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

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Summary for an audience of scientific peers

Maintaining long-term climate manipulation experiments to study the long-term effects of warming at ecosystem level is extremely expensive. Hence, very few long-term studies exist and many of those are simulation studies, extrapolating current climate response models. Here, changes in plant productivity and ecosystem carbon (C) and nitrogen (N) stocks along natural short-term (i.e.: ~5 years) and long-term (i.e.: very likely minimum of 3 centuries) soil warming gradients in the sub-arctic grassland system were studied to shed some light on the subject. Increased soil temperature (T_S) is expected to cause changes in the cycling of C and N through the soil, which influences plant productivity and ultimately results in changes of ecosystem C and N stocks. However, the increased changes in C and N cycling might also be transient. Whether changes are transient or not was studied by comparing the short- and longterm temperature gradients. In order to better understand the mechanisms behind changes in plant productivity and in ecosystem C and N stocks, several components of the ecosystem were studied along the soil temperature gradients which all included study plots at the following T_S elevations: ambient T_S (i.e.: control) and approximately 1°C, 3°C, 5°C and 10°C above ambient T_S. After short-term warming, soil C and N stocks were significantly lower and (root) biomass significantly decreased at high T_S elevation (i.e.: +5°C and +10°C). These results suggest that decomposition rates increased, resulting in increased emission of C- an Nbased greenhouse gases. The much smaller root system at high T_S elevation might have exacerbated the loss of N by failing to immobilize the increasing soil N availabilities. After long-term warming soil C stocks were also significantly lower at high T_S elevation. However, in this case soil N stocks showed no significant differences and the productivity of vascular plants even increased with increasing T_S. These results indicate that after long-term warming the warmed plants and microbes are able to maintain the high N stocks and availability, supporting enhanced plant productivity. However, this warming induced increase in productivity is too small to compensate for the soil C stocks, which strongly declined. This thesis thus supports the hypothesis that global warming will result in large C losses from northern soils that can be expected to feedback positively to global warming.

Summary for a general audience

Recent climate projections estimate that air temperature will most likely rise by 3°C up to 5°C in land areas of the Arctic regions by the year 2100. To learn about the short-term and longterm consequences of warming, changes in plant growth and ecosystem carbon (C) and nitrogen (N) stocks were studied along natural short-term (i.e.: ~5 years) and long-term (i.e.: ~3 centuries) soil warming gradients in a sub-arctic grassland system. Nitrogen is the element that plants are lacking most for growth in northern regions, while C plays an important role as greenhouse gas (when occurring in the atmosphere as carbon dioxide: CO_2). In this study, we studies whether or not the ongoing climate warming would increase the availability of N for plants, which would then sustain faster plant growth, and whether it would result in losses of soil C, which would then imply higher CO₂ concentrations in the air and thereby faster global warming. Ecologists typically study the effect of warming on the ecosystem by warming them. This is, however, very expensive and has the serious drawback that one can never be sure whether the observed changes in ecosystem function are due to the imposed disturbance, or are in fact the long-term equilibrium responses. The natural warming gradients occurring on Iceland provide not only free temperature experiments, the presence of both young and old temperature gradients also allow us to study whether changes are transient or not. Some changes in the ecosystem seemed to be transient. After short-term warming, strong soil warming (i.e.: +5°C and +10°C) caused a strong decrease in plant biomass, supposedly due to heat stress. However, after long-term warming biomass no longer decreased and productivity of vascular plants even increased. This suggests elimination of temperature-sensitive species and/or adaptation to higher temperatures. However, even after long-term warming, the increased plant productivity was not able to compensate for the strongly decreased soil C stocks caused by rapidly increased C emissions in the earlier phase of warming. This study thus suggests that warming of northern soils is likely to increase plant growth, but also to transfer large amounts of soil carbon to the atmosphere, thereby further strengthening global warming.

1 Introduction

Yoggi Berra once spoke the confusing but accurate words: "In theory there is no difference between theory and practice. In practice there is." We now know from practice and experience that the climate is changing and that the planet is warming (ACIA 2004). We are still learning from practice how further warming might affect different ecosystems in the near future by conducting field and laboratory studies. However, how this will affect ecosystems decades and centuries later is mostly theoretic. In the sub-arctic grasslands of Hveragerði (Iceland), nature designed an experiment for us that can turn this theory into practice. As a result of geothermal activity, grasslands are naturally warmed at certain locations (i.e.: hotspots), creating natural temperature gradients of varying age (ranging from several years to several centuries), depending on when warming initiated.

Recent projections estimate that air temperature will rise by 3°C up to 5°C in land areas of the Arctic regions by the end of this century, including Iceland (ACIA, 2004). In order to know what the consequences of climate change (including changes in precipitation, CO_2 concentrations, extreme weather events and temperature) will be, we need to understand both how changes in individual factors affect ecosystems and how they interact with each other. In this study we will address the first problem and study how short-term and long-term increased soil temperature (T_s) (resulting from natural geothermal activity) affects a sub-arctic grassland ecosystem.

Until now, the effects of increased soil temperature have been less studied than the effect of increased air temperature. However, it strongly affects soil and vegetation processes and should not be overseen (Gavito et al. 2001). Sub-arctic soils contain large amounts of carbon stored as soil organic matter compared to warmer regions due to the low temperatures preventing decomposition and subsequent respiration of CO_2 (McGuire et al. 2009). However, under scenarios of rising T_S (together with increasing air temperature) they constitute a large potential source for greenhouse gas emissions (mainly CO_2 , CH_4 and N_2O ; Mosier A.R. 1998; Conrad R. 1996). Greenhouse gas emissions can induce positive feedback to global warming, leading to further increase of temperature due to the predicted net emission of C and N with increasing temperature in the form of greenhouse gases (Kirschbaum M.U.F. 1995; Woodwell et al. 1998; Cox at al. 2000; Follet et al. 2012).

Studies on long-term (i.e.: decades to centuries) effects of warming at ecosystem level are rare and are often simulation studies based on modelling due to time and funding constraints. Several questions remain with important implications for the predicted reinforcing feedbacks of affected ecosystem carbon (C) and nitrogen (N) stocks to global warming (Kirschbaum M.U.F. 1995; Woodwell et al. 1998; Cox at al. 2000; Follet et al. 2012). The prevailing uncertainty about long-term effects of warming is the reason why this study on plant productivity and ecosystem C and N stocks along short-term (i.e.: approximately 5 years; see § 2.2.1) and long-term (i.e.: approximately 3 centuries; see § 2.2.1) soil warming gradients is not only interesting but also necessary.

The research aims raised above are rather general. Therefore, they are split up in several testable research questions (§ 1.1 to § 1.6) based on the following main hypothesis: increased soil temperature (T_S) causes changes in the cycling of C and N through the soil, which influences productivity and nutrient uptake by plants and ultimately results in changes of total C and N stocks of the ecosystem. In order to get a better understanding of the changes in plant productivity and ecosystem C and N stocks after short-term and long-term warming, the ecosystem was subdivided into components which will be compared at different T_S elevations (see § 2.4).

1.1 Is there an increase in productivity with increasing T_S along a natural soil warming gradient after 5 years? Are these changes in productivity transient or do they remain after centuries of soil warming?

Changes in productivity can directly (e.g.: through exudate production) and indirectly (e.g.: through necromass production) alter ecosystem C and N stocks (Fan et al. 2007; de Graaf et al. 2006; Herbert et al. 1999). De Graaf et al. (2006) found that soil C input through plant growth is the main driver of soil C sequestration. Changes in biomass (i.e.: a proxy of productivity; Shaver et al. 1996) can thus play an important role in the ultimate effect of warming on ecosystem C and N stocks by mitigating C and N losses from the soil.

We expect increase of T_S to cause increased mineralization rates (Yue L et al. 2014; Zaman et al. 2006; Rustad et al. 2001; Pendall et al. 2004). This would result in an increase of plant available N (Chapin et al. 1995; Melillo et al. 2002; Natali et al. 2012; Guntiñas et al. 2012). A meta-analysis by Lee et al. (2010) looked for general trends of biomass allocation in response to higher N availability in grasslands. Based on this research (Lee et al. 2010), we expect a <u>small</u> negative temperature effect on root biomass, due to decreased belowground nutrient competition (Wilson S.D. & Tilman D. 1993), and a <u>large</u> positive temperature effect on aboveground vascular biomass due to increased N availability (DeMarco et al. 2014; Flanagan et al. 2011; Dieleman et al. 2012; Walker et al. 2006; Rustad et al. 2001). This would result in an increased total biomass of <u>vascular</u> vegetation (i.e.: monocotyledons, dicotyledons and equiseta) (Jonasson et al. 1999).

Due to a shift from nutrient to light competition (Wilson S.D. and Tilman D. 1993; Lee et al. 2010) resulting from the expected increase of aboveground vascular biomass we expect a decrease of <u>non-vascular</u> vegetation biomass (i.e.: mosses and lichens) with increasing T_s (Chapin F.S. and Shaver G.R. 1985; Zhang et al. 1996; Chapin et al. 1995; Van Wijk et al. 2003).

Due to the expected smaller negative temperature effect on root biomass compared to the larger positive temperature effect on aboveground vascular biomass and the longer growing season for all vegetation types at higher T_S (Leblans et al., unpublished data; Natali et al. 2011) we expect total vegetation biomass (i.e.: proxy for grassland productivity; Shaver et al. 1996) to increase with increasing T_S .

1.2 How do C:N ratios of biomass and necromass change as a result of changed nutrient availability in a nutrient limited ecosystem after 5 years of soil warming? Are these changes transient or do they remain after centuries of soil warming?

C:N ratios (i.e.: approximation of necromass quality;: Larcher W. 2004) can influence C and N stocks by delaying or accelerating litter decomposition rate and the resulting C and N input to the soil (Berg B, 2000). This in turn could lead to altered plant available N, thereby affecting several processes in the ecosystem C and N cycle.

Increased N availability can accumulate in the vegetation if it is available in excess and additional growth is limited by other factors (Larcher W. 2004; Chapin et al. 1995; Knecht M.F. and Göransson A. 2004). However, due to the strong N limitation in this sub-arctic region (Sigurdsson B.D. 2001; Óskarsson H. 2010) we do not expect higher N concentrations (%) at higher T_S elevations. Thus, assuming C uptake increases proportionally with biomass increase (Gough L. and Hobbie S.E. 2003), we expect no change in C:N ratio of biomass with increasing T_S . Higher N availability could result in lower pressure on plants to take N back up from biomass during senescence and thus a decrease in C:N ratio in the necromass.

1.3 How do biomass (i.e.: live vegetation) stocks of C and N change along a natural soil warming gradient after 5 years? Are these changes transient or do they remain after centuries of soil warming?

As total vegetation C and N stocks are the combined result of changes in productivity, C and N concentration (%) and we do not expect C or N concentration to differ with T_s , a proportionate increase or decrease of stocks with changes in biomass is expected (Gough L. and Hobbie S.E. 2003). Therefore, we expect increased C and N stocks in total vascular biomass and aboveground vascular biomass, decreased C and N stocks in root biomass and

non-vascular biomass and increased C and N stocks in total vegetation with increasing T_S (see § 1.1).

1.4 How do necromass (i.e.: litter) and necromass stocks of carbon and nitrogen change along a natural soil warming gradient after 5 years? Are these changes transient or do they remain after centuries of soil warming?

Aboveground necromass provides one of the primary inputs of C to soil (together with belowground root necromass) (Davidson E.A. and Janssens I.A. 2006; Kätterer et al. 2011). Increased necromass input due to increased aboveground productivity and turnover rates could lead to more C input to the soil where it can be stabilised for a long time as soil organic matter (Dieleman et al., unpublished).

Due to increased turnover rate at higher T_S elevation (Chapin F.S. 1980; Chapin et al. 1995), we expect higher necromass <u>input</u> with increasing T_S . However, we expect a net decrease in necromass with increasing T_S due to increased decomposition rates in the warmer plots (Aerts R. 2006) and higher temperature sensitivity of decomposition compared to productivity (Kirschbaum M.U.F. 1995; Woodwell et al. 1998). As with biomass, differences in necromass C stocks are expected to follow differences in the amount of necromass between different T_S elevations. However, due to the predicted decreased pressure to take N back up from senescing leaves at higher T_S (§ 1.2), we expect slightly higher N stocks at higher T_S elevations.

1.5 How do soil stocks of C and N change along a natural soil warming gradient after 5 years? Are these changes transient or do they remain after centuries of soil warming?

Soil processes can directly and indirectly mitigate or reinforce global warming by net absorption (sink) or net emission (source) of mainly CO₂, CH₄ and N₂O (Mosier A.R. 1998; Conrad R. 1996). Mechanisms of production and consumption of these C- and N-based greenhouse gases in soil are numerous and are all affected by temperature (Conrad R. 1996; Zaman M. and Chang S.X. 2004; Aerts R. 2006). On top of that, bidirectional relations between soil processes and vegetation processes can positively or negatively feedback on soil C and N stocks in response to warming (see § 1.1 to § 1.4) resulting in further mitigation or reinforcement of global warming. We do not expect drought effects on the vegetation resulting from higher T_S elevations in this sub-arctic grassland (high average annual precipitation: see § 2.1). Therefore, we based our expectations on observed changes in soil C and N stocks under circumstances of non-limiting soil moisture. Decomposition and the resulting heterotrophic respiration of CO_2 (i.e.: one of the main drivers of the C cycle; Schindlebacher et al. 2011) is sensitive to changes in T_S. More specifically, decomposition increases in soils with increasing T_S (Rustad et al. 2001; Pendall et al. 2004; Zaman M. and Chang S.X. 2004; Schindlebacher et al. 2011). Although increased plant productivity with increasing T_S can mitigate this increased C emission from the soil by increasing C input to the soil (see § 1.1, § 1.3 and § 1.4), we expect a net loss of soil C (in case of non-limiting availability of decomposable substrate) due to the higher temperature sensitivity of decomposition compared to productivity (Follet et al. 2012; Kirschbaum M.U.F. 1995; Woodwell et al. 1998). Continued warming during winter resulting in (low) microbial activity equally increases chance of a net CO₂ loss (Oechel et al. 2000). Additionally, Luo et al (2013) found that any deviation from average temperature will likely result in decreased CH₄ uptake, possibly reinforcing the decrease of soil C stocks with increased T_S elevation.

Linked to increased microbial activity (Zak et al. 1999) and linked decomposition with increasing T_S, mineralization is expected to increase with increasing T_S (Zaman M. and Chang S.X. 2006; Li et al. 2014; Rustad et al. 2001; Pendall et al. 2004). This would lead to increased (plant available) soil N stocks (Melillo et al. 2002). However N that was previously bound to metal ions (i.e.: typical for Andosol type soils; García-Rodeja et al. 2004), to other soil particles or fixed in undecomposed soil organic matter and necromass (rendering them unavailable for uptake, denitrification or leaching) will become increasingly available as well, due to the higher energy availability for soil microbiota at higher T_S elevation. It is then sensitive to several mechanisms that lead to N removal from the soil, especially in grassland soil (Lang et al. 2010). Plants are expected to take up part of the newly plant available N, leading to increased productivity (see § 1.1 to 1.4) which will eventually positively feedback to soil N stocks through increased litter input. Stimulation of denitrification at higher T_S elevations, resulting increased N₂O flux to the atmosphere (Luo et al. 2013), would cause decreased soil N stocks. Leaching could further reduce the increasingly mobile soil N stocks at higher T_S elevations. Overall, we expect a net decrease of soil N stocks with increasing T_S elevation due to decomposition being more temperature sensitive than productivity (Follet et al. 2012; Kirschbaum M.U.F. 2000, Woodwell et al. 1998).

1.6 How do ecosystem stocks of C and N change along a natural soil warming gradient after 5 years? Are these changes transient or do they remain after centuries of soil warming?

Soils contain the largest stocks of C and N in the grassland ecosystem (Johnston et al. 2004) and considering the high temperature sensitivity of soil processes (Kirschbaum M.U.F. 1995; Woodwell et al. 1998) will most likely determine the balance of in- and outgoing C and N fluxes. Therefore, we expect decreasing C and N stocks with increasing T_S elevation at ecosystem level (see § 1.5).

2 Material and methods

2.1 Research area

All soil warming gradients were located in grasslands in the vicinity of Hveragerði, a village in the south-west of Iceland (63°59'54.27"N, 21°11'58.80"W). The area has an average annual air temperature of 4°C and an average annual precipitation of 1373 mm (Icelandic Met Office, IMO). The synoptic weather station IMO is located in Eyrabakki, which is the closest and has the most comparable weather conditions to Hveragerði. The growing season starts half May and ends late August. The soil in this volcanic area is classified as Andosol type soil, which is characterised by low bulk density and high water retention (Arnalds Ó. 2004, a). Present vegetation is characterised by perennial monocotyledons and mosses and the absence of woody vegetation. Sampling of vegetation and soil was done in July, 2013.

