituted by *Sanionia uncinata*, *Tomentypnum nitens*, *Polytrichastrum alpinum, Hylocomium splendes*, and *Dicranium* spp. MO- and WE-habitats lacked both rushes and evergreen dwarf shrubs. MO-habitats were dominated by grasses such as *A. magellanica* and *Calamagrostis neglecta*, the deciduous dwarf shrub *S. polaris*, the vascular cryptogam *Equisetum arvense*, and forbs such as *B. vivipara*. Other graminoids found in WE-habitats, such as *Dupontia fisheri* and *Eriophorum scheuchzeri*, occurred in MO-habitats only in small quantities. The moss compartment was mainly characterized by species from the genus *Aulacomnium*, *Dicranium,* and *T. nitens*. In WE-habitats, the vegetation was largely dominated by grasses (predominantly *D. fisheri* and *C. neglecta*) and the sedge *E. scheuchzeri*. Other species, such as *S. polaris* and *B. vivipara*, occurred only occasionally. *Calliergon* spp. and *Scorpidium* spp. were the dominant genera of the moss compartment in WE-habitats. Vascular plant names follow the Pan-Arctic Flora (http://nhm2.uio.no/paf), whereas moss names follow Frisvoll and Elvebakk (1996).

To investigate chemical responses of tundra ecosystem compartments (i.e. vascular plants, mosses, and soil) to herbivory and warming, we used a full-factorial randomized block-design with simulated spring goose disturbance (two levels: disturbed [D] and undisturbed [U]) and summer warming (two levels: warming [W] and ambient [A]), for a total of four treatment combinations imposed in all three habitats (Figure 2D). Within each habitat, an experimental block was established in a patch with homogenous vegetation that was large enough to include all plots. Four plots (80 × 80 cm) were laid out at least 2 m apart in each block (minimum 80% of the plot had to be vegetated), for a total of 84 plots. Treatment combinations were randomly assigned to plots. Due to a flooding in early summer 2016, one of the selected blocks in WE-habitats had to be excluded (WE-habitats, n = 6).

Spring goose disturbance simulation: the treatment was applied in the period 5-12 June in 2016 and 15-22 June in 2017, at the peak of the grubbing season. To simulate natural pink-footed goose beak-sized bites, which are typically found to a depth of 2-8 cm across different habitats (Fox et al. 2006), we used a sharpened steel tube (20 mm Ø) that was inserted to a depth of approximately 50 mm and twisted to remove plant and soil material from the plot, following Speed et al. (2010a). Grubbing was implemented in a regular fashion to approximately 33% of the plot surface (Figure 1C-E). We then added 120 g of fresh goose faeces (approx. 45 g dry-weight, hereafter termed %dw) uniformly spread out as single droppings. Faeces were collected each spring within the experimental area. Faeces addition was based upon detailed observations of the faeces distribution patterns within naturally-grubbed areas found in Adventdalen. To quantify how much N was added in ‘disturbed’ plots, twenty fresh goose droppings were randomly selected each spring and analysed for C- and N-content (means %dw ±SD in 2016: C = 37.8 ± 6.7, N = 1.71 ± 0.62; in 2017: C = 36.8 ± 3.0, N = 1.73 ± 0.30) using a CN analyser (Vario EL Cube, Elementar Analysesysteme GmbH, Hanau, Germany). Consequently, approx. 1.2 g m^{-2} of N was added to ‘disturbed’ plots through goose droppings in both spring 2016 and 2017.

The intensity of our spring goose disturbance treatment was comparable to what we observed in naturally-grubbed areas typically found in MO-habitats. We observed somewhat lower intensity of goose disturbance in ME-habitats and somewhat higher intensity in WE-habitats, as reported in previous studies (e.g. Speed et al. 2009). However, by keeping the same goose disturbance intensity, we were able to compare chemical responses of ecosystem compartments across habitats (see Q2) (cf. Speed et al. 2010a). Moreover, following their population increase, pink-footed geese have already started exploiting less suitable, drier habitats (Pedersen et al. 2013a, Pedersen et al. 2013b). Thus, the intensity of our simulation may be a likely scenario for ME-habitats in the coming years if the pink-footed goose population will continue to rise (Jensen et al. 2008, Jensen et al. 2014).

Summer warming simulation: the treatment was implemented by hexagonal open-top chambers (OTCs, made of LEXAN© polycarbonate – 1.4 m Ø), following the guidelines in the International Tundra Experiment (ITEX) protocol (Molau and Mølgaard 1996, Henry and Molau 1997). We used larger OTCs than the plot size to minimize edge effects. To prevent potential confounding effects of uneven snow accumulation (Bokhorst et al. 2011), OTCs were removed before the first snowfall in autumn and throughout the winter period. In 2016, OTCs were set up during the implementation of the goose disturbance treatment (i.e. period 5-12 June), when all plots had already been snow-free for 7-10 days. In 2017, OTCs were set up as soon as snow conditions allowed (i.e. period 1-7 June). OTCs are passive warming devices that have been successfully used in numerous ecological studies to increase air and soil temperatures at the plot level (see e.g. the review and synthesis by Elmendorf et al. 2012). Throughout the summer, OTCs generally increase average air temperature by 1.5-1.9 ˚C and maximum air temperature by ~3.5 ˚C, whereas changes in average soil temperature range from 0.6 ˚C to 1.1 ˚C (Marion et al. 1997, Hollister and Webber 2000). However, OTCs may also alter other microclimatic variables, such as soil moisture and air humidity (see Marion et al. 1997, Hollister and Webber 2000 for further details concerning OTC performances).

To avoid natural herbivory as a possible confounding factor, cages were set up on all plots at the same time as setting up the OTCs. We used cages made of metal net (90×90 cm area × 50 cm height; mesh-size 1.9×1.9 cm) to exclude herbivore activities from ‘ambient’ plots. We used larger cages than the plot size to minimize edge effects. In ‘warmed’ plots, a metal net (mesh-size 1.9×1.9 cm) was used to close the open top part of each OTC, which then acted as an herbivore exclosure throughout the summer. Cages were removed during winter.

Micro-environment monitoring

We measured soil moisture at the peak of the growing season in 2016 and 2017 within each plot using a soil moisture probe attached to a moisture logger (ML3 Theta Probe and HH2 Moisture Meter Logger, Delta-T Devices Ltd., Cambridge, UK). Air temperature at 5 cm above the moss surface was registered in three ‘ambient’ and three ‘warmed’ plots per habitat every 30 min throughout the two growing seasons using temperature loggers (U23-003/UA-001 HOBO, Onset Computer Corporation, Bourne, Massachusettes, USA; accuracy: ±0.2 ˚C) equipped with solar-radiation shields. Temperature loggers (DS1921G-F5 Thermochron iButtons, Homechip Ltd, Milton Keynes, UK; accuracy: ±1.0 ˚C) were also used to register surface moss-mat (−2 cm from the moss surface) and vascular-plant rooting zone (−7 cm) temperatures in three to four plots of each treatment per habitat every 2 h throughout the two growing seasons. We obtained average and maximum July temperatures by extracting mean and maximum daily temperatures from each temperature logger and by averaging these values for the period 1-31 July.

Sample collection and processing

To quantify chemical responses of tundra ecosystem compartments to spring goose disturbance and summer warming, we collected vascular plant, moss, and soil samples from each plot and determined their C- and N-content (%dw), and C:N ratio. All samples were collected at the peak of the growing seasons (periods 19-29 of July and 20-28 of July in 2016 and 2017, respectively).

Vascular plant C and N contents

Vascular plant sampling was designed to minimise disturbance and was performed randomly within each plot by placing a metal frame (50×50 cm) with 25 evenly-distributed points in the centre of each plot. Nine woody sticks (numbered from 1 to 9) were dropped down vertically to the tundra from 9 randomly-selected points within the frame (Figure 2E). From stick 1 to 9, the species for which the uppermost leaf had touched the stick was registered. The same species was not registered twice. Hence, the second closest species to a stick was registered when the stick would have provided a species already registered. Three to five fresh leaves of each registered species were collected at each plot, starting from the first stick at which the species was registered and continuing with the subsequent sticks. The sampling led to a total of 6-27 leaves per plot depending on the number of dominant species found in that plot. Across habitats, we collected leaves from 14 species belonging to seven broadly-classified plant functional types (PFTs), namely forbs, grasses, rushes, sedges, deciduous and evergreen dwarf shrubs, and horsetails (Supplementary Table S2). Their combined biomass encompassed in average over 99% (range: 89-100%) of the vascular plant biomass within plots (own data; see below and Supplementary Figure S1). In this study, we used the collected leaves to calculate C and N community-weighted contents (%dw) of the vascular plant compartment as a whole (see below).

During fieldwork, the leaves from each species and plot were stored together in a tea-filter bag and flattened with a plant-press within 1 to 10 hours after collection. After 72 h, all leaf samples were oven-dried at 60 ˚C for 48 h. All sampled leaves were analysed for C- and N-content (%dw) with Near Infrared Reflectance Spectroscopy (NIRS) using a FieldSpec 3 (ASD Inc., Boulder, Colorado, USA) in 350‒2500 nm range and equipped with a 4 mm light-adapter for full-leaf scanning. For each leaf, between 3 and 10 measurements were taken (on average 3.7 ± 1.2), depending on the size of the leaf. Each measurement was converted to C- and N-content using the prediction models based on milled and tableted plant samples

(Murguzur et al. 2019) and with correction factors for full leaves (Petit Bon et al. 2020a). We first calculated the median of the replicate measurements of each leaf and then averaged the medians of the leaves to obtain mean C- and N-content for each species within a plot. Additional details on sample collection and chemical analyses with NIRS are provided in Supplementary Appendix 2A (for a similar approach, see Petit Bon et al. 2020b).

To derive vascular-plant C and N community-weighted means, we assessed above-ground vascular-plant biomass by using point intercept frequency method (PIM - Bråthen and Hagberg 2004). Two subplots (25×25 cm) were randomly selected within each plot after snowmelt in 2016 and PIM was performed within these at peak-season in both 2016 and 2017 by using a sampling frame with 25 evenly-distributed intercepts. Due to time constraints, 12 intercepts were randomly selected and used in 2016, whereas all 25 intercepts were used in 2017. Such difference is assumed to be irrelevant for the results since a pin density of 12 pins per ~0.06 m^{-2} area (the area of our subplots) is already largely above the threshold after which an increase in number of pins had a negligible effect on the accuracy of biomass estimates (Bråthen and Hagberg 2004). Within each subplot, we vertically lowered a stick (3 mm Ø) through the selected intercepts and counted the number of contacts between the stick and each live vascular plant species. Point intercepts for each species were first averaged between the 2 subplots within plot and converted into biomass values (g m^{-2}) (see below).

A total of seventeen 25×25 cm plots were selected for destructive harvesting within the study area at the peak of the growing season in 2016. Plots were selected to encompass the three habitats and a high degree of variation in both vascular-plant biomass and species (and PFT) composition. At each plot, we performed PIM by using a frame (25 × 25 cm) consisting of 50 evenly-distributed intercepts. Live above-ground vascular-plant biomass from each plot was harvested and sorted in PFTs, oven-dried at 60 ˚C for 48 h, and weighted with an accuracy of

±0.001 g. By fitting weighted linear-regression models (Bråthen and Hagberg 2004), we calculated the relationships between PIM data and grams of biomass (g m^{-2}) for each PFT (Supplementary Table S3). These PFT-specific relationships were used to calculate plant-species biomass (g m^{-2}) in our experimental plots.

Finally, vascular-plant C and N community-weighted contents were obtained following Garnier et al. (2004):

Vascular plant C- or N-content (%dw) = $\sum_{i=1}^{n} = p_i \times (C\text{- or }N\text{-content})_i$

where n represents the number of species in a plot, p_i is the relative contribution of species i to the overall plot live biomass, and (C- or N-content)$_i$ are C- and N-content (%dw) of species i, respectively.

Moss C and N contents

We randomly collected moss shoots within each plot using the same metal frame employed for vascular-plant sampling (see above; Figure 2E). Moss shoots were collected where the nine sticks used to sample vascular-plant leaves touched the moss layer. We collected about the same amount of moss material at each stick (i.e. 7-10 moss shoots, for a total of 63-90 shoots per plot). Moss shoots were oven-dried at 60 °C for 48 h and shoots of the same plot were pooled prior to analyses. For each moss sample, we separated the green, photosynthetically-active part of the shoots from the brown, nearly-decomposed part and only the former was used in the analyses. Samples were milled using a ball mill (Retsch Mixer Mill MM 400, Haan, Germany) (milling time: 40 min; milling intensity: 15 Hz). After milling, a 4-6 mg subsample was analysed for its C- and N-content (%dw) using a CN analyser. Since we collected any moss species that was randomly hit by the sticks and about the same number of shoots from each stick, estimates of C- and N-content are considered approximate community-weighted contents of the moss compartment.

Soil C and N contents

The organic soil layer in Svalbard is thin and often forms a continuum with the lower part of the moss-mat, which can be found at different stages of decomposition. Hence, the distinction between soil and moss compartments can be challenging due to heterogeneous profiles, which also vary between habitats. Here, the moss-soil interface was defined as the point where the moss tissue was no longer distinguishable by eye from the organic soil. Three samples of organic soil were collected from each plot using a soil sample cylinder (20 mm Ø and 30-40 mm deep) (Figure 2E). Spots for soil sample collection within each plot were randomly selected by throwing a pencil over the shoulder. The moss layer was removed before inserting the soil corer into the ground. Soil samples were stored in a refrigerator at 3-4 ˚C within 1 to 10 hours after collection. Within one week, the three samples of organic soil belonging to a plot were pooled together and oven-dried at 60 ˚C for 48 h. Samples were first mixed and homogenised and subsequently sieved with a 2-mm mesh-size soil sieve. A 5-9 mg subsample was analysed for its C- and N-content (%dw) using a CN analyser.

Statistical analyses

We analysed the data using linear mixed-effects models (LMM) fitted with the lme-function from the nlme package (Pinheiro et al. 2015) in the R environment version 3.6.1 (https://www.r-project.org). We initially used 'spring goose disturbance' (two-level factor: undisturbed [U] and disturbed [D]), 'summer warming' (two-level factor: ambient [A] and warming [W]), 'year' (two-level factor: 2016 and 2017), and their interactions as predictors in full models with C- and N-content (%dw) and C:N ratio as response variables, separately for each ecosystem compartment (vascular plants, mosses, and soil) and habitat (ME, MO, and WE), for a total of 27 models. In all LMMs, we specified 'site' as random term to account for the hierarchical spatial structure of the study design. Moreover, 'plot' was nested within 'site' to account for the repeated sampling in 2016 and 2017. Response variables in each model were $log_e(y)$-transformed prior to analyses in order to achieve homogeneity in the residual variance. Using other transformations such as the square-root transformation yielded comparable results.

By using likelihood ratio test on full LMMs (fitted using maximum likelihood – ML), we simplified the fixed-effects structure of each model following a two-step approach. First, we attempted to select the better, but common, fixed-effects structure for all the analyses in order to compare effect sizes obtained for different response variables, ecosystem compartments, and habitats across all 27 models. The final model structure for common LMMs included 'spring goose disturbance', 'summer warming', and 'year' as additive fixed-terms and 'spring goose disturbance × year' and 'summer warming × year' as interaction fixed-terms. However, these interactions were found to be statistically significant in only 4 out of the 27 models. Such across-year statistical consistency in C, N, and C:N ratio responses of ecosystem compartments to treatments was considered of interest in light of our experimental questions (Q3). Thus, we selected the better, most parsimonious fixed-effects structure for each LMM and decided to present results from these models. Results from common LMMs are provided in Supplementary Tables S4-S6 and displayed in Supplementary Figures S2-S3.

In the presentation of the results, we focus on C, N, and C:N ratio responses of vascular plants, mosses, and soil to spring goose disturbance and summer warming, separately for the three habitats. We display the main effects of our experimental treatments since their interaction was not found to be statistically significant in any of the models. We also report natural variation in ecosystem-compartment C- and N-content and C:N ratio between 2016 and 2017 to allow comparisons with treatment effects.

We focus on each response by providing absolute values of standardized estimates for the model parameters, which allow comparing more easily effect sizes of positive and negative responses. The direction (i.e. positive or negative) of each response is also provided. Standardized estimates have the standard deviation (SD) as their unit, and thus indicate of how many SDs the response variable changes in response to treatments for every change of 1 SD of the response variable in un-manipulated control plots. Standardized estimates were obtained by extracting standardized coefficients from model outputs (fitted by restricted ML) following Gelman (2008).

Statistically significant effects were defined as having their 95% confidence interval (CI) not crossing 0. In addition, close-to-significant trends were also evaluated and defined as presenting their 90% CI not crossing 0. Since effect sizes of close-to-significant responses were similar to those of significant responses, we consider both these responses as biologically meaningful in the *Results*. Finally, we validated each model by assessing normality and homogeneity of variances in the residuals for the fixed-effects and checking for approximate linearity between observed and fitted values. Model estimates for the most parsimonious LMMs are provided in Supplementary Tables S7-S9.

Results

Micro-environmental characteristics

Micro-environmental characteristics measured throughout the warmest month (July) varied between habitats and years and were modified to a different extent by experimental treatments (Figure 3).

Soil moisture showed the highest variation between habitats (ME: ~43%, MO: ~67%, WE: ~95%; average across years and treatments), whereas differences between years and treatments were comparatively small (Figure 3A).

Across years and habitats, average air temperature in warmed plots was ~0.7 ˚C higher compared to ambient plots, whereas maximum air temperature was increased by ~3.7 ˚C (Figure 3B). Warming also increased moss-mat temperatures and, to a lesser extent, vascular-plant rooting-zone temperatures (Figure 3C-D). Average and maximum moss-mat temperatures in warmed plots were ~0.8 ˚C and ~1.4 ˚C higher, respectively, than ambient plots. Average and maximum vascular-plant rooting-zone temperatures were increased with warming by ~0.4 ˚C in undisturbed plots only. Overall, disturbance in ambient plots increased maximum moss-mat temperature by ~0.6 ˚C (Figure 3C). Despite this general pattern, disturbance had more variable between-year and between-habitat effects than warming (Figure 3C-D). OTCs had negligible effects on air relative humidity and soil pH (Figure S4).

