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Short-term and long-term changes of vegetation biomass in response to natural soil warming and nitrogen availability in a subarctic grassland

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Table of contents

Scientific summary

In recent years, awareness of potential feedback mechanisms between global warming and primary productivity has increased. These feedbacks illustrate the importance of warming experiments, which have yielded important knowledge during the past decades. However, a thorough understanding on long-term warming effects on primary productivity and the effect of soil warming is still lacking. This study aimed to investigate short-term and long-term soil warming effects on vegetation biomass (proxy for primary productivity) and to explore the mechanisms behind the responses (direct temperature effects vs. indirect effects via warming-induced increased nitrogen (N) availability). The study took place at the ForHot research site, in the neighborhood of Hveragerði, Iceland. At this research site, subarctic grasslands on natural geothermal soil temperature gradients of different age are studied. One grassland had been warmed for approximately 8 years and was used to study short-term soil warming effects. The second grassland had been warmed for at least 50 years (probably for centuries), and was used to study long-term soil warming effects.

Contrary to our expectations, soil warming decreased total vegetation biomass in both the short-term and the long-term warmed grassland. This was caused by a strong decrease of belowground biomass along the soil warming gradient in both grasslands presumably due to increased root turnover or direct temperature stress at high temperatures. Aboveground biomass was not affected by soil warming. The increasing shoot/root ratio with warming indicates that the aboveground productivity was not nutrient or water limited. Therefore, we hypothesize that the aboveground biomass was light limited, instead of temperature or nutrient limited. We conclude that long-term warming could decrease the carbon (C) sink capacity (primary production) of these northern grasslands by decreasing its belowground biomass stock if soil warming gives a reliable estimate of the effect of total ecosystem warming.

Wetenschappelijke samenvatting

In de afgelopen jaren is het besef over de mogelijke terugkoppelingsmechanismen tussen de opwarming van de aarde en de primaire productiviteit gestegen. Deze mechanismen illustreren het belang van opwarmingsexperimenten en hebben reeds belangrijke kennis opgeleverd in de afgelopen decennia. Er is echter een gebrek aan kennis omtrent de effecten van bodemopwarming en vooral lange termijn opwarming op primaire productiviteit. Deze studie had als doel om de korte en lange termijn bodemopwarming-effecten op de vegetatiebiomassa (proxy voor de primaire productiviteit) en de mechanismen achter de reacties (directe temperatuureffecten versus indirecte effecten via een warmte geïnduceerde verhoging van stikstof beschikbaarheid) te onderzoeken. Het experiment vond plaats in het ForHot onderzoeksterrein, in de buurt van Hveragerði, IJsland. Op dit onderzoeksterrein werden twee subarctische graslanden bestudeerd gelegen op natuurlijke bodemtemperatuurgradiënten van verschillende ouderdom. Het eerste grasland was ongeveer voor 8 jaar opgewarmd en werd gebruikt om de korte termijn bodem warmingseffecten te bestuderen. Het tweede grasland was minstens reeds 50 jaar (en waarschijnlijk reeds eeuwen) opgewarmd, en werd gebruikt om de lange termijn bodemopwarmingseffecten te bestuderen.

In tegenstelling tot onze verwachtingen, zorgde de bodemopwarming voor een daling in de totale vegetatiebiomassa in zowel het korte als lange termijn opgewarmd grasland. De oorzaak was een sterke afname van de ondergrondse biomassa langs de bodemopwarmingsgradiënt. Deze afname kwam vermoedelijk door de kortere levensduur van wortels of door temperatuurstress bij hoge temperaturen. De bovengrondse biomassa werd niet beïnvloed door de bodemopwarming. De toenemende *shoot/root ratio* van bovengrondse op ondergrondse biomassa geeft aan dat de bovengrondse productiviteit niet nutriënt- of water-gelimiteerd was. We kunnen dus veronderstellen dat de bovengrondse biomassa waarschijnlijk licht gelimiteerd was in plaats van de temperatuur- of nutriënt-gelimiteerd. We concluderen dat lange-termijn opwarming mogelijk de koolstof (C) opslagcapaciteit (primaire productie) van deze noordelijke graslanden verlaagt door de afname van de ondergrondse biomassa, tenminste indien bodemopwarming een betrouwbare proxy is van het effect van de totale ecosysteemopwarming.

Layman's summary

For several decades now scientists have been trying to understand the effects of climate change on natural ecosystems. In northern regions, warming will be more pronounced which will result in the melting of ice and frozen soils, affecting not only local ecosystems, but also the rest of the world. Iceland is a northern island situated on the edge between the continental plates of America and Eurasia. Volcanoes frequently occur on such borders and create geothermal systems. Geothermal systems are a unique opportunity to explore the effects of climate change with a natural soil temperature gradient and the presence of both young and old temperature gradients allows us to study whether changes are transient or not.

Short-term and long-term consequences of warming, changes in plant growth and N availability were studied along natural short-term (warmed for ~8 years) and long-term (warmed for at least 50 years) soil warming gradients in a subarctic grassland system. 6 different soil temperature gradients are used to collect data, namely control, +1°C, +3°C, +5°C, +10°C and +20°C. Increased soil warming caused a strong decrease in plant biomass, supposedly due the warming technique that is used in this study, the increased root turnover or direct temperature stress at high temperatures. The sharp decline in root biomass and constant aboveground biomass suggests that the grasslands are limited in light instead of nutrients or water. N availability did not seem affected by the increased soil temperature probably due to N competition with the measuring method and increase in N losses.

1. Introduction

1.1. Climate change

Climate is changing all around the world (Rosenzweig et al. 2008; IPCC 2013). The global cover of land- and sea ice is declining and more extreme weather conditions are occurring (Masciopinto & Liso 2016). An important aspect of climate change is the increase in global surface temperatures. Global predictions give us an idea of the effect climate change will have globally. However, it gives little information about the changes on the regional scale and it is precisely at this level that the impact will be felt the most. For example, climate models predict that for (sub)arctic areas the mean annual temperature will increase between 2.5 and 8.3°C by the end of the century, which is twice as much as the predicted average global warming (IPCC 2013). The stronger climate change at higher latitudes compared to the rest of the globe is due to a phenomenon called 'Polar amplification'. Polar amplification results primarily from positive feedbacks from the retreat of ice and snow (Bekryaev et al. 2010).

Climate change is a wildly discussed topic in the media nowadays because of the potential adverse effects it can have on our ecosystems and society. Amongst others, it is likely to affect plant carbon assimilation, growth, biomass allocation, and nutrient uptake (Gavito et al. 2001). It is important to understand how plant production responds to climate change, because of its importance in the carbon cycle, which can induce powerful feedbacks to the climate system itself. Net primary productivity, the yield of dry matter production of a plant community, is linked to the carbon (C) and nitrogen (N) cycle (de Graaff et al. 2006; Fan et al. 2008). Global warming is predicted to impact ecosystem function and global primary production (Cramer et al. 2001; Zhao & Running 2010). It is hard to predict the net effect climate change is going to have on primary productivity because it depends on many direct and indirect effects. Moreover, in order to predict the impact of warming on plant productivity, we need to know how it will affect the productivity of different components of the ecosystem (such as aboveground vs. belowground productivity) (Bellard et al. 2012).

1.2. Effects of climate change on ecosystem C stocks at high northern latitudes

Subarctic soils contain one third of the global terrestrial C stock even if they only cover 5% of the terrestrial surface (White et al. 2000). The reason for this large C storage is that the low temperatures prevent decomposition of dead plant material and subsequent release of respired CO_2 (McGuire et al. 2012). Therefore, a larger part of the C taken up during primary productivity is stored for a long time span in the soil. However, if the temperature would increase in this region, this large C stock has the potential to become a source for greenhouse gasses because of the increase in decomposition (Woodwell et al. 1998; Follett et al. 2012), causing the stored C to escape to the atmosphere (Davidson & Janssens 2006). On the other hand, climate warming could stimulate productivity at high northern latitudes via direct or indirect mechanisms (Fig.1), which could increase the C uptake in this region if the plant-derived inputs to the soil are increased more than the decomposition (Weedon et al. 2013).

Direct positive warming effects on primary productivity include (1) temperature stimulation of the photosynthetic system, which causes a faster C uptake (Prentice et al. 2001) and (2) an extension of the growing season, as the period with suitable conditions for growth extends with warming at high northern latitudes (Xu et al. 2013). The indirect positive effect of warming on primary productivity is a release of the N limitation of primary productivity, which is typical for high northern latitude ecosystems (Vitousek & Howarth 1991; Aerts & Chapin 2000), and is a consequence of the warming-induced increase in decomposition and thereby in N availability. Nitrogen limitation results from the transient nature of biologically available forms of N (Vitousek et al. 2002). Once N is fixed in the ecosystem, it becomes very susceptible to being removed by leaching through the soil or volatilizing to the atmosphere (LeBauer & Treseder 2008). Studies have shown that nutrient availability is expected to increase with higher temperatures (Liski et al. 1999; Jarvis & Linder 2000).

Changes in plant biomass, the total mass represented by all living plants, can be used as an estimate of productivity (Downing & Leibold 2002). Therefore, biomass stocks can be used as a proxy to study the effects of temperature increase on ecosystem productivity. The current uncertainty on the effects of long-term warming on primary productivity (reasons: see § 1.3), is one of the main reasons why this study on plant productivity and N stocks along long- and short-term temperature elevations is important.

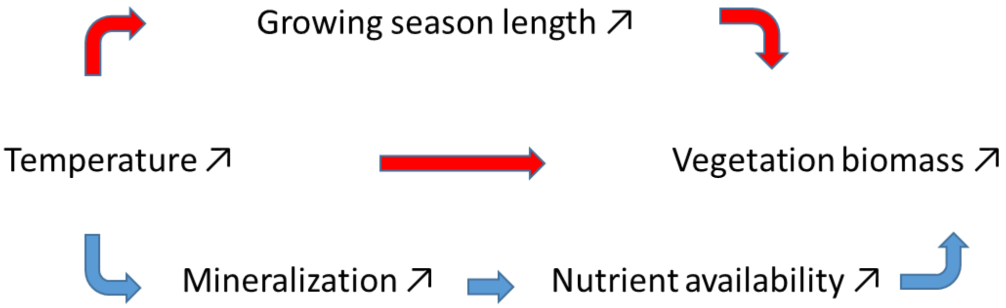

Figure 1: Direct and indirect effects of increased temperature on vegetation biomass (red: direct effects and blue: indirect effects).

1.3. Natural geothermal soil warming gradients as an alternative for climate manipulation studies

The effect of warming on primary productivity is generally studied using warming manipulation experiments. These experiments have yielded many new insights and a better understanding of the functioning of warming ecosystems. However, climate manipulation experiments are often limited in time and in size due to logistical and financial constraints (O'Gorman et al. 2014; De Boeck et al. 2015). They typically run for a short time period, thus if the response is not linear in time or if a new stable state is not reached after the experimental period, it will not be a good proxy for long-term effects. They also have limited temperature elevations and limited replicates which results in reduced detectability of small (but perhaps important) responses (De Boeck et al. 2015). Further, the small number of warming treatments also limits the possibility of detecting non-linear responses (De Boeck et al. 2015).

One option to overcome many problems that arise when using climate manipulation experiments, is the use of natural geothermal soil warming gradients. Geothermal systems are present for a long time period which makes it possible to study long-term warming effects (O'Gorman et al. 2014). On the other hand, these systems are dynamic, sometimes shifting toward previously unwarmed areas, making it possible to also study short-term warming effects (O'Gorman et al. 2014). This combination is very interesting as it allows to compare short-term (and possibly transient) from long-term (permanent) soil warming effects and to observe the dynamics of the warming response in time. This is very interesting because knowledge on long-term warming effects on plant-productivity are lacking. It is important to fill this knowledge gap because warming effects on plant productivity are not necessarily linear in time, and extrapolations of short-term observations might lead to a substantial over- or underestimation of the effect. (O'Gorman et al. 2014).

Another benefit of geothermal systems is the broad soil temperature gradient (from unwarmed soils, to soil temperatures up to the boiling point) that is covered by these geothermal systems. This makes it possible to select the most relevant temperature range for your specific research question. Further, geothermal soil temperature gradients are continuous, making it possible to observe non-linear responses along the gradient. Moreover, the small distances between the soil warming levels (typically ≤ 50 m between unwarmed soils and warming levels of +20°C) have the advantage that other environmental variables (e.g. precipitation) remain constant (Sigurdsson et al. 2016. Finally, natural geothermal soil warming gradients offer a low-cost alternative for climate manipulating 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 (De Boeck et al. 2015). The gradients are all located in volcanic areas, and are thus underlain by volcanic soils (andosols), which have particular soil properties. One of these properties is the strong bounds with soil organic matter, resulting in high C sequestration potential and high water holding capacity (Arnalds 2004). The most important restriction of such geothermal soil warming gradients is the decoupling of soil warming from air warming. This is certainly important when the investigated processes are driven more by air temperature. Nevertheless, soil temperature is, in comparison to air temperature, still a relatively unexplored area in science

1.4 Subarctic grasslands as study ecosystem

Subarctic grasslands were chosen as study ecosystems in this study because of their wide distribution (ca. 40 % of the global terrestrial surface are grasslands, whereof 25 % are located at northern high latitudes; Chapin et al. 2002). Moreover, grasslands have a high C sink capacity and are therefore very relevant to study in the context of warming effects on plant C uptake (Soussana et al. 2007; Yoshitake et al. 2015). Also, grasslands are underrepresented in literature studies because most of the time the focus of research is centered on forests.

1.5. Aim of study

The aim of this thesis was to study short-term and long-term soil warming effects on plant productivity in subarctic grassland ecosystems and to investigate whether the response is a direct temperature response, or indirect response via increased N availability as a consequence of temperature stimulation of decomposition. The specific hypotheses that were tested were:

1. **Effects of soil warming on total vegetation biomass**
    a. We hypothesized that increased soil temperature would increase total vegetation biomass of subarctic grasslands after both short-term and long-term natural soil warming.
    b. We hypothesized that there would be a threshold warming level up to where a positive soil warming effect on the total vegetation biomass would occur. Above this threshold, we expected a negative impact of soil warming on total vegetation biomass because the northern vegetation is not adapted to high temperatures.
    c. We hypothesized that the threshold would be higher in the short-term than in the long-term warmed grassland because of adaptation of the species present in the long-term grassland or the income of new heat tolerant species.
2. **Direct and indirect effects on total vegetation biomass**
    a. We hypothesized that the positive soil warming effect on vegetation biomass stocks would act mainly indirectly through increasing N availability as a consequence of the temperature stimulation of decomposition, thereby stimulating plant growth.
    b. We hypothesized that the N availability would be higher in the short-term warmed grassland than in the long-term warmed because of the recent exposure to higher soil temperatures, and would cause a stronger stimulation of the total vegetation biomass.

