Plant communities and global change: adaptation by changes in present species composition or adaptation in plant traits. A case study in Iceland.

By MICHIELSEN Lieven

Promotor **Prof. Dr. JANSSENS Ivan A.**

Co-promotor **LEBLANS Niki**

Year **2013-2014**

Master thesis submitted to obtain the degree of Master of Science in Biology, specialization Ecology and Environment

PLECO
Plant & Vegation Ecology
University of Antwerp

Universiteit
Antwerpen

Preface

A thesis is written by one person, but cannot be realized by one person only. Therefore, it is not more than appropriate to pay tribute to several people who were indispensable in making this thesis.

Without the expertise and the talent for organization of my promoter Prof. Dr. Ivan Janssens, my thesis had never been possible. With his passion for examining the effects of global change and sharing this passion during his classes, he encouraged me to become very interested in the drivers and impacts of this phenomenon. It was because of his contacts in Iceland that I was able to go there and take part of this international project. Even though, he is a very busy man, he managed to make time for me when I needed it most, to introduce me to very competent and helpful people and to correct my thesis very scrupulously.

My co-promoter, Niki Leblans was always prepared to help with a smile and undying optimism. Even when she was implementing her own research in Iceland, she made time for my questions, suggested new lines of thought and critically corrected multiple "final versions". The things I learned from her will be extremely useful in the future, in science and elsewhere.

I would like to thank the Agricultural University of Iceland for my stay at the university campus during the fieldwork. Special thanks to Prof. Dr. Bjarni Sigurdsson, who was always very and enthusiastic about my contribution to the project. He was also responsible for our pleasant accommodation in the "headmaster's villa". I was pleased to stay there in the company of Niki Leblans, Katherine Vande Velde, Dr. Freja Dreesen, Raffaële Thuys and Pieter Roefs. Thank you guys for the unforgettable birthday!

I am Dr. James Weedon and Joanna Horemans very grateful for helping me with the statistical background and retrieving the maximum out of my data.

I would like to thank my parents for giving me the opportunity to study biology, supporting me in everything I do and always being there for me.

Special thanks to my brother, friends from college and "De Zolder" for listening to the twaddle about my thesis and offering welcome distraction when I needed it most.

Table of contents

Abstract

Climate change is expected to have the largest impact on northern ecosystems at high latitudes. Models predict that temperature increase in these areas will be twofold greater compared to the rest of the world.

The geothermal conditions in southern Iceland offer an exceptional opportunity to study the impact of warming on northern high-latitude ecosystems. Hot springs and fumaroles induce temperature gradients in different valleys. These temperature gradients are excellent natural manipulation experiments that allow studying the response of these northern grassland ecosystems to temperature increases. In one of the two studied valleys, these natural temperature gradients have been present for centuries, but an earthquake in 2008 resulted in new geothermal activity and associated temperature gradients in an adjacent valley. This difference in the warming time frame makes it possible to study differences in short term and long term warming on similar vegetation.

The main research question was whether soil warming would induce a change in plant community structure or would result in adaptations of plant traits. For the latter aim, three target species (*Agrostis capillaris*, *Poa pratensis* and *Ranunculus acris*) were studied.

Our results indicate that soil warming induces a decrease in overall biodiversity and favours plant species preferring a nitrogen-richer environment. Total plant coverage and the plant height for the three target species solely experience a positive temperature effect in the century-long warmed valley. These results indicate that the two strategies to adapt to warming cannot be seen as separate consequences of warming. Moreover, our results suggest different main drivers for adaptation in the two studied grasslands. We speculate these drivers to be temperature stress in the short term warmed grassland and the temperature-induced nitrogen-increase the driver in the century-long warmed grassland. Species adaptation to higher temperatures in the long term could explain this difference.

Samenvatting

Men verwacht dat klimaatverandering het meest uitgesproken zal zijn op de noordelijke hoge breedtegraden. Modellen voorspellen dat de temperatuurstijging in deze gebieden tegen het einde van deze eeuw het dubbele zal zijn in vergelijking met de rest van de wereld.

De omstandigheden in de buurt van Hveragerði, een dorp in het zuiden van IJsland, bieden een uitgelezen kans om de impact van klimaatverandering op noordelijke ecosystemen te bestuderen. Warmwaterbronnen en fumarolen induceren temperatuurgradiënten in verschillende valleien. Deze temperatuurgradiënten zijn uitstekende natuurlijke manipulatie-experimenten die toelaten om de respons van noordelijke graslandecosystemen op temperatuurstijging te bestuderen. In één van de twee bestudeerde valleien zijn al eeuwen zulke gradiënten aanwezig, maar een aardbeving in 2008 veroorzaakte nieuwe geothermische activiteit en bijbehorende temperatuurgradiënten in een naburige vallei. Het verschil in duur van de bodemopwarming tussen de twee valleien maakt het mogelijk om de effecten op zowel korte als op lange termijn te bestuderen.

We onderzochten of bodemopwarming een verandering veroorzaakt in soortensamenstelling of eerder adaptaties teweegbrengt in geselecteerde planteneigenschappen. Voor dit laatste werden drie doelsoorten (*Agrostis capillaris*, *Poa pratensis* en *Ranunculus acris*) bestudeerd.

Onze resultaten tonen aan dat bodemopwarming een afname van de algemene biodiversiteit veroorzaakt en een soortensamenstelling bevoordeelt die een stikstofrijkere omgeving verkiest. Een toename van totale plantenbedekking en planthoogte voor alle drie de doelsoorten wordt enkel aangetoond in de eeuwenlang verwarmde vallei. Deze resultaten geven aan dat de twee strategieën om te reageren op opwarming niet als twee aparte gevolgen van bodemopwarming mogen gezien worden. Bovendien suggereren deze resultaten een andere drijfveer voor adaptaties op korte en op lange termijn. We vermoeden dat temperatuurstress de drijfveer is voor verandering in het recent verwarmde grasland en temperatuur-geïnduceerde stikstoftoename de drijfveer voor adaptaties in het eeuwenlang verwarmde grasland. Adaptatie aan hogere bodemtemperaturen op lange termijn zou dit verschil kunnen verklaren.

Abstract for laymen

Across the world, a general temperature increase has been observed. This increase will cause more extreme weather conditions such as longer periods of drought and stronger hurricanes. This is called "global change". In northern regions warming will be pronounced and this will cause the melting of ice and frozen soils, affecting not only local ecosystems, but also the rest of the world. This makes it interesting to study the effect of increased temperatures in northern regions on the present plant species.

Iceland is a northern island situated on the edge between the continental plates of America and Eurasia. Volcanoes frequently occur on such borders and, therefore, appear all over Iceland. Volcanic activity may also heat water, creating hot springs and steam holes, inducing temperature gradients in the soil. These soil temperature gradients are studied as a prediction of the effect of Global warming on the present vegetation. Two grasslands are examined. The soil of the first grassland has been warmed by hot springs and steam holes for centuries and the second grassland has been warmed since an earthquake in 2008. This makes it possible for us to study whether there is a difference between short-term and long-term soil warming.

Soil warming causes a decrease in the number of species in both grasslands, but only in the century-long warmed grasslands plants are forced to grow taller and plant coverage is increased. These results indicate that vegetation in general is affected by the increase of soil temperature and by the different length of warming. In the grassland, warmed since 2008, plants suffer from the changed temperature. This causes less stress resistant plants to disappear, while species seem to have adapted to higher temperatures in the century-long warmed grassland. There some species grow taller in warmer areas, forcing smaller species to disappear.

1. Introduction

1.1 Climate change in the arctic and subarctic region

The global climate system has been changing since the industrial revolution, mainly due to anthropogenic activities (Vitousek et al., 1997). In the twelve years between 1995 and 2006, eleven were ranked in the twelve warmest years since the beginning of the measurements in 1850 (IPCC, 2007). Global cover of mountain glaciers and snow caps is declining and more extreme weather conditions are but a few indications for global change. Climate models predict an average global temperature increase between 1.4 and 5.8 °C (IPCC, 2007). The impact of climate change is hard to predict and depends on many direct and indirect effects, from the increase of greenhouse gases until changes in nutrient cycles and sea level rise. The largest impact is expected in the northern high latitudes (Figure 1). During the last decades, temperature increases in the northern regions were twice those of the rest of the world (ACIA, 2004). Averaged over all global climate models participating in the IPCC Fifth Assessment report (IPCC, 2013) and the RCP 8.5 trajectory of atmospheric CO_2 concentrations, which assumes continued increases of greenhouse gases or "the business as usual scenario", temperature averages in the northern high latitudes could increase up to more than 8°C towards the end of the century (Figure 1)(Riahi et al., 2011; Meehl et al., 2012; IPCC, 2013).
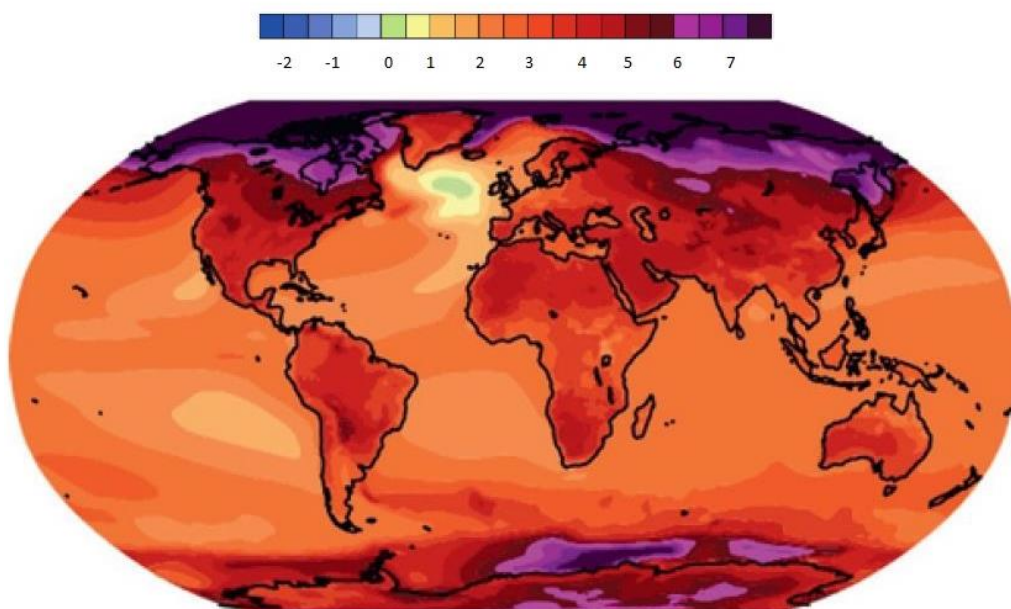
Figure 1: *Surface air temperature differences for the end of the 21st century, calculated in relation to temperature date of the period 1986-2005 according to the RCP 8.5 scenario (source: Meehl et al., 2012).*

A lot of research, using remote sensing and Earth System models, has been done to predict the temperature rises across the globe and especially in the High North. The decrease of the sea ice extent in late summer and autumn reduces the albedo effect in the arctic sea and increases the heat transferred to the seawater and long wave radiation by its surface (Serreze et al., 2009; Screen & Simmonds, 2010). Warmer seawater changes the vertical temperature structure of the atmosphere by heating the air layers close to the surface (Graversen et al., 2008) and increasing the amount of atmospheric water vapour and cloud formation, important factors in the greenhouse effect (Liu et al., 2008). These effects are most likely the cause of the northern warming amplification and are incorporated in climate models (Koenigk et al., 2012).

Changes in the northern high latitudes will affect ecosystems on a global scale. The melt water from sea and land ice will increase the evaporation rate of seawater and decrease its density (Overland & Wang, 2010). Less dense seawater is lighter, more likely to float and less capable of pulling warmer water towards the arctic seas. This northward movement of seawater drives the ocean currents. When this process slows down, it will affect the climates of all the regions depending on these currents. More interesting for this thesis is the warming of the permafrost layer and the soil in general. The thawed organic matter in the permafrost layer will start to decompose, releasing nutrients and increasing the amount of greenhouse gases in the atmosphere, which both initiate a cascade of secondary ecosystem responses (ACIA, 2004; Kaplan & New, 2006). In order to obtain more grip on the global scale, it is important to understand the impact of climate change on a more local scale.

1.2 Warming experiments in northern high latitudes ecosystems

Since the awareness of climate change has grown, numerous papers have been published on the effects of temperature increase on a wide variety of taxonomic groups from across the globe (Parmesan, 2006). This thesis focuses on the impact of climate change on grasslands on northern high latitudes. Experiments on the impact of temperature on nitrogen availability and decomposition rates of soil organic carbon, both limited in environments with colder temperatures, are especially interesting in northern environments (Giblin & Nadelhoffer, 1991; Mack et al., 2004; Sistla et al., 2012). Nitrogen is an important building brick for proteins and consequently for essential enzymes, which makes that limitation of this element has a non-negligible effect on the plant metabolism (Aerts & Chapin, 2000). The availability of nitrogen in the soil determines their productivity as well as which plant species and communities can thrive on a certain location (Ordoñez et al., 2009). If plant

productivity alters by decreasing the present nitrogen limitation, this could have crucial effects on the global carbon balance.

Temperature increase is expected to increase the decomposition rate by microbes, increasing the C release in the atmosphere through respiration (Jenkinson et al., 1991; Davidson & Janssens, 2006; Hartley et al., 2007; McGuire & Anderson, 2009; Sistla et al., 2012). The hypothesis that this increase in C release can be compensated by a greater plant productivity, ameliorating the C storage capacity, has been discussed intensively but is considered less likely (Hyvönen et al., 2007). However, models using the international goal of 2°C of warming, predict a forest expansion of 55% or a northward shift of the tree line of 400km, at the expense of tundra vegetation. This would increase the C storage, though it is unlikely that this effect is direct and can outweigh the additional C release from the soil (Kaplan & New, 2006).

Climate change experiments on tundra vegetation noticed an increased nitrogen availability and nutrient accumulation, suggesting this might be an important indirect effect of warming (Chapin et al., 1995). Increased availability of nitrogen would lead to a shift in nutrient limitation to more light-limiting conditions, favouring more competitive species at the expense of more rare understory or stress-tolerant species (Young et al., 2011).

Another experiment was done in a boreal forest in southern Norway. In this case the soil was heated using a raster of electric heating cables in underneath the litter layer. This setup gave rise to one heated temperature step varying between +3°C and +5°C without warming the overlaying air layer (Lükewille & Wright, 1997). From this experiment it appeared that the soil warming led to higher concentrations of both NO_3 and NH_4 in the runoff. This confirms the increased availability of nitrogen, but the plants are unable to fix the larger part of this extra available nitrogen components, creating an outflow of nitrogen (also McHale et al., 1996). Other soil warming experiments focusing on individual trees, noticed a direct effect of warming on physiological processes and plant traits like stomatal conductance, growth rate and nutrient translocation (Dang & Cheng, 2004).

Research on temperature effects in plant communities (Chapin et al., 1995) incorporate only one or two additional temperature treatments besides a control (Bergh & Linder, 1999; Hartley et al., 2007; Day et al., 2008; Lavoie et al., 2011), yielding no information on the shape of the temperature response function. Even the method used to achieve warming can influence the outcome (Aerts, 2006). Some experiments using transparent, open-top chambers, warming the surface air temperatures, even reported a negative response of the soil temperature attributed to an increase in leaf area index and a subsequent decrease of solar radiation reaching the soil surface (Jonsdottir et al., 2005). These warming experiments varied in length from short term to longer term, but are rarely

longer than a decade (see Dieleman et al. 2012 for a review). Short-term impacts of warming are not fool proof in predicting long-term effects (Hobbie et al., 2002).

Many of these experiments pointed out that some temperature effects, like changes in litter quality and in species dominance relations, appear gradually, stressing the importance of long term field experiments (Chapin et al., 1995; Arft et al., 1999). Aside from the passive warming technique that uses screens to block nocturnal infrared emissions and typically achieve a warming of less than 1°C, temperature manipulation experiments are extremely expensive, explaining why warming experiments are typically short-term. Erroneous conclusions arising from analysing transient responses in short-term experiments differences can be overcome by studying ecosystems from locations with different temperatures across spatial climate gradients (latitudinal or altitudinal gradients). These analyses do incorporate the long term effect, but have the disadvantage that there is hardly standardization of species, soil composition, stress factors and many other environmental variables (Aerts, 2006). Spatial gradients are therefore not perfect either.

1.3 Natural small-scale temperature gradients in Iceland as predictors for temperature impact

Soil conditions in the neighbourhood of the small town of Hveragerði, situated in southern Iceland, offer a third possibility of studying the impact of the predicted temperature raise, countering the disadvantages of the previous two methods. Iceland is an island formed due to its position on the Mid-Atlantic ridge, the divergence zone between the American and Eurasian continental plates. Magma from the asthenosphere emerges along this ridge, pushing the two continental plates apart and creating an elongated mountain range on the ocean floor. Iceland is one of the submerging parts of this range, explaining the large geothermal activity on the island (Halldórsson & Sigbjörnsson, 2009). The area around Hveragerði is extremely geothermal active, because it is situated in the direct surroundings of the Hengill volcano system (Zakharova & Spichak, 2012), illustrated by the over 100,000 micro-earthquakes reported in the region between 1994 and 2007 (Jakobsdóttir, 2008). The geothermal activity manifests itself as hot springs and fumaroles (Tobler et al., 2008). These phenomena act as hot spots that warm the surrounding soil layers, creating a temporally constant, yet spatially decreasing soil temperature gradient when moving further away. As a result, these rather rare thermal features offer the opportunity to study the occurring ecosystems as a "natural" warming experiment with as many different temperature levels as required.

In this thesis two grassland ecosystems with such geothermal temperature gradients are compared. The first grassland is situated in a parallel valley of the valley of Reykjadalur or 'steam valley', the major tourist attraction of Hveragerði. Both valleys are known to have housed fumaroles and hot rivers for centuries. The first written reference to this valley comes from the Icelandic farm register dating back from 1708 (Magnússon, 1708); more precise land registers are available from the 1980ties and these confirm the current location of the hot spots. If one assumes that the current thermal sites were constant during the last century or at least during the last decades, the grasslands in this valley can be considered a case study for the effects of long time exposure to increased temperatures, because most soil processes as well as grassland plant species communities can be considered to be near equilibrium after many centuries of warming. One remark has to be made, up to the year before we conducted our study, this valley was extensively grazed by sheep, which can have profound effects in shaping the plant community structure in grassland ecosystems (Brooker & van der Wal, 2003; van der Wal et al., 2004; Olofsson, 2006; Evju & Austrheim, 2009).

The second grassland ecosystem that we studied is located in the valley adjacent to the "steam valley" and is even more peculiar. In May 2008, a shallow crustal earthquake occurred with its epicentres between the towns of Selfoss and Hveragerði and a strength of 6.3 on the Richter magnitude scale. The earthquake did not cause severe structural damage, but changed the course of underwater systems. New fumaroles appeared and the general thermal activity increased around the university campus of Hveragerði (Sigbjörnsson et al., 2008; Halldórsson & Sigbjörnsson, 2009). As in the adjacent valley that was probably warmed for centuries, new soil temperature gradients arose on the slopes around the campus. This sequence of events provides the perfect opportunity to consider these sites as short term (5-6 years), natural heating experiments. The grassland ecosystem that has been warmed since at least 1708, is from now on referred to as "Grassland Old" (GO) and the grasslands in the newly formed temperature gradients described here are called "Grassland New" (GN). To avoid confusion, the assumption is that both grassland ecosystems have the same age, only the length of geothermal heating differs greatly.

The temperature gradients in this part of Iceland have a lot of advantages in comparison with human induced warming experiments. Numerous temperature steps or treatments are possible between a control plot and a chosen maximal temperature, the warming costs are zero, no problems exist with standardizing the soil characteristics, plant species and environmental conditions, to name a few. However, one must bear in mind that these study sites are not a means to simulate the impact of climate change as a whole. This is because global warming is associated with an increase in atmospheric CO_2 concentrations, which also affect ecosystems directly and interacts strongly with

warming (Dieleman et al. 2012). Moreover, the ICCP-models as well as weather observations suggest that greenhouse gases are not only heating the atmosphere, but also increase the prevalence of extreme events. Nonetheless, the natural temperature gradients in Iceland offer an excellent opportunity to study the impact of temperature on many ecosystem processes and –states and to discover important knowledge to be implemented in Earth System models.

Two perspectives regarding the impact of soil warming and an expected change in nitrogen availability on the present grassland vegetation, are studied in this thesis. This impact can be translated into an alteration in species composition or biodiversity or can manifest itself by driving the present species in modifying their plant traits.

A change in temperature can change the plant species composition directly by favouring species with higher temperature optima, but also indirectly by its positive effect on nutrient cycling, yielding an advantage for competitive species at the expense of more stress-tolerant species (Chapin et al., 1995; Young et al., 2011; Soudzilovskaia et al., 2013). Increasing nutrient concentrations therefore usually lead to lower diversity (Barot & Gignoux, 2004). This change in plant species diversity is the starting point for the first perspective. Estimating the total cover of all present species allows to examine the shift in dominance of one species to another (Jost, 2006). From this data the biodiversity can be evaluated for each vegetation plot. Used parameters are the Shannon-Wiener index and its two determining factors, the absolute diversity and the evenness (Jost, 2006; Heip et al., 1998).