2.2 Experimental design

2.2.1 The old and recent warming gradients

Iceland contains several high-temperature geothermal areas, including Hveragerði which is located at the margin of an active volcano belt (Arnórsson S. 1995). The heat source in Hveragerði originates from magmatic underlying dyke swarms (Arnórsson S. 1995). At several locations the heat can penetrate up to the surface, where a hotspot is formed. The soil temperature decreases as distance from the hotspot increases, resulting in defined soil temperature gradients (Fig. 1). Historic records, that report warming in the area of what we will call the <u>"Old Grassland" (GO)</u>, date back as far as 1708 (Magnússon A & Vídalín P. 1708). Therefore, the long-term effects of soil warming on plant productivity, and C and N stocks were studied along soil temperature gradients in the GO. New hotspots can occur as a result of seismic activity in the area of what we will call the <u>"New Grassland" (GN)</u> (Weedon J. 2012). This is where we studied short-term effects of soil warming on plant productivity, C and N stocks.

2.2.2 Defining the experimental plots

In both the GO and the GN, 20 x 50cm study plots were placed at five T_S levels in five replicate transects (n = 25 in each system: Fig. A.1). The five temperature levels consisted of ambient T_S (i.e.: control) and approximately 1°C, 3°C, 5°C and 10°C above ambient T_S on average (further referred to as control, +1°C, +3°C, +5°C and +10°C plots). Temperatures were measured at 10 cm depth. Transects were alternately orientated uphill and downhill

where there was an elevation profile in order to avoid artefacts caused by water transport through the soil. Grazers were excluded from the study area in the GO by fencing (no grazers were present in the GN).



Fig. 1: Isotherms surrounding a hotspot, resulting from geothermal activity in the area of Hveragerði. Numbers represent soil temperature of the associated isotherm. (Oddsdottír et al. 2013)

2.3 Data gathering of dependent variables

Samples were collected of (1) aboveground vascular vegetation (separate dicotyledons, monocotyledons and equiseta), (2) non-vascular vegetation (separate mosses and lichens), (3) necromass, (4) roots and (5) soil.

2.3.1 Aboveground biomass and necromass sampling

Samples of aboveground vascular vegetation were clipped in the 20 x 50 cm study plots and separated in dicotyledons, monocotyledons and equiseta in the field. Subsequently, non-vascular vegetation, lichens and necromass were collected in a 20 x 20 cm area within these study plots. All samples were dried at 65°C for 48h to obtain the dry weight. After weighing, sub samples of ~2g were grinded at 13 000 rpm through a 0.05 mm grinding sieve with an ultra-centrifugal mill (Retsch, ZM 200 Ultra Centrifugal Mill). Where insufficient plant biomass of a certain vegetation type was present for C and N analyses (i.e.: < 1 g), samples of the same T_s elevation and grassland were joined.

2.3.2 Fine root biomass and soil sampling

Two soil cores were taken from the 20 x 20 cm area within the 20 x 50 cm study plot, using a hand drill with a core diameter of 5.3 cm. The samples were taken until a depth of 30 cm or until the bedrock was reached. Both soil cores were subdivided in sections from 0 - 5 cm, 5 - 5

10 cm, 10 - 20 cm and 20 - 30 cm depth. After sampling, the soil cores were stored at -18° C, awaiting further processing.

The first of the two cores was used to estimate both the living fine root biomass (further referred to as "root biomass") and the fraction of soil with particle size larger than 2 mm. To separate the root biomass from the soil, the soil cores were suspended in water and were subsequently sieved (maize diameter 0.5 mm) several times until the small particle size soil was washed out. Then, the floating roots were separated from the sinking heavier soil particles. Next the remaining soil particles (< 0.5 mm) were sieved over a 2 mm sieve to obtain the soil fraction with particle size larger than 2 mm.

Afterwards the roots and soil fraction with particle size larger than 2 mm were dried for 48h at 65°C and weighed. Finally, subsamples of ~2g of the roots were grinded at 13 000 rpm through a 0.05 mm grinding sieve with an ultra-centrifugal mill (Retsch, ZM 200 Ultra Centrifugal Mill) as preparation for further C and N analyses.

The second soil core was used to obtain the soil fraction with particle size smaller than 2 mm. Sub samples of approximately 5 g wet weight were sieved out using a small brush and a 2 mm sieve. These subsamples were dried, weighed and grinded as described for the root biomass.

2.3.3 *C* and *N* analyses of biomass, necromass and soil samples

After grinding all samples, C and N concentrations were determined by flash combustion using a NC[®]2100 element analyser (Carlo Erba Instruments, Italy), a system used to determine total C and N concentration simultaneously. Data were processed by Eager[®]200, a WindowsTM based data handling system. This was done in the PLECO laboratory at the University of Antwerp. The flash combustion temperature used was 1700°C to 1800°C and the reduction temperature used was 750°C. Before analysis of the samples a calibration curve was set up using atropine, which has a similar concentration of C and N as the samples that were to be analysed (i.e.: 70.54% C and 4.84% N).

2.3.4 Origin of used data and data processing prior to statistical analyses

2.3.4.1 Biomass and necromass

All data were transformed to weight per square meter (g m⁻²). Unfortunately, due to a miscommunication, a part of the root samples from the GO were lost. However, spring root biomass of the same growing season (sampled in April) from both the GN and the GO was available, which could be used as a proxy for summer root biomass (sampled in July). Therefore, a close similarity in T_s effect on spring and summer root biomass was assumed. One drawback was that spring roots were sample until 10 cm depth compared to 30 cm for

summer roots. However, several studies showed that most of the root biomass in grassland ecosystems is located in the upper 10 cm (Suseela V. & Dukes J.S. 2013; Pucheta et al. 2004; Mezhunts et al. 2005; Fan et al. 2007; Li et al. 2011). Therefore, differences in the upper layers were assumed to be representative for differences in total root biomass.

To verify these assumptions regarding the use of spring root biomass as a proxy for summer root biomass the interaction between the effect of T_S and sampling period on root biomass was tested and a depth profile of root biomass was constructed (see § 2.5). To make the depth profile of root biomass, we used the summer root samples from the GN (of which the measured dry weight was not lost due to the mentioned miscommunication), because of the deeper sampling depth (i.e.: until 30 cm where bedrocks were deep enough). Although total root biomass was expected to be influenced by soil depth, it was not possible to normalize root biomass for depth. As spring roots were sampled up to 10 cm depth (or until the bedrock was reached) root data were unequally spread in function of depth, and the linear trend line fitted through the plotted data could not be used to extrapolate root biomass. The same applies to C and N stocks in root biomass. Spring root biomass from the GN had only two repeats in the control plots due to missing samples in the other transects.

2.3.4.2 C and N stocks in biomass and necromass

The C and N stock in biomass and necromass (g m⁻²) was calculated by combining biomass and necromass stocks and their C and N concentration (%). Due to their small weight, root samples from the same treatment (the same T_S elevation and the same grassland) were joined to obtain large enough samples for the C and N analyses. Therefore the C and N stocks in roots should be interpreted carefully. This is also the reason why no statistical analysis of root N concentration could be done in § 3.3 (i.e.: only one replicate for C and N concentrations per T_S elevation was available in each grassland).

2.3.4.3 C and N stocks in the soil

Carbon and N stocks in soil are generally calculated by multiplying bulk density (BD) with the respective C and N concentration. However, due to a miscommunication, the major part of the soil samples for the BD was lost. Therefore a literature-based estimate of BD was used. According to Arnalds et al. (2004, b) the research area contains brown andosol type soils which have an average BD of 0.69 g cm⁻³ (Óskarsson et al. 2004).

To overcome the problem that soil samples were taken up to different depths (as a consequence of different bedrock depths) and to allow comparison of soil C and N stocks between different T_S elevations and between the GN and the GO, the total soil C and N stocks

were normalized for soil depth. Therefore, the total soil C and N stocks were assumed to be correlated with depth of the soil core. Further, soil depth was assumed not to be correlated with T_S , as this would lose information about the T_S effect on soil C and N stocks by normalizing them for soil depth. The validity of these assumptions was verified by testing the effect of T_S on soil depth, the correlation between soil C and N stocks and soil depth, and the interaction between the effect of soil depth and T_S on soil C and N stocks (see § 2.5). Samples were normalized for depth by plotting soil C and N stocks in function of total soil core depth in the GN and the GO separately, using Microsoft Excel[®]. The function of the linear trend line was used to estimate the soil stocks in a soil layer of 30 cm depth.

2.4 Ecosystem components

To obtain a better understanding of the causality of changes in plant productivity and ecosystem C and N stocks after short-term and long-term warming, the ecosystem was divided in different components (Table 1).

ECOSYSTEM COMPONENT	SUBDIVISIONS			
	Vascular vegetation		Monocotyledons	
		Aboveground	Dicotyledons	
Diamage			Equiseta	
Biomass		Fine roots (belowground)		
	Non-vascular vegetation	A h ann ann an d	Mosses	
		Aboveground	Lichens	
Necromass	Not separated			
	0 to 5 cm depth			
S ~ 1	5 to 10 cm depth			
5011	10 to 20 cm depth			
	20 to 30 cm depth			

Table 1: Overview of different ecosystem components and their subdivisions

2.5 Statistical analyses

All statistical tests and accompanying figures were made using RStudio version 0.98.501 (© 2009-2013 RStudio, Inc.). Figure 4 and figure 14 were made using Origin version 7.0 (© OriginLab). Differences in biomass (dependent variable) between T_S elevations (categorical explanatory variable with 5 levels; control, $+1^{\circ}$ C, $+3^{\circ}$ C, $+5^{\circ}$ C and $+10^{\circ}$ C) and between the duration of warming (categorical explanatory variable with 2 levels: GN and GO) were first tested by performing a two-way ANOVA (p > 0.1: non-significant). However, due the low number of repeats, the power of the tests was low. Therefore, differences in biomass between

T_s elevations were always tested using one-way ANOVAs in the GN and the GO separately to ensure that no differences between grasslands were overlooked (p > 0.1: non-significant). Two-by-two comparisons were made using the Tukey multi comparison of means test (p > 0.1: non-significant), which corrects for multiple comparisons. However, before performing parametric tests (i.e.: two-way and one-way ANOVAs) conditions of normality of residuals (Shapiro Wilkinson: W > 0.90) and homoscedacity (Fligner-Killeen test: p > 0.05) were tested. If conditions were not met, logarithmic transformation was applied to the dependent variable. If conditions after logarithmic transformation were not met, box cox transformation was applied to the dependent variable (using the following RStudio-packages: Matrix, car, MASS and graphics). In the rare case that assumptions were not met after box cox transformation, non-parametric Kruskal-Wallis chi-squared test was applied (p > 0.1: nonsignificant). The non-parametric alternative was used as last option, as there are, to our knowledge, no non-parametric alternatives for the two-way ANOVAs available in RStudio. Therefore, opting for a non-parametric alternative would require splitting the test in two oneway ANOVAs. The same statistical procedure was used for all dependent variables listed in Table 2.

To test the applicability of spring root biomass as a proxy for summer root biomass, a twoway ANOVA was performed in the same way as described above but with T_S and sampling period (categorical explanatory variable 2 levels: April and July) as explanatory variables. To test the representativeness of spring root biomass from 0 to 10 cm depth for total root biomass, a depth profile of the summer root biomass (which was sampled from 0 to 30 cm depth in the GN) was made. This was done by performing a one-way ANOVA in the same way as described above but with soil layer as categorical explanatory variable (6 levels: [0-5], [5-10], [10-15], [15-20], [20-25] and [25-30] cm depth). To test the correlation between soil depth and T_S, a one-way ANOVA was applied in the same way as described above. To test the correlation between C and N stocks and soil depth and the interaction between the effect of soil depth (continuous explanatory variable) and T_S on C and N stocks, an ANCOVA (p > 0.01: non-significant) was performed. To test whether soil depth was significantly correlated with T_s, a one-way ANOVA was performed in the same way as described above. Finally, a two-way ANOVA was used to compare N concentration (%) between different vegetation types and between grasslands, with grassland and vegetation type as categorical explanatory variable (5 levels: mosses, monocotyledons, dicotyledons, equiseta and lichens).

Table 2: Overview of dependent variables compared between different T_s elevations and between the GN and the GO as described in § 2.5.

DEPENDENT VARIABLES					
	Vascular biomass	A 1		Monocotyledon biomass (g m ⁻²)	
		Aboveground vascular biomass (g m ⁻²)		Dicotyledon biomass (g m ⁻²)	
Total biomass	(g m ⁻²)			Equiseta biomass (g m ⁻²)	
(g m)		Root biomass (g m ⁻²)			
	N 1 1: (- ²)			Moss biomass (g m ⁻²)	
	Non-vascular biomass (g m ⁻)			Lichen biomass (g m ⁻²)	
Necromass (g m ⁻²)					
Monotyledon N concentrat	tion (%)				
Dycotyledon N concentration	ion (%)				
Moss N concentration (%)					
C:N ratio total vegetation					
C:N ratio necromass					
Total vegetation C stock (g m^{-2})	Vascular vegetation C stock (g m ⁻²)			Aboveground vascular vegetation C stock (g m ⁻²)	
(g)	Non-vascular vegetation C stock (g m ⁻²)				
Necromass C stock (g m ⁻²)					
Total vegetation N stock $(q m^2)$	Vascular vegetation N stock (g m ⁻²)			Aboveground vascular vegetation N stock (g m ⁻²)	
	Non-vascular vegetation N stock (g m ⁻²)				
Necromass N stock (g m ⁻²)					
	Soil C stock: 0 to 5 cm depth $(g m^{-2})$				
Soil C stock $(g m^{-2})$	Soil C stock 5 to 10 cm depth (g m^{-2})				
- non-normalized - normalized	Soil C stock 10 to 20 cm depth (g m ⁻²)				
	Soil C stock 20 to 30 cm depth (g m ⁻²)				
	Soil N stock: 0 to 5 cm depth (g m ⁻²)				
Soil N stock (g m ⁻²)	Soil N stock 5 to 10 cm depth (g m ⁻²)				
- non-normalized - normalized	Soil N stock 10 to 20 cm depth (g m ⁻²)				
	Soil N stock 20 to 30 cm depth (g m ⁻²)				

3 Results

3.1 Testing assumptions

3.1.1 T_s effect on root biomass in spring (April) and summer (July) in the GN

There was a significant T_S effect on root biomass (Table A.4: <u>tables located in annex are</u> <u>always referred to as Table A.x</u>) and no significant interaction between the effect of T_S and the sampling period on root biomass from 0 to 10 cm soil depth in the GN (p = 0.65; Table A.4). Although there was a significant difference in root biomass between the two sampling periods (Table A.4), with the amount in the July being 383 g m⁻² higher on average, root biomass in different sampling periods followed similar trends when exposed to the same T_S elevations and spring root biomass could be used as a proxy for summer root biomass. Since we had complete biomass and C and N stock analyses for roots sampled in spring but not for roots sampled in summer, we continued working with spring root data to allow comparison between grasslands and to stay consistent throughout the research. (Fig. A.4: figures located in annex are always referred to as Fig. A.x)

3.1.2 Root biomass distribution

We analysed the root biomass distribution in summer, which was sampled to deeper depths (0 to 30 cm soil depth). On average $89.4 \pm 3.8 \%$ (n = 24) of summer root biomass was located in the upper 10 cm of the soil in the GN. There was significantly more root biomass stored in the surface layer of the soil (0 to 5 cm depth) compared to subsurface soil layers in the GN at all T_s elevations (Table A.5). From 5 to 10 cm depth there was also significantly more root biomass than from 20 to 25 cm and 25 to 30 cm depth (Table A.5). Because 90% or root biomass was located in the upper 10 cm of the soil, with no differences among T_s elevations, it was faire to assess the variation in root biomass using only the data from the upper 10 cm, which was available for both grasslands. (Fig. A.5)

3.1.3 Depth effect on soil C and N stocks and T_S effect on soil depth

Not all soils were of the same depth and, as expected, deeper soils contained significantly higher C and N stocks for all T_S elevations (Table A.6(1)) (Fig. A.6(1)). Because our aim was to compare soil C and N stocks among different T_S elevations we needed to normalize the soil stocks to a common depth. We therefore first analysed the interaction between soil depth and T_S effect and the relation between soil depth and T_S . There was no significant interaction between the effect of soil depth and T_S on C and N stocks (p = 0.45; Table A.6(1)) (Fig. A.6(1)). Also, there were no significant differences in depth for different T_S elevations (p =

0.67 in the GN and p = 0.88 in the GO; Table A.6(2) and Table A.6(3)) (Fig. A.6(2)). Because of these results it was possible to normalize the soil C and N stocks to a common depth, as explained in § 2.3.4.3.