2. Material and methods

2.1 Study sites

Our study sites were located in the surroundings of Hveragerði (63°59'54.27"N, 21°11'58.80"W), and are part of the FORHOT research site (www.forhot.is). Hveragerði is a town in the southwest of Iceland, located 45 km to the east of Reykjavík. Hveragerdi is very suitable for our research because the geothermal activity occurring in this area creates naturally warmed subarctic grasslands. The volcanism present in Iceland is a consequence of its location on the divergence of the North American and Eurasian plates (Sigmundsson 2006). Hveragerði is located at the margin of an active volcano belt. Heat escapes from magnetic underlying dykes and penetrates to the surface, forming hotspots (Arnalds 2004). Geothermally warmed deep groundwater that flows underneath the rocks causes them to heat up. The rocks than heat the soil that lies on top, without contamination of the superficial ground water (O'Gorman et al. 2014). The closer it is to a hotspot, the warmer the soil is, which causes defined soil warming gradients. The climate of South West Iceland is maritime with cool summers and mild winters due to the cold East Greenland Current and the warm North Atlantic Current (Einarsson 1984). Hveragerði has a mean annual temperature of 4.4°C and the mean annual precipitation amounts to 1403 mm (Icelandic Meteorological Office, IMO). The growing season in Iceland begins half May and ends late August. The soil type at our sites is a brown andosol (a soil type with a volcanic origin), which typically has a high C storage capacity, high water retention capacity and a low bulk density (Arnalds 2004). The vegetation at our study sites consists of grasses, sedges, herbs and mosses with dominant species *Agrostis capillaris, Anthoxantum odoratum, Poa pratensis, Carex nigra, Gallium boreale and Ranunculus acris*, which are all species with a circumpolar distribution in boreo-arctic and temperate regions (Kristinsson & Sigurdsson 2010).

2.2. Experimental design

We selected two areas that are part of the ongoing FORHOT research project (www.forhot.is), with clear soil warming gradients (Fig.2). The FORHOT project investigates how changes in soil temperature affect various ecosystem processes in both natural grasslands and a planted 50-year old Sitka spruce forest stand in Iceland. The project was started as a pilot project in autumn 2011 and as a full-scale research project in spring 2013. It is coordinated by the Agricultural University of Iceland (www.forhot.is).

The short-term warmed grassland has been warmed for approximately 8 years. In 2008 an earthquake created new hotspots, exposing previously ambient ecosystems to warmer temperatures and so generating opportunities for research into transient responses to rapid warming (Halldorsson & Sigbjörnsson 2009). The grassland was used to investigate the effects of short-term warming on plant productivity and N cycling. In the short-term warmed grassland, the dominant species were *Agrostis capillaris, Anthoxantum odoratum Equisetum pratense* and *Ranunculus acris.*

The long-term warmed grassland, was situated in ‘Grændalur’ or ‘Green valley’ 2 km northwest of Hveragerði (Fig.3). This area could be used to study the effects of long-term soil warming on productivity and N cycling because the duration of the warming was ongoing for at least 50 years and probably for centuries (Kristján Sæmundsson, pers. comm.; Thorbjörnsson et al. 2009; Daebeler et al. 2014). The first time the name ‘Grændalur’ was mentioned into a historical document was in 1708 (Magnússon & Vídalín 1918-1921), and shows that its vegetation was more green than that of its surroundings (which points to geothermal activity) already 300 years ago. Further, a survey of geothermal activity in 1963-1965 showed that the grasslands have been more or less constant in soil temperature over the past 50 years (Kristján Sæmundsson, pers. comm.). More benefits to this valley are the regular soil temperature measurements that were recorded in various hotspot in the area since 2005 (Daebeler et al. 2014) and a detailed geothermal mapping of the valley in 2008 (Thorbjörnsson et al. 2009). The dominant species in the long-term warmed grassland were *Agrostis capillaris, Poa pratensis, Potentilla anserina, Galium boreale, Equisetum pratense* and *Equisetum arvense.*

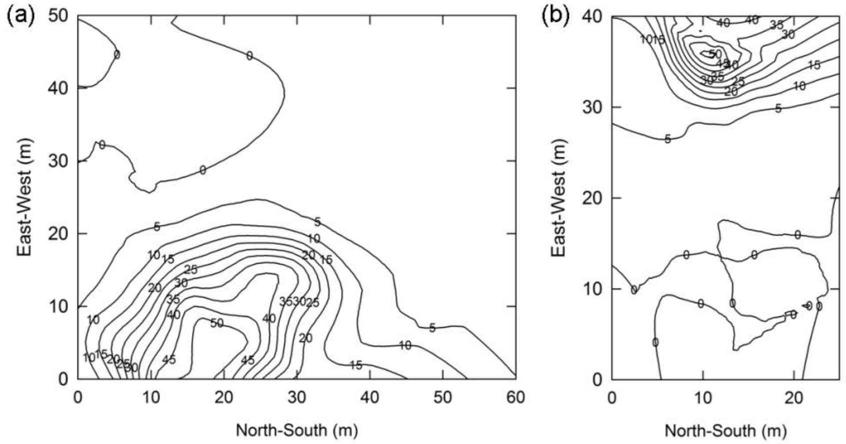

Figure 2: Schematic diagram showing the spatial distribution of geothermal soil warming at two of the ForHot grassland sites. Isoclines show differences in soil temperature (°C) at 10 cm depth between unwarmed and warmed areas. (a) One of the grassland sites which had been exposed to geothermal warming since an earthquake in 2008; (b) One of the grassland sites that had been geothermally heated for a at least 50 years and probably for centuries (Gorman et al 2014).

Figure 3: Left: short-term warmed grassland; Right: long-term warmed grassland (Pictures: Wendelien Meynzer, 2015)

In each area (the short-term and the long-term warmed grassland), five replicate transects were established along the temperature gradients. Each transect consisted of six temperature levels: one control plot with ambient soil temperature and five plots with approximately: +1, 3, 5, 10 and 20°C above the ambient soil temperature. Temperatures were measured hourly at 10 cm depth, using HOBO TidbiT v2 Water Temperature Data Loggers (Onset Computer Corporation, USA). The soil warming was relatively stable throughout the year, resulting in normal seasonal fluctuations in soil temperature (Appendix, Fig. A.1).

Each main plot of 2x2m, where non-destructive measurements were performed was accompanied by an adjacent 0.5x0.5 m subplot, where destructive measurements, such as biomass harvesting, were conducted. Transects were chosen to be alternately orientated uphill and downhill in order not to have water transport influence the results. Fences were used to exclude grazers out of the long-term grassland. In the short-term warmed grassland, no grazing occurred.

2.3. Data collection

2.3.1. Aboveground vascular and non-vascular biomass sampling

Vegetation samples were taken at the end of July in 2015, close to the moment of maximum standing biomass. All living vascular aboveground biomass was cut in the subplot within a 20x50 cm frame. The non-vascular vegetation (moss and lichens) and the litter were collected in a 20x20 cm subdivision of the 20x50 cm frame. After cutting, the samples of the living vascular aboveground biomass and non-vascular vegetation were separately stored in labelled paper bags. Afterwards, the samples were transported to the lab where they were separated in monocotyledons, dicots, equiseta, moss, lichens and litter. Next, the samples were dried at a temperature of 105 °C for 24h and weighed to determine the dry weight. All data were then transformed to weight per square meter (g m-2) by correcting for the respective sampling area. Homogenized subsamples of approximately 2 g were weighted with an accuracy of ± 0.01 g (A&D company Ltd, GX-600-EC, England). The subsamples were grinded at 13 000 rpm through a 0.05 mm grinding sieve with an ultra-centrifugal mill (Thermo Scientific, Flash 2000 elementanalysator, Italy) as preparation for CN analysis with flash combustion (Flash 2000 elementanalysator, Thermo Scientific, Italy).

2.3.2. Soil sampling

Two soil samples were taken within the vegetation harvest plot in early August 2015. The cores were obtained using a soil corer with a diameter of 5.12 cm. The cores were drilled to a depth of 30 cm, where possible, or until bedrock was reached. After this, the samples were separated into 0-5; 5-10; 10-20 and 20-30 cm depth segments and stored in the freezer at a temperature of -18 °C.

2.3.2.1 Fine root biomass

The first core was used to determine living fine root biomass (<2mm). The root biomass was separated through suspending the cores in water and by sieving several times over a sieve with

0.5 mm mesh size. This caused the floating roots to be separated from the sinking soil particles. The roots were then dried at 105 °C for 24 h and weighed to obtain the dry weight of the fine root biomass. All data were then transformed to weight per square meter (g m-2) correcting for the respective sampling area. The root samples were then prepared for CN analyses using the same method as described with the aboveground vascular and non-vascular vegetation (see § 2.3.1).

2.3.2.2. Total soil N stock

The second soil core was used to determine the soil N stock. The core was placed in the oven at 40 °C for 48 h. Then, a sieve with a mesh size of 2 mm and a fine brush were used to obtain representative subsamples of approximately 2 g. These subsamples were then prepared for CN analysis with the previous mentioned technique. The remainder of the core was dried at 105 °C for 24 h and weighted to obtain the dry weight of the soil sample.

2.3.3. N availability

N availability was measured by inserting plant Root Simulator probes (PRS™ probes, Western Ag Innovations Inc.; Saskatoon, SK, Canada) within the subplots for one week in October 2015. The probes are ion exchange resin membranes held in plastic supports that are inserted into the upper 10 cm of the soil to measure ion supply in situ with minimal disturbance. The probes are placed in the upper 10 cm of the soil where the major part of the roots are located, and where the major part of biological activity takes place. Four replicate samples were taken for each plot, which were pooled to obtain a reliable estimation of the N availability over the total plot surface. After the collection, the probes were cleaned and send to Western Ag Innovations Inc. for further analyses.

2.3.4. N stocks in vegetation and soil

The N stocks (ton ha-1) of aboveground vegetation and roots were calculated by multiplying the respective N concentrations (determined by flash combustion, see § 2.3.1. and 2.3.2) with the dry weight of the sample and correcting for the respective sampling size. The N stocks of the soil where calculated by multiplying the respective N concentration with the bulk density of the soil (derived from Leblans, 2016) and transformed to the same units by correcting for the respective core area.

2.3.5. Teabag decomposition study

As a proxy for the mineralization rate of organic matter, and thus of the release rate of nutrients, the temperature effect on decomposition rate (mass loss over time) was determined by the standardized teabag decomposition protocol (http://www.decolab.org/tbi/). Teabags were buried in the upper 10 cm of the soil from a period of June to September 2014 for 110 days. For each measurement plot, four replicates of green tea (easily decomposable litter) were used. Data are presented as average mass loss (%) per plot over the total burial period.

2.3.6. Soil moisture

The moisture of the soil was determined by taking a subsample (15-25 g) of the soil core. The wet sample was weighed and then placed in the oven to dry at the temperature of 105 °C for 24h. Afterwards it was weighed again to derive the dry weight of the sample. The difference between the wet weight and the dry weight yields the percentage of water in the soil.

2.3.7. Statistical analysis

Linear mixed models were used to test the effect of environmental factors (soil warming, N availability, and total soil N stock) on vegetation biomass (aboveground, belowground, total and the different aboveground vegetation classes, i.e. monocotyledons, dicots, equiseta, moss and lichens), using the environmental factor and warming duration (short-term vs. long-term) as explanatory variables. Further, the effects of soil warming on N availability, total soil N stocks and decomposition (easy decomposable litter), were tested with linear mixed models using soil temperature and warming duration (short-term vs. long-term) as explanatory variables. Principal component analysis (PCA) was performed to evaluate the relative importance of the different factors on vegetation biomass and to reveal overall differences between short-term and long-term warming. The PCA's were visualized by using ggbiplot2 graphs. All tests were performed in R software (RStudio Team, 2015) and null hypotheses were rejected at $p < 0.05$.

3. Results

3.1. Soil warming effects on vegetation biomass

3.1.1. Total vegetation biomass

Soil warming had no effect on the total aboveground biomass (which includes monocotyledons, dicotyledons, equiseta, mosses and lichens). The belowground biomass, on the contrary, did decrease with soil warming. The decrease amounted to 4.48 ± 0.65 % $°C^{-1}$ of the root biomass under unwarmed conditions and did not differ significantly between the short-term and long-term warmed grassland (Fig.4)

Total vegetation biomass, including both aboveground and belowground biomass, decreased significantly in both the short-term and the long-term warmed grassland. The decrease amounted to 3.64 ± 0.51% $°C^{-1}$ of the total vegetation biomass under unwarmed conditions (Fig.4) and did not differ significantly between the short-term and long-term warmed grassland.

