Masterthesis submitted to obtain the degree of Master in Biology, specialisation
Biodiversity: conservation and restoration

The effect of temperature and nitrogen on plant community structure in Icelandic subarctic grassland ecosystems

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Abstract

Temperatures are rising, especially at high northern altitudes, which makes these ecosystems particularly interesting to study. Changes in plant community structure has been studied in several short-term warming experiments, but long-term studies are lacking. These are needed because changes in plant community structure are slow and many biogeochemical processes, which also influence community structure, are altered by temperature at different rates.

This thesis addresses long-term and short-term changes in plant community structure in response to changes in soil temperature along natural soil warming gradients in an Icelandic subarctic grassland ecosystem. The aspects of the plant community structure that were investigated are species richness, species evenness, Shannon-Wiener diversity index, species composition and species presence. In addition to temperature, soil water content (SWC) and nitrogen (N) were considered in the statistical analyses. N availabilities were incorporated in this study, because, as Northern ecosystems are typically nitrogen-limited, increases in nitrogen availability induced by higher temperature may mask the true temperature effect. Soil water content (SWC) was considered, as this has been found to decrease with warming and can alter plant community structure.

Species richness decreased exponentially along the soil warming gradient. The species that disappeared along the warming gradient were cold-adapted species, which in the short term were replaced by ruderal species, and in the long term by cosmopolitan species. Soil warming was found to increase N cycling and decrease SWC, but these were not the main drivers behind the changes in vegetation structure along the temperature gradient. SWC had no effect on plant community structure and N availabilities had only minor effects.

Generally, global warming is expected to induce local extinctions in subarctic ecosystems, but the decrease in species number is expected to be less severe, because Iceland has a limited species pool due to its isolated location. On the short term, warming may promote the spread of invasive species, threatening natural ecosystems. On the long-term, systems will find a new equilibrium.

Samenvatting

Er wordt verwacht dat de gemiddelde globale temperaturen 2 – 5 °C zullen stijgen dor klimaatverandering aan het einde van de 21ste eeuw. In ecosystemen in het hoge noorden lopen de verwachtte temperatuurstijgingen zelfs op tot meer dan 8 °C, wat hen bijzonder interessant maakt om te bestuderen. Er zijn reeds verscheidene korte termijn studies betreffende het effect van verwarming op vegetatiesamenstelling, maar algemene trends en lange-termijn studies ontbreken. Deze zijn van belang omdat verandering in vegetatiesamenstelling een traag proces is en temperatuur verscheidene biogeochemische processen beïnvloed, die elk ook invloed hebben op de vegetatiesamenstelling en op elkaar.

Deze thesis gaat over de veranderingen in vegetatiesamenstelling als reactie op veranderingen in bodemtemperatuur over een natuurlijke gradiënt in bodemtemperatuur in een IJslands subarctisch grasland ecosysteem. De aspecten van de vegetatiesamenstelling die in beschouwing worden genomen zijn soortenrijkdom, soorten ‘evenness’, diversiteit, berekend a.d.h.v. de Shannon-Wiener index, soortensamenstelling en de aanwezigheid van soorten. Stikstofbeschikbaarheid is opgenomen in de analyse omdat subarctische ecosystemen nutriënten gelimiteerd zijn en veranderingen in stikstofbeschikbaarheid hierdoor het temperatuureffect kunnen maskeren. Bodemvochtigheid werd ook toegevoegd aan de analyse omdat opwarming het vochtgehalte van de bodem doet afnemen, wat ook effect kan hebben op de soortensamenstelling.

De soortenrijkdom nam exponentieel af met toenemende bodemtemperatuur. De soorten die verdwenen waren koud-geadapteerde soorten, die op korte termijn vervangen werden door ruderale soorten, maar op lange termijn door kosmopolieten. Bodemverwarming deed de stikstofcyclering toenemen en de bodemvochtigheid dalen, zoals verwacht, maar dit bleek niet de reden dat de vegetatiesamenstelling veranderd over de temperatuurgradiënt. Het vochtgehalte van de bodem had geen effect op de vegetatiesamenstelling en stikstofbeschikbaarheden hadden slechts een kleine invloed.

Over het algemeen wordt er verwacht dat klimaatopwarming lokale extincties zal induceren in subarctische ecosystemen, maar de afname in soortenrijkdom wordt verwacht minder drastisch te zijn, aangezien IJsland een beperkte soortenpoel heeft door zijn geïsoleerde locatie.

Layman's summary

For several decades now, scientists have been trying to understand the effects of climate change on natural ecosystems. At high northern altitudes temperatures are rising more rapidly than anywhere else in the world, which makes these ecosystems particularly interesting to study. Changes in plant community structure has been studied in several short-term warming experiments, but long-term studies are lacking. These are needed because changes in plant community structure are slow.

This thesis is about changes in plant community structure in response to soil warming. The study was done in two naturally warmed grasslands in Iceland; one that has been warmed for 7 and the other for more than 50 years. Finally, since warming can affect other environmental factors, we tried to relate these indirect temperature effects to the changes in plant community structure.

Our results showed that with increasing temperature, less species could be found. The species that disappeared along the warming gradient were cold-adapted species, which in the short term were replaced by species who take advantage of disturbed systems, and in the long term by species that can be found all over the world. Soil warming was found to increase nitrogen cycling and decrease soil water content, but these were not the main drivers behind the changes in plant community structure along the temperature gradient. Soil water content had no effect on plant community structure and nitrogen availabilities had only minor effects.

Generally, global warming is expected to induce local extinctions in these kind of ecosystems, but the decrease in species number is expected to be less severe, because Iceland has a limited species pool due to its isolated location.

1. Introduction

1.1 Climate change

Fossil fuel combustion, land-use change and other anthropogenic activities have increased atmospheric concentrations of greenhouse gases such as methane (CH_4), carbon dioxide (CO_2) and nitrous oxide (N_2O) dramatically since the beginning of the industrial revolution (IPCC 2013). These gases have the capacity to trap heat in the atmosphere by absorbing infrared radiation emitted from the earth's surface (Rustad et al. 2001). This greenhouse effect is expected to increase global mean air temperature by 2 – 5 °C by the end of the 21st Century compared to preindustrial values (Rogelj et al. 2014). This change of the earth's climate inevitably changes our natural ecosystems. Even though the effects of temperature on ecosystems are much more investigated than those of elevated CO_2 concentrations, predictions on the matter are much more difficult. This is because temperature impacts virtually all chemical and biological processes, whereas the direct influence of CO_2 is almost entirely limited to photosynthesis (Koch & Mooney 1997). Moreover, the projected temperature increase shows greater spatial and temporal variations compared to the relatively uniform and continued increase in atmospheric CO_2 (Shaver et al. 2000). Especially at northern high latitudes, temperatures are rising more rapidly than anywhere else in the world and are expected to increase by more than 8 °C by the end of the century (Riahi et al. 2011; Meehl et al. 2012; IPCC 2013).

1.2 Observed effects of short-term warming on vegetation composition

Short-term studies have shown that species diversity, evenness, and richness decreased in response to warming (walker et al. 2006). Walker et al. (2006) stated that the decrease in evenness indicates a change in the dominance structure of the plant communities, where fewer species produce a larger proportion of the cover and that this consistent response may represent a first step toward local species extinction. Changes in community composition have been ascribed to alterations in competitive hierarchies and relative dominance of different plant species under warming (Chapin et al. 1995; Harte & Shaw 1995; Klanderud & Totland 2005; Niu & Wan 2008). Because of their intrinsic thermal sensitivity, plant species and functional groups can show different responses to warming, contributing to the shifts in the competitive ability and relative dominance among species and functional groups (Chapin et al. 1995; Harte & Shaw 1995; Walker et al. 2006a).

1.3 Temperature effect depends on initial conditions

Shaver et al. (2000) stated that, for many ecosystems, the indirect effects of a temperature increase are likely to be more important than the direct effects. To substantiate this statement, they cited examples of nutrient-limited ecosystems, in which the changes in nutrient cycling induced by temperature were of more substantial influence than temperature itself, and of water-limited ecosystems, in which the drying effect overweighted that of temperature. This indicates that ecosystem responses to warming are strongly affected by initial physical and chemical conditions. Consequently, when trying to understand changes in plant community structure to soil temperature rise, changes in abiotic conditions also need to be monitored.

Many biogeochemical processes have an optimum temperature. Therefore, changes in temperature will cause different responses in different processes, depending on initial temperatures (Shaver et al. 2000). At high northern latitudes, initial conditions are below 0 °C most of the year and growing season temperatures are only a few degrees higher. Even a small increase in average temperature at higher latitudes could increase both the length of the unfrozen period and degree-day accumulations by large percentages (Billings 1987). Therefore, northern ecosystems are particularly vulnerable to temperature changes, which makes them an interesting and useful research topic.

1.4 Indirect warming effects – changes in the nitrogen cycle

1.4.1 The nitrogen cycle in a nutshell

Nitrogen (N) is a crucial element in the plant metabolism, as it is linked to vegetative growth and reproduction. Although the majority of N is located in the atmosphere, plants mainly take up N from the soil in the form of ammonium (NH_4^+) and nitrate (NO_3^-). Inorganic N enters the soil by atmospheric fixation and wet and/or dry deposition. Organic N is recycled within the ecosystem and added to the soil as dead organic N, where soil organisms mineralise it into ammonium (NH_4^+), a process called ammonification or mineralisation. Ammonification occurs under both aerobic and anaerobic conditions. Nitrification, a second and strictly aerobic process, converts ammonium into nitrate (NO_3^-). (Butterbach-Bahl et al. 2011). The N cycle is influenced by many factors such as temperature, water availability, soil type and the composition of plant and microbial communities (Godbold & Lukac 2011; Osman 2013). A schematic representation of the N cycle can be seen in figure 1.

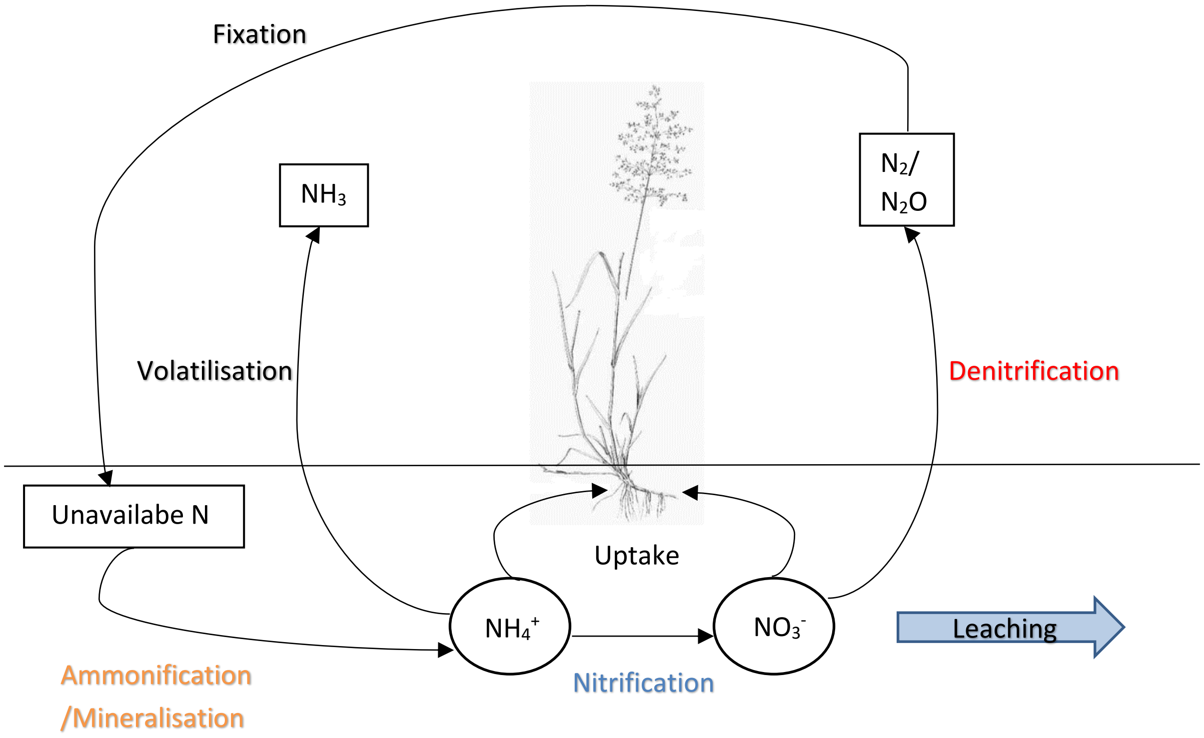


Figure 1: Simplified representation of the nitrogen cycle. Blue represents aerobe processes, red represents anaerobe processes, green represents processes which can occur under both aerobe and anaerobe conditions. Nitrification is strictly aerobe.

1.4.2 Temperature and the nitrogen cycle

At high-latitude ecosystems, decomposition is usually constrained by cold and wet conditions, resulting in soils with high N stocks, but low N availability (Chapin et al. 1995). Warming is known to stimulate the N cycle, as it accelerates and increases many processes such as net N mineralisation and net nitrification (Bai et al. 2013; Rustad et al. 2001). This results in a higher nutrient ($NH_4^{+,}$ NO_3^-) availability (Bai et al. 2013; Jarvis & Linder 2000; Liski et al. 1999), which co-determines plant community structure (Stevens et al. 2004; van den Berg et al. 2011; Clark & Tilman 2008; Song et al. 2011). Changes in nutrient availability induced by temperature may therefore mask the true temperature effect.

Ecosystem responses to warming are also strongly affected by the dominant form of available N in the soil (Shaver et al. 2000). When nitrification is slow and the system has a predominant ammonium economy, no significant loss of N from the ecosystem is expected under warming conditions. If the system has a nitrate economy or a mixed ammonium-nitrate economy, N can be lost from the system by leaching or denitrification and thereby reduce the N stocks (Shaver et al. 2000). Warming effects on N pools and fluxes are interrelated and interdependent. Increased net N mineralization and nitrification as a result of warming, which converts the organic soil N stock (which is largely unavailable for plants) into inorganic N (which is available for plants), may lead to higher N losses from the soil. However, a meta-analysis from Bai et al. (2013) did not show any correlation between soil N stocks and soil warming. This might be because these pools are much larger than the soil inorganic N pool (Galloway et al. 2008) and it may take much longer for them to respond to warming.

To discover a change in nutrient cycling, it is also interesting to investigate the ratio of available N to stored N. When N stocks are low, N availability is also low when the rate of nutrient cycling stays constant. Another way to investigate changes in nutrient cycling is by looking at the nutrient preferences of the species present by calculating the community-weighted Ellenberg indicator value for soil fertility (Ellenberg et al. 1991). The indicator values place plant species on a simple ordinal classification according to their realised ecological niche along an environmental gradient.

1.5 Indirect warming effects – Changes in soil water content

1.5.1 Temperature and soil water content

An additional environmental parameter, which is known to affect plant community structure, is the soil water content (SWC). Plants need different traits to cope with wet or dry conditions. Plants usually do not possess traits for both conditions, so changes in the water regime will favour different plant species and/or plant traits adapted to the area’s water new regime, changing the plant community composition.

Two separate meta-analyses on ecosystem warming effects (Bai et al. 2013; Rustad et al. 2001) showed that in general, SWC was reduced by warming. Bai et al. (2013) believe that the interaction between temperature and other abiotic factors, especially moisture, is an important determinant with regard to ecosystem responses to warming. They observed a greater reduction in SWC in grassland ecosystems compared to other ecosystems, which may offset the temperature effect in this ecosystem type (Brzostek et al. 2012). Since this study was conducted in a grassland, it is important to incorporate SWC in our analysis.

Whether drying induces significant biological or biochemical responses depends largely on the initial hydrological conditions of the site. To disentangle whether observed changes in plant community structure with soil warming are caused by the warming itself, or by drought, it is necessary to look at the environmental preferences of the present plant species. For N, this is done by looking at the community-weighted average Ellenberg value for moisture and investigating whether there is a relation with SWC.

1.5.2 Influence of soil water content on the nitrogen cycle

The N cycle is influenced by water availability. Excess of water in the soil decelerates the cycling of nutrients by inducing anaerobic conditions, since aerobic microbes require oxygen to process N during the nitrification process. Lower SWC caused by warming could therefore promote nitrification and reduce denitrification as a result of increased soil aeration and O_2 content (Smith et al. 2003). The nitrification rate is highest at moderate SWCs (Szukics et al. 2010). When the SWC approaches this optimal value for nitrification as a result of warming, the nitrification rate may increase; however, if initial conditions are dry, it may also decrease (Bai et al. 2013)

Mineralisation rates are also influenced by SWC. Wet or moist soils are found to be positively correlated with N mineralisation rates (Hong 2014; Rustad et al. 2001). This means that, while higher temperatures typically increase N mineralisation, it might be that N mineralisation is inhibited by drought induced by increasing temperature. Changes in the SWC can thus alter nutrient cycling and thereby indirectly change the plant community structure.