Differences in ambient average temperatures between years were greater than within-year effects of warming. Across habitats, air temperature was ~1.6 ˚C higher in 2016 than 2017 (Figure 3B), moss-mat temperature was ~1.2 ˚C higher in 2016 than 2017 (Figure 3C), and vascular-plant rooting-zone temperature was ~1.4 ˚C higher in 2016 than 2017 (Figure 3D). Conversely, between-year differences in ambient maximum temperatures were smaller than within-year effects of OTCs, except for the ~1.5 ˚C higher vascular-plant rooting-zone temperature in 2016 compared to 2017 (Figure 3D).

Chemical composition of ecosystem compartments in un-manipulated controls

Ecosystem compartments varied widely in their chemical composition (Figure 4). C-content was much lower in soil (average ±SD: 7.1% ±3.8%) than the two vegetation compartments (vascular plants: 43.7% ±1.3%; mosses: 42.4% ±2.3%) (Figure 4 – upper panels). N-content was lowest in soil (0.44% ±0.26%), intermediate in mosses (1.04% ±0.25%), and highest in vascular plants (2.86% ±0.40%) (Figure 4 – mid panels). C:N ratio was high in mosses (43.4

±11.9) and comparable between soil (16.4 ±2.1) and vascular plants (15.6 ±2.4) (Figure 4 – bottom panels).

Chemical composition of ecosystem compartments showed strong significant differences between years (Figure 4). In all habitats, C- and N-content of vascular plants were higher and C:N ratio was lower in 2017 than 2016 (Figure 4 – left panels). Mosses had lower C-content in 2017 than 2016 in all habitats, whereas N-content was higher in 2017 compared to 2016 in WE-habitats and C:N ratio was lower in 2017 than 2016 in MO- and WE-habitats (Figure 4 – mid panels). Soil had lower C-content in 2017 than 2016 in MO- and WE-habitats, lower N-content in 2017 compared to 2016 in WE-habitats, and lower C:N ratio in 2017 than 2016 in all habitats (Figure 4 – right panels).

Both within and across habitats, differences in chemical composition between years were larger in vascular plants and comparable between mosses and soil, as expressed by standardized effect sizes (Figure 4, Table 1). Across ecosystem compartments, differences in chemical composition between years were greatest in WE-, intermediate in MO-, and smallest in ME-habitats (Figure 4, Table 1). This overall pattern was driven by mosses and soil, whilst between-year differences in chemical composition of vascular plants had similar standardized effect size in all three habitats (Table 1).

Chemical responses of ecosystem compartments to disturbance and warming

Out of a total of 58 treatment effects assessed across chemical variables, ecosystem compartments, and habitats, we found 9 effects having their 95% CI not crossing 0 and 3 effects having their 90% CI not crossing 0 [21% of the total] (Figure 5). Out of the 12 responses, 8 were detected in vascular plants [67%], 3 in soil [25%], and 1 in mosses [8%]. While vascular plants responded to both goose disturbance (3 responses) and warming (5 responses), mosses and soil only responded to disturbance. Goose disturbance consistently increased and warming decreased N-content in vascular plants (Figure 5B), thus leading to lower and higher vascular-plant C:N ratio in disturbed and warmed plots, respectively (Figure 5C).

When considering all treatment effects, chemical responses of vascular plants were stronger (as expressed by standardized effect sizes) compared to those of mosses and soil (Table 1). However, chemical responses of ecosystem compartments to treatments had comparable standardized effect sizes when only considering the 12 significant responses (Table 1). Differences in chemical composition of ecosystem compartments between years (see above) were up to three-fold larger than their chemical responses to treatments (cf. Figures 4 and 5; Table 1).

Ecosystem compartments differed between habitats in their chemical responses to treatments (Figure 5). Vascular plants responded to goose disturbance in ME- and WE-, but not MO-habitats, and all responses were consistent across years. Vascular-plant responses to warming occurred in ME- and MO-, but not WE-habitats; whereas they were consistent across years in MO-habitats, they all became apparent in ME-habitats following two years of elevated temperatures (Figure 5c-d – bottom panel). All responses of soil to disturbance occurred in ME-habitats and whilst C- and N-content only responded in the first year (Figure 5a-b – bottom panel), C:N ratio was consistently affected across years. The only response of mosses was instead detected in WE-habitats, where disturbance affected their C-content in both years.

Both within and across ecosystem compartments, chemical responses to treatments had comparable standardized effect sizes in all three habitats (Table 1). In ME-habitats, chemical responses of ecosystem compartments to treatments had similar standardized effect sizes than their differences in chemical composition between years (Table 1). Yet, between-year differences in chemical composition of ecosystem compartments in MO- and WE-habitats were up to three-fold larger than their chemical responses to treatments (Table 1).

Discussion

The novelty of this study is that we directly compared whether major compartments of tundra ecosystems (i.e. vascular plants, mosses, and soil) differ in their chemical responses to herbivory and warming. The key result is that vascular plants, mosses, and soil differed in their short-term C, N, and C:N ratio responses to our manipulations (vascular plants > soil > mosses), suggesting that herbivory and elevated temperatures may be having important effects on the immediate functioning of tundra ecosystems. However, we found that chemical responses differed between the three habitats (mesic > moist > wet habitats), indicating that these perturbations likely alter ecosystem processes and functions across the tundra landscape at different rates. Overall, the consistent chemical responses of vascular plants, mosses, and soil found across experimental years that widely differed in temperature conditions demonstrate that herbivores and warming are predictable modifiers of biogeochemical processes in Arctic ecosystems. Yet, larger between-year differences in ecosystem-compartment C and N contents and C:N ratio compared to treatment effects suggest that stronger responses should be expected as the intensity of perturbations increases.

We set out to explore the possibility of that goose disturbance in spring interacts with higher summer temperatures in determining C, N, and C:N ratio responses of vascular plants, mosses, and soil. Indeed, the importance of considering herbivory to understand tundra-ecosystem responses to warming has been repeatedly emphasised: studies indicate that herbivores can counteract the effects of higher temperatures on shrub encroachment (e.g. Post and Pedersen 2008) and influence warming-induced changes in the C balance of tundra ecosystems (e.g. Ylänne et al. 2015). However, whether herbivores can affect chemical responses of tundra ecosystems to warming had not been previously tested. We did not find any signs of interactions between our experimental manipulations across all chemical variables, ecosystem compartments, and habitats. Thus, a key finding of this study is that goose disturbance and warming, at least in the short-term, influence the chemical composition of major tundra-ecosystem compartments in an additive way. Yet, the observation that our manipulations generally had opposing effects on N-content and C:N ratio of vascular plants points to that their net effects on the chemical composition of the vascular-plant compartment may still be neutral.

In accordance to our prediction, the chemical composition of vascular plants was the most affected by our short-term manipulations, and this was generally verified across all chemical variables and habitats. Relatively fast changes in C and N contents and C:N ratio of tundra vascular-plant species following goose herbivory (Bazely and Jefferies 1985, Beard et al. 2019) and elevated temperatures (Welker et al. 2005, Doiron et al. 2014) have been previously documented. Here, by encompassing all the dominant vascular plant species structuring our tundra-plant communities, we show that goose disturbance and summer warming also affect the immediate chemical composition of the whole vascular-plant compartment. Interestingly, this was not verified for the moss compartment, whose chemical composition was found relatively unresponsive and, against our expectation, less affected compared to that of soil. Considering the well-known capacity of mosses to quickly sequester additional nutrients in tundra ecosystems (Jónsdóttir et al. 1995), their general unresponsiveness to goose disturbance (here including faeces deposition) was particularly surprising. It seemed that the moss layer dried out in experimentally-disturbed plots as the summer progressed (*personal observation*). Since mosses are unable to remain physiologically active when dry (Proctor et al. 2007), the disturbance caused by our simulation may have prevented their nutrient absorption. These findings, together with higher N-content of vascular plants found in disturbed plots, have also implications for the role of mosses in constraining the fertilizing effects of animal-excreta on forage species. Indeed, whereas an intact moss layer can disrupt the N return from goose faeces to vascular plants (Pouliot et al. 2009, Sjögersten et al. 2010), this might not be the case when mosses are concomitantly affected by other goose-related activities.

We hypothesised goose disturbance to mainly affect ecosystem-compartment chemical composition in relatively dry compared to wetter habitats. In agreement with this, we found N-content and C:N ratio of vascular plants in mesic habitats to respond to our simulation, whereas the vascular-plant compartment was not affected in moist habitats. However, N-content of vascular plants in wet habitats also responded to goose disturbance and the effect size of the change in their C:N ratio could indicate a forthcoming response. Thus, although plant communities in wetter habitats may be less impacted by goose disturbance than those found in drier habitats (Speed et al. 2010a), the chemical composition of their vascular-plant compartment is indeed affected in the short-term. Also in line with our hypothesis, the chemical composition of the soil compartment was affected by goose disturbance only in mesic habitats. It is likely that our manipulation disturbed the soil in mesic, but not in moist and wet, habitats, where the moss-mat is thicker and eventually protects the soil from grubbing geese. However, soil C and N contents were increased by goose disturbance only during the first experimental year, suggesting that these responses may be either transient or more likely contingent on differences in abiotic conditions between years.

We hypothesised warming to mainly affect ecosystem-compartment chemical composition in relatively moist compared to drier habitats. In line with this, responses of the vascular-plant compartment to warming in moist habitats concerned all three chemical variables and were consistent in the two years. Conversely, warming-induced changes in vascular-plant N-content and C:N ratio in mesic habitats only became apparent following two years of manipulation. This pattern suggests that the chemical composition of tundra plant communities in moister habitats can be affected by experimental warming to a greater extent compared to that of plant communities in drier habitats (Welker et al. 2005). However, the chemical composition of the vascular-plant compartment in mesic habitats was indeed impacted by warming. Elevated temperatures did not affect the chemistry of either moss or soil, and this was consistent across all habitats. These short-term results align with longer-term findings (Sorensen et al. 2012, Alatalo et al. 2017), suggesting that also short-term warming may be a relatively weak driver of the chemical composition of these ecosystem compartments in our high-Arctic ecosystem.

The chemical composition of the three ecosystem compartments largely differed between years. Moreover, the between-year variation in ecosystem-compartment chemical composition was much higher compared to changes in chemical composition induced by our manipulations, and this was consistent across all chemical variables, ecosystem compartments, and habitats. Arctic regions are characterized by large year-to-year variations in climatic conditions (Førland et al. 2011, Pelt et al. 2016), which in turn cause strong between-year variability in e.g. biomass production (Van der Wal and Stien 2014) and biogeochemical processes (Jonasson et al. 2001). The two years encompassed by this study markedly differed in their climatic conditions, as shown by our plot-level temperature data. These data also reveal that the effects of our short-term perturbations were much weaker compared to differences between an extreme year (2016) and a more average year (2017). Combined, these observations suggest that vascular plants, mosses, and soil can all be chemically affected by changes in temperature conditions, but that moss and soil compartments may require a stronger temperature increase than that applied in our warming treatment before such short-term responses become apparent. Thus, the predicted temperature increase of about 2-4 ˚C for the Arctic regions over the next century (Post et al. 2019) may be expected to lead to major changes in the chemical composition of the major compartments of tundra ecosystems.

We simulated herbivore disturbance by mimicking spring goose grubbing in a regular fashion and by subsequently adding fresh faeces. Despite habitats on Svalbard vary in the natural level of goose disturbance they experience (Speed et al. 2009), our consistent manipulation (based on naturally-grubbed tundra-patches observed in moist habitats) allowed us to compare chemical responses of vascular plants, mosses, and soil across habitats. Our perturbation can be regarded as a fairly realistic disturbance, as opposed to more extreme ones such as the complete removal of the moss layer (e.g. Gornall et al. 2007, Gornall et al. 2009, Speed et al. 2010a). Yet, we expect our findings to be conservative as goose disturbance was experimentally controlled and hence did not accommodate possible herbivore patch choice decisions that may have further strengthened grazer-impact. Furthermore, the increase in summer temperatures caused by OTCs was also minor compared to the future temperature changes expected for the Arctic regions. In spite of this, our results indicate that short-term herbivory and warming can influence the immediate chemical composition of major compartments of Arctic ecosystems. Importantly, these compartments were characterized by a different sensitivity to herbivory and warming, which also varied among habitats, indicating that changes in the biogeochemistry of tundra ecosystems may depend on the factor of disturbance and be spatially heterogeneous. Our study sets the context for understanding the extent to which ecosystem processes may be modified by herbivore- and warming-induced changes in ecosystem-compartment chemical composition. Importantly, the large difference in vascular-plant, moss, and soil chemical composition found between our two contrasting experimental seasons highlights the need of further research in order to identify how tundra ecosystems may respond to environmental changes.

Acknowledgements

We thank Anton Hochmuth, Calum Bachell, and numerous other students at UNIS for assistance during fieldwork. We are also grateful to Majsofie Christensen and Henninge Torp Bie for processing moss and soil samples in 2017. Funding for field assistance and field material was provided by the Svalbard Environmental Protection Fund (grant project number 15/128) and the Research Council of Norway (Arctic Field Grant, project number 269957) (both to Matteo Petit Bon).

References

Aerts, R. et al. 2009. Seasonal climate manipulations result in species-specific changes in leaf nutrient levels and isotopic composition in a sub-arctic bog. – Funct. Ecol. 23: 680-688.

Alatalo, J. M. et al. 2017. Impacts of twenty years of experimental warming on soil carbon, nitrogen, moisture and soil mites across alpine/subarctic tundra communities. – Sci. Rep. 7: 44489.

Bardgett, R. D. et al. 2007. Temporal variability in plant and soil nitrogen pools in a high-Arctic ecosystem. – Soil Biol. Biochem. 39: 2129-2137.

Barrio, I. C. et al. 2016. Biotic interactions mediate patterns of herbivore diversity in the Arctic. – Glob. Ecol. Biogeog. 25: 1108-1118.

Bazely, D. R. and Jefferies, R. L. 1985. Goose faeces: a source of nitrogen for plant growth in a grazed salt marsh. – J. Appl. Ecol. 22: 693-703.

Beard, K. H. et al. 2019. Migratory goose arrival time plays a larger role in influencing forage quality than advancing springs in an Arctic coastal wetland. – PloS One 14: e0213037.

Biasi, C. et al. 2008. Initial effects of experimental warming on carbon exchange rates, plant growth and microbial dynamics of a lichen-rich dwarf shrub tundra in Siberia. – Plant Soil 307: 191-205.

Bokhorst, S. et al. 2011. Microclimate impacts of passive warming methods in Antarctica: implications for climate change studies. – Polar Biol. 34: 1421-1435.

Bråthen, K. A. and Hagberg, O. 2004. More efficient estimation of plant biomass. – J. Veg. Sci. 15: 653-660.

Cornelissen, J. H. C. et al. 2007. Global negative vegetation feedback to climate warming responses of leaf litter decomposition rates in cold biomes. – Ecol. Lett. 10: 619-627.

Deane-Coe, K. K. et al. 2015. Experimental warming alters productivity and isotopic signatures of tundra mosses. – Ecosystems 18: 1070-1082.

Doiron, M. et al. 2014. Effects of experimental warming on nitrogen concentration and biomass of forage plants for an arctic herbivore. – J. Ecol. 102: 508-517.

Elmendorf, S. C. et al. 2012. Global assessment of experimental climate warming on tundra vegetation: heterogeneity over space and time. – Ecol. Lett. 15: 164-175.

Førland, E. J. et al. 2011. Temperature and precipitation development at Svalbard 1900-2100. – Adv. Meteorol. 2011: 1-14.

Fox, A. et al. 2006. Diet and habitat use of Svalbard pink-footed geese *Anser brachyrhynchus* during arrival and pre-breeding periods in Adventdalen. – Ardea 94: 691-699.

Fox, A. D. and Madsen, J. 2017. Threatened species to super-abundance: The unexpected international implications of successful goose conservation. – Ambio 46: 179-187.

Frisvoll, A. and Elvebakk, A. 1996. A catalogue of Svalbard plants, fungi, algae and cyanobacteria. Part 2. Bryophytes. – Skrifter-Norsk Polarinstitutt: 57-172.

Garnier, E. et al. 2004. Plant functional markers capture ecosystem properties during secondary succession. – Ecology 85: 2630-2637.

Gelman, A. 2008. Scaling regression inputs by dividing by two standard deviations. – Stat. Med. 27: 2865-2873.

Giblin, A. et al. 1991. Biogeochemical diversity along a riverside toposequence in arctic Alaska. – Ecol. Monogr. 61: 415-435.

Gornall, J. et al. 2009. Herbivore impacts to the moss layer determine tundra ecosystem response to grazing and warming. – Oecologia 161: 747-758.

Gornall, J. L. et al. 2007. Arctic mosses govern below-ground environment and ecosystem processes. – Oecologia 153: 931-941.

Henry, G. H. R. and Molau, U. 1997. Tundra plants and climate change: the International Tundra Experiment (ITEX). – Glob. Change Biol. 3: 1-9.

Hobbie, S. E. et al. 2002. A synthesis: The role of nutrients as constraints on carbon balances in boreal and arctic regions. – Plant Soil 242: 163-170.

Hollister, R. D. and Webber, P. J. 2000. Biotic validation of small open-top chambers in a tundra ecosystem. – Glob. Change Biol. 6: 835-842.

Jefferies, R. L. and Rockwell, R. F. 2002. Foraging geese, vegetation loss and soil degradation in an Arctic salt marsh. – Appl. Veg. Sci. 5: 7-16.

Jensen, G. H. et al. 2014. Snow conditions as an estimator of the breeding output in high-Arctic pink-footed geese *Anser brachyrhynchus*. – Polar Biol. 37: 1-14.

Jensen, R. A. et al. 2008. Prediction of the distribution of Arctic-nesting pink-footed geese under a warmer climate scenario. – Glob. Change Biol. 14: 1-10.

Jonasson, S. et al. 2001. Biogeochemistry in the Arctic: patterns, processes, and controls. – In: Ernst-Detlef, S. et al. (eds), Global biogeochemical cycles in the climate system. Elsevier, pp. 139-150.

Jónsdóttir, I. S. et al. 1995. Fate of added nitrogen in a moss-sedge Arctic community and effects of increased nitrogen deposition. – Sci. Total Environ. 160-161: 677-685.

Madsen, J. et al. 2017. Svalbard Pink-footed Goose. – Population Status Report 2016-2017. Aarhus University, DCE – Danish Centre for Environment and Energy, pp. 14.

Marion, G. M. et al. 1997. Open-top designs for manipulating field temperature in high-latitude ecosystems. – Glob. Change Biol. 3: 20-32.