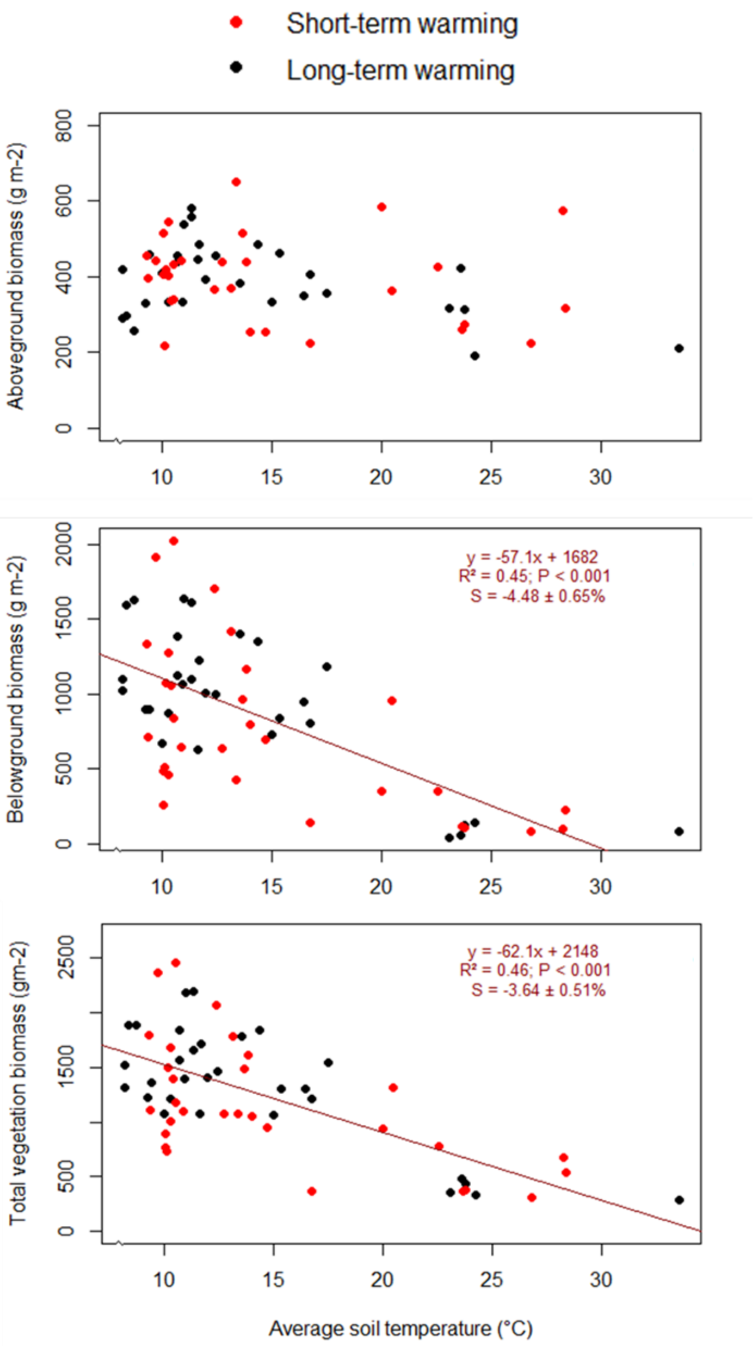

Figure 4: Soil warming effects on aboveground (upper panel), belowground (central panel) and total vegetation biomass (lower panel) in the short-term warmed grassland (red dots) and the long-term warmed grassland (black dots). The average soil temperature was calculated over the period of 1 May 2013 until 30 September 2015. Because the warming-duration x warming interaction was not statistically significant for neither the belowground biomass nor the total vegetation biomass, (P = 0.66 and 0.55 respectively), the short- and long term warmed grassland were combined in the linear regression analysis (dark red line). The sensitivity (S) was calculated as the linear change per °C as % of the at ambient soil temperature. Uncertainty levels (±) refer to SE's.

3.1.2. Aboveground biomass classes

The effect of soil warming on the different aboveground biomass classes did not vary significantly between the short-term and long-term warmed grassland. There was no significant soil warming effect on the separate categories: monocotyledons, dicotyledons, equiseta, moss and lichens (Fig.5).

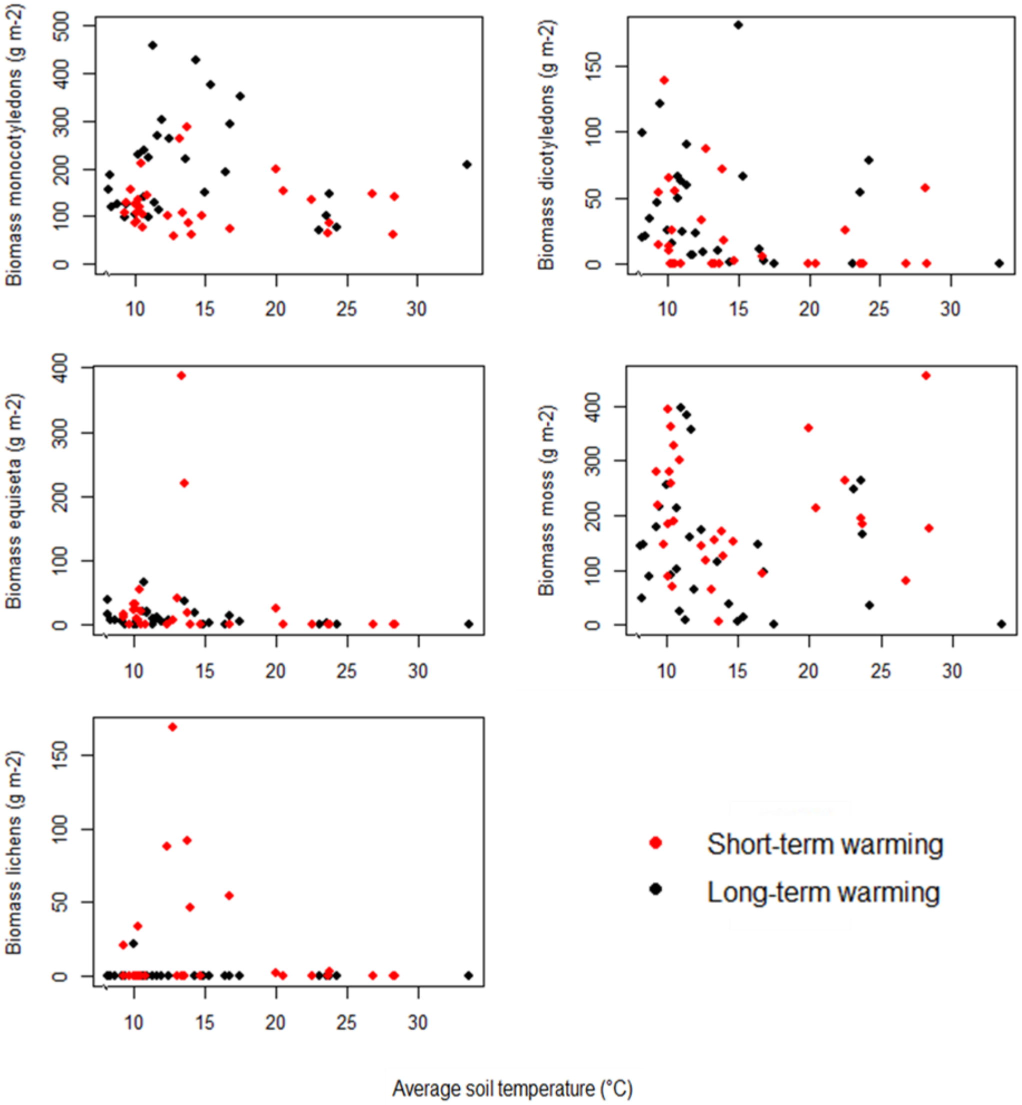

Figure 5: Soil warming effects on different aboveground vegetation classes in the short-term warmed grassland (red dots) and the long-term warmed grassland (black dots). The average soil temperature was calculated over the period of 1 May 2013 until 30 September 2015. Note the different scale on the y-axes.

3.2. Soil warming effects on vegetation biomass distribution

3.2.1. Shoot/root ratio

The shoot/root ratio increased under increasing soil warming in both the short-term and long-term warmed grassland. However, the shoot/root of the short-term warmed grassland increased with 0.04 units per °C while it increased with 0.08 units per °C for the long-term warming grassland (Fig. 6). The shoot/root ratio only includes the vascular vegetation so to investigate both vascular and non-vascular vegetation, the aboveground/belowground ratio will also be examined (see § 3.2.2).

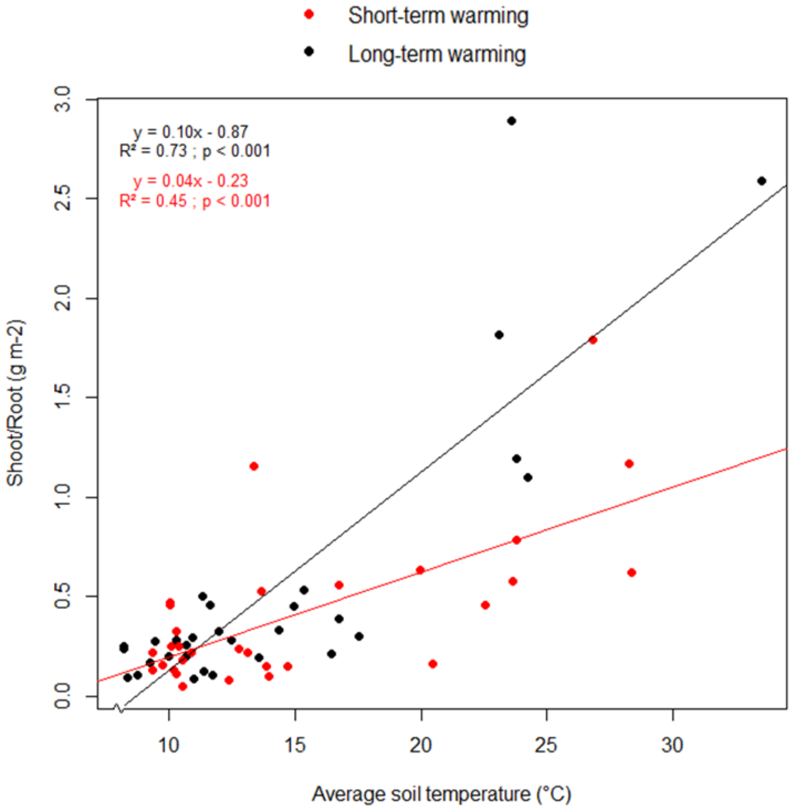

Figure 6: Soil warming effects on the shoot/root ratio the short-term warmed grassland (red dots) and the long-term warmed grassland (black dots). The average soil temperature was calculated over the period of 1 May 2013 until 30 September 2015. Regression line: red = short-term warmed grassland; black = long-term warmed grassland. The sensitivity (S) was calculated as the linear change per °C as % of the at ambient soil temperature. Uncertainty levels (±) refer to SE's.

3.2.2. Total aboveground biomass/belowground biomass ratio

Soil warming significantly increased the total aboveground biomass/belowground biomass ratio, with 0.17 units per °C. Total aboveground biomass included monocotyledons, dicotyledons, equiseta, moss and lichens. The effect of soil warming on the total aboveground biomass/belowground biomass ratio did not differ significantly between the short-term and long-term warmed grassland (Fig.7).

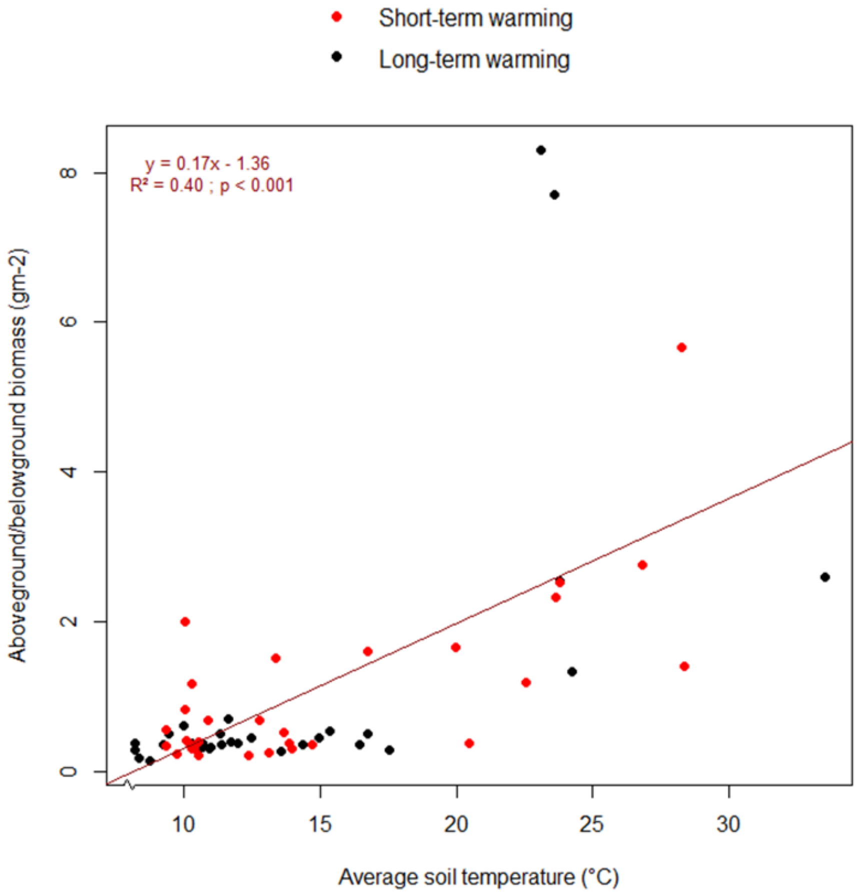

Figure 7: Soil warming effects on the total aboveground biomass/belowground biomass ratio in the short-term warmed grassland (red dots) and the long-term warmed grassland (black dots). The average soil temperature was calculated over the period of 1 May 2013 until 30 September 2015. Because the warming-duration x warming interaction was not statistically significant, (P = 0.20), the short- and long term warmed grassland were combined in the linear regression analysis (dark red line). The sensitivity (S) was calculated as the linear change per °C as % of the at ambient soil temperature. Uncertainty levels (±) refer to SE's.

3.3. Soil warming effects on N availability

3.3.1. N availability

The effect of soil temperature on N availability, measured with PRS probes, did not differ significantly between the short-term and long-term warmed grassland. Figure 8 shows that that there was no significant soil warming effect on N availability.