3.2 **Productivity**

3.2.1 Total vascular vegetation (monocotyledons, dicotyledons, equiseta)

When only considering vascular vegetation in the GN, biomass was significantly lower in the $+10^{\circ}$ C plots compared to the $+1^{\circ}$ C plots where highest vascular biomass was found (Table A.10(2)). In contrast to the GN, the GO showed no significant differences between different T_s elevations (Table A.10(3)) and had significantly more aboveground vascular biomass than the GN (Table A.10(1)). (Fig. 2)

3.2.2 Root biomass of vascular vegetation

Plots with high T_s elevation (+10°C) had significantly lower root biomass than +1°C plots in the GN (Table A.10(2)). In the GO no significant differences were detected (Table A.10(3)). Again, the GO had significantly more root biomass than the GN (Table A.10(1)). (Fig. 2)

3.2.3 Aboveground vascular and non-vascular vegetation (mosses, lichens)

In contrast to the GN (where no significant differences were detected; Table A.10(2)), the GO showed a positive temperature effect on aboveground vascular biomass (Table A.10(3)). In +10°C plots aboveground vascular biomass was significantly higher (270 g m⁻²) than in control and +1°C plots, where biomass was approximately half of the +10°C plots. The GO had significantly more aboveground vascular biomass than the GN (Table A.10(1)). (Fig. 2) As expected, the pattern observed when considering non-vascular vegetation contrasted with the pattern observed when considering aboveground vascular vegetation. Differences in non-vascular biomass between T_S elevations were not significantly lower in the warmest plots (+10°C) compared to the +1°C plots (Table A.10(3)). No significant difference in non-vascular biomass was found when comparing the two grasslands (Table A.10(1)). (Fig. 2)

3.2.4 Total live vegetation

In the GN highest biomass was observed just above control T_s in +1°C plots. In +5°C and +10°C plots biomass was significantly lower in the GN (Table A.10(2)). Biomass in +10°C plots decreased to less than half of what was observed at the +1°C plots, going from 1200 g m⁻² in +1°C plots to 580 g m⁻² in +10°C plots. The GO showed no significant differences between different T_s elevations (Table A.10(3)) and contained significantly more

biomass than the GN (Table A.10(1)). The difference in biomass between the GN and the GO was 640 g m⁻² on average. (Fig. 3 and 4)



Fig. 2: Biomass in the two grasslands at different T_s elevations compared to control T_s (i.e.: ambient T_s) A: Total vascular vegetation; **B**: Aboveground vascular biomass; **C**: Vascular root biomass; **D**: Non-vascular biomass. Error bars indicate SE. Letters indicate significant differences in biomass compared at different T_s elevations within grasslands separately. Significance codes: p > 0.1: n.s.; p < 0.1: °; p < 0.05: *; p < 0.01: **; p < 0.001: ***.



Fig. 3: Total vegetation biomass in the two grasslands at different T_s elevations compared to control T_s (i.e.: ambient T_s). Error bars indicate SE. Letters indicate significant differences in total vegetation biomass compared at different T_s elevations within grasslands separately. Significance codes: p > 0.1: n.s.; p < 0.1: °; p < 0.05: *; p < 0.01: **; p < 0.001: ***.



Fig. 4: Total vegetation biomass divided in different vegetation components: roots, mosses, lichens, aboveground monocotyledons ("monocotyledons"), aboveground dicotyledons ("dicotyledons") and aboveground equiseta ("ferns"). These are compared in the two grasslands at different T_s elevations compared to control T_s (i.e.: ambient T_s) **A**: GN; **B**: GO. Error bars indicate SE.

3.3 N concentration and C:N ratios

The temperature effect on N concentration (%) depended on the vegetation type considered. There was no apparent increased accumulation of N with increasing T_S nor in aboveground vascular vegetation (i.e.: monocotyledons and dicotyledons) nor in roots (Tables A.11(2) and A.11(3)). However, the N concentration in aboveground dicotyledon biomass went down significantly in +1°C, +3°C, +5°C and +10°C plots compared to control plots in the GN (Table A.11(2)). N concentration in aboveground dicotyledon biomass was significantly higher in the GO compared to the GN in +1°C, +3°C and +5°C plots (Table A.11(1)). This could not be assessed for plots at +10°C because only one replicate was available for the GO. In non-vascular vegetation of the GN (i.e.: mosses) N concentration was significantly higher in +10°C plots compared to +1°C plots (Table A.11(2)). (Fig. 5)

The C:N ratio of total live vegetation showed no significant differences between different T_S elevations in the GN (Table A.12(2)). However in the GO, C:N ratio at T_S elevation of +5°C was significantly higher than at +10°C (Table A.12(3)). (Fig. 6)

The C:N ratio of necromass was significantly higher at $+1^{\circ}$ C compared to other T_S elevations in the GN (Table A.12(2)). In the GO C:N ratio of necromass was significantly higher in control, $+3^{\circ}$ C and $+5^{\circ}$ C plots compared to $+10^{\circ}$ C plots (Table A.12(3)). (Fig. 6)



Fig. 5: N concentration of biomass in the two grasslands at different T_s elevations compared to control T_s (i.e.: ambient T_s). A: Aboveground biomass monocotyledons; B: aboveground biomass dicotyledons; C: roots; D: moss. Error bars indicate SE. Letters indicate significant differences in N concentration compared at different T_s elevations within grasslands separately. Significance codes: p > 0.1: n.s.; p < 0.1: °; p < 0.05: *; p < 0.01: **; p < 0.001: ***; n.a.: not assessable (only one replicate per T_s elevation for root N concentration in each grassland; see § 2.3.4.2).



Fig. 6: C:N ratio in the two grasslands at different T_s elevations compared to control T_s (i.e.: ambient T_s). A: Total biomass; B: Necromass. Error bars indicate SE. Letters indicate significant differences in C:N ratio compared at different T_s elevations within grasslands separately. Significance codes: p > 0.1: n.s.; p < 0.1: °; p < 0.05: *; p < 0.01: **; p < 0.001: ***.

3.4 C and N stocks in biomass

The C in biomass showed similar patterns as the biomass at different T_S elevations in both grasslands and are therefore joined in annex (Fig. A.9(1) and A.9(2)). However, changes in N

stocks showed some deviations from the pattern observed in biomass and are therefore included in this section (Fig. 7 and 8).

3.4.2 Stocks in total vascular vegetation

As when looking at biomass, C stocks in total vascular vegetation in the GN decreased significantly in the +10°C plots compared to +1°C plots (Table A.13(2)). However, in the GO no significant differences in C stocks between T_s elevations were detected (Table A.13(3)) and stocks were significantly higher than in the GN (Table A.13(1)). (Fig. A.9(1))

As with C stocks, N stocks in total vascular vegetation decreased significantly in the $+10^{\circ}$ C plots compared to the $+1^{\circ}$ C plots in the GN (Table A.14(2)). Again, no significant differences in N stocks were detected in the GO (Table A.14(3)) and stocks were significantly higher than in the GN (Table A.14(1)). (Fig. 7)

3.4.3 Stocks in root biomass (from 0 to 10 cm soil depth)

In the GN, C stocks in roots up to 10 cm depth were significantly lower at T_S elevations of +5°C and +10°C compared to +1°C plots (Table A.13(2)). In the GO no significant differences between different T_S elevations were detected (Table A.13(3)) and stocks were significantly higher compared to the GN (Table A.13(1)). (Fig. A.9(1))

N stocks in roots were significantly lower at a T_S elevation of +10°C compared to +1°C plots in the GN (Table A.14(2)). Again, no significant differences were detected in the GO (Table A.14(3)) and stocks were significantly higher compared to the GN (Table A.14(1)). (Fig. 7)

3.4.4 Stocks in aboveground vascular and non-vascular vegetation

C stocks in aboveground vascular vegeation in the GN stayed relatively stable. Concordantly, no significant differences were detected (Table A.13(2)). However, in the GO a positive temperature effect could be observed with a significant difference between highest T_S elevation plots (+10°C) and colder control and +1°C plots containing less C (Table A.13(3)). The GO had a significantly higher C stocks than the GN (Table A.13(1)). (Fig. A.9(1))

As with C stocks, N stocks in aboveground vascular vegetation did not differ significantly in the GN (Table A.14(2)). In the GO highest N stocks were observed at +10°C plots. However, the difference with lower T_S elevation plots was not significant (Table A.14(3)). Again, the GO had significantly higher N stocks compared to the GN (Table A.14(1)). (Fig. 7)

As with biomass, C stocks in non-vascular vegetation contrasted with the C stocks in above ground vascular vegetation. No significant differences in C stocks of non-vascular vegetation between different T_S elevations were detected in the GN nor the GO (Table A.13(2) and A.13(3)). The GO had significantly lower C stocks than the GN (Table A.13(1)). (Fig. A.9(1))

No significant differences in N stocks of non-vascular vegetation between different T_s elevations were detected in the GN nor the GO (Table A.14(2) and A.14(3)). However, N stocks were highly variable at different T_s elevations, especially in the GN. (Fig. 7)

3.4.1 Stocks in total biomass

As expected, C stocks were a reflection of the total biomass production. In the GN highest C stocks were observed at T_s elevation of +1°C (477 g m⁻²). C stocks at T_s elevation of +10°C (235 g m⁻²) were significantly lower compared to +1°C plots (Table A.13(2)). In the GO no significant differences in C stocks between different T_s elevations were detected (Table A.13(3)) and stocks were significantly higher compared to the GN (Table A.13(1)) with a difference of 230 g m⁻² on average. (Fig. A.9(2))

For N stocks the difference between the highest stock in $+1^{\circ}$ C plots (11.0 g m⁻²) and the lowest stocks in $+10^{\circ}$ C (6.8 g m⁻²) plots was not significant and no other significant differences were detected in the GN (Table A.14(2)). As for C stocks, in the GO no significant differences between N stocks at different T_s elevations were detected (Table A.14(3)) and stocks were significantly higher compared to the GN (Table A.14(1)) with a difference of 5.7 g m⁻² on average. (Fig. 8)



Explanation Fig. 7: see p. 25

Fig. 7: N stocks of biomass in the two grasslands at different T_s elevations compared to control T_s (i.e.: ambient T_s). A: total vascular vegetation; B: aboveground vascular vegetation; C: roots; D: non-vascular vegetation. Error bars indicate SE. Letters indicate significant differences in N stocks of biomass compared at different T_s elevations within grasslands separately. Significance codes: p > 0.1: n.s.; p < 0.1: °; p < 0.05: *; p < 0.01: **; p < 0.001: ***.



Fig. 8: N stocks of total biomass in the two grasslands at different T_s elevations compared to control T_s (i.e.: ambient T_s). Error bars indicate SE. Letters indicate significant differences in N stocks of total biomass compared at different T_s elevations within grasslands separately. Significance codes: p > 0.1: n.s.; p < 0.1: °; p < 0.05: *; p < 0.01: **; p < 0.001: ***.

3.5 Aboveground necromass and C and N stocks in aboveground necromass

In the GN necromass showed a decreasing trend at T_S elevations higher than +1°C and was significantly lower in +5°C plots compared to +1°C plots (Table A.15(2)). However, in the GO no significant differences were detected (Table A.15(3)) and necromass was significantly lower compared to the GN (Table A.15(1)). (Fig. 9)

The C stocks in necromass showed a similar decreasing trend at T_s elevation higher than +1°C and were again significantly lower in +5°C plots (170 g m⁻²) compared to +1°C plots (370 g m⁻²) in the GN (Table A.16(2)). In the GO, C stocks remained stable with no significant differences (Table A.16(3)) and were significantly <u>lower</u> compared to the GN (Table A.16(1)) with a difference of 110 g m⁻² on average. (Fig. 10)

N stocks showed no significant differences at different T_S elevations in the GN as well as in the GO (Table A.17(2) and A.17(3)). As with C stocks, N stocks were significantly <u>lower</u> in the GO (Table A.17(1)) with a difference of 2.6 g m⁻² on average. (Fig. 10)



Fig 9: Aboveground necromass in the two grasslands at different T_s elevations compared to control T_s (i.e.: ambient T_s). Error bars indicate SE. Letters indicate significant differences in C and N stocks compared at different T_s elevations within grasslands separately. Significance codes: p > 0.1: n.s.; p < 0.1: °; p < 0.05: *; p < 0.01: **; p < 0.001: ***.



Fig. 10: **A**: C stocks of necromass in the two grasslands at different T_s elevations compared to control T_s (i.e.: ambient T_s). **B**: N stocks of necromass in the two grasslands at different T_s elevations compared to control T_s (i.e.: ambient T_s). Error bars indicate SE. Letters indicate significant differences in C and N stocks compared at different T_s elevations within grasslands separately. Significance codes: p > 0.1: n.s.; p < 0.1: °; p < 0.05: *; p < 0.01: **; p < 0.001: ***.

3.6 C and N stocks in soil

There was more C stored in the soil than in biomass, with average values ranging from 2900 to 9300 g m⁻² (not normalized for soil depth) compared to average values ranging from 240 to 670 g m⁻² in total biomass (Fig 14). C stocks in the $+5^{\circ}$ C and $+10^{\circ}$ C plots were significantly lower than in the control plots in the GN, for non-normalized as well as normalized data (Table A.18(2) and A.19(2)). In the GO total soil C stocks were significantly lower in $+10^{\circ}$ C plots compared to $+3^{\circ}$ C plots (Table A.18(3)). Normalized for soil depth, soil C stocks in $+10^{\circ}$ C plots were significantly lower than stocks in both control and $+3^{\circ}$ C plots and C stocks in $+5^{\circ}$ C plots were significantly lower than in $+3^{\circ}$ C plots (Table A.19(3)). (Fig. 11)

As for C, more N was stored in the soil than in the biomass (Fig 14). Soil N stocks in the GN showed the same pattern as soil C stocks in the GN (Table A.20(2)). Except in $+10^{\circ}$ C plots when using normalized data, where N stocks were significantly lower than stocks in $+1^{\circ}$ C and $+3^{\circ}$ C plots as well (Table A.21(2)). No significant differences in soil N stocks were detected for non-normalized and normalized data in the GO (Table A.20(3) and A.21(3)). (Fig. 12)



Fig. 11: Soil C stocks in the two grasslands at different T_s elevations compared to control T_s (i.e.: ambient T_s). **A**: not normalized for soil depth; **B**: normalized for soil depth. Error bars indicate SE. Letters indicate significant differences in C stocks compared at different T_s elevations within grasslands separately. Significance codes: p > 0.1: n.s.; p < 0.1: °; p < 0.05: *; p < 0.01: **; p < 0.001: ***.



Fig. 12: Soil N stocks in the two grasslands at different T_s elevations compared to control T_s (i.e.: ambient T_s). A: not normalized for soil depth; **B**: normalized for soil depth. Error bars indicate SE. Letters indicate significant differences in N stocks compared at different T_s elevations within grasslands separately. Significance codes: p > 0.1: n.s.; p < 0.1: °; p < 0.05: *; p < 0.01: **; p < 0.001: ***.

3.7 C and N stocks in ecosystem

When biomass, necromass and soil stocks are joined, differences in ecosystem C stocks between T_S elevations were no longer significant in the GN nor for non-normalized nor for normalized data for soil depth (Table A.22(2) and A.23(2)). Although the effect of temperature was marginally significant (p < 0.1), pairwise comparisons detected no significant differences. In the GO, ecosystem C stocks in control and $+3^{\circ}$ C plots were significantly higher than in $+10^{\circ}$ C plots (Table A.22(3)). When data was normalized for soil depth, ecosystem C stocks in $+10^{\circ}$ C plots were also significantly lower than in $+1^{\circ}$ C plots (Table A.23(3)). Ecosystem C stocks in the GO were significantly higher than in the GN for normalized as well as non-normalized data for soil depth (Table A.22(1) and A.23(1)). (Fig. 13)

When looking at ecosystem N stocks in the GN, no significant differences between T_S elevations were found (Table A.24(2)). However, when data was normalized for soil depth ecosystem N stocks in +10°C plots were significantly lower than in control and +1°C plots (Table A.25(2)). In the GO neither non-normalized nor normalized ecosystem N stocks showed significant differences between different T_S elevations (Table A.24(3) and A.25(3)). Again, ecosystem N stocks were significantly higher in the GO compared to the GN (Table A.24(1)) but not for normalized ecosystem N stocks (Table A.25(1)). (Fig. 13)



Fig. 13: Total ecosystem C and N stocks in the two grasslands at different T_s elevations compared to control T_s (i.e.: ambient T_s). A: C stocks not normalized for soil depth; B: C stocks normalized for soil depth; C: N stocks not normalized for soil depth; D: N stocks normalized for soil depth. Error bars indicate SE. Letters indicate significant differences in total stocks compared at different T_s elevations within grasslands separately. Significance codes: p > 0.1: n.s.; p < 0.1: °; p < 0.05: *; p < 0.01: ***; p < 0.001: ***.



Fig. 14: Upper figure: Ecosystem C stocks divided in different components: soil C stocks normalized for soil depth, necromass C stocks and biomass C stocks. A: GN; B; GO. Lower figure: Ecosystem N stocks divided in different components: soil N stocks normalized for soil depth, necromass N stocks and biomass N stocks. A: GN; B: GO. Error bars indicate SE.