Molau, U. and Mølgaard, P. 1996. International tundra experiment (ITEX) manual. – Danish Polar Center, Copenhagen, Denmark.

Mulder, C. P. H. 1999. Vertebrate herbivores and plants in the Arctic and subarctic: effects on individuals, populations, communities and ecosystems. – Perspect. Plant Ecol. Evol. Syst. 2: 29-55.

Murguzur, F. J. A. et al. 2019. Towards a global arctic-alpine model for Near-infrared reflectance spectroscopy (NIRS) predictions of foliar nitrogen, phosphorus and carbon content. – Sci. Rep. 9: 8259.

Nadelhoffer, K. J. et al. 1991. Effects of temperature and substrate quality on element mineralization in six Arctic soils. – Ecology 72: 242-253.

Pedersen, Å. et al. 2013a. Prevalence of pink-footed goose grubbing in the arctic tundra increases with population expansion. – Polar Biol. 36: 1569-1575.

Pedersen, Å. Ø. et al. 2013b. Spatial patterns of goose grubbing suggest elevated grubbing in dry habitats linked to early snowmelt. – Polar Res. 32: 1-9.

Pelt, W. et al. 2016. Multidecadal climate and seasonal snow conditions in Svalbard. – J. Geophys. Res. Earth Surf. 121: 2100-2117.

Petit Bon, M. et al. 2020a. One leaf for all: Chemical traits of single leaves measured at the leaf surface using Near infrared-reflectance spectroscopy (NIRS). – Methods Ecol. Evol., doi: https://doi.org/10.1111/2041-210X.13432.

Petit Bon, M. et al. 2020b. Interactions between winter and summer herbivory affect spatial and temporal plant nutrient dynamics in tundra grassland communities. – Oikos, doi: https://doi.org/10.1111/oik.07074.

Pinheiro, J. et al. 2015. nlme: Linear and nonlinear mixed effects models. R package version, 3(1), pp. 111.

Post, E. and Pedersen, C. 2008. Opposing plant community responses to warming with and without herbivores. – Proc. Natl. Acad. Sci. 105: 12353-12358.

Post, E. et al. 2019. The polar regions in a 2° C warmer world. – Sci. Adv. 5: eaaw9883.

Pouliot, R. et al. 2009. Moss carpets constrain the fertilizing effects of herbivores on graminoid plants in arctic polygon fens. – Botany 87: 1209-1222.

Proctor, M. C. et al. 2007. Desiccation-tolerance in bryophytes: a review. – Bryologist 110: 595-621.

Ravolainen, V. et al. 2020. High Arctic ecosystem states: Conceptual models of vegetation change to guide long-term monitoring and research. – Ambio 49: 666-677.

Rønning, O. I. 1996. The Flora of Svalbard. – Norwegian Polar Institute.

Rustad, L. et al. 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. – Oecologia 126: 543-562.

Shaver, G. R. and Chapin, F. S. 1980. Response to fertilization by various plant growth forms in an Alaskan tundra: nutrient accumulation and growth. – Ecology 61: 662-675.

Shaver, G. R. et al. 1992. Global change and the carbon balance of arctic ecosystems: Carbon/nutrient interactions should act as major constraints on changes in global terrestrial carbon cycling. – BioScience 42: 433-441.

Shaver, G. R. et al. 2000. Global warming and terrestrial ecosystems: A conceptual framework for analysis. – Bioscience 50: 871-882.

Sjögersten, S. et al. 2010. Nitrogen transfer between herbivores and their forage species. – Polar Biol. 33: 1195-1203.

Sorensen, P. L. et al. 2008. Nitrogen uptake during one year in subarctic plant functional groups and in microbes after long-term warming and fertilization. – Ecosystems 11: 1223-1233.

Sorensen, P. L. et al. 2012. Moss-specific changes in nitrogen fixation following two decades of warming, shading, and fertilizer addition. – Plant Ecol. 213: 695-706.

Speed, J. D. et al. 2019. Trophic interactions and abiotic factors drive functional and phylogenetic structure of vertebrate herbivore communities across the Arctic tundra biome. – Ecography 42: 1152-1163.

Speed, J. D. M. et al. 2010a. Plant community properties predict vegetation resilience to herbivore disturbance in the Arctic. – J. Ecol. 98: 1002-1013.

Speed, J. M. et al. 2010b. Extrapolating herbivore-induced carbon loss across an arctic landscape. – Polar Biol. 33: 789-797.

Speed, J. M. et al. 2009. Predicting habitat utilization and extent of ecosystem disturbance by an increasing herbivore population. – Ecosystems 12: 349-359.

Tolvanen, A. and Henry, G. H. 2001. Responses of carbon and nitrogen concentrations in high arctic plants to experimental warming. – Can. J. Bot. 79: 711-718.

Turetsky, M. R. et al. 2012. The resilience and functional role of moss in boreal and arctic ecosystems. – New Phytol. 196: 49-67.

Van der Wal, R. and Stien, A. 2014. High-arctic plants like it hot: a long-term investigation of between-year variability in plant biomass. – Ecology 95: 3414-3427.

Walker, D. A. 2000. Hierarchical subdivision of Arctic tundra based on vegetation response to climate, parent material and topography. – Glob. Change Biol. 6: 19-34.

Welker, J. M. et al. 2005. Leaf mineral nutrition of Arctic plants in response to warming and deeper snow in northern Alaska. – Oikos 109: 167-177.

Ylänne, H. et al. 2015. Vegetation shift from deciduous to evergreen dwarf shrubs in response to selective herbivory offsets carbon losses: evidence from 19 years of warming and simulated herbivory in the subarctic tundra. – Glob. Change Biol. 21: 3696-3711.

Tables

Table 1. Average standardized effect sizes for between-year differences in chemical composition of ecosystem compartments (cf. Figure 4) and their chemical responses to treatments (cf. Figure 5), sorted according to ecosystem compartments (vascular plants, mosses, and soil) and habitats (ME, MO, and WE). Average standardized effect sizes for 'All responses' were calculated by averaging all effects addressed in this study, and thus represent the average response strength of ecosystem compartments to either between-year variability or treatments. Average standardized effect sizes for '95% and 90% CI responses' were calculated by averaging only significant and close-to-significant effects, and thus represent the average maximum strength that ecosystem compartments reach in response to either between-year variability or treatments. Empty cells for '95% and 90% CI responses' indicate that no significant or close-to-significant responses were detected.

Ecosystem compartments		Mesic (ME)		Moist (MO)		Wet (WE)		Overall (across habitats)	
		Between-year variability	Treatment effects	Between-year variability	Treatment effects	Between-year variability	Treatment effects	Between-year variability	Treatment effects
Vascular plants	All responses	0.41	0.21	0.42	0.17	0.41	0.12	0.41	0.16
	95% and 90% CI responses	0.41	0.26	0.42	0.27	0.41	0.21	0.41	0.26
Mosses	All responses	0.09	0.13	0.22	0.04	0.29	0.10	0.20	0.09
	95% and 90% CI responses	0.20		0.31		0.29	0.24	0.28	0.24
Organic Soil	All responses	0.10	0.09	0.19	0.06	0.42	0.12	0.24	0.09
	95% and 90% CI responses	0.24	0.23	0.26		0.42		0.31	0.23
Overall (across ecosystem compartments)	All responses	0.20	0.15	0.28	0.09	0.37	0.11	0.28	0.12
	95% and 90% CI responses	0.33	0.25	0.34	0.27	0.37	0.23	0.35	0.25

Figures

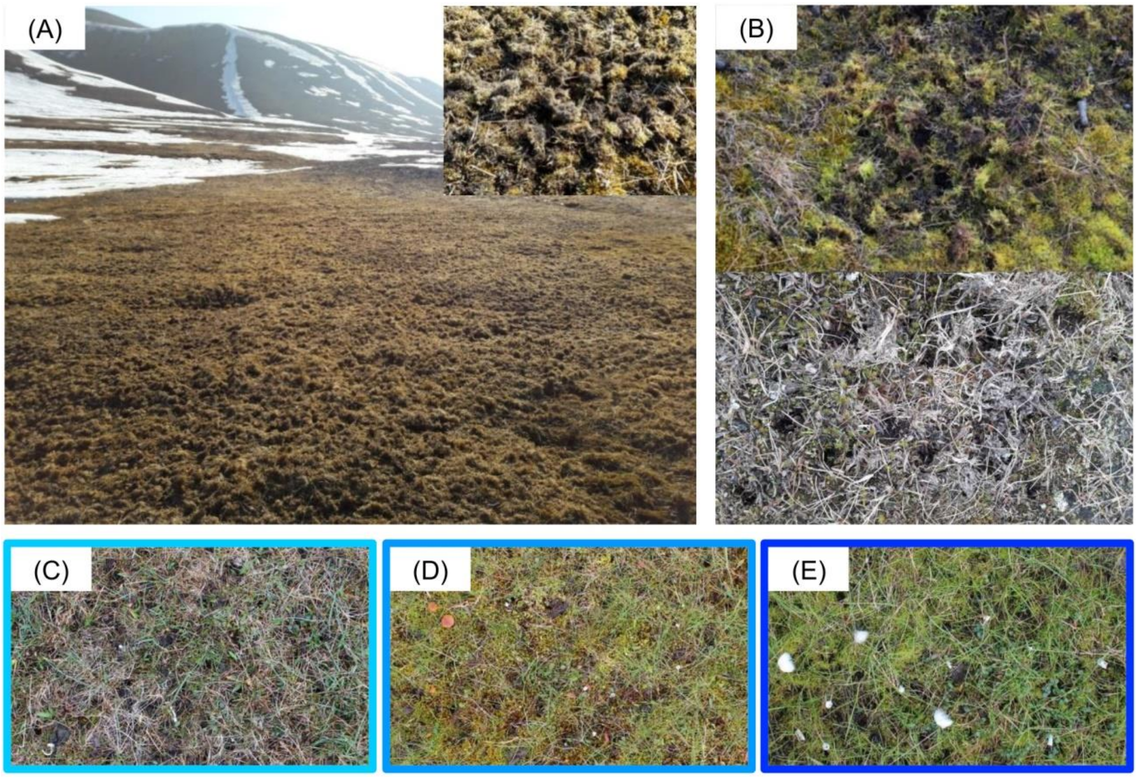


Figure 1. Natural and simulated spring grubbing by pink-footed geese. (A) Extensive natural grubbing as found in many pre-breeding sites in Svalbard. In the inset, a closer visualization of a heavily grubbed tundra-patch is shown. (B) Natural beak-sized bites in a relatively moist [top] and dry [bottom] tundra-patch. Examples of our goose disturbance treatment in a (C) mesic [ME], (D) moist [MO], and (E) wet [WE] habitat. Pictures were taken (A-B) within the study area in spring 2017 and (C-E) in experimental plots during fieldwork in July 2017.

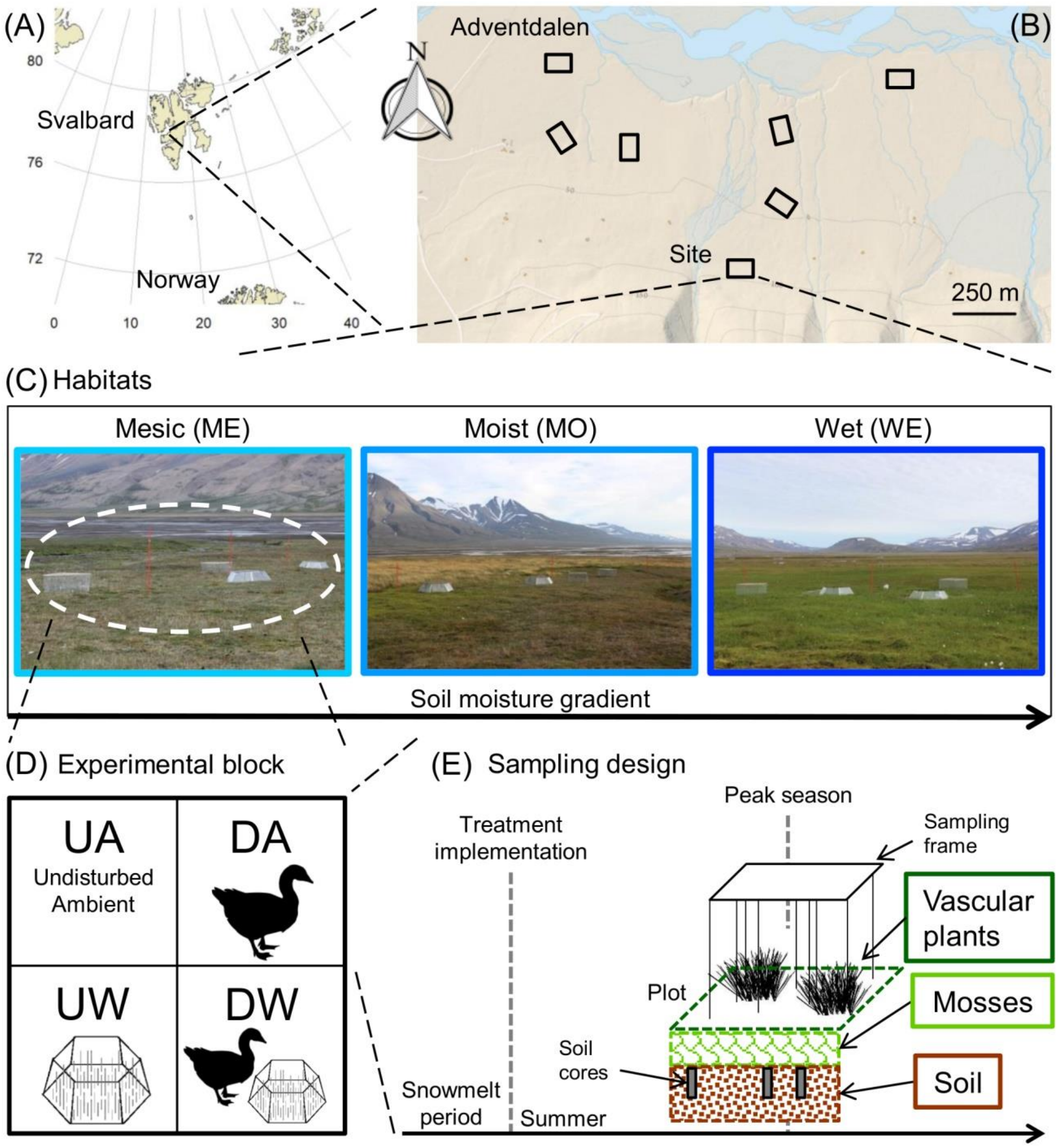


Figure 2. Study and sampling design. (A) Study location, (B-D) hierarchical spatial structure of the study design, and (E) sampling design adopted for vascular-plant, moss, and organic-soil sample collection in summer 2016 and 2017. Colour coding for (C) habitats (mesic [ME], moist [MO], and wet [WE]) and (E) ecosystem compartments (vascular plants, mosses, and organic soil) and (D) treatment abbreviations (UA = undisturbed/ambient, DA = disturbed/ambient, UW = undisturbed/warming, and DW = disturbed/warming) and *silhouettes* presented in this figure will be consistent throughout the manuscript. Pictures in (C) were taken during fieldwork in July 2016.

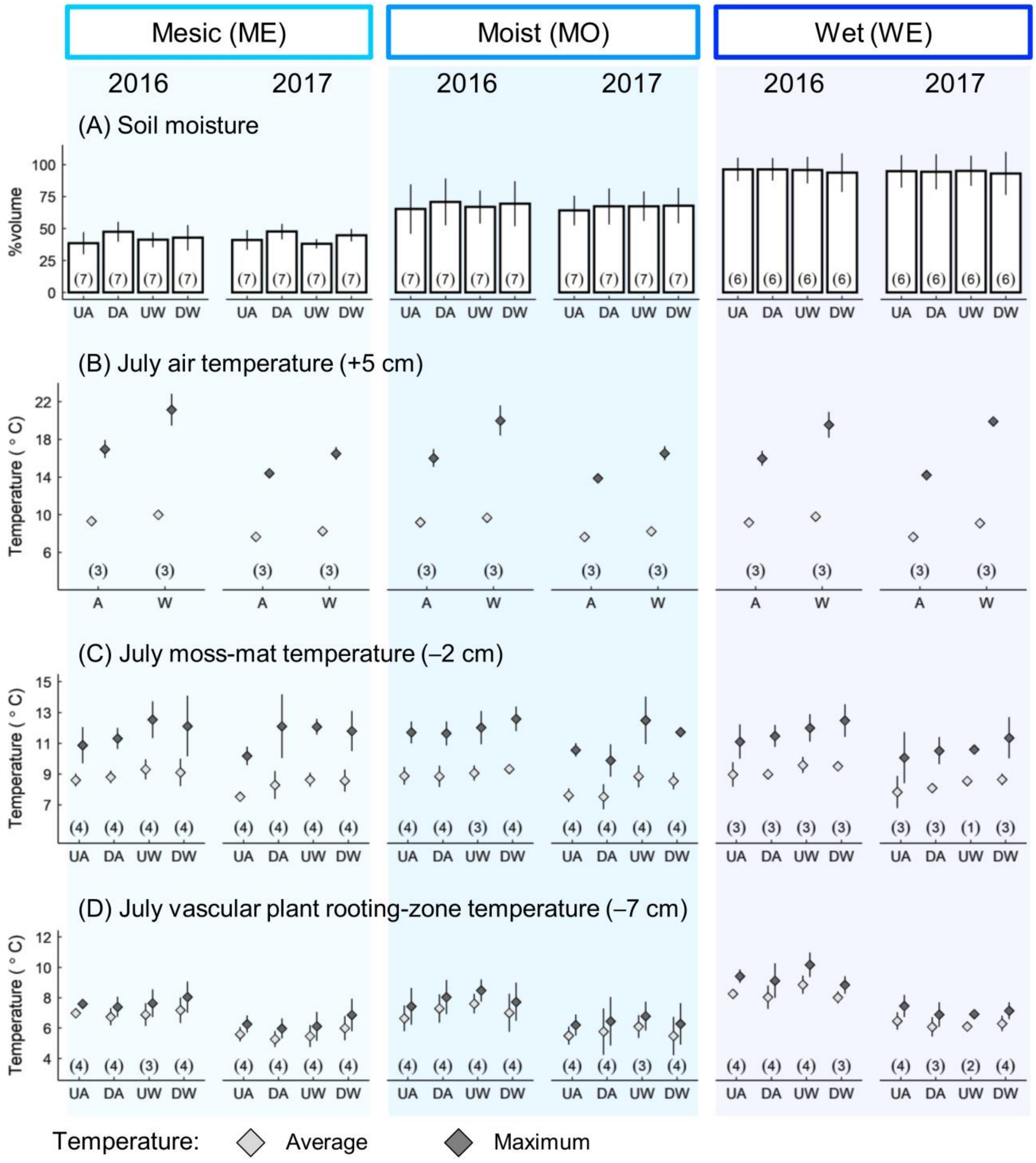


Figure 3. Micro-environmental characteristics of habitats and treatments in 2016 and 2017. (A) Peak-season soil moisture (%volume). Average and maximum (B) air temperature, (C) moss-mat temperature, and (D) vascular-plant rooting-zone temperature throughout the warmest month (July) in 2016 and 2017. Error bars represent the standard deviation of the mean. A few temperature loggers showed malfunctioning, and thus data were excluded before calculating the summary statistics presented here (numbers at the base of each panel refer to the number of monitored plots).