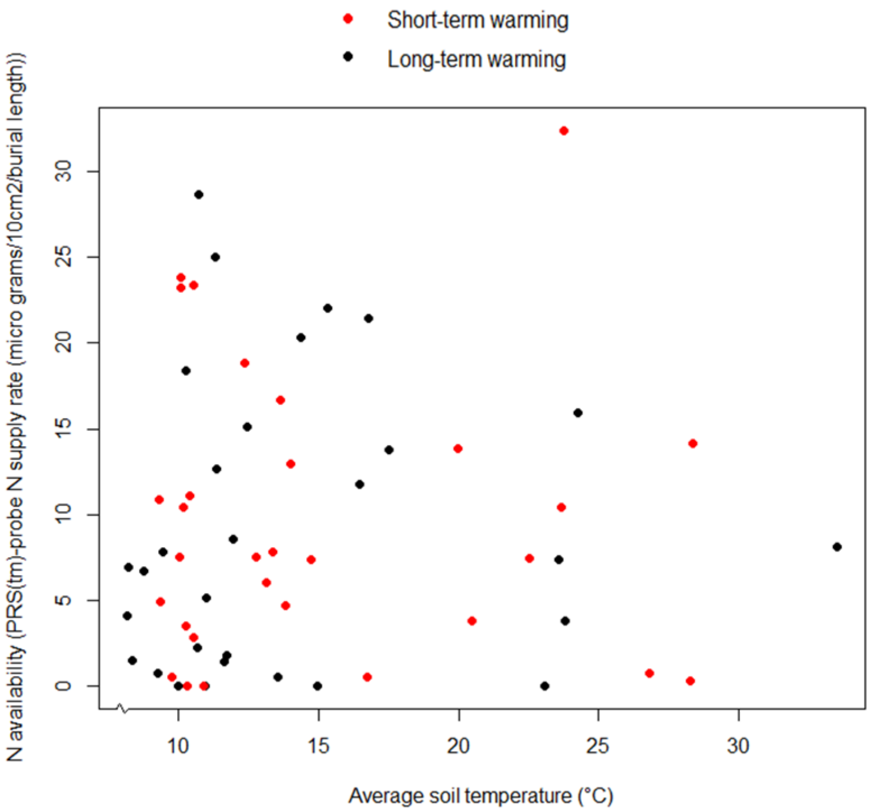

Figure 8: Relationship between N availability and average soil temperature in the short-term warmed grassland (red dots), and the long-term warmed grassland (black dots). Average soil temperature was calculated over the period of 1 May 2013 until 30 September 2015.

3.3.2. Soil N stocks

There was a significant effect of soil warming on the total soil N stock of the study sites. Soil warming significantly decreased the total soil N stocks, with 2.99 ± 0.51% $°C^{-1}$ of the total soil N stock under unwarmed conditions and there was no significant difference between the short-term and long-term warmed grassland (Fig.9).

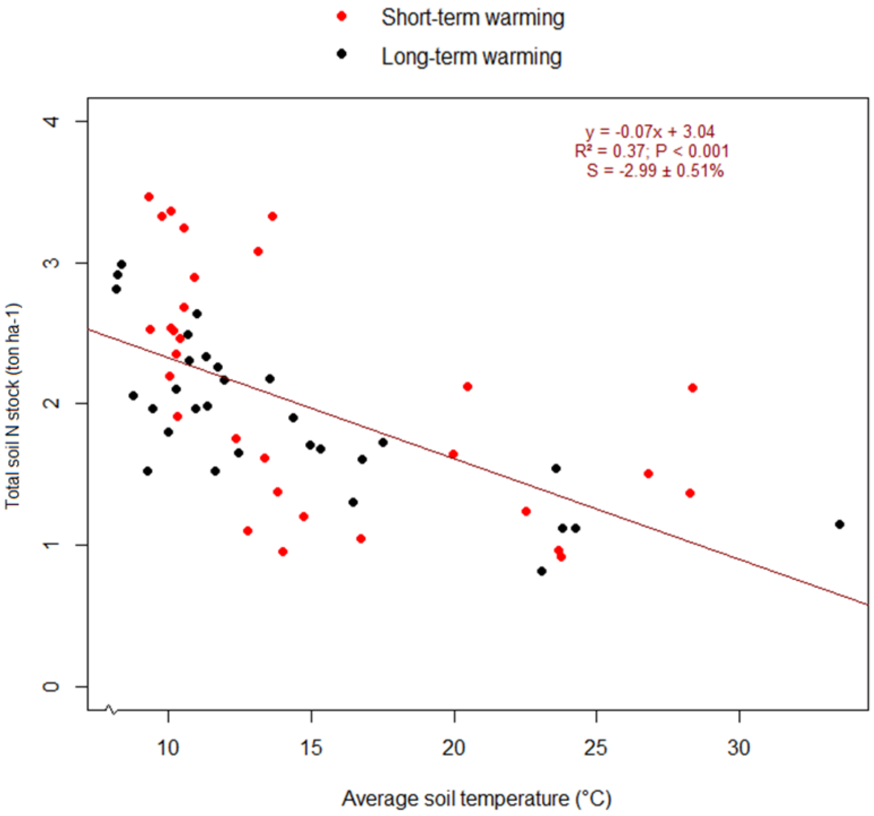

Figure 9: Relationship between total soil N stocks and soil temperature in the short-term warmed grassland (GN; red dots) and the long-term warmed grassland (GO; black dots). Average soil temperature was calculated over the period of 1 May 2013 until 30 September 2015. Because the warming-duration x warming interaction was not statistically significant (P = 0.68), the short- and long term warmed grassland were combined in the linear regression analysis (dark red line). The sensitivity (S) was calculated as the linear change per °C as % of the at ambient soil temperature. Uncertainty levels (±) refer to SE's.

3.3.3. Biomass N stocks

There was a significant negative soil temperature effect on biomass N stocks, amounting to -3.37 ± 0.49 % °C^{-1} of the biomass N stock under unwarmed conditions (fig.10). The soil warming effect did not differ between the short-term and long-term warmed grassland.

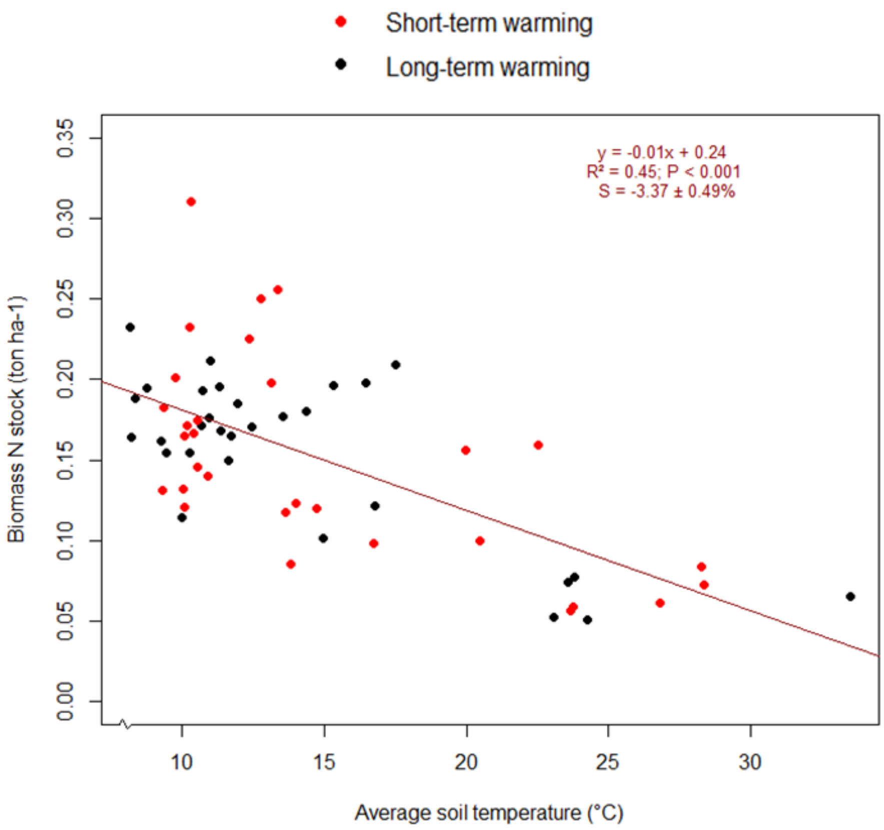

Figure 10: Relationship between biomass N stocks and average soil temperature in the short-term warmed grassland (red dots) and the long-term warmed grassland (black dots). Average soil temperature is calculated over the period of 1 May 2013 until 30 September 2015. Because the warming-duration x warming interaction was not statistically significant (P = 0.93), the short- and long term warmed grassland were combined in the linear regression analysis (dark red line). The sensitivity (S) was calculated as the linear change per °C as % of the at ambient soil temperature. Uncertainty levels (±) refer to SE's.

3.3.4. Mineralization

Mineralization (measured as mass loss of field-incubated easily decomposable litter) increased significantly under increased soil warming in the short-term and long-term warmed grassland. However, the rate of mass loss was slightly higher after short-term warming (1.60 ± 0.33 % $°C^{-1}$) than after long-term warming (0.68 ± 0.22 % $°C^{-1}$) compared to the mass loss under unwarmed conditions (Fig.11).

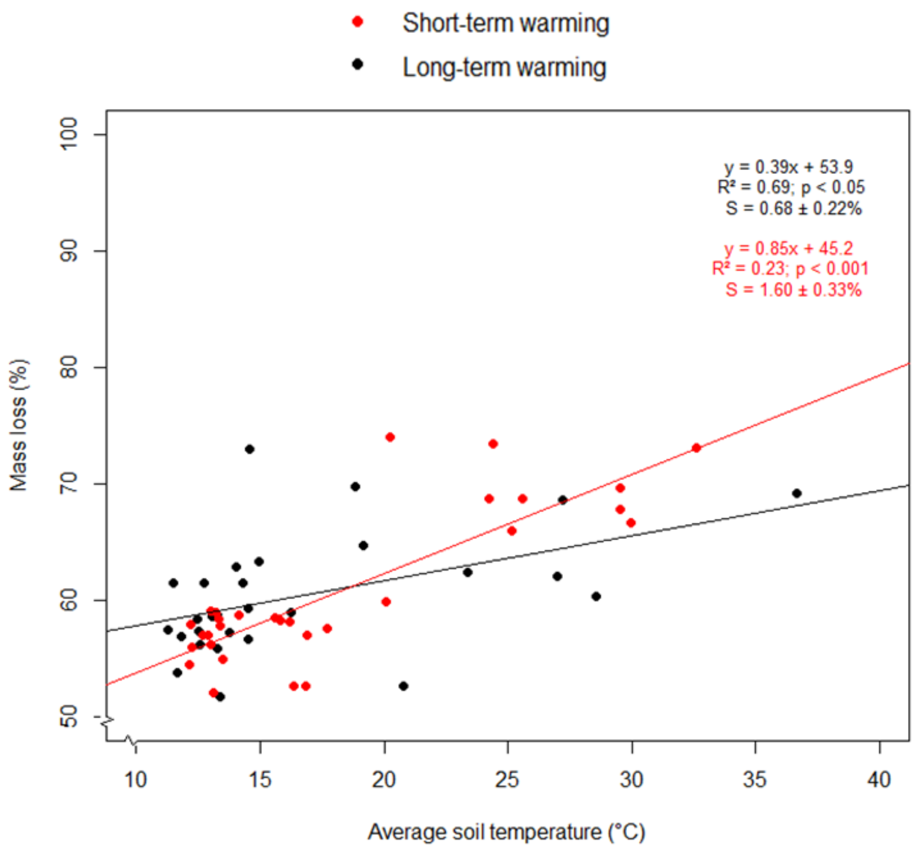

Figure 11: Relationship between mass loss (as a proxy for decomposition rate) and average soil temperature for the short-term warmed grassland (red dots) and the long-term warmed grassland (black dots). Average soil temperature was calculated over the period of 1 May 2013 until 30 September 2015. Easily decomposable litter was used (green tea, which contains a high proportion of easily degraded compounds as sugars, starches and proteins) to exanimate the decomposition. Regression line: red = short-term warmed grassland; black = long-term warmed grassland. The sensitivity (S) was calculated as the linear change per °C as % of mass loss at ambient soil temperature. Uncertainty levels (±) refer to SE's.

3.4. Effect of N status on total vegetation biomass

3.4.1. N availability

3.4.1.1. Total vegetation biomass

N availability had no effect on the aboveground, belowground or total biomass (Fig. 12) and neither on the different aboveground biomass classes (monocotyledons, dicotyledons, equiseta, moss and lichens) (data not shown).

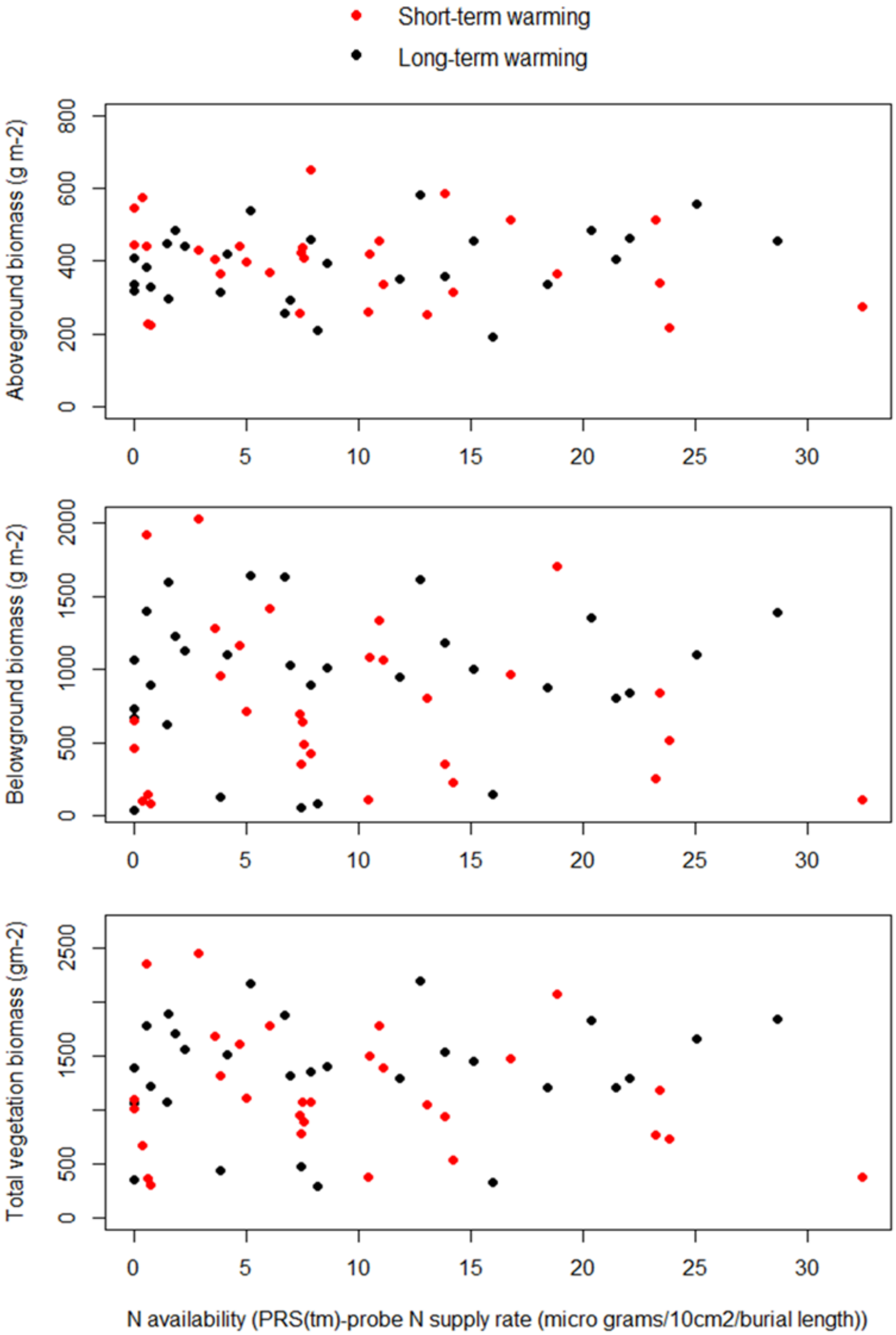

Figure 12: Relationship between aboveground (upper panel), belowground (central panel) and total biomass (lower panel) on the one hand and N availability on the other hand in the short-term warmed grassland (red dots), and the long-term warmed grassland (black dots).