3.8 Hypothesis testing: vegetation type biomass and N concentration

In the GN no significant differences in biomass of different vegetation types were detected between T_s elevations (Table A.26(2)). In the GO monocotyledons had significantly higher biomass in +10°C plots compared to control and +1°C plots and mosses had significantly lower biomass in +10°C compared to +1°C plots (Table A.26(3)). No other significant differences in biomass were found in the GO (Table A.26(3)). (Fig. 15)

N concentration was significantly lower in mosses compared to all other vegetation types in both the GN and the GO (Table A.27(2) and A.27(3)). In the GN, N concentration increased significantly in the following order: mosses < monocotyledons < dicotyledons < equiseta < lichens (Table A.27(2)). In the GO, N concentration was significantly lower in monocotyledons compared to dicotyledons, equiseta and lichens (Table A.27(3)). (Fig. 16)



Fig. 15: Aboveground biomass in the two grasslands at different T_s elevations compared to control T_s (i.e.: ambient T_s). A: monocotyledons; B: dicotyledons; C: Equiseta; D: lichens; E: mosses. Error bars indicate SE. Letters indicate significant differences in biomass compared at different T_s elevations within grasslands separately. Significance codes: p > 0.1: n.s.; p < 0.1: °; p < 0.05: *; p < 0.01: **; p < 0.001: ***; n.a.: not assessable (no non-parametric alternative for two-way ANOVAs in RStudio).



Fig. 16: Average N concentration (%) of different vegetation types in the GN and the GO. Moss = mosses; VA-M = Monocotyledons; VA-H = dicotyledons; FE = Equiseta; Lichens = lichens. Letters indicate significant differences in N concentration compared at different T_s elevations. Error bars indicate SE.

4 Discussion

In this section, first changes in plant productivity with increasing T_S will be discussed and compared among different vegetation components (total vegetation, total vascular vegetation, aboveground vascular vegetation, roots, non-vascular vegetation and necromass) and between short-term and long-term soil warming gradients (§ 4.1). Changes in N concentration linked to T_S and resulting C:N ratios will be considered briefly (§ 4.2). Subsequently, changes in C and N stocks will be compared among different ecosystem components (i.e.: biomass, necromass and soil) (§ 4.3 to 4.5) before determining whether there is a loss or gain in total C and total N from the ecosystem due to direct and indirect effects of increasing T_S (§ 4.6). Again, the two grasslands will be compared in order to determine whether the observed changes after 5 years in the GN are transient or in equilibrium. Finally assumptions, artefacts and prospects for future research will be discussed (§ 4.7).

4.1 Effect of elevated T_S on productivity

Changes in total vegetation biomass (i.e.: proxy for grassland productivity; Shaver et al. 1996) can directly and indirectly alter total ecosystem C and N stocks and the balance of net ecosystem C and N fluxes in response to warming (Fan et al. 2007; de Graaff et al. 2006; Herbert et al. 1999). We expected that increased T_S would have a positive effect on productivity, mainly through its positive effect on the length of the growing season (Leblans et al., unpublished data) and on increased plant available N, due to the warming-induced stimulation of microbial activity and the associated increase in decomposition and mineralization rates (Zak et al. 1999; Zaman et al. 2004; Li et al., 2014; Rustad et al. 2001; Pendall et al. 2004). However, this was not confirmed by our results. In contrast, in the GN even a negative temperature effect was observed at high T_S elevations (+5°C and +10°C) (Table A.10(2)) (Fig 3). This unexpected result suggests that biomass might not function as a C sink after short-term warming and as a result, may not buffer the expected increased atmospheric C input due to increased soil respiration (Rustad et al. 2001; Pendall et al. 2004; Schindlbacher et al. 2011) and the supposedly decreased methane uptake (Luo et al. 2013) at high T_S elevations (+3° to +10°C) (see § 4.5).

As the largest component of biomass, it is not surprising that total biomass of vascular plants showed similar temperature responses than total biomass as a whole: no significant increase with increasing T_s and even significant decreases at +10°C compared to +1°C (Table A.10(2)) (Fig 2). The latter was mainly due to an apparent negative temperature effect on root biomass. Our results are in contrast with other warming experiments (also not experiencing

drought effects), which found an increase of total vascular biomass or of productivity with increasing temperature (Jonasson et al. 1999; Lee et al. 2010; DeMarco et al. 2014; Wu et al. 2011; Shaw et al. 2002; Flanagan L.B. & Adkinson A.C. 2011). Moreover, although we predicted a decrease of root biomass in response to an increased shoot:root ratio, we did not expect such a strong negative temperature effect on root biomass nor a lack of a positive temperature effect on aboveground vascular biomass, which both go against general observations (DeMarco et al. 2014; Flanagan L.B. 2012; Dieleman et al. 2012; Walker et al. 2006; Rustad et al. 2001).

However, almost all temperature manipulation studies have altered air temperature (T_A) (Flanagan L.B. & Adkinson A.C. 2011; Shaw et al. 2002; Walker et al. 2006) and only few studies intentionally manipulated T_S . Among studies that did consider the T_S , the majority of the methods for temperature manipulation resulted merely in slight T_S elevations of less than or approximately 2°C (Chapin et al. 1995; Arft et al. 1999; Jonasson et al. 1999; Shaver G.R. & Jonasson S. 1999; DeMarco et al. 2014; Klein et al. 2005), which is approximately equivalent to our +1°C and +3°C plots, where no significantly negative temperature effect was detected either. The lack of climate change studies with high T_S elevations could explain why only very few studies report a decrease of total biomass with increasing T_S .

Similar field studies that reported a decrease of total or aboveground biomass with increasing temperature often explained this by drought effects occurring as a result of the increased temperature (Carlyle et al. 2013; Epstein et al. 1997; Walker et al. 2006; Dieleman et al., unpublished), which offset the positive temperature effect. However, in our study, drought stress is unlikely to have occurred during the growing season because it rained very frequently and evaporative demand was smaller than precipitation (Table A.2(2); Flanagan L.B. & Adkinson A.C. 2011).

The most likely explanation for the strongly decreased root biomass at high T_S elevations, especially in the GN, could be direct temperature stress (Ferris et al. 1998; Ebrahim et al. 1998; Rachmilevitch et al 2006; Xu Q. & Huang B. 2000). Not many field studies on elevated soil temperature and its effect on (root) biomass have been conducted in sub-arctic grasslands and to our knowledge the <u>soil</u> temperature at which heat injury usually occurs (i.e.: heat injury limit) in sub-arctic grasslands has not been determined yet. However, plants adapted to cold-climate temperatures are more sensitive to temperature stress (Hongmei et al. 2009) and thus more likely to show detrimental effects at relatively low T_S elevations compared to what is found in other climates. Especially since high T_S appears to be more detrimental than high T_A (Xu Q. & Huang B. 2000). Due to higher T_S in warmed plots, T_S has a higher chance of
surpassing a heat injury limit in those plots, especially during summer when the highest atmospheric temperatures occur (Table A.2(1)).

Temperature stress could have a negative feedback effect on soil N stocks in the GN by lowering (root) biomass (Ferris et al. 1998; Ebrahim et al. 1998; Rachmilevitch; Xu Q. & Huang B. 2000) and thereby reducing the plant uptake of the assumed initial increase in plant available N with increasing T_S (Chapin et al. 1995; Natali et al. 2012; Guntiñas et al. 2012). Once N is mineralised, it becomes vulnerable to leaching and denitrification mechanisms if it is not taken up immediately by vegetation (see § 1.5). Reduced N immobilization by roots and increased N leaching or volatilization could thus be the mechanism explaining the decreasing soil N stocks (and thus total ecosystem N stocks) at high T_s elevations in the GN (see § 4.5).

As we hypothesized, soil N stocks (including plant available and unavailable N) decreased and were significantly lower in the highest T_S plots of the GN (+5° and +10°C) (Table A.20(2) and A.21(2)) (Fig. 12). However, these total soil N stocks also include the plant <u>unavailable</u> N. Therefore, plant <u>available</u> N could still have been higher at high T_S elevations, even if total soil N stocks were lower and no positive temperature effect on aboveground vascular biomass was detected at high T_S elevations. The available N may simply not have been immobilized by the much smaller root system, but lost to ground water or the atmosphere.

Interestingly, <u>in the GO</u> total biomass did not differ between different T_S elevations (Table A.10(2)) (Fig. 3), which suggests that negative temperature effects, as experienced by vascular vegetation at high T_S elevation in the GN, are a transient phenomenon. It may be that in the long-term, temperature sensitive plant species, or temperature sensitive individuals from certain species, are outcompeted by less sensitive species or individuals. An indication for this was given in the Master Thesis by Lieven Michielsen (Michielsen L. 2014), who found that typical cold-preferring boreal plant species were lost from the communities growing at higher temperature. In addition to loss of temperature-sensitive species, plants may also have adapted to long-term warming. Rachmilevitch et al. (2006) found that acclimation of the respiratory carbon metabolism is important for root survival under chronically high <u>soil</u> temperatures when comparing grass species adapted to growing in geothermal areas with non-geothermal grass species. This adaptation could possibly be achieved by stronger expression of Heat Shock Proteins (HSP) in vascular plants of the GO (Stout R.G. & Al-Niemi T.S. 2002; Stout et al. 1997).

While the GN showed very surprising responses of plant biomass to temperature increase, observations in the GO were more in line with our expectations: a non-significant decreasing trend in root biomass coincided with a significant positive temperature effect on aboveground vascular biomass at highest T_s elevation (Table A.10(3)) (Fig. 2) (Lee et al. 2010). However, there was no overall temperature effect on total vascular vegetation (including vascular roots) (Table A.10(3)) (Fig. 2). This was caused partly by the proportionally higher root biomass compared to aboveground vascular biomass (Fig. 4) and the higher turnover rates of aboveground biomass (Chapin F.S. 1980; Chapin et al. 1995). Moreover, the more frequent freeze-thaw events as a consequence of the earlier snow-melt in the warmer plots could have play a role as well (Baptist et al 2010).

As we expected, non-vascular vegetation decreased at T_S elevations where aboveground vascular biomass increased (Fig. 2), presumably due to a shift from nutrient to light competition (Wilson S.D. & Tilman D. 1993; Lee et al. 2010). This lead to constant aboveground biomass across all temperature (Table A.8(1), A.8(2) and A.8(3)) (Fig. A.8). This result agreed with several studies reporting shifts in community structure in response to increased aboveground biomass of vascular vegetation at higher T_S elevations (Chapin F.S. & Shaver G.R. 1985; Zhang Y. & Welker J.M. 1996; Chapin et al. 1995; Cornelissen et al. 2001; Van Wijk et al. 2003; DeMarco et al. 2014; Walker et al. 2006).

Total vegetation biomass was higher in the GO than in the GN (Table A.10(1)) (Fig. 3). This was partly due to the lack of a negative temperature effect on the root biomass in the GO (Table A.10(3)) (Fig. 3). However, the higher biomass in control plots of the GO compared to the control plots in the GN suggests additional differences between the two grasslands. This difference between control plots is mainly caused by the shallower soil in the GN as compared to the GO (Fig. A.7). The more shallow soil at all T_S elevations could possibly have restricted root growth more in the GN (Larcher W. 2004). If root data would have been normalized for depth, the difference between root biomass in the GN and the GO would probably have been smaller at all T_S elevations. However normalization of root biomass and stocks was not possible as explained in § 2.3.4.1.

4.2 T_S effect on C:N ratios

C:N ratios can influence C and N cycling by delaying or accelerating litter decomposition rate and the resulting C and N input to the soil (Berg B. 2000). This in turn could lead to altered plant available N, thereby affecting several processes in the ecosystem C and N cycle.

The temperature effect on N concentration (%) depended on the vegetation type considered (i.e.: aboveground monocotyledons, aboveground dicotyledons, vascular roots, mosses) (Table A.11(1)) (Fig. 5). Because we assumed that any increase in plant N would result in plant growth and hence increased C, we expected no apparent temperature effect on N concentration of any of the biomass pools: aboveground vascular biomass (i.e.: monocotyledons and dicotyledons), root biomass and non-vascular biomass. This was indeed observed in the GO (Table A.11(3)) (Fig. 5).

In the GN however, N concentration declined significantly in dicotyledon biomass at all T_S elevations compared to control plots (Table A.11(2)) (Fig. 5). This might be the result of monocotyledons having the advantage in the competition for N due to their higher initial abundance (DeMarco et al. 2014). Further, in N limited systems dilution of N in plant tissue at moments of increased growth in N limited systems has been reported before (Gavito et al. 2001; Flanagan L.B. & Adkinson, A.C. 2011). However, there was no significant increase of dicotyledon biomass at different T_S elevations compared to control, which excludes that this dilution mechanism plays a role (Table A.26(2)) (Fig. 15).

The decrease of N concentration with elevated T_s in dicotyledons did not cause a significant increase of <u>C:N ratio in biomass</u>, which remained stable <u>in the GN</u> (Table A.12(2)) (Fig. 6). This is presumably due to the lower amount of dicotyledons compared to other vegetation types, reducing its influence on the C:N ratio of total biomass (Fig. 4 and 5). In contrast to what we expected, <u>necromass</u> had a significantly higher <u>C:N ratio</u> at +1°C compared to control and higher T_s elevations after short-term warming (Table A.12(2)) (Fig. 6). Although the significantly lower N concentration of dicotyledons could increase the C:N ratio compared to control, it cannot be the sole explanation as they had only a low abundance (Fig. 4 and 5). Increased productivity resulting in increased senescence of N-poor vegetation types (e.g.: monocotyledons and moss; Fig. 16) due to higher turnover rates of biomass at +1°C could be another possible explanation as is explained in § 4.4.

The lower C:N ratio in total vegetation at $+10^{\circ}$ C compared to $+5^{\circ}$ C in the GO, could indicate a slight N accumulation at $+10^{\circ}$ C (Table A.12(3)) (Fig. 6). Even when N is no longer limiting, accumulation of excess N usually remains very low (Knecht M.F. & Göransson A. 2003). This slight decrease of C:N ratio in total vegetation could indicate that N availability is no longer limiting at $+10^{\circ}$ C in the GO. Although no significant increases in N concentration were found, the highest average N concentration in different vegetation components was always found at $+10^{\circ}$ C in the GO (Fig. 5), which could result in significantly lower C:N ratios of biomass when accumulated. The significantly lower C:N ratio of necromass at $+10^{\circ}$ C further supports the release of N limitation at $+10^{\circ}$ C in the GO (Table A.12(3)) (Fig. 6). However, such a decrease of C:N ratios could also be caused by increased abundance of more N-rich vegetation types (e.g.: lichens, equiseta, dicotyledons; Fig. 16). Abundance of these Nrich vegetation types was rather patchy and although the $+10^{\circ}$ C plots contained higher dicotyledon biomass, this was not significant due to large variability (Table A.26(3)) (Fig. 15). Nonetheless, we cannot exclude the possibility that a species shift could in part explain the lower C:N ratio found at $+10^{\circ}$ C in the GO.

4.3 T_S effect on biomass C and N stocks

As explained in previous sections (4.1 and 4.2) changes in C and N stocks of biomass can play an important role in C and N cycling by acting as C sources or sinks and by altering decomposition rates of necromass (Fan et al. 2009, Kätterer et al. 2011, Dieleman et al., unpublished; Herbert et al. 1999; Berg B. 2000). N stocks in biomass could also give a rough indication of long-term changes in N availability in the soil, based on increased or decreased N uptake by vegetation (Koerselman W. & Meuleman A.F.M. 1996).

As we expected, C stocks in total biomass and C stocks of different biomass components followed the same pattern as biomass at different T_S elevations (Annex 13). Differences between T_S elevations can be explained by mechanisms mentioned in § 4.1. However, N stocks in total biomass showed some deviations from what was found in biomass at different T_S elevations suggesting that the response of N uptake to increased T_S is more variable among different biomass components.

In the GN, despite significant decreases in total biomass at high T_S elevations no such differences were found in N stocks (Table A.14(2)) (Fig. 8), suggesting that there was no significant decrease of <u>plant available</u> N in the soil at high T_S elevations (Koerselman W. & Meuleman A.F.M. 1996). This deviation from the pattern found in biomass is especially striking in non-vascular N stocks, which showed high peeks at +3°C to +10°C with large SE indicating high variability of N stocks (Fig. 7). This patchy distribution of plots with exceptionally high abundances of usually less abundant N-rich vegetation types could be due to competition effects, with less abundant plants taking advantage when they can of "weakened" dominant vegetation types (monocotyledons and mosses) at high T_S elevations due to direct heat stress (§ 4.1).