1.6 The use of natural geothermal gradients as a proxy for climate change

In this study, we made use of natural geothermal temperature gradients at a high northern latitude, where ecosystems are particularly prone to undergo drastic climatic changes. The use of natural geothermal soil warming gradients has several advantages and forms a valuable addition to other types of warming experiments. Firstly, the permanent nature of natural geothermal soil temperature gradients allows for the study of long-term effects of soil warming on ecosystems and the evolution of ecosystem responses in time, on which there is a large research gap. This is an advantage compared to conventional artificially heated warming experiments, which are usually restricted to the duration of the research project. Short-term warming effects can also be studied in these systems when shifts in the underground streams occur, heating previously unheated areas (Gorman et al. 2015). This allows for comparison between short-term and long-term ecosystem responses, which may be very different from one another due to slow structural changes and interactions between responding processes, nutrient pools and communities (above- and belowground). Long-term studies may on the other hand also validate the results of the many other, mostly short-term climate change studies and experiments.

Further, many studies looking at warming effects on the vegetation composition at high northern latitudes have few warming levels, which are often lower than the predictions for the next century in these areas. The wide range of the temperatures in natural geothermal areas makes it possible to use a regression approach and investigate the shape of the temperature responses and detect threshold temperatures. Moreover, because these wide ranges of temperatures occur on a spatially small area, other environmental variables than temperature, such as precipitation, irradiation, day length, bedrock etc. remain constant (Sigurdsson et al. 2016). Finally, natural geothermal soil warming gradients offer a low-cost alternative for climate manipulation experiments.

It should be noted, however, that there are also certain drawbacks linked to the use of these natural geothermal gradients as a proxy for climate change. The gradients are all located in volcanic areas, and are thus underlain by volcanic soils (Andosols), which have high water holding capacity (Arnalds 2008).

The most important restriction of such geothermal soil warming gradients is the decoupling of soil warming from air warming. Nonetheless, there are studies that indicate that soil warming has a stronger effect on ecosystems than increases in air temperature (Xu & Huang 2000).

1.7 The need of long-term warming studies and the scope of this study

Long-term existing geothermal gradients provide the opportunity to assess the temporal stability of an ecosystem response to warming by looking at slow structural adaptations such as vegetation composition (Beier et al. 2012). Changes in vegetation composition take time because of resilience and longevity of individual plants and population turnover rates of dominant plant species. Changes in plant community structure may subsequently alter biogeochemical processes, nutrient pools and belowground microbial community structure and functioning, which in itself react to warming, interact with one another and occur at different rates (Bai et al. 2013; Bassirirad 2000; Jansson & Berg 1985; MacDonald et al. 1995; Malhi et al. 1990; Meentemeyer 1978; Radujkovic 2016; Raich & Schlesinger 1992; Zak et al. 2003). This leads to feedback, which can be positive or negative (Suttle et al. 2007; Wardle et al. 2003; Zak et al. 2003). All this prolongs ecosystem responses to warming and the magnitude and direction of net changes in the vegetation composition of ecosystems may therefore vary over time. Thus, the principal long-term ecosystem changes may be very different to the short-term ecosystem changes (Shaver et al. 2000). These long-term changes and interactions are still poorly understood. Moreover, many studies focus on one aspect of the ecosystem response to warming, hence, more knowledge is required on the structural and functional response of ecosystems under future climatic conditions.

The geothermal area where the study was conducted was based around Hveragerdi, which lies in Southwest Iceland. The area is characterised by large temperature gradients occurring in a natural environment dominated by grassland and Spruce forest ecosystems. Icelandic grasslands are relatively species rich (Kristinsson & Sigurdsson 2010). Most of the common vascular plant species have a circumpolar distribution in boreo-arctic and temperate regions (Preston et al. 2002), meaning these species are adapted to cold temperatures and will most likely be adversely influenced by a rise in temperature, which could lead to a decrease in species richness and diversity.

An earthquake in 2008 caused new geothermal activity and associated temperature gradients in adjacent grasslands. This created a unique opportunity for ecologists to study how various ecosystem processes are affected by temperature, which was seized by several researchers. They created the ForHot research consortium, a group of expert scientists in plant-soil responses to warming. This allowed us to investigate long-term effects of soil warming as well as the short-term effects on the vegetation composition of subarctic grasslands.

The scope of this thesis addresses changes in plant community structure to changes in soil temperature along a natural soil warming gradient in a subarctic grassland ecosystem. The aspects of the plant community structure that were investigated are species richness, species evenness, Shannon-Wiener diversity index, species composition and species presence.

Temperature effects depend on initial conditions of the site and interacts with abiotic factors such as SWC and nutrient cycling (Shaver et al. 2000), which are influential in determining the plant community structure (Gentry 1988). Therefore, in order to improve our understanding, we incorporate abiotic factors in the statistical analyses. As the study site is N-limited, changes in N availability induced by temperature may mask the true temperature effect. This is why, in this study, particularly with its N-limited ecosystem, it is very important to incorporate and monitor N availability.

1.8 Hypotheses

In this thesis, we aim to verify the following hypotheses:

1. Species assemblages in the long-term warmed grassland are expected to be adapted to warmed conditions, contrary to the short-term warmed grassland, where species assemblages are still adjusting to the new conditions. Therefore, species richness, evenness and the Shannon-Wiener diversity index are expected to change more along the soil temperature gradient in the long-term warmed grassland compared to the short-term warmed grassland.
2. Species richness, evenness and diversity are expected to decrease and plant species presence and composition will change with temperature.
3. We expect these responses as a direct temperature effect because Icelandic subarctic grasslands are adapted to cold temperatures and will therefore suffer from heat stress.
4. We hypothesize that, apart from the direct temperature effects, indirect effects on nutrient cycling will be a major explanatory variable for the observed responses. Temperature is expected to increase the N availability through increased mineralisation and nitrification rates.
5. Increased N availability is expected to decrease species richness and evenness and alter species composition and presence.
6. Soil N stocks are expected to decrease with temperature as result of the increased mineralisation and nitrification rates. Other studies did not show any correlation between soil N stocks and soil warming because these pools are large and take a long time to respond to warming. In this study, the ecosystem is exposed to warming for a longer period. Therefore, soil N stocks are expected to decrease with temperature.
7. Soil N stock itself is not expected to alter plant community structure.
8. Soil water content is expected to decrease with warming.

9. SWC it is not expected to influence the vegetation composition in any way, not directly through drought stress, nor indirectly through changes in the nitrogen cycle since these influences are expected to be minor and will be masked by the main temperature influences. This because of the initial wet conditions of the site. Conditions are relatively wet because the site has high annual precipitation rates, high water storage potential of the soil and relatively short summers (Sigurdsson et al. 2016). However, it is important to include SWC in the analyses, since there could be interaction effects.

1.8.1 Summary of the hypotheses:

We expect species richness, species evenness and species diversity to decrease and species composition and presence to change with increasing temperature, primarily through loss of species by heat stress. Further we expect that these changes will partly occur indirectly through increase in N availability and possibly through decrease in soil water content (Fig. 2).

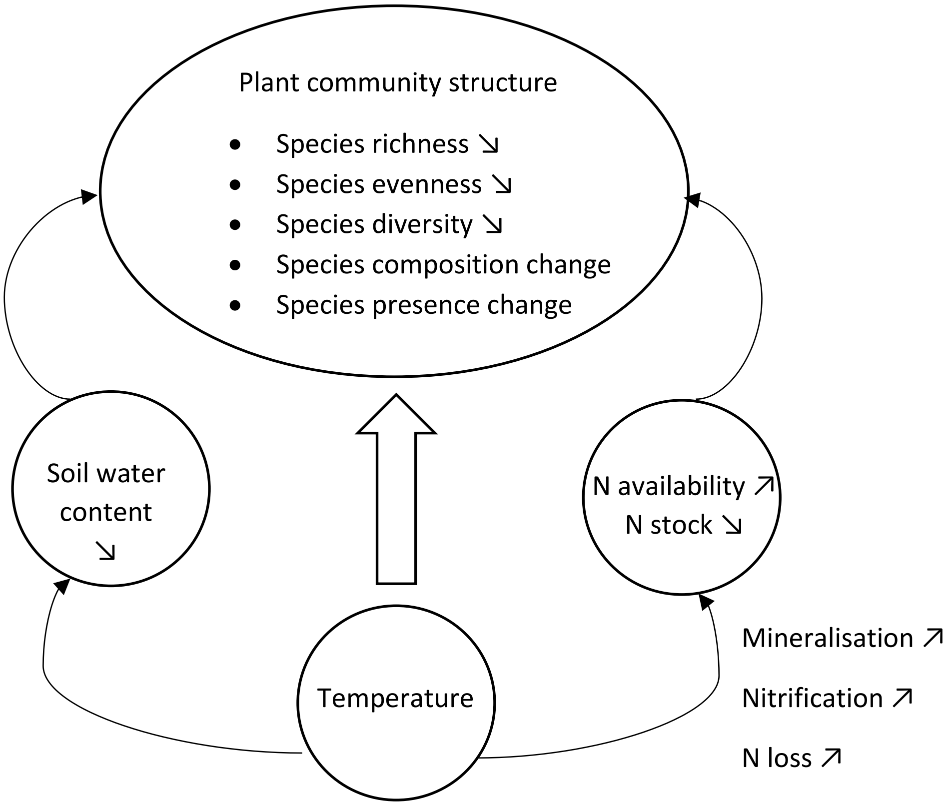


Figure 2: Schematic representation of the hypotheses.

2 Materials and methods

2.1 Study area

The study sites were located in the hills around Hveragerdi, Southwest Iceland (63.9995° N, 21.1797° W). Iceland is an oceanic island close to the Arctic Circle. It is located on a volcanic hotspot on the Mid-Atlantic Ridge, where the Eurasian and North American plates are moving apart. This geological process is responsible for its intense volcanic activity. Despite its northerly position Iceland knows a cool temperate climate, as it is moderated by the North Atlantic current, with cool summers (+5 to +15 °C) and mild winters (-10 to +5 °C) (Icelandic Met Office, 2016). However, the often strong North Atlantic winds can make the wind chill temperature a lot lower. The mean yearly precipitation measured at the nearest weather station in Eyrarbakki was 1423, 4 mm over the last 10 years (Icelandic Met Office, 2016). The soil in this volcanic area is classified as Andosol, which is characterised by its high carbon storage capacity, high water retention and low bulk density (Arnalds 2008). Because of its position near the Arctic Circle, the solar angle is never high in Iceland, and the difference in day length between summer and winter is large. The growing season is quite short, starting late May and ending in half August, with most plants flowering in July.

2.1.1 The ForHot research site

I was able to do my work at the ForHot research site, where short-term and long-term warming gradients occur in adjacent valleys, allowing the study of the effects of higher temperatures on grassland (and forest) ecosystems. This research site is located in a geothermally active area with hotspots in places where the heat is able to reach the surface (Arnórsson 1995). The highest temperatures are found next to the hotspots, but further away the temperature gradually decreases, thereby forming a geothermal soil temperature gradient.

We studied the effect of natural soil warming in several grasslands at two areas with different warming duration, allowing us to compare long-term warming effects and short-term warming effects on vegetation composition. Long-term warming effects are investigated in grasslands that have been warmed for at least 50 years, and probably for centuries (Magnússon & Vídalín 1918-1921). These grasslands, hereafter ‘Old grassland’ (GO), are located 2.5 kilometres north of Hveragerði (Fig. 3 b).

In May 2008, an earthquake altered the underground hot water systems, initiating geothermal activity in grasslands close to the university of Hveragerði (Halldórsson & Sigbjörnsson 2009), hereafter ‘new grasslands’ (GN) (Fig. 3 a). These grasslands were not geothermally active prior the earthquake and the earthquake did not affect geothermal activity and soil warming in a grassland in an adjacent grassland (Thorbjörnsson et al. 2009).

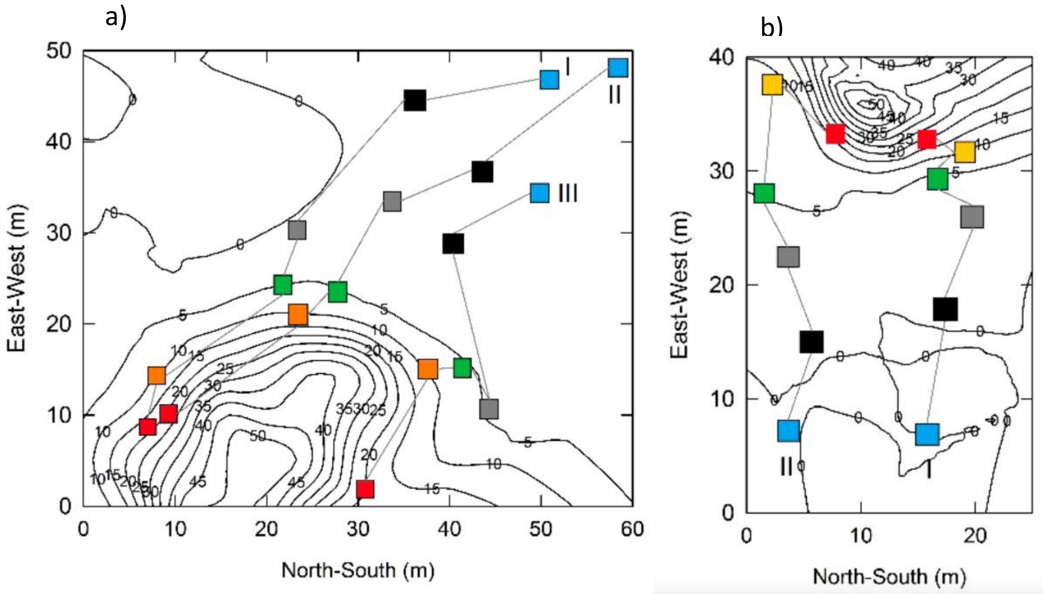


Figure 3 Spatial distribution of geothermal soil warming at the ForHot site. Isolines show differences in soil temperature (°C) at 10 cm depth between unaffected and warmed areas. (a) Grassland site which has been exposed to geothermal warming since an earthquake in 2008 (GN) with three transect as examples; (b) Grassland site that has been geothermally heated for at least 50 years (GO) with two transects as example. Warming levels are unwarmed control (blue), black (+1°C), grey (+3°C), green (+5°C), (orange (+10°C) and (red (+20°C).; from Sigurdsson et al. 2016.

2.2 Experimental setup

In each set of grasslands (GN and GO), 5 replicate soil warming gradients (transects) with 6 different warming levels were established in May 2013. The target soil warming levels at 10 cm soil depth were +0°C (control), +1, +3, +5, +10 and +20°C. The temperatures in these plots fluctuate throughout the year, but the difference in temperature between the warming levels remains fairly constant (Fig. 4). Each plot measured 2 x 2 m (1 x 1 m for the +20°C plots) and was accompanied by a 55 x 55 cm side plot for destructive measurements. The +20°C plots, which were established in May 2014, were smaller because the soil temperature gradient was much steeper at the warmer end of the transects. An additional 30 plots, located in GN, were established in October 2014. Of these plots, 25 are unheated (+0 °C) and 5 have a target temperature of +10 °C. These plots are incorporated as additional data for GN. In total, we investigated the plant community structure of 90 plots: 60 in GN and 30 in GO.

When choosing the location of these plots, SWC, slope, elevation, soil depth, etc. were kept as constant as possible, to avoid confounding factors (Sigurdsson et al. 2016). Also pH is similar for all plots in both types of grasslands.

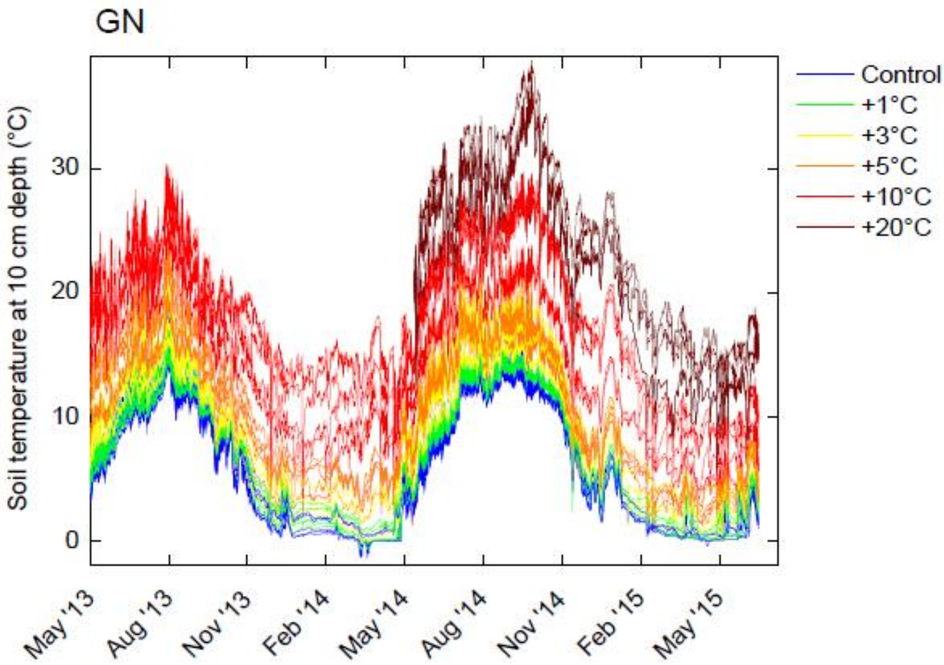


Figure 4: soil temperature (10 cm) fluctuation throughout the year in all plots, illustrating that the difference between the plots remains constant.