3.4.2. Total soil N stock

3.4.2.1. Total vegetation biomass

Total soil N stock had no significant effect on the aboveground biomass (including monocotyledons, dicotyledons, equiseta, moss and lichens). The belowground biomass increased significantly with increasing soil N stocks for both the short-term and the long-term warmed grassland. However, the effect of total soil N stocks on belowground biomass was smaller in the short-term than in the long-term warmed grassland. In the short-term warmed grassland, the increase of belowground biomass amounted to 119 ± 32.0 % per ton N ha^{-1} (compared to the belowground biomass at the lowest total soil N stocks), while in the long-term warmed grassland it amounted to 337 ± 48.5 % per ton N ha^{-1} (compared to the belowground biomass at the lowest total soil N stocks) (Fig.13). Total soil N stock had a significant effect on the total vegetation biomass in the short-term and long-term warming grassland. Also here, the soil N stock effect on the biomass N stocks varied between the two sites. The short-term warmed grassland had an increase in total vegetation biomass of 59.4 ± 15.3 % per ton N ha^{-1} while the long-term grassland ad a strong increase of 128 ± 22.3 % per ton N ha^{-1} (both compared to the total vegetation biomass at the lowest total soil N stocks).

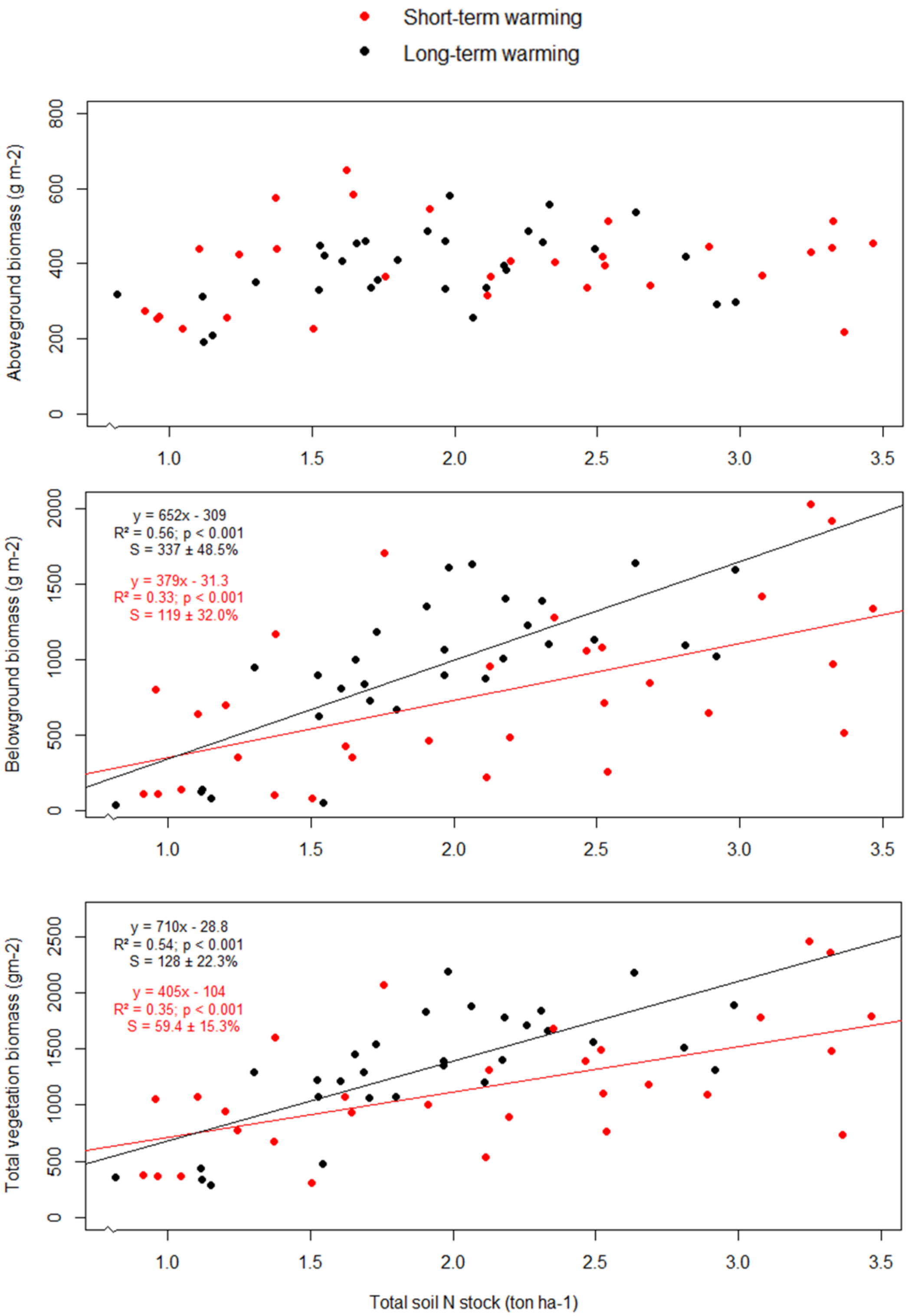

Figure 13: Relationship between aboveground (upper panel), belowground (central panel) and total vegetation biomass (lower panel) on the one hand and total soil N stock on the other hand in the short-term warmed grassland (red dots) and the long-term warmed grassland (black dots). Regression lines: red = short-term warmed grassland; black = long-term warmed grassland. The sensitivity (S) was calculated as the linear change in vegetation biomass per ton N ha^{-1} as % of the value at the lowest N stock. Uncertainty levels (±) refer to SE's.

3.4.2.2. Aboveground biomass classes

In the short-term warmed grassland, aboveground monocotyledon biomass increased significantly with increasing total soil N stock, with 9.01 ± 6.18 % per ton N ha^{-1} (compared to the to the aboveground monocotyledon biomass at the lowest total soil N stocks). In the long-term warmed grassland, however, no increase of monocotyledon biomass with increasing total soil N stock was found. Further, there were no significant effects of total soil N stock on the other vegetation classes (dicotyledons, equiseta, moss and lichens) (Fig. 14).

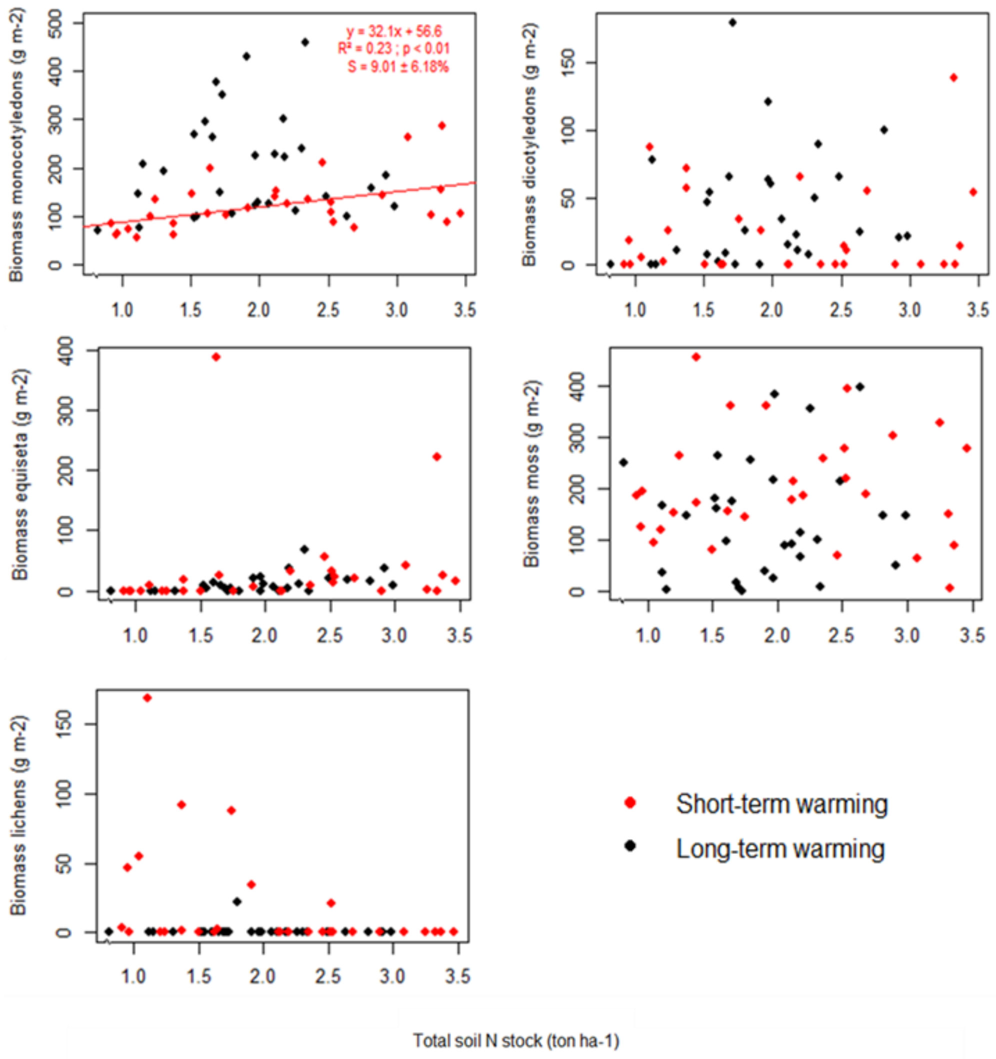

Figure 14: Relationship between the different aboveground biomass classes and total soil N stock in the short-term warmed grassland (red dots) and the long-term warmed grassland (black dots). Regression lines: red = short-term warmed grassland. The sensitivity (S) was calculated as the linear change in vegetation biomass per ton N ha^{-1} as % of the value at the lowest total soil N stock. Uncertainty levels (±) refer to SE's. Note the different scale on the y-axes.

3.5. Relative importance of the effects of soil warming, N status and moisture on vegetation biomass

For the short-term warmed grassland, the results of the PCA analysis showed a clear gradient of the different temperature classes along the first axis (Fig. 15). The first axis, which explained 57.0% of the total variation, was associated with soil temperature, total soil N stock and soil moisture. No differentiation between the different temperature treatments occurred along the second axis, which was associated with N availability and explained 25.0 % of the total variation.

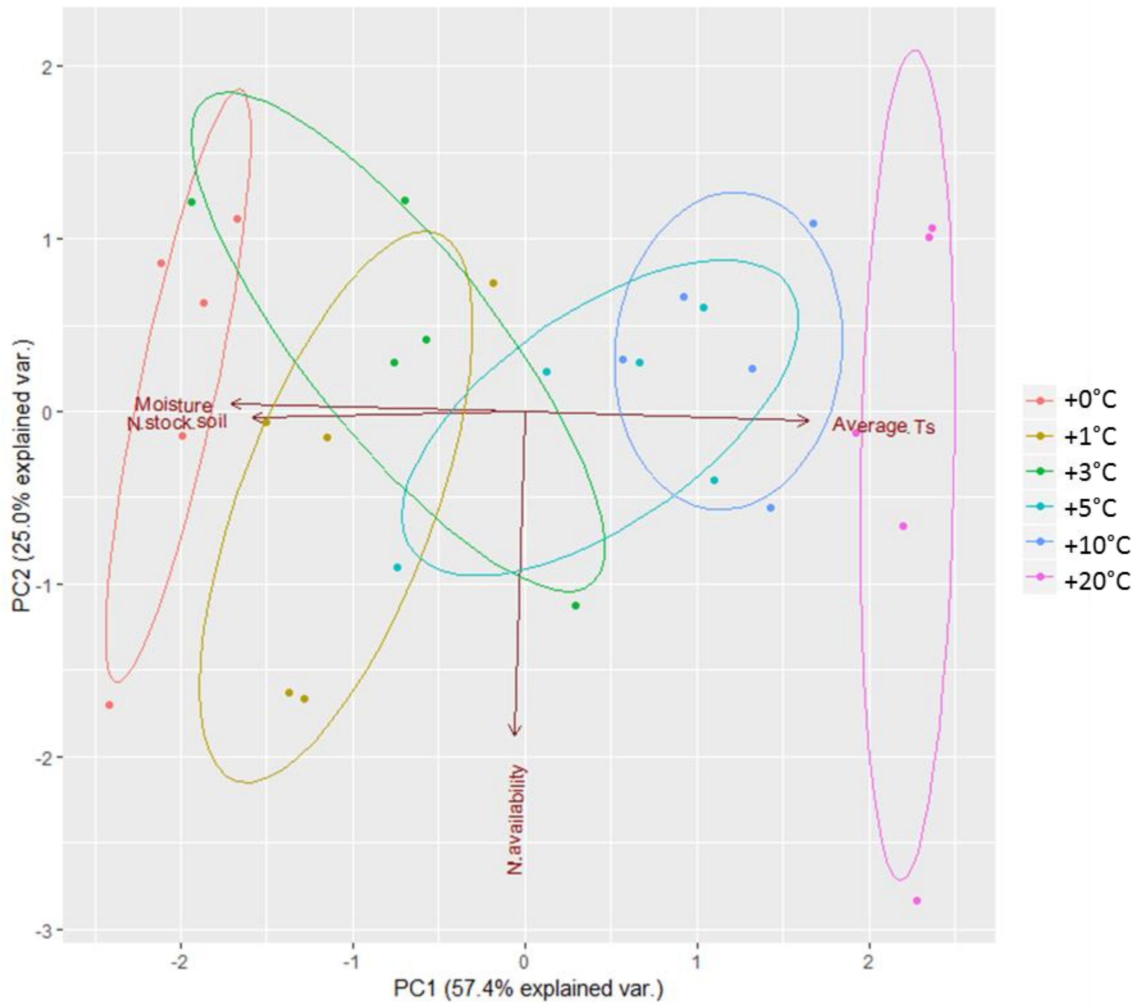

Figure 15: Principal component analysis of investigated environmental factors (average soil temperature, N availability, total soil N stock soil and soil moisture) in the short-term warmed grassland. The two first principal component axes explain 57.0 and 25.0% of the variance respectively. Dots (representing the individual plots) and the corresponding ellipses are colored according to their respective soil temperature elevation classes.