Ellenberg indicator values are a useful tool to estimate the ecological preferences of species within the temperature gradient (Hill et al., 1999). These indicator values are estimated as the mean of a given variable across the ecosystem where the species grows best. Highest growth values can be considered at the optimal environmental conditions for a species (Violle et al., 2007). A shift in species composition is generally the result of a change in the present environmental conditions. Therefore, the community-weighted Ellenberg values make it possible to assign this change fairly accurately, to the appropriate environmental parameter. In this study, we used Ellenberg values for four different environmental conditions; temperature, moisture, acidity and soil nitrogen availability. A change in the community-weighted Ellenberg value of a certain plot can be caused by introduction and disappearance of species as well as by shifts in the abundance of present species.

In addition to the effect of the environment on species, species in turn can also affect ecosystem properties as a reaction to temperature change (Chapin, 2003). A typical example is the relation between nutrient cycling and litter composition. Plants adapted to stress or nutrient-limited

conditions strengthen these conditions by producing nutrient-poor and recalcitrant litter. The reverse is true for plant species adapted to nutrient-rich soils (Díaz et al., 2004; Ordoñez et al., 2009). These feedbacks towards the environment are determined by the characteristics of the occurring species, which introduces the second perspective: assessing the impact of warming on the ecosystem functionality by measuring well-chosen plant traits (Chapin, 2003). A "trait" is in its most simple definition a surrogate for the condition of an organism (Darwin, 1859).

The usage of traits in plant ecology evolved towards evaluating the functionality but also the community structure of ecosystems (Lavorel & Garnier, 2002; Eviner & Chapin, 2003). Violle and colleagues (2007) therefore defined a plant trait as "any morphological, physiological or phenological feature measurable at the individual level, from the cell to the whole-organism level, without reference to the environment or any other level of organization." A plant trait can be a surrogate for a function or the function itself, like photosynthesis. In this way, a plant trait has an indirect effect on the fitness of an individual by its effect on biomass accumulation, survival and reproduction (Violle et al., 2007). Four plant traits were selected to examine the impact of soil warming on the target species; plant height, leaf stoichiometry, specific leaf area and stomatal conductance. We compared the variation of these traits in three target species that occurred in all study plots. The selected plants included two grass species (*Agrostis capillaris* L. and *Poa pratensis L.*) and one herbaceous dicot (*Ranunculus acris* L.). These three common grassland species provided a good representation of the occurring vegetation and its feedbacks with major ecosystem processes like nutrient cycling (Grime, 1998; Díaz et al., 2004).

The first studied plant trait, plant height can be directly measured in the field and its relative appearance for a certain species is seen as a strong indication for the applied growth strategy and environmental conditions (Westoby, 1998). Temperature experiments have demonstrated that there is a plasticity in growth response with temperature. Increased soil temperature brings along an increased mineralization of soil organic matter, which results in an increase of nutrients available for plants. Nutrients become less important as a limiting factor for plant growth and as a result, plants in mesic environments such as Iceland, are expected to start competing for light, making individual plants grow taller to compete against their neighbours (Arft et al., 1999; Aerts & Chapin, 2000; Mokany et al., 2006).

Leaf stoichiometry or the concentration and ratios of different chemical elements are highly informative regarding present limitations of these elements in the soil and therefore studied as second plant trait. The leaf N:P ratio is the most widely used proxy for nitrogen or phosphorus

limitation in the soil (Koerselman & Meuleman, 1996). It is a general accepted rule of thumb that N:P ratios lower than 14 indicate nitrogen limitation and ratios above 16 suggest a phosphorus limitation (Aerts & Chapin, 2000). On a global scale, the N:P ratio increases from the poles towards the equator, which is associated with younger soils (more P and less N in young minerals), warmer temperatures (more N_2-fixation in warmer climates), and lower atmospheric N deposition at higher latitudes (Reich & Oleksyn, 2004; Galloway et al., 2004; He et al., 2008). Northern ecosystems are characterized by stress-tolerant species, adapted to nutrient-poor conditions and having low leaf nitrogen and phosphorus concentrations (Grime, 1997; Reich et al., 1998; Reich et al., 2007). Assessing the impact of recent and long term temperature gradients in the soil on the leaf stoichiometry of species, could give greater insight in nutrient availability and acquisition (Güsewell, 2004).

The leaf chemical elements analysed in the context of this thesis are the biologically most relevant ones: carbon (C), nitrogen (N), calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), phosphor (P), sulphur (S), iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn). Besides N and P, K has a big influence on plant physiology and appearance when this element is limited. High K^+ concentration in the cytosol of plant cells prevents an excessive uptake of Na^+. Na^+ is toxic for cytosolic enzymes when entering the cytosol in too high concentrations and therefore internal K^+ is essential for maintaining cellular metabolism (Zhu, 2003). Previous research, however, did not find a significant predictive strength of leaf K concentration for other plant traits (Wright et al., 2004). Plants limit excessive uptake of N and P which makes their leaf concentrations only useful in determining whether or not they are limiting. Uptake of Ca, Mg and K is far less down regulated, uptake of Ca is for most species even directly related to its concentration in the external environment (Knecht & Göransson, 2004).

The Specific Leaf Area (SLA) is the third plant trait examined in this thesis. SLA is the ratio between the leaf area and its mass. Thicker leaves with a higher storage of carbohydrates lead to smaller SLA values and mostly indicate a bigger investment against herbivores (Cornelissen et al., 2003). The amount of energy and nutrients invested in a leaf is proportional to the leaf mass and the amount of light captured is correlated with the leaf area. These two relations make SLA a useful index for the potential return upon investment (Cunningham, 1999), which makes SLA a good benchmark in predicting other plant traits and deducing plant strategies (Díaz et al., 2004; Reich et al., 2007). In general there is a declining trend in SLA with decreasing nutrient availability, precipitation and light availability (Cunningham, 1999; Ordoñez et al., 2009; Hodgson et al., 2011). In other words, plants seem to invest more units dry weight per leaf area when under stress condition. These leaves have a higher cost than high SLA-leaves and therefore need to remain functional during a longer time interval (Reich et al., 1997). In an earlier warming experiment in pine trees, SLA was found to vary with temperature, with the highest SLA at the species' optimal temperature (Dang & Cheng, 2004).

Both SLA and N concentration in the leaf influence photosynthesis. Photosynthetic activity is improved when one of these two factors increases and the other factor remains constant or improves as well (Wright et al., 2004). Enzymes like Rubisco, essential for the conversion of light into energy, need N as one of their building stones. Therefore, a higher concentration of N is typically indicative for a higher concentration of photosynthetic proteins and higher photosynthesis rates (Evans, 1989). However, also structural proteins include N. Their function is to strengthen the leaf, resulting in thicker leaves (lower SLA) and reducing the photosynthetic efficiency by hampering CO_2 influx towards internal cells (Parkhurst, 1994, Epron et al. 1995). The combination of both plant traits results in a good prediction of the maximal photosynthetic capacity.

As the main controllable resistance between the internal environment of the leaf and the surrounding atmosphere, stomatal conductance is a measure for CO_2 uptake of and loss of H_2O by diffusion. This makes stomatal conductance a way of examining the plant's water status and metabolism, giving a greater insight in its adaptation to altered environmental conditions, such as increased temperature (Parkhurst, 1994; Way & Oren, 2010) and therefore, measured as a last plant trait in this thesis. An increase in nutrient availability will increase the plant's metabolism, resulting in a higher demand for CO_2. This would induce an increase in stomatal conductance. However, when the temperature increase induces drought stress, plants will lower their stomatal conductance to prevent excessive water loss, countering the positive effect of increased nutrient availability on photosynthesis and plant growth (Dang & Cheng, 2004; Sellers et al., 1997).

1.4 Hypotheses

The basic assumption in this thesis is that soil warming will have a positive effect on soil nutrient cycling, thereby increasing the availability of N for the plants. N is the most growth-limiting element in northern ecosystems and is therefore expected to have a strong influence on the present vegetation. The question remains if this impact will express itself as a change in the community structure or will lead to adaptations in the present species. Based on the known processes and impacts of temperature, the following hypotheses were formulated:

1. Total plant cover will increase and lead to a shift from N-limitation towards light limitation. Species will increase their aboveground biomass to make optimal use of the available space and to outcompete their neighbours.

2. Increases in soil fertility regularly go hand in hand with lower biodiversity through competitive species that outcompete small and more stress-tolerant species. We therefore hypothesize a loss of species in the warmer temperature plots.

3. Rising temperature brings along species preferring relatively warmer environments. This shift in species takes time and therefore, we hypothesize that this community composition shift will be more pronounced in GO than in GN. Because of the abundant rainfall in the region, moisture preference is not expected to change among the present species. Increased N-availability in turn would lead to species preferring a more fertile area.

4. Increasing competition for light will force the plants to grow taller to compete with their neighbours. Since temperature indirectly controls this competition, plant height is expected to increase along the temperature gradient.

5. Leaf N and P concentrations reflect their availably in the soil, and we therefore hypothesize a positive trend with increasing temperature. The N:P ratio is hypothesized to increase because N becomes less limiting.

6. The SLA is expected to be maximal at the optimal temperature for the considered species as a consequence of the increased availability of nutrients and subsequent increase of leaf area.

7. Despite the higher evapotranspiration at higher soil temperature, we assume that the stomatal conductance will not be altered by water depletion, because the ample rainfall prevents soil drying. Therefore, we hypothesize an increase of stomatal through a higher demand of CO_2, resulting from the higher nutrient availability and increased plant metabolism.

2. Material and Methods

2.1 Description of study area

2.1.1 Situation

Iceland is a volcanic island situated on the conjuncture of the Atlantic and Arctic ocean close to the Arctic Circle between the latitudes 62°23'N and 66°32'N and longitudes 13°30'W and 24°32'W. Because of its high latitude, the solar angle is never high and there is a large difference in day length between summer and winter. The climate of South West Iceland is maritime with cool summers and mild winters influenced by the cold East Greenland Current and the warm North Atlantic Current (Einarsson, 1984). The island itself is a dome uplift of the Mid-Atlantic ridge with mountain peaks of up to more than 2000 m. Glaciers cover about 11.5% of Iceland's total area (Zakharova & Spichak, 2012). Its position on the Mid-Atlantic ridge makes it a very active volcanic area which played a major role in shaping the island together with the erosive power of the past and present glacial cover (Óskarsson et al., 2012).

2.1.2 Study sites

The two grasslands studied in this thesis are situated in the South West part of Iceland, near the town of Hveragerði. The mean annual temperature is 4.1°C, with a mean of -1.1°C in the coldest month (January) and 10.8°C in the warmest (July), and an annual precipitation of 1372mm (Icelandic Met Office, 2014). The soil type is a brown andosol with a volcanic origin. This is a freely drained soil type, rich in allophane clay minerals and ferrihydrates, evolved from eolian and tephra materials originating from neighbouring active volcanos (Arnalds, 1999; Arnalds, 2004). The geothermal activity occurring in this area, mainly in the form of hot springs and fumaroles, originates from the Hengill volcanic system. This system is situated on the intersect of three volcanic zones, the Hengill system, the Hrómundartindur system and the Hveragerdi system (Geptner et al., 2006; Zakharova & Spichak, 2012).

The GO ecosystem is situated in a valley several kilometres north of Hveragerði, known for its geothermal activity for centuries (Magnússon, 1708). The transects in this area will be studied as a proxy for long term effects of soil warming. The other study site, GN, has only been warmed since an earthquake in May 2008 modified the underground hot water systems (Halldórsson & Sigbjörnsson, 2009). The transects of this grassland are situated near the university campus of Hveragerði and will be examined as an indicator for short term effects of soil warming on vegetation.

In general, each transect consists out of 5 soil temperature levels, indicated by its deviation of the normal soil temperature. The temperature levels were as follows: 1 control plot with a normal soil temperature and 4 plots with averages of 1°C, 3°C, 5°C and 10°C above the normal average soil temperature. With a mean average soil temperature of 5°C, the warmest plots are more or less three times higher than the normal temperature. According to the RCP 8.5 estimates, a warming of 10°C is not exaggerated and ecologically relevant to be studied (IPCC, 2013).

2.1.3 Defining plots

Before the onset of this study, it had been decided to establish five replicate transects along the temperature gradients that occurred in each of the two studied systems and therefore were evenly distributed amongst vegetation that has been growing on heated soil for a long time and vegetation with a short exposure to geothermal heating. Each temperature gradient replicate consisted out of five different soil temperature levels.

To determine the location of the five transects, the soil temperature was measured throughout the two study areas to make a soil temperature maps. A series of variables was taken into account to avoid bias of the results through other factors than the difference in temperature. Because this project takes place in a hilly setting, we tried to keep the orientation of the slopes as similar as possible for all transects. That way, sunshine should be evenly distributed over the vegetation in all the plots. The same applies to soil moisture. Another important condition was that the three target species for the measurements of the plant traits (the most common species across all the plots: *Agrostis capillaris*, *Poa pratensis* and *Ranunculus acris*), could be found in the transect plots or in their direct vicinity. Moreover, the selected study areas had to be undisturbed and free from erosion for a long time, so that the vegetation was not limited in its growth by physical constraints. Since extensive grazing with sheep occurs in the old grassland study site, it meant that the transects in this study area needed to be fenced to avoid influence of grazing or trampling, no matter how small.

The temperature levels were represented by vegetation plots of 2x2m in which the average temperature corresponds with one of the five temperature levels that were discussed above. In these plots non-destructive measurements and repetitive observations were done, such as plant height and stomatal conductance. Close to these large plots, smaller plots of 0.2 x 0.5 m were indicated for destructive measurements such as harvesting of biomass and sampling for the leaf stoichiometry of the three target species (Appendix 1). These subplots are assumed to be a good representation of the large plots with the same characteristics. An overview of the measurements can be found in Table 1.

Table 1: *An overview of the measurements and on which part of the plots, they were conducted, whether the measurements were done on the main plots (2x2m) or subplots (0.2x0.5m) and the number of replicates. Remarks: (1) All temperature levels have 5 replicates (or plots) in both grassland ecosystems. If there was more than 1 measurement within one plot, this number is put between brackets. (2) These measurements are discussed in detail in the thesis of Katherine Vande Velde. (3) Due to technical restrictions the stomatal conductivity of Poa pratensis was not measured in all the temperature plots.*

Measurement	Main plot	Subplot	Plot level measurement	Species level measurement	n[1]
Vegetation coverage and diversity		X	X		5
Biomass[2]		X	X		5
Stoichiometry		X		X	5
Soil samples[2]		X	X		5(2)
Specific Leaf Area	X			X	5(3)
Plant height	X			X	5(5)
Stomatal conductivity[3]	X			X	5

2.2 Sampling period

Samples were taken mid-July, close to the moment of maximum standing biomass, according to NDVI (Normalized Difference Vegetation Index) measurements. It was ensured that all measurements alternated between the GN-transects and the GO-transects as much as the circumstances allowed it. Simultaneously, the time lapse between the sampling of the first transect and the last was kept as short as possible to avoid too much influence of the different timing in the growing season of the data collection.

2.3 Vegetation coverage and species diversity

A frame of 0.2x0.5 m was used to define the borders of the vegetation inside the plots in which the vegetation and species diversity was determined (Table 1). Therefore, all the species present within this frame were determined and their total coverage was estimated. Six vegetation categories were recognized: (1) monocots, (2) dicots, (3) ferns and equiseta, (4) mosses, (5) lichen, (6) litter. The first three categories were determined up to species level. Due to different vegetation layers, the sum of the species specific coverage percentages can differ from 100%.

The data for the total cover were used to calculate the Shannon-Wiener diversity index (H) and the evenness (E) of each individual plot using equation 1 and 2 (Heip et al., 1998; Jost, 2006).

Shannon-Wiener index: $H = -\sum_{i=1}^{s} (p_i \ln(pi))$ (eq. 1)

Evenness index: $E = \dfrac{H}{-\ln(1/S)}$ (eq. 2)

With pi the coverage proportion of a species i and S the total number of species in a plot.

Besides these indices, the Ellenberg indicator values of the occurring species for thermal optimum, nitrogen content of the soil, moisture and acidity preference were looked up in databases. Thereafter the weighted averages of the four indicator values (Í) were calculated for each plot taking into account the coverage of the concerning species (Hill et al., 1999; Ökologische Zeigerwerte). An example is given by equation 3.

$$\text{Í}_{temperature} = \frac{\sum_{i=1}^{s} \text{Í}_{temperature\ i} \times cover_i}{s}$$ (eq.3)

2.4 Plant traits

2.4.1 Plant height

For each of the three target species, five individual plants were chosen in each main plot (n=5x5 per treatment per area) from which the height was measured with a folding ruler (Table 1). The height of *Ranunculus acris* individuals was measured until the flower, if absent until the highest leaf. The height of the grass species *Poa pratensis* and *Agrostis capillaris* was obtained by measuring until its maximum height, i.e. a leaf tip or, if present, the flower tip.

2.4.2 Stoichiometry

As stated before, the presence of *Agrostis capillaris*, *Poa pratensis* and *Ranunculus acris* was one of the conditions to create a vegetation plot on a certain location. These three target species were used to analyse their chemical composition. Besides the leaf element concentration, there was looked at the element:N ratio, also referred to as leaf stoichiometry. The N:P ratio is an important ratio in the ecology through being a good proxy for N or P limitation for a studied species. So it is worth to focus in particular on this ratio.

After the determination of the surface cover and total cover, leaves of each of the three target species were harvested in the 0.2x0.5 m subplot so as to obtain 2 g dry weight (Table 1). Adult, non-discoloured leaves were selected to prevent divergent results due to other factors than soil temperature. In cases that the subplot did not contain sufficient leave biomass for the analysis, the harvest was completed with additional leaves from the direct surroundings of the plot, so as to prevent too much deviation from the aimed soil temperature.

The samples were dried at a standard temperature of 50°C during 48 hours.

The total C and N were analysed by using dry combustion with an NC2100 C/N analyser (Carlo Erba Instruments, Italy). The remaining elements (Ca, Mg, K, Na, P, S, Fe, Mn, Cu en Zn) were analysed using a different method. The dry matter of the samples was weighed before and after they were dried for a couple of hours at 105°C. Of each sample, 0.1 g was dissolved in boiling nitric acid for 20 hours. Subsequently the samples were diluted with water and measured in Inductively Coupled Plasma (ICP) equipment.

2.4.3 Specific Leaf Area

Leafs of the three target species were collected for SLA determination (Table 1). Three leafs of each species were gathered in each main plot (n = 3x5 per treatment per studied system). In the field, each individual leaf was placed on a white sheet of paper with a reference black square of 3x3 cm. A picture was taken of the leaf together with the reference square. Afterwards the leafs were dried for 48 hours on a temperature of 40°C and weighed to obtain the dry weight.

The computer program Adobe Photoshop CS6 was used to acquire the amount of pixels of both the reference square and the leaf. With the known surface of the reference square, the leaf surface was obtained using equation 5:

$$Area_{leaf} = (\#pixels_{leaf} / \#pixels_{reference}) \times Area_{reference} \qquad (eq. 5)$$

The specific leaf area was obtained by dividing the calculated leaf area by its corresponding leaf dry weight.

$$SLA = Area_{leaf} / Mass_{leaf} \qquad (eq. 6)$$

2.4.4 Stomatal conductivity

The stomatal conductivity was measured on the three target species. These measurements were done in the main plots or in their direct vicinity if the target species did not occur inside the plot (Table 1). The measurements were performed on two separate days, under the right weather conditions (dry and clear sky).

The stomatal conductance was measured using an AP4 Leaf Porometer. In each plot one healthy leaf of each target species was measured (n = 5 per treatment per area). There was made sure that the leaf filled the complete sensor chamber in order to obtain correct values. Large veins were avoided, in particular concerning *Ranunculus acris*. For each measurement, the stomatal conductivity was noted as well as the temperature in the sensor chamber, the difference with the air temperature and the relative humidity.

2.5 Statistical analyses

All statistical analyses were started from the complete statistical model containing the response variable (or variables when dealing with multivariate analyses), grassland type, soil temperature elevation and species (for the plant traits) as fixed explanatory variables. The five transects in both grasslands were assumed to be replicates and therefore not incorporated as (random) explanatory variables. Grassland type is a discrete variable, but added soil temperature can be considered both as a continuous and as a discrete variable. In most studies, temperature can be regarded as a continuous variable whereby the relative magnitude of the values is important. As an illustration; 3°C of added temperature in comparison with the control, represents three times more warming than 1°C of added temperature. However due to the characteristics of the permanent field setup for this thesis, temperature can also be incorporated as a discrete variable. The plots represent a certain temperature level, without random intermediate values (Appendix1). Consequently, the five temperature levels can also be seen as five different treatments. Instead of choosing one of the two manners of looking at the temperature, there was decided to use both with a proper argumentation.

For the statistical analysis of the leaf stoichiometry, the concentration data for the different chemical elements was standardized and normalized to be able to compare them.

$$sX = X / (max_{element}) \hspace{5cm} (eq. 4)$$

Using equation 4 results in standardized values 'sX' for a certain element by dividing the original value by the highest value for this element. The element:N ratios were standardized using the same procedure.

Most response variables were tested with temperature level as a continuous causal variable, which results in the use of ANCOVAs. Soil temperature elevation was regarded as continuous variable because most plotted data showed a linear trend with rising temperature. In case the dataset showed little or no trend, the analysis was redone regarding soil temperature elevation as discrete variable, which allows to detect any difference between any set of two or more temperature levels. Normal distribution of the datasets was tested with the Shapiro-Wilk test ($W>0.9$). If this condition was met, a two-way ANCOVA or ANOVA was performed. When a two-way interaction appeared to be significant, the dataset was split and considered as different datasets for the rest of the analysis. Therefore the two study areas or the target species were analysed separately in some cases. If the interactions were not statistically significant, the model could be simplified by removing explanatory variables until only those remained that explained significant differences for the analysed response variable. This is called model reduction. Afterwards pairwise comparisons were done when

applicable, by using the Tukey test for the simplified ANOVA models. Data that were not normally distributed, were analysed using the non-parametric Spearman's rank correlation test for the correlation between soil temperature elevation and the corresponding factor within the individual study areas (Hollander & Wolfe, 1973).