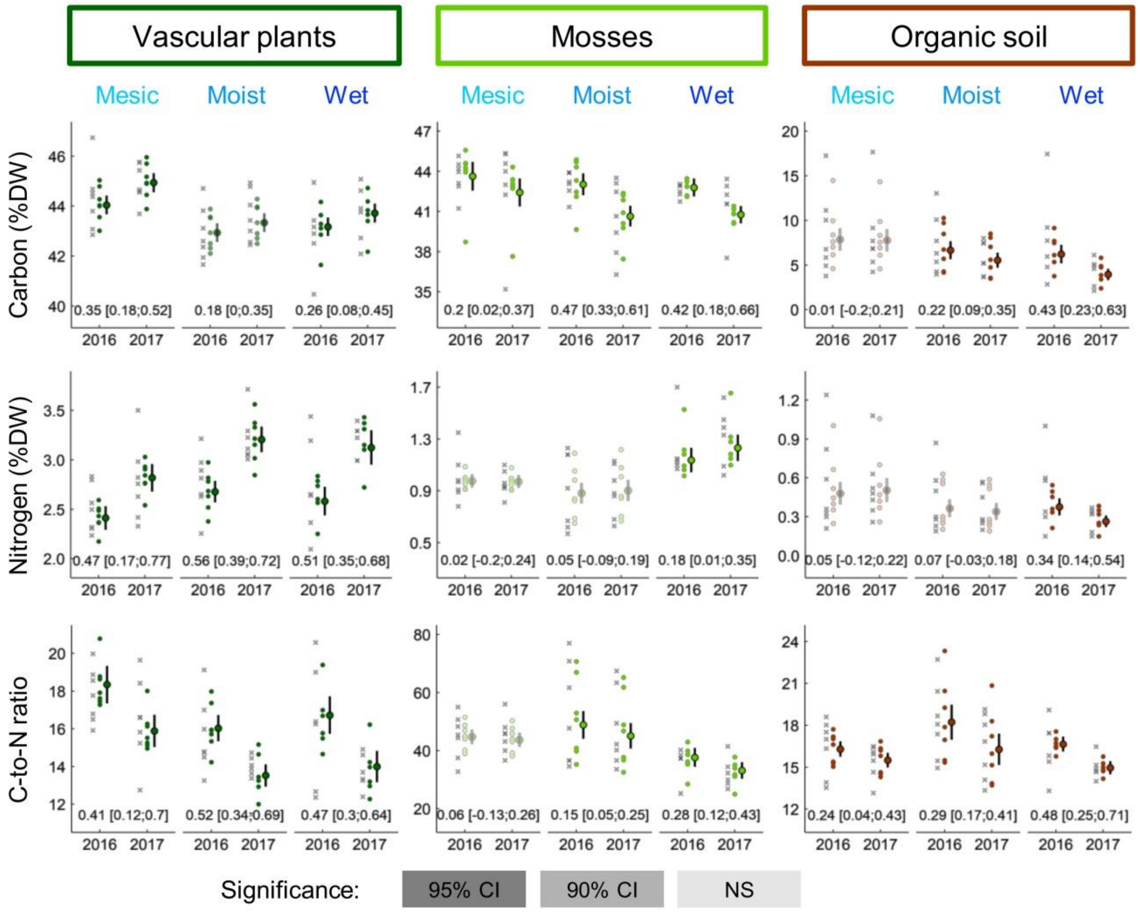


Figure 4. Chemical composition of the three ecosystem compartments in the un-manipulated controls of the three habitats in 2016 and 2017. Predicted and raw values of carbon (C) and nitrogen (N) content (% of dry weight – %dw) and C:N ratio are presented for vascular plants, mosses, and organic soil in un-manipulated controls within the three habitats (mesic [ME], moist [MO], and wet [WE]). Data were back-transformed from the $log_e(y)$-scale and are presented in their original scale; note the different scales of y-axis. Main dots represent model predictions ± their standard error (SE), coloured dots represent fitted values, and grey dots represent raw values. Predictions (and their SE) in bold colours highlight statistically significant differences (i.e. 95% confidence interval [CI] not overlapping 0). Predictions (and their SE) in half-shaded colours highlight close-to-significant differences (i.e. 90% CI not overlapping 0). Predictions (and their SE) in shaded colours highlight non-statistically significant differences. At the base of each panel, absolute values of standardized model

estimates (and their 95% CI) for the difference between the two years are reported in order to allow comparisons with treatment effects (cf. Figure 5).

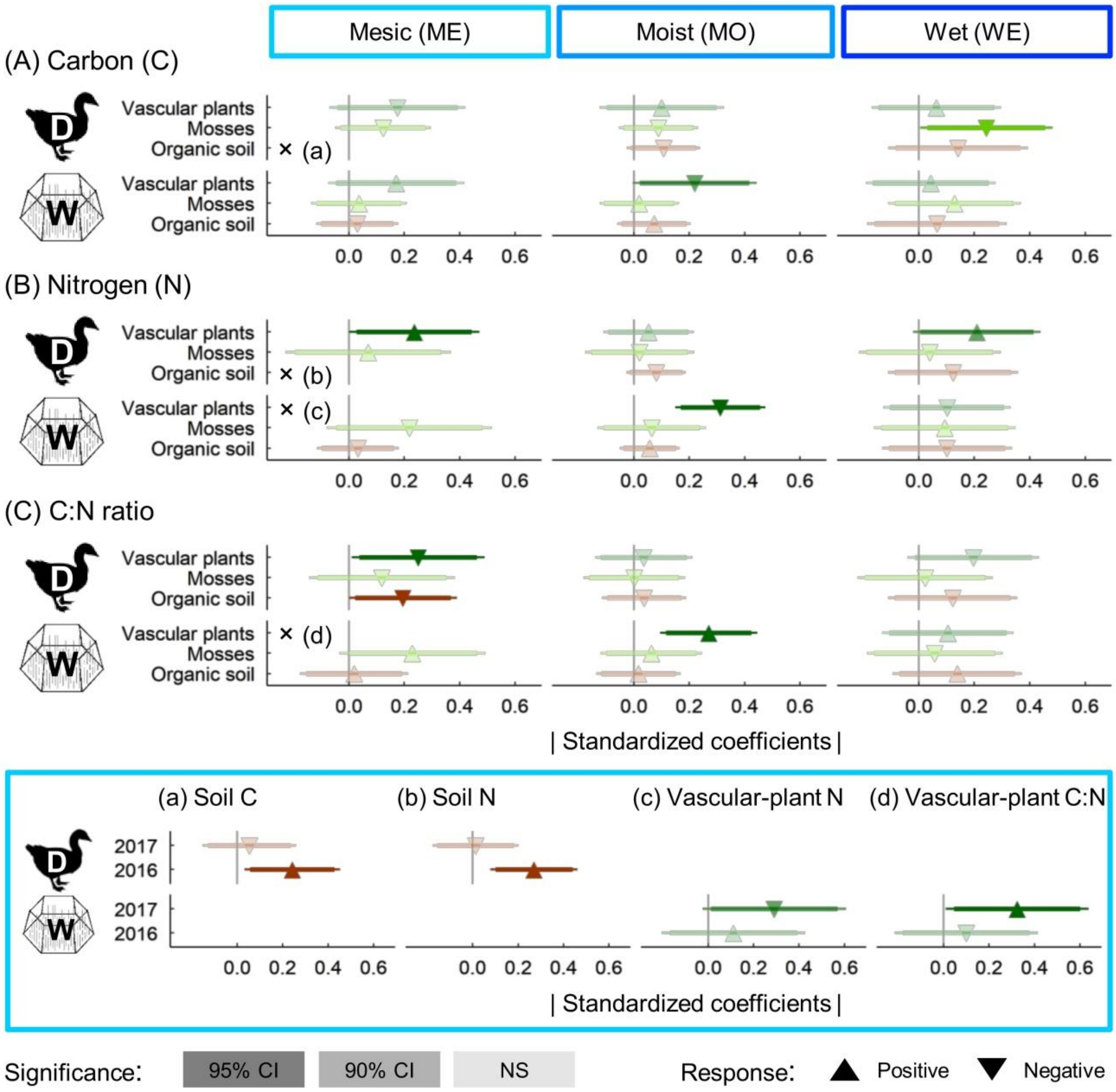


Figure 5. Change in chemical composition of the three ecosystem compartments in response to treatments. Absolute values of standardized model estimates for changes in (A) carbon [C], (B) nitrogen [N], and (C) C:N ratio of vascular plants, mosses, and organic soil in response to spring goose disturbance (D *versus* undisturbed plots [U – the reference level]) and summer warming (W *versus* ambient plots [A – the reference level]) in the three habitats (mesic [ME], moist [MO], and wet [WE]). The symbol '×' indicates that a significant 'treatment × year' interaction was found and results are presented separately for each year in the bottom panel; letters in parentheses refer to the sub-panel in which estimates are displayed. Treatment-specific standardized effect sizes, their 90% confidence interval [CI] (thick line), and their 95% CI (thin line) are given for the main effects of spring goose disturbance and summer

warming; at the base of each panel, absolute values of standardized effect sizes are presented. The reference level is denoted with the grey line at 0 effect size. Estimates (and their CIs) in bold colours highlight statistically significant effects (i.e. 95% CI not overlapping 0). Estimates (and their CIs) in half-shaded colours highlight close-to-significant effects (i.e. 90% CI not overlapping 0). Estimates (and their CIs) in shaded colours highlight non-statistically significant effects. Upward-pointing triangles denote positive standardized effect sizes (i.e. positive responses), whereas downward-pointing triangles denote negative standardized effect sizes (i.e. negative responses).

Supplementary Material

Below are the supplementary methods, tables, figures supporting the study "**Variable chemical responses of three major ecosystem compartments to herbivory and warming in high-Arctic tundra**".

Matteo Petit Bon[1,2,*], Hanna Böhner[1,2], Kari Anne Bråthen[2], Virve Tuulia Ravolainen[3], Ingibjörg Svala Jónsdóttir[4]

[1] Department of Arctic Biology, University Centre in Svalbard (UNIS), PO Box 156, N-9171 Longyearbyen, Norway

[2] Department of Arctic and Marine Biology, Faculty of Biosciences, Fisheries, and Economics, Arctic University of Norway (UiT), N-9037 Tromsø, Norway

[3] Norwegian Polar Institute (NPI), Fram Centre, PO Box 6606 Langnes, N-9296 Tromsø, Norway

[4] Institute of Life and Environmental Sciences, University of Iceland, Sturlugata 7, IS-101 Reykjavik, Iceland

[*] Corresponding author. E-mail: matteo.petitbon@unis.no; Fax: (+47) 79 02 33 01; Phone: (+47) 966 59 147; ORCID-ID: https://orcid.org/0000-0001-9829-8324

HB: hanna.bohner@uit.no

KAB: kari.brathen@uit.no (ORCID-ID: https://orcid.org/0000-0003-0942-1074)

VTR: virve.ravolainen@npolar.no (ORCID-ID: https://orcid.org/0000-0001-8411-7238)

ISJ: isj@hi.is (ORCID-ID: https://orcid.org/0000-0003-3804-7077)

Supporting Tables

Environmental characteristics (1st July — 31st July)	Year 2016			Year 2017		
	Average	St.dev	Range [min-max]	Average	St.dev	Range [min-max]
Air temperature (˚C)	8.7	± 1.35	[6.7 - 11.6]	7.0	± 1.55	[3.0 - 10.6]
Air relative humidity (%)	80.5	± 12.3	[53.9 - 100]	82.2	± 6.8	[63.9 - 94.7]
Photosynthetically active radiation - PAR (mol / m²sec)	351	± 178	[108 - 723]	347	± 125	[150 - 630]
Solar radiation (W / m²sec)	158	± 82	[48 - 327]	156	± 57	[66 - 283]

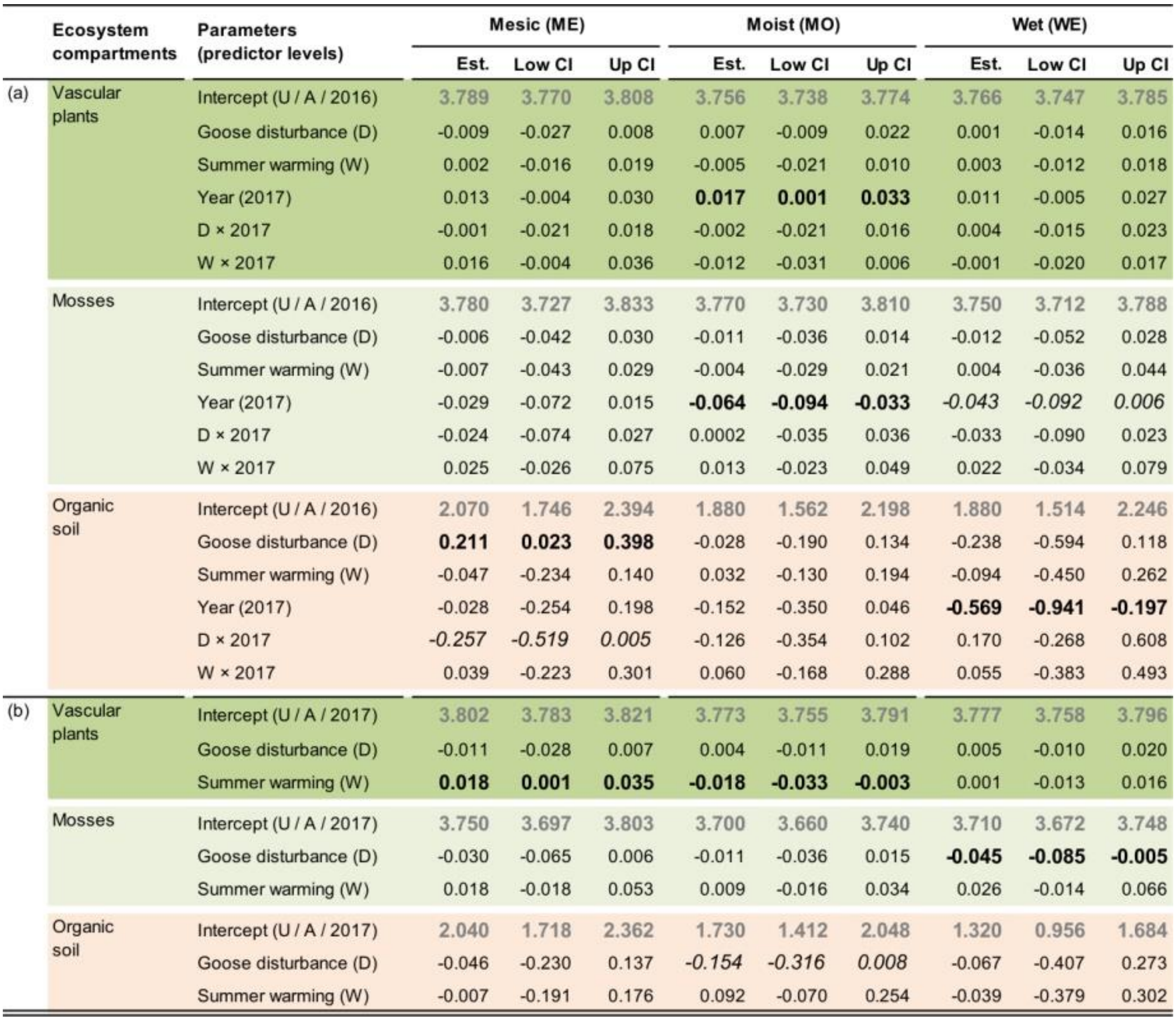
Table S1. Environmental characteristics measured *in-situ* during summers 2016 and 2017. Daily average (and its standard deviation and range [minimum; maximum]) for air temperature, air relative humidity, photosynthetically active radiation (PAR), and solar radiation measured *in-situ* throughout the warmest month (July) in 2016 and 2017. Measurements (logging interval: 15 min) were registered at 2 m above-ground by two fully-automated weather stations located within the study area and their average values are provided in the table. The average for each environmental variable was obtained by calculating mean daily values within each data logger and averaged these values for the period 1-31 July. Weather stations were equipped with a 4-channel Micro Station data logger (H21-002 HOBO Onset Computer Corporation, Bourne, Massachusetts, USA) and respective sensors for environmental data collection (Air temperature sensor [S-THB-M002]: accuracy ±0.2 ˚C; Air relative humidity sensor [S-THB-M002]: accuracy ±2.5%; PAR sensor [S-LIA-M003]: accuracy ±5 µmol/m²/sec; solar radiation sensor [S-LIB-M003]: accuracy ±10 W/m²). Sensors for measuring air temperature and air relative humidity were equipped with solar-radiation shields.

Species	**Plant Functional Type (PFT)**
Bistorta vivipara	Forb
Coptidium lapponicum	Forb
Oxyria digyna	Forb
Stellaria longipes	Forb
Alopecurus ovatus	Grass
Calamagrostis neglecta	Grass
Dupontia fisheri	Grass
Poa arctica	Grass
Luzula nivalis	Rush
Luzula wahlenbergii	Rush
Eriophorum scheuchzeri	Sedge
Salix polaris	Deciduous dwarf shrub
Dryas octopetala	Evergreen dwarf shrub
Equisetum arvense	Horsetail

Table S2. Vascular plant species and the plant functional type (PFT) they belonged to. List of the 14 vascular plant species and the PFT they belonged to from which we collected plant-leaf samples to assess C and N community-weighted means of the vascular-plant compartment (see main text for details). Their combined biomass encompassed in average over 99% (range: 89-100%) of the vascular plant biomass within plots (see also Figure S1).