2.3 Data collection

2.3.1 Vegetation surveys

We measured species richness, species evenness, Shannon-Wiener diversity index, species composition and species presence in the summer of 2015. Present vegetation is characterised by perennial monocotyledons and mosses. All measurements were performed between the 30th of July and the 6th August, which is the peak of the growing.

In each plot, the presence of all species was recorded. This allowed us to determine the total number of species (species richness, s) and compose a presence-absence matrix. Determination of species was done using the book 'Flowering plants and ferns of Iceland' by Hörður G. Kristinsson (2010).

Species composition, species evenness and biodiversity (Shannon-Wiener diversity index) were determined based on species cover estimations in two 20 x 50 cm subplots within the main plot. The subplots were placed at two opposite sides of the plots, as in figure 5. The abundance of each species in these subplots was estimated using the Braun-Blanquet scale (table 1, (Poore, M. E. D. 1955). The total cover could exceed 100 % when the vegetation was multi-layered.

Species evenness (E) and the Shannon-Wiener diversity index (Hs) were calculated with equation 1 and 2 (Heip et al. 1998), respectively.

Species Evenness: $$E = \frac{Hs}{-\ln\left(\frac{1}{s}\right)} \quad \text{(eq. 1)}$$

Shannon-Weiner diversity index: $$Hs = \sum_{i=1}^{s} \left(pi * \ln(pi)\right) \quad \text{(eq. 2)}$$

With *pi*= coverage proportion of species *i* & *s*= species richness

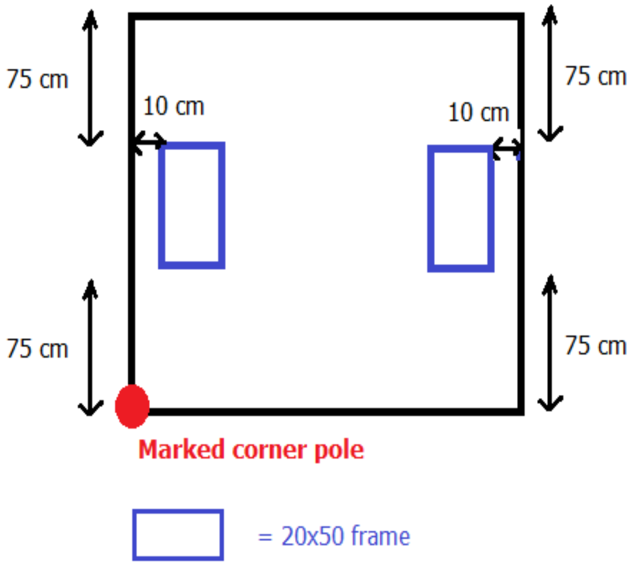


Figure 5: experimental setup for vegetation surveys.

Table 1: Braun-Blanquet scale used for estimations of total vegetation covers. Average abundance (%) is used in analyses.

Symbol	Estimated Abundance (%)	Average abundance (%)
r	0-0.5	0.25
+	0.5-1	0.75
1	1-5	3
2a	5-15	10
2b	16-25	20.5
3	26-50	38
4	51-75	63
5	76-100	87

2.3.2 Ellenberg values

The Ellenberg values used in this study are those for soil fertility (N) and moisture (F) and were obtained from ECOFACTS (Hill et al. 1999a). In this report, scales for temperature (T) and continentally (K) are omitted because they were found unreliable and unsatisfactory in an oceanic climate (Hill et al. 1999b). All Ellenberg values were averaged based on the species abundances in the plots (Eq. 3). This way, the Ellenberg value of more abundant species had a bigger influence on the average Ellenberg value of the plot.

Community-weighted Ellenberg indicator value: $\frac{\sum Ellenberg\ value(\text{species i}) * cover(\text{species i})}{total\ cover}$ (eq. 3)

Ellenberg values for soil fertility (N) run from 1 to 9, with lower values indicating infertile sites and high values indicating rich fertile sites. Those for moisture run from 1 to 12, with 1 indicating extreme dryness and 12 as indicator for plants which are permanently or almost constantly submerged under water.

2.3.3 Environmental parameters

2.3.3.1 Temperature

Soil temperature at 10 cm depth was measured on an hourly basis using HOBO TidbiT v2 Water Temperature Data Loggers (Onset Computer Corporation, USA). We calculated the mean of these measured temperatures from the 1st of May 2015 until the 30th of September 2015.

2.3.3.2 Nitrogen availability

N availability was measured by placing Plant Root Simulator (PRS) probes in the plots for a week in October 2015. These probes measured nitrate (NO^-_3) and ammonium (NH^+_4) availabilities by means of anion and cation exchange. Four replicate samples were taken per plot and sent to Western Ag Innovations Inc. (Saskatoon, SK, Canada) for further analyses.

2.3.4 Nitrogen stocks

For half of the plots in GN and all the plots in GO, the N stock had already been measured in 2013 (Michielsen 2014; Vande Velde 2014). Since Leblans et al. (2016) found little interannual variation in the plot-specific N stocks, we used the 2013 data for this thesis. We quantified the N stock of the other half of the GN plots (25 unheated plots and 5 plots of approximately +10°C) in the summer of 2015. A soil core was taken with a corer (diameter: 5.3 cm) in the side plots after removing all aboveground vegetation. The soil core was taken up to 30 cm or until bedrock was reached. This core was divided into depth intervals (0-5, 5-10, 10-20 and 20-30 cm). Afterwards, the samples were dried for 48h at 40°C or until weight loss stopped.

Of each sample, 2 g of soil was sieved with a 2 mm sieve and milled using a ball mill (Retsch MM301 Mixer Mill, Haan, Germany) as preparation for N analysis by flash combustion using a NC®2100 element analyser (Carlo Erba Instruments, Italy).

2.3.5 Additional data: soil water content

SWC was measured by taking soil samples in the side plots and determining the percentage of water loss after drying 15-25 g of soil at 105°C for 24h. These analyses were done in the framework of the master thesis of Dajana Radujkovic (2016).

2.4 Statistical analysis

All statistical analyses were performed in R Studio (Version 0.99.486 – © 2009-2015 RStudio, Inc.).

2.4.1 Univariate analysis

First, the effect of temperature on the different community parameters (species richness, species evenness and the Shannon-Wiener diversity index) and on the measured environmental parameters (N availability, soil N stock and SWC) was tested. Hereafter, the effect of the environmental parameters was tested on the community parameters which had been found to be significantly altered by temperature.

For each model, the effect of warming duration on the correlation was tested as a fixed explanatory variable by means of an ANOVA. This ANOVA also tested the potential interaction with the independent variable. Non-significant explanatory variables were removed from the model. Prior to these analyses, normal distribution of the dataset was tested using the Shapiro-Wilkinson test.

When data showed a non-linear pattern, the data were log-transformed and a new linear model was fitted. Afterwards, the best model was selected by comparing this logarithmic model with the first linear model, using the AKAIKE information criterion. It evaluates the goodness of fit, while keeping in mind the complexity of the model.

2.4.1.1 Sensitivity

For each regression, the sensitivity (S) was calculated. This is the relative change in the dependent variable per unit of the independent variable. For the linear regressions, this was calculated by means of equation 4. The error on this sensitivity was calculated by means of equation 5.

$$S = \frac{slope}{intercept} * 100 \qquad \text{(eq. 4)}$$

$$Error = \frac{slope + SD\ slope}{intercept} * 100 \qquad \text{(eq. 5)}$$

With *SD* being the standard error of the slope.

Because measured independent variables rarely went as low as 0, an estimated value for the dependent variable was calculated based on the obtained regression model for the lowest recorded independent variable. This value was then used in the equation instead of the intercept.

For the logarithmic models, the sensitivity was calculated over a certain interval by means of equation 6.

$$S = \frac{\frac{y2 - y1}{y1} * 100}{x2 - x1} \qquad \text{(eq. 6)}$$

With x_1 being the independent variable at the start of the interval and x_2 being the independent variable at the end of the interval. y_1 and y_2 are the estimated dependent variables calculated for these independent variables by the obtained regression model. For the sensitivities shown in the graphs, the interval is the range of measured independent variables, meaning that $x_1 = x_{min}$ and $x_2 = x_{max}$.

2.4.2 Multivariate analysis

Non-metric Multidimensional scaling (NMDS) was used to visualize vegetation composition. This method is commonly regarded as the most robust unconstrained ordination method in community ecology (Minchin 1987). Non-metric multidimensional scaling (NMDS) is an indirect gradient analysis approach which produces an ordination based on, in this case, a Bray-Curtis dissimilarity matrix. Unlike methods which attempt to maximise the variance or correspondence between objects in an ordination, NMDS attempts to represent as accurately as possible, the pairwise dissimilarity between objects in a low-dimensional space.

In NMDS plots, objects that are ordinated closer to one another are likely to be more similar than those further apart. However, the scale of the axes and the orientation of the plot are arbitrary.

To visualise the possible relation of the plots to a given variable, isoclines can be represented on these ordinations. In this case, the isoclines connects points with the same soil temperature (Fig. 14 & 15). Polygons can be drawn around objects with the same factor level, in this case, temperature level.

The function “vectorfit” finds directions in the ordination space towards which the environmental vectors change most rapidly and to which they have maximal correlations with the ordination configuration. These vectors depart from the centroid of the data cluster. The longer the arrows, the more important this variable is in explaining the variation. When vectors lie close to each other or opposite of one another, these variables covary the most and are likely to be correlated to each other.

3 Results

3.1 Direct soil warming effects on vascular plant diversity

3.1.1 Soil warming effects on species richness

Overall, there was a significant negative effect of soil warming on species richness ($p < 0.001$), where the species richness was consistently higher in GO compared to GN (Fig. 6). This is in acccordance with what was hyothesised in hypothesis 1 and 2. However, the loss in species richness per °C was similar in both types of grasslands (ANOVA, no interaction $p = 0.67$). In both cases 72 ± 3 % of the number of species that occur in the unwarmed plots were lost over the full warming gradient. This translates to an average decrease of 2.72 ± 0.12 % species per °C. However, this decrease was not linear, but exponential, as was shown with AKAIKE comparison between the linear and logaritimic fit. When the temperature range was split up in different soil warming intervals (+0-1; 1-3; 3-5; 5-10 and 10-20°C), it was clear that more species were lost per °C at lower than at higher warming levels (Table 1).

Thus, species richness was negatively influenced by soil temperature and more species are lost at low warming levels compared to higher warming levels. Further, GO was species richer than GN.

Table 2: Decrease in species richness in % per °C at different intervals of soil warming. The warming ranges refer to the average warming levels of the plots compared to the control plots, using average temperatures from the 1st of May 2015 until the 30th of September 2015.

Warming range	Sensitivity (%/ °C)
0-1	- 8.19 ± 0.63
1-3	- 7.19 ± 0.58
3-5	- 6.31 ± 0.50
5-10	- 4.91 ± 0.37
10-20	- 3.27 ± 0.23

3.1.2 Soil warming effects on species evenness & Shannon-Wiener diversity index

Overall, species evenness was not significantly influenced by soil temperature ($p = 0.74$), nor was there a significant difference in species evenness between both types of grasslands ($p = 0.15$) (Fig. 7 a). This was not in accordance with what was hypothesised (hypothesis 1 and 2). However, the ANOVA showed a significant interaction effect of soil temperature and grassland type ($p < 0.05$). Looking at both types of grasslands separately, GN showed no effect of soil temperature on species evenness, while in GO, there was a negative trend ($p = 0.08$), with a 1.20 ± 0.65 % decrease in species evenness per °C (Fig. 7 a).

There was a significant negative effect of soil temperature on the Shannon-Wiener diversity index ($p < 0.05$; Fig. 7 b), which was in accordance with hypothesis 2. The type of grassland had a significant effect on the index ($p < 0.05$), even though the Tukey test did not show a significant difference between the indices of both grasslands (TukeyHSD, $p = 0.08$). Additionally, there was a significant interaction effect of grassland type and soil temperature ($p < 0.05$). In GN, the effect of soil temperature was not significant ($p = 0.30$), in contrast to GO, where there was a negative effect of soil warming on the Shannon-Wiener index ($p < 0.01$; Fig. 7 b). Here, the Shannon-Wiener diversity index decreased by 2.17 ± 0.60 % per °C.

In conclusion, there was no significant soil temperature effect on species evenness or the Shannon-diversity index in GN. In GO, on the other hand, both decreased with increasing temperature.

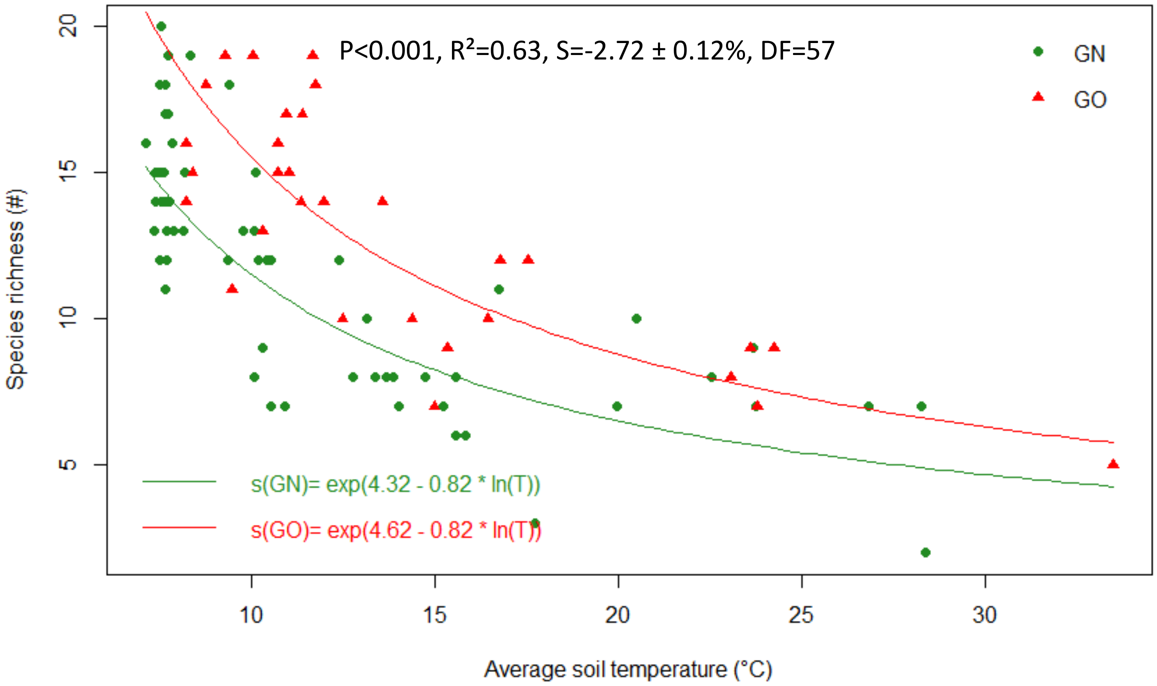
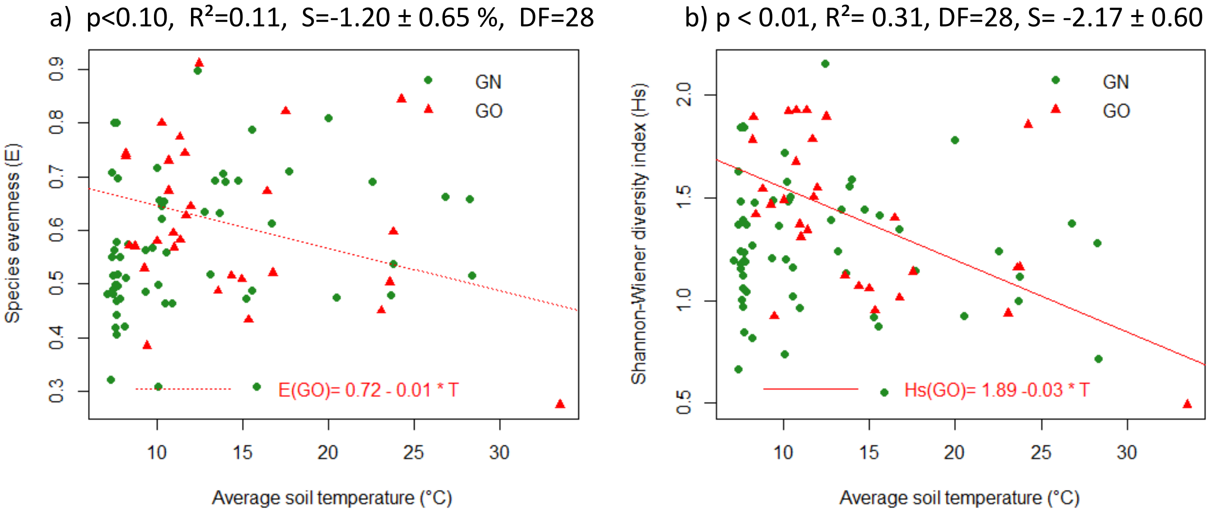