For the analyses with more than one response variable, multivariate tests were applied. This was the case for total coverage of individual species, a part of the analysis for the Ellenberg's indicator values and leaf stoichiometry. For these three datasets it was appropriate to do the statistical analysis with two different methods. A standard multivariate analysis (MANOVA) could be applied for the Ellenberg's indicator values dataset and the stoichiometry dataset. The total coverage of the individual species per plot however, requires a different analysis. This dataset consists of percentages, many of which were zero values by reason of species not being present in certain plots. This results in a high chance of a non-normally distributed dataset. A permutational MANOVA using Bray-Curtis measure is best suited to gain insight in the this kind of dataset, and consequently is able to describe ecological differences between plant communities (McArdle & Anderson, 2001).

Further, three different methods for graphical representation were used to visualize the multivariate analyses, according to the type of data. For each test, there was chosen for the most relevant and revealing method. The data for the total coverage of the different species required Non-metric Multidimensional scaling (NMDS), an unconstrained correlation analysis. This method is commonly regarded as the most robust unconstrained ordination method in community ecology and shows best all the present variance along the newly created NMDS-axes.

A constrained correspondence analysis (CCA) was performed for the Ellenberg's indicator values, which makes it possible to include "soil temperature elevation" and "grassland" as constrained parameters for the explanation of variance. Consequently, CCA does not explain all the variation in the data but only the variation explained by the constraints, which are in this case, the most interesting parameters.

At last a Principal Component Analysis (PCA) was applied on the data for leaf stoichiometry. This analysis makes it possible to study the relevance of the different chemical elements and their mutual relation by their position towards the principal components and each other.

R version 3.0.2 was used as statistical processing software together with Rstudio version 0.98.501.

3. Results

This section gives a clear overview of the data obtained from the field and the statistical analysis performed on these data. It is important to notice that the results can be subdivided in two major themes according to the major research question, '*does warming of the soil causes a change in species composition or adaptations of present species?*' The first section shows the tests on possible effects of soil heating on parameters for species composition and community structure. The second part shows the outcomes for the effect of the soil temperature levels on the selected plant traits of the three selected species. According to the statistical relevance, some parts will be displayed in more detail than others (Appendix 3).

3.1 Effects of soil warming on species composition and community structure

An overview of all the significant effects of temperature on species composition and community structure can be found in Appendix 3 (Table 3A(1)).

3.1.1 Effect of soil warming on vascular plant species total coverage

The average total cover per vascular plant species is illustrated by two bar charts, one for both study systems (Appendix 2). This graphs also give an overview of which species can be found in the two grassland types and their average contribution to the total plant coverage. In general 13 species were found in the GN-plots and 19 in the GO-plots.

Looking at the average total coverage of vascular plats per temperature level there was a distinct difference in response between the old grassland and the new grassland (p<0.001). Increasing soil warming had a positive effect on the plant total coverage in GO which results in a positive trend, but no trend could be found in GN(Figure 2).
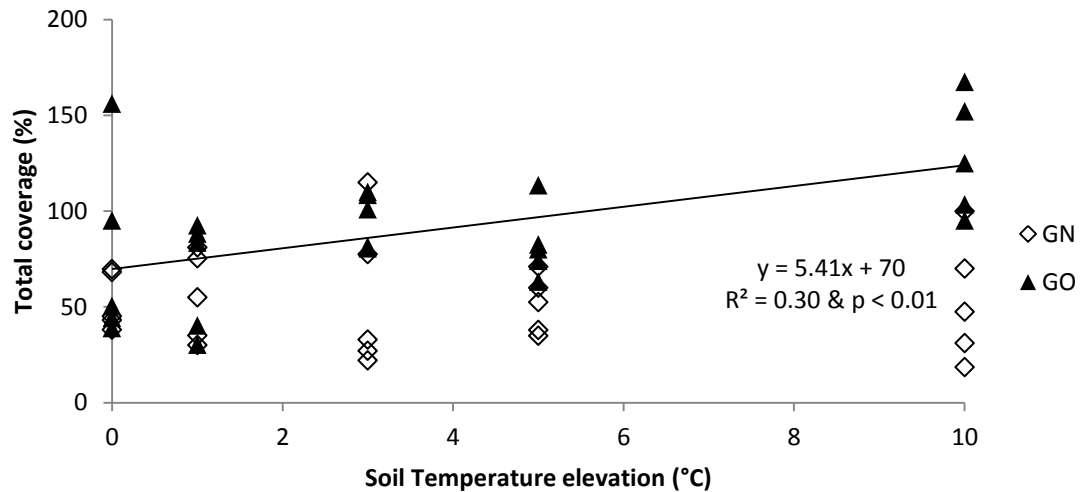
Figure 2: *Scatter plot of soil temperature elevation as continuous variable and total coverage of vascular species. GO and GN differ significantly (p<0.001). The trend line for GO is significant (p<0.01), total coverage in GN did not show a trend (p=0.9).*

The total coverage of individual species per temperature level allowed to investigate differences in community structure. There was a highly significant difference for grassland type (p<0.001) and also a significant effect of soil temperature (p<0.05) in both grasslands. There was no difference in soil temperature effect between GN and GO (p=0.2).

Figure 3A illustrates the significant difference between the two grassland types (p<0.001), illustrated by the two polygons. The position of the species represents their relative importance to certain plots and therefore also to GN or GO. *Festuca vivipara*, *Agrostis capillaris* and *Poa pratensis* are associated with both grasslands, though *Viola palustris* and the Gallium species for example are more associated with the old grassland and *Ranunculus acris* and *Equisetum pratense* with the new grassland*.* Figure 3B is generated with the same statistical model from the same dataset to show the effect of added soil temperature on the vascular plant species coverage. A slight shift to the right with increasing soil temperature can be noticed. The warmest and coldest plots of both grasslands are the furthest apart.
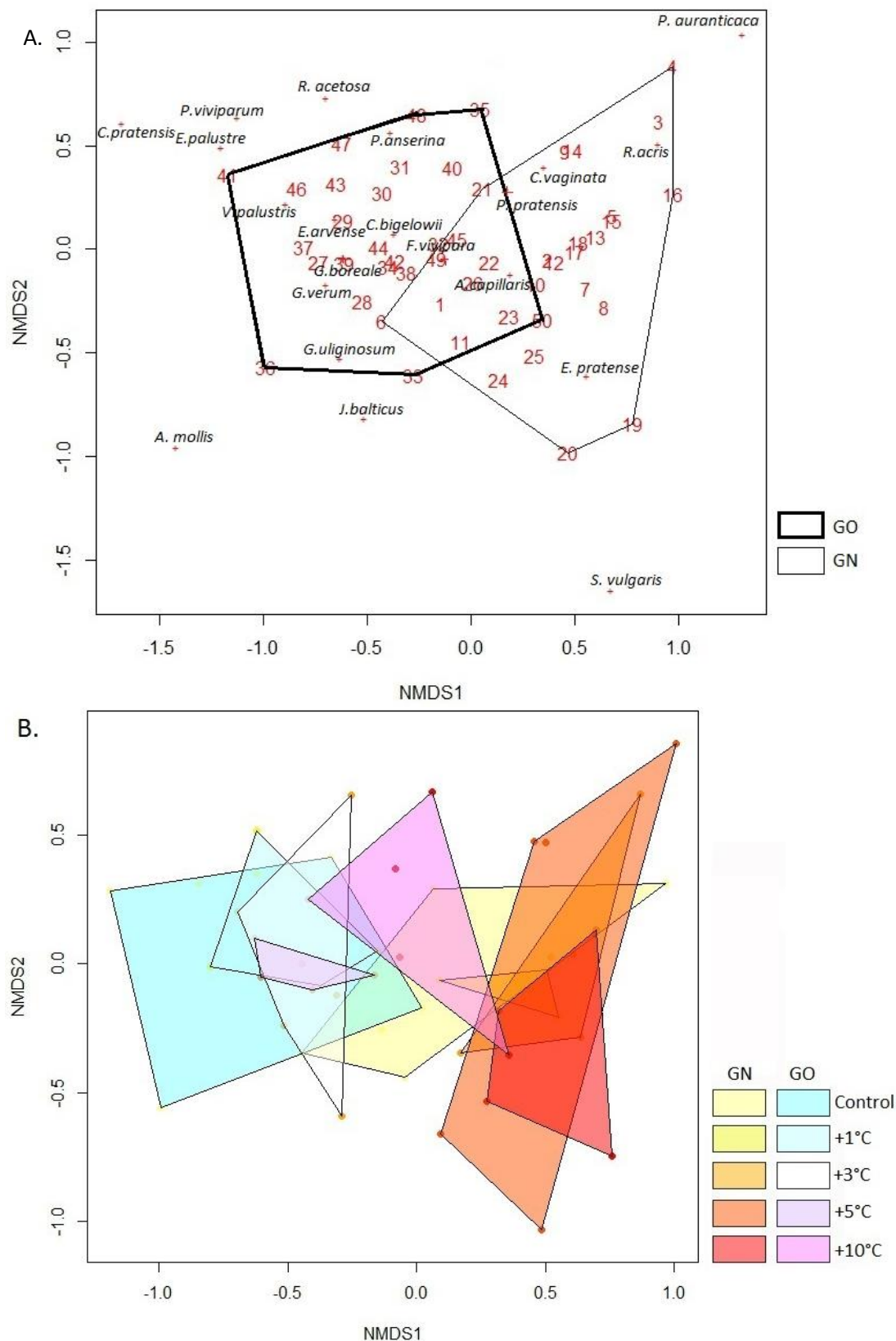
Figure 3: *NMDS-graphical representation of the total cover per individual vascular plant species per individual plot. Both figures have the same properties and represent the same dataset, but focus on a different aspect. Stress of this model is 0.19, which is acceptable for an NMDS-model (Clarke & Warwick, 2001). (A) The numbers correspond to the plots, plot number 1 until 25 are situated in the*

new grassland and 26 until 50 in the old grassland. The two polygons connect the outermost plots of the two grassland types representing the significant difference between the two (p<0.001). All plant species are automatically added as weighted averages. The exact position in the figure is marked by a '+'-sign. (B) The polygons connect the outermost plots of the same soil temperature level within the same grassland type. GO and GN have their own colour range with changing temperature as is shown by the legend, illustrating the significant effect of temperature on the species composition (p<0.05).

The two axes of the plot cannot be judged individually, but only the configuration of them together makes sense. That is why the quality of fit of an NMDS-model is expressed by a stress factor and not by an explanation of variance by the different axis. One can interpret this stress as a percentage of variation not explained by the used dimensions. The stress for this model is 0.19, which is not optimal, but according to the rules of thumb of Clarke & Warwick (2001), an acceptable representation of the dataset.

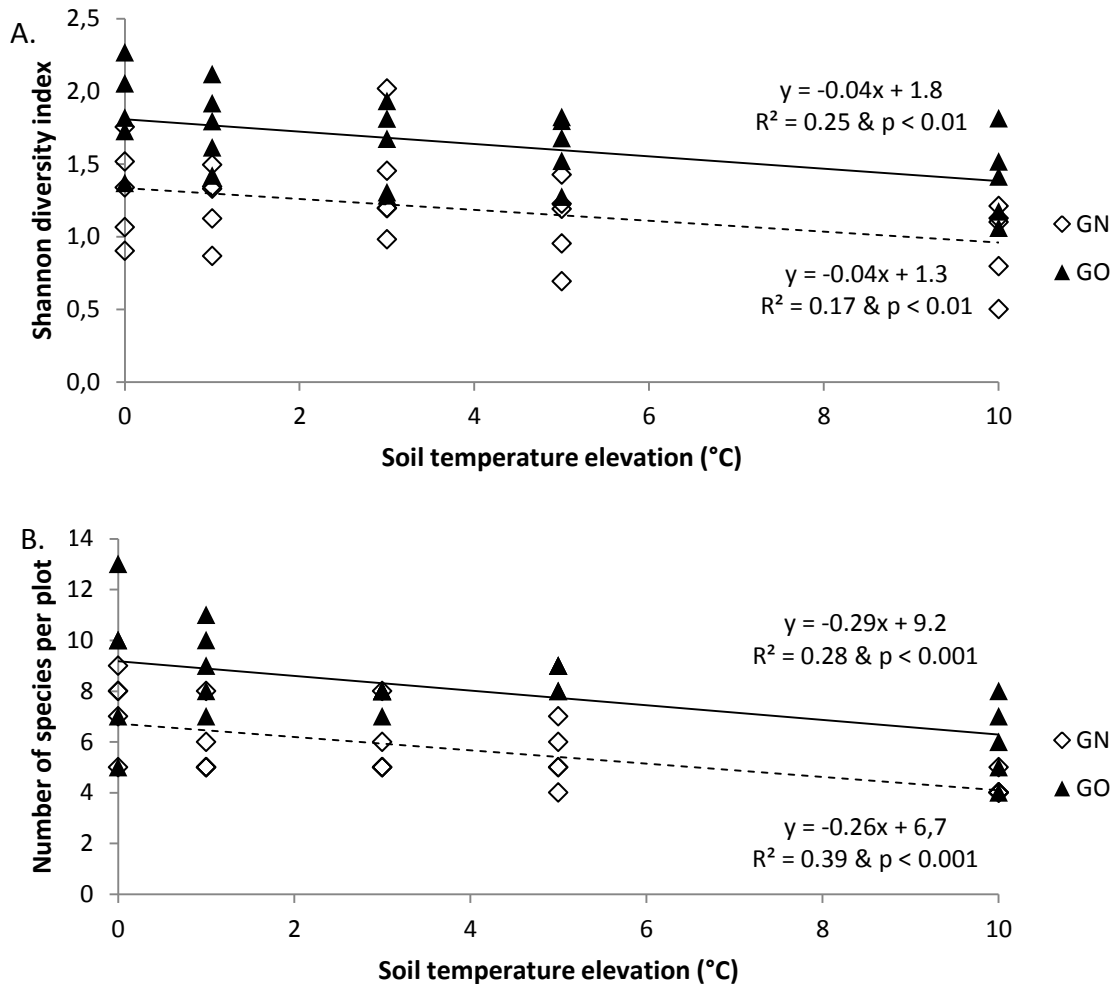### 3.1.2 Effect on vascular plant species diversity

Soil warming has a distinct influence on species diversity according to the results for the Shannon-Wiener index (Figure 4A). Both for GN as for GO, the soil temperature gradient has a significant negative effect with rising temperature on the diversity index (p<0.01). The magnitude of this effect is the same for both grasslands, but on average, the Shannon-Wiener index is significantly higher in the plots of GO than in plots of GN (p<0.001).

The Shannon-Wiener index gives a combined value based on the absolute number of species and the evenness or relative abundance of the present species. This value allows to calculate trends in diversity, but due to the combined nature of the value it is not obvious to draw conclusions about the cause of changes in diversity. Therefore, the same analysis was performed with species abundance (the absolute number of species) and species evenness. This made it possible to find out which aspect of the Shannon-Wiener index that caused this negative trend.

The statistical analysis for the absolute number of species per plot (Figure 4B) results in a similar output as for the Shannon-Wiener index. Both grassland types have a significant decreasing trend (slope = -0.275) in number of species per plot along the soil temperature gradient (p<0.001). For GN this means that on average 37% of the species were lost by adding 10°C to the normal soil temperature. In GO 31% of the species were lost. The number of species per plot was significantly higher in the GO than in GN (p<0.001).

The second aspect of the Shannon-Wiener index, the evenness of the vegetation plots, shows far less impressive results. The only significant outcome is once again a difference between the two grassland types (p<0.01) (Appendix 4, Figure 4A). The evenness of the different species is not altered by soil temperature.

A.

y = -0.04x + 1.8
R² = 0.25 & p < 0.01 ▲

◇ GN

y = -0.04x + 1.3
R² = 0.17 & p < 0.01 ◇

▲ GO

B.

y = -0.29x + 9.2
R² = 0.28 & p < 0.001

◇ GN

▲ GO

y = -0.26x + 6,7
R² = 0.39 & p < 0.001

Figure 4: *(A) Shows the outcome of the ANCOVA on the Shannon-Wiener diversity index. Both trend lines are significant and have an equal slope. The average Shannon-Wiener index of GO is significantly higher than the index of GN (p<0.001). (B) The graphical output of the ANCOVA for the absolute number of species. The temperature effect on the number of species is significant and similar in both grasslands. The trend lines have the same slope and the average number of species is significantly higher in the old grassland than in the new grassland (p<0.001).*

3.1.3 Effect on the ecological state according to Ellenberg indicator values

Combining the dataset with total coverage per vascular plant species with two datasets containing the four chosen Ellenberg indicator values (Moisture, Thermal optimum, Acidity and Nitrogen content) (Appendix 5, Table 5A) and applying equation 3, results in a new dataset with weighted

indicator values per temperature plot. Analysing the obtained weighted Ellenberg value dataset with multivariate statistics clarifies the general trends. GN and GO were significantly different from each other but the temperature gradient had a similar effect in both grasslands. The nature and the indicator most responsible for this effect will become clear hereafter.
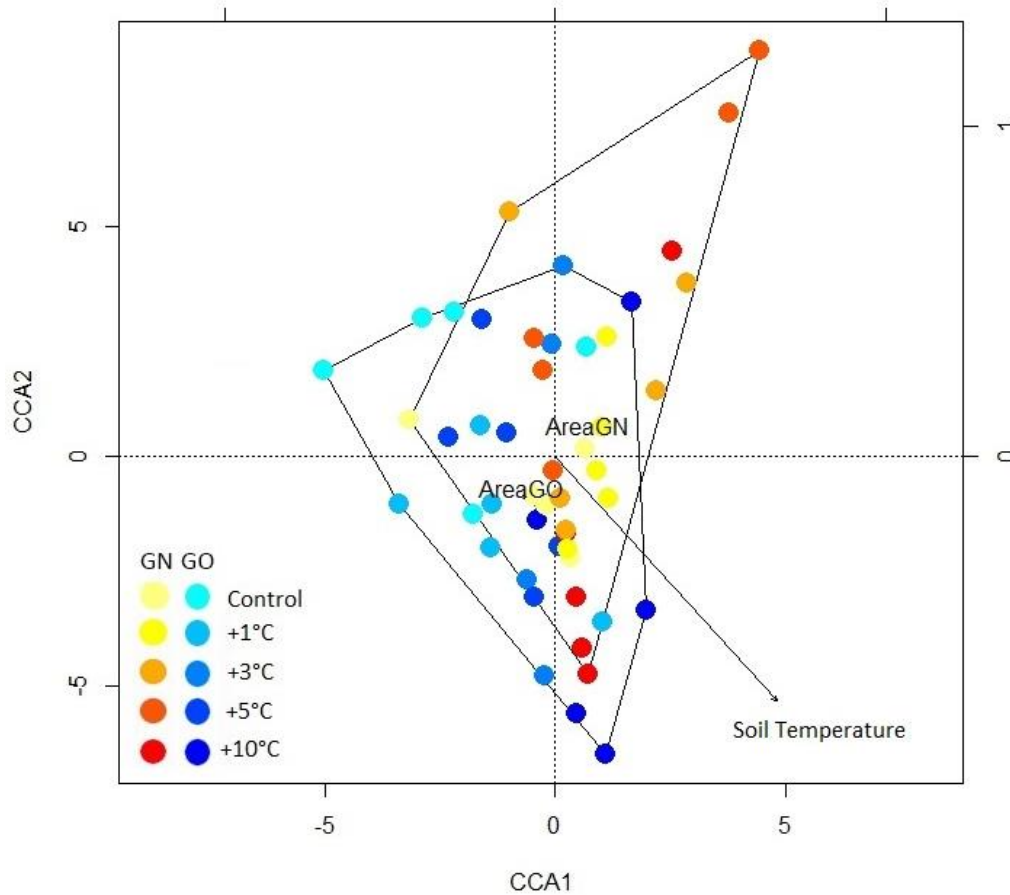
Figure 5: *The constrained correspondence analysis output of the multivariate analysis for the weighted Ellenberg's indicator values of thermal optimum, moisture, nitrogen content of the soil and acidity preference. This type of representing multivariate data displays only the variation for the used constrains, which are in this case "Temperature treatment" and "Grassland type". Both had a significant effect within the MANOVA (Temperature treatment: p<0.01 and Grassland: p<0.05). This constrained model explained 13.6% of the variance compared to the unconstrained model, 10.6% of this variance is explained by CCA1 and 2.8% by CCA2. The dots represent the different plots. The two colour ranges are explained in the legend and represent the different temperature levels in the two grasslands.*

The constrained model explained 13.6% of the variance that could have been explained when it was transformed to an unconstrained model (Figure 5). The explained variance is rather small, but the constraints have both a statistically significant effect in the dataset which justifies their choice as constrained factors.

Since the graphical output of a constrained correspondence analyses is in general not very clear on the significant differences, the different indicator values were analysed separately with ANCOVAs. Plotting the different indicator values in different graphs allows us to interpret this dataset in more detail (Figure 6). Nitrogen content of the soil appeared to be the most influencing ecological indicator value for this study. There was no significant difference between the two grasslands (p=0.2), but the effect of temperature on this indicator value was highly significant (p<0.001, R^2=0.27). The interaction between grassland type and added soil temperature was a borderline (p=0.055).