4.4 T_S effect on necromass C and N stocks

Aboveground necromass provides one of the primary inputs of C to soils (together with root necromass) (Davidson E.A. & Janssens, I.A. 2006; Käterer et al. 2011). Increased necromass

due to increased aboveground productivity and turnover rates could lead to more C input to the soil, where it can be stabilised for a long time as soil organic matter (Dieleman et al., unpublished). Although necromass seems to follow a decreasing trend at $T_S > +1^{\circ}C$ in the <u>GN</u>, the difference is significantly lower only at +5°C compared to +1°C (Table A.15(2)) (Fig. 9). This decrease of necromass at +5°C is probably due to a combination of decreased productivity (§ 4.1) and increased decomposition rate at high T_S elevations (Dieleman et al., unpublished; Zaman M. & Chang S.X. 2004, Li et al. 2014; Aerts R. 2006) rather than to a lower temperature sensitivity of productivity compared to decomposition as we hypothesized in § 1.4.

The peak of necromass at +1°C (Fig. 9) in the GN could either suggest higher productivity of aboveground (vascular) vegetation (DeMarco et al. 2014; Flanagan L.B. & Adkinson A.C. 2011; Dieleman et al. 2012; Walker et al. 2006; Rustad et al. 2001) or decreased decomposition rates due to lower necromass quality (Berg B. 2000) compared to control. Aboveground vascular vegetation at +1°C is not significantly different from control (Table A.10(2)) (Fig. 2). However, this could be the result of higher turnover rates resulting in increased litter input with aboveground biomass staying approximately constant. On the other hand decreased decomposition could be due to higher C:N ratios of litter at +1°C (Table A.12(2)) (Fig. 6) (Berg B. 2000). However, Hobbie S.E. (1996) stated that differences in rates of litter decomposition were more related to C quality than to nitrogen concentration. Increased C quality could be caused by shifts in vegetation types leading to increased abundance of litter with low lignin content (Berg B. 2000). However, control and +1°C do not differ significantly in abundance of vegetation types in the GN (Table A.26(2)) (Fig. 15). Therefore, it is unlikely that decreased necromass quality was the main reason for the higher stock of necromass at $+1^{\circ}$ C, especially since increased T_S would promote decomposition through direct temperature effects (Aerts R. 2006). Therefore, higher aboveground productivity seems more probable as the main reason of the higher necromass at +1°C in the GN as is supported by several climate change studies (DeMarco et al. 2014; Flanagan L.B & Adkinson A.C. 2011; Dieleman et al. 2012; Walker et al. 2006; Rustad et al. 2001). Additionally, higher turnover rates of N-poor vegetation types (e.g.: monocotyledons and moss; Fig. 16) could partly explain the higher C:N ratio of necromass at $+1^{\circ}$ C.

Unexpectedly, necromass remained stable at different T_S elevations <u>in the GO</u> instead of decreasing due to supposed increased decomposition at higher T_S elevations (Table A.15(3)) (Fig. 9). This suggests that increased productivity (and linked turnover rates; Chapin F.S. 1980; Chapin et al. 1995) at higher T_S elevation, causing higher necromass input,

compensates for the increased decomposition rates at higher T_S elevation. We found no significant difference in <u>total</u> vascular biomass in the GO (including roots) (see § 4.1), suggesting no change in productivity of vascular vegetation. However, significantly higher aboveground vascular biomass at high T_S elevation suggests a positive temperature effect on productivity of vascular plants, which would result in higher turnover rates (Table A.10(3)) (Fig. 2) (Chapin F.S. 1980; Chapin et al. 1995). Moreover, vascular vegetation types have a higher turnover rate than moss (Chapin, et al. 1995). This could result in increased litter input and could possibly compensate for the increased decomposition rates at higher T_S elevations in the GO (+5°C and +10°C) (see § 4.5).

As in biomass, necromass C stocks follow the pattern found in necromass more closely than necromass N stocks (Table A.16(2) and A.16(3)) (Fig. 10). N stocks are lower than expected at $+1^{\circ}$ C in the GN (Table A.17(2)) (Fig. 10), which is in accordance with the lower necromass quality found at $+1^{\circ}$ C (Fig. 6) (see § 4.2).

4.5 T_S effect on soil C and N stocks

As explained in § 1.6, soil processes are likely to determine the balance of in- and outgoing fluxes that result in increased or decreased C and N stocks at the ecosystem level. Soil processes can directly and indirectly mitigate or reinforce global warming by net absorption (sink) or net emission (source) of CO₂, CH₄ and N₂O (Mosier A.R. 1998; Conrad R. 1996). Mechanisms of production and consumption of these C- and N-based greenhouse gases in soil are numerous and are all affected by temperature (Conrad R. 1996; Zaman M. & Chang S.X. 2004; Aerts R. 2006). On top of that, bidirectional relations between soil processes and vegetation processes can induce a positive or negative feedback on soil C and N stocks in response to warming (see § 4.1 to § 4.4) resulting in further mitigation or reinforcement of global warming. Temperature induced changes in soil C and N stocks can indicate whether soils will ultimately act as (C- and N-based) greenhouse gas sinks or sources as a result of the multiple direct and indirect effects of increasing T_S .

The unchanged (+1°C and +3°C) or decreased (+5°C and +10°C) soil C stocks with increasing T_S in the GN suggest that soils do not function as a sink for C-based greenhouse gases after short-term warming in sub-arctic grasslands (Table A.18(2)) (Fig. 11). In contrast, the reduction of soil C stocks at high T_S elevations indicates that soils act as a source for C-based greenhouse gases. As we expected (see § 1.5), soil C stocks (both non-normalized and normalized for depth) decreased significantly at high T_S elevations (+5°C and +10°C) in the GN, suggesting higher soil respiration rates and decomposition rates (Table A.18(2) and

A.19(2)) (Fig. 11). The net decrease of (normalized) soil C stocks is not surprising given that plant productivity increased only little (and only at +1°C) in the GN (see § 4.4) and could not partly compensate for the soil C losses due to accelerated decomposition rates of litter and soil organic matter (in contrast to what we hypothesized in §1.5). Additionally, the presumably stress related strong decrease of root biomass at higher T_S elevations (see § 4.1) will have added to the lower C-inputs through decreasing root exudates and root turnover. The combined decreased productivity and increased decomposition at high T_S elevations in the GN, resulted in a larger risk to lose the increasing fraction of mobile N from the soil by leaching and denitrification (as hypothesized in § 1.5). This hypothesized loss of soil N was confirmed in the GN, where (normalized) soil N stocks decreased significantly at +5°C and +10°C (Table A.20(2) and A.21(2)) (Fig. 12).

Also in the GO, normalized C stocks decreased significantly at higher T_S elevation (Table A.18(3) and A.19(3)) (Fig. 11). This indicates that soil C loss remained higher than soil C input in the long-term, despite the fact that C input increased with T_S due to higher productivity of vascular plants (§ 4.4). Alternatively, C losses may all have occurred during the initial warming phase, with current C losses in equilibrium with current C inputs. In the latter, more likely -yet not testable- case, soil C stocks at high T_S elevations would no longer decrease but remain lower compared to soil stocks at lower T_S elevations due to soil C losses in the past. Several studies found that increased respiration rates due to warming were only transient and decreased again after long-term warming (i.e.: 10 years or longer) resulting in stable soil C stocks regardless of the T_S elevation (Rustad L.E. 2001; Eliasson at al. 2005; Selmants et al. 2014; Luo et al. 2001; Melillo et al. 2002; Sistla et al. 2013). Davidson E.A. & Janssens I.A. (2006) summarised the following three main interpretations for this. One being that soil microbiota acclimate to the higher T_S elevations, returning respiration rates back to "normal" levels comparable to control plots after long-term warming (Bradford et al. 2008; Malcolm et al. 2008). Another mechanism could be that the remaining fraction of soil organic matter contains more physically or chemically stabilized soil C, which is not being decomposed, and is therefore not responsive to changes in temperature. Under these conditions, respiration rates would return back to "normal" once the smaller fraction of labile soil organic matter was depleted. Finally, a variant of this last mechanism is that the larger fraction of recalcitrant soil C would not be completely insensitive to changes in temperature but would decompose much more slowly (Mellilo et al. 2002; Balser T.C. & Wixon D.L.

2009). This would result in increased decomposition rates that remain below detection limits and cannot be measured (Eliasson et al. 2005; Knorr et al. 2005; Kirschbaum M.U.F. 2004).

The fact that significant decreases in normalized soil C stocks did still occur after centuries of soil warming at highest T_S elevations could be interpreted in two ways, based on the review by Davidson E.A. & Janssens I.A. (2006). Firstly, it could be that increased decomposition rates of recalcitrant soil organic matter are only detectable if the T_S increase is high enough (+10°C increases of T_S were thus far not considered in climate change studies, although predictions for the far North are up to +8°C by the end of the century; IPCC 2007). This is further supported by recent studies finding that environmental factors (such as T_S) determine soil organic matter stability more than the molecular structure of soil organic matter (Schmidt et al. 2011). Secondly, it could be that acclimation of soil microbiota may not have occurred yet, even after a time-span of approximately three centuries at high T_S elevations. However, Bradford et al. (2008) found that some microbial communities already show signs of adaptation to climate warming in grasslands after 15 years. Moreover, in a lab incubation experiment, Wei et al. (2014) even observed acclimation of soil microbes after two months of incubation at elevated temperature. Therefore, we regard the second mechanism as unlikely.

We hypothesized in § 1.5 that changes in productivity may affect the N retention capacity of soils due to changes in immobilization of N by the vegetation. This mechanism seemed to be supported by our results. In contrast to the GN, no difference was found between the normalized soil N stocks at different temperatures in the GO (Table A.20(3) and A.21(3)) (Fig. 12). This might be the result of the suggested acclimation of vegetation after centuries of soil warming releasing the stress response of the vegetation (see § 4.1) and resulting in increased productivity at higher T_S elevations (see § 4.4). It is indeed more likely that the more extensive root system at higher T_S elevations in the GO was more efficient in immobilizing more of the increased productivity (possibly combined with increased N fixation by soil bacteria) makes vegetation more able to compensate for increased denitrification at higher T_S elevation after long-term warming.

4.6 T_S effect on ecosystem C and N stocks

As we expected (see § 1.6) changes in soil C and N stocks determined the overall pattern of ecosystem C and N stocks in response to warming (Fig. 13 and 14). However, in the GN, ecosystem C stocks were not significantly lower at highest T_S elevations (Table A.22(2) and A.23(2)) while soil C stocks significantly decreased (see 4.5). This non-significant decrease

on ecosystem level was caused by the C stock from other ecosystem components (i.e.: necromass and biomass) which partly compensated for the soil C losses at high T_S elevations. Normalized ecosystem N stocks, on the other hand, were significantly lower at higher T_S elevations (Table A.25(2)) (Fig.13). Thus the decreased soil N stock could not be compensated for by other ecosystem components.

Lower (normalized) ecosystem C stocks at highest T_S elevations in the GO (Table A.22(3) and A.23(3)) (Fig. 13) shows that increased productivity of vascular plants (see § 4.4) could not compensate for the lower soil C stocks at highest T_S elevations after long-term warming. Mechanisms for this significantly lower soil C stocks at highest T_S elevations are discussed in § 4.5. Our results suggest that the more extensive root system in the GO increases immobilization of N in the soil, which may also explain why there was no significant decrease of ecosystem N stocks in the GO (in contrast to the GN) (Table A.24(3) and A.25(3)) (Fig. 13).

4.7 Validation of assumptions, artefacts and future research

4.7.1 Spring root biomass as proxy for summer root biomass

Our results confirmed that differences in spring root biomass could be used as a proxy for differences in summer root biomass (Annex 6). Season-dependent differences in fine root responses to warming have been shown in a mesic ecosystem. Those were, however, attributed to differential drought effects (Suseela V. & Dukes J.S. 2013). In our systems, drought effects were not likely to have occurred during the growing season (Table A.2(2)). Further, lack of interaction between T_S effect on root biomass and timing of sampling suggests that root biomass in spring and summer is affected by T_S elevation in a similar way (Table A.6(1)).

4.7.2 Location of root biomass

We assumed that most root biomass would be situated in the upper soil layer from 0 to 10 cm depth (Suseela V. & Dukes J.S. 2013; Pucheta et al. 2004; Mezhunts et al., 2005; Fan et al. 2007; Li et al. 2011) and that differences in the upper layers would be representative for differences in total root biomass. This was confirmed in the GN where on average 89.4 ± 3.8 % (n = 24) of summer root biomass was located in the upper 10 cm of the soil (Table A.5) (Fig. A.5). The GO had deeper bedrocks than the GN (Fig. A.6(2)) and the percentage of roots situated in the upper 10 cm would be lower than in the GN (where it often contained 100% of root biomass due to shallow soil depth). Li et al. (2011) found that the upper 10 cm of soil (130 cm deep) contained about 60% of root biomass in alpine meadows, alpine

steppes, desert grasslands and that root biomass decreased exponentially with depth. We assume therefore that the majority of the root biomass in the GO was located in the upper 10 cm as well. Probably even more than was found by Li et al. (2011), assuming that the GO was less exposed to drought stress than the systems of Li X et al. (2011) and felt therefore less pressure to expand the root system in search of water (Chaves et al. 2002).

4.7.3 Correlation between depth and T_s elevation and normalizations for soil depth No correlation between T_s and depth was found nor an interaction between the effect of depth and T_s on C and N stocks (Table A.6(1)) (Fig. A.6(1)). We can therefore assume that we can normalize stocks for soil depth and allow comparison between grasslands without losing information on the T_s effect on C and N stocks. However, we must keep in mind that the soils in the GN are often shallower than 30 cm – the standard measuring depth – and nonnormalized soil stocks therefore represent an approximation of the total availability of N to vegetation. Therefore, the non-normalized stocks should be considered as well when analysing the effect of changes in soil N stocks on plant productivity. Where soils were deeper than 30 cm non-normalized soil N stocks still represent a good approximation of availability to vegetation because most of the root biomass is situated the upper 10 cm of the soil (see § 4.7.2) (Pucheta et al. 2004; Mezhunts et al. 2005; Garcia-Pausas et al. 2010; Li et al., 2011).

4.7.4 Research artefacts and weaknesses

We are aware that our research results may be influenced by some artefacts and weaknesses.

- In contrast to the GN, <u>the GO was grazed</u> during the growing season <u>in previous years</u>, including the year prior to our research. Although grazers were excluded from the research plots by fencing, there could still be negative lag effect from previous grazing on soil C stocks and plant productivity (Martinsen et al. 2011; Fan et al. 2013, Garcia-Pausas et al. 2010; Larcher W. 2004). However, assuming that grazers grazed equally in all plots in previous years, their influence should not have caused the differences observed between T_s elevations in the GO.
- <u>Plots were not separated physically</u> by impenetrable barriers for roots. Therefore, we cannot exclude lateral growth of roots and associated mycorrhizal fungi towards other T_S elevations. However, significant differences between T_S elevations suggest that lateral root growth did not have a large effect on dependent variables.
- Although <u>drought effects</u> were unlikely to have occurred as a result of soil warming (Table A.2(2)), we cannot exclude drought effects on vegetation (Tezara et al. 1999) and

soil microbial processes (Manzoni et al. 2012) until soil moisture content is monitored during the complete growing season.

- <u>Bulk density</u> values of the soil were retrieved from literature (see § 2.3.4.3). Lower BD than assumed (linked to differences in soil fraction with soil particle size > 2 mm) may lead to an overestimation of C and N stock (and vice versa). Additionally temperature might affect BD.
- Due to the loss of a part of the samples, <u>spring root samples were used as proxy</u> for summer roots (see § 2.3.4.1). Although this was justified in § 4.7.1, the difference in highest root biomass between spring (at +1°C) and summer (in control plots) suggests differences between sampling periods. This could be due to an earlier start of the growing season in +1°C compared to control (Leblans et al., unpublished data) which may have led to a higher root biomass in +1°C plots at time of the sampling in spring.
- <u>Approximate T_S data instead of true T_S data</u> might have influenced results. However, Leblans Niki (Unpublished data) found that the real yearly average T_S differences between the plots corresponded closely to the estimated average T_S differences (Fig. A.3(1) and A.3(2)).

4.7.5 *Recommendations for future research*

This research can be considered as a pilot study on short-term and long-term warming effects along natural warming gradients in grasslands. The same experimental setup can be used for further research, provided that additional measurements are conducted. Bulk density of all soil samples should be determined and soil cores over total soil depth should be taken in order to determine soil C and N stocks more accurately. To rule out the role of water stress in the warmed plots, water use efficiency should be monitored by measuring CO₂ and H₂O vapour fluxes as described by Emmerich W.E. (2007). More repeats should be taken of the measurements at all T_S elevation and the true T_S (measured by soil temperature loggers in the plots) should be used as explanatory variable to decrease variation and increase statistical power of the analyses. To determine whether the assumed increased mineralization in warmer plots leads to higher plant available N, Plant Root Simulator (PRS)TM probes (Western AG) could be used as described by Wiederholt R. (2008). Additionally, the vacant holes after removing soil cores could be filled by ingrowth cores to estimate root production (Steingrobe et al. 2000) and standardized litter bags could be buried in the plots to determine the T_S effect on decomposition rates more accurately.