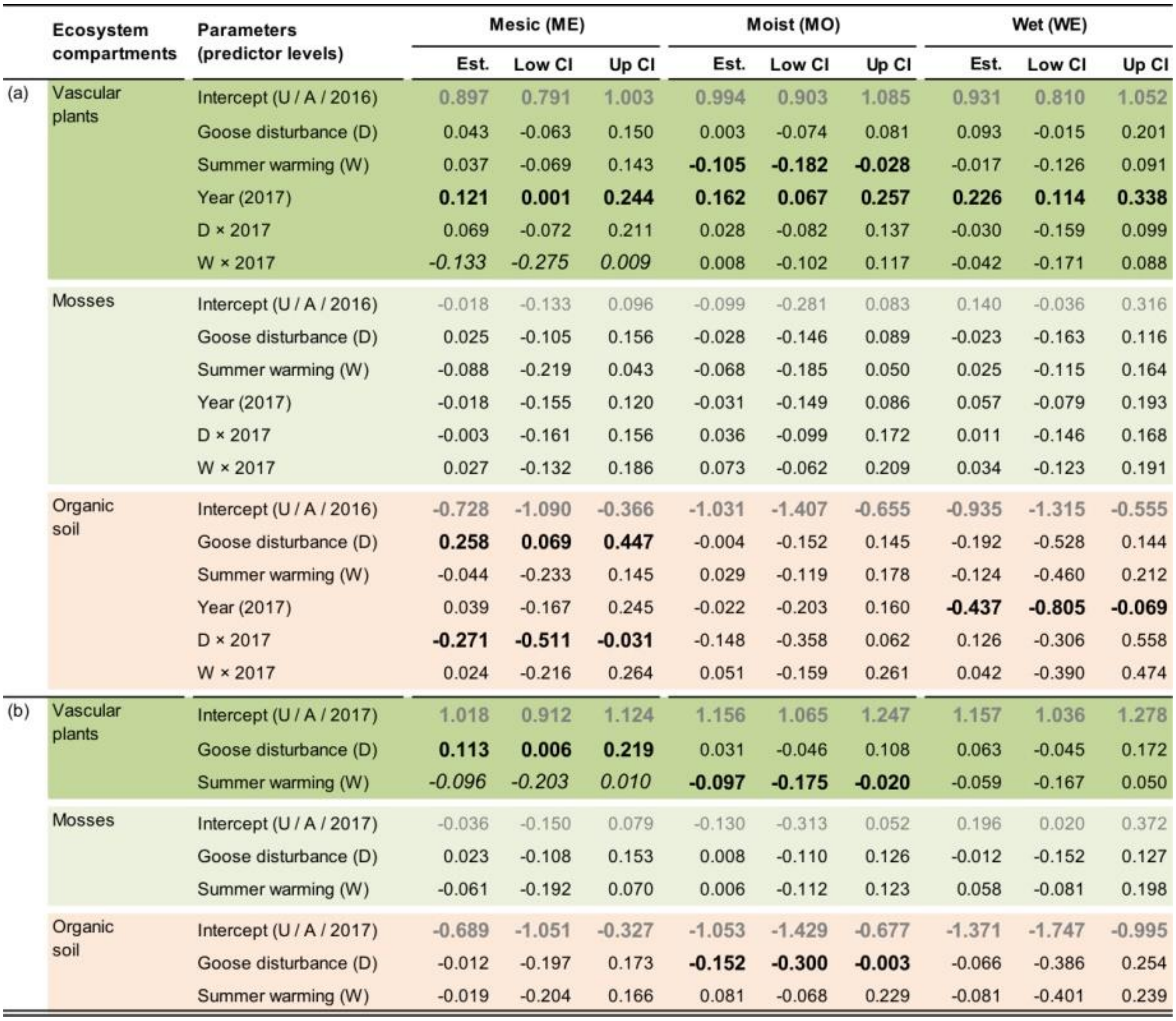
Plant Functional Type (PFT)		b	±SD	CV	n
Forbs		2.21	0.16	0.24	14
Grasses		4.09	0.19	0.12	17
Rushes		3.23	0.20	0.18	8
Sedges		2.71	0.17	0.23	3
Deciduous dwarf shrubs	All	3.26	0.15	0.13	17
	Leaves	2.04	0.20	0.14	17
Evergreen dwarf shrubs	All	11.45	0.13	0.24	6
	Leaves	7.03	0.13	0.18	6
Horsetails		2.68	0.19	0.12	13

Table S3. Relationships between point intercept (PI) frequency data and grams of live above-ground plant biomass (g m^{-2}). These relationships were achieved by fitting weighted linear-regression models separately for each plant functional type (PFT) following Bråthen and Hagberg (2004). Each PFT-specific relationship was used to convert PI data obtained for each species (Table S2) in its respective live above-ground biomass (g m^{-2}). For woody PFTs (i.e. deciduous and evergreen dwarf shrubs), relationships were calculated for both live above-ground biomass as a whole (“All”) and live leaf above-ground biomass only (“Leaves”); the latter has been used in the calculations. Slope coefficients (b), standard deviation (SD), coefficient of variation (CV), and number of sampled plots for each PFT (n) are shown.



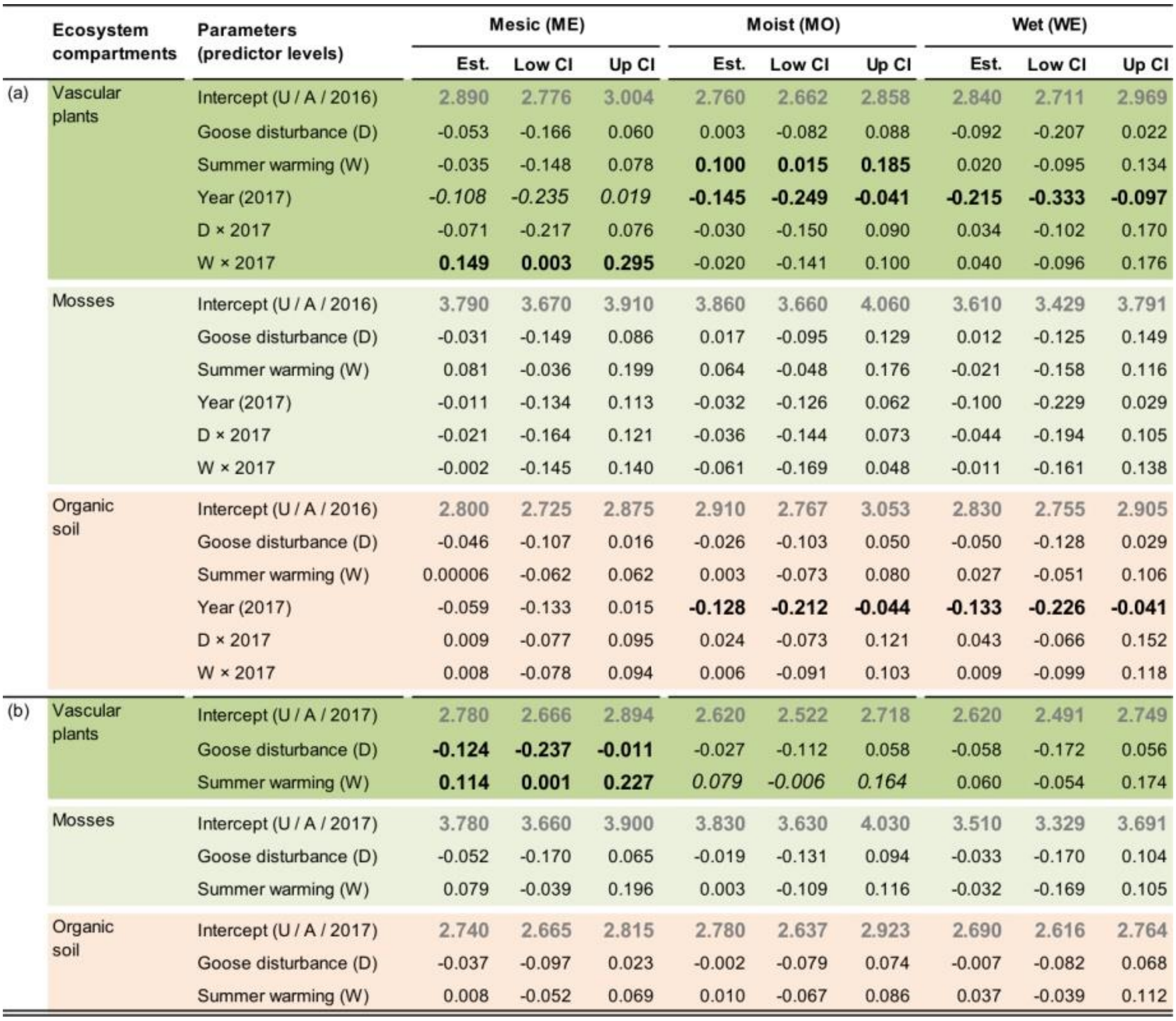
	Ecosystem compartments	Parameters (predictor levels)	Mesic (ME)			Moist (MO)			Wet (WE)		
			Est.	Low CI	Up CI	Est.	Low CI	Up CI	Est.	Low CI	Up CI
(a)	Vascular plants	Intercept (U / A / 2016)	3.789	3.770	3.808	3.756	3.738	3.774	3.766	3.747	3.785
		Goose disturbance (D)	-0.009	-0.027	0.008	0.007	-0.009	0.022	0.001	-0.014	0.016
		Summer warming (W)	0.002	-0.016	0.019	-0.005	-0.021	0.010	0.003	-0.012	0.018
		Year (2017)	0.013	-0.004	0.030	**0.017**	**0.001**	**0.033**	0.011	-0.005	0.027
		D × 2017	-0.001	-0.021	0.018	-0.002	-0.021	0.016	0.004	-0.015	0.023
		W × 2017	0.016	-0.004	0.036	-0.012	-0.031	0.006	-0.001	-0.020	0.017
	Mosses	Intercept (U / A / 2016)	3.780	3.727	3.833	3.770	3.730	3.810	3.750	3.712	3.788
		Goose disturbance (D)	-0.006	-0.042	0.030	-0.011	-0.036	0.014	-0.012	-0.052	0.028
		Summer warming (W)	-0.007	-0.043	0.029	-0.004	-0.029	0.021	0.004	-0.036	0.044
		Year (2017)	-0.029	-0.072	0.015	**-0.064**	**-0.094**	**-0.033**	*-0.043*	*-0.092*	*0.006*
		D × 2017	-0.024	-0.074	0.027	0.0002	-0.035	0.036	-0.033	-0.090	0.023
		W × 2017	0.025	-0.026	0.075	0.013	-0.023	0.049	0.022	-0.034	0.079
	Organic soil	Intercept (U / A / 2016)	2.070	1.746	2.394	1.880	1.562	2.198	1.880	1.514	2.246
		Goose disturbance (D)	**0.211**	**0.023**	**0.398**	-0.028	-0.190	0.134	-0.238	-0.594	0.118
		Summer warming (W)	-0.047	-0.234	0.140	0.032	-0.130	0.194	-0.094	-0.450	0.262
		Year (2017)	-0.028	-0.254	0.198	-0.152	-0.350	0.046	**-0.569**	**-0.941**	**-0.197**
		D × 2017	*-0.257*	*-0.519*	*0.005*	-0.126	-0.354	0.102	0.170	-0.268	0.608
		W × 2017	0.039	-0.223	0.301	0.060	-0.168	0.288	0.055	-0.383	0.493
(b)	Vascular plants	Intercept (U / A / 2017)	3.802	3.783	3.821	3.773	3.755	3.791	3.777	3.758	3.796
		Goose disturbance (D)	-0.011	-0.028	0.007	0.004	-0.011	0.019	0.005	-0.010	0.020
		Summer warming (W)	**0.018**	**0.001**	**0.035**	**-0.018**	**-0.033**	**-0.003**	0.001	-0.013	0.016
	Mosses	Intercept (U / A / 2017)	3.750	3.697	3.803	3.700	3.660	3.740	3.710	3.672	3.748
		Goose disturbance (D)	-0.030	-0.065	0.006	-0.011	-0.036	0.015	**-0.045**	**-0.085**	**-0.005**
		Summer warming (W)	0.018	-0.018	0.053	0.009	-0.016	0.034	0.026	-0.014	0.066
	Organic soil	Intercept (U / A / 2017)	2.040	1.718	2.362	1.730	1.412	2.048	1.320	0.956	1.684
		Goose disturbance (D)	-0.046	-0.230	0.137	*-0.154*	*-0.316*	*0.008*	-0.067	-0.407	0.273
		Summer warming (W)	-0.007	-0.191	0.176	0.092	-0.070	0.254	-0.039	-0.379	0.302

Table S4. Parameter estimates for common linear mixed-effects models on ecosystem-compartment carbon (C) content. Parameter estimates (Est.) and their 95% confidence interval (CI – lower and upper bounds) for common models on C-content fitted separately for each ecosystem compartment (vascular plants, mosses, and soil) and habitat (mesic [ME], moist [MO], and wet [WE]), in which predictors are (i) ‘spring goose disturbance’ (two-level factor: undisturbed [U] and disturbed [D]), ‘summer warming’ (two-level factor: ambient [A] and warming [W]), and ‘year’ (two-level factor: 2016 and 2017) as additive fixed-terms and (ii) ‘spring goose disturbance × year’ and ‘summer warming × year’ as interaction fixed-terms. We retained a common model structure to be able to compare effect sizes obtained for different response variables (see also Tables S5-S6), ecosystem compartments, and habitats (see main text for details). Estimates and CIs are given on the $log_e(y)$-scale. To provide all the contrasts of interest, intercept (in grey colour) was calculated for (a) undisturbed plots [U], ambient plots [A], and year 2016 and (b) undisturbed plots [U], ambient plots [A], and year 2017 (Figure S2 [upper panel] and Figure S3A in Supplementary Figures). Est. and CIs for (i) the main effect of 'year' and (ii) the interaction effects of 'spring goose disturbance × year' and 'summer warming × year' on ecosystem-compartment C-content are presented in relation to (a) undisturbed plots [U], ambient plots [A], and year 2016. Est. (and CIs) for such model terms would be the same, but with opposite sign, if presented for (b) undisturbed plots [U], ambient plots [A], and year 2017 (not shown – refer to (a) for these model terms). Estimates (and their CIs) in bold highlight statistically significant effects (i.e. 95% CI not overlapping 0), whereas estimates (and their CIs) in italic highlight close-to-significant effects (i.e. 90% CI not overlapping 0).