Acidity preference had no significant effect, and, contrary to the hypothesis, both indicator values for Thermal optimum and Moisture reveal no effect, but differ significantly between GO and GN (p<0.05 and p<0.05). The species occurring in GO prefer on average warmer and dryer conditions than species in GN.

A.

B.

C.
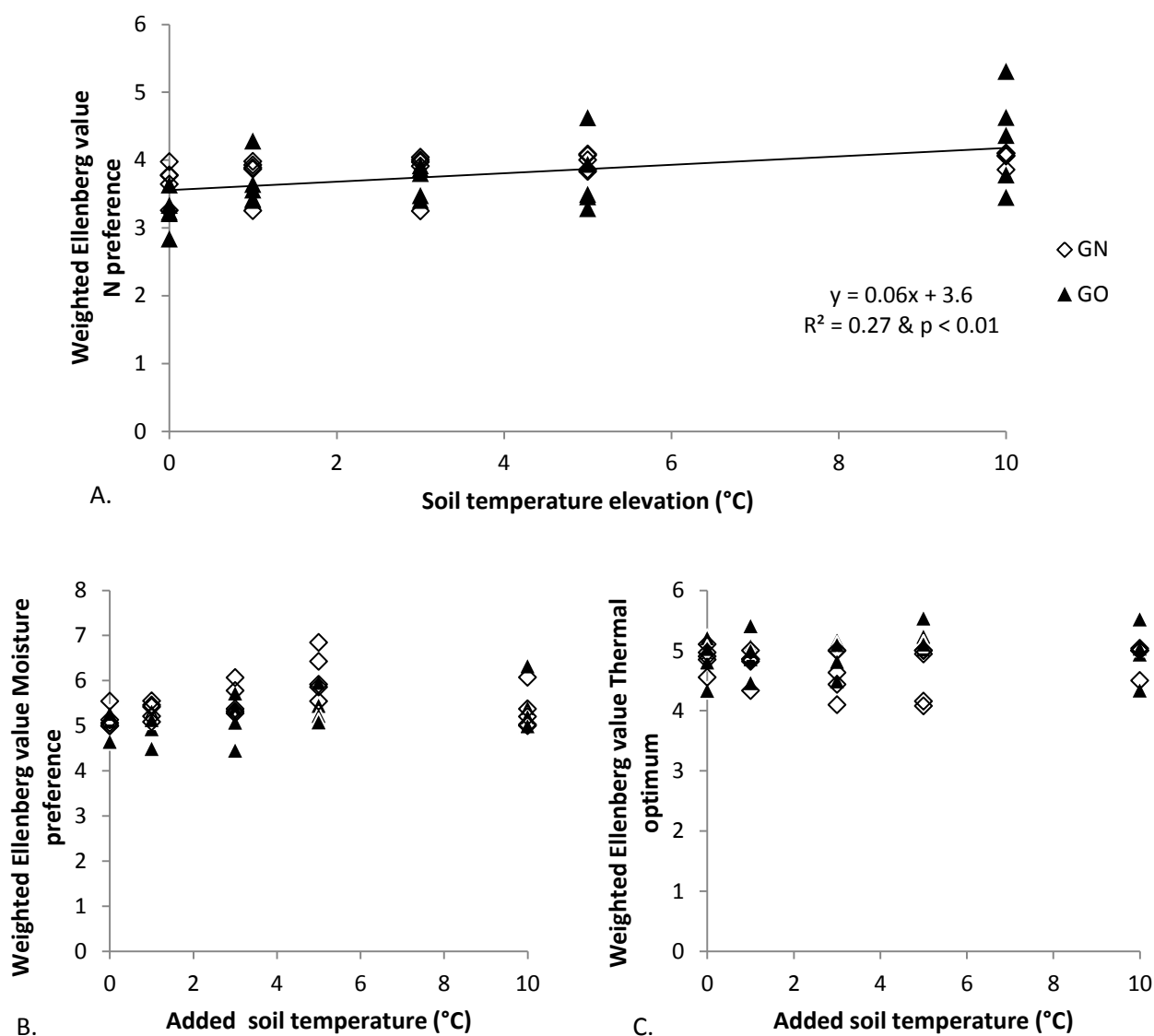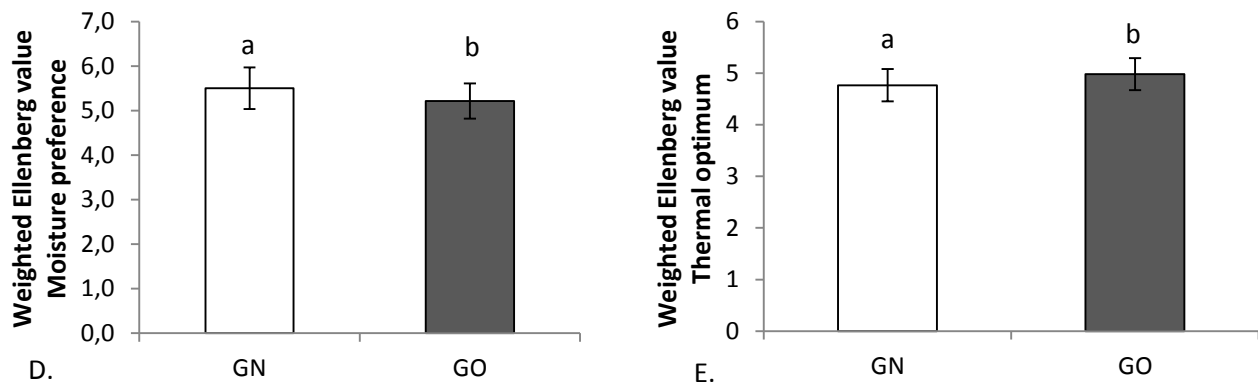
Figure 6: *Scatter plots for the individual Ellenberg's indicator values for (A) N content, (B) Moisture, (C) Optimal temperature preference, and bar charts for the indices for Moisture (D) and Optimal temperature (E). The soil temperature effect on the environmental index for nitrogen content is significant, but the scatter plots for moisture and thermal optimum show no soil temperature effect. However the difference between the two grassland types is significant for both environmental indices (moisture: $p<0.05$ and Temperature: $p<0.05$) indicated by 'a' and 'b'.*

3.2 Effect of soil warming on plant traits of target species

An overview of all the significant effects of soil temperature on the discussed plant traits can be found in Appendix 3 (Table 3A(2) and followings).

3.2.1 Effect on plant height

The plant height of *Agrostis capillaris* showed an increase with rising added soil temperature. Consequently there was chosen to add temperature as a continuous variable. This trend appeared to be highly significant ($p<0.001$, $R^2=0.16$) (Figure 7A). There was no difference between the grasslands.

For *Poa pratensis* the data were subdivided per grassland type because of the significant interaction between grassland type and soil temperature level ($p<0.001$). Only a minor significant difference between two temperature levels was shown for (Appendix 6, Figure A6(1)). In GO there was a significant positive trend along the temperature gradient ($p<0.001$, $R^2=0.22$) (Figure 7B).

The plant height dataset for *Ranunculus acris* also required to split the data in a GN and GO-dataset. The GN showed some highly significant effects between temperature levels ($p<0.001$, $R^2=0.15$), but no trend (Appendix 6, Figure A6(2)). Contrary to GO, which did suggest a trend ($p<0.05$) but without much explanation of variance ($R^2=0.05$) (Figure 7C).

Overall, plant height did not give consequent results for all three species. The grass species suggested an effect of increasing temperature on aboveground growth, *Agrostis capillaris* for both grasslands, *Poa pratensis* and *Ranunculus acris* only for GO (Appendix 3, Table 3A(2)).

A.

B.

C.
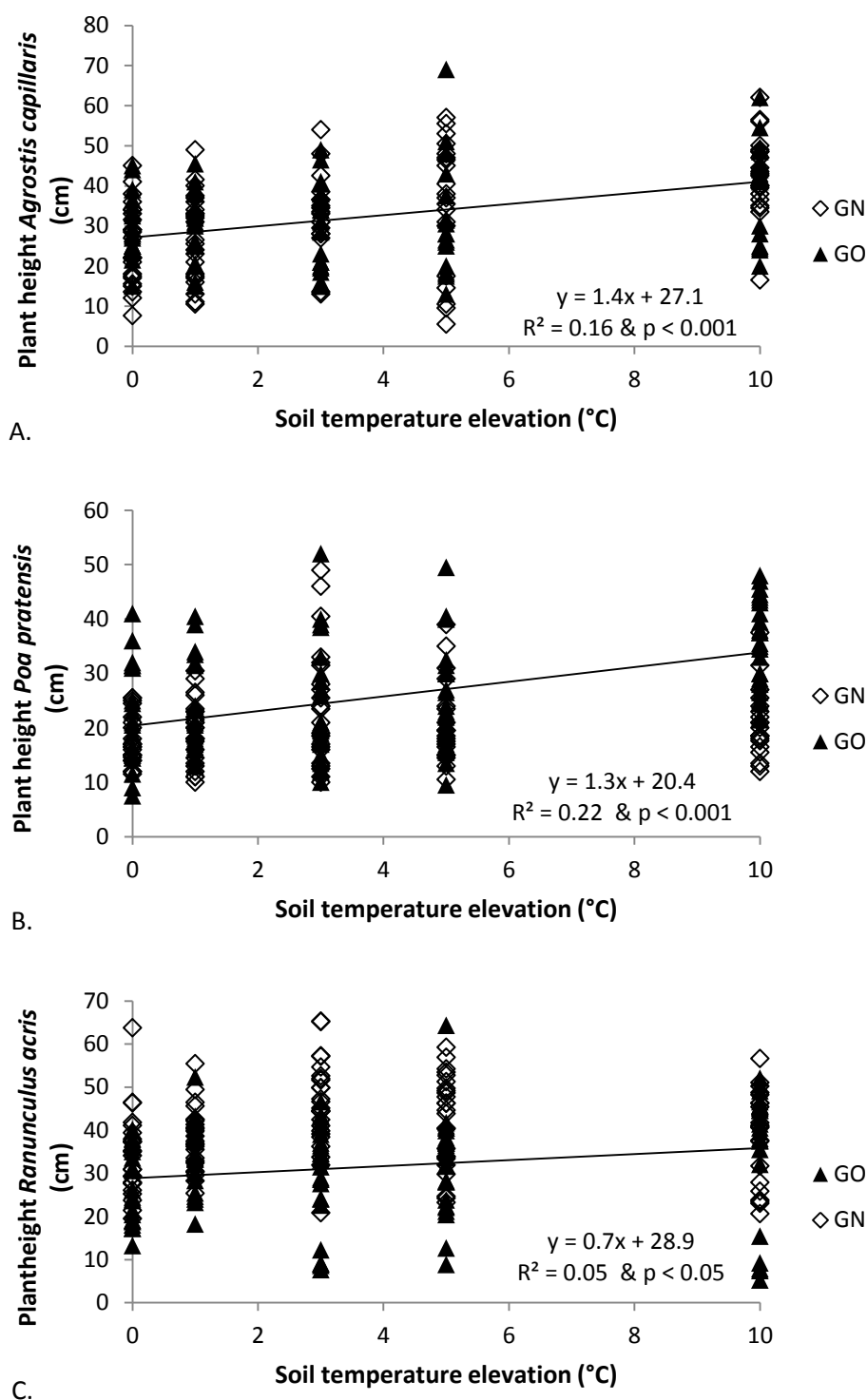
Figure 7: *Graphical representation of the relevant plant height measurements of Agrostis capillaris (A), Poa pratensis (B) and Ranunculus acris (C). (A) Plant height of Agrostis capillaris showed a significant positive trend with increasing soil temperature (p<0.001) for both grasslands together. (B)*

Plant height of Poa pratensis showed a significant positive trend for GO but not for GN (p=0.3). (C) Plant height of Ranunculus acris showed a positive trend with increasing soil temperature for GO but not for GN (p=0.4).

3.2.2 Effect on leaf stoichiometry

Statistics were performed on all 12 individual element concentrations and all 11 element:N ratio's using ANCOVAs and Spearman's rank correlation tests when the data were not normally distributed. To keep the overview, only the data that show a significant soil temperature effect are shown in Table 2.

Table 2: *Overview of the statistical outputs for the effects of soil warming on leaf element concentrations and element:N ratios. Significance notation; ○: 0.10<p<0.05, ●: p<0.05, ●●: p<0.01, ●●●: p<0.001. The R^2-value is given for the final ANCOVA model. Data not showing a normal distribution, were analysed using Spearman's rank correlation test and ρ is given for the correlations. Only the significant temperature effects or correlations are given. If 'grassland' is left empty, the temperature effect is the same in both grasslands.*

Element	Species	Grassland	p-value	R^2/ ρ	Test
C	*Agrostis capillaris*	GN	●	0.46	Spearman
Mg	*Agrostis capillaris*	GO	●	0.2	Ancova
	Poa pratensis		●●●	0.4	Ancova
	Ranunculus acris		●●●	0.2	Ancova
K	*Agrostis capillaris*	GO	●	0.3	Ancova
Na	*Poa pratensis*	GN	●	-0.44	Spearman
P	*Ranunculus acris*	GO	●●●	0.5	Ancova
S	*Poa pratensis*	GN	●	-0.41	Spearman
	Ranunculus acris	GO	●	-0.47	Spearman
Zn	*Poa pratensis*	GN	●●●	-0.72	Spearman
Cu	*Poa pratensis*	GN	●●	0.3	Ancova
Mg/N	*Agrostis capillaris*	GO	●	0.2	Ancova
	Ranunculus acris		●●●	0.2	Ancova
K/N	*Poa pratensis*		●●●	0.4	Ancova
P/N	*Agrostis capillaris*	GO	●	0.1	Ancova
	Ranunculus acris	GO	●●●	0.4	Ancova
S/N	*Ranunculus acris*		●	0.3	Ancova
Mn/N	*Ranunculus acris*	GN	●●	-0.53	Spearman
Zn/N	*Poa pratensis*		●	0.1	Ancova

There was no chemical element concentration or ratio that experienced the same temperature effect for all three species. In fact there was only one chemical element, Mg, that showed a temperature effect for all three species however the temperature effect for *Agrostis capillaris* is only significant in GO, while the other two species experience a temperature effect in both grasslands. On the other

hand, all species show multiple significant temperature effects regarding their leaf stoichiometry. Looking at the grassland type, six temperature effects are found in GN, seven in GO and six temperature effects are found for elements and ratios without distinction between the two grasslands. Therefore, there is no consistency in the effect of soil temperature gradients on leaf stoichiometry in one of the two grassland types.
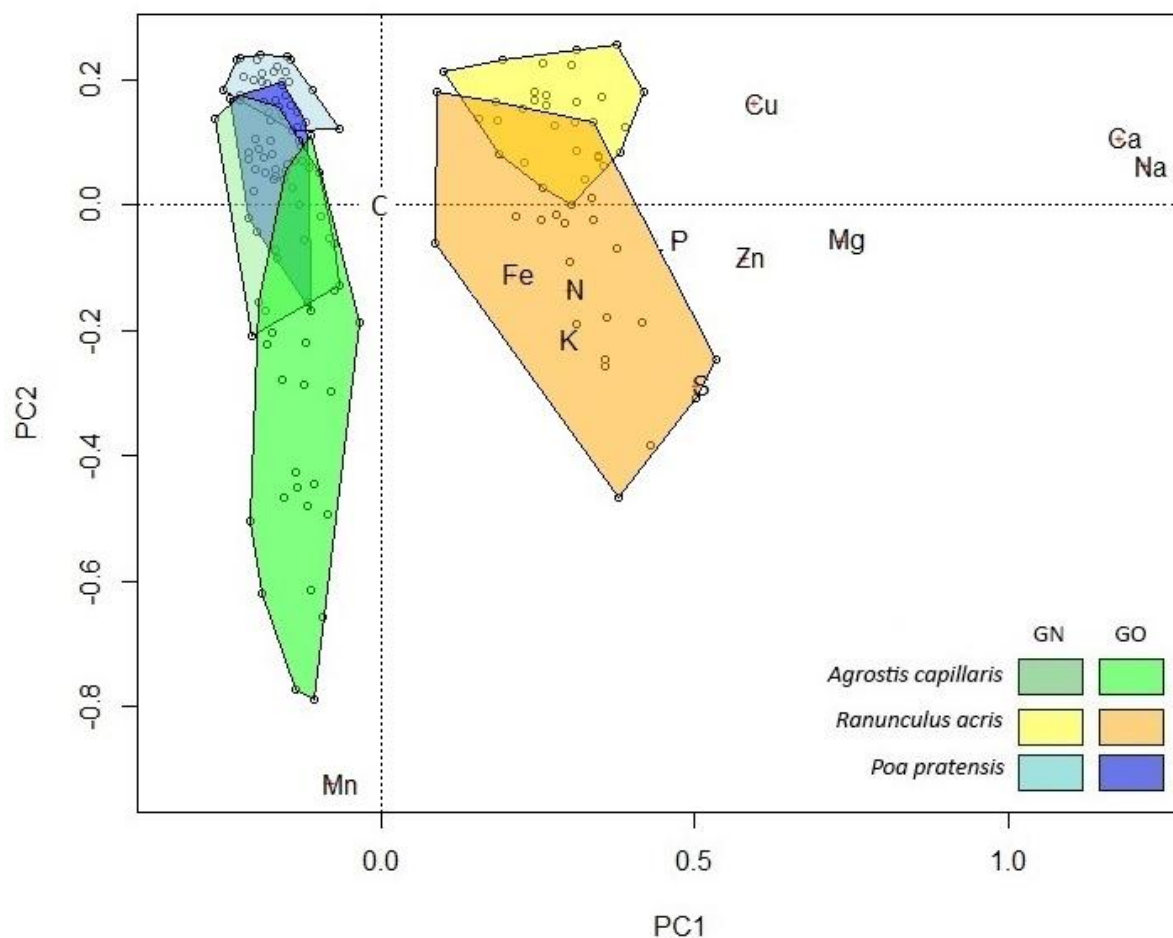
Leaf element concentration is besides the leaf stoichiometry, the only plant trait also analysed with multivariate analysis. Each data point is represented by 12 chemical elements or response variables. Using multivariate analysis for this dataset can reveal patterns that are not visible when concentrating on each individual element or ratio. Preforming a MANOVA on the stoichiometry dataset with all the species included, resulted in distinct interaction effects of species with grassland type ($p<0.001$) and soil temperature ($p<0.01$) (Table A3(3), Appendix3).

The data are graphically shown with the help of a redundancy analysis or optionally principal component analysis (Figure 8). The first principal component accounts for 64.4% of the variance in the model and the second principal component explains 14.2%, the third, not shown on the figure, explains 5%. There was a clear separation between *Ranunculus acris* and the grass species along PC1. PC2, on the other hand, allowed to distinguish the two grassland types for all three species and separated the grass species *Poa pratensis* and *Agrostis capillaris*. This figure gives some suggestions for the relative importance of the chemical elements for the differentiation of the species and grassland types. In Appendix3, Table A3(4), the scores for the different chemical elements can be found. These scores express the importance of each element with the corresponding PC. The leaf concentrations of Ca, Na and Mg were the most important parameters for PC1, but were rather trivial for PC2. For this principal component leaf concentrations of Mn, Cu, S, K were more important. The sequel of the multivariate analysis was done separately for the three species.

The multivariate analysis for *Agrostis capillaris* revealed a significant interaction between grassland type and temperature ($p<0.05$) leading to a further subdivision of the data in GN and GO. For GO this resulted in a significant effect of temperature on the leaf stoichiometry ($p<0.05$). The dataset for GN gave no additional information.

The statistical output of *Poa pratensis* gave a highly significant interaction between GN and GO ($p<0.001$) as well, indicating a different effect of temperature on the chemical element composition in the two grasslands. A separated analysis showed that there was a significant effect of the present soil temperature gradient in both grasslands (GN: $p<0.01$, GO: $p<0.05$).

Ranunculus acris showed roughly similar results as *Agrostis capillaris*. A significant interaction (p<0.05) led to a separated analysis for both grasslands and there was a soil temperature effect on the leaf stoichiometry in GO (p<0.01), which was not reflected in GN.

Figure 8: *A Principal Component Analysis (PCA) for the leaf elemental concentrations. The two axis or the two first principal components in this case, explain 51.0% and 11.8% of the variance. The dots represent the individual plots and the polygons connect the outermost plots of the corresponding species and grassland type as given in the legend. The chemical elements were added as weighted averages and can be interpreted as follows: The further away from the dotted intersection, the more important for explaining the shown variation. The imaginary line between an element and the intersection, gives the direction of its importance. Ranunculus acris and the two grass species were clearly separated by PC1, consequently the elements Ca and Na explain the most variance. Poa pratensis and Agrostis capillaris are more or less distinguishable by PC2. For all species PC2 has the most influence on the separation of both grassland types. Elements like Mn, S and K were more important for PC2.*

Besides the scores of the chemical elements and significances, the scores of the measuring points are another useful output of the multivariate analysis. In principle, the scores of the PCA are the coordinates of each data point on the principal component axes. Preforming ANCOVAs on the scores of the three first principal components (PC1, PC2 and PC3) of the mutual analysis in relation with soil temperature, allows to answer the question which principal component determines most the temperature effect (Appendix 3, TableA3(4)). Comparing these statistical results with the scores of the chemical elements, one can determine which chemical element concentrations are most affected by temperature. These analyses were done because the significant soil temperature effects are not visible on Figure 8.