4.8 Conclusion

Our results indicate that plant productivity increases at low T_S elevation (+1°C) after shortterm warming. At higher T_S elevations negative effects occur, presumably due to temperature stress reducing plant productivity and especially root production in the warmed plots (Ferris et al. 1998; Ebrahim et al. 1998; Rachmilevitch et al. 2006; Xu Q. & Huang B. 2000). These results suggest that biomass will not function as a C sink or buffer for the increased C emission from the soil after short-term warming at moderate T_S elevations (+3°C). At highest T_S elevations (+5°C and +10°C) biomass may even function as C source.

Total vegetation N stocks were not significantly different among T_S elevations after shortterm warming, suggesting that plant available N did not decrease at higher T_S elevations, in contrast to the total soil N stocks (including plant available and plant unavailable N) which were significantly lower at high T_S elevations (+5°C and +10°C). This may be due to the much smaller root system at highest T_S elevations, failing to immobilize the increasingly mobile N resulting from increased decomposition rates at highest T_S elevations and consequential leaching.

Decreased soil C stocks at $+5^{\circ}$ C and $+10^{\circ}$ C indicate increased decomposition rates after short-term warming at high T_S elevations. Increased decomposition rates would result in higher respiration rates and thus decreased soil C stocks (Rustad et al. 2001; Pendall et al. 2004; Zaman M. and Chang S.X. 2004; Schindlebacher et al. 2011). This suggests that soils will act as a source for C-based greenhouse gases after short-term warming at high T_S elevations.

In contrast to short-term warming, long-term warming does not seem to negatively affect productivity at high T_S elevations. This suggests that negative temperature effects, as experienced by vascular vegetation at high T_S elevation in the GN, are a transient phenomenon. This could be caused by competition effects resulting in removal of temperature sensitive plants species, or temperature sensitive individuals from certain species from the community (Michielsen L. 2014). Additionally, plants may also have adapted to long-term warming (Rachmilevitch et al. 2006; Stout R.G. & Al-Niemi T.S. 2002; Stout et al. 1997). Further, our results suggest an increased productivity with increasing T_S after long-term warming and confirm that increased aboveground productivity of vascular biomass coincides with decreased productivity of non-vascular biomass, presumably due to a shift from nutrient to light competition (Wilson S.D. and Tilman D. 1993; Lee et al. 2010).

However, the hypothesized increased C-input to the soil resulting from increased aboveground productivity of vascular plants did not seem to be large enough to restore the C

losses that may already have occurred during the initial warming phase. Current C losses from the soil seem in equilibrium with current C inputs if the latter interpretation of decreased soil C stocks after long-term warming would be true.

These results indicate that after long-term warming the warmed plants and microbes are able to maintain the high N stocks and availability, supporting enhanced plant productivity. However, this warming-induced increase in productivity is too small to compensate for the soil C stocks, which strongly declined. This thesis thus supports the hypothesis that global warming will result in large C losses from northern soils that can be expected to feedback positively to global warming.

Ecosystem C and N stocks confirm that changes in soil stocks determine the overall temperature response of the ecosystem both after short-term and long-term warming. This emphasizes the importance of conducting more studies on long-term warming effects on soil processes and the mechanisms behind the temperature response of these soil processes.

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ANNEX 1: EXPERIMENTAL SETUP



Fig. A.1: Schematic representation of the experimental setup in Hveragerði (Iceland). Temperatures indicate the approximate T_s at 10 cm depth above ambient T_s on average. C = control plots (i.e.: ambient T_s).

ANNEX 2: CLIMATIC BACKGROUND DATA (2013)

Table A.2(1): Air temperature data measured in 2013 by the Icelandic Let Office (IMO), the closest
and most comparable synoptic station situated in Eyrabakki. Air temperature was measured at 2 m
above the soil surface.

	Mean monthly air	Mean monthly daily	Maximum air
Month	temperature	max air temperature	temperature reading
	(°C)	(°C)	during month (°C)
January	2.2	4.7	8.5
February	3.8	5.6	8.5
March	0.1	4.7	8.6
April	1.4	6.3	10.0
May	6.0	9.5	13.0
June	9.8	12.4	16.6
July	11.1	14.0	19.0
August	10.4	13.5	19.0
September	6.9	9.7	12.5
October	3.3	6.5	9.8
November	1.7	4.7	8.5
December	-0.5	2.0	8.2

Table A.2(2): Precipitation data measured in 2013 by the Icelandic Met Office (IMO), the closest and most comparable synoptic station situated in Eyrabakki. Precipitation was measured at 1.5 m above the soil surface.

Month	Total monthly precipitation	Maximum daily precipitation
Wonth	(mm)	(mm)
January	178.1	21.5
February	156.2	22.0
March	51.3	28.5
April	76.4	21.0
May	108.5	21.0
June	115.8	14.2
July	157.4	35.0
August	152.5	31.0
September	152.4	30.5
October	108.1	30.0
November	143.1	30.0
December	83.8	15.0





Fig. A.3(1): A: T_s measured by temperature loggers at 10 cm depth in the GN for the year 2013. B: Estimated difference and real difference between ambient T_s (in control plots) and warmed plots in the GN. (Leblans et al., unpublished data)



Fig. A.3(2): A: T_s measured by temperature loggers at 10 cm depth in the GO for the year 2013. B: Estimated difference and real difference between ambient T_s (in control plots) and warmed plots in the GO. (Leblans et al., unpublished data)

ANNEX 4: T_S EFFECT ON ROOT BIOMASS IN SPRING (APRIL) AND SUMMER (JULY)



Fig. A.4: Root biomass in the two sampling periods at different T_s elevations compared to control T_s (i.e.: ambient T_s). Error bars indicate SE. Letters indicate significant differences in root biomass compared at different T_s elevations. Significance codes: p > 0.1: n.s.; p < 0.1: °; p < 0.05: *; p < 0.01: ***; p < 0.001: ***.

Table A.4: two-way ANOVA of main treatment effects and treatment interactions for root biomass. Treatments: T_S (5 levels: control, +1°C, +3°C, +5°C and +10°C); sampling periods (2 levels: April and July).

Treatment	p-value	F-value	df	$R^{2}(\%)$
Necromass C stock				
sampling period	< 0.01	4.69	F(1,39)	27.41
Ts	< 0.01	10.66	F(4,39)	15.58
sampling period x T _s	0.65	0.62	F(4,35)	3.77

ANNEX 5: ROOT BIOMASS DISTRIBUTION (DEPTH PROFILE)



Fig. A.5: Summer root biomass in different soil layers in the GN. Error bars indicate SE.

Table A.5: One-way ANOVA and Tukey multi comparison of means test of soil depth effect	for
summer root biomass in the GN. Treatment: soil depth (6 levels: [0-5], [5-10], [10-15], [15-20],	[20-
25], [25-30] cm). (F(5,59); $R^2 = 48.98\%$; p < 0.001)	

	Comparison	GN			
Dependent variable	temperature	Mean Δ	95% CI		
	elevations	(b – a)	lower bound	Upper bound	p-value
	[0-5] - [5-10]	-4.49	-8.03	-0.94	< 0.01
	[0-5] - [10-15]	-7.00	-12.09	-1.91	< 0.01
	[0-5] - [15-20]	-7.00	-12.09	-1.91	< 0.01
	[0-5] - [20-25]	-12.19	-19.44	-4.93	< 0.001
	[0-5] - [25-30]	-12.19	-19.44	-4.93	< 0.001
Soil depth	[5-10] - [10-15]	-2.52	-7.69	2.65	0.71
Son depth	[5-10] - [15-20]	-2.52	-7.69	2.65	0.71
(Box cox-	[5-10] - [20-25]	-7.70	-15.01	-0.39	< 0.05
transformed)	[5-10] - [25-30]	-7.70	-15.01	-0.39	< 0.05
	[10-15] - [15-20]	0.00	-6.33	6.33	1.00
	[10-15] - [20-25]	-5.18	-13.36	3.00	0.43
	[10-15] - [25-30]	-5.18	-13.36	3.00	0.43
	[15-20] - [20-25]	-5.18	-13.36	3.00	0.43
	[15-20] - [25-30]	-5.18	-13.36	3.00	0.43
	[20-25] - [25-30]	1.42e-14	-9.68	9.68	1.00

ANNEX 6: DEPTH EFFECT ON SOIL C AND N STOCKS AND T_{S} EFFECT ON SOIL DEPTH



Fig. A.6(1): **A**: Soil C stock in function of depth at different T_s elevation. **B**: Soil N stock in function of depth at different T_s elevations.

Table A.6(1): ANCOVA of main treatment effects and treatment interactions for soil C stock. Treatments: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C); depth (continuous explanatory variable).

Treatment	p-value	F-value	df	$R^{2}(\%)$
Soil C stock				
depth	< 0.001	39.39	F(1,44)	36.12
T _s	< 0.001	6.42	F(4,44)	23.53
depth x T _s	0.45	0.93	F(4,40)	3.45



Fig. A.6(2): Soil depth in the two grasslands at different T_s elevations compared to control T_s (i.e.: ambient T_s). Error bars indicate SE. Letters indicate significant differences in soil depth compared at different T_s elevations within grasslands separately. Significance codes: p > 0.1: n.s.; p < 0.1: °; p < 0.05: *; p < 0.01: **; p < 0.001: ***; n.a.: not assessable (to our knowledge, there is no non-parametric alternative for two-way ANOVAs in RStudio; see § 2.5).

Table A.6(2): One-way ANOVA and Tukey multi comparison of means test of T_s effect for soil depth in the GN. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C). (F(4,20); $R^2 = 10.69\%$; p = 0.67)

	Comparison	GN				
Dependent variable	temperature	Mean Δ	95% CI		p-value	
	(b – a)	lower bound	Upper bound			
	Control - +1°C	-0.43	-1.40	0.53	0.67	
	Control - +3°C	-0.18	-1.14	0.79	0.98	
	Control - $+5^{\circ}C$	-0.32	-1.28	0.65	0.86	
Soil depth	Control - +10°C	-0.07	-1.04	0.89	1.00	
1	+1°C - +3°C	0.26	-0.71	1.22	0.93	
(Log-transformed)	$+1^{\circ}C - +5^{\circ}C$	0.12	-0.85	1.08	1.00	
	$+1^{\circ}C - +10^{\circ}C$	0.36	-0.61	1.33	0.80	
	$+3^{\circ}C - +5^{\circ}C$	-0.14	-1.11	0.83	0.99	
	+3°C - +10°C	0.10	-0.86	1.07	1.00	
	$+5^{\circ}C - +10^{\circ}C$	0.24	-0.72	1.21	0.94	

Table A.6(3): Kruskal-Wallis chi-squared test and Multiple comparison test after Kruskal-Wallis of T_s effect for soil depth in the GO. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C). (F(4,20); $R^2 = 10.69\%$; p = 0.88)

	Comparison	GO			
Dependent variable	temperature elevations	Mean Δ	95% CI		
		(b – a)	lower bound	Upper bound	p-value
	Control - +1°C	0.60	NA	NA	> 0.10
	Control - +3°C	2.50	NA	NA	> 0.10
	Control - $+5^{\circ}C$	1.70	NA	NA	> 0.10
Soil depth	Control - +10°C	1.60	NA	NA	> 0.10
1	+1°C - +3°C	1.90	NA	NA	> 0.10
(Non-parametric)	$+1^{\circ}C - +5^{\circ}C$	2.30	NA	NA	> 0.10
	$+1^{\circ}C - +10^{\circ}C$	1.00	NA	NA	> 0.10
	$+3^{\circ}C - +5^{\circ}C$	4.20	NA	NA	> 0.10
	$+3^{\circ}C - +10^{\circ}C$	0.90	NA	NA	> 0.10
	$+5^{\circ}C - +10^{\circ}C$	3.30	NA	NA	> 0.10

ANNEX 7: SAMPLING DEPTH OF ROOTS IN THE GN AND THE GO



Fig. A.3: Percentage (%) of spring root samples until 5 cm depth and until 10 cm depth in the GN and the GO.

ANNEX 8: TOTAL ABOVEGROUND BIOMASS



Fig. A.8: Aboveground biomass in the two grasslands at different T_s elevations compared to control T_s (i.e.: ambient T_s). Error bars indicate SE. Letters indicate significant differences in aboveground biomass compared at different T_s elevations within grasslands separately. Significance codes: p > 0.1: n.s.; p < 0.1: °; p < 0.05: *; p < 0.01: **; p < 0.001: ***.

Table A.8(1): two-way ANOVA of main treatment effects and treatment interactions for aboveground biomass. Treatments: T_S (5 levels: control, +1°C, +3°C, +5°C and +10°C); grassland (2 levels: GN and GO).

Treatment	p-value	F-value	df	R ² (%)
Soil C stock				
Ts	0.900	0.26	F(4,39)	2.86
grassland	0.915	0.01	F(1,39)	0.03
T _s x grassland	0.880	0.30	F(4,39)	2.86

Table A.8(2): One-way ANOVA and Tukey multi comparison of means test of T_s effect for aboveground biomass in the GN. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

Dependent variable	Comparison temperature elevations	GN				
		$Mean \Delta (b-a)$	95% CI		n valuo	
			lower bound	Upper bound	p-value	
Aboveground biomass	Control - +1°C	9.14	-217.83	236.12	1.00	
	Control - +3°C	44.80	-182.18	271.77	0.97	
	Control - $+5^{\circ}C$	-36.71	-277.46	204.04	0.99	
	Control - +10°C	15.67	-211.31	242.64	1.00	
	+1°C - +3°C	35.65	-191.33	262.63	0.99	
	$+1^{\circ}C - +5^{\circ}C$	-45.85	-286.60	194.89	0.98	
	$+1^{\circ}C - +10^{\circ}C$	6.52	-220.46	233.50	1.00	
	$+3^{\circ}C - +5^{\circ}C$	-81.51	-322.25	159.24	0.84	
	$+3^{\circ}C - +10^{\circ}C$	-29.13	-256.11	197.85	0.99	
	$+5^{\circ}C - +10^{\circ}C$	52.38	-188.37	293.12	0.96	

Dependent variable	Comparison temperature elevations	GO				
		$\begin{array}{l} \text{Mean } \Delta \\ (b-a) \end{array}$	95% CI		n velue	
			lower bound	Upper bound	p-value	
Aboveground biomass (box cox- transformed)	Control - +1°C	4.14e-06	-6.95e-06	1.52e-05	0.80	
	Control - +3°C	2.01e-06	-9.08e-06	1.31e-05	0.98	
	Control - +5°C	1.58e-06	-9.51e-06	1.27e-05	0.99	
	Control - +10°C	-1.04e-06	-1.21e-05	1.01e-05	1.00	
	+1°C - +3°C	-2.13e-06	-1.32e-05	8.96e-06	0.98	
	$+1^{\circ}C - +5^{\circ}C$	-2.56e-06	-1.36e-05	8.53e-06	0.96	
	$+1^{\circ}C - +10^{\circ}C$	-5.17e-06	-1.63e-05	5.91e-06	0.64	
	$+3^{\circ}C - +5^{\circ}C$	-4.32e-07	-1.15e-05	1.07e-05	1.00	
	$+3^{\circ}C - +10^{\circ}C$	-3.05e-06	-1.41e-05	8.04e-06	0.92	
	+5°C - +10°C	-2.62e-06	-1.37e-05	8.47e-06	0.95	

Table A.8(3): One-way ANOVA and Tukey multi comparison of means test of T_s effect for aboveground biomass in the GO. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).


ANNEX 9: FIGURES: BIOMASS C STOCKS

Fig. A.9(1): C stocks of biomass in the two grasslands at different T_s elevations compared to control T_s (i.e.: ambient T_s). A: total vascular vegetation; B: aboveground vascular vegetation; C: roots; D: non-vascular vegetation. Error bars indicate SE. Letters indicate significant differences in C stocks of biomass compared at different T_s elevations within grasslands separately. Significance codes: p > 0.1: n.s.; p < 0.1: °; p < 0.05: *; p < 0.01: **; p < 0.001: ***.



Fig. A.9(1): C stocks of total biomass in the two grasslands at different T_s elevations compared to control TS (i.e.: ambient T_s). Error bars indicate SE. Letters indicate significant differences in C stocks of total biomass compared at different T_s elevations within grasslands separately. Significance codes: p > 0.1: n.s.; p < 0.1: °; p < 0.05: *; p < 0.01: **; p < 0.001: ***.

ANNEX 10: STATISTICAL RESULTS: BIOMASS

Table A.10(1): Two-way ANOVA of main treatment effects and treatment interactions for total vascular biomass, root biomass, aboveground vascular biomass, non-vascular biomass and total biomass. Treatments: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C); Grassland (2 levels: GN and GO).