Figure 6: Logarithmic relationship between species richness and temperature. For the soil temperature, the average is taken over the period from the 1st of May 2015 until the 30th of September 2015. s= species richness, GN= short-term warmed grasslands, GO= old grasslands. The sensitivity (S) was calculated as the linear change in species richness per °C as % of the ambient value. DF=degrees of freedom.



a) p<0.10, R^2=0.11, S=-1.20 ± 0.65 %, DF=28

b) p < 0.01, R^2= 0.31, DF=28, S= -2.17 ± 0.60

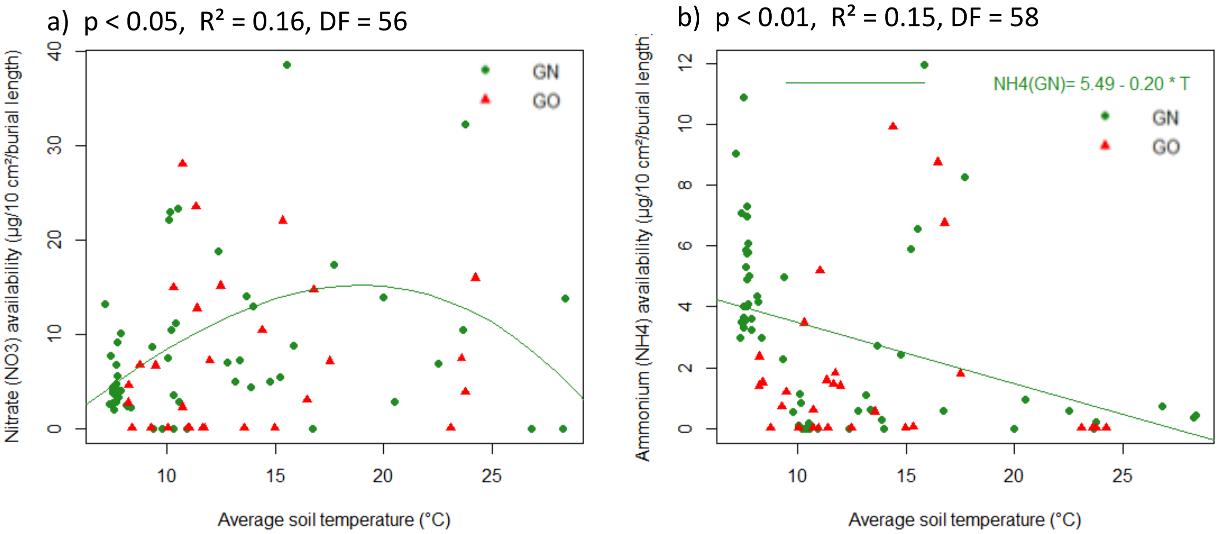
Figure 7: Species evenness (a) and the Shannon-Wiener diversity index (b) in relation to average soil temperatures over the period of the 1st of May 2015 until the 30th of September 2015. E= species Evenness, Hs= Shannon-Wiener diversity index, GN= short-term warmed grasslands, GO= long-term warmed grasslands. The sensitivity (S) was calculated as the linear change in species evenness or the Shannon-Wiener index per °C as % of the ambient value. DF=degrees of freedom.

3.2 Temperature effects on abiotic factors

3.2.1 Nitrogen

3.2.1.1 *Available nitrogen*

Soil warming was expected to stimulate decomposition and increase the amount of available N for plants (hypothesis 4). However, this was not the case. Total N availability did not change along the soil temperature gradient in neither GN, nor GO ($p = 0.66$, Appendix 1). Nitrate concentration in the soil did not show a significant linear relation with soil temperature ($p = 0.09$), however, there was a significant hump shaped trend in NO_3^-=availability in GN ($p < 0.05$, $R^2 = 0.16$ Fig. 9 a), albeit not in GO ($p = 0.72$, $R^2 = 0.05$). Ammonium concentrations decreased significantly along the soil temperature gradient in GN ($p < 0.05$, $R^2 = 0.15$, Fig 9 b), but not in GO ($p = 0.82$, $R^2 = 0.002$).


a) $p < 0.05$, $R^2 = 0.16$, DF = 56

b) $p < 0.01$, $R^2 = 0.15$, DF = 58

Figure 8: Nitrate (a) and ammonium (b) availability in relation to average soil temperature over the period of the 1st of May 2015 until the 30th of September 2015. GN= short-term warmed grasslands, GO= old grasslands.

3.2.1.2 *Soil Nitrogen Stocks*

Soil N stocks, on the other hand, decreased logarithmically with increasing soil temperature in both GN and GO ($p < 0.001$, Fig. 10), which was in accordance with hypothesis 6. Soil N stock differed significantly between both types of grasslands (TukeyHSD $p < 0.001$), whereas the N stocks were consistently lower in GO. However, the loss in soil N stock per °C was the same for both types of grasslands (no interaction, ANOVA, $p = 0.40$).

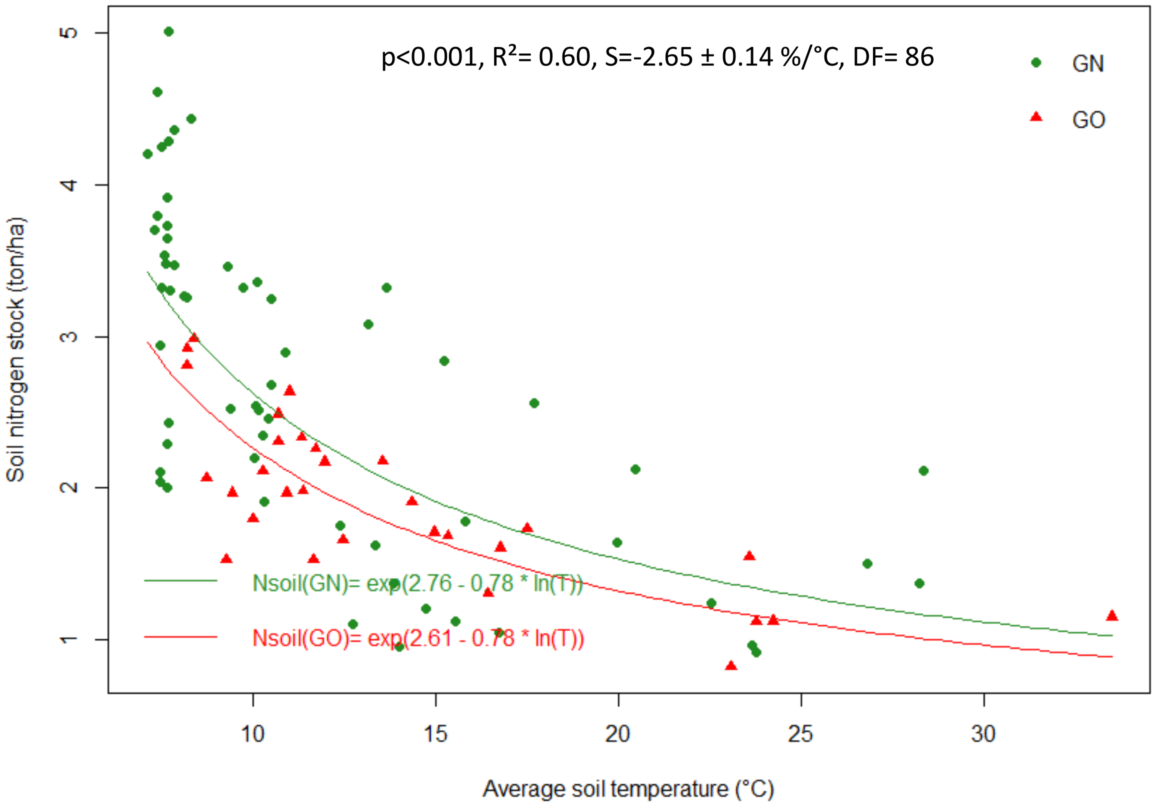


Figure 9: logarithmic relation between the average soil temperature between the 1st of May 2015 until the 30th of September 2015 and the soil nitrogen stock. GN= short-term warmed grasslands, GO= long-term warmed grasslands, Nsoil (GN) = soil nitrogen stock in short-term warmed grasslands, Nsoil (GO) = soil nitrogen stock in long-term warmed grasslands, p=significance R^2=, coefficient of determination S=sensitivity, DF=degrees of freedom.

Consequently, the proportion of the soil N stock that was available for plants increased ($p < 0.01$, Appendix 2). This relation did not differ between both types of grasslands (no interaction between soil temperature and grassland type: $p = 0.46$; no effect of grassland type $p=0.48$).

3.2.2 Effect of Nitrogen availability on species richness

Species richness decreased significantly with increasing nitrate concentrations ($p < 0.05$, Fig. 11 a), as hypothesised (hypothesis 5), but increased significantly with increasing ammonium concentrations, which was unexpected ($p < 0.05$, Fig. 11 b). However, both correlations had a very low fit ($R^2 = 0.07$ & 0.09, respectively).

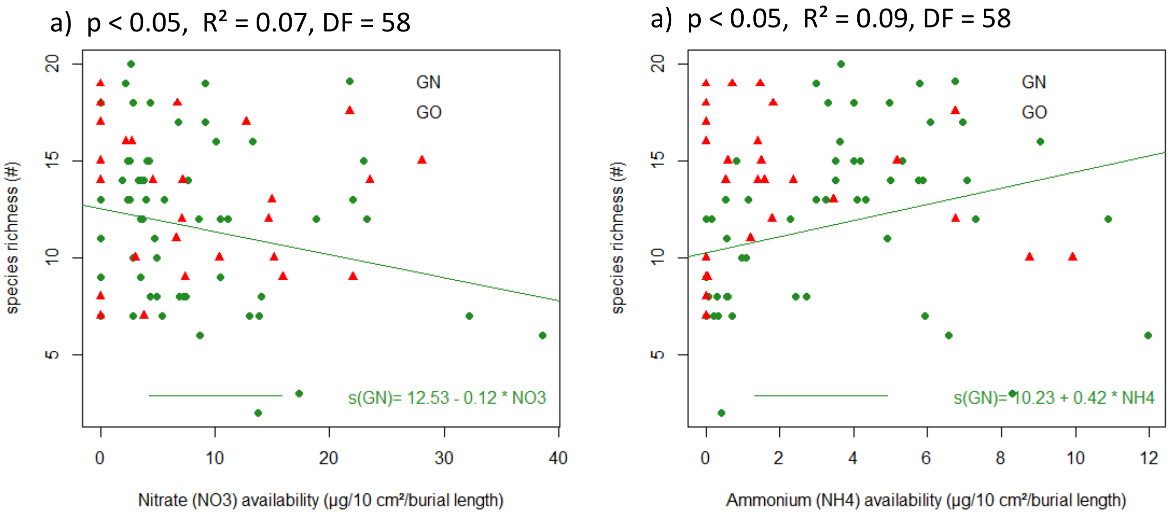


Figure 10: Relationship between species richness and nitrate availability (a) and ammonium availability (b). s= species richness, GN= short-term warmed grasslands, GO= long-term warmed grasslands. DF=degrees of freedom.

3.2.3 Ellenberg value for soil fertility

Ellenberg values for soil fertility (N) range between 1 to 9, with lower values indicating infertile sites and high values indicating rich fertile sites. Most plots had an Ellenberg value between 3 (more or less infertile soils) and 5 (intermediate fertility). Some of the lowest temperature plots in GN had a lower value (2: low fertility), while some of the plots in GO had a higher value (7: richly fertile).

The average Ellenberg value for soil fertility (N) of the plots increased along the soil temperature gradient ($p < 0.001$; Fig. 12), suggesting that soils became more fertile with increasing soil temperature. This increase in Ellenberg values for soil fertility with soil temperature was similar for both grasslands types (no interaction $p = 0.24$). However, there was a significant difference between the types of grasslands (TukeyHSD, $p < 0.01$), where soil fertility was consistently higher in GO (ANOVA, $p < 0.05$). Here too, a hump shaped trend fitted significantly for GN ($p < 0.001$, $R^2 = 0.49$, Appendix 3), but not for GO ($p = 0.76$, $R^2 = 0.04$).

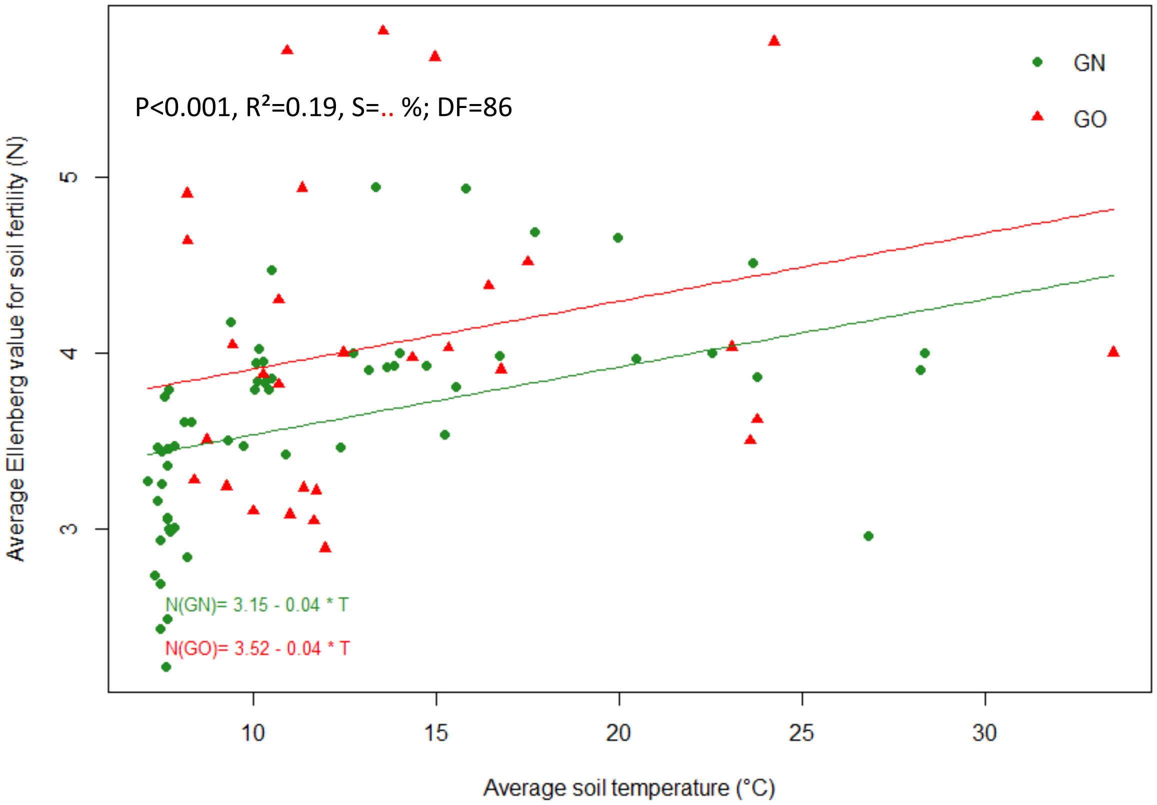


Figure 11: Relationship between average Ellenberg value for soil fertility (N) and the average soil temperature measured over the period of the 1st of May 2015 until the 30th of September 2015. GN= short-term warmed grasslands, GO= long-term warmed grasslands. The sensitivity (S) was calculated as the linear change in the average Ellenberg value for soil fertility per °C as % of the ambient value. DF= degrees of freedom.

3.2.4 Soil water content

Soil water content (SWC) declined logarithmically with soil temperature, as was expected (hypothesis 8) ($p < 0.001$; Based on the Akaike comparison test). In the unheated plots, the SWC did not differ significantly between GN and GO ($p = 0.78$). However at higher soil temperatures, the soil dried more rapidly in GN compared to GO ($p < 0.001$; Fig. 8).