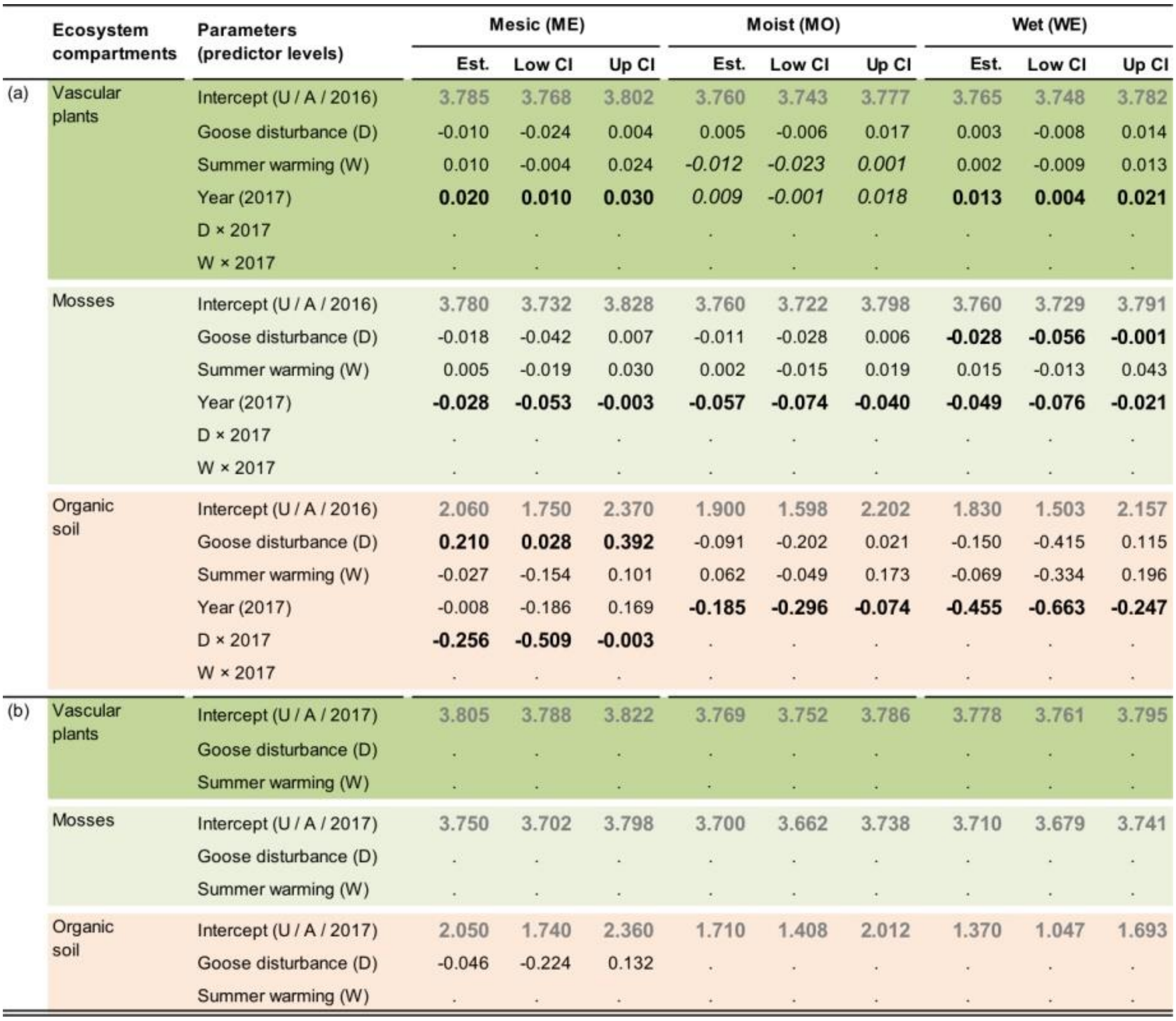
	Ecosystem compartments	Parameters (predictor levels)	Mesic (ME)			Moist (MO)			Wet (WE)		
			Est.	Low CI	Up CI	Est.	Low CI	Up CI	Est.	Low CI	Up CI
(a)	Vascular plants	Intercept (U / A / 2016)	0.897	0.791	1.003	0.994	0.903	1.085	0.931	0.810	1.052
		Goose disturbance (D)	0.043	-0.063	0.150	0.003	-0.074	0.081	0.093	-0.015	0.201
		Summer warming (W)	0.037	-0.069	0.143	**-0.105**	**-0.182**	**-0.028**	-0.017	-0.126	0.091
		Year (2017)	**0.121**	**0.001**	**0.244**	**0.162**	**0.067**	**0.257**	**0.226**	**0.114**	**0.338**
		D × 2017	0.069	-0.072	0.211	0.028	-0.082	0.137	-0.030	-0.159	0.099
		W × 2017	*-0.133*	*-0.275*	*0.009*	0.008	-0.102	0.117	-0.042	-0.171	0.088
	Mosses	Intercept (U / A / 2016)	-0.018	-0.133	0.096	-0.099	-0.281	0.083	0.140	-0.036	0.316
		Goose disturbance (D)	0.025	-0.105	0.156	-0.028	-0.146	0.089	-0.023	-0.163	0.116
		Summer warming (W)	-0.088	-0.219	0.043	-0.068	-0.185	0.050	0.025	-0.115	0.164
		Year (2017)	-0.018	-0.155	0.120	-0.031	-0.149	0.086	0.057	-0.079	0.193
		D × 2017	-0.003	-0.161	0.156	0.036	-0.099	0.172	0.011	-0.146	0.168
		W × 2017	0.027	-0.132	0.186	0.073	-0.062	0.209	0.034	-0.123	0.191
	Organic soil	Intercept (U / A / 2016)	-0.728	-1.090	-0.366	-1.031	-1.407	-0.655	-0.935	-1.315	-0.555
		Goose disturbance (D)	**0.258**	**0.069**	**0.447**	-0.004	-0.152	0.145	-0.192	-0.528	0.144
		Summer warming (W)	-0.044	-0.233	0.145	0.029	-0.119	0.178	-0.124	-0.460	0.212
		Year (2017)	0.039	-0.167	0.245	-0.022	-0.203	0.160	**-0.437**	**-0.805**	**-0.069**
		D × 2017	**-0.271**	**-0.511**	**-0.031**	-0.148	-0.358	0.062	0.126	-0.306	0.558
		W × 2017	0.024	-0.216	0.264	0.051	-0.159	0.261	0.042	-0.390	0.474
(b)	Vascular plants	Intercept (U / A / 2017)	1.018	0.912	1.124	1.156	1.065	1.247	1.157	1.036	1.278
		Goose disturbance (D)	**0.113**	**0.006**	**0.219**	0.031	-0.046	0.108	0.063	-0.045	0.172
		Summer warming (W)	*-0.096*	*-0.203*	*0.010*	**-0.097**	**-0.175**	**-0.020**	-0.059	-0.167	0.050
	Mosses	Intercept (U / A / 2017)	-0.036	-0.150	0.079	-0.130	-0.313	0.052	0.196	0.020	0.372
		Goose disturbance (D)	0.023	-0.108	0.153	0.008	-0.110	0.126	-0.012	-0.152	0.127
		Summer warming (W)	-0.061	-0.192	0.070	0.006	-0.112	0.123	0.058	-0.081	0.198
	Organic soil	Intercept (U / A / 2017)	-0.689	-1.051	-0.327	-1.053	-1.429	-0.677	-1.371	-1.747	-0.995
		Goose disturbance (D)	-0.012	-0.197	0.173	**-0.152**	**-0.300**	**-0.003**	-0.066	-0.386	0.254
		Summer warming (W)	-0.019	-0.204	0.166	0.081	-0.068	0.229	-0.081	-0.401	0.239

Table S5. Parameter estimates for common linear mixed-effects models on ecosystem-compartment nitrogen (N) content. Parameter estimates (Est.) and their 95% confidence interval (CI – lower and upper bounds) for common models on N-content fitted separately for each ecosystem compartment (vascular plants, mosses, and soil) and habitat (mesic [ME], moist [MO], and wet [WE]), in which predictors are (i) ‘spring goose disturbance’ (two-level factor: undisturbed [U] and disturbed [D]), ‘summer warming’ (two-level factor: ambient [A] and warming [W]), and ‘year’ (two-level factor: 2016 and 2017) as additive fixed-terms and (ii) ‘spring goose disturbance × year’ and ‘summer warming × year’ as interaction fixed-terms. We retained a common model structure to be able to compare effect sizes obtained for different response variables (see also Tables S4 and S6), ecosystem compartments, and habitats (see main text for details). Estimates and CIs are given on the $log_e(y)$-scale. To provide all the contrasts of interest, intercept (in grey colour) was calculated for (a) undisturbed plots [U], ambient plots [A], and year 2016 and (b) undisturbed plots [U], ambient plots [A], and year 2017 (Figure S2 [middle panel] and Figure S3B in Supplementary Figures). Est. and CIs for (i) the main effect of 'year' and (ii) the interaction effects of 'spring goose disturbance × year' and 'summer warming × year' on ecosystem-compartment N-content are presented in relation to (a) undisturbed plots [U], ambient plots [A], and year 2016. Est. (and CIs) for such model terms would be the same, but with opposite sign, if presented for (b) undisturbed plots [U], ambient plots [A], and year 2017 (not shown – refer to (a) for these model terms). Estimates (and their CIs) in bold highlight statistically significant effects (i.e. 95% CI not overlapping 0), whereas estimates (and their CIs) in italic highlight close-to-significant effects (i.e. 90% CI not overlapping 0).



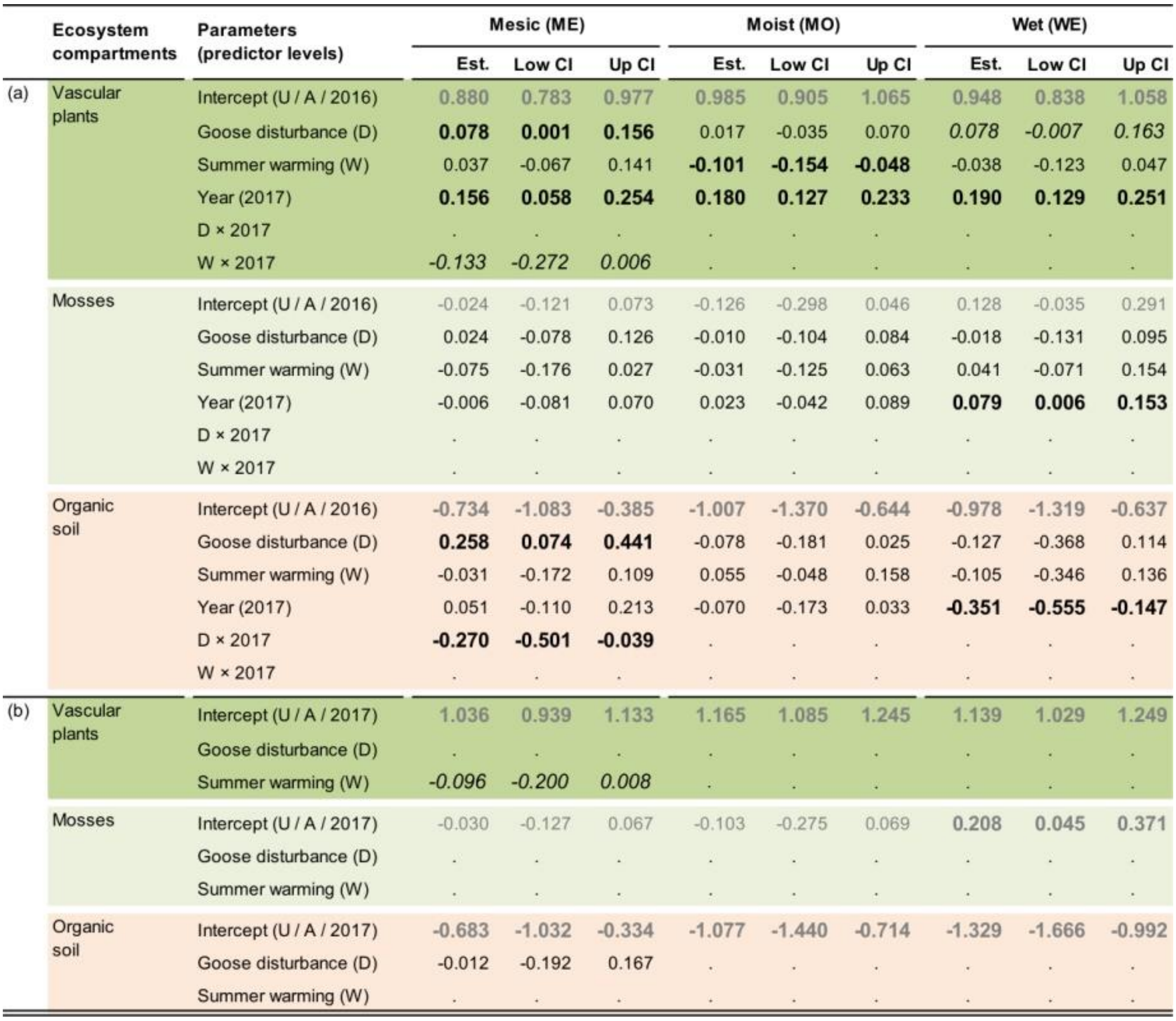
	Ecosystem compartments	Parameters (predictor levels)	Mesic (ME)			Moist (MO)			Wet (WE)		
			Est.	Low CI	Up CI	Est.	Low CI	Up CI	Est.	Low CI	Up CI
(a)	Vascular plants	Intercept (U / A / 2016)	2.890	2.776	3.004	2.760	2.662	2.858	2.840	2.711	2.969
		Goose disturbance (D)	-0.053	-0.166	0.060	0.003	-0.082	0.088	-0.092	-0.207	0.022
		Summer warming (W)	-0.035	-0.148	0.078	**0.100**	**0.015**	**0.185**	0.020	-0.095	0.134
		Year (2017)	*-0.108*	*-0.235*	*0.019*	**-0.145**	**-0.249**	**-0.041**	**-0.215**	**-0.333**	**-0.097**
		D × 2017	-0.071	-0.217	0.076	-0.030	-0.150	0.090	0.034	-0.102	0.170
		W × 2017	**0.149**	**0.003**	**0.295**	-0.020	-0.141	0.100	0.040	-0.096	0.176
	Mosses	Intercept (U / A / 2016)	3.790	3.670	3.910	3.860	3.660	4.060	3.610	3.429	3.791
		Goose disturbance (D)	-0.031	-0.149	0.086	0.017	-0.095	0.129	0.012	-0.125	0.149
		Summer warming (W)	0.081	-0.036	0.199	0.064	-0.048	0.176	-0.021	-0.158	0.116
		Year (2017)	-0.011	-0.134	0.113	-0.032	-0.126	0.062	-0.100	-0.229	0.029
		D × 2017	-0.021	-0.164	0.121	-0.036	-0.144	0.073	-0.044	-0.194	0.105
		W × 2017	-0.002	-0.145	0.140	-0.061	-0.169	0.048	-0.011	-0.161	0.138
	Organic soil	Intercept (U / A / 2016)	2.800	2.725	2.875	2.910	2.767	3.053	2.830	2.755	2.905
		Goose disturbance (D)	-0.046	-0.107	0.016	-0.026	-0.103	0.050	-0.050	-0.128	0.029
		Summer warming (W)	0.00006	-0.062	0.062	0.003	-0.073	0.080	0.027	-0.051	0.106
		Year (2017)	-0.059	-0.133	0.015	**-0.128**	**-0.212**	**-0.044**	**-0.133**	**-0.226**	**-0.041**
		D × 2017	0.009	-0.077	0.095	0.024	-0.073	0.121	0.043	-0.066	0.152
		W × 2017	0.008	-0.078	0.094	0.006	-0.091	0.103	0.009	-0.099	0.118
(b)	Vascular plants	Intercept (U / A / 2017)	2.780	2.666	2.894	2.620	2.522	2.718	2.620	2.491	2.749
		Goose disturbance (D)	**-0.124**	**-0.237**	**-0.011**	-0.027	-0.112	0.058	-0.058	-0.172	0.056
		Summer warming (W)	**0.114**	**0.001**	**0.227**	*0.079*	*-0.006*	*0.164*	0.060	-0.054	0.174
	Mosses	Intercept (U / A / 2017)	3.780	3.660	3.900	3.830	3.630	4.030	3.510	3.329	3.691
		Goose disturbance (D)	-0.052	-0.170	0.065	-0.019	-0.131	0.094	-0.033	-0.170	0.104
		Summer warming (W)	0.079	-0.039	0.196	0.003	-0.109	0.116	-0.032	-0.169	0.105
	Organic soil	Intercept (U / A / 2017)	2.740	2.665	2.815	2.780	2.637	2.923	2.690	2.616	2.764
		Goose disturbance (D)	-0.037	-0.097	0.023	-0.002	-0.079	0.074	-0.007	-0.082	0.068
		Summer warming (W)	0.008	-0.052	0.069	0.010	-0.067	0.086	0.037	-0.039	0.112

Table S6. Parameter estimates for common linear mixed-effects models on ecosystem-compartment carbon-to-nitrogen (C:N) ratio. Parameter estimates (Est.) and their 95% confidence interval (CI – lower and upper bounds) for common models on C:N ratio fitted separately for each ecosystem compartment (vascular plants, mosses, and soil) and habitat (mesic [ME], moist [MO], and wet [WE]), in which predictors are (i) 'spring goose disturbance' (two-level factor: undisturbed [U] and disturbed [D]), 'summer warming' (two-level factor: ambient [A] and warming [W]), and 'year' (two-level factor: 2016 and 2017) as additive fixed-terms and (ii) 'spring goose disturbance × year' and 'summer warming × year' as interaction fixed-terms. We retained a common model structure to be able to compare effect sizes obtained for different response variables (see also Tables S4-S5), ecosystem compartments, and habitats (see main text for details). Estimates and CIs are given on the $log_e(y)$-scale. To provide all the contrasts of interest, intercept (in grey colour) was calculated for (a) undisturbed plots [U], ambient plots [A], and year 2016 and (b) undisturbed plots [U], ambient plots [A], and year 2017 (Figure S2 [lower panel] and Figure S3C in Supplementary Figures). Est. and CIs for (i) the main effect of 'year' and (ii) the interaction effects of 'spring goose disturbance × year' and 'summer warming × year' on ecosystem-compartment C:N ratio are presented in relation to (a) undisturbed plots [U], ambient plots [A], and year 2016. Est. (and CIs) for such model terms would be the same, but with opposite sign, if presented for (b) undisturbed plots [U], ambient plots [A], and year 2017 (not shown – refer to (a) for these model terms). Estimates (and their CIs) in bold highlight statistically significant effects (i.e. 95% CI not overlapping 0), whereas estimates (and their CIs) in italic highlight close-to-significant effects (i.e. 90% CI not overlapping 0).