Table A3(5) (Appendix 3) gives an overview of the ANCOVAs performed on the scores of the multivariate analysis. In general they confirmed the visual assumptions. PC1 showed significant differences between *Ranunculus acris* and the two grass species ($p < 0.001$). PC2 separated the different grassland types resulting from the significant differences between the two grassland types for the different species ($p < 0.001$). Further, PC2 separated the grass species from each other and from *Ranunculus acris*. Returning to the question in which principal component the temperature effect was incorporated, the answer is clear. PC1 is the only component that showed a significant temperature effect, though its significance is much lower than the significance of the interspecies difference. Looking at the loadings of the same analysis as these scores, Na, Ca and Mg were the three element concentrations most affected by temperature.

An analogous multivariate analysis for the element:nitrogen ratios was performed as for the leaf element concentrations (Appendix 3, Table A3(6)). The MANOVA on the element:nitrogen ratios of the three target species also resulted in the expected significant effects of the interactions between 'species' and 'grassland type' ($p < 0.001$) and between 'species' and 'temperature' ($p < 0.05$). Figure 9 gives a graphical expression of the relation between the different species and grassland types based on the leaf stoichiometry. This figure is very similar to Figure 8 and the same distinctions can be made. The first axis explains 56.0% of the variance, the second 13.3% and the third axis, not shown in the figure, explains 10.2%. The ratio scores confirm what can be concluded from Figure 9; Ca:N, Na:N and Mg:N are most important for PC1 and Mn:N, S:N, K:N and Cu:N for PC2 (Table A3(7), Appendix 3). These ratios contain the elements that were also important in the PCA-model for the element concentrations.

The leaf element:N ratios for *Agrostis capillaris* show only a significant effect between the two grasslands ($p < 0.001$). The same analysis for *Poa pratensis* on the other hand, resulted in a significant

effect for the interaction (p<0.01) and a soil temperature effect in both grasslands (GN p<0.01; GO p<0.01) was found. The element:nitrogen ratios of *Ranunculus acris* showed a significant soil temperature effect in GO but not in GN. In comparison with the raw element concentration analysis, the soil temperature effect for *Agrostis capillaris* was lost by working with element:nitrogen ratios, the other significances are the same.
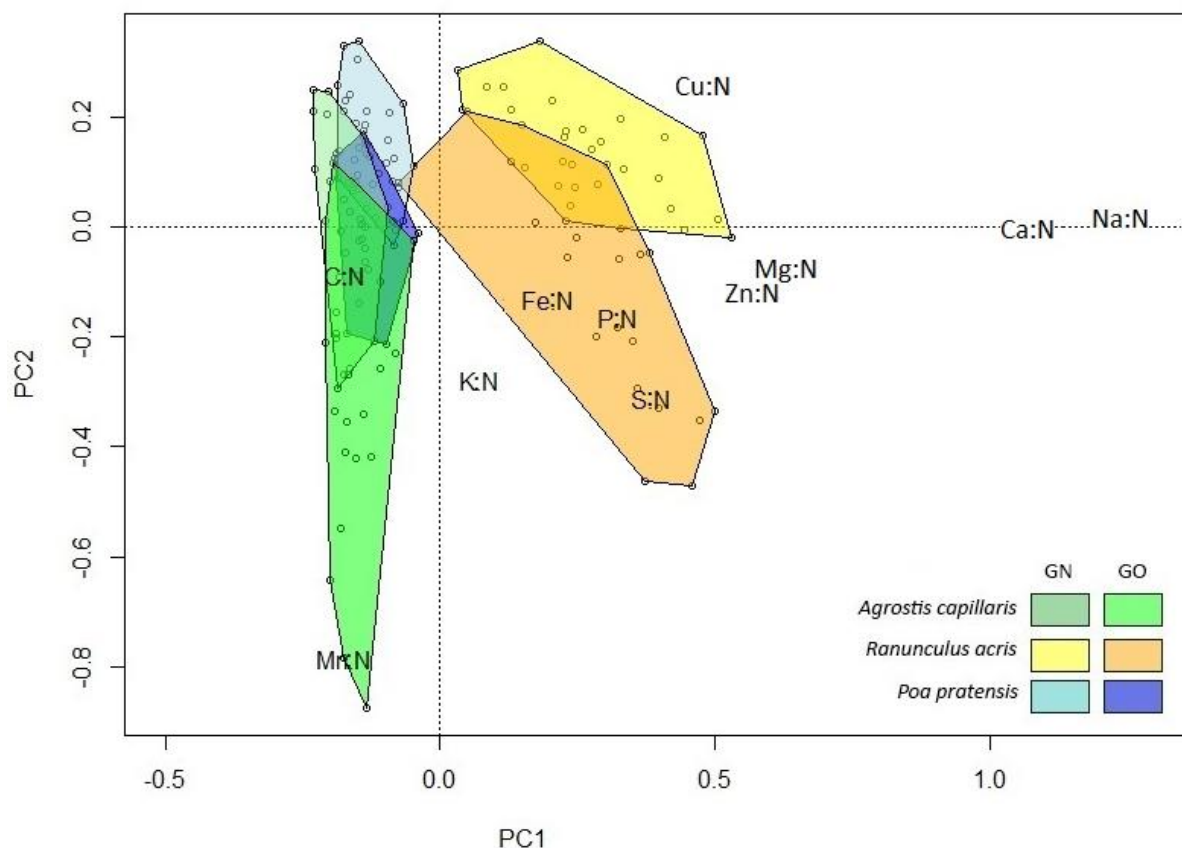
Figure 9: *Graphical output of the principal component analysis (PCA) on the element:nitrogen ratio dataset. The figure can be interpreted as Figure 8. The grass species are separated from Ranunculus acris along principal component 1 (PC1) and the grassland types show a shift from GN to GO along principal component 2 (PC2). The ratios Ca:N and Na:N are the most important for PC1 and Mn:N for PC2 with less importance for S:N, K:N and Cu:N.*

For the same reason as for the stoichiometry, ANCOVAs on the scores of the element:nitrogen multivariate analysis were performed (Appendix A3, Table A3(8)). The statistical output shows high similarities with the results of the element concentrations; PC1 splits *Ranunculus acris* from the grass species and PC2 distinguishes the grassland types of the three species. The soil temperature effect however, is no longer shown on the first axis, but on the second axis and only for *Ranunculus acris*. PC3 shows a species effect for this analysis, with significant differences between *Poa pratensis* and the two other target species.

In Table 2 can be found that P:N ratio is the only ratio that shows some consistency. There is a temperature effect on this ratio in the GO for both *Agrostis capillaris* and *Ranunculus acris*. The ANOVA on N:P ratio led to the same effects.

Figure 10 show the N:P ratios of the three species, and the soil temperature trend found for *Ranunculus acris* and *Agrostis capillaris*. The temperature effect is positive for *Ranunculus acris* and *Agrostis capillaris.* The R^2-value for *Agrostis capillaris* is very low, probably through the two outliers and +1°C and +3°C.
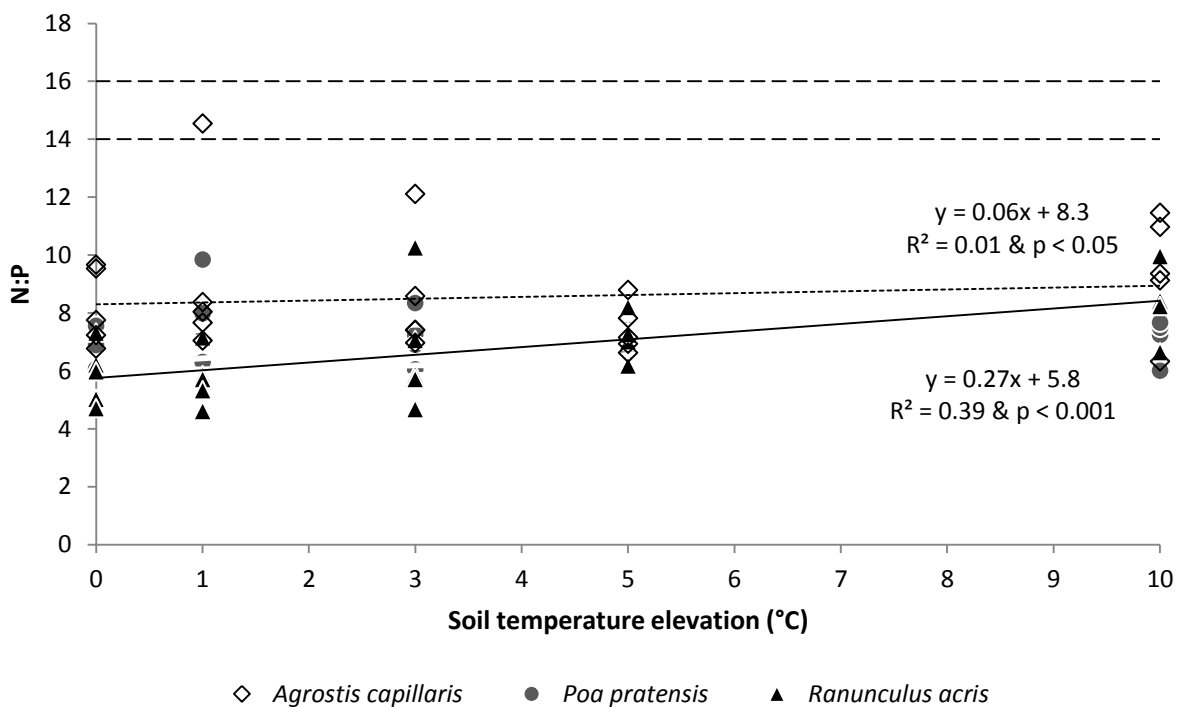
Figure 10: *Graphical representation of the N:P-ratio for the three species in the old grassland. Ranunculus acris and Agrostis capillaris both show a significant effect of increasing temperature (Agrostis capillaris: dotted trend line; Ranunculus acris: full trend line). The limits for nitrogen and phosphorus limitation at respectively 14 and 16 are indicated with two dashed lines.*

3.2.3 Effect on Specific Leaf Area

The SLA was for *Agrostis capillaris* in all the vegetation plots very similar and showed no statistical differences. *Poa pratensis* and *Ranunculus acris* differed only significantly in SLA between the two grassland types (p<0.001 and p<0.05 respectively) (Appendix 6, Figure 6A(3)) In both cases, GO had the highest SLA-values. The SLA-data of *Ranunculus acris* showed an additional difference between the control plot and the +10°C-temperature level (Appendix 6, Figure 6A(5)), with higher values for +10°C.

3.2.4 Effect on Stomatal conductivity

For all three target species a similar significant effect was obtained, more specifically a significant difference between the two grassland types. Contrary to the two other species, the conductivity of *Ranunculus acris* was the lowest in the old grassland. There were no differences found between the different soil temperature levels, neither with soil temperature level as continuous or discrete variable (Appendix 3, Table A3(6)). Figure A6(5) (Appendix 6) gives a graphical representation of the data. The standard errors for *Poa pratensis* were relatively large.

4. Discussion

4.1 Effects of soil warming on community structure and biodiversity

4.1.1 Effect on vascular plant species total coverage

The increasing trend in total plant coverage with soil temperature in GO, confirms the first hypothesis, which stated that higher soil temperatures would induce an increase of total plant cover (Figure 2). We assume that this increase was caused by a positive effect of warming on N-cycling, as was shown by Chapin et al. (1995), which resulted in a shift from belowground competition via roots, to aboveground competition via leaf coverage (Young et al., 2011). If a shift in limitation is the only process involved, this would have led to an increase of plant coverage in both grasslands. However, the plant height results for GN contradict our hypothesis. In GN there is no proof for an increase in plant coverage with rising soil temperature.

This deviation from what was hypothesized, can be explained by the occurrence of off-setting mechanisms and the difference in soil warming duration between the two grasslands (Arft et al., 1999; Chapin et al., 1995). Soil temperature itself could be a stress factor for the species in GN, reducing their ability to profit from the changed conditions. This is supported by a drastic decrease in root biomass with increasing temperature in GN (MSc Thesis Katherine Vande Velde, 2014). Such a large decrease in root surface, might limit N uptake and prevent the shift from N limitation to light limitation. A combination of adaptation and directional selection in GO could have led to plants that are used to warmer soil temperatures and higher N availability, and are able to benefit from this conditions. In contrast to GO, plants in GN might be less adapted to warmer soils and less capable of immobilizing the temperature-induced surplus of N in the soil and converting it into aboveground biomass, which would result in N leaching or volatilization (McHale et al., 1996; Lükewille & Wright, 1997). Anyway, this explanation remains purely speculative and future research is definitely needed to understand this apparent difference between GN and GO.

The increased coverage in the warmer plots of GO is mainly due to the continued presence of *Agrostis capillaris* and the strong emergence of *Poa pratensis* and *Potentilla anserina* (Figure A2, Appendix 2). Grasses like *Agrostis capillaris* and *Poa pratensis* are known to respond quickly to increased soil temperature (Brooker & van der Wal, 2003). Grasses increase their total live biomass especially by increasing the shoot size rather than the shoot number, resulting in an augmented total coverage. The increased coverage of *Potentilla anserina* contradicts earlier findings, where the biomass of this species declined with increased temperature (Shi et al., 2010). This response

however, was ascribed to drought stress associated with warming in open-top chambers (Jonsdottir et al., 2005; Aerts, 2006; Shi et al., 2010). Drought stress is, however, absent or rare in this region due to the abundant rainfall and point measurements in spring and summer of 2013 did not reveal any changes in soil moisture with temperature (Leblans Niki, personal communication). *Potentilla anserina* could have an advantage through its reproductive strategy. It can reproduce asexually by stolons or runners, making it possible for this species to colonize its direct vicinity when fully adapted to the environmental conditions. This strategy was often observed in the field, although in general, an increase in competition induces more investment in sexual than in clonal reproduction (Rautiainen et al., 2004).

Looking at the individual species coverage, which is a measure for species shifts and community structure, there are two interesting features that require more attention. Firstly, the species composition, taking into account their individual coverage, differs significantly between the two grasslands (Figure 3A). This could be a confirmation that species selection has occurred throughout the centuries in GO.

A second point of interest is shown by Figure 3B, which illustrates the similar impact of soil temperature on the vascular plant community structure. In addition to the clear positive effect of rising soil temperature on total coverage in GO, it is obvious that temperature also affected the individual species coverage. However, despite the fact that temperature did not influence total cover in GN, Figure 3B does suggest some effect of soil warming on GN community structure. Therefore, there must be another explanation for the impact of soil temperature on species composition in GN besides an increase in leaf coverage. This could be the inability to adapt to increased soil temperature at short notice.

Worth noticing is, that there is a clear difference in species coverage between the control plots of GN and GO (Figure 3B), stressing that these plots cannot be regarded as ten replicates of the same temperature level but as separate control plots within the temperature gradients of GN and GO respectively.

4.1.2 Effect on vascular plant species diversity

The second hypothesis, stating that soil warming would decrease plant species diversity, was well substantiated. The decline in the Shannon-Wiener index with increased heating of the soil, confirms this hypothesis (Figure 4A). The increase in light-competition, related to the assumed warming-induced increase in N availability (Chapin et al., 1995), would be the main reason for the loss of species diversity (Barot & Gignoux, 2004). Competitive species, making optimal use of the increased availability of soil nutrients, outcompete the more stress-tolerant species in their search for light.

This has been shown to result in a denser cover (Chapin et al., 1995; Young et al., 2011; Soudzilovskaia et al., 2013). From our own total coverage estimations we can conclude that this statement is only true for one grassland, GO. The total coverage of GN did not show the expected positive trend with rising soil temperature (Figure 2) and therefore, increased competition for light cannot be the mechanism, driving the loss of diversity at higher temperatures in GN.

The explanation for the declining biodiversity in GN might be found in the duration of the warming, in other words: the age of the temperature gradients. One knows for sure that the temperature gradients in GN exist a bit over five years (at the time of fieldwork), making them good representatives for short time warming (Halldórsson & Sigbjörnsson, 2009). GO meanwhile, has been warmed for centuries and maybe even longer (Magnússon, 1708). This time span is long enough for plants to adapt structurally and physiologically to this higher soil temperature. Five years of warming in GN is may have been too short to produce such adaptations. This allows us to hypothesize that the present species loss with temperature increase, is not the result of increased nutrient availability, as most likely is the case in GO, but a result of temperature stress for the unadapted plants.

To examine which aspect of the biodiversity index causes the decline in biodiversity, both evenness and species number were tested. Evenness was not altered by soil temperature (data not shown), revealing that species loss is the main factor driving the negative temperature effect on the Shannon-Wiener index (Figure 4B).

Kaplan et al. (2006) found that warming in the arctic was associated with increased nutrient availabilities and led to a succession. The extensive grazing occurring in GO could give an explanation for the absence of succession in this study. The presence of grazing in arctic regions with dwarf shrub vegetation, usually leads to a transition towards grassland vegetation (Díaz et al., 2007). Grazing favours the presence of grassland species over mosses and the grazing itself removes seedlings of dwarf shrubs (Van der Wal et al., 2004). Previous research pointed out that after removing the grazing pressure, it takes decades before the grazing effect is undone and a dwarf shrub ecosystem is established (Olofsson, 2006). So, despite the fact that the plots in GO were fenced at the beginning of the growing season of 2013, it will take years before a real equilibrium is established if the grazing pressure was big enough to have a significant impact on the vegetation.

4.1.3 Effects of soil warming on the ecological state according to Ellenberg's indicator values

Ellenberg indicator values are scores to quantify the ecology of a species. One could say that the Ellenberg values of a species illustrate the environmental conditions to which a species is best

adapted. These scores apply to all individuals within a species and, as a consequence, cannot be used as an indication for an adaptation to specific conditions. Therefore, these values cannot prove whether directional selection within a species has taken place after centuries of soil warming in GO. They do, however, suggest which environmental parameters may have been the main drivers of an observed species shift, as they reveal the optimal environmental conditions for the present species in the different studied treatments (Violle et al., 2007).

The multivariate analysis for the different community weighted Ellenberg indicators seemed to support the general hypothesis that rising temperatures will favour species with a preference for warmer temperatures and a N-richer environment and having a higher drought-resistance (Figure 5). A general difference in community-weighted Ellenberg values between the two grasslands is then a consequence of the difference in species composition in GN and GO. It is likely that the significant temperature effect on both species composition and Ellenberg indicator values was caused by the same driver, since the community-weighted Ellenberg values per plot were based on the total coverage per species, and therefore both datasets are related.

The analysis performed on the N-preference indicator value of the present species, resulted in a common trend for GN and GO, indicating that on average more N-preferring species occurred in the warmer plots, indirectly proving that N-availability was most likely enhanced (at least when integrating over long periods) (Figure 6A). However, the absence of a positive trend in total coverage with rising temperature in GN, makes it harder to explain the increase of more N-preferring species in GN. The total coverage data suggested that temperature was a stress factor for plants instead of improving their chance in gathering N. The combination of Figure A2 and Table A5 (Appendix 2 and 5), offers a logical explanation for the apparent increase of N-preferring species in GN. It appears that there was no increase in N-preferring species with increasing soil temperature, but a disappearance of indicator species for N-poor conditions, like *Galium boreale*, *Carex bigelowii* and *Pilosella aurantia.* This species loss led to the establishment of a very species-poor community (only 4 species remain), without large differences in dominance structure. The remaining species were associated with moderate N-availability, and were apparently not able use the additional soil N to produce more aboveground biomass, resulting in a status quo in total coverage across the entire studied soil temperature gradient in GN.

In GO the same trend of losing indicator species for N-limiting conditions was found for GO (*Galium boreale, Festuca vivipara*). In the warmest temperature plots of the GO gradient, there was an additional trend. The coverage share of *Poa pratensis* and *Potentilla anserina* increased sharply. Both species have a relatively high Ellenberg value for soil N-preference and therefore, contributed to the

increased community-weighted N-preference indicator score. One can thus conclude that the cause of the elevated community-weighted preference in more N-rich environments is twofold. On the one hand a loss of N-limiting indicator species, which occurred in both GN and GO, and on the other hand an increased dominance of N-preferring species in GO.

The weighted Ellenberg values for the thermal optimum and moisture preference revealed something unexpected (Figure 6B-E). We hypothesized that an increase of the soil temperature would attract species with a higher temperature preference in both grasslands, albeit more pronounced in GO, because species establishment is a slow process. However, there was no statistical evidence for a trend in thermal optimum with rising temperature (Figure 6B). We expected a positive effect of soil temperature for moisture preference through a higher demand of CO_2 as a consequence of increased nutrient availability. Abundant rainfall in the region would prevent water stress caused by increased evapotranspiration. This hypothesis is rejected, the moisture preference is constant in both grasslands but differs significantly between GN and GO (Figure 6C). However, there is an inversed statistical difference between thermal optimum and moisture preference in the two grasslands (Figure 6D-E). The highest weighted averages for thermal optimum appear in GO as well as the lowest values for moisture preference. One can conclude that, in general, the vegetation in GO prefers warmer temperatures and has a higher drought resistance. Earlier research proved that species preferring warmer temperatures, in general are more resistant to drought stress (Dang & Cheng, 2004; Easterling & Apps, 2005). This suggests a temperature induced species selection throughout the whole valley of GO in comparison with GN. If the old temperature gradients in GO gave certain species the ability to adapt to higher soil temperatures, this would give these species an advantage towards unadapted species, increasing their relative abundance in the valley. This reasoning is not applicable for species in GN, because an adaptation needs time to establish itself throughout a population by natural selection (Byars et al., 2007). As stated before, Ellenberg values are not a proof for adaptations within a species, but they do not exclude adaptations either. Species that are able to persist in the warmer areas through being better adapted, have a higher chance of developing additional adaptations, which will further improve their survival. This is what could have occurred in GO. Again, this is pure speculation, but an interesting research question to be developed in the coming years.

Concerning the community-weighted indicator value of soil pH, no effect of soil temperature was found. Further, no difference in community-weighted pH Ellenberg value was observed between the studied grasslands.