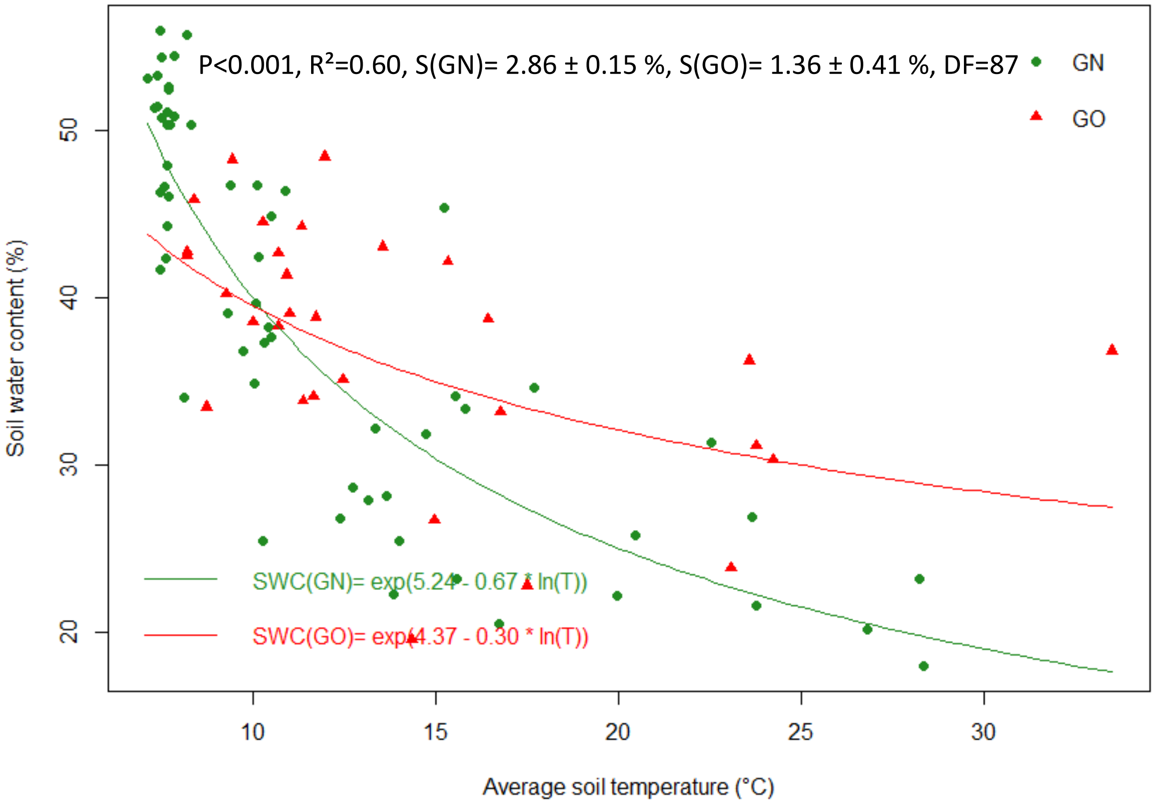


Figure 12: Logarithmic relation between soil water content (%) (SWC) and average soil temperature over the period of the 1st of May 2015 until the 30th of September 2015. GN= short-term warmed grasslands, GO= old grasslands. The sensitivity (S) was calculated as the linear change in SWC per °C as % of the ambient value. DF=degrees of freedom.

3.2.5 Effect of soil water content on species richness

Species richness increased significantly with SWC ($p < 0.001$), contrary to what was expected (hypothesis 9) and the increase did not differ between GN and GO ($p = 0.46$; Fig. 13). However, species richness was consistently higher in GO compared to GN ($p < 0.01$). Since SWC and species richness both decreased exponentially with increasing temperature, this relation might be a hidden temperature effect.

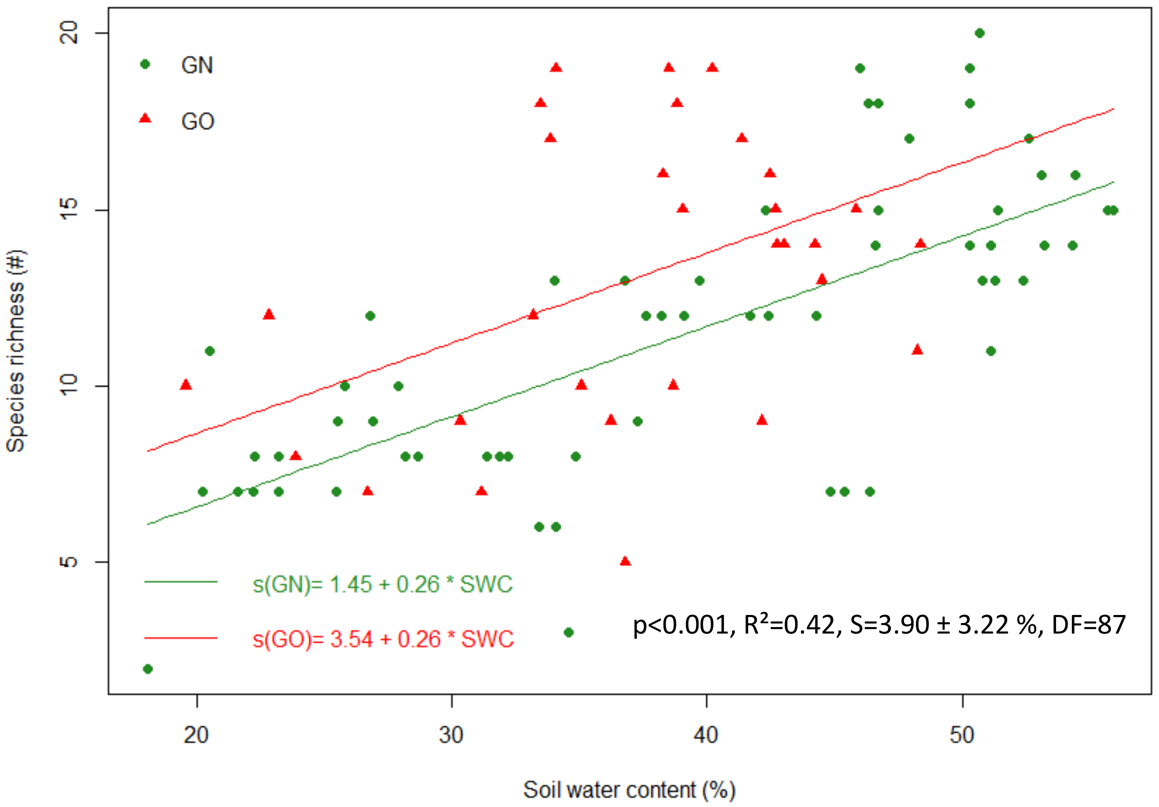


Figure 13: Correlation between soil water content and species richness. s= species richness, GN= short-term warmed grasslands, GO= old grasslands, SWC = soil water content. The sensitivity (S) was calculated as the linear change in species richness per % change in soil water content as % of the ambient value. DF=degrees of freedom.

3.2.6 Ellenberg value for moisture

The average Ellenberg value for soil moisture did not show any relationship with measured SWC in neither GN, nor GO (Appendix 4).

3.3 Species composition and species presence (beta-diversity)

3.3.1 Influence of warming duration on species composition and species presence

When looking at the overall species composition, there was a large overlap between GN and GO (Fig. 14 a). Although the PERMANOVA test showed a highly significant difference between these sites ($p < 0.001$), the low R^2 value (0.05) revealed that only a small portion of variance is explained by the overall differences between the two types of grasslands. There was a significant fit ($p < 0.001$) of the temperature vector onto the NMDS ordination for the species composition, with a moderate R^2 value for GN (0.56, Table 2), and a rather low R^2 value for GO (0.33, Table 2).

The NMDS plot that shows the variance in the data based on the presence-absence matrix (Fig. 14 b) showed again a large overlap between GN and GO. This indicates that the species occurring in GN and GO were very similar. However, also here, the PERMANOVA test showed a significant difference between the two ($p < 0.001$). The R^2 value was slightly higher (0.08), but still indicated that variance in the dataset could only for a small portion be explained by differences between the two types of grasslands. There was a significant fit ($p < 0.001$) of the temperature vector onto the NMDS ordination for the species presence, with very high R^2 values for both types of grasslands (0.79 and 0.76 for GN and GO respectively, Table 2). These vectors were orientated differently onto the NMDS ordination plot, implying that the effect of soil temperature on species presence differed between the two types of grasslands.

In summary, even though GN and GO differed significantly in species composition and presence, the difference between both types of grasslands was not adequate in explaining variation in the data. Thus, the same species occurred in both types of grasslands, their composition was similar, but their response to temperature was different.

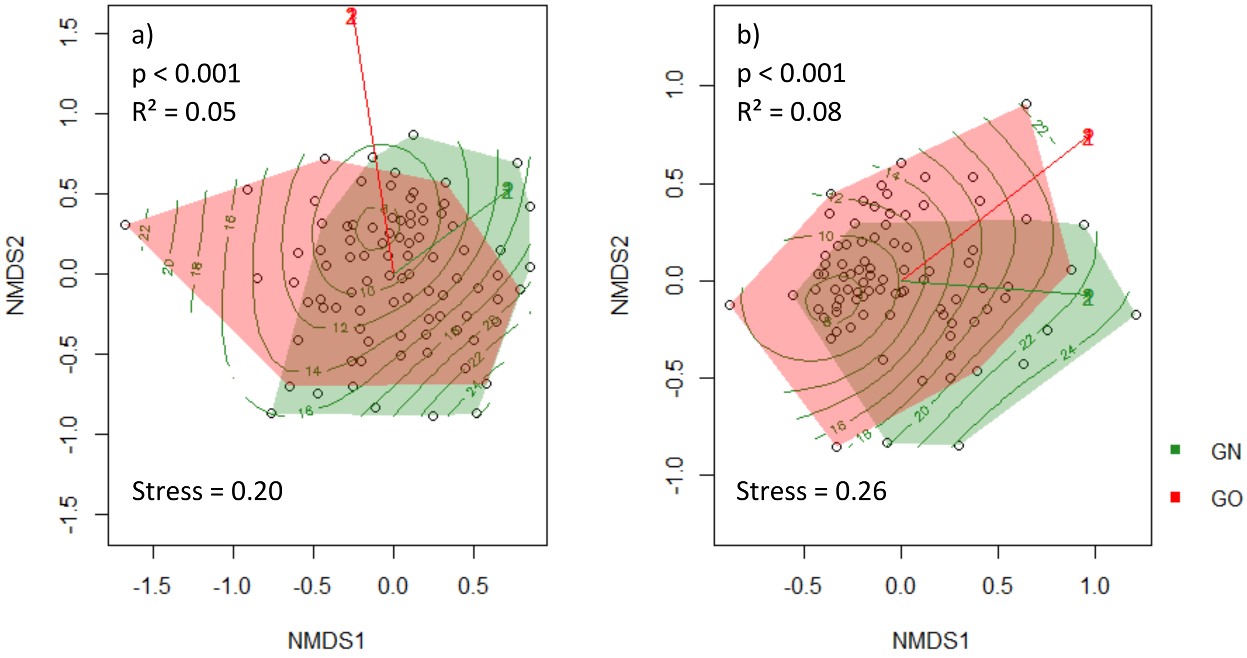


Figure 14: Non-metric multidimensional scaling (NMDS) plot for species composition (a) and species presence (b) for the short-term warmed grasslands (green) and the long-term warmed grasslands (red). Points represent plots. Arrows represent the temperature effect in both valleys.

Table 3: p-value and R^2 values for the fit of temperature vector onto the NMDS ordination.

	Species composition				**Species presence**			
	NMDS1	**NMDS2**	**p-value**	**R²**	**NMDS1**	**NMDS2**	**p-value**	**R²**
GN	0.80	0.60	< 0.001	0.56	0.996	-0.08	< 0.001	0.79
GO	-0.16	0.99	< 0.005	0.33	0.79	0.61	< 0.001	0.76

3.3.2 Proportional importance of different environmental parameters for species composition and presence

GN showed a gradual transition in species composition from the cold plots (+ 0 °C) to the warmest plots (+ 20 °C; Fig. 15 a). However, there was a large overlap between the different soil warming levels. Three environmental vectors fitted significantly onto the ordination (Fig. 15 a & Table 3): soil temperature, SWC and soil N stock ($p < 0.001$). The temperature vector fitted rather well onto the ordination ($R^2 = 0.56$), as well as those of soil N stock and SWC ($R^2 = 0.43$ and 0.61 respectively). Both vectors laid opposite to that of soil temperature, suggesting a negative relation of both environmental factors with soil temperature. N availability did not significantly fit onto the ordination of species composition in GN (Table 3), but the vector of ammonium availability did ($p < 0.001$, $R^2 = 0.40$), and showed no apparent relation with any of the environmental factors.

When the NMDS plot was based on the presence-absence matrix of the entire 2 x 2 m plots (instead of 0.2 x 0.5 m subplots), the differentiation between the species composition of different soil warming levels became more profound (Fig. 15 b). Both the lowest (+ 0 °C) and the highest (+ 20 °C) warming level were easily distinguishable from the rest. At intermediate warming levels (+ 1 °C, + 3 °C, + 5°C), there was an overlap between the polygons, indicating that these communities were more similar, but a clear gradient could still be observed. In this case, five environmental vectors fitted significantly into the ordination (Fig. 15 b & Table 3): soil temperature, SWC, soil N stock (all $p<0.001$), nitrate availability ($p < 0.05$) and ammonium availability ($p < 0.01$). However, the latter two fit rather poorly ($R^2 = 0.11$ & 0.23, respectively). The main explanatory variable was soil temperature, with a vector that fitted rather well onto the ordination ($R^2 = 0.79$) and correlated maximally to the first NMDS axis (0.9996). (Visually, this can be derived from figure 15 b, as the soil temperature vector lies parallel to the first axis and the temperature isoclines are perpendicular to this axis). SWC and soil N stock had a similar influence on species composition, but opposite (Fig. 15 b & Table 3), which indicates a strong negative relation between both factors and soil temperature.

For GO, there was no clear change in species composition along the soil temperature gradient (Fig. 15 c). Temperature isoclines were not parallel, implying there was no linear relation of temperature to the NMDS ordination. The vector of soil temperature fitted rather poorly onto the ordination ($R^2 = 0.31$), but was nonetheless the best of all measured environmental factors (Table 4). The soil temperature vector coincided with the secondary axis, NMDS2 (Fig. 15 c & Table 4). Here too, the NDMS plot indicates that there was a negative relationship between soil temperature and soil N stock and SWC (Fig. 15 c & Table 4).

As in GN, the presence-absence data followed a clear temperature gradient (Fig. 15 d). At lower temperatures (+ 0 °C, + 1 °C, + 3 °C, + 5 °C), there were large overlaps, but the higher soil temperatures

(+ 10 °C and especially + 20 °C) were clearly separated from the rest. Here, the same four environmental vectors fitted significantly into the ordination. Again, soil temperature was the main explanatory variable (Table 4). The SWC vector fitted remarkably weaker onto the ordination of species presence compared to in GN ($R^2 = 0.20$, compared to $R^2 = 0.70$). Again, there was a negative relation between soil temperature and SWC and soil N stock.

In summary, soil temperature was the main explanatory variable for variation in species composition and species presence in both GN and GO. Its effects were more profound in GN than in GO. Additionally, the presence of species seems to be more determined by soil temperature than their relative abundance in both types of grasslands. The vectors of Soil N stock and SWC fitted very well onto the ordination as well, but were both strongly negatively related to temperature.

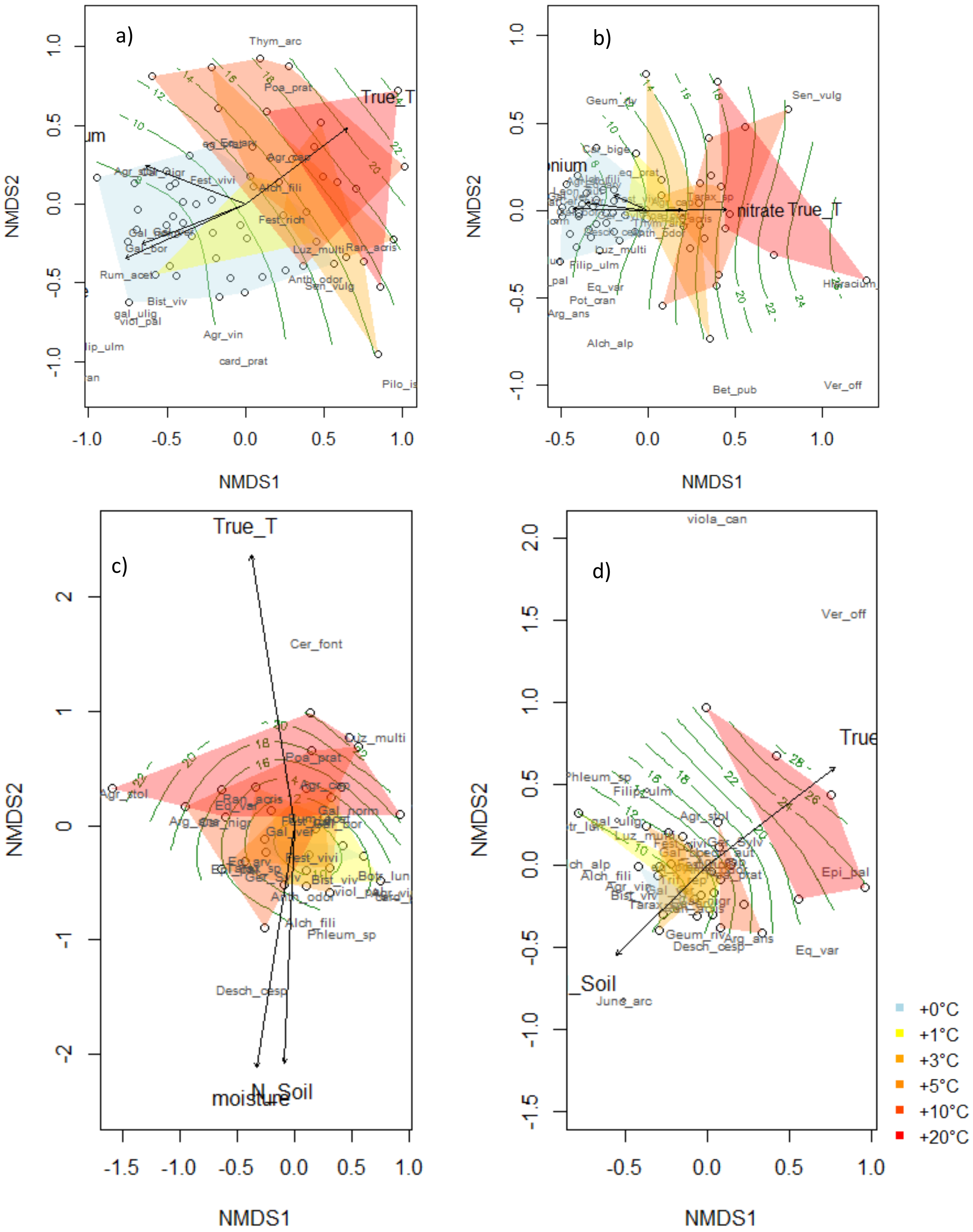


Figure 15: a) NMDS plot of species composition in the short-term warmed grasslands with the environmental factors (arrows) that were significantly correlated to changes in vegetation composition between plots. b) NMDS plot of species presence in the short-term warmed grasslands and the environmental factors (arrows) that were significantly correlated to changes in species presence between plots. c) NMDS plot of species composition in the long-term warmed grasslands with the environmental factors (arrows) that were significantly correlated to changes in vegetation composition between plots. d) NMDS plot of species presence in the long-term warmed grasslands and the environmental factors (arrows) that were significantly correlated to changes in species presence between plots. True_T= average soil temperature measured over the period of the 1st of May 2015 until the 30th of September 2015, moisture= soil water content (%), N_Soil= nitrogen stock of the soil, ratio.Nav.Nst= ratio of Nitrogen availability to soil nitrogen stock. Colors indicate the different soil warming levels.