For the long-term warmed grassland, the results of the PCA analysis also showed a gradient of the different waring treatments, although less clear than in the short-term warmed grassland (Fig.

13). The colder plots were more grouped together. As in the short-term warmed grassland, the first axis, which explained 54.5 % of the variation, was associated with soil temperature, total soil N stock and soil moisture. No differentiation between the different temperature treatments occurred along the second axis, which was associated with N availability and explained 25.0 % of the total variation.

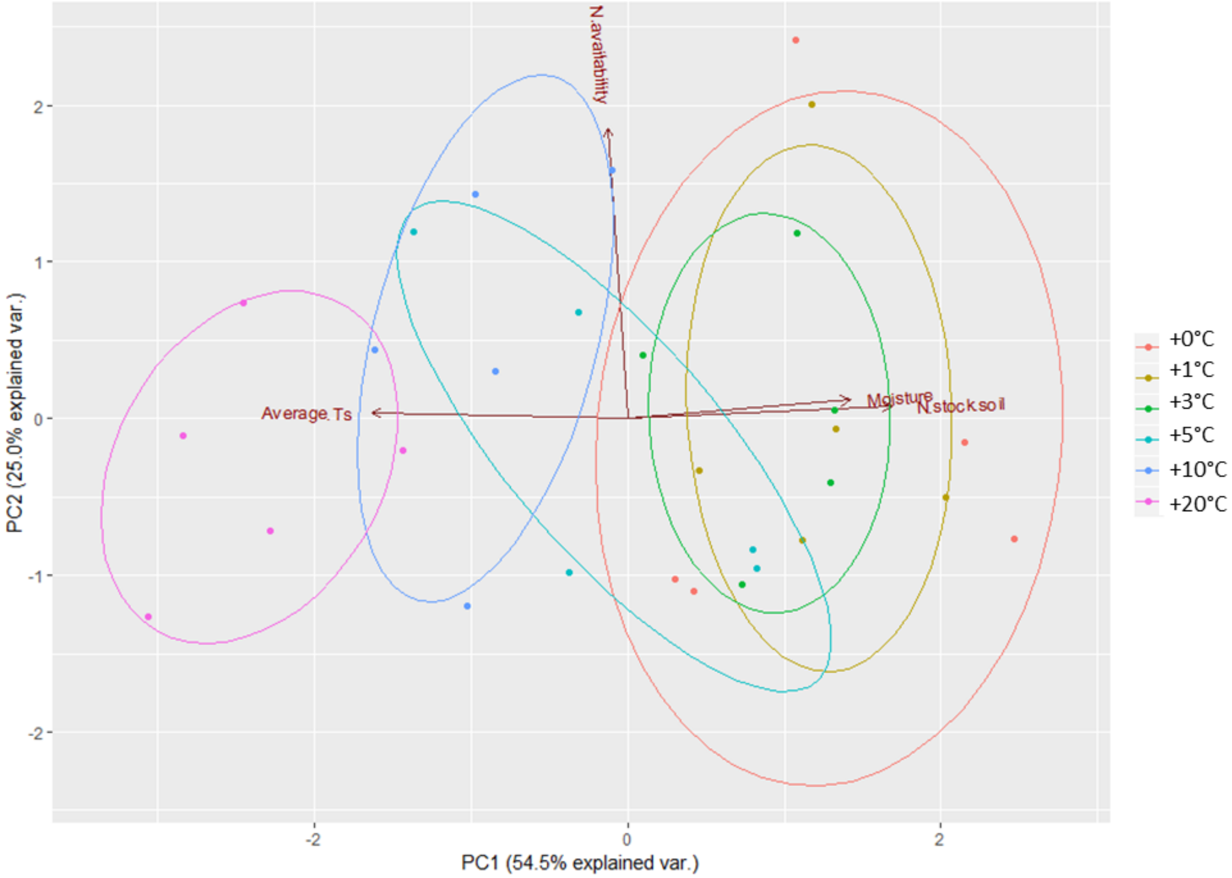

Figure 16: Principal component analysis of investigated environmental factors (average soil temperature, N availability, total soil N stock soil and soil moisture) in the long-term warmed grassland. The two first principal component axes explain 57.0 and 25.0% of the variance respectively. Dots (representing the individual plots) and the corresponding ellipses are colored according to their respective soil temperature elevation classes.

4. Discussion

Our study aimed to investigate the effect of elevated soil temperature on plant biomass (as a proxy for plant productivity; Downing & Leibold 2002) and to disentangle the direct temperature effects from indirect effects from accelerated N cycling. To investigate changes in vegetation biomass, samples were taken from naturally warmed grasslands with soil temperature elevations ranging from +0 to +20°C. Soil warming levels up to +10°C were used as natural climate change experiments, as the predicted warming in (sub)arctic areas by 2100 ranges from +2.2 to +8.3°C (IPCC 2013). Soil warming elevations between +10-20°C were mainly studied to uncover the underlying mechanisms of the responses. Further, short-term responses (+/- 8 years) of plant biomass to soil warming and N statues could be compared to long-term (≥50 years) responses, to investigate the time-dependence of the response.

4.1. Soil warming effects on vegetation biomass

4.1.1. Total vegetation biomass

Contrary to our expectations, total vegetation biomass did not increase with increasing soil temperature (Fig.4). This is in contrast to most warming studies, which reported a positive effect on total vegetation biomass with increasing temperatures in the absence of drought stress (Jonasson et al. 1999; Lee et al. 2010; Flanagan & Adkinson 2011; Wu et al. 2011; DeMarco et al. 2014). A positive soil warming effect was expected up until a certain threshold because of a direct positive effect of temperature on photosynthesis, a longer growing season and higher mineralization rates, which would increase the plant N availability (see also § 4.3, 4.4 and 4.5) (Rustad et al. 2001; Zaman & Chang 2004; Menzel et al. 2006; Kim et al. 2012). At higher temperatures, plants were expected to experience stress, resulting in a negative effect. Below, a closer look is taken of the separate vegetation classes and of the aboveground and belowground biomass to investigate were the effect was the most pronounced.

4.1.2. Aboveground biomass

In contrast to the negative response of total biomass to soil warming, no soil warming effect on aboveground biomass occurred. Also when looking at separate vegetation categories (monocotyledons, dicotyledons, equiseta, moss and lichens) no soil warming effect was found

(Fig.5). This indicates that aboveground biomass stayed constant with the increasing soil temperature which is contradictory to what most other warming studies on productivity at high northern latitudes have found (Xu et al. 2016). The discrepancy between our study and most other studies (no response vs. a positive response of vegetation biomass to warming) could have been caused by a difference in the limitation of the plant productivity. While the vegetation in most other studies was nutrient limited (and warming alleviated this limitation), there are indications that the vegetation in our study had reached its maximum leaf area index (LAI). Maximum LAI suggests that the system was likely light limited which would restrict the possibility for aboveground biomass to increase. This hypothesis is discussed in more detail in § 4.2.1. It must be noted, however, that an increase in productivity with warming could have been masked by increasing plant cycling, as the aboveground growing season length strongly increased with increasing soil temperature (Leblans, 2016). This possibility should be investigated in more detail in future research.

4.1.3. Belowground biomass

Strikingly, root biomass seemed to be the most affected biomass component. Even if some other field warming studies in (sub)arctic terrestrial ecosystems also observed a decline in root biomass with increasing soil temperature, this was usually caused by drought stress (Walker & Wahren 2006; Carlyle et al. 2013). However, the region where our experiment was conducted has abundant rainfall, indicating that drought stress is probably not the reason behind this observation (Flanagan & Adkinson 2011). Indeed, the soil water content at the ForHot research site was always well above the wilting point between 2013 and 2016 (Sigurdsson et al. 2016). In the absence of drought, most manipulation warming studies in northern ecosystems found increasing root biomass with increasing temperature (Rustad et al. 2001; Walker & Wahren 2006; Flanagan & Adkinson 2011; DeMarco et al. 2014).

One potential explanation for the contrast between our study and most other studies could be the difference in the warming technique. While most warming studies on grasslands primarily manipulate the air temperature, in our study, it was mainly the soil temperature that was elevated (Walker & Wahren 2006; Flanagan & Adkinson 2011). The few studies that did manipulate soil temperature, only elevated it for less than 2°C (Jonasson et al. 1999; Klein et al.

2005; DeMarco et al. 2014). The lack of studies featuring soil warming, combined with the typically much smaller temperature increase, can explain why decreases in root biomass have not been reported yet.

Another explanation for the decrease in root biomass, can be an increased root turnover. Soil temperature has a strong role in determining root phenology, given that the start of root production is often keyed by soil warming in the spring (Gill & Jackson 2000). The cost of root maintenance is also believed to increase exponentially with temperature. Potentially, if soils warm as a result of climate change, maintenance costs of roots and nutrient availability might increase and contribute to higher rates of root turnover (see $4.3.4) (Gill & Jackson 2000). This possibility will be studied in more detail in the future, using minirhizotron tubes that were installed at the ForHot research site in spring 2015.

Additionally, the warming-induced decrease in root biomass in this study could be caused by direct temperature stress (Ferris et al. 1998; Rachmilevitch et al. 2006). As previously mentioned, information on elevated soil temperature effects is scarce in the literature, thus our knowledge on heat injury of plant roots is limited. Hongmei et al. (2009) reported that cold-adapted plants are more sensitive to changes in temperature conditions. It is thus possible that the subarctic grassland vegetation in our study was affected at relatively low temperature elevations in comparison to other ecosystems. Moreover, the root biomass was less responsive to the lowest temperature elevations, which fell within the inter-annual variation in soil temperature, while the strongest decrease in root biomass occurred at warming higher levels (> +5-10°C), which are outside the range of normal soil temperatures of these subarctic grasslands. This supports the stress hypothesis. In addition, there are findings indicating that elevation of soil temperature has a stronger effect on ecosystems than increases in air temperature (Xu & Huang 2000).

Finally, it is important to note that the short-term warmed and long-term warmed grasslands were subjected to the large negative effect of soil warming on root biomass to the same extent. This indicates that the negative temperature effect is not a transient phenomenon as was assumed in earlier research (Vande Velde 2014), but that it lasts for many decades and maybe even centuries.

4.2. Soil warming effects on biomass distribution

Biomass allocation, which is typically assessed by the shoot to root ratio at the individual plant level, or by the belowground to aboveground biomass ratio at the ecosystem level, reflects the strategy of plants or ecosystems to partition photosynthates between belowground and aboveground tissues (Kang et al. 2013).

4.2.1. Shoot/root ratio

The shoot/root ratio of an ecosystem gives information about the nutrients or water limitation of the ecosystem, in this case at different soil warming levels. Low nutrient or water availability promotes investment in belowground plant parts (Chu et al., 2006). Figure 6 shows that the shoot/root ratio increased, both after short-term and long-term warming, indicating either a drop in below biomass or an increase in aboveground biomass. In this case, the aboveground biomass remained constant while the belowground biomass declined significantly (see § 4.1). The increase in shoot/root ratio indicates that the vegetation was not nutrient limited (see also § 4.3) or water limited, resulting the maintaining of the same aboveground biomass with a smaller amount of roots. Moreover, the lack of increase in the aboveground biomass, while nutrients or water were not limited, suggests that the aboveground biomass production might be light limited. This could be investigated in the future by analyzing ecosystem-averaged Ellenberg values for light requirement along the soil warming gradients (Szymura et al. 2014).

4.2.2. Aboveground/belowground biomass ratio

The ecosystem aboveground/belowground ratio includes both vascular and non-vascular vegetation, while shoot/root ratio only includes the vascular vegetation. Under nutrient-poor and/or water deficit conditions, ecosystems typically decrease the aboveground/belowground ratio to take up limited belowground resources (Kang et al. 2013). However, in extremely limiting conditions, plants that can acquire their nutrients and moisture from the air (nonvascular plants) will start to dominate, thereby strongly increasing the aboveground/belowground ratio (Turetsky 2003). In this study, the same positive trend as with the shoot/root ratio was observed (Fig. 7). As there was no effect of soil warming on the nonvascular plants, this increase in aboveground/belowground ratio can be fully attributed to the decrease in root biomass. This confirms the lack of nutrient or water limitation that was concluded from the shoot/root data (see § 4.2.1).

4.3. Importance of warming-induced acceleration of the N cycle on vegetation biomass

In this paragraph I investigate whether the observed temperature effects were mainly caused by direct temperature effects or rather indirect by increased N cycling (§ 4.3.3). To investigate this, however, it is important to start with examining the temperature effect on the N status of the ecosystems (§ 4.3.1 and 4.3.2).

4.3.1. Soil warming effects on N availability

In contrast to our expectations, N availability was not affected by soil warming (Fig.8). Soil warming was expected to cause an acceleration of decomposition, and thereby an increase in N availability. This was in turn expected to results in a higher vegetation biomass, as high northern latitude ecosystems are generally strongly N limited (Aerts and Chapin, 2000). The observed result is peculiar because many other studies indicate that N availability increases with higher soil warming (Liski et al. 1999; Jarvis & Linder 2000; Bai et al. 2013; Salmon et al. 2016; Dawes et al. 2017).