4.2 Effect of soil warming on plant traits

In the previous part, it was shown that soil warming induced changes on the community level, both in GO as in GN. Here the question arises whether temperature effects are also seen in the plant traits of individual species. The next sections will discuss the impact of soil warming on plant height, leaf stoichiometry, SLA and stomatal conductance of three plant species, occurring in both grasslands and across the entire soil temperature gradients.

4.2.1 Effect of soil warming on plant height

Only one target species confirmed hypothesis number four, which stated that increased light competition at higher temperatures would force plants to grow taller to compete for sunlight. More specifically, *Agrostis capillaris* showed an increasing trend with soil temperature, in both GN and GO (Figure 7A). The explanation for the different responses among the three target species can likely be found in the differences of their dominance status. *Agrostis capillaris* is the only species that could be called dominant in nearly all the vegetation plots of both grasslands (Figure A2, Appendix 2). *Agrostis capillaris* is the only species that is able to profit from the increased availability of soil nutrients, induced through soil warming, in both the short and the long term. This makes him a good light competitor and a dominant species, confirming previous research and our hypothesis (Mokany et al., 2006; Arft et al., 1999).

Ranunculus acris and *Poa pratensis* were able to increase their maximal height with increasing temperature in GO, but failed to do so in GN (Figure 7B-C). For *Ranunculus acris*, this trend is unlikely to be ecologically relevant given its low coefficient of determination (R^2-value), contrary to *Poa pratensis*. The latter species is rather rare throughout the whole valley of GN, but has a more prominent presence in GO, and its cover increased with rising soil temperature (Figure A2, Appendix 2). The following is pure speculation about the underlying mechanisms. *Poa pratensis* may have been able to adjust its physiology to the increased soil temperature in the long run and subsequently became more common in these areas. Therefore, this species is able to explain the increase of total cover in GO with soil temperature, while this trend is absent in GN. If we assume that the starting situation of both grasslands was the same, the soil temperature effect for *Poa pratensis* is not (yet) visible after the first five years of natural soil warming in GN. Since the plant height in the colder GO plots is still relatively low and similar to GN, this adaptation could have triggered the plasticity of plant height to adjust more properly to soil temperature changes and N-availability (Byars et al., 2007).

Ranunculus acris has a reversed status of dominance in comparison with *Poa pratensis* (Figure A2, Appendix2)*. Ranunculus acris* can be called common in GN, but in GO, the species seems to suffer from the large coverage of *Agrostis capillaris*, *Poa pratensis* and *Potentilla anserina* in the warmest plots, and the overall large species diversity in the colder plots (Figure A2). This is expressed as a general lower plant height in GO compared to GN.

Considering plant height of *Ranunculus acris* in GN as an indicator for the optimal temperature for growth, it appears that the optimum temperature is around +3°C (Figure A6(2), Appendix 6). However, this conclusion should be substantiated with results from other growth-related measurements (Dang & Cheng, 2004). *Poa pratensis* showed a similar, albeit less pronounced, pattern with a maximal height around +3°C (Figure A6(1), Appendix 6).

4.2.2 Effect on leaf stoichiometry

Leaf stoichiometry, both individual leaf element concentrations and leaf element:N ratios, gives insight in potential nutrient limitations (Aerts & Chapin, 2000; Koerselman & Meuleman, 1996). Since Northern high latitude ecosystems are characterized by nutrient-limiting conditions through lower temperatures (Peñuelas et al., 2013), we hypothesized that the leaf concentrations of essential elements, such as N and P would increase and N:P ratios would increase, indicating less N-limiting conditions with increasing soil temperature. The individual analysis for the different elements, however, did not confirm this hypothesis (Table 2). Nonetheless, eight out of twelve tested leaf element concentrations showed for at least one of the three target species a significant positive trend with rising soil temperature. On the other hand, no clear pattern was detected for the absolute concentrations and element:N ratios in neither of the two grasslands, like increased concentrations across all three target species or in both grasslands. Our results for absolute leaf concentrations not only contradict our hypothesis, but also the results of Zhang et al. (2012) who concluded that temperature was one of the key variables determining leaf element concentrations. Sardans and colleagues (Submitted) on the other hand confirmed our results for element:N ratio. In their study on leaf stoichiometry in tree species across Europe, they concluded that leaf stoichiometry is an evolutionary-determined characteristic of a species, with only minor flexibility according to the present climate and applied strategy.

The multivariate analysis on the whole-leaf elemental concentrations and the same analysis for the leaf element:N ratios resulted in a clear distinction between the three target species (Figure 8 and Figure 9). This was expected since these species are non-related species with their own accumulation and allocation strategies (Sardans & Peñuelas, 2012). A major difference in elemental composition is shown between *Ranunculus acris* and the two grass species along the first axis of the principal

component analysis. The two grass species were separated on the second axis (Figure 8 and 9). Also the two grasslands were separated along the second component for each of the three species. The latter could suggest that the species apply slightly different strategies in GN and GO, or may indicate differences in soil mineral concentrations between both grasslands. There are indications for the latter (Niki Leblans, personal communication). Nonetheless, the divergence between species on the first two axes of the principal component analysis was by far larger than the divergence between the two grasslands within each species. This is the result of the different acquisition and allocation strategies of the three species, which have a larger effect on leaf-elemental concentration than the possible difference in soil composition and element availability between GN and GO.

The scores of both multivariate analyses revealed which elements were causing the observed differences. Ca, Na, Mg, Zn and Cu were mainly responsible for the difference between *Ranunculus acris* and the two grass species, while Mn and Mn:N were most different between *Agrostis capillaris* and *Poa pratenis*.

Both leaf element concentrations and leaf element:N ratios revealed a temperature effect (Figure 8 and Figure 9). A multivariate analysis on the separate species, resulted in trends with temperature for all species in GO and for *Poa pratensis* also in GN. Nonetheless, the analysis of the individual scores revealed that the temperature effect was but of minor significance compared to the species effect and the effect of the two grasslands (Appendix 3, Table 5 and 8). This agrees with the lack of consistent effects of temperature on the individual elements and element:N ratios (Table 2). We assume that the few temperature effects observed are due to the plasticity in leaf element concentrations and the rather small range of variability in species-specific stoichiometry as was found by (Sardans et al., submitted).

Since N:P ratios and their ecological relevance are well documented in literature, it is interesting to take a look at the N:P ratio of the three target species across the temperature gradient. There was a positive trend for both *Agrostis capillaris* and *Ranunculus acris* with increasing soil temperature in GO. However, this trend was absent for *Poa pratensis* (Table 2, Figure 10). All ratios but one for *Agrostis capillaris* are lower than 14, suggesting that the environment is N-limiting for the growth of these species; ratios above 16 would have indicated phosphor shortage (Koerselman & Meuleman, 1996; Aerts & Chapin, 2000). Our results confirm that northern ecosystems are characterized by N-limitation (Aerts & Chapin, 2000). This limitation reduces with rising soil temperature for *Agrostis capillaris* and *Ranunculus acris*, but remains present, even in the warmest plots.

4.2.3 Effect on SLA

The SLA was hypothesized to increase with rising soil temperature as a result of the more favourable nutrient conditions (Cunningham, 1999; Ordoñez et al., 2009). Our results do not confirm this hypothesis. The SLA of *Agrostis capillaris* did not show any significant trend. The SLA of *Poa pratensis* and *Ranunculus acris* did not change with temperature, but differed between the two grasslands (Figure 6A (3 and 4), Appendix 6). In both cases, the SLA was higher for plants in GO than in GN, suggesting better conditions in GO. Bearing in mind previous results, this could be another indication of the occurrence of adaptations in GO to the altered soil temperature, which is not (yet) achieved in GN.

Our findings do not necessarily contradict previous research. Previous papers on SLA mainly discuss the global pattern of SLA linked to climate conditions and applied plant strategies. This usually involves a shift in species with different average SLA-values, rather than an adaptation or plasticity within a species (Reich et al., 1997; Cunningham, 1999; Reich et al., 2007; Ordoñez et al., 2009; Soudzilovskaia et al., 2013).

4.2.4 Effect on stomatal conductance

As was stated in hypothesis number seven, none of the three target species showed a decreasing stomatal conductance with rising soil temperature. There was a significant difference between GN and GO for all three species, but this difference was not consistent across the three species. Stomatal conductance was higher in GO than GN for *Agrostis capillaris* and *Poa pratensis*, but the reverse was seen for *Ranunculus acris* (Figure A6 (5), Appendix 6).

If stomatal conductance is a measure of water stress (Sellers et al., 1997; Dang & Cheng, 2004), *Agrostis capillaris* and *Poa pratensis* experienced less water availability in GN, while the stomatal conductance of *Ranunculus acris* suggested more water stress in GO. It is, however, unlikely that any of the measured plants was suffering from water deficit, due to the abundant rainfall in the area.

Stomatal conductance also depends of internal CO_2-concentration. When the plant metabolism is elevated as a consequence of increased nutrient availability, the internal CO_2-concentration will drop, inducing a higher stomatal conductivity to improve the uptake of CO_2 (Sellers et al., 1997). This makes stomatal conductivity a measure for general stress, with higher values for plants well adapted to their environment. Consequently, these data confirm the measurements of plant height and the dominance status of *Poa pratensis* and *Ranunculus acris*. *Poa pratensis* is more abundant in GO with higher plant heights, while the opposite is true for *Ranunculus acris*, suggesting that *Poa pratensis* is better adapted than *Ranunculus acris* in GO compared to GN.

5. Conclusion

Our results show that soil warming has a distinct impact on the present grassland vegetation. Soil warming induced changes in variables such as species richness and diversity, community-weighted N-preference and plant height. Other variables such as leaf stoichiometry and SLA, however, were not affected by soil temperature.,

Coming back to the main research question whether soil warming induces a change in species composition or rather an adaptation of present species, we can conclude that our results indicate that these two strategies cannot be seen as separate possible consequences. It is, however, essential to separate between the consequences of short term and long term soil warming, represented by GN and GO respectively. An adaptation, which is beneficial for a species under the prevailing conditions, takes time to spread through a population. This is illustrated by the large differences often found between GN and GO, indicating that plants in GN lacked the time needed to develop favourable adaptations.

Adaptations on species level were absent in GN, but our results showed that the community structure of both grasslands was affected by increased soil temperature. In both GN and GO, soil warming caused the plant biodiversity to decline as a consequence of a decrease in species richness. Nonetheless, it is short-sighted to extrapolate community changes observed in short term experiments to long term experiments, for it appears that this decline in biodiversity was induced by two different drivers in the different time scales of warming. In the short term, soil warming was experienced as a stress factor, eliminating all species that were not able to persist the higher temperatures. When species were given the time to adapt to the higher temperatures, as was the case in GO, plants were able to utilize the increased availability of nutrients, which caused a shift from soil-nutrient competition to light competition. This competition shift caused the decline in species richness, but an increase of total plant coverage on warmed soil in the long term.

A welcome extension to our results, would be a characterization of GN and GO in the field of soil composition incorporating nutrient availability. Attempts have been made to obtain direct evidence that warmer plots are associated with higher N-availability, but this has not been proven yet, only indirectly by our results. It would be very interesting for future research to examine whether our assumption that adaptations occurred on species level is valid. The results of *Poa pratensis* revealed it as a good target species to test the new research questions that were posed in this thesis.

References

ACIA, & Hassol, S. J. (2004). *Arctic climate impact assessment* (p. 146). Cambridge: Press Syndicate of the University of Cambridge. Retrieved from http://brage.bibsys.no/npolar/retrieve/138/Internrapport14.pdf

Aerts, R. (2006). The freezer defrosting: global warming and litter decomposition rates in cold biomes. *Journal of Ecology*, *94*(4), 713–724. doi:10.1111/j.1365-2745.2006.01142.x

Aerts, R., & Chapin, F. (2000). The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. *Advances in Ecological Research*. Retrieved from http://www.sciencedirect.com/science/article/pii/S0065250408600161

Arft, A., Walker, M., & Gurevitch, J. (1999). Responses of tundra plants to experimental warming: meta-analysis of the international tundra experiment. *Ecological …*, *69*(June 1998), 491–511. Retrieved from http://www.esajournals.org/doi/abs/10.1890/0012-9615(1999)069%5B0491:ROTPTE%5D2.0.CO%3B2

Arnalds,O., Hallmark,C.T., Wilding,L.P., (1995). Andisols from Four Different Regions of Iceland. Soil Sci. Soc. Am. J. 59, 161-169.

Arnalds, O. (1999). Soil survey and databases in Iceland, (1960), 181–186. Retrieved from http://139.191.1.96/ESDB_Archive/eusoils_docs/esb_rr/n09_soilresources_of_europe/Iceland. pdf

Arnalds, O. (2004). Volcanic soils of Iceland. *Catena*, *56*(1-3), 3–20. doi:10.1016/j.catena.2003.10.002

Barot, S., & Gignoux, J. (2004). Mechanisms promoting plant coexistence: can all the proposed processes be reconciled? *Oikos*, 185–192. Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.0030-1299.2004.13038.x/full

Bergh, J., & Linder, S. (1999). Effects of soil warming during spring on photosynthetic recovery in boreal Norway spruce stands. *Global Change Biology*, 245–253. Retrieved from http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2486.1999.00205.x/full

Brooker, R., & van der Wal, R. (2003). Can soil temperature direct the composition of high arctic plant communities? *Journal of Vegetation Science*, (1996), 535–542. Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.1654-1103.2003.tb02180.x/full

Byars, S. G., Papst, W., & Hoffmann, A. a. (2007). Local adaptation and cogradient selection in the alpine plant, Poa hiemata, along a narrow altitudinal gradient. *Evolution; International Journal of Organic Evolution*, *61*(12), 2925–41. doi:10.1111/j.1558-5646.2007.00248.x

Chapin, F. S. (2003). Effects of Plant Traits on Ecosystem and Regional Processes: a Conceptual Framework for Predicting the Consequences of Global Change. *Annals of Botany*, *91*(4), 455–463. doi:10.1093/aob/mcg041

Chapin, F., Shaver, G., & Giblin, A. (1995). Responses of arctic tundra to experimental and observed changes in climate. *Ecology*, *76*(3), 694–711. Retrieved from http://www.esajournals.org/doi/abs/10.2307/1939337

Clarke, K. R. and Warwick, R. M. (2001). Change in marine communities: an approach to statistical analysis and interpretation. PRIMER-E, Plymouth.

Cornelissen, J. H. C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D. E., … Poorter, H. (2003). A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany*, *51*(4), 335. doi:10.1071/BT02124

Cunningham, S. (1999). Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. *Ecological …*, *69*(4), 569–588. Retrieved from http://www.esajournals.org/doi/abs/10.1890/0012-9615(1999)069%5B0569:EDILSA%5D2.0.CO%3B2

Dang, Q.-L., & Cheng, S. (2004). Effects of soil temperature on ecophysiological traits in seedlings of four boreal tree species. *Forest Ecology and Management*, *194*(1-3), 379–387. doi:10.1016/j.foreco.2004.03.004

Darwin, C. (1859). On the origin of species. John Murray.

Davidson, E. a, & Janssens, I. a. (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, *440*(7081), 165–73. doi:10.1038/nature04514

Day, T. a., Ruhland, C. T., & Xiong, F. S. (2008). Warming increases aboveground plant biomass and C stocks in vascular-plant-dominated Antarctic tundra. *Global Change Biology*, *14*(8), 1827–1843. doi:10.1111/j.1365-2486.2008.01623.x

Díaz, S., Hodgson, J. G., Thompson, K., Cabido, M., Cornelissen, J. H. C., Jalili, a., … Zak, M. R. (2004). The plant traits that drive ecosystems: Evidence from three continents. *Journal of Vegetation Science*, *15*(3), 295. doi:10.1658/1100-9233(2004)015[0295:TPTTDE]2.0.CO;2

Díaz, S., Lavorel, S., de Bello, F., Quétier, F., Grigulis, K., & Robson, T. M. (2007). Incorporating plant functional diversity effects in ecosystem service assessments. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(52), 20684–9. doi:10.1073/pnas.0704716104

Easterling, W., & Apps, M. (2005). Assessing the consequences of climate change for food and forest resources: a view from the IPCC. *Increasing Climate Variability and Change*, 165–189. Retrieved from http://link.springer.com/chapter/10.1007/1-4020-4166-7_8

Einarsson, M. (1984). Climate of Iceland. *World Survey of Climatology*. Retrieved from http://m.en.vedur.is/media/loftslag/myndasafn/frodleikur/Einarsson.pdf

Eviner, V. T., & Chapin III, F. S. (2003). Functional matrix: A Conceptual Framework for Predicting Multiple Plant Effects on Ecosystem Processes. *Annual Review of Ecology, Evolution, and Systematics*, *34*(1), 455–485. doi:10.1146/annurev.ecolsys.34.011802.132342

Evju, M., & Austrheim, Æ. G. (2009). Grazing responses in herbs in relation to herbivore selectivity and plant traits in an alpine ecosystem, 77–85. doi:10.1007/s00442-009-1358-1

Galloway, J., Dentener, F., & Capone, D. (2004). Nitrogen cycles: past, present, and future. *Biogeochemistry*, 153–226. Retrieved from http://link.springer.com/article/10.1007/s10533-004-0370-0

Geptner, a. R., Ivanovskaya, T. a., & Pokrovskaya, E. V. (2006). Hydrothermally altered clayey sediments in the rift zone of Iceland (influence of microbiota on accumulation of minor elements). *Lithology and Mineral Resources*, *41*(4), 332–343. doi:10.1134/S0024490206040043

Giblin, A., & Nadelhoffer, K. (1991). Biogeochemical diversity along a riverside toposequence in arctic Alaska. *Ecological Monographs*, *61*(4), 415–435. Retrieved from http://www.esajournals.org/doi/abs/10.2307/2937049

Graversen, R. G., Mauritsen, T., Tjernström, M., Källén, E., & Svensson, G. (2008). Vertical structure of recent Arctic warming. *Nature*, *451*(7174), 53–6. doi:10.1038/nature06502

Grime, J. P. (1997). ECOLOGY: Biodiversity and Ecosystem Function: The Debate Deepens. *Science*, *277*(5330), 1260–1261. doi:10.1126/science.277.5330.1260

Grime, J. P. (1998). Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *Journal of Ecology*, *86*, 902–910.

Güsewell, S. (2004). N : P ratios in terrestrial plants: variation and functional significance. *New Phytologist*, *164*(2), 243–266. doi:10.1111/j.1469-8137.2004.01192.x

Halldórsson, B., & Sigbjörnsson, R. (2009). The Ölfus earthquake at 15:45 UTC on 29 May 2008 in South Iceland: ICEARRAY strong-motion recordings. *Soil Dynamics and Earthquake Engineering*, *29*(6), 1073–1083. doi:10.1016/j.soildyn.2008.12.006

Hartley, I. P., Heinemeyer, A., & Ineson, P. (2007). Effects of three years of soil warming and shading on the rate of soil respiration: substrate availability and not thermal acclimation mediates observed response. *Global Change Biology*, *13*(8), 1761–1770. doi:10.1111/j.1365-2486.2007.01373.x

He, J.-S., Wang, L., Flynn, D. F. B., Wang, X., Ma, W., & Fang, J. (2008). Leaf nitrogen:phosphorus stoichiometry across Chinese grassland biomes. *Oecologia*, *155*(2), 301–10. doi:10.1007/s00442-007-0912-y

Heip, C. H. R., Herman, P. M. J., & Soetaert, K. (1998). Indices of diversity and evenness. *Vliz.be*, *24*(2459), 61–87. Retrieved from http://www.vliz.be/imisdocs/publications/221019.pdf&a=bi&pagenumber=1&w=100

Hill, M., Mountford, J., Roy, D., & Bunce, R. (1999). Ellenberg's indicator values for British plants, 1999. *… and Regions (NERC), Grange-over-Sands, UK*. Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Ellenberg's+indicator+values +for+british+plants#5

Hobbie, S., Nadelhoffer, K., & Högberg, P. (2002). A synthesis: the role of nutrients as constraints on carbon balances in boreal and arctic regions. *Plant and Soil*, 163–170. Retrieved from http://link.springer.com/article/10.1023/A:1019670731128

Hodgson, J. G., Montserrat-Martí, G., Charles, M., Jones, G., Wilson, P., Shipley, B., … Royo Pla, F. (2011). Is leaf dry matter content a better predictor of soil fertility than specific leaf area? *Annals of Botany*, *108*(7), 1337–45. doi:10.1093/aob/mcr225

Hollander, M. & Wolfe, D. A. (1973), Nonparametric Statistical Methods. New York: John Wiley & Sons. Pages 185–194 (Kendall and Spearman tests).

Hyvönen, R., Agren, G. I., Linder, S., Persson, T., Cotrufo, M. F., Ekblad, A., … Wallin, G. (2007). The likely impact of elevated [CO2], nitrogen deposition, increased temperature and management on carbon sequestration in temperate and boreal forest ecosystems: a literature review. *The New Phytologist*, *173*(3), 463–80. doi:10.1111/j.1469-8137.2007.01967.x

Icelandic Met Office (2014). Climate archives for weather stations. Downloaded on Feb. 15 2014 from http://www.vedur.is/vedur/vedurfar/medaltalstoflur/.

Jakobsdóttir, S. (2008). Seismicity in Iceland: 1994–2007. *Jökull*, (58), 1994–2007. Retrieved from http://earthice.hi.is/sites/jardvis.hi.is/files/Pdf_skjol/Jokull58_pdf/jokull58-jakobsdottir.pdf

Jenkinson, D. S., Adams, D. E. &Wild (1991), A.Model estimates of CO2 emissions fromsoil in response to global warming. Nature 351, 304–306.