Table 4: Directions in the ordination space towards which the environmental vectors change most rapidly and to which they have maximal correlations with the ordination configuration with their significance and coefficient of determination in the short-term warmed valley (GN). Temperature = average soil temperature measured over the period of the 1st of May 2015 until the 30th of September 2015, (%), N stock Soil= nitrogen stock of the soil, Nav/ Nstock = ratio of Nitrogen availability to soil nitrogen stock, SWC= Soil water content (%).

GN	**Species composition**				**Species presence**			
	NMDS1	**NMDS2**	**p-value**	**R^2**	**NMDS1**	**NMDS2**	**p-value**	**R^2**
Temperature	0.80	0.60	< 0.001	0.56	1.00	0.01	< 0.001	0.79
Total N availability	-0.35	0.94	0.42	0.03	0.95	0.31	0.23	0.05
NO^-availability	0.80	0.61	0.39	0.03	0.90	0.44	< 0.05	0.11
NH_4^+availability	-0.93	0.36	< 0.001	0.40	-0.85	0.52	< 0.01	0.23
N stock soil	-0.93	-0.36	< 0.001	0.43	-0.99	0.11	< 0.001	0.49
SWC	-0.91	-0.41	< 0.001	0.61	-1.00	0.02	< 0.001	0.70

Table 5: Directions in the ordination space towards which the environmental vectors change most rapidly and to which they have maximal correlations with the ordination configuration with their significance and coefficient of determination in the long-term warmed valley (GO). Temperature = average soil temperature measured over the period of the 1st of May 2015 until the 30th of September 2015, (%), N stock Soil= nitrogen stock of the soil, N av/ Nstock = ratio of Nitrogen availability to soil nitrogen stock, SWC= Soil water content (%).

GO	**Species composition**				**Species presence**			
	NMDS1	**NMDS2**	**p-value**	**R^2**	**NMDS1**	**NMDS2**	**p-value**	**R^2**
Temperature	-0.14	0.99	< 0.01	0.32	0.79	0.61	< 0.001	0.76
Total N availability	-0.67	0.74	0.15	0.13	0.69	-0.72	0.14	0.14
NO^-availability	-0.77	0.64	< 0.10	0.17	0.68	-0.74	< 0.10	0.17
NH_4^+availability	0.94	0.35	0.76	0.02	-0.14	0.99	0.98	0.001
N stock soil	-0.07	-1.00	< 0.05	0.24	-0.71	-0.70	< 0.001	0.47
SWC	-0.18	-0.98	< 0.05	0.25	-0.78	-0.62	< 0.10	0.20

4 Discussion

4.1 Hypothesis 1 - Short-term warming vs long-term warming

Even though GO was in general more species rich than GN, as hypothesised, this could not be derived from the total species list per grassland type. The multivariate analysis also showed that, even though there was a significant difference between both types of grasslands, there was large overlap between them. A lot of species occur in both types of grasslands, but in lower numbers in GN (e.g. *Geranium sylvaticum, trifolium repens, Potentilla anserina, Equisetum arvense, Rumex acetosa, Deschampsia cespitosa and Agrostis stolonifera*). On the other hand, typical cold-adapted plants such as *Alchemilla filicaulis, Gallium boreale* and *Luzula multiflora* have similar incidences in both types of grasslands. In other words, while cold-adapted plants are equally present in both types of grasslands, more cosmopolitan species are more frequently present in GO, leading to overall higher species numbers for GO.

Most graminoid species have a higher incidence in GN than in GO (*Festuca richardsonii, Poa pratensis, Anthoxanthum odoratum, Agrostis vinealis, Agrostis capillaris,* and *Juncus trifidus*). Short-term warming experiments have indeed shown that graminoid species respond positively to warming, in contrast to non-graminoid species (Brooker & van der Wal 2003). It seems that this positive effect on graminoid species did not hold in the long term. This could be caused by the ability of non-graminoid species with a higher temperature tolerance to invade the area after a longer period of warming. We speculate that the short-term warmed areas are still moving towards a new equilibrium in vegetation composition, which has been reached in the long-term warmed areas. The warm-adapted species might already be present in the system, but they are still slowly forming a new balance.

4.2 Hypothesis 2 - Direct of soil warming on species richness, species evenness and the Shannon-Wiener diversity index

Both types of grasslands showed a very similar exponential decline in species richness. This decline in species richness with warming is in accordance with what was hypothesised and several other studies (Gedan & Bertness 2009; Harmens et al. 2004; Klein et al. 2004; Harte & Shaw 1995; Yang et al. 2011). However, some other studies have found increases in species richness with temperature (Zhang et al. 2015).

For the multivariate analysis, correlations were more significant and fit better for species presence than for species composition, suggesting that the presence of species was more determined by soil temperature than their composition in both grasslands. If the dominant species remain the same, and less abundant species come in, or disappear, the species presence shows large shifts, while the community composition stays nearly unaffected. This also shows in the observation that species evenness did not change, while species richness strongly declined. As a consequence there was only a moderate temperature effect on the Shannon-Wiener diversity index after long-term warming and no effect after short-term warming. This unresponsiveness of the Shannon-Wiener diversity (which combines both species richness and evenness in a single index), was probably due to a lack of change in species evenness, even if the species richness declined.

Even though the finding that species evenness and diversity were not affected by soil warming was in accordance with several studies (Kardol et al. 2010; Yang et al. 2011; Harmens et al. 2004; Zhang et al. 2015), other studies did find warming-induced reductions in Shannon-Wiener diversity, caused by a combined decline in species richness and evenness (Chapin et al. 1995; Gedan & Bertness 2009; Walker et al. 2006b). However, Zhang et al. (2015) reported a strong interannual variability in species evenness, which complicates the interpretation of trends in species evenness at one specific moment in time. Another reason for the unresponsiveness of species evenness to warming might be that changes in species abundances cancel each other out in terms of community diversity, as was suggested by Jónsdóttir et al. (2005), who found a significant change in the abundance of dominant growth forms, but no change in diversity measures in two contrasting tundra communities in Iceland, although they accounted for species abundance.

A final important note on the subject, is that species evenness, Shannon-Wiener diversity index and species composition were all calculated based on observations in two 20 x 50 cm subplots, whereas species richness and presence was counted over the total 2 x 2 m plot. Since the vegetation typically grows in patches in subarctic grasslands and the subplots were so small that samples comprised sections of patches of some species at random, variation between subplots amongst the same temperature treatments might have been too large for comparison. Ideally, species evenness, diversity and composition should also be measured over the entire 2 x 2 m plot.

4.3 Hypothesis 3 - Speculations on the mechanisms that drive community structural changes

The source pool of species in Iceland is mainly restricted to (sub) arctic and boreal species, due to its northern latitude. Such cold-adapted plants are more sensitive to changes in temperature conditions (Hongmei et al. 2009). At a certain warming level, these species cannot resist the heat stress and will disappear. At the same time, there are not many warm-tolerant species that can colonise the area. Only the most resilient species can tolerate the heat stress. Indeed, after short-term warming, cold-adapted species with a circumboreal distribution (e.g. *Alchemilla filicaulis, Gallium boreale, Luzula multiflora*) disappeared along the temperature gradient, while more cosmopolitan species (e.g.

Deschampsia cespitosa, Veronica officinalis, Agrostis capillaris, and *Festuca sp*.) remained. Moreover, ruderal species, typical for disturbed soils (e.g. *Taraxacum sp., Hieracium sp., Senecio vulgaris* and *Veronica officinalis*) took advantage of the voids left by disappearing species. *Taraxacum sp., Hieracium sp.* and *Senecio vulgaris* are included in the global invasive species database because of their ability to thrive in a wide range of conditions and their rapid dispersal capacities (Global invasive species database 2016). Therefore, a rise in temperature makes these kind of systems more vulnerable for alien invasive species in the short-term. The finding that global warming makes systems more vulnerable to invasive species has been reported by several other studies (Mooney & Hobbs 2000).

After long-term warming, the warmest plots (+20 °C) were not occupied by ruderal species. While *Hieracium sp*. and *Senecio vulgaris* did not occur at all, *Taraxacum sp.* was found, but not in the warmest plots. *Veronica officinalis* was also present, but in far lower numbers than after short-term warming. In general, there were few species that flourished exclusively at high temperatures after long-term warming, but there were a multitude of species that occurred at all temperature levels, including the warmest level (+ 20°C).

The loss of species with warming can be a general trend for northern ecosystems, but in this specific case it could also be partly caused by the limited species pool in Iceland, caused by its isolated location. Northern boreal areas on the mainland, which experience warming through climate change, may not have this problem. Heat tolerant species from the south might expand their range and replace the cold-adapted species in the long term. Therefore, on a global level, species richness may not necessarily decrease with soil temperature on the long run, but species distributions are largely predicted to shift poleward as global temperatures increase, which has already been observed across a range of taxonomic groups (Chen et al. 2011; Hickling et al. 2006). However, since structural changes in vegetation are slow, at first, more species will disappear than that will immigrate, favouring ruderal or even invasive species on the short term (Sandel & Dangremond 2012). Additionally, many rare and endangered species have restricted distributions and may be particularly vulnerable to climate change, leading to extinctions.

4.4 Soil warming effects on Soil water content and Nitrogen

4.4.1 Effects of soil warming on nitrogen cycling

Contrary to our expectations and to what was found in other studies (Bai et al. 2013; Jarvis & Linder 2000; Liski et al. 1999), there was no relationship between temperature and total N availability: N availability did not change significantly with short- or long-term soil warming. The soil N stock, however, did decrease exponentially along the soil temperature gradient. This means that the same amount of N was available for plants, for a much lower N stock, suggesting that the N cycling did indeed increase. This is clearly shown by the increasing ratio of soil N availability to soil N stock along the soil temperature gradient.

4.4.1.1 Hypothesis 4 - Effects of soil warming on Nitrogen availability

The observation that a maximal amount of nitrate was available at a warming level of about 20°C in GN, is in accordance with results for other subarctic regions. The optimum temperature for nitrification is correlated with the geographic origins of the soil (Bai et al. 2013). Warmer climatic regions support nitrifying microbes, with an optimum at 35°C, and this optimum temperature can drop to 30°C in colder regions (Dalias et al. 2002; Mahendrappa et al. 1966) or even to 20°C in subarctic regions such as Alberta, Canada (Dalias et al. 2002; Malhi et al. 1990). A possible explanation for the observation that GO did not show a temperature optimum for nitrate concentrations is that nitrifying microbial communities in the warmer plots of GO had shifted towards communities with higher nitrification optimum temperatures, but that microbial communities did not make this transition yet after short-term warming. Further research on the matter is necessary.

The decrease of ammonium concentrations along the soil temperature gradient in GN might be an effect of the drying of the soil. In moist conditions, ammonium cannot be converted to nitrate, because this is a strict aerobe process, while ammonification can occur under both aerobic and anaerobic conditions. This might lead to ammonium accumulation. Moreover, wetter soils have been shown to be positively correlated with N mineralisation rates (Hong 2014; Rustad et al. 2001). Indeed, the ammonium concentrations showed a positive relationship with SWC in GN (Appendix 5). In GO, however, deeper soils dampened soil drying, which might explain the absence of such a trend here.

4.4.1.2 Hypothesis 5 - Effects of changes in the nitrogen status

While total N availability did not alter plant community structure in any way, nitrate and ammonium each separately did influence the community structure in GN. However, their environmental vectors fit poorly onto the ordinations and also in relation to species richness, the fit was rather low. This suggests that N availabilities do alter community structure, but have a relatively low impact compared to temperature.

In order to find out whether changes of plant community structure with N availabilities in GN were induced by warming, it was necessary to look at the environmental preferences of the species along the temperature gradient, by looking into the community weighted Ellenberg indicator values. As the trend in Ellenberg values and available nitrate concentrations in GN was very similar (hump shaped), it is likely that nitrate was the source of available N which has the strongest impact on the community composition.

This clear hump shape that was seen after short-term warming did not occur after long-term warming. This suggests that soil warming did indeed indirectly alter species composition through changes in the N cycle in the short-term, but not in the long-term. We speculate that microbial communities seem to have adapted to warmer conditions after long-term warming, creating similar nutrient conditions over the entire warming gradient, thereby cancelling out its effects on plant communities. Because the maximum nitrate concentrations lie at intermediate warming levels after short-term warming, and the species richness exponentially decreases with soil warming, we conclude that the change in species richness with temperature were not driven by changes in nutrient availability.

4.4.1.3 *Hypothesis 6 - Effect of soil warming on soil nitrogen stocks*

The observation that soil N stocks decreased drastically with soil warming, both after short-term and long-term warming, shows that N was lost from the system soon after the start of the warming. Leaching and gaseous N emissions from nitrification and denitrification are the two possible pathways for ecosystem N loss (Bai & Houlton 2009). Laboratory incubation studies found that increased temperature can indeed result in an increase of denitrification (Braker et al. 2010).

The volcanic soils (andosols) in the research area have a high water storage capacity (Sigurdsson et al. 2016), which reduces the chance of N leaching from the soil (Vitosh et al. 1995). However, the soils were not very deep, often reaching bedrock less than 30 cm below the surface. This, in combination with high precipitation rates, might on the other hand have promoted leaching.

A possible explanation for the large decrease in soil N stocks along the soil temperature gradient might be winter N leaching. A growing number of studies have revealed the potential importance of winter processes in influencing annual N budgets (e.g. Brooks & Williams 1999; Groffman et al. 2001). Warming-induced increased N mineralization during the winter season at a time when plant roots are largely inactive, coupled with an increased frequency of soil freeze-thaw cycles, may increase soil N leaching (Turner & Henry 2010).

4.4.1.4 *Hypothesis 7 – relation between soil N stock and species richness*

Since the majority of soil N is unavailable for plants, the positive relation of soil N stock with species richness, composition and presence is unlikely to be due to the effects of soil N stocks on the plant community structure. However, it might be the other way around. Soil N stocks have been shown to increase with plant diversity (Fornara & Tilman 2008; Cong et al. 2014). Therefore, the loss of species with warming might be another reason for the loss of N from the system, as the loss of species may reduce the number of N immobilising plant species. However, this relationship could also be a result from the fact that both are correlated to soil temperature. Further research on N immobilising plant species is needed to clarify this matter.

4.4.2 Hypothesis 8 - Effects of soil warming on soil water content

As expected, soil warming caused a decrease in SWC in both the short-term and the long-term warmed grasslands. The fact that the warming-induced drying was somewhat stronger in the GN was likely due to its shallower soil, which leads to a lower water storage capacity.

However, one has to bear in mind that SWC samples were taken after an exceptionally dry period, likely leading to an overestimation of the mean annual effect of temperature on SWC. Such dry-spells in mid- to late summer are infrequent and usually soils are wetter due to high precipitation rates, saturating the plots with water and reducing inter-plot differences. Consequently, the role of SWC may be overestimated. This is supported by Sigurdsson et al. 2016, who state that very little drying is detected if many measurement campaigns are taken into account

4.4.2.1 Hypothesis 9 - Indirect soil warming effects - Effects of changes in soil water content

The observation that there was a significant positive relation between SWC and species richness, species composition and species presence, whereas the multivariate analysis indicated that the effect of SWC was opposite to that of temperature, could either be a result from the relation between temperature and SWC or be a real effect of SWC on the plant community. To disentangle whether the observed plant community responses were caused by soil warming or by a decrease in SWC, we made use of the environmental preferences of the species present. This was done by looking at the community weighted Ellenberg values for moisture preference. If the decrease in species richness would be caused by drought, the community weighted Ellenberg value for moisture preference would decrease with decreasing SWC. This was not the case, indicating that, even though SWC decreased along the soil temperature gradient, the drying effect did not alter vegetation structure in itself. This could be because the SWC is almost always higher than the permanent wilting point (Leblans et al. 2017), suggesting that effects of the drying for plants will be minimal. Moreover, SWC is measured in the top 5 cm of the soil, so this dry-spell may not necessarily cause physiological stress for plants, especially not those that have roots that extend below 5 cm. Earlier research by Michielsen, L. in 2014 on stomatal conductance confirmed that deep-rooted species (*Ranunculus acris, Poa pratensis* and *Agrostis capillaris*) show no significant drought-induced stomatal closure, neither in the GN nor in GO. The stomatal conductance of shallow-rooted *Poa* and *Agrostis* grasses, however, was lower in GN than in GO. This fits with the measurements of the water status of both valleys (Leblans et al. 2016). Climate change might increase the frequency of these dry periods, which might favour drought-tolerant or deep-rooted species in the warmest plots in the future. Whether drying induces significant biological or biochemical responses depends largely on the initial hydrological conditions of the site. Therefore, in other, dryer ecosystems, drought induced by warming may have more profound effects.