However, the mass loss of easily decomposable litter (proxy for decomposition rate) increased in both the short- and long-term warmed grassland. This suggests that there is indeed an acceleration of the decomposition and thus the N cycling (Fig.11) due to acceleration of chemical reactions under higher soil temperatures. The warming-induced stimulation of decomposition was somewhat higher after short-term warming. A reason for this observation could be that the community structure of organisms that drive decomposition (fungi, bacteria, nematods, arthropods) is strongly altered after short-term warming, but comes back to normal (except for the highest warming levels) after long-term warming. The new bacterial community structures after short-term warming might be somewhat more efficient in decomposing easily decomposable litter.

A potential reason why the increase in decomposition rate is not seen in our N availability measurements, could be the measuring method that was used during the experiment. The N availability was measured by inserting PRS probes in the soil for one week. However, it has been shown that the real plant availability N might be masked when using this technique as a consequence of competition between PRS probes with plant roots and microbes for the available N (Kuzyakov & Xu 2013).

As an alternative for PRS probes, long-term N availability can also be estimated from N stocks in the biomass, based on increased or decreased N uptake by vegetation (Koerselman & Meuleman 1996). Figure 10 shows that the N stocks in the vegetation decrease with higher soil temperatures. This could indicate that the N availability decreased with increasing soil temperature. However, if this would be the case, this would lead to more severe N limitation with increasing soil warming, and looking at the shoot/root ratios (§ 4.2.1), this was not the case. While the belowground biomass decreased, the aboveground biomass stayed constant (Fig.4). This means that the same aboveground biomass could be maintained with less roots. This indicates that the decline in the biomass N stocks is probably caused by the decreasing root biomass and not by decreasing N availability. In conclusion, there is no clear effect of soil warming on N availability.

4.3.2. Soil warming effects on N stocks

There was a negative effect of soil warming on total soil N stock (Fig.9). This decrease in total soil N stock must be caused by increased N losses from the soil. The faster N cycling (higher decomposition rate, Fig.11) has the possibility to lead to larger N losses, which was not expected in these N limited ecosystems. Indeed, there are indications that the N loss occurred via both (a) leeching and (b) volatilization (Leblans, 2016; Appendix, Fig. A.2), which are the two most important pathways for soil N losses (Rillig et al., 2001). Both N_2O volatilization as NO_3^- leaching increased strongly with increasing temperature. Additionally, it is possible that soil warming causes extra N losses in winter. As plants are not active during this period, they do not take up the available N, while soil warming still increases decomposition (Ineson et al. 1998; Schuerings et al. 2014). This might trigger N losses from the ecosystems by N leaching or gaseous losses.

4.3.3. N availability effect on vegetation biomass

We expected that, in these N limited ecosystems, increasing N availability would lead to increased biomass. The results, however, indicate that this was not the case in these investigated short-term- and long-term warmed grassland. However, vegetation biomass (at least belowground vegetation biomass) increased with total soil N stock (Fig.13).

This is as expected because the acceleration of the mineralization (Fig.11) results in more nitrate leaching or being converted into NO_x, N_2O and/or N_2, which leads to volatile N losses. This hypothesized loss of soil N was confirmed by Leblans, 2016, where N losses exponentially increased with higher soil temperatures for both grasslands (Appendix, Fig.A.2). The increase in N losses probably drove the observed decline of the N stocks at higher temperatures. At the same time the faster mineralization also created higher availabilities of ammonium and nitrate, resulting in the vegetation needing fewer roots (Mo et al. 2007).

4.4. Relative importance of temperature and N availability (PCA's)

Finally, the relative importance of the investigated environmental factors was investigated, both for the short-term and long-term grassland. For both grasslands, all environmental factors (soil temperature, N availability, total soil N stock and soil moisture) had a significant explanatory value when explaining the variation between the samples (Fig. 15; 16). Total soil N stock and soil moisture were both important in the short-term as long-term warming, but their effect seems to counteract each other. The short-term warmed grassland follows a clear temperature gradient along the first axis, but that this is less so for the long-term warmed grassland. This result could indicate that the long-term grassland is slowly reverting back to its original state with the exception of the highest soil warming elevations.

4.5. Evaluation of the tested hypotheses

To conclude we evaluate the hypotheses which were drafted at the beginning of the experiment and tested in this thesis.

Hypotheses 1a and 1b, stating that increased soil temperature would increase total vegetation biomass of subarctic grasslands after both short-term and long-term natural soil warming and that there would be a threshold warming level up to where a positive soil warming effect on the total vegetation biomass would occur, were rejected. A strong negative soil warming effect was observed on the root biomass, while the aboveground biomass was not affected. No threshold warming level was observed in the short- and long-termed grassland.

Hypothesis 1c, stating that the threshold above which negative effects occur would be higher in the short-term than in the long-term warmed grassland, was also rejected. No difference was found between the short- and long-termed warming grassland indicating that the vegetation of the long-term warmed grassland was not adapted to the higher soil temperatures.

Hypotheses 2a and 2b, stating that the positive soil warming effect on vegetation biomass stocks would act mainly indirectly through increasing N availability and that the N availability would be higher in the short-term warmed grassland than in the long-term warmed, were not seen in the results. We presume that the effect was masked because decomposition did increase with temperature, which indicates that organically-bound N is converted faster into available N.

We propose a new hypothesis, namely that increasing soil warming decreases the vegetation biomass of these subarctic grasslands due to a decrease in the belowground biomass (possibly because of an increased nutrient availability and/or an increase in root turnover) and a lack of response of the aboveground biomass (possibly because of light limitation, i.e. maximum LAI) (fig.16).

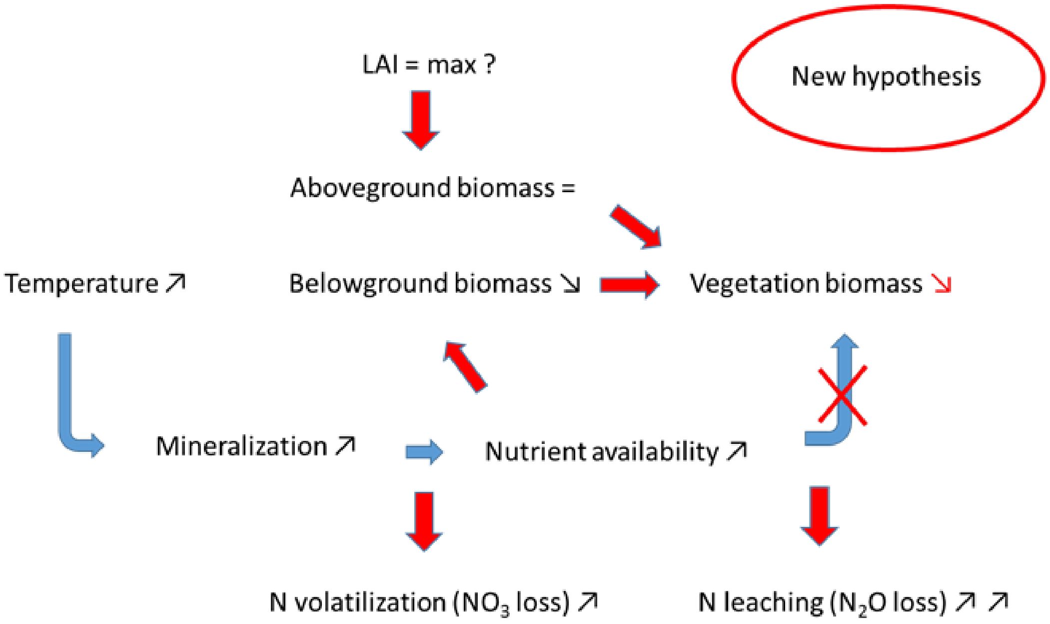

Figure 17: Proposal new hypothesis for future research. Red arrows: changes to previous hypothesis.

4.6. Evaluation of research

Like any research that is conducted, there are factors that can influence the results. In this paragraph an evaluation is made of these factors. The long-term grassland was extensively grazed, unlike the short-term grassland, during the growing season in the years preceding our measurements. This may have resulted in a positive lag effect on plant productivity in the long-term grassland, namely faster nutrient cycle, removal of litter, etc. (Larcher W. 2004). If the grassland was evenly grazed (no preferential grazing at certain soil temperature elevations), the influence can be neglected for the results within the long-term warmed grassland. Another drawback could that the plots were not separated physically, allowing the roots to grow laterally. The significant differences with increasing soil temperature, however, indicates that this factor probably did not have a great influence on the variables.

4.7. Future research

The results of the experiment have given more ideas for further investigation about the effects of soil warming on subarctic ecosystems. One uncertainty that remains is whether the lack of temperature response of the aboveground biomass was real, or that it was masked by accelerated plant cycling. This could be studied by measuring the litter production along the temperature gradients in detail. Further, the higher rates of root turnover, as an explanation for decrease in root biomass, could be studied in more detail, using the minirhizotron tubes that were installed at the FORHOT research site in spring 2015 (Cheng et al. 1991). Also the unexpected observation that the ecosystem might not be nutrient limited but light limited should be further investigated. This could for instance be done by comparing ecosystem-averaged Ellenberg values for light requirement along the soil warming gradients (Szymura et al. 2014).

5. Conclusion

Contrary to our expectations, total vegetation biomass decreased with increasing soil temperature in both the short-term and long-term warmed grassland. This was caused by a strong negative soil warming effect on the root biomass, while the aboveground biomass was not affected presumably due to increased root turnover of more active roots. The observation that the same amount of aboveground biomass could be maintained by a smaller amount of roots at higher soil warming levels (i.e. increasing shoot/root ratio) indicates that the vegetation was not nutrient or water limited. This contrasts with our expectation that the vegetation would be strongly limited by N. We hypothesize that it was instead light limited, thus that the leaf area index (LAI) of the ecosystem has reached a maximum. This could explain why the aboveground vegetation was unresponsive to warming in this study, while it typically increases with increasing temperature in warming studies at high northern latitudes where the vegetation was in general N limited. We conclude that long-term warming could decrease the C sink capacity (primary production) of these northern grasslands by decreasing its belowground biomass stock if soil warming gives a reliable estimate of the effect of total ecosystem warming.

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References

Aerts, R. & Chapin, F.S., 2000. The Mineral Nutrition of Wild Plants Revisited: A Re-evaluation of Processes and Patterns. *Advances in Ecological Research*, 30(C), pp.1–67.

Arnalds, O., 2004. Volcanic soils of Iceland. *Catena*, 56(1–3), pp.3–20.

Bai, E. et al., 2013. A meta-analysis of experimental warming effects on terrestrial nitrogen pools and dynamics. *New Phytologist*, 199(2), pp.431–440.

Bekryaev, R. V., Polyakov, I. V. & Alexeev, V.A., 2010. Role of polar amplification in long-term surface air temperature variations and modern arctic warming. *Journal of Climate*, 23(14), pp.3888–3906.

Bellard, C. et al., 2012. Impacts of climate change on the future of biodiversity. *Ecology letters*, 15(4), pp.365–377.

De Boeck, H.J. et al., 2015. Global Change Experiments: Challenges and Opportunities. *BioScience*, 65(9), pp.922–931.

Carlyle, C.N., Fraser, L.H. & Turkington, R., 2013. Response of grassland biomass production to simulated climate change and clipping along an elevation gradient. *Oecologia*, pp.1065–1073.

Chapin, F.S., Matson, P. a & Mooney, H. a, 2002. *Principles of Terrestrial Ecosystem Ecology*, New York: Springer-Verlag New York, Inc.

Cheng, W., Coleman, D.C. & Box, J.E., 1991. Measuring root turnover using the minirhizotron technique. *Agriculture, Ecosystems & Environment*, 34(1), pp.261–267.

Chu, Y., Yu, F. H. & Dong, M., 2006. Clonal plasticity in response to reciprocal patchiness of light and nutrients in the stoloniferous herb Glechoma longituba L. *Journal of Integrative Plant Biology*, 48, pp.400-408.

Cramer, W. et al., 2001. Global response of terrestrial ecosystem structure and function to CO 2 and climate change: results from six dynamic global vegetation models. *Global Change Biology*, 7(4), pp.357–373.

Daebeler, A. et al., 2014. Interactions between Thaumarchaea, Nitrospira and methanotrophs modulate autotrophic nitrification in volcanic grassland soil. *The ISME Journal*, 8(10), pp.2397–2410.

Davidson, E.A. & Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, 440(7081), pp.165–173.

Dawes, M.A. et al., 2017. Soil warming opens the nitrogen cycle at the alpine treeline. *Global change biology*, 23(1), pp.421–434.

DeMarco, J., Mack, M. & Bret-Harte, M., 2014. Long-term experimental warming and nutrient additions increase productivity in tall deciduous shrub tundra. *Ecosphere*, 5(June), pp.1–22.

Downing, A.L. & Leibold, M.A., 2002. Ecosystem consequences of species richness and composition in pond food webs. *Nature*, 416(6883), pp.837–841.

Einarsson, M.Á., 1984. Climate of Iceland. *World Survey of Climatology: 15: Climates of the Oceans*, H. van LOON (Ed.) pp.673–697.

Fan, J. et al., 2008. Carbon storage in the grasslands of China based on field measurements of above- and below-ground biomass. *Climatic Change*, 86(3–4), pp.375–396.

Ferris, R. et al., 1998. Effect of High Temperature Stress at Anthesis on Grain Yield and Biomass of Field-grown Crops of Wheat. *Annals of Botany*, 82(5), pp.631–639.

Flanagan, L.B. & Adkinson, A.C., 2011. Interacting controls on productivity in a northern Great Plains grassland and implications for response to ENSO events. *Global Change Biology*, 17(11), pp.3293–3311.

Follett, R.F. et al., 2012. Effects of climate change on soil carbon and nitrogen storage in the US Great Plains. *Journal of Soil and Water Conservation*, 67(5), pp.331–342.

Gavito, M.E. et al., 2001. Interactive effects of soil temperature, atmospheric carbon dioxide and soil N on root development, biomass and nutrient uptake of winter wheat during vegetative growth. *Journal of experimental botany*, 52(362), pp.1913–1923.

Gill, R.A. & Jackson, R.B., 2000. Global patterns of root turnover for terrestrial ecosystems. *New Phytologist*, 147(1), pp.13–31.

de Graaff, M.-A. et al., 2006. Interactions between plant growth and soil nutrient cycling under elevated CO2: a meta-analysis. *Global Change Biology*, 12(11), pp.2077–2091.

Halldorsson, B. & Sigbjornsson, R. 2009. The M(w)6.3 Olfus earthquake at 15:45 UTC on 29 May 2008 in South Iceland: ICEARRAY strong-motion recordings. Soil Dynamics and Earthquake Engineering, 29, 1073-1083.