Treatment	p-value	F-value	df	$R^{2}(\%)$
Total vascular biomass				
Ts	< 0.05	3.20	F(4,39)	11.82
Grassland	< 0.001	56.55	F(1,39)	52.18
T _s x grassland	0.90	0.26	F(4,35)	1.57
Root biomass				
Ts	< 0.01	4.80	F(4,39)	17.29
Grassland	< 0.001	52.82	F(1,39)	47.58
T _s x grassland	0.96	0.14	F(4,35)	0.57
Above-ground vascular				
biomass				
Ts	< 0.10	2.25	F(4,43)	15.04
Grassland	< 0.01	7.77	F(1,43)	13.00
T _s x grassland	0.26	1.37	F(4,39)	8.86
Non-vascular biomass				
Ts	0.58	0.72	F(4,40)	5.99
Grassland	0.11	2.63	F(1,40)	5.45
T _s x grassland	0.61	0.67	F(4,40)	5.60
Total biomass				
Ts	< 0.001	5.98	F(4,39)	21.02
Grassland	< 0.001	50.90	F(1,39)	44.72
T _s x grassland	0.93	0.20	F(4,39)	0.78

Table A.10(2): One-way ANOVA and Tukey multi comparison of means test of T_s effect for total vascular biomass, root biomass, aboveground vascular biomass, non-vascular biomass and total biomass in the GN. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GN				
Dependent variable	temperature	Mean Δ	95%	5 CI		
	elevations	(b – a)	lower bound	Upper bound	р	
	Control - +1°C	284.91	-482.13	1051.94	0.78	
	Control - +3°C	-3.41	-797.37	790.55	1.00	
	Control - +5°C	-162.91	-956.87	631.05	0.97	
	Control - +10°C	-332.19	-1099.23	434.85	0.67	
Total vascular	$+1^{\circ}C - +3^{\circ}C$	-288.31	-903.31	326.69	0.61	
biomass	$+1^{\circ}C - +5^{\circ}C$	-447.82	-1062.82	167.18	0.22	
	$+1^{\circ}C - +10^{\circ}C$	-617.10	-1196.92	-37.27	< 0.05	
	$+3^{\circ}C - +5^{\circ}C$	-159.51	-807.77	488.76	0.94	
	+3°C - +10°C	-328.79	-943.79	286.21	0.49	
	$+5^{\circ}C - +10^{\circ}C$	-169.28	-784.28	445.72	0.91	
D (1)	Control - $+1^{\circ}C$	243.89	-484.44	972.22	0.84	
	Control - $+3^{\circ}C$	5.07	-748.83	758.96	1.00	
ROOT DIOMASS	Control - +5°C	-220.05	-973.94	533.84	0.89	
	Control - +10°C	-392.34	-1120.67	335.99	0.48	

	$+1^{\circ}C - +3^{\circ}C$	-238.82	-822.79	345.14	0.72
	+1°C - +5°C	-463.94	-1047.90	120.02	0.15
	$+1^{\circ}C - +10^{\circ}C$	-636.22	-1186.79	-85.66	< 0.05
	$+3^{\circ}C - +5^{\circ}C$	-225.12	-840.67	390.43	0.79
	$+3^{\circ}C - +10^{\circ}C$	-397.41	-981.37	186.56	0.27
	$+5^{\circ}C - +10^{\circ}C$	-172.29	-756.25	411.68	0.89
	Control - +1°C	1.07	-2.23	2.53	1.00
	Control - +3°C	-1.18	-2.80	2.01	0.98
Above-ground	Control - $+5^{\circ}C$	1.27	-1.97	3.17	0.93
vascular biomass	Control - +10°C	1.14	-2.09	2.69	0.99
(Log transformed)	+1°C - +3°C	-1.26	-2.98	1.89	0.93
(Log-transformed)	+1°C - +5°C	1.19	-2.10	2.98	0.98
	+1°C - +10°C	1.06	-2.23	2.53	1.00
	$+3^{\circ}C - +5^{\circ}C$	1.49	-1.67	3.74	0.68
	+3°C - +10°C	1.34	-1.77	3.17	0.85
	$+5^{\circ}C - +10^{\circ}C$	-1.12	-2.79	2.24	1.00
	Control - +1°C	-3.03	-301.45	295.40	1.00
	Control - +3°C	51.82	-246.61	350.25	0.98
	Control - $+5^{\circ}C$	3.06	-295.37	301.49	1.00
	Control - +10°C	-15.64	-314.06	282.79	1.00
Non veccular	$+1^{\circ}C - +3^{\circ}C$	54.84	-243.58	353.27	0.98
Inon-vascular	$+1^{\circ}C - +5^{\circ}C$	6.08	-292.34	304.51	1.00
	$+1^{\circ}C - +10^{\circ}C$	-12.61	-311.04	285.82	1.00
	$+3^{\circ}C - +5^{\circ}C$	-48.76	-347.19	249.67	0.99
	+3°C - +10°C	-67.45	-365.88	230.97	0.96
	$+5^{\circ}C - +10^{\circ}C$	-18.69	-317.12	279.73	1.00
	Control - $+1^{\circ}C$	105.32	-609.60	820.24	0.99
	Control - +3°C	-103.17	-843.18	636.84	0.99
	Control - $+5^{\circ}C$	-404.48	-1144.49	335.53	0.47
	Control - +10°C	-524.39	-1239.31	190.53	0.21
Total vagatation	+1°C - +3°C	-208.49	-781.70	364.72	0.79
Total vegetation	$+1^{\circ}C - +5^{\circ}C$	-509.80	-1083.01	63.41	< 0.10
	$+1^{\circ}C - +10^{\circ}C$	-629.71	-1170.14	-89.28	< 0.05
	$+3^{\circ}C - +5^{\circ}C$	-301.31	-905.53	302.91	0.55
	+3°C - +10°C	-421.22	-994.43	151.99	0.21
	$+5^{\circ}C - +10^{\circ}C$	-119.91	-693.12	453.30	0.96

Table A.10(3): One-way ANOVA and Tukey multi comparison of means test of T_s effect for total vascular biomass, root biomass, aboveground vascular biomass, non-vascular biomass and total biomass in the GN. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GO					
Dependent variable	temperature	Mean Δ	95% CI				
	elevations	(b – a)	lower bound	Upper bound	р		
	Control - +1°C	136.46	-564.19	837.11	0.98		
	Control - +3°C	29.23	-671.42	729.88	1.00		
Total vascular	Control - +5°C	-113.39	-814.04	587.26	0.99		
biomass	Control - +10°C	-178.68	-879.33	521.97	0.94		
	+1°C - +3°C	-107.23	-807.88	593.42	0.99		
	$+1^{\circ}C - +5^{\circ}C$	-249.85	-950.50	450.80	0.82		

	$+1^{\circ}C - +10^{\circ}C$	-315.14	-1015.79	385.51	0.67
	+3°C - +5°C	-142.62	-843.27	558.03	0.97
	+3°C - +10°C	-207.91	-908.56	492.74	0.90
	+5°C - +10°C	-65.29	-765.94	635.36	1.00
	Control - +1°C	154.48	-514.10	823.07	0.96
	Control - +3°C	-7.51	-676.10	661.07	1.00
	Control - +5°C	-122.19	-790.78	546.39	0.98
	Control - +10°C	-295.77	-964.35	372.82	0.68
D 11	+1°C - +3°C	-162.00	-830.58	506.59	0.95
Root biomass	+1°C - +5°C	-276.68	-945.26	391.91	0.73
	+1°C - +10°C	-450.25	-1118.84	218.33	0.29
	+3°C - +5°C	-114.68	-783.26	553.91	0.99
	+3°C - +10°C	-288.26	-956.84	380.33	0.70
	+5°C - +10°C	-173.58	-842.16	495.01	0.93
	Control - +1°C	-1.01	-1.97	1.94	1.00
	Control - +3°C	1.38	-1.42	2.70	0.61
	Control - +5°C	1.19	-1.65	2.32	0.94
Above-ground	Control - +10°C	2.00	-1.02	3.92	0.04
vascular biomass	+1°C - +3°C	1.39	-1.41	2.72	0.59
(Log transformed)	+1°C - +5°C	1.19	-1.64	2.34	0.93
(Log-transformed)	+1°C - +10°C	2.02	-1.03	3.95	< 0.05
	+3°C - +5°C	-1.16	-2.28	1.68	0.96
	+3°C - +10°C	1.45	-1.35	2.84	0.48
	$+5^{\circ}C - +10^{\circ}C$	1.69	-1.16	3.31	0.17
	Control - +1°C	0.37	-0.86	1.60	0.89
	Control - +3°C	0.02	-1.20	1.25	1.00
	Control - +5°C	0.08	-1.15	1.31	1.00
Non-vascular	Control - +10°C	-0.85	-2.08	0.37	0.27
biomass	+1°C - +3°C	-0.35	-1.58	0.88	0.91
(Log_transformed)	$+1^{\circ}C - +5^{\circ}C$	-0.29	-1.52	0.94	0.95
(Log transformed)	$+1^{\circ}C - +10^{\circ}C$	-1.23	-2.46	0.00	< 0.05
	$+3^{\circ}C - +5^{\circ}C$	0.06	-1.17	1.28	1.00
	$+3^{\circ}C - +10^{\circ}C$	-0.88	-2.11	0.35	0.24
	$+5^{\circ}C - +10^{\circ}C$	-0.93	-2.16	0.29	0.19
	Control - +1°C	164.08	-487.45	815.62	0.94
	Control - +3°C	-45.89	-697.42	605.64	1.00
	Control - $+5^{\circ}C$	-156.81	-808.34	494.72	0.95
	Control - +10°C	-348.97	-1000.50	302.57	0.51
Total biomass	$+1^{\circ}C - +3^{\circ}C$	-209.98	-861.51	441.56	0.87
I otal biomass	$+1^{\circ}C - +5^{\circ}C$	-320.90	-972.43	330.64	0.59
	$+1^{\circ}C - +10^{\circ}C$	-513.05	-1164.58	138.48	0.17
	$+3^{\circ}C - +5^{\circ}C$	-110.92	-762.45	540.61	0.99
	$+3^{\circ}C - +10^{\circ}C$	-303.07	-954.61	348.46	0.64
	$+5^{\circ}C - +10^{\circ}C$	-192.15	-843.69	459.38	0.90

ANNEX 11: STATISTICAL RESULTS: N CONCENTRATION

Table A.11(1): Two-way ANOVA of main treatment effects and treatment interactions for aboveground monocotyledon N percentage, aboveground dicotyledon N percentage, root N percentage, moss N percentage. Treatments: T_S (5 levels: control, +1°C, +3°C, +5°C and +10°C); Grassland (2 levels: GN and GO). NA = not assessable (only one replicate of root N percentage at each T_S elevation; see § 2.3.4.2).

Treatment	p-value	F-value	df	$R^{2}(\%)$
Aboveground				
monocotyledon N %				
Ts	0.67	0.59	F(4,39)	5.20
Grassland	0.36	0.87	F(1,39)	1.93
T _s x grassland	0.53	0.80	F(4,39)	7.08
Aboveground				
dicotyledon N %				
Ts	< 0.001	8.90	F(4,28)	30.15
Grassland	< 0.001	32.82	F(1,28)	27.79
T _s x grassland	< 0.01	5.42	F(4,28)	18.35
Root N %				
Ts				
Grassland	NA	NA	NA	NA
T _s x grassland	NA	NA	NA	NA
	NA	NA	NA	NA
Moss N %				
Ts	< 0.05	3.59	F(4,44)	24.62
Grassland	0.17	1.96	F(1,43)	3.29
T _s x grassland	0.50	0.86	F(4,39)	5.86

Table A.11(2): One-way ANOVA and Tukey multi comparison of means test of T_S effect for aboveground monocotyledon N percentage, aboveground dicotyledon N percentage, root N percentage, moss N percentage in the GN. Treatment: T_S (5 levels: control, +1°C, +3°C, +5°C and +10°C). NA = not assessable (only one replicate of root N percentage at each T_S elevation; see § 2.3.4.2).

		GN				
Dependent variable	Comparison	Mean Δ	95% CI			
		(b – a)	lower bound	Upper bound	p-value	
	Control - +1°C	-0.24	-1.05	0.58	0.91	
	Control - +3°C	-0.17	-0.98	0.65	0.97	
	Control - +5°C	-0.37	-1.24	0.50	0.71	
Aboveground	Control - +10°C	-0.32	-1.14	0.50	0.76	
monocotyledon	+1°C - +3°C	0.07	-0.75	0.89	1.00	
N %	$+1^{\circ}C - +5^{\circ}C$	-0.13	-1.00	0.74	0.99	
	$+1^{\circ}C - +10^{\circ}C$	-0.09	-0.90	0.73	1.00	
	+3°C - +5°C	-0.20	-1.07	0.67	0.95	
	+3°C - +10°C	-0.16	-0.98	0.66	0.98	
	$+5^{\circ}C - +10^{\circ}C$	0.05	-0.82	0.91	1.00	
Aboveground	Control - +1°C	-1.70	-2.81	-0.59	< 0.001	
dicotyledon N %	Control - +3°C	-1.37	-2.58	-0.17	< 0.05	
	Control - +5°C	-1.65	-2.86	-0.45	< 0.01	

	Control - +10°C	-1.64	-2.84	-0.43	< 0.01
	+1°C - +3°C	0.33	-0.93	1.59	1.00
	+1°C - +5°C	0.04	-1.22	1.30	1.00
	+1°C - +10°C	0.06	-1.20	1.32	1.00
	+3°C - +5°C	-0.28	-1.63	1.06	1.00
	+3°C - +10°C	-0.27	-1.62	1.08	1.00
	$+5^{\circ}C - +10^{\circ}C$	0.02	-1.33	1.36	1.00
	Control - +1°C	NA	NA	NA	NA
	Control - $+3^{\circ}C$	NA	NA	NA	NA
	Control - +5°C	NA	NA	NA	NA
	Control - +10°C	NA	NA	NA	NA
Root N %	+1°C - +3°C	NA	NA	NA	NA
	$+1^{\circ}C - +5^{\circ}C$	NA	NA	NA	NA
	+1°C - +10°C	NA	NA	NA	NA
	+3°C - +5°C	NA	NA	NA	NA
	+3°C - +10°C	NA	NA	NA	NA
	+5°C - +10°C	NA	NA	NA	NA
	Control - +1°C	-0.15	-0.46	0.15	0.57
	Control - $+3^{\circ}C$	-0.04	-0.35	0.27	0.99
	Control - $+5^{\circ}C$	0.04	-0.27	0.34	1.00
	Control - +10°C	0.18	-0.13	0.49	0.42
Moss N %	+1°C - +3°C	0.11	-0.19	0.42	0.80
	$+1^{\circ}C - +5^{\circ}C$	0.19	-0.12	0.50	0.38
	$+1^{\circ}C - +10^{\circ}C$	0.34	0.03	0.65	< 0.05
	$+3^{\circ}C - +5^{\circ}C$	0.08	-0.23	0.39	0.94
	$+3^{\circ}C - +10^{\circ}C$	0.22	-0.08	0.53	0.23
	$+5^{\circ}C - +10^{\circ}C$	0.15	-0.16	0.46	0.62

Table A.11(3): One-way ANOVA and Tukey multi comparison of means test of T_S effect for aboveground monocotyledon N percentage, aboveground dicotyledon N percentage, root N percentage, moss N percentage in the GO. Treatment: T_S (5 levels: control, +1°C, +3°C, +5°C and +10°C). NA = not assessable (only one replicate of root N percentage at each T_S elevation; see § 2.3.4.2).

	Comparison		GO				
Dependent variable	temperature	Mean Δ	95% CI		1		
	elevations	(b – a)	lower bound	Upper bound	p-value		
	Control - $+1^{\circ}C$	-0.02	-0.37	0.33	1.00		
	Control - +3°C	0.04	-0.31	0.40	1.00		
	Control - +5°C	-0.08	-0.44	0.27	0.96		
Aboveground	Control - +10°C	0.20	-0.15	0.55	0.46		
monocotyledon	+1°C - +3°C	0.06	-0.29	0.41	0.99		
N %	+1°C - +5°C	-0.06	-0.42	0.29	0.98		
	$+1^{\circ}C - +10^{\circ}C$	0.22	-0.14	0.57	0.37		
	+3°C - +5°C	-0.12	-0.48	0.23	0.84		
	+3°C - +10°C	0.16	-0.20	0.51	0.67		
	+5°C - +10°C	0.28	-0.07	0.63	0.16		
Aboveground	Control - +1°C	0.04	-0.84	0.92	1.00		
dicotyledon N %	Control - +3°C	0.00	-0.88	0.88	1.00		
	Control - +5°C	-0.19	-1.13	0.74	0.93		

	+1°C - +3°C	-0.04	-0.92	0.84	1.00
	$+1^{\circ}C - +5^{\circ}C$	-0.23	-1.17	0.70	0.89
	$+3^{\circ}C - +5^{\circ}C$	-0.19	-1.13	0.74	0.93
	Control - +1°C	NA	NA	NA	NA
	Control - +3°C	NA	NA	NA	NA
	Control - +5°C	NA	NA	NA	NA
	Control - +10°C	NA	NA	NA	NA
Root N %	$+1^{\circ}C - +3^{\circ}C$	NA	NA	NA	NA
	$+1^{\circ}C - +5^{\circ}C$	NA	NA	NA	NA
	+1°C - +10°C	NA	NA	NA	NA
	$+3^{\circ}C - +5^{\circ}C$	NA	NA	NA	NA
	$+3^{\circ}C - +10^{\circ}C$	NA	NA	NA	NA
	$+5^{\circ}C - +10^{\circ}C$	NA	NA	NA	NA
	Control - +1°C	0.11	-0.38	0.60	0.96
	Control - +3°C	0.09	-0.40	0.58	0.98
	Control - +5°C	0.06	-0.43	0.56	0.99
	Control - +10°C	0.46	-0.06	0.98	0.10
Moss N %	$+1^{\circ}C - +3^{\circ}C$	-0.02	-0.51	0.47	1.00
	$+1^{\circ}C - +5^{\circ}C$	-0.04	-0.54	0.45	1.00
	$+1^{\circ}C - +10^{\circ}C$	0.35	-0.17	0.87	0.30
	$+3^{\circ}C - +5^{\circ}C$	-0.02	-0.51	0.47	1.00
	+3°C - +10°C	0.37	-0.15	0.89	0.25
	$+5^{\circ}C - +10^{\circ}C$	0.39	-0.13	0.91	0.20

Table A.12(1): Two-way ANOVA of main treatment effects and treatment interactions for total vegetation C:N ratio and necromass C:N ratio. Treatments: T_S (5 levels: control, +1°C, +3°C, +5°C and +10°C); Grassland (2 levels: GN and GO).