5 Conclusion

This thesis studied the long-term and short-term changes in plant community structure in response to changes in soil temperature along natural soil warming gradients in an Icelandic subarctic grassland ecosystem. Apart from the response of plant communities, it was also investigated whether this response was a direct temperature effect, or an indirect temperature effect through increased nitrogen cycling or soil drying.

Species richness was higher in the long-term warmed grasslands than in the short-term warmed grasslands, as hypothesised. This was likely because communities in the short-term warmed grasslands are still moving towards a new equilibrium, which has already been reached on the long-term.

Contrary to what was hypothesised, species evenness and diversity did not change with warming. Species richness, on the other hand, decreased exponentially along the soil warming gradient. The species that disappeared along the warming gradient were cold-adapted species, which in the short term were replaced by ruderal species, and in the long term by cosmopolitan species.

Soil warming was found to increase N cycling and decrease SWC, as expected, but these were not the main drivers behind the changes in vegetation structure along the temperature gradient. SWC had no effect on plant community structure and N availabilities had only minor effects. Therefore, it can be concluded that the loss of species with warming is a pure temperature effect.

On a global level, local extinctions may occur due to warming, but the decrease in total species number may be less dramatic, as Iceland is an isolated island with a limited species pool, impeding species migration. On the short term, warming may promote the spread of invasive species, threatening natural ecosystems. On the long-term, systems will find a new equilibrium.

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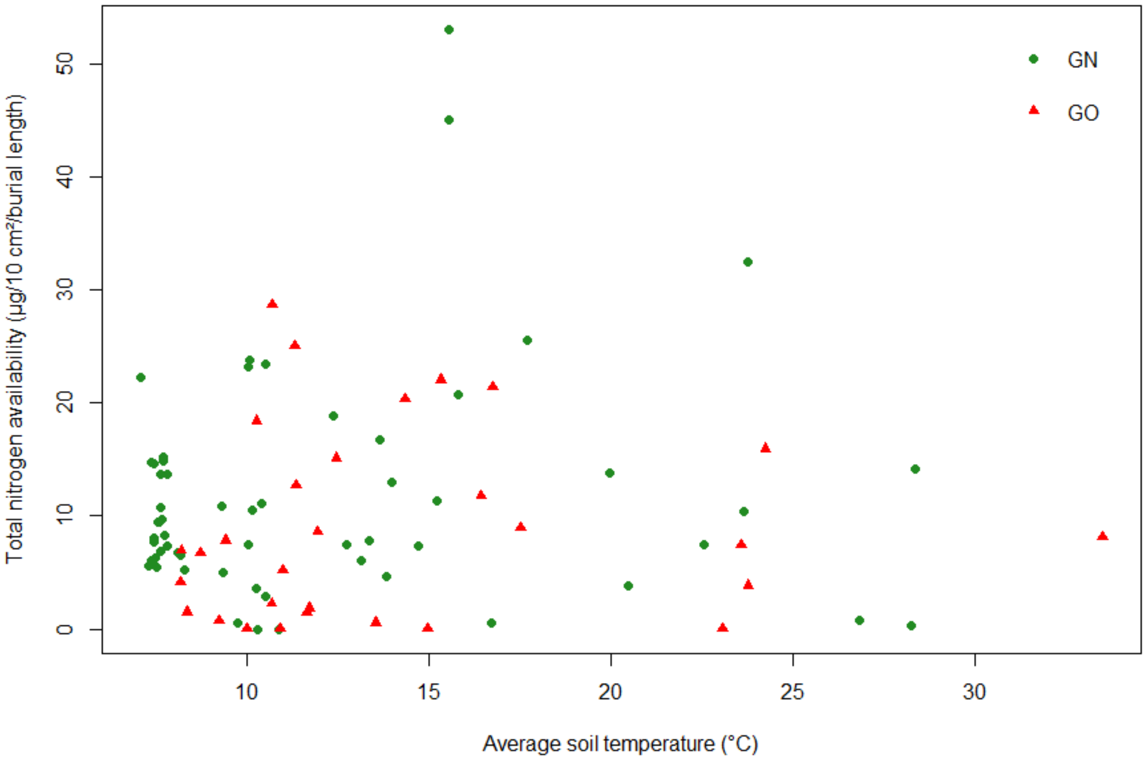
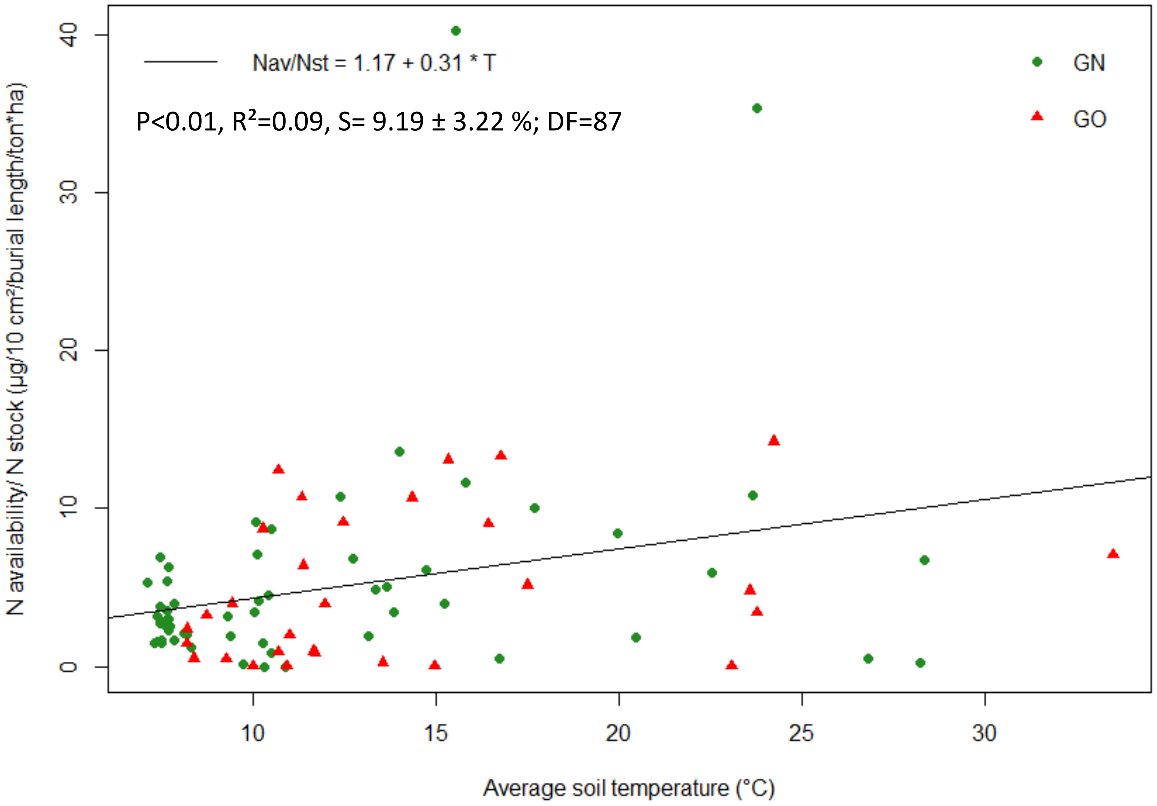
7 Appendix

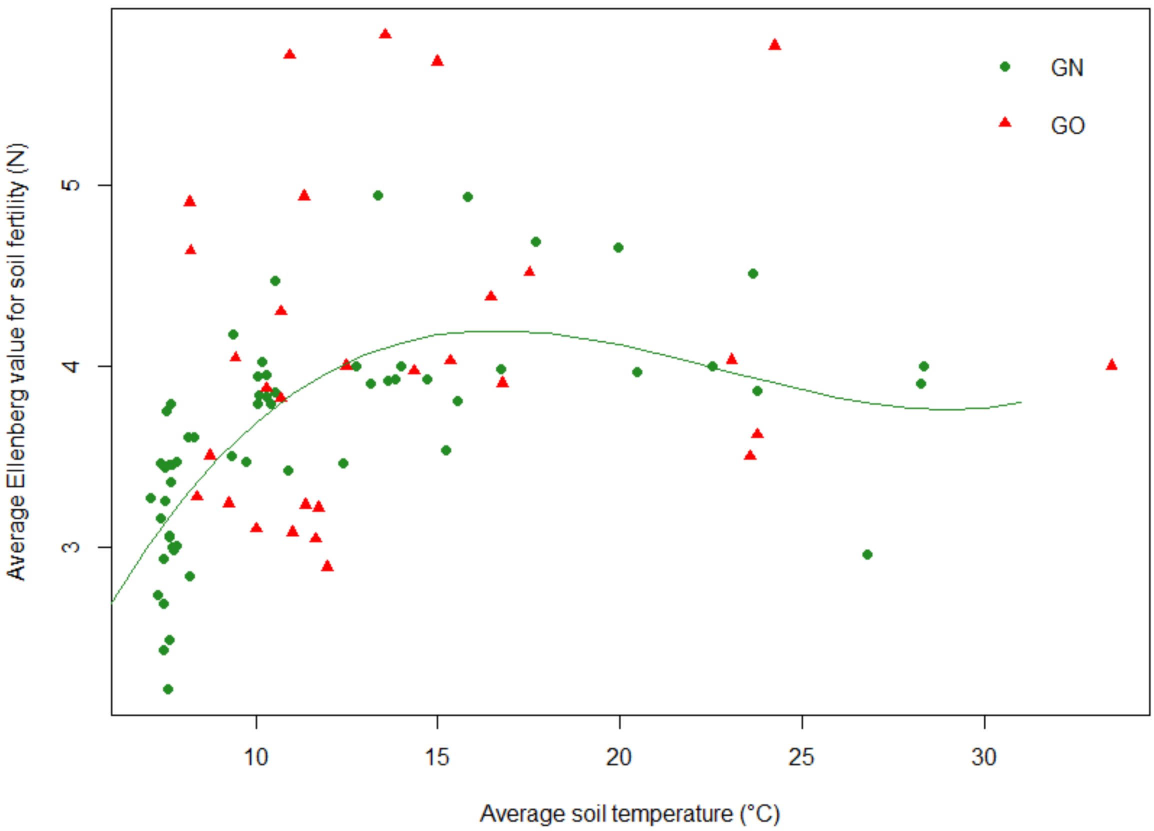


Appendix 1: Total nitrogen availability in relation to average soil temperature over the period of the 1st of May 2015 until the 30th of September 2015.

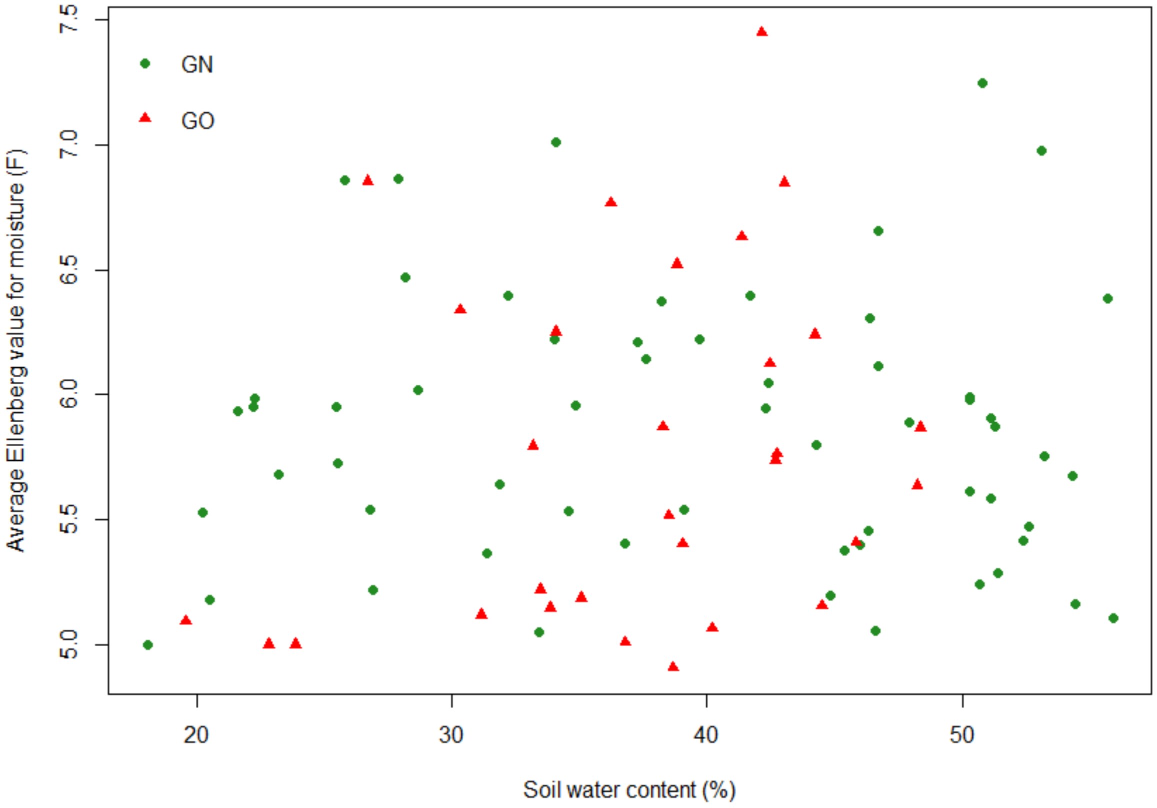


Appendix 2: Relationship between the ratio of soil nitrogen availability to soil nitrogen stock and average soil temperature measured over the period of the 1st of May 2015 until the 30th of September 2015. Nav/Nst= ratio of soil nitrogen availability to soil nitrogen stock, T= average soil temperature, GN= short-term warmed grasslands, GO= long-term warmed grasslands, the sensitivity (S) was calculated as the linear change in N availability / N stock per °C as % of the ambient value. DF=degrees of freedom.