Jonsdottir, I. S., Magnusson, B., Gudmundsson, J., Elmarsdottir, A., & Hjartarson, H. (2005). Variable sensitivity of plant communities in Iceland to experimental warming. *Global Change Biology*, *11*(4), 553–563. doi:10.1111/j.1365-2486.2005.00928.x

Jost, L. (2006). Entropy and diversity. *Oikos*, *2*. Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.2006.0030-1299.14714.x/full

Kaplan, J. O., & New, M. (2006). Arctic climate change with a 2 ∘C global warming: Timing, climate patterns and vegetation change. *Climatic Change*, *79*(3-4), 213–241. doi:10.1007/s10584-006-9113-7

Knecht, M. F., & Göransson, A. (2004). Terrestrial plants require nutrients in similar proportions. *Tree Physiology*, *24*(4), 447–60. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14757584

Koenigk, T., Brodeau, L., Graversen, R. G., Karlsson, J., Svensson, G., Tjernström, M., … Wyser, K. (2012). Arctic climate change in 21st century CMIP5 simulations with EC-Earth. *Climate Dynamics*, *40*(11-12), 2719–2743. doi:10.1007/s00382-012-1505-y

Koerselman, W., & Meuleman, A. (1996). The vegetation N: P ratio: a new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology*, *33*(6), 1441–1450. Retrieved from http://www.planta.cn/forum/files_planta/1452_1189569238.pdf_169.pdf

Lavoie, M., Mack, M. C., & Schuur, E. a. G. (2011). Effects of elevated nitrogen and temperature on carbon and nitrogen dynamics in Alaskan arctic and boreal soils. *Journal of Geophysical Research*, *116*(G3), G03013. doi:10.1029/2010JG001629

Lavorel, S., & Garnier, E. (2002). Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. *Functional Ecology*, 545–556. Retrieved from http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2435.2002.00664.x/full

Liu, Y., Key, J. R., & Wang, X. (2008). The Influence of Changes in Cloud Cover on Recent Surface Temperature Trends in the Arctic. *Journal of Climate*, *21*(4), 705–715. doi:10.1175/2007JCLI1681.1

Lükewille, A., & Wright, R. F. (1997). Experimentally increased soil temperature causes release of nitrogen at a boreal forest catchment in southern Norway. *Global Change Biology*, 13–21. Retrieved from http://onlinelibrary.wiley.com/doi/10.1046/j.1365-2486.1997.00088.x/full

Mack, M.C., Schuur, E.A.G., Bret-Harte, M.S., Shaver, G.R., Chapin III, F.S., (2004). Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. Nature 431, 440e443

Magnússon, Árni & Vídalín, Páll. (1918-1921) Jarðabók. Annað bindi [The farm register of Iceland. 2nd Volume], Copenhagen (Originally published in 1708).

McArdle, B., & Anderson, M. (2001). Fitting multivariate models to community data: a comment on distance-based redundancy analysis. *Ecology*, *82*(1), 290–297. Retrieved from http://www.esajournals.org/doi/abs/10.1890/0012-9658(2001)082[0290:FMMTCD]2.0.CO;2

McGuire, A., & Anderson, L. (2009). Sensitivity of the carbon cycle in the Arctic to climate change. *Ecological …*, *79*(4), 523–555. Retrieved from http://www.esajournals.org/doi/abs/10.1890/08-2025.1

McHale, P., Mitchell, M., Raynal, D., & Bowles, F. (1996). Increasing soil temperature in a northern hardwood forest: effects on elemental dynamics and primary productivity. Retrieved from http://www.treesearch.fs.fed.us/pubs/13406

Meehl, G. a., Washington, W. M., Arblaster, J. M., Hu, A., Teng, H., Tebaldi, C., … White, J. B. (2012). Climate System Response to External Forcings and Climate Change Projections in CCSM4. *Journal of Climate*, *25*(11), 3661–3683. doi:10.1175/JCLI-D-11-00240.1

Mokany, K., Raison, R. J., & Prokushkin, A. S. (2006). Critical analysis of root : shoot ratios in terrestrial biomes. *Global Change Biology*, *12*(1), 84–96. doi:10.1111/j.1365-2486.2005.001043.x

Olofsson, J. (2006). Short-and long-term effects of changes in reindeer grazing pressure on tundra heath vegetation. *Journal of Ecology*, *94*(2), 431–440. Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2745.2006.01100.x/full

Ordoñez, J. C., van Bodegom, P. M., Witte, J.-P. M., Wright, I. J., Reich, P. B., & Aerts, R. (2009). A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. *Global Ecology and Biogeography*, *18*(2), 137–149. doi:10.1111/j.1466-8238.2008.00441.x

Óskarsson, B. V., Riishuus, M. S., & Arnalds, Ó. (2012). Climate-dependent chemical weathering of volcanic soils in Iceland. *Geoderma*, *189-190*, 635–651. doi:10.1016/j.geoderma.2012.05.030

Overland, J. E., & Wang, M. (2010). Large-scale atmospheric circulation changes are associated with the recent loss of Arctic sea ice. *Tellus A*, *62*(1), 1–9. doi:10.1111/j.1600-0870.2009.00421.x

Parkhurst, D. (1994). Diffusion of CO2 and other gases inside leaves. *New Phytologist*, (65). Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.1469-8137.1994.tb04244.x/abstract

Parmesan, C. (2006). Ecological and Evolutionary Responses to Recent Climate Change. *Annual Review of Ecology, Evolution, and Systematics*, *37*(1), 637–669. doi:10.1146/annurev.ecolsys.37.091305.110100

Parry, M., Canziani, O., & Palutikof, J. (2007). Climate change 2007: The physical science basis. *Climate Change*. Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:IPCC,+Summary+for+Policym akers#3

Peñuelas, J, Poulter, B, Sardans, J, Ciais, P, van der Velde, M, Bopp, L, Boucher, O, Godderis, Y, Hinsinger, P, Llusia, J, Nardin, E, Vicca, S, Obersteiner, M & Janssens, I.A. (2013). Human-induced nitrogen-phosphorus imbalances alter natural and managed ecosystems across the globe. Nature Communications 4, 2934, doi: 10.1038/ncomms3934.

Rautiainen, P., Koivula, K., & Hyvärinen, M. (2004). The effect of within-genet and between-genet competition on sexual reproduction and vegetative spread in Potentilla anserina ssp. egedii. *Journal of Ecology*, 505–511. Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.0022-0477.2004.00878.x/full

Reich, P. B., Ellsworth, D. S., & Walters, M. B. (1998). Leaf structure (specific leaf area) modulates photosynthesis-nitrogen relations: evidence from within and across species and functional groups. *Functional Ecology*, *12*(6), 948–958. doi:10.1046/j.1365-2435.1998.00274.x

Reich, P. B., & Oleksyn, J. (2004). Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(30), 11001–6. doi:10.1073/pnas.0403588101

Reich, P. B., Walters, M. B., & Ellsworth, D. S. (1997). From tropics to tundra: global convergence in plant functioning. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(25), 13730–4. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=28374&tool=pmcentrez&renderty pe=abstract

Reich, P. B., Wright, I. J., & Lusk, C. H. (2007). Predicting leaf physiology from simple plant and climate attributes: a global GLOPNET analysis. *Ecological Applications : A Publication of the Ecological Society of America*, *17*(7), 1982–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/17974336

Riahi, K., Rao, S., Krey, V., Cho, C., Chirkov, V., Fischer, G., … Rafaj, P. (2011). RCP 8.5—A scenario of comparatively high greenhouse gas emissions. *Climatic Change*, *109*(1-2), 33–57. doi:10.1007/s10584-011-0149-y

Sardans, J., Janssens, I. a., Alonso, R., Veresoglou, S. D., Rilling, M. C., Sanders, T., … Peñuelas, J. (n.d.). Elemental composition of European forest tree species results from evolutionary traits and the present environmental and competitive conditions, 1–32.

Sardans, J., & Peñuelas, J. (2012). The role of plants in the effects of global change on nutrient availability and stoichiometry in the plant-soil system. *Plant Physiology*, *160*(4), 1741–61. doi:10.1104/pp.112.208785

Screen, J. a, & Simmonds, I. (2010). The central role of diminishing sea ice in recent Arctic temperature amplification. *Nature*, *464*(7293), 1334–7. doi:10.1038/nature09051

Sellers, P., Hall, F., Kelly, R., & Black, A. (1997). BOREAS in 1997: Experiment overview, scientific results, and future directions. *Journal of Geophysical …*, *102*(97), 731–769. Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:BOREAS+in+1997:+Experime nt+overview,+scientific+results,+and+future+directions#0

Serreze, M., Barrett, A., & Stroeve, J. (2009). The emergence of surface-based Arctic amplification. *The Cryosphere Discuss*, *3*(1), 11–19. Retrieved from http://www.the-cryosphere.net/3/11/2009/tc-3-11-2009.pdf

Shi, F. S., Wu, Y., Wu, N., & Luo, P. (2010). Different growth and physiological responses to experimental warming of two dominant plant species Elymus nutans and Potentilla anserina in an alpine meadow of the eastern Tibetan Plateau. *Photosynthetica*, *48*(3), 437–445. doi:10.1007/s11099-010-0058-8

Sigbjörnsson, R., Snæbjörnsson, J. T., Higgins, S. M., Halldórsson, B., & Ólafsson, S. (2008). A note on the M w 6.3 earthquake in Iceland on 29 May 2008 at 15:45 UTC. *Bulletin of Earthquake Engineering*, *7*(1), 113–126. doi:10.1007/s10518-008-9087-0

Sistla, S. a., Asao, S., & Schimel, J. P. (2012). Detecting microbial N-limitation in tussock tundra soil: Implications for Arctic soil organic carbon cycling. *Soil Biology and Biochemistry*, *55*, 78–84. doi:10.1016/j.soilbio.2012.06.010

Soudzilovskaia, N. a, Elumeeva, T. G., Onipchenko, V. G., Shidakov, I. I., Salpagarova, F. S., Khubiev, A. B., … Cornelissen, J. H. C. (2013). Functional traits predict relationship between plant abundance dynamic and long-term climate warming. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(45), 18180–4. doi:10.1073/pnas.1310700110

Stocker, D. (2013). Climate change 2013: The physical science basis. *… of the Intergovernmental Panel on Climate Change, …*. Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Climate+Change+2013:+The+ Physical+Science+Basis#0

Tobler, D. J., Stefánsson, A., & Benning, L. G. (2008). In-situ grown silica sinters in Icelandic geothermal areas. *Geobiology*, *6*(5), 481–502. doi:10.1111/j.1472-4669.2008.00179.x

Vande Velde, Katherine (2014), Changes in plant productivity and stocks of carbon and nitrogen along natural soil temperature gradients in Iceland (Hveragerði).

Violle, C., Navas, M.-L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., & Garnier, E. (2007). Let the concept of trait be functional! *Oikos*, *116*(5), 882–892. doi:10.1111/j.2007.0030-1299.15559.x

Vitousek, P., Mooney, H., Lubchenco, J., & Melillo, J. (1997). Human domination of Earth's ecosystems. *Science*, *277*(July). Retrieved from http://www.sciencemag.org/content/277/5325/494.short

Wal, R. Van der, Bardgett, R., Harrison, K., & Stien, A. (2004). Vertebrate herbivores and ecosystem control: cascading effects of faeces on tundra ecosystems. *Ecography*, *2*(September 2003). Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/j.0906-7590.2004.03688.x/full

Way, D. a, & Oren, R. (2010). Differential responses to changes in growth temperature between trees from different functional groups and biomes: a review and synthesis of data. *Tree Physiology*, *30*(6), 669–88. doi:10.1093/treephys/tpq015

Westoby, M. (1998). A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant and Soil*, 213–227. Retrieved from http://link.springer.com/article/10.1023/A:1004327224729

Wiedermann, R., Karrer, G. Ökologische Zeigerwerte, www.boku.ac.at, last consulted 14/04/2014.

Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., … Villar, R. (2004). The worldwide leaf economics spectrum. *Nature*, *428*(6985), 821–7. doi:10.1038/nature02403

Young, H. S., McCauley, D. J., & Dirzo, R. (2011). Differential responses to guano fertilization among tropical tree species with varying functional traits. *American Journal of Botany*, *98*(2), 207–14. doi:10.3732/ajb.1000159

Zakharova, O. K., & Spichak, V. V. (2012). Geothermal fields of Hengill Volcano, Iceland. *Journal of Volcanology and Seismology*, *6*(1), 1–14. doi:10.1134/S074204631201006X

Zhang, S.-B., Zhang, J.-L., Slik, J. W. F., & Cao, K.-F. (2012). Leaf element concentrations of terrestrial plants across China are influenced by taxonomy and the environment. *Global Ecology and Biogeography*, *21*(8), 809–818. doi:10.1111/j.1466-8238.2011.00729.x

Zhu, J.-K. (2003). Regulation of ion homeostasis under salt stress. *Current Opinion in Plant Biology*, *6*(5), 441–445. doi:10.1016/S1369-5266(03)00085-2

Appendix

Appendix 1: Field setup
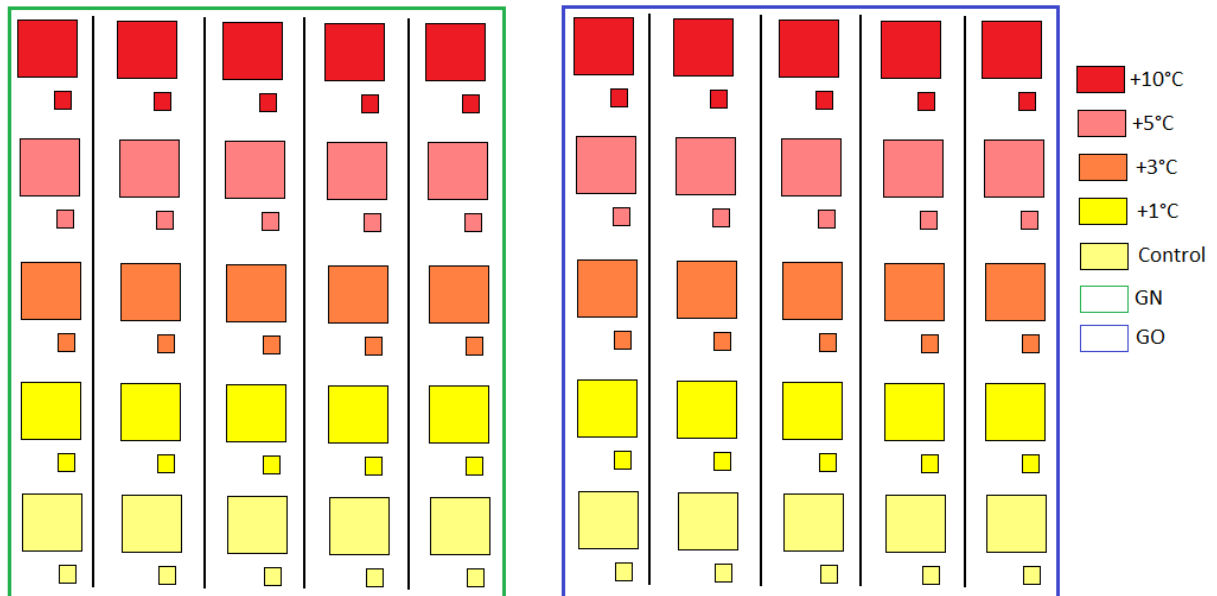
Figure A1: *Shows a schematic overview of the field setup near the university campus of Hveragerði or the new grassland (GN) and the valley of Reykjadalur or the old grassland (GO). The figure shows the five different temperature levels within all ten transects, five transects belonging to GN and five to GO. The large squares represent the main plots and the smaller squares refer to the subplots.*

Appendix 2: Average species coverage
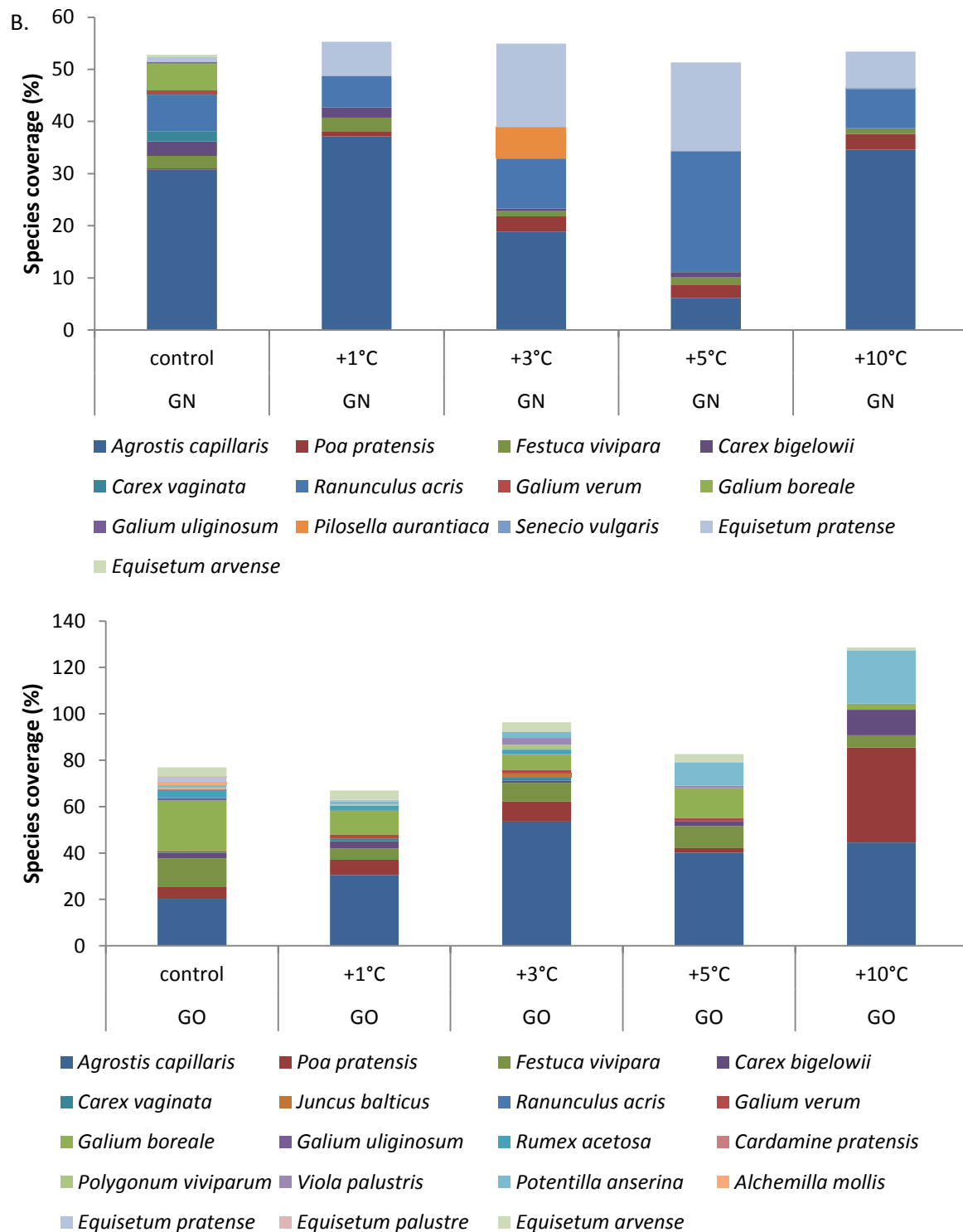
Figure A2: *Average species coverage per soil temperature level for both new grassland (a) and old grassland (b). The whole bar shard gives the average total vascular plant cover per soil temperature level. The legend underneath both bar chards gives all the species occurring in the corresponding grassland.*

Appendix 3: Overview of statistical outputs

Table A3(1): *Overview of the statistical outputs for the effects of soil warming on community structure and species composition. Significance notation; ○: 0.10<p<0.05, ●: p<0.05, ●●: p<0.01, ●●●: p<0.001. Only the relevant comparisons with a p-value smaller than 0.1 are included in the table. The R^2-value is given for the final ANCOVA model.*

Response variable		Explanatory variable	p-value	R^2	Test
Total coverage		Grassland:Temperature	●		ANCOVA
		Grassland type	●●●		
	GN	Temperature	NS		
	GO	Temperature	●●	0.30	
Coverage individual species		Grassland:Temperature	NS		MANOVA
		Grassland type	●●●		
		Temperature	●		
Shannon-Wiener index		Grassland:Temperature	NS		ANCOVA
		Grassland type	●●●		
		Temperature	●●	0.51	
Species number per plot		Grassland:Temperature	NS		ANCOVA
		Grassland type	●●●		
		Temperature	●●●	0.54	
Evenness		Grassland:Temperature	NS		ANCOVA
		Grassland type	●●		
		Temperature	NS	0.16	
Weighted Ellenberg's indicator values		Grassland:Temperature	NS		MANOVA
		Grassland type	●		
		Temperature	●●		
Weighted Moisture preference indicator value		Grassland:Temperature	NS		ANCOVA
		Grassland type	●		
		Temperature	○	0.10	
Weighted Thermal optimum preference indicator value		Grassland:Temperature	NS		ANCOVA
		Grassland type	●		
		Temperature	NS	0.11	
Weighted Acidity preference indicator value		Grassland:Temperature	NS		ANCOVA
		Grassland type	NS		
		Temperature	NS	0.01	
Weighted Nitrogen content preference indicator value		Grassland:Temperature	○		ANCOVA
		Grassland type	NS		
		Temperature	●●●	0.27	