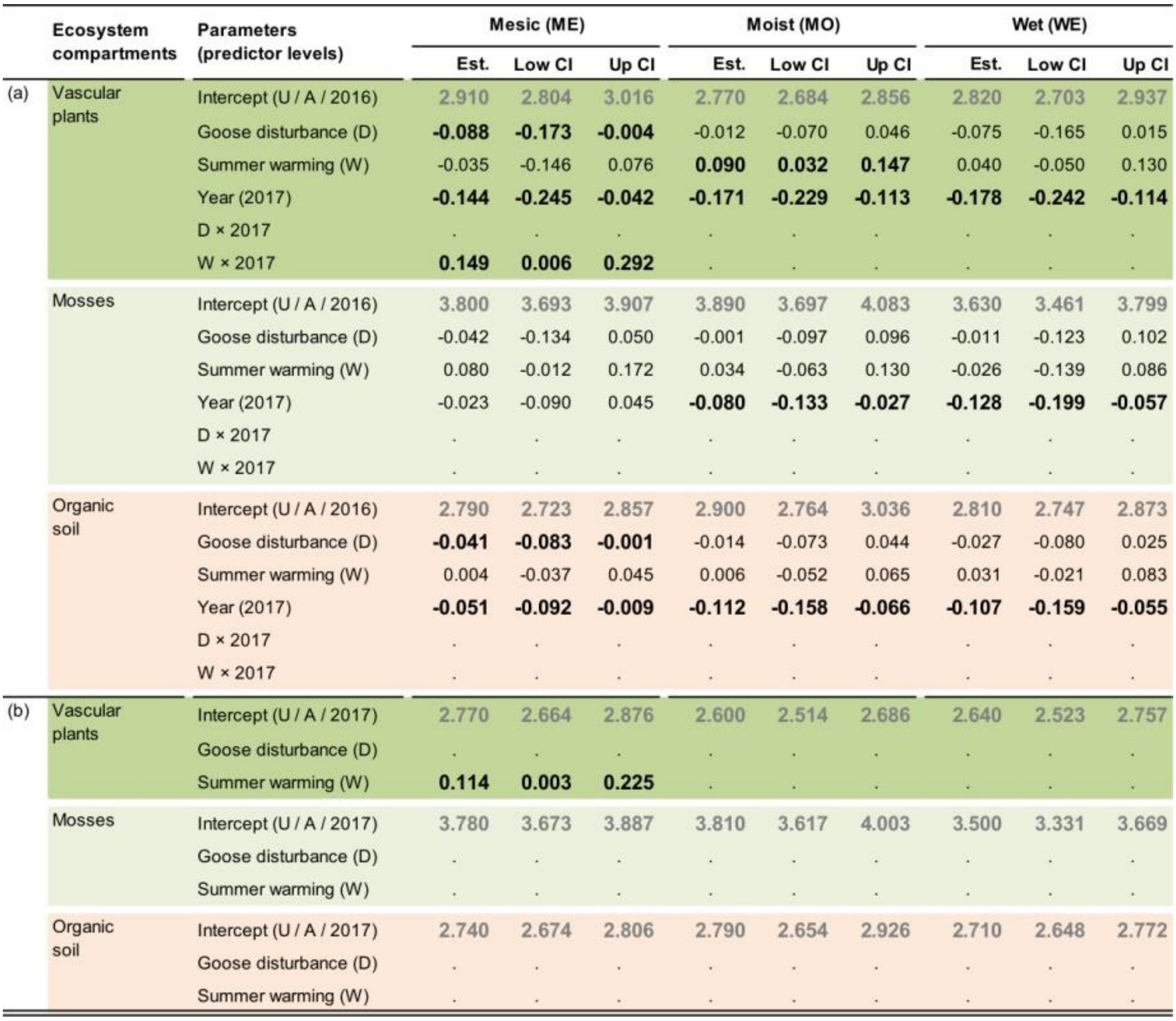
	Ecosystem compartments	Parameters (predictor levels)	Mesic (ME)			Moist (MO)			Wet (WE)		
			Est.	Low CI	Up CI	Est.	Low CI	Up CI	Est.	Low CI	Up CI
(a)	Vascular plants	Intercept (U / A / 2016)	3.785	3.768	3.802	3.760	3.743	3.777	3.765	3.748	3.782
		Goose disturbance (D)	-0.010	-0.024	0.004	0.005	-0.006	0.017	0.003	-0.008	0.014
		Summer warming (W)	0.010	-0.004	0.024	*-0.012*	*-0.023*	*0.001*	0.002	-0.009	0.013
		Year (2017)	**0.020**	**0.010**	**0.030**	*0.009*	*-0.001*	*0.018*	**0.013**	**0.004**	**0.021**
		D × 2017	.	.	.	.	.	.	.	.	.
		W × 2017	.	.	.	.	.	.	.	.	.
	Mosses	Intercept (U / A / 2016)	3.780	3.732	3.828	3.760	3.722	3.798	3.760	3.729	3.791
		Goose disturbance (D)	-0.018	-0.042	0.007	-0.011	-0.028	0.006	**-0.028**	**-0.056**	**-0.001**
		Summer warming (W)	0.005	-0.019	0.030	0.002	-0.015	0.019	0.015	-0.013	0.043
		Year (2017)	**-0.028**	**-0.053**	**-0.003**	**-0.057**	**-0.074**	**-0.040**	**-0.049**	**-0.076**	**-0.021**
		D × 2017	.	.	.	.	.	.	.	.	.
		W × 2017	.	.	.	.	.	.	.	.	.
	Organic soil	Intercept (U / A / 2016)	2.060	1.750	2.370	1.900	1.598	2.202	1.830	1.503	2.157
		Goose disturbance (D)	**0.210**	**0.028**	**0.392**	-0.091	-0.202	0.021	-0.150	-0.415	0.115
		Summer warming (W)	-0.027	-0.154	0.101	0.062	-0.049	0.173	-0.069	-0.334	0.196
		Year (2017)	-0.008	-0.186	0.169	**-0.185**	**-0.296**	**-0.074**	**-0.455**	**-0.663**	**-0.247**
		D × 2017	**-0.256**	**-0.509**	**-0.003**	.	.	.	.	.	.
		W × 2017	.	.	.	.	.	.	.	.	.
(b)	Vascular plants	Intercept (U / A / 2017)	3.805	3.788	3.822	3.769	3.752	3.786	3.778	3.761	3.795
		Goose disturbance (D)	.	.	.	.	.	.	.	.	.
		Summer warming (W)	.	.	.	.	.	.	.	.	.
	Mosses	Intercept (U / A / 2017)	3.750	3.702	3.798	3.700	3.662	3.738	3.710	3.679	3.741
		Goose disturbance (D)	.	.	.	.	.	.	.	.	.
		Summer warming (W)	.	.	.	.	.	.	.	.	.
	Organic soil	Intercept (U / A / 2017)	2.050	1.740	2.360	1.710	1.408	2.012	1.370	1.047	1.693
		Goose disturbance (D)	-0.046	-0.224	0.132	.	.	.	.	.	.
		Summer warming (W)	.	.	.	.	.	.	.	.	.

Table S7. Parameter estimates for most parsimonious linear mixed-effects models on ecosystem-compartment carbon (C) content. Parameter estimates (Est.) and their 95% confidence interval (CI – lower and upper bounds) for most parsimonious models on C-content fitted separately for each ecosystem compartment (vascular plants, mosses, and soil) and habitat (mesic [ME], moist [MO], and wet [WE]), in which predictors are 'spring goose disturbance' (two-level factor: undisturbed [U] and disturbed [D]), 'summer warming' (two-level factor: ambient [A] and warming [W]), and 'year' (two-level factor: 2016 and 2017). Estimates and CIs are given on the *log*$_e$(*y*)-scale. To provide all the contrasts of interest, intercept (in grey colour) was calculated for (a) undisturbed plots [U], ambient plots [A], and year 2016 and (b) undisturbed plots [U], ambient plots [A], and year 2017 (Figure 4 [upper panel] and Figure 5A in the main text). Est. and CIs for (i) the main effect of ‘year’ and (ii) the interaction effects of ‘spring goose disturbance × year’ and ‘summer warming × year’ on ecosystem-compartment C-content are presented in relation to (a) undisturbed plots [U], ambient plots [A], and year 2016. Est. (and CIs) for such model terms would be the same, but with opposite sign, if presented for (b) undisturbed plots [U], ambient plots [A], and year 2017 (not shown – refer to (a) for these model terms). When no interactions were detected, only (a) is used since model terms would be the same if presented for (b). Estimates (and their CIs) in bold highlight statistically significant effects (i.e. 95% CI not overlapping 0), whereas estimates (and their CIs) in italic highlight close-to-significant effects (i.e. 90% CI not overlapping 0). Empty cells indicate that an interaction coefficient was not statistically significant, thus it was removed from the model.



	Ecosystem compartments	Parameters (predictor levels)	Mesic (ME)			Moist (MO)			Wet (WE)		
			Est.	Low CI	Up CI	Est.	Low CI	Up CI	Est.	Low CI	Up CI
(a)	Vascular plants	Intercept (U / A / 2016)	0.880	0.783	0.977	0.985	0.905	1.065	0.948	0.838	1.058
		Goose disturbance (D)	**0.078**	**0.001**	**0.156**	0.017	-0.035	0.070	*0.078*	*-0.007*	*0.163*
		Summer warming (W)	0.037	-0.067	0.141	**-0.101**	**-0.154**	**-0.048**	-0.038	-0.123	0.047
		Year (2017)	**0.156**	**0.058**	**0.254**	**0.180**	**0.127**	**0.233**	**0.190**	**0.129**	**0.251**
		D × 2017	.	.	.	.	.	.	.	.	.
		W × 2017	*-0.133*	*-0.272*	*0.006*	.	.	.	.	.	.
	Mosses	Intercept (U / A / 2016)	-0.024	-0.121	0.073	-0.126	-0.298	0.046	0.128	-0.035	0.291
		Goose disturbance (D)	0.024	-0.078	0.126	-0.010	-0.104	0.084	-0.018	-0.131	0.095
		Summer warming (W)	-0.075	-0.176	0.027	-0.031	-0.125	0.063	0.041	-0.071	0.154
		Year (2017)	-0.006	-0.081	0.070	0.023	-0.042	0.089	**0.079**	**0.006**	**0.153**
		D × 2017	.	.	.	.	.	.	.	.	.
		W × 2017	.	.	.	.	.	.	.	.	.
	Organic soil	Intercept (U / A / 2016)	-0.734	-1.083	-0.385	-1.007	-1.370	-0.644	-0.978	-1.319	-0.637
		Goose disturbance (D)	**0.258**	**0.074**	**0.441**	-0.078	-0.181	0.025	-0.127	-0.368	0.114
		Summer warming (W)	-0.031	-0.172	0.109	0.055	-0.048	0.158	-0.105	-0.346	0.136
		Year (2017)	0.051	-0.110	0.213	-0.070	-0.173	0.033	**-0.351**	**-0.555**	**-0.147**
		D × 2017	**-0.270**	**-0.501**	**-0.039**	.	.	.	.	.	.
		W × 2017	.	.	.	.	.	.	.	.	.
(b)	Vascular plants	Intercept (U / A / 2017)	1.036	0.939	1.133	1.165	1.085	1.245	1.139	1.029	1.249
		Goose disturbance (D)	.	.	.	.	.	.	.	.	.
		Summer warming (W)	*-0.096*	*-0.200*	*0.008*	.	.	.	.	.	.
	Mosses	Intercept (U / A / 2017)	-0.030	-0.127	0.067	-0.103	-0.275	0.069	**0.208**	**0.045**	**0.371**
		Goose disturbance (D)	.	.	.	.	.	.	.	.	.
		Summer warming (W)	.	.	.	.	.	.	.	.	.
	Organic soil	Intercept (U / A / 2017)	-0.683	-1.032	-0.334	-1.077	-1.440	-0.714	-1.329	-1.666	-0.992
		Goose disturbance (D)	-0.012	-0.192	0.167	.	.	.	.	.	.
		Summer warming (W)	.	.	.	.	.	.	.	.	.

Table S8. Parameter estimates for most parsimonious linear mixed-effects models on ecosystem-compartment nitrogen (N) content. Parameter estimates (Est.) and their 95% confidence interval (CI – lower and upper bounds) for most parsimonious models on N-content fitted separately for each ecosystem compartment (vascular plants, mosses, and soil) and habitat (mesic [ME], moist [MO], and wet [WE]), in which predictors are ‘spring goose disturbance’ (two-level factor: undisturbed [U] and disturbed [D]), ‘summer warming’ (two-level factor: ambient [A] and warming [W]), and ‘year’ (two-level factor: 2016 and 2017). Estimates and CIs are given on the $log_e(y)$-scale. To provide all the contrasts of interest, intercept (in grey colour) was calculated for (a) undisturbed plots [U], ambient plots [A], and year 2016 and (b) undisturbed plots [U], ambient plots [A], and year 2017 (Figure 4 [middle panel] and Figure 5B in the main text). Est. and CIs for (i) the main effect of ‘year’ and (ii) the interaction effects of ‘spring goose disturbance × year’ and ‘summer warming × year’ on ecosystem-compartment N-content are presented in relation to (a) undisturbed plots [U], ambient plots [A], and year 2016. Est. (and CIs) for such model terms would be the same, but with opposite sign, if presented for (b) undisturbed plots [U], ambient plots [A], and year 2017 (not shown – refer to (a) for these model terms). When no interactions were detected, only (a) is used since model terms would be the same if presented for (b). Estimates (and their CIs) in bold highlight statistically significant effects (i.e. 95% CI not overlapping 0), whereas estimates (and their CIs) in italic highlight close-to-significant effects (i.e. 90% CI not overlapping 0). Empty cells indicate that an interaction coefficient was not statistically significant, thus it was removed from the model.



	Ecosystem compartments	Parameters (predictor levels)	Mesic (ME)			Moist (MO)			Wet (WE)		
			Est.	Low CI	Up CI	Est.	Low CI	Up CI	Est.	Low CI	Up CI
(a)	Vascular plants	Intercept (U / A / 2016)	2.910	2.804	3.016	2.770	2.684	2.856	2.820	2.703	2.937
		Goose disturbance (D)	**-0.088**	**-0.173**	**-0.004**	-0.012	-0.070	0.046	-0.075	-0.165	0.015
		Summer warming (W)	-0.035	-0.146	0.076	**0.090**	**0.032**	**0.147**	0.040	-0.050	0.130
		Year (2017)	**-0.144**	**-0.245**	**-0.042**	**-0.171**	**-0.229**	**-0.113**	**-0.178**	**-0.242**	**-0.114**
		D × 2017	.	.	.	.	.	.	.	.	.
		W × 2017	**0.149**	**0.006**	**0.292**	.	.	.	.	.	.
	Mosses	Intercept (U / A / 2016)	3.800	3.693	3.907	3.890	3.697	4.083	3.630	3.461	3.799
		Goose disturbance (D)	-0.042	-0.134	0.050	-0.001	-0.097	0.096	-0.011	-0.123	0.102
		Summer warming (W)	0.080	-0.012	0.172	0.034	-0.063	0.130	-0.026	-0.139	0.086
		Year (2017)	-0.023	-0.090	0.045	**-0.080**	**-0.133**	**-0.027**	**-0.128**	**-0.199**	**-0.057**
		D × 2017	.	.	.	.	.	.	.	.	.
		W × 2017	.	.	.	.	.	.	.	.	.
	Organic soil	Intercept (U / A / 2016)	2.790	2.723	2.857	2.900	2.764	3.036	2.810	2.747	2.873
		Goose disturbance (D)	**-0.041**	**-0.083**	**-0.001**	-0.014	-0.073	0.044	-0.027	-0.080	0.025
		Summer warming (W)	0.004	-0.037	0.045	0.006	-0.052	0.065	0.031	-0.021	0.083
		Year (2017)	**-0.051**	**-0.092**	**-0.009**	**-0.112**	**-0.158**	**-0.066**	**-0.107**	**-0.159**	**-0.055**
		D × 2017	.	.	.	.	.	.	.	.	.
		W × 2017	.	.	.	.	.	.	.	.	.
(b)	Vascular plants	Intercept (U / A / 2017)	2.770	2.664	2.876	2.600	2.514	2.686	2.640	2.523	2.757
		Goose disturbance (D)	.	.	.	.	.	.	.	.	.
		Summer warming (W)	**0.114**	**0.003**	**0.225**	.	.	.	.	.	.
	Mosses	Intercept (U / A / 2017)	3.780	3.673	3.887	3.810	3.617	4.003	3.500	3.331	3.669
		Goose disturbance (D)	.	.	.	.	.	.	.	.	.
		Summer warming (W)	.	.	.	.	.	.	.	.	.
	Organic soil	Intercept (U / A / 2017)	2.740	2.674	2.806	2.790	2.654	2.926	2.710	2.648	2.772
		Goose disturbance (D)	.	.	.	.	.	.	.	.	.
		Summer warming (W)	.	.	.	.	.	.	.	.	.

Table S9. Parameter estimates for most parsimonious linear mixed-effects models on ecosystem-compartment carbon-to-nitrogen (C:N) ratio. Parameter estimates (Est.) and their 95% confidence interval (CI – lower and upper bounds) for most parsimonious models on C:N ratio fitted separately for each ecosystem compartment (vascular plants, mosses, and soil) and habitat (mesic [ME], moist [MO], and wet [WE]), in which predictors are ‘spring goose disturbance’ (two-level factor: undisturbed [U] and disturbed [D]), ‘summer warming’ (two-level factor: ambient [A] and warming [W]), and ‘year’ (two-level factor: 2016 and 2017). Estimates and CIs are given on the $log_e(y)$-scale. To provide all the contrasts of interest, intercept (in grey colour) was calculated for (a) undisturbed plots [U], ambient plots [A], and year 2016 and (b) undisturbed plots [U], ambient plots [A], and year 2017 (Figure 4 [lower panel] and Figure 5C in the main text). Est. and CIs for (i) the main effect of ‘year’ and (ii) the interaction effects of ‘spring goose disturbance × year’ and ‘summer warming × year’ on ecosystem-compartment C:N ratio are presented in relation to (a) undisturbed plots [U], ambient plots [A], and year 2016. Est. (and CIs) for such model terms would be the same, but with opposite sign, if presented for (b) undisturbed plots [U], ambient plots [A], and year 2017 (not shown – refer to (a) for these model terms). When no interactions were detected, only (a) is used since model terms would be the same if presented for (b). Estimates (and their CIs) in bold highlight statistically significant effects (i.e. 95% CI not overlapping 0), whereas estimates (and their CIs) in italic highlight close-to-significant effects (i.e. 90% CI not overlapping 0). Empty cells indicate that an interaction coefficient was not statistically significant, thus it was removed from the model.

Supporting Figures

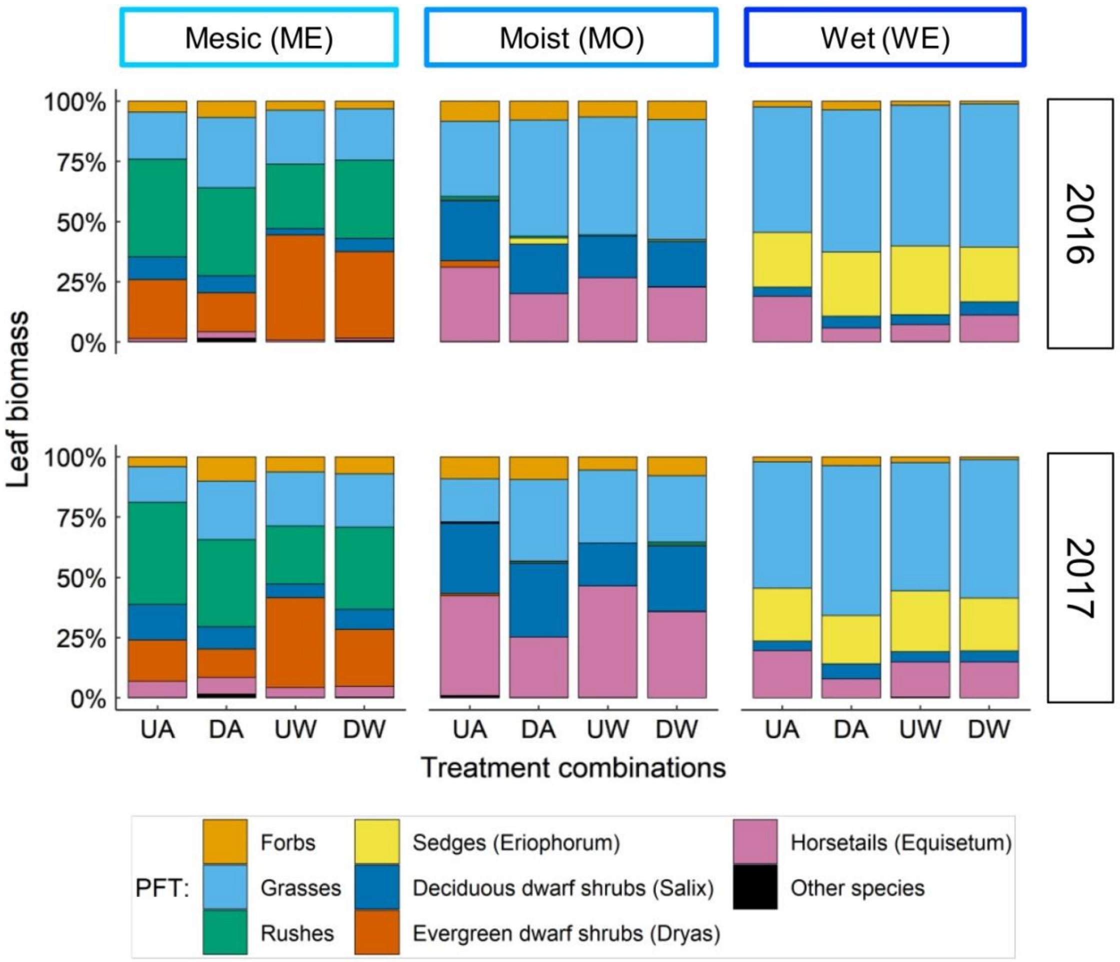


Figure S1. Relative contribution of plant functional types (PFTs) to overall live leaf above-ground vascular-plant biomass. Relative contribution (expressed as %) of the 14 vascular plant species sampled within the experiment (here grouped in PFTs – see Table S2) to the overall live leaf above-ground biomass, separately for each habitat (mesic [ME], moist [MO], and wet [WE]), year (2016 and 2017), and treatment combination (UA = undisturbed/ambient [un-manipulated controls], DA = disturbed/ambient, UW = undisturbed/warming, and DW = disturbed/warming). Their combined biomass encompassed in average over 99% (range: 89-100%) of the vascular plant biomass within plots. ‘Other species’ denotes the relative contribution to overall live leaf above-ground vascular-plant biomass of those species from which we did not collect samples for chemical content analyses (see main text for details).