Hongmei, D., Wang, Z., & Huang, B., 2009. Differential responses of warm-season and cool-season turfgrass species to heat stress associated with antioxidant enzyme activity. *J. Amer. Soc. Sci.*, 134(4), 417 - 422.

Ineson, P. et al., 1998. Effects of climate change on nitrogen dynamics in upland soils. 2. A soil warming study. *Global Change Biology*, 4(2), pp.153–161.

IPCC 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. In: [STOCKER, T. F., D. QIN, G.-K. PLATTNER, M. TIGNOR, S.K. ALLEN, J. BOSCHUNG, A. NAUELS, Y. XIA, V. BEX AND P.M. MIDGLEY & (EDS.)] (eds.) Cambridge University Press. Cambridge, United Kingdom and New York, NY, USA.

Jarvis, P. & Linder, S., 2000. Botany: Constraints to growth of boreal forests. *Nature*, 405(6789), pp.904–905.

Jonasson, S. et al., 1999. Responses in microbes and plants to changed temperature, nutrient, and light regimes in the arctic. *Ecology*, 80(6), pp.1828–1843.

Kang, M. et al., 2013. Biomass and Its Allocation in Relation to Temperature, Precipitation, and Soil Nutrients in Inner Mongolia Grasslands, China. *PLoS ONE*, 8(7), pp.1–7.

Kim, Y. et al., 2012. Satellite detection of increasing Northern Hemisphere non-frozen seasons from 1979 to 2008: Implications for regional vegetation growth. *Remote Sensing of Environment*, 121, pp.472–487. Available at: http://dx.doi.org/10.1016/j.rse.2012.02.014.

Klein, J. a., Harte, J. & Zhao, X.Q., 2005. Dynamic and complex microclimate responses to warming and grazing manipulations. *Global Change Biology*, 11(9), pp.1440–1451.

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), pp.1441–1450.

Kuzyakov, Y. & Xu, X., 2013. Competition between roots and microorganisms for nitrogen: Mechanisms and ecological relevance. *New Phytologist*, 198(3), pp.656–669.

Larcher, W., 2003 Physiological plant ecology. Ecophysiology and stress physiology of functional groups. Vol. 4th, pp. 1 - 67 ; 185 - 229 ; 232 - 296. Germany: Springer.

LeBauer, D.S. & Treseder, K.K., 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology*, 89(2), pp.371–379.

Leblans, Niki I. W., 2016. Natural gradients in temperature and nitrogen: Iceland represents a unique environment to clarify longterm global change effects on carbon dynamics. Joint PhD thesis between Agricultural University of Iceland and University of Antwerp. Defended on Novemer 14, 2016, Agric. Univ. Iceland, Reykjavik, Iceland.

Lee, M. et al., 2010. A global comparison of grassland biomass responses to CO2 and nitrogen enrichment. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 365(1549), pp.2047–2056.

Liski, J. et al., 1999. CO2; Emissions from Soil in Response to Climatic Warming Are Overestimated: The Decomposition of Old Soil Organic Matter Is Tolerant of Temperature. *Ambio*, 28(2), pp.171–174.

Magnússon, Á. & Vídalín, P., 1918-1921. Jardbók. Annad bindi [The farm register of Iceland. 2nd Volume], Copenhagen (Originally published in 1708) (In Icelandic).

Masciopinto, C. & Liso, I.S., 2016. Assessment of the impact of sea-level rise due to climate change on coastal groundwater discharge. *Science of the Total Environment*, 569–570, pp.672–680.

McGuire, A.D. et al., 2012. An assessment of the carbon balance of Arctic tundra: Comparisons among observations, process models, and atmospheric inversions. *Biogeosciences*, 9(8), pp.3185–3204.

Menzel, A. et al., 2006. European phenological response to climate change matches the warming pattern. *Global Change Biology*, 12(10), pp.1969–1976.

MO, J. et al., 2007. Nitrogen addition reduces soil respiration in a mature tropical forest in southern China. *Global Change Biology*, 14(2), pp.403–412.

O'Gorman, E.J. et al., 2014. Climate change and geothermal ecosystems: Natural laboratories, sentinel systems, and future refugia. *Global Change Biology*, 20(11), pp.3291–3299.

Prentice, I., Farquhar, G. & Fasham, M., 2001. The carbon cycle and atmospheric carbon dioxide. *Climate Change 2001: The Scientific Basis*, pp.183–237.

Rachmilevitch, S., Lambers, H. & Huang, B., 2006. Root respiratory characteristics associated with plant adaptation to high soil temperature for geothermal and turf-type Agrostis species. *Journal of Experimental Botany*, 57(3), pp.623–631.

Rillig, M. C., Wright, S. F., Nichols, K. A., Schmidt, W. F. & Torn, M. S. 2001. Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. Plant and Soil, 233, 167-177.

Rosenzweig, C. et al., 2008. Attributing physical and biological impacts to anthropogenic climate change. *Nature*, 453(7193), pp.353–7.

Rustad, L.E. et al., 2001. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia*, 126(4), pp.543–562.

Salmon, V.G. et al., 2016. Nitrogen availability increases in a tundra ecosystem during five years of experimental permafrost thaw. *Global change biology*, 22(5), pp.1927–41.

Schuerings, J. et al., 2014. Increased winter soil temperature variability enhances nitrogen cycling and soil biotic activity in temperate heathland and grassland mesocosms. *Biogeosciences*, 11(23), pp.7051–7060.

Sigmundsson, F., 2006. Iceland geodynamics: Outlook. In *Iceland Geodynamics: Crustal Deformation and Divergent Plate Tectonics*. Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 175–176.

Sigurdsson, B.D. et al. 2016. Geothermal ecosystems as natural climate change experiments: the ForHot research site in Iceland as a case study. Icelandic Agricultural Sciences, 29, pp. 53-71

Soussana, J.F. et al., 2007. Full accounting of the greenhouse gas (CO2, N2O, CH4) budget of nine European grassland sites. *Agriculture, Ecosystems and Environment*, 121(1–2), pp.121–134.

Szymura, T.H., Szymura, M. & Macioł, A., 2014. Bioindication with Ellenberg's indicator values: A comparison with measured parameters in Central European oak forests. *Ecological Indicators*, 46(November), pp.495–503.

Thorbjörnsson, P., Saemundsson, K., Kristinsson, S. G., Kristjansson, B. R. & Ágústsson, K., 2009. Southern lowland earthquakes 29th May 2008, effect of groundwater level, geothermal activity and fractures. (In Icelandic). In: ORKURANNSÓKNIR, Í. (ed.).

Turetsky, M.R., 2003. The Role of Bryophytes in Carbon and Nitrogen Cycling. *The Bryologist*, 106(3), pp.395–409.

Vande Velde, K., 2014. *Effects of short-term and long-term natural soil warming gradients on plant productivity, carbon and nitrogen stocks of a sub-arctic grassland.*

Vitousek, P.M. et al., 2002. Nitrogen and nature. *Ambio*, 31(2), pp.97–101.

Vitousek, P.M. & Howarth, R.W., 1991. Nitrogen limitation on land and in the sea: How can it occur? *Biogeochemistry*, 13(2), pp.87–115.

Walker, M. & Wahren, C., 2006. Plant community responses to experimental warming across the tundra biome. *Proceedings of the …*, 103(5), pp.1342–6.

Weedon, J.T. et al., 2013. Temperature sensitivity of peatland C and N cycling: Does substrate supply play a role? *Soil Biology and Biochemistry*, 61, pp.109–120.

White, A., Cannell, M. & Friend, A., 2000. The high-latitude terrestrial carbon sink: a model analysis. *Global Change Biology*, 6, pp.227–245.

Woodwell, G.M. et al., 1998. Biotic feedbacks in the warming of the earth. *Climatic Change*, 40(3–4), pp.495–518.

Wu, Z. et al., 2011. Responses of terrestrial ecosystems to temperature and precipitation change: A meta-analysis of experimental manipulation. *Global Change Biology*, 17(2), pp.927–942.

Xu, L. et al., 2013. Temperature and vegetation seasonality diminishment over northern lands. *Nature Climate Change*, 3(3), pp.581–586.

Xu, M. et al., 2016. Warming effects on plant biomass allocation and correlations with the soil environment in an alpine meadow, China. *Journal of Arid Land*, 8(5), pp.773–786.

Xu, Q. & Huang, B., 2000. Growth and Physiological Responses of Creeping Bentgrass to Changes in Air and Soil Temperatures. *Crop Science*, 40(5), p.1363.

Yoshitake, S. et al., 2015. Soil microbial response to experimental warming in cool temperate semi-natural grassland in Japan. *Ecological Research*, 30(2), pp.235–245.

Zaman, M. & Chang, S.X., 2004. Substrate type, temperature, and moisture content affect gross and net N mineralization and nitrification rates in agroforestry systems. *Biology and Fertility of Soils*, 39(4), pp.269–279.

Zhao, M. & Running, S.W., 2010. Drought-Induced Reduction in Global. *Science*, 329(5994), pp.940–943.

Appendix

Appendix 1: Soil temperature in study plots (May 2013 to May 2015)

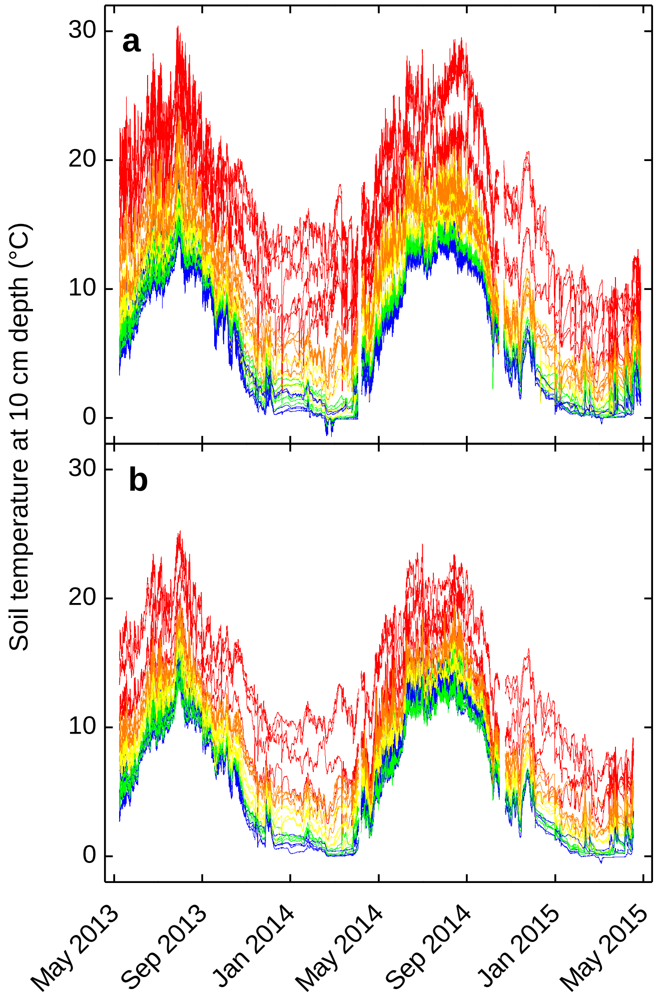

Figure A.1: Soil temperature elevations measured hourly at 10 cm depth from May 2013 to May 2015 in every measurement plot in (a) the short-term warmed grassland and (b) the long-term warmed grassland. Colors show targeted soil temperature elevations: blue for unwarmed soils, green for +1°C, yellow for +3°C, orange for +5°C and red for +10°C (Leblans 2016).

Appendix 2: N losses in short-term and long-term warmed grassland

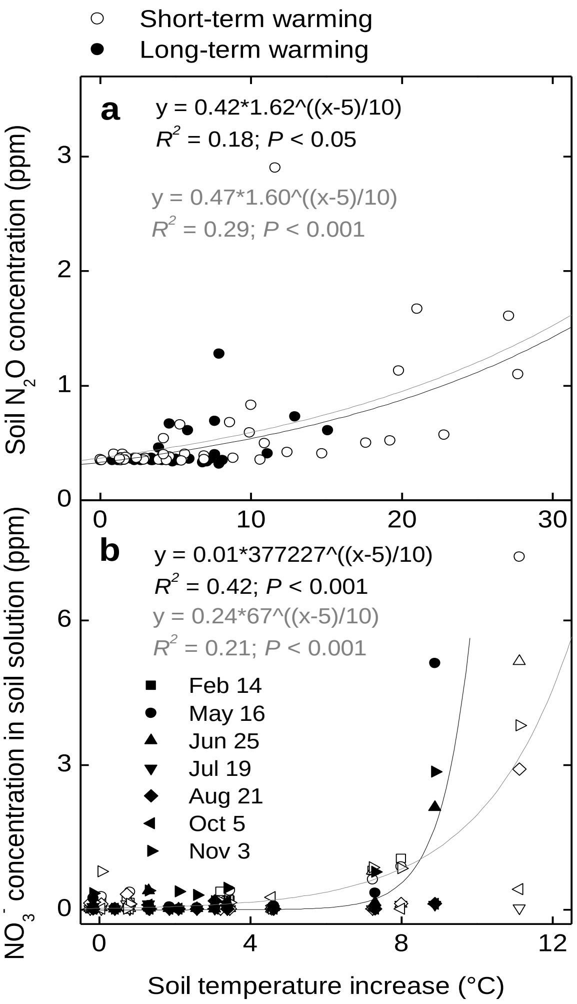

Figure A.2: Increasing gaseous and liquid N concentrations with increasing soil temperature. Soil temperature increase is expressed relative to ambient soil temperature (at 10 cm depth), averaged from May 2013 to May 2015. (a) Soil N_2O concentrations and (b) nitrate (NO_3^-) concentrations in the soil solution at different collection dates. Equations show the Q10 relationships with soil temperature for short-term warming (grey) and long-term warming (black). Relationships were considered statistically significant at $P < 0.05$ (Leblans 2016).

Appendix 3: Owner list data

Table A.1: List of data and collectors.

Data	**Collector**
Vegetation biomass	Van Loock Stephanie
Biomass N stocks	Van Loock Stephanie
Root N stocks	Van Loock Stephanie
N availability	Leblans Niki
Teabag decomposition	Leblans Niki
Soil moisture	Leblans Niki