Table A3(2): *Overview of the statistical outputs for the effects of soil warming on plant height. Significance notation; ○: 0.10<p<0.05, ●: p<0.05, ●●p<0.01, ●●●: p<0.001. The R²-value is given for the final ANOVA or ANCOVA model. Only the relevant comparisons with a p-value smaller than 0.1 are included in the table. C indicates the control plots without added soil temperature.*

Species	Comparison	Explanatory variable	p-value	R²	Test
Agrostis capillaris		Grassland:Temperature	NS		ANCOVA
		Grassland	NS		
		Temperature	●●●	0.17	
Poa pratensis		Grassland:Temperature	●●●		ANOVA
		Grassland	●●●		
	GN	Temperature	NS	0.01	ANCOVA
	GN	Temperature	●	0.08	ANOVA
	C - +3°C	Temperature	●		
	GO	Temperature	●●●	0.22	ANCOVA
Ranunculus acris		Grassland:Temperature	●●		ANOVA
		Grassland	●●●		
	GN	Temperature	NS	0.004	ANCOVA
	GN	Temperature	●●●	0.15	ANOVA
	C - +5°C	Temperature	●●●		
	C - +3°C	Temperature	●		
	+1°C - +3°C	Temperature	●		
	+3°C - +10°C	Temperature	●		
	GO	Temperature	●	0.05	ANCOVA

Table A3(3): *Overview of the multivariate outputs for the effects of soil warming on leaf stoichiometry . Significance notation; ○: 0.10<p<0.05, ●: p<0.05, ●●: p<0.01, ●●●: p<0.001.*

Species		Explanatory variable	p-value	Test
All		Grassland:Species	●●●	MANOVA
		Species:Temperature	●●	
Agrostis capillaris		Grassland:Temperature	●	MANOVA
		Grassland	●●●	
	GN	Temperature	NS	
	GO	Temperature	●	
Poa pratensis		Grassland:Temperature	●●●	MANOVA
		Grassland	●●●	
	GN	Temperature	●●	
	GO	Temperature	●	
Ranunculus acris		Grassland:Temperature	●	MANOVA
		Grassland	●●	
	GN	Temperature	NS	
	GO	Temperature	●●	

Table A3(4)↓: *Table with the score outputs of the PCA on the leaf element concentration. The values are the of the elements on the corresponding PC of Figure 8 expressing the relative importance of each element for this PC.*

Element	PC1	PC2	PC3
N	0.30082	-0.13247	0.35912
C	-0.01284	0.01111	0.01775
Ca	1.17342	0.10916	-0.17500
Mg	0.73421	-0.05611	0.06060
K	0.28898	-0.21119	0.14027
Na	1.21969	0.06457	-0.21119
P	0.46594	-0.05322	0.01502
S	0.50202	-0.28513	0.10833
Fe	0.20894	-0.10825	-0.01081
Mn	-0.08111	0.92001	-0.09350
Zn	0.58104	-0.08172	-0.00176
Cu	0.59551	0.16470	0.34999

Table A3(5): *Overview of the output of the ANCOVAs performed on the scores of the three first PC of the multivariate analysis for leaf element concentration. Significance notation; ○: 0.10<p<0.05, ●: p<0.05, ●●: p<0.01, ●●●: p<0.001. The R²-value is given for the final ANOVA or ANCOVA model. Only relevant comparisons with a p-value smaller than 0.1 are included in the table.*

PC	Species	Comparison	Explanatory variable	p-value	R²	Test
PC1	All		Grassland:Species	NS		ANCOVA
			Species:Temperature	NS		
			Grassland:Temperature	NS		
			Temperature	●		
			Grassland	●		
			Species	●●●	0.92	
		Ran-Agr	Species	●●●		ANOVA
		Ran-Poa	Species	●●●		
PC2	All		Grassland:Species	●●●		ANCOVA
			Species:Temperature	NS		
		Agr-Poa	Species	●●●		ANOVA
		Agr-Ran	Species	●●●		
		Poa-Ran	Species	●		
	Agrostis capillaris		Grassland:Temperature	NS		ANCOVA
			Grassland	●●●		
			Temperature	NS	0.54	
	Poa pratensis		Grassland:Temperature	NS		ANCOVA
			Grassland	●●●		
			Temperature	NS	0.44	
	Ranunculus acris		Grassland:Temperature	NS		ANCOVA
			Grassland	●●●		
			Temperature	NS	0.43	
PC3	All		Grassland:Species	NS		ANCOVA
			Species:Temperature	NS		
			Grassland:Temperature	NS		
			Temperature	NS		
			Grassland	NS		
			Species	NS		

Table A3(6): *Overview of the multivariate output for the effects of soil warming on the leaf chemical element:N ratios. Significance notation; ○: 0.10<p<0.05, ●: p<0.05, ●●: p<0.01, ●●●: p<0.001.*

Species		Explanatory variable	p-value	Test
All		Grassland:Species	●●●	MANOVA
		Species:Temperature	●	
Agrostis capillaris		Grassland:Temperature	○	MANOVA
		Grassland	●●●	
		Temperature	NS	
Poa pratensis		Grassland:Temperature	●●	MANOVA
		Grassland	●●●	
	GN	Temperature	●●	
	GO	Temperature	●●	
Ranunculus acris		Grassland:Temperature	●	MANOVA
		Grassland	●●●	
	GN	Temperature	NS	
	GO	Temperature	●	

Table A3(7): *Table with the loading output of the PCA on the leaf element:N concentration. The values are the coordinates of the elements on the corresponding PC of Figure 9 expressing the relative importance of each element for this PC.*

Ratio	PC1	PC2	PC3
C:N	-0.18845	-0.088319	0.46163
Ca:N	1.07035	0.009552	0.03192
Mg:N	0.60810	-0.091723	0.02875
K:N	0.05381	-0.279306	0.26126
Na:N	1.24217	0.018865	-0.27523
P:N	0.30550	-0.164225	0.47433
S:N	0.36731	-0.312361	0.04641
Fe:N	0.18001	-0.131305	0.03824
Mn:N	-0.19277	-0.785631	-0.19698
Zn:N	0.54808	-0.101277	0.06462
Cu:N	0.44062	0.256711	0.27449

Table A3(8): *Overview of the output of the ANCOVAs performed on the scores of the three first principal components (PC) of the multivariate analysis for leaf element:N ratios. Significance notation; ○: 0.10<p<0.05, ●: p<0.05, ●●p<0.01, ●●●: p<0.001. The R²-value is given for the final ANOVA or ANCOVA model. Only the relevant comparisons with a p-value smaller than 0.1 are included in the table.*

PC	Species	Comparison	Explanatory variable	p-value	R²	Test
PC1	All		Grassland:Species	NS		ANCOVA
			Species:Temperature	NS		
			Grassland:Temperature	NS		
			Temperature	NS		
			Grassland	NS		
			Species	●●●	0.85	
		Ran-Agr	Species	●●●		ANOVA
		Ran-Poa	Species	●●●		
PC2	All		Grassland:Species	●●●		ANCOVA
			Species:Temperature	NS		
		Agr-Poa	Species	●●●		ANOVA
		Agr-Ran	Species	●●●		
	Agrostis capillaris		Grassland:Temperature	NS		ANCOVA
			Grassland	●●●		
			Temperature	NS	0.39	
	Poa pratensis		Grassland:Temperature	NS		ANCOVA
			Grassland	●●●		
			Temperature	NS	0.23	
	Ranunculus acris		Grassland:Temperature	NS		ANCOVA
			Grassland	●●●		
			Temperature	●	0.43	
PC3	All		Grassland:Species	NS		ANCOVA
			Species:Temperature	NS		
			Grassland:Temperature	NS		
			Temperature	NS		
			Grassland	NS		
			Species	●●●	0.35	
		Poa-Agr	Species	●●●		ANOVA
		Poa-Ran	Species	●●●		

Table A3(9): *Overview of the statistical outputs for the effects of soil warming on SLA. Significance notation; ○: 0.10<p<0.05, ●: p<0.05, ●●: p<0.01, ●●●: p<0.001. The R²-value is given for the final ANOVA model. Only the relevant comparisons with a p-value smaller than 0.1 are included in the table. C indicates the control plots without added soil temperature.*

Species	Comparison	Explanatory variable	p-value	R²	Test
Agrostis capillaris		Grassland:Temperature	NS		ANOVA
		Grassland	NS		
		Temperature	NS	0.02	
Poa pratensis		Grassland:Temperature	NS		ANOVA
		Grassland	●●●		
		Temperature	NS	0.08	
Ranunculus acris[1]		Grassland:Temperature	○		ANOVA
		Grassland	●●		
		Temperature	●	0.13	
	C - +3°C	Temperature	○		
	C - +10°C	Temperature	●		

(1) Dataset was transformed by taking the logarithm of all SLA-values which resulted in a normal distribution.

Table A3(10): *Overview of the statistical outputs for the effects of soil warming on stomatal conductivity. Significance notation; ○: 0.10<p<0.05, ●: p<0.05, ●●: p<0.01, ●●●: p<0.001. Only the relevant comparisons with a p-value smaller than 0.1 are included in the table. The R²-value is given for the final ANOVA model.*

Species	Comparison	Explanatory variable	p-value	R²	Test
Agrostis capillaris		Grassland:Temperature	NS		ANOVA
		Grassland	●●●		
		Temperature	○	0.26	
Poa pratensis		Grassland:Temperature	NS		ANOVA
		Grassland	●		
		Temperature	NS	0.10	
Ranunculus acris		Grassland:Temperature	NS		ANOVA
		Grassland	●●●		
		Temperature	NS	0.27	

Appendix 4: Extra figures species composition and community structure
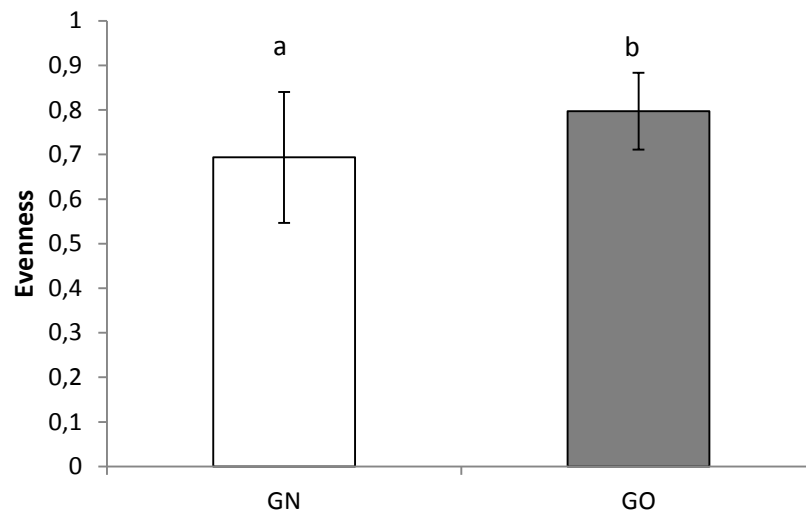
Figure 4A: *Average evenness for the data of the two grassland types, GO and GN. Error bars indicate the standard deviation with n = 25. Letters show statistical significant differences. (a-b: p<0.01)*

Appendix 5: Weighted Ellenberg's values

Table A5: *Overview table of the occurring species on the study site and their Ellenberg's indicator values for N content, pH, optimal temperature and moisture. An increase of value stands for an increase of the corresponding parameter except for pH. A rising score for pH means the species prefers less acid or more basic environments. (Source: Hill et al., 1999; Ökologische Zeigerwerte).*

| Species	Ellenberg's indicator value			
	Nitrogen content	pH	Optimal temperature	Moisture
Agrostis capillaris	4	4	5	5
Poa pratensis	5	6	5	5
Festuca vivipara	2	3	NA	6
Carex bigelowii	2	2	3	5
Carex vaginata	3	6	3	3
Juncus balticus	2	5	4	8
Ranunculus acris	4	6	5	6
Galium verum	2	6	6	4
Galium boreale	3	7	6	5
Galium uliginosum	4	6	5	9
Rumex acetosa	4	5	5	5
Pilosella aurantiaca	2	6	3	4
Senecio vulgaris	7	7	6	5
Cardamine pratensis	8	5	5	4
Polygonum viviparum	2	4	2	5
Viola palustris	2	3	5	9
Potentilla anserina	6	7	6	7
Alchemilla mollis	3	4	5	5
Equisetum pratense	4	5	4	7
Equisetum palustre	3	6	5	8
Equisetum arvense	6	6	5	6

Appendix 6: Extra figures plant traits
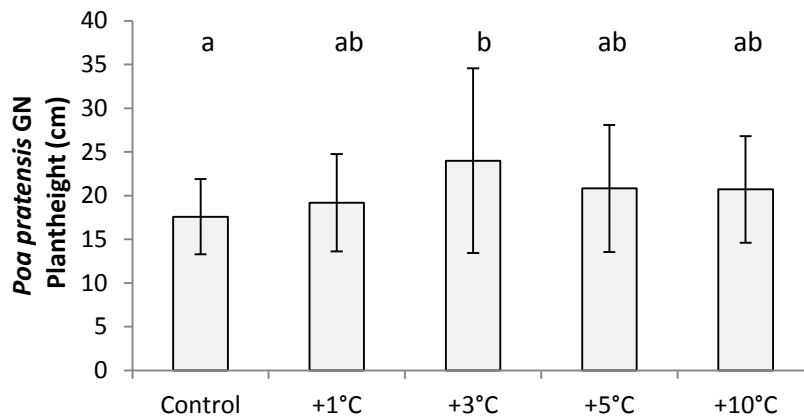
Figure A6(1): *Average plant height of Poa pratensis in the different soil temperature levels of GN. Error bars indicate the standard deviation of the average plant height with n = 5. The plots of +3°C added soil warmth differed significantly from the control plots (p=0.03). Letters show statistical significant differences. (a-b: p < 0.05).*
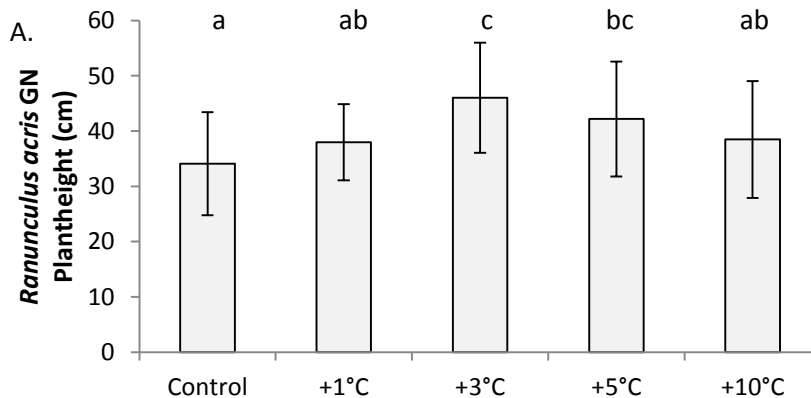

Figure 6A(2): *Average plant height of Ranunculus acris for GO. Error bars indicate the standard deviation of the average plant height with n = 5. For GN, the control plot differed significantly from +5°C (p=0.04) and +3°C (p<0.001). +3°C contains the highest Ranunculus individuals because this plots also differed significantly from the plots of +1°C (p<0.05) and +10°C (p<0.05). Letters show statistical significant differences.*
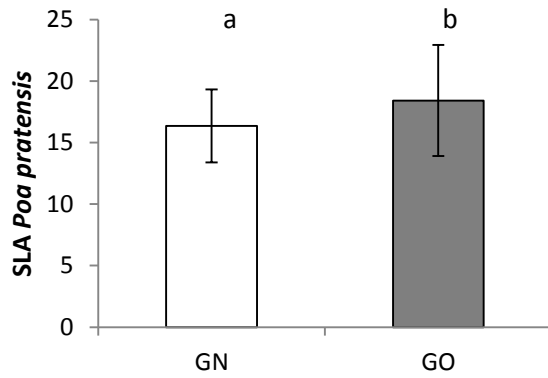
Figure 6A(3): *Average Specific Leaf Area (SLA) of Poa pratensis for both grassland types. Error bars indicate standard deviation. Letters show statistical significant differences (a – b: p<0.001).*
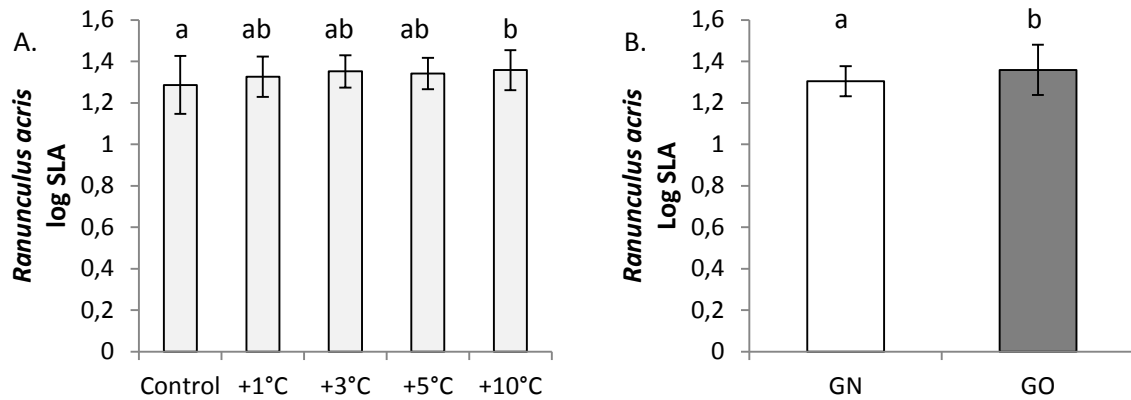
Figure 6A(4): *(A) Average log SLA of Ranunculus acris at different soil temperature levels. Error bars indicate the standard deviation for the averages of log SLA with n = 30 for the temperature level differences and Letters show statistical significant differences (a – b: p<0.05)(B) Average log SLA of Ranunculus acris at different grassland types. Error bars indicate the standard deviation for the averages of log SLA with n = 75 for the grassland types. Letters show statistical significant differences (a – b: p<0.01).*
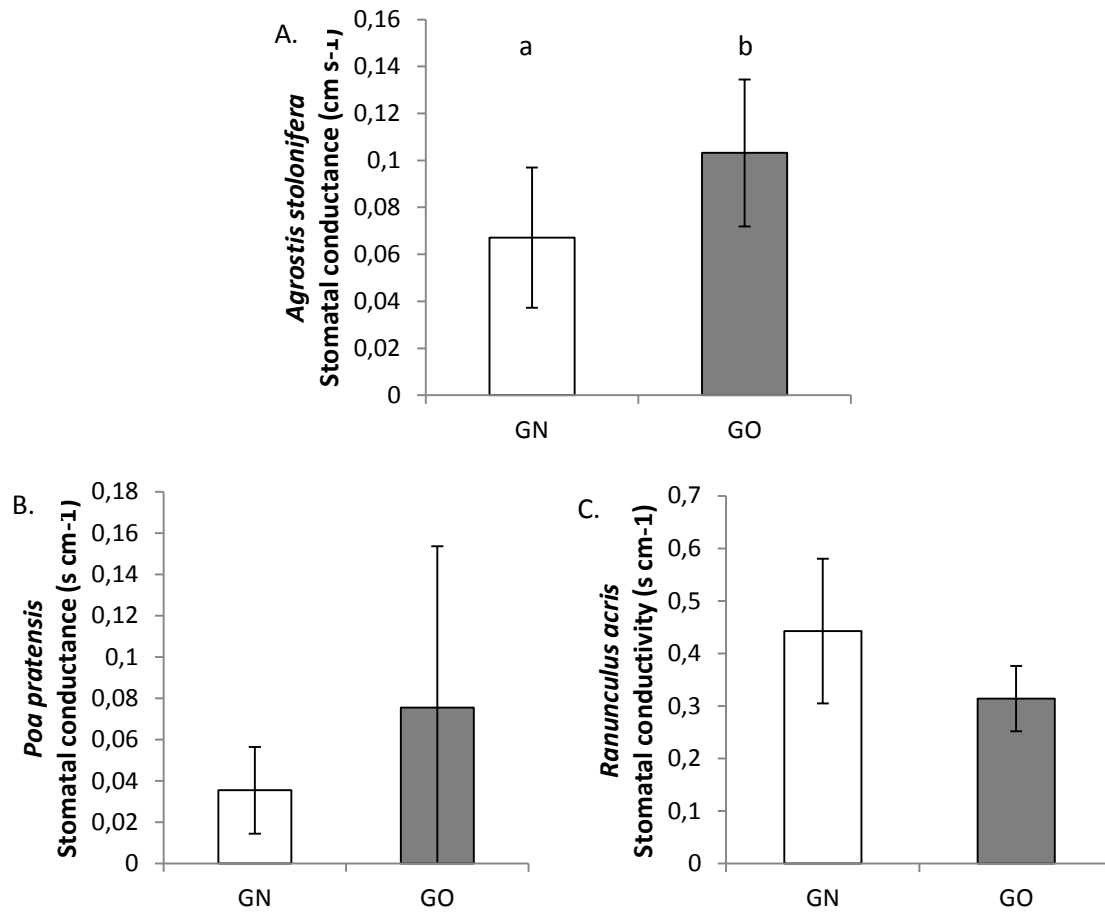
Figure 6A(5): *Bar charts of the average stomatal conductivity of the three target species. Error bars indicate the standard deviation for the average stomatal conductance with n = 25. (A) Ranunculus acris; letters show statistical significant differences (a-b: p<0.001), (B) Poa pratensis; letters show statistical significant differences (a-b: p<0.04), (C) Ranunculus acris; letters show statistical significant differences (a-b: p<0.001). Note the different scale for the different species.*