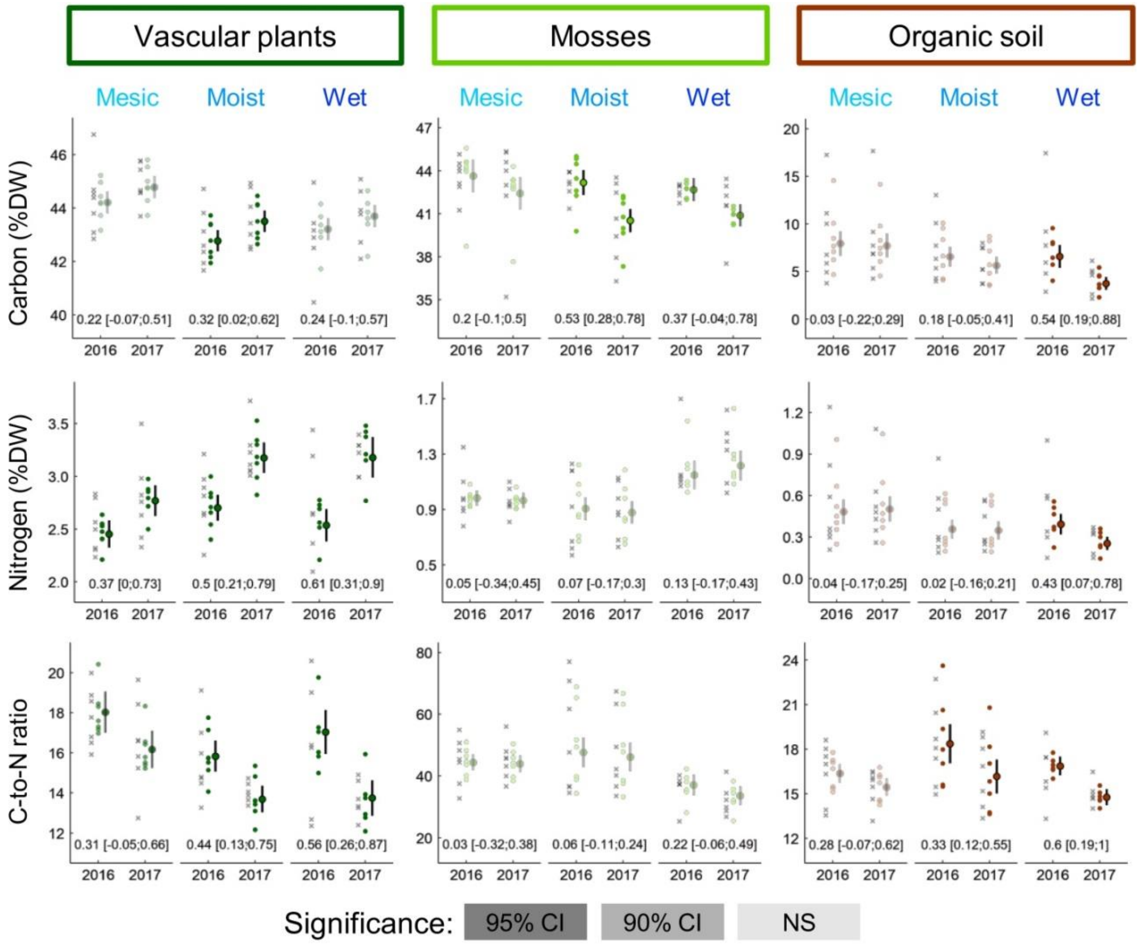


Figure S2. Baseline chemical composition of the three ecosystem compartments in the three habitats for the two years. Differences between 2016 and 2017 in carbon (C) and nitrogen (N) content (% of dry weight – %DW) and C:N ratio of vascular plants, mosses, and organic soil in undisturbed/ambient [UA] plots (un-manipulated controls) within the three habitats (mesic [ME], moist [MO], and wet [WE]). Data were back-transformed from the $log_e(y)$-scale and are presented in their original scale; note the different scales of y-axis. Main dots represent model predictions ± their standard error (SE) as derived by common linear mixed-effects models (see main text for details), coloured dots represent fitted values, and grey dots represent raw values. Predictions (and their SE) in bold colours highlight statistically significant differences (i.e. 95% confidence interval [CI] not overlapping 0), predictions (and their SE) in half-shaded colours highlight close-to-significant differences (i.e. 90% CI not overlapping 0), and predictions (and their SE) in shaded colours highlight non-statistically significant differences. At the base of each panel, absolute values of standardized model estimates (and their 95% CI) for the difference between the two years are reported in order to allow comparisons with treatment effects (cf. Supplementary Figure S3). Model estimates are presented in Supplementary Tables S3-S6.

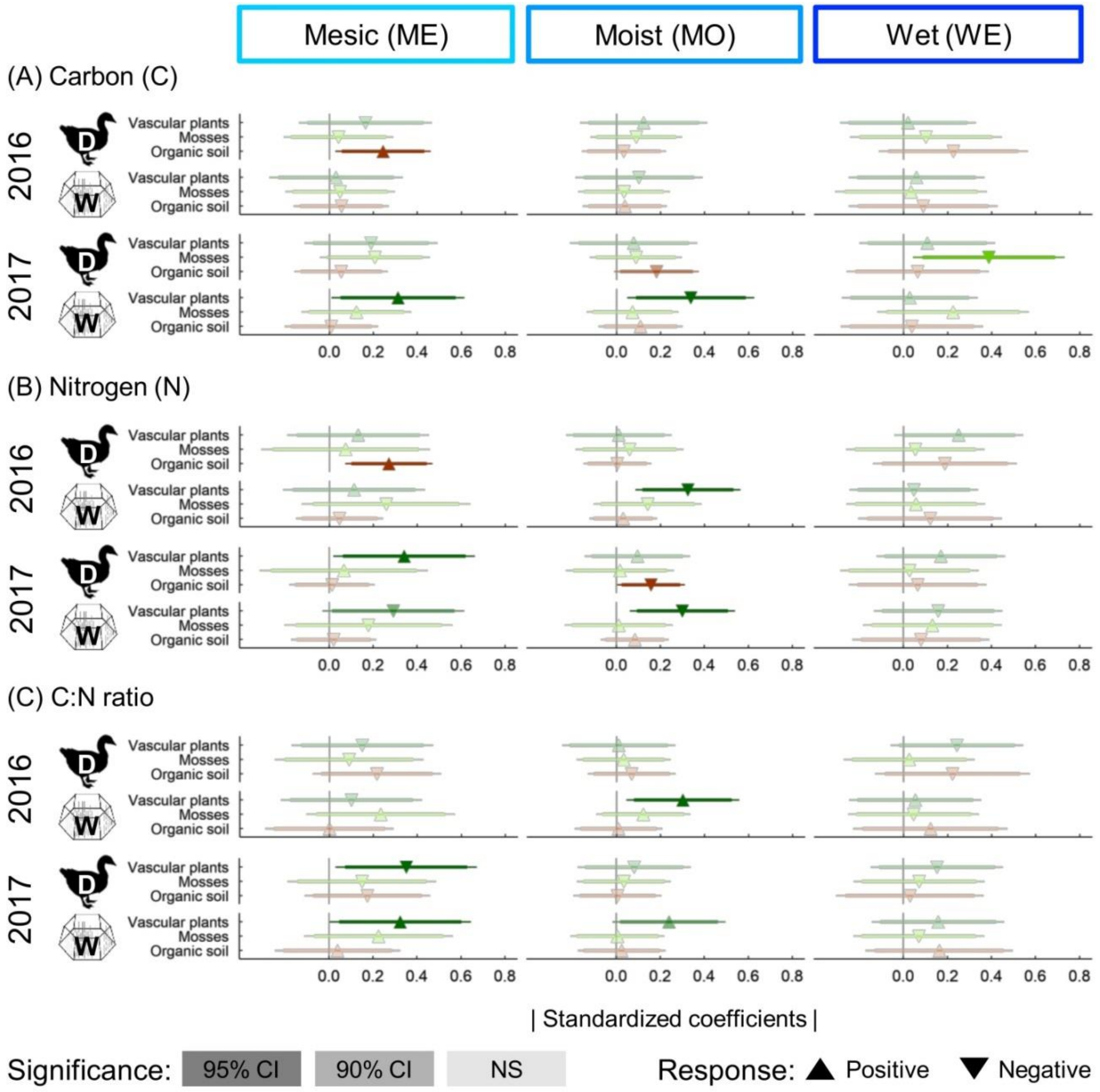


Figure S3. Chemical responsiveness and response strength of the three ecosystem compartments to treatments as derived by common linear mixed-effects models. Absolute values of standardized model estimates for changes in (A) carbon [C], (B) nitrogen [N], and (C) C:N ratio of vascular plants, mosses, and organic soil in response to spring goose disturbance (D *versus* undisturbed plots [U – the reference level]) and summer warming (W *versus* ambient plots [A – the reference level]) in the three habitats (mesic [ME], moist [MO], and wet [WE]), separately for 2016 and 2017. Treatment-specific standardized effect sizes, their 90% confidence interval [CI] (thick line), and their 95% CI (thin line) are given for the main effects of spring goose disturbance and summer warming; at the base of each panel, absolute values of standardized effect sizes are presented. The reference level is denoted with the grey line at 0 effect size. Estimates (and their CIs) in bold colours highlight statistically significant effects (i.e. 95% CI not overlapping 0), estimates (and their CIs) in half-shaded colours highlight close-to-significant effects (i.e. 90% CI not overlapping 0), and estimates (and their CIs) in shaded colours highlight non-statistically significant effects. Upward-pointing triangles denote positive standardized effect sizes (i.e. positive responses), whereas downward-pointing triangles denote negative standardized effect sizes (i.e. negative responses). Model estimates are presented in Supplementary Tables S3-S6.

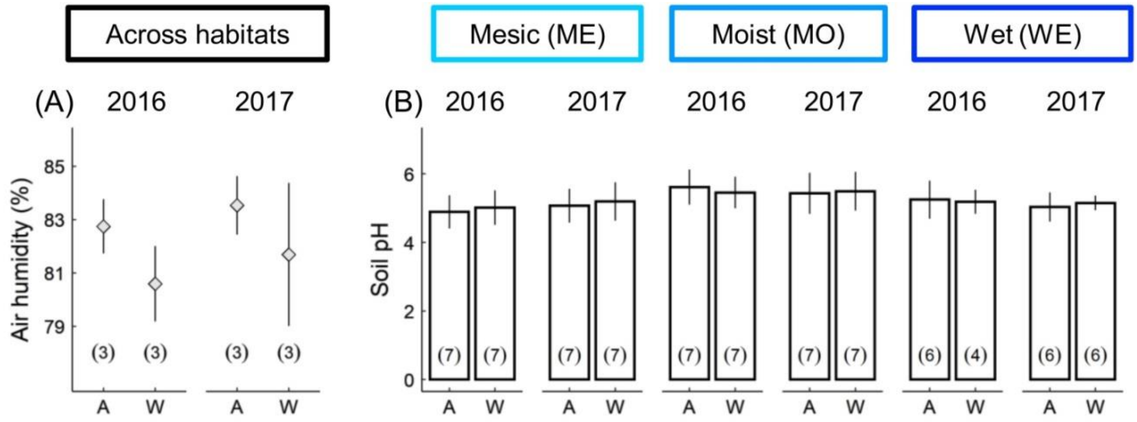


Figure S4. Effects of summer experimental warming on air relative humidity and soil pH. (A) Average air relative humidity throughout the warmest month (July) in 2016 and 2017, within ambient [A] and warming [W] plots. Data were registered at 5 cm above the surface in one A and W plot per habitat (mesic [ME], moist [MO], and wet [WE]) every 30 min throughout the two growing seasons using data loggers (U23-003 HOBO, Onset Computer Corporation, Bourne, Massachusettes, USA; accuracy: ±2.5%) equipped with solar-radiation shields. Average air relative humidity was obtained by calculating mean daily air relative humidity within each data logger and averaged these values for the period 1-31 July. (B) Peak-season soil pH in the three habitats in 2016 and 2017, within ambient [A] and warming [W] plots. Soil pH was measured on the remaining part of organic soil not utilized in the assessment of soil C- and N-content (see main text for details) by using a pH electrode (SympHony, VWR, Radnor, Pennsylvania, USA). Lines represent the standard deviation of the mean. Numbers at the base of each panel refer to sample sizes.

Appendix 1A: The Svalbard trophic system, with particular reference to Adventdalen

In winter, the Svalbard trophic system is relatively simple (Hansen et al., 2013) and includes only three herbivores, namely the wild Svalbard reindeer (*Rangifer tarandus platyrhynchus*), Svalbard rock ptarmigan (*Lagopus muta hyperborea*), and sibling vole (*Microtus levis*), as well as the Arctic fox (*Vulpes lagopus*), which is the only terrestrial predator. Whilst reindeer and ptarmigan are sympatric species and widely distributed across the Svalbard archipelago, the sibling vole is only found in a small bird cliff area (Yoccoz and Ims, 1999) and is absent from our study area in Adventdalen. The few resident terrestrial vertebrates and the lack of small rodents make the Svalbard terrestrial food-web relatively simple compared to many other Arctic terrestrial ecosystems (Ims and Fuglei, 2005).

In summer, the Svalbard trophic system becomes more complex due to the arrival of many species of migratory birds. The majority of these species fall in the ‘category’ of sea-birds, which feed on marine ecosystems and thus influence terrestrial ecosystems only in the proximity of coast lines through the transport of nutrients from sea to land. Our study area in Adventdalen is not influenced by sea birds. Three migratory goose species nest and feed on the terrestrial ecosystems of the archipelago, namely the pink-footed goose (*Anser brachyrhynchus*), barnacle goose (*Branta leucopsis*), and brent goose (*Branta bernicla*). Whilst the brent goose is not commonly found within the study area, both pink-footed geese and barnacle geese heavily utilize Adventdalen as feeding ground during the pre-breeding period (mid May-mid June). During this period, pink-footed geese feed almost exclusively on below-ground plant parts (i.e. roots and rhizomes of vascular plants) through grubbing (see main text for details). Conversely, barnacle geese mostly feed by grazing above-ground plant material, and mosses are an important part of their diet (Fox and Bergersen, 2005). Thus, there is little overlap in the feeding ecology and diet of these two goose species during their pre-breeding period in early spring (Fox and Bergersen, 2005). In this experiment, spring goose disturbance treatment was modelled on grubbing by pink-footed geese in order to assess how this goose species affects chemical responsiveness and response strength of vascular plants, mosses, and organic soil in Svalbard across a range of different habitats (see main text for details).

Appendix 2A: Details on sample collection and chemical analyses with NIRS

We collected in total 2244 leaves from 14 vascular plant species (Table S2, Figure S1). The number of species sampled within a plot ranged between a minimum of three and a maximum of nine; on average 5.4 ±1.3 species. The variability in the number of sampled species per plot reflects the variability in the dominant species found in that plot. We collected a total of 857 independent plant samples.

After pressing and oven-drying the leaf samples, we stored them in their original tea-filter bags in a dry and dark place at room temperature. Prior to analyses, leaf samples were oven-dried again at 60 ˚C for 2 h in order to remove eventual traces of water, which can cause distortion in the light absorbance and undermine predictions of leaf chemical contents (Smis et al., 2014). Subsequently, leaf samples were cooled down in a desiccator until scanning by Near Infrared Reflectance Spectroscopy (NIRS) (see main text for details). Moreover, we visually inspected each leaf and removed particles that could cause distortion in the light absorbance (Smis et al., 2014). Narrow leaves, such as the ones characterizing graminoids, were cut and stacked together in order to cover the minimum area needed for analyses (4 mm Ø). NIRS analyses led to a total of 8410 spectra, which were converted to C- and N-content and finally utilized to calculate vascular-plant C, N, and C:N ratio community-weighted means (see main text for details).

References for Supplementary material

Bråthen, K. A. and Hagberg, O. 2004. More efficient estimation of plant biomass. – J. Veg. Sci. 15: 653-660.

Fox, A. D. and Bergersen, E. 2005. Lack of competition between barnacle geese Branta leucopsis and pink-footed geese Anser brachyrhynchus during the pre-breeding period in Svalbard. – J. Avian Biol. 36: 173-178.

Hansen, B. B. et al. 2013. Climate events synchronize the dynamics of a resident vertebrate community in the high Arctic. – Science 339: 313-315.

Ims, R. A. and Fuglei, E. 2005. Trophic interaction cycles in tundra ecosystems and the impact of climate change. – Bioscience 55: 311-322.

Smis, A. et al. 2014. Determination of plant silicon content with near infrared reflectance spectroscopy. – Front. Plant Sci. 5, 496.

Yoccoz, N. G. and Ims, R. A. 1999. Demography of small mammals in cold regions: the importance of environmental variability. – Ecol. Bull. 47: 137-144.

Paper V

Nutrient contents and nutrient pools of high-Arctic plant communities: Opposing effects of herbivory and warming and plant functional type dependent responses