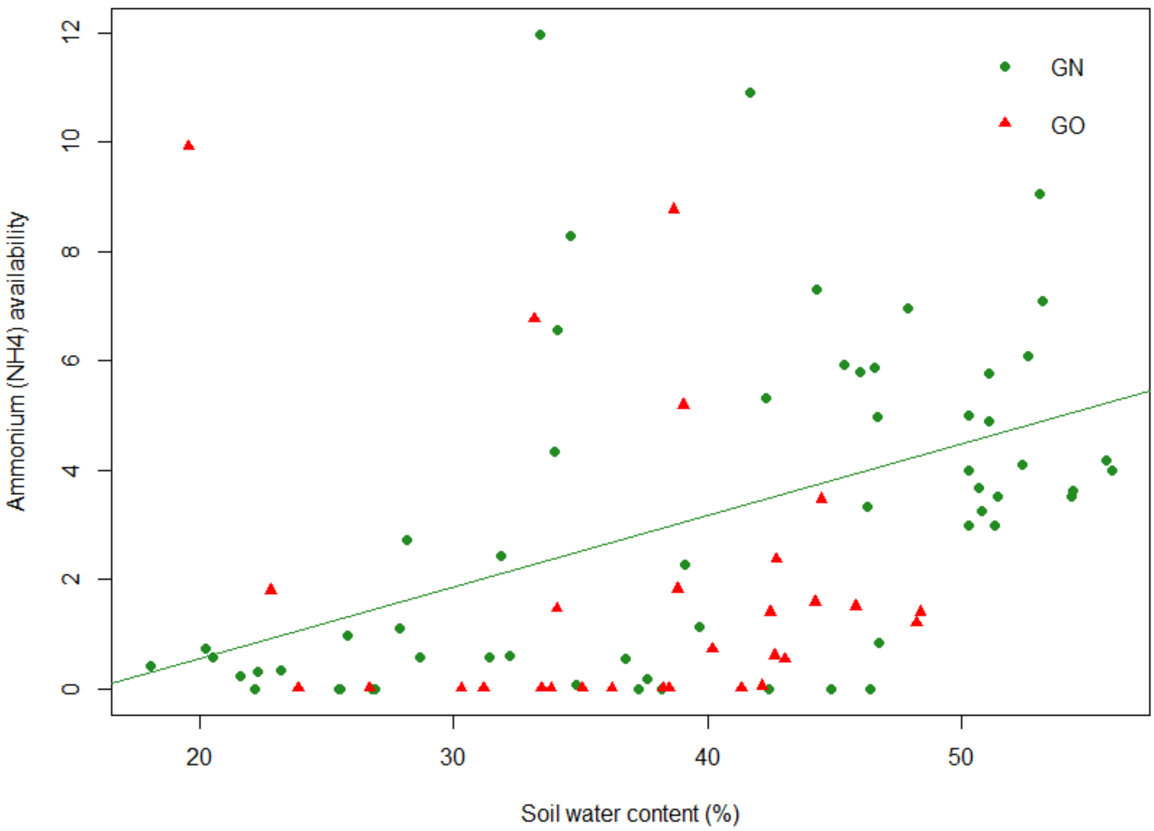
$P < 0.001$, $R^2 = 0.49$, DF= 56



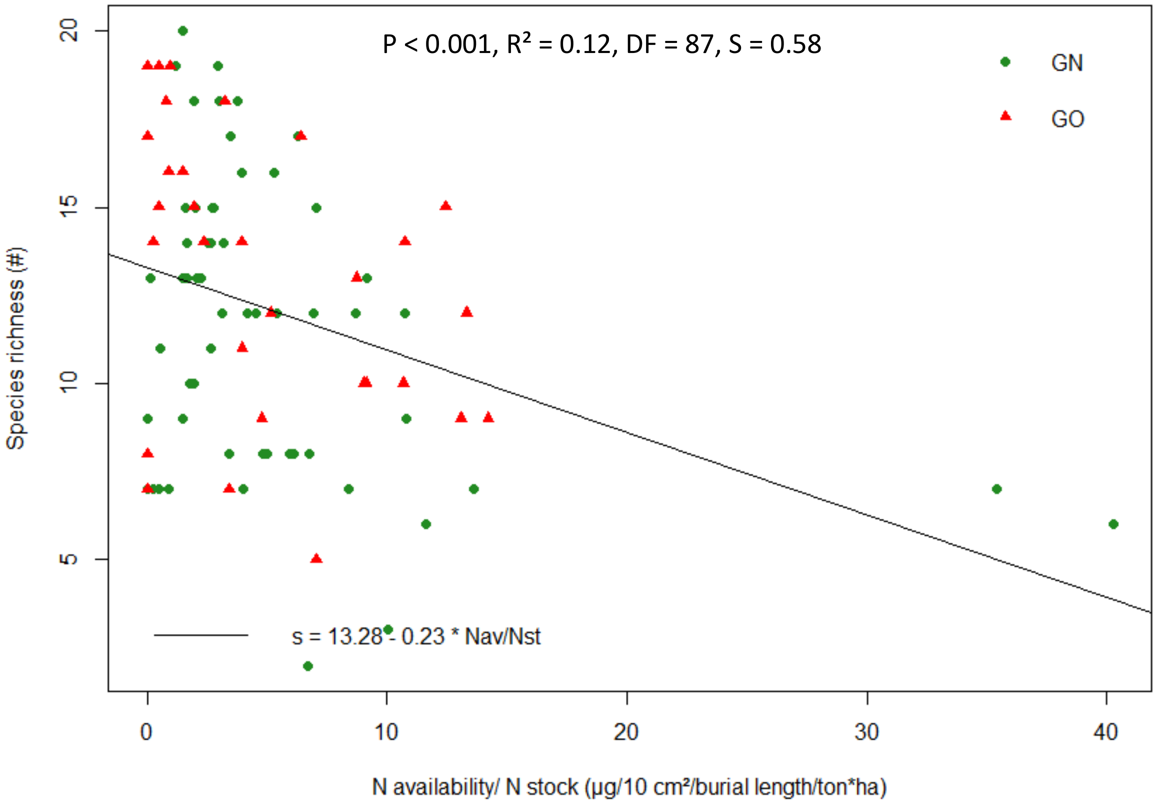
Appendix 3: Relationship between average Ellenberg value for soil fertility (N) and the average soil temperature measured over the period of the 1st of May 2015 until the 30th of September 2015. GN= short-term warmed grasslands, GO= long-term warmed grasslands. The sensitivity (S) was calculated as the linear change in the average Ellenberg value for soil fertility per °C as % of the ambient value. DF=degrees of freedom.



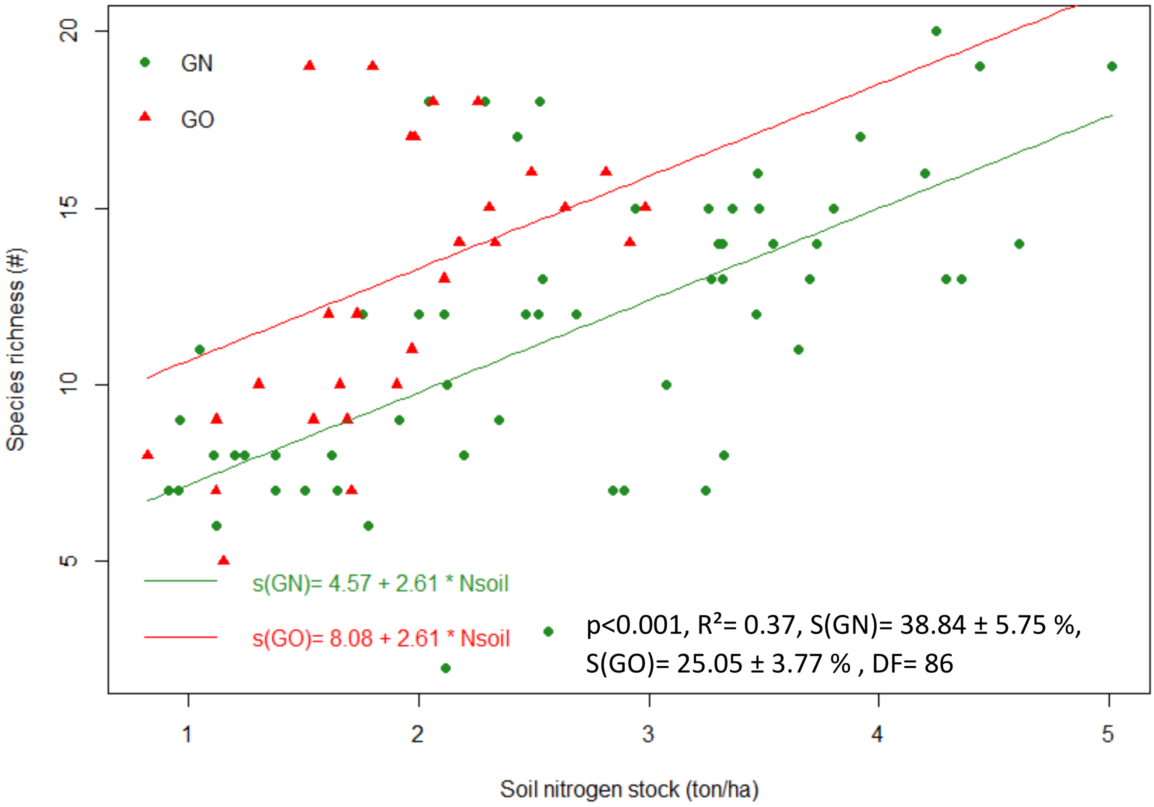
Appendix 4: Average Ellenberg value for moisture in function of soil water content. GN= short-term warmed grasslands, GO= long-term warmed grasslands.



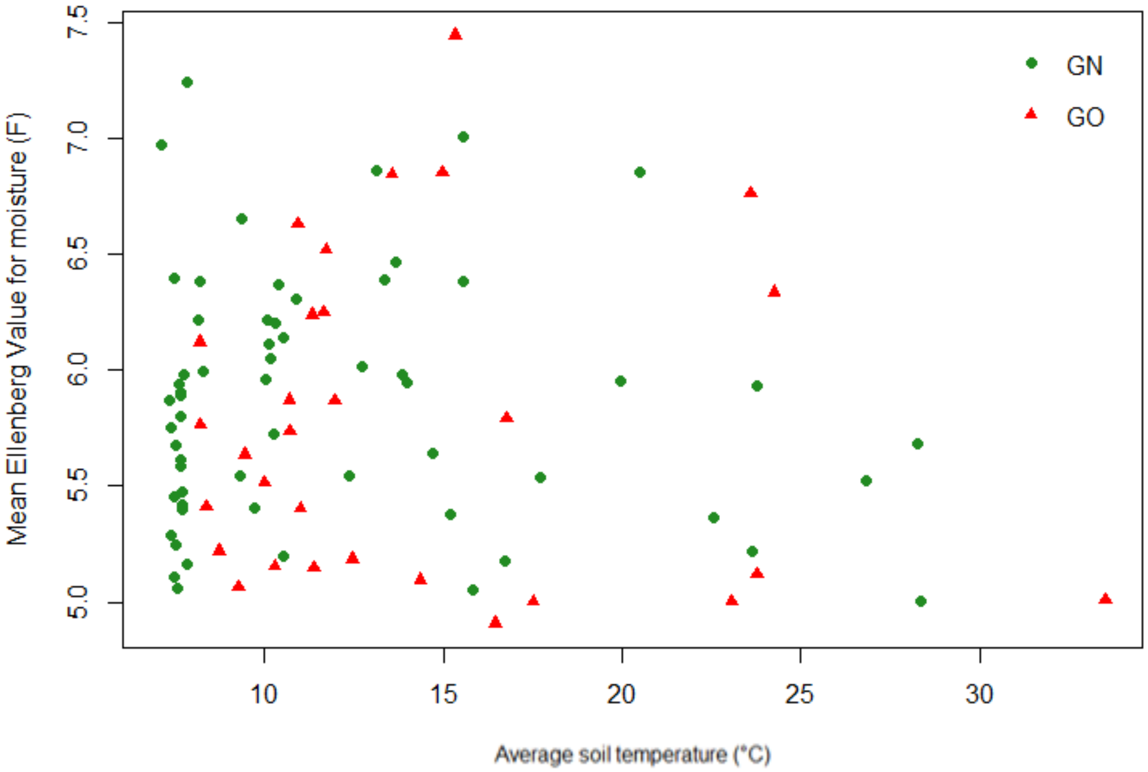
Appendix 5: relation between Ammonium (NH_4^+) availability and soil water content (%) in GN. GN= short-term warmed grasslands, GO= long-term warmed grasslands.



Appendix 6: Relationship between species richness and the ratio of soil nitrogen availability to soil nitrogen stock. Nav/Nst= ratio of soil nitrogen availability to soil nitrogen stock, s= species richness, GN= short-term warmed grasslands, GO= long-term warmed grasslands. The sensitivity (S) was calculated as the linear change in species richness per µg as % of the lowest value. DF=degrees of freedom.



Appendix 7: Relationship between species richness and soil nitrogen stock.GN= short-term warmed grasslands, GO= long-term warmed grasslands, s= species richness, Nsoil= soil nitrogen stock, p=significance R²=, coefficient of determination S=sensitivity, DF=degrees of freedom.



Appendix 8: community weighted Ellenberg value for moisture in relation to average soil temperature over the period of the 1st of May 2015 until the 30th of September 2015. GN= short-term warmed grasslands, GO= long-term warmed grasslands.

Appendix 9: Incidence of each species occurring in the long-term warmed grassland per warming level. Incidence is the number of plots in which the species was observed per warming level divided by the total amount of plots in that warming level.

GO	+ **0** °C	+ **1** °C	+ **3** °C	+ **5** °C	+ **10** °C	+ **20** °C
Agrostis capillaris	1	1	1	1	1	1
Anthoxanthum odoratum	1	1	1	1	1	0,8
Festuca richardsonii	1	0,8	1	0,8	0,6	0,4
Carex nigra	1	0,8	1	0,8	0,6	0,2
Festuca vivipara	1	0,6	0,8	0,6	0,4	0,6
Poa pratensis	0,8	0,8	1	1	1	0,8
Equisetum pratense	0,8	0,8	0,4	0,8	0,2	0,2
Galium boreale	0,6	0,8	0,8	1	0,8	0,2
Potentilla anserina	0,6	0,6	0,6	0,2	0,6	0,4
Luzula multiflora	0,6	0,4	0,8	0,4	0	0,2
Galium normanii	0,4	0,2	0,6	0,2	0,2	0,2
Leontodon autumnalis	0,2	0	0,2	0,2	0	0,2
Agrostis stolonifera	0	0,4	0,4	0,2	0,4	0,2
Geranium sylvaticum	0	0,2	0,4	0,2	0,4	0,2
Trifidus repens	0	0,2	0,2	0,4	0	0,2
Rumex acetosa	1	1	0,4	0,8	0,4	0
Equisetum arvensis	1	0,6	1	1	0,6	0
Galium verum	1	0,4	0,8	0,2	0,4	0
Ranunculus acris	0,8	0,6	0,6	0,6	0,8	0
Alchemilla filicaulis	0,4	0,4	0,2	0,2	0	0
Agrostis vinealis	0,4	0,4	0,2	0	0,2	0
Deschampsia cespitosa	0,4	0,4	0	0,2	0,4	0
Botrichium lunaria	0,4	0,2	0,2	0,2	0	0
Taraxacum sp.	0,4	0	0,8	0,4	0	0
Viola palustre	0,2	0,4	0,6	0,2	0	0
Galium uligunosum	0,2	0,4	0,6	0,2	0	0
Cardamine pratensis	0,2	0,2	0	0	0	0
Alchemilla alpina	0,2	0	0	0	0	0
Bistorta vivipara	0,8	0,6	0	0	0	0
Thymus arcticus	0	0,2	0	0	0	0
Prunella vulgaris	0	0,2	0	0	0	0
Geum rivale	0	0	0,2	0	0	0
Filipendula ulmaria	0	0	0,2	0	0	0
Epilobium ciliatum	0	0	0,2	0	0	0
Juncus arcticus	0	0	0,2	0	0	0
Phleum sp.	0	0	0	0,2	0	0
Equisetum variegatum	0	0,2	0	0	0	0,2
Epilobium palustre	0	0	0	0,2	0	0,4
Cerastium fontanum	0	0	0	0	0	0,4
Veronica officinalis	0	0	0	0	0	0,4
Viola canina	0	0	0	0	0	0,2
Carex bigelowii	0	0	0	0	0	0,2

Appendix 10: Incidence of the species occurring in the short-term warmed grassland per warming level. Incidence is the number of plots in which the species was observed per warming level divided by the total amount of plots in that warming level.

GN	+ 0 °C	+ 1 °C	+ 3 °C	+ 5 °C	+ 10 °C	+ 20 °C
Agrostis capillaris	0,966667	1	1	1	1	1
Anthoxanthum odoratum	0,966667	0,8	0,8	1	0,6	0,8
Equisetum pratense	0,966667	1	0,8	0,8	0,7	0,2
Festuca richardsonii	0,9	1	0,8	1	0,8	0,6
Festuca vivipara	1	1	0,8	0,4	0,6	0,8
Poa pratensis	0,9	0,8	0,8	1	0,8	0,6
Ranunculus acris	0,533333	1	1	1	0,6	0,6
Luzula multiflora	0,866667	0,8	0,6	0,4	0,5	0
Carex nigra	0,9	0,6	0	0,4	0,3	0
Galium verum	0,966667	0,6	0,4	0	0	0
Galium boreale	0,866667	0,6	0,2	0,2	0	0
Equisetum arvense	0,366667	0,2	0,2	0,2	0,1	0
Alchemilla filicaulis	0,266667	0,4	0,2	0	0,1	0
Senecio vulgaris	0	0	0	0	0,5	0,4
Thymus arcticus	0,2	0	0	0	0,2	0,4
Taraxacum sp.	0,066667	0,2	0	0	0,1	0,4
Deschampsia cespitosa	0,2	0	0	0	0,1	0,2
Veronica officinalis	0	0	0,2	0	0,1	0,2
Hieracium sp.	0	0	0	0	0,1	0,2
Geum rivale	0,033333	0,2	0,2	0	0	0
Agrostis stolonifera	0,2	0	0	0,2	0	0
Potentilla anserina	0,033333	0	0	0	0,1	0
Betula pubescens	0	0	0,4	0	0	0
Potentilla crantzii	0,166667	0	0,2	0	0	0
Leontodon autumnalis	0,066667	0,2	0	0	0	0
Filipendula ulmaria	0,033333	0,2	0	0	0	0
Geranium sylvaticum	0	0,2	0	0	0	0
Pilosella islandica	0	0	0,2	0	0	0
Rumex acetosa	0,533333	0,2	0	0	0	0
Car exbigelowii	0,133333	0,2	0	0,4	0	0
Alchemilla alpina	0,1	0,2	0,2	0	0	0
Plantoga maritima	0	0	0,2	0,2	0,1	0
Galium normanii	0,8	0	0	0	0	0
Equisetum variegatum	0,066667	0	0	0	0	0
Viola palustre	0,133333	0	0	0	0	0
Botrichium lunaria	0,066667	0	0	0	0	0
Salix herbacea	0,066667	0	0	0	0	0
Cerastium fontanum	0,033333	0	0	0	0	0
Epilobium ciliatum	0,033333	0	0	0	0	0
Galium uligunosum	0,033333	0	0	0	0	0
Juncus trifidus	0,033333	0	0	0	0	0
Bistorta vivipara	0,533333	0	0	0	0	0

Agrostis vinealis	0,2	0	0	0	0	0
Cardamina pratensis	0,466667	0	0	0	0	0

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