Treatment	p-value	F-value	df	$R^{2}(\%)$
Total vegetation C:N				
ratio				
Ts	< 0.10	2.25	F(4,32)	17.21
Grassland	0.83	0.05	F(1,32)	0.08
T _s x grassland	< 0.05	2.83	F(4,32)	21.61
Necromass C:N ratio				
Ts	< 0.001	9.52	F(4,40)	37.36
Grassland	0.52	0.42	F(1,40)	0.41
T _s x grassland	< 0.001	5.85	F(4,40)	22.98

Table A.12(2): One-way ANOVA and Tukey multi comparison of means test of T_S effect for total vegetation C:N ratio and necromass C:N ratio in the GN. Treatment: T_S (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GN				
Dependent variable	temperature	Mean Δ	95%	5 CI	1	
	elevations	(b – a)	lower bound	Upper bound	p-value	
	Control - +1°C	-1.77	-19.16	15.62	1.00	
	Control - +3°C	-7.15	-25.16	10.85	0.74	
	Control - $+5^{\circ}C$	-11.65	-29.65	6.36	0.31	
	Control - +10°C	-9.94	-27.33	7.45	0.43	
Total vegetation	$+1^{\circ}C - +3^{\circ}C$	-5.38	-19.33	8.56	0.76	
C:N ratio	$+1^{\circ}C - +5^{\circ}C$	-9.88	-23.82	4.07	0.24	
	$+1^{\circ}C - +10^{\circ}C$	-8.17	-21.32	4.98	0.35	
	$+3^{\circ}C - +5^{\circ}C$	-4.49	-19.19	10.21	0.88	
	$+3^{\circ}C - +10^{\circ}C$	-2.79	-16.73	11.16	0.97	
	$+5^{\circ}C - +10^{\circ}C$	1.71	-12.24	15.65	1.00	
	Control - +1°C	19.72	6.79	32.65	< 0.01	
	Control - +3°C	3.37	-9.56	16.30	0.93	
	Control - +5°C	-1.80	-14.74	11.12	0.99	
	Control - +10°C	-5.37	-18.30	7.56	0.73	
Necromass	$+1^{\circ}C - +3^{\circ}C$	-16.35	-29.28	-3.42	< 0.01	
C:N ratio	$+1^{\circ}C - +5^{\circ}C$	-21.53	-34.46	-8.60	< 0.001	
	$+1^{\circ}C - +10^{\circ}C$	-25.09	-38.02	-12.16	< 0.001	
	$+3^{\circ}C - +5^{\circ}C$	-5.17	-18.10	7.76	0.75	
	$+3^{\circ}C - +10^{\circ}C$	-8.74	-21.67	4.19	0.29	
	$+5^{\circ}C - +10^{\circ}C$	-3.56	-16.49	9.37	0.92	

Table A.12(3): One-way ANOVA and Tukey multi comparison of means test of T_s effect for total vegetation C:N ratio and necromass C:N ratio in the GO. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GO				
Dependent variable	temperature	Mean Δ	95%	5 CI	1	
	elevations	(b – a)	lower bound	Upper bound	p-value	
	Control - +1°C	-0.72	-5.91	4.48	0.99	
	Control - +3°C	-1.05	-6.25	4.14	0.97	
	Control - +5°C	3.59	-1.92	9.10	0.32	
	Control - +10°C	-5.27	-11.27	0.72	0.10	
Total vegetation	$+1^{\circ}C - +3^{\circ}C$	-0.34	-5.53	4.86	1.00	
C:N ratio	$+1^{\circ}C - +5^{\circ}C$	4.31	-1.20	9.82	0.17	
	$+1^{\circ}C - +10^{\circ}C$	-4.56	-10.56	1.44	0.19	
	$+3^{\circ}C - +5^{\circ}C$	4.64	-0.87	10.15	0.12	
	+3°C - +10°C	-4.22	-10.22	1.78	0.25	
	$+5^{\circ}C - +10^{\circ}C$	-8.86	-15.14	-2.59	< 0.01	
	Control - $+1^{\circ}C$	-0.41	-11.30	10.49	1.00	
	Control - +3°C	2.00	-8.90	12.89	0.98	
	Control - $+5^{\circ}C$	3.72	-7.18	14.62	0.84	
	Control - +10°C	-9.75	-20.65	1.15	< 0.10	
Necromass	$+1^{\circ}C - +3^{\circ}C$	2.40	-8.50	13.30	0.96	
C:N ratio	$+1^{\circ}C - +5^{\circ}C$	4.12	-6.77	15.02	0.79	
	+1°C - +10°C	-9.34	-20.24	1.55	0.12	
	$+3^{\circ}C - +5^{\circ}C$	1.72	-9.17	12.62	0.99	
	+3°C - +10°C	-11.75	-22.64	-0.85	< 0.05	
	$+5^{\circ}C - +10^{\circ}C$	-13.47	-24.36	-2.57	< 0.05	

ANNEX 13: STATISTICAL RESULTS: BIOMASS C STOCKS

Table A.13(1): Two-way ANOVA of main treatment effects and treatment interactions for total vascular C stock, root C stock, aboveground vascular C stock, non-vascular C stock and total vegetation C stock. Treatments: T_S (5 levels: control, +1°C, +3°C, +5°C and +10°C); Grassland (2 levels: GN and GO).

Treatment	p-value	F-value	df	$R^{2}(\%)$
Total vascular C stock				
Ts	< 0.05	3.48	F(4,37)	13.50
Grassland	< 0.001	52.13	F(1,37)	50.60
T _s x grassland	0.76	0.47	F(4,33)	1.93
Root C stock				
Ts	< 0.01	4.04	F(4,39)	14.58
Grassland	< 0.001	55.54	F(1,39)	50.18
T _s x grassland	0.75	0.48	F(4,35)	1.85
Aboveground vascular C				
stock				
Ts	0.27	1.33	F(4,41)	9.72
Grassland	< 0.01	8.56	F(1,45)	15.59
T _s x grassland	0.22	1.52	F(4,37)	10.54
Non-vascular C stock				
T _s	0.82	0.36	F(4,43)	3.07
Grassland	< 0.05	4.57	F(1,47)	8.87
T _s x grassland	0.66	0.60	F(4,39)	5.12
Total vegetation				
C stock				
T _s	< 0.001	5.86	F(4,36)	22.88
Grassland	< 0.001	43.08	F(1,36)	42.01
T _s x grassland	0.83	0.37	F(4,32)	1.56

Table A.13(2): One-way ANOVA and Tukey multi comparison of means test of T_S effect for total vascular C stock, root C stock, aboveground vascular C stock, non-vascular C stock and total vegetation C stock in the GN. Treatment: T_S (5 levels: control, $+1^{\circ}C$, $+3^{\circ}C$, $+5^{\circ}C$ and $+10^{\circ}C$).

	Comparison	GN				
Dependent variable	temperature	Mean Δ	95%	5 CI	n valua	
	elevations	(b – a)	lower bound	Upper bound	p-value	
	Control - +1°C	132.33	-161.53	426.20	0.64	
	Control - +3°C	4.30	-299.88	308.48	1.00	
	Control - +5°C	-61.49	-365.67	242.68	0.97	
Total vascular	Control - +10°C	-108.39	-402.26	185.47	0.78	
C stock	$+1^{\circ}C - +3^{\circ}C$	-128.03	-363.65	107.58	0.47	
	+1°C - +5°C	-193.83	-429.44	41.79	0.13	
	$+1^{\circ}C - +10^{\circ}C$	-240.73	-462.87	-18.59	< 0.05	
	$+3^{\circ}C - +5^{\circ}C$	-65.79	-314.15	182.57	0.92	
	+3°C - +10°C	-112.69	-348.31	122.92	0.59	
	$+5^{\circ}C - +10^{\circ}C$	-46.90	-282.52	188.71	0.97	
	Control - +1°C	116.17	-163.20	395.55	0.70	
Root C stock	Control - +3°C	7.78	-281.40	296.96	1.00	
	Control - +5°C	-84.38	-373.56	204.79	0.89	
	Control - +10°C	-132.92	-412.29	146.45	0.60	

	$+1^{\circ}C - +3^{\circ}C$	-108.40	-332.39	115.60	0.58
	+1°C - +5°C	-200.56	-424.55	23.44	< 0.10
	+1°C - +10°C	-249.10	-460.28	-37.91	< 0.05
	+3°C - +5°C	-92.16	-328.27	143.95	0.75
	+3°C - +10°C	-140.70	-364.70	83.29	0.34
	+5°C - +10°C	-48.54	-272.54	175.45	0.96
	Control - +1°C	0.05	-0.79	0.89	1.00
	Control - +3°C	-0.18	-1.01	0.66	0.97
Above-ground	Control - +5°C	0.23	-0.66	1.11	0.94
vascular C stock	Control - +10°C	0.13	-0.71	0.96	0.99
	+1°C - +3°C	-0.23	-1.07	0.61	0.92
	+1°C - +5°C	0.18	-0.71	1.06	0.97
(Log-transformed)	+1°C - +10°C	0.08	-0.76	0.91	1.00
	$+3^{\circ}C - +5^{\circ}C$	0.40	-0.48	1.29	0.66
	+3°C - +10°C	0.30	-0.53	1.14	0.81
	$+5^{\circ}C - +10^{\circ}C$	-0.10	-0.99	0.79	1.00
	Control - +1°C	0.19	-120.21	120.59	1.00
	Control - +3°C	24.20	-96.20	144.60	0.97
	Control - $+5^{\circ}C$	4.58	-115.83	124.98	1.00
	Control - +10°C	-0.91	-121.31	119.49	1.00
Non-vascular C	+1°C - +3°C	24.01	-96.39	144.41	0.97
Stock	$+1^{\circ}C - +5^{\circ}C$	4.38	-116.02	124.78	1.00
	$+1^{\circ}C - +10^{\circ}C$	-1.10	-121.50	119.30	1.00
	$+3^{\circ}C - +5^{\circ}C$	-19.63	-140.03	100.78	0.99
	$+3^{\circ}C - +10^{\circ}C$	-25.11	-145.51	95.29	0.97
	$+5^{\circ}C - +10^{\circ}C$	-5.49	-125.89	114.92	1.00
	Control - +1°C	62.28	-218.17	342.73	0.96
	Control - +3°C	-31.70	-322.00	258.60	1.00
	Control - $+5^{\circ}C$	-153.88	-444.18	136.41	0.50
	Control - +10°C	-179.55	-460.00	100.91	0.32
Total vegetation C	+1°C - +3°C	-93.98	-318.84	130.88	0.70
Stock	$+1^{\circ}C - +5^{\circ}C$	-216.16	-441.02	8.70	0.06
	$+1^{\circ}C - +10^{\circ}C$	-241.83	-453.83	-29.83	< 0.05
	$+3^{\circ}C - +5^{\circ}C$	-122.18	-359.21	114.84	0.52
	$+3^{\circ}C - +10^{\circ}C$	-147.85	-372.71	77.01	0.30
	$+5^{\circ}C - +10^{\circ}C$	-25.67	-250.53	199.20	1.00

Table A.13(3): One-way ANOVA and Tukey multi comparison of means test of T_s effect for total vascular C stock, root C stock, aboveground vascular C stock, non-vascular C stock and total vegetation C stock in the GO. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	_ Comparison		GO				
Dependent variable	temperature elevations	Mean Δ	95%	n voluo			
		(b – a)	lower bound	Upper bound	p-value		
	Control - +1°C	30.74	-236.54	298.02	1.00		
Total vascular	Control - $+3^{\circ}C$	10.38	-256.91	277.66	1.00		
C stock	Control - $+5^{\circ}C$	-52.57	-336.07	230.92	0.98		
	Control - +10°C	-56.11	-339.61	227.38	0.97		
	+1°C - +3°C	-20.36	-287.65	246.92	1.00		
	$+1^{\circ}C - +5^{\circ}C$	-83.31	-366.81	200.18	0.90		

	+1°C - +10°C	-86.85	-370.35	196.64	0.88
	+3°C - +5°C	-62.95	-346.44	220.55	0.96
	+3°C - +10°C	-66.49	-349.98	217.01	0.95
	+5°C - +10°C	-3.54	-302.37	295.29	1.00
	Control - +1°C	37.48	-205.53	280.49	0.99
	Control - +3°C	-5.84	-248.85	237.17	1.00
	Control - +5°C	-25.27	-268.28	217.74	1.00
	Control - +10°C	-91.24	-334.25	151.76	0.79
Root C stock	+1°C - +3°C	-43.32	-286.33	199.69	0.98
	+1°C - +5°C	-62.75	-305.76	180.26	0.94
	+1°C - +10°C	-128.72	-371.73	114.28	0.52
	+3°C - +5°C	-19.43	-262.44	223.58	1.00
	$+3^{\circ}C - +10^{\circ}C$	-85.40	-328.41	157.60	0.83
	$+5^{\circ}C - +10^{\circ}C$	-65.97	-308.98	177.04	0.92
	Control - +1°C	0.00	-0.70	0.70	1.00
	Control - $+3^{\circ}C$	0.33	-0.37	1.03	0.62
	Control - $+5^{\circ}C$	0.16	-0.59	0.90	0.97
Above ground	Control - $+10^{\circ}C$	0.73	-0.01	1.47	< 0.10
vascular C stock	$+1^{\circ}C - +3^{\circ}C$	0.33	-0.37	1.03	0.62
vuseului e stoek	$+1^{\circ}C - +5^{\circ}C$	0.16	-0.59	0.90	0.97
	$+1^{\circ}C - +10^{\circ}C$	0.73	-0.02	1 47	0.06
	$+3^{\circ}C - +5^{\circ}C$	-0.17	-0.92	0.57	0.95
	$+3^{\circ}\text{C} - +10^{\circ}\text{C}$	0.40	-0.35	1 14	0.55
	$+5^{\circ}C - +10^{\circ}C$	0.10	-0.21	1 36	0.22
	1000000000000000000000000000000000000	28.33	-60.33	117.00	0.22
	Control $-+3^{\circ}C$	-14.05	-102.72	74 62	0.99
	Control - $+5^{\circ}C$	0.45	-88.21	89.12	1.00
	Control - $+10^{\circ}$ C	-37.90	-131 94	56.15	0.74
Non-vascular	$+1^{\circ}C - +3^{\circ}C$	-42.38	-131.05	46.28	0.61
C stock	$+1^{\circ}C - +5^{\circ}C$	-27.88	-116 55	60.79	0.88
	$+1^{\circ}C - +10^{\circ}C$	-66.23	-160.28	27.81	0.00
	$+3^{\circ}C - +5^{\circ}C$	14 50	-74.16	103.17	0.25
	$+3^{\circ}C - +10^{\circ}C$	-23.85	-117 89	70.20	0.94
	$+5^{\circ}C - +10^{\circ}C$	-38.35	-132.39	55.69	0.74
	$\frac{19 \text{ Control} - 10 \text{ C}}{10 \text{ C}}$	59.07	-190 55	308.70	0.74
	Control $-+3^{\circ}C$	-3 67	-253 30	245.95	1.00
	Control $-+5^{\circ}C$	-52.55	-255.50	243.93	0.97
	Control $-+10^{\circ}$ C	-79.06	-367.30	212.22	0.97
Total vegetation	$+1^{\circ}C - +3^{\circ}C$	-79.00	-307.30	186.88	0.92
C stock	$+1^{\circ}C +5^{\circ}C$	111.62	-312.37	153.15	0.74
	$+1^{\circ}C = +10^{\circ}C$.128.12	-370.33	155.15	0.70
	$+3^{\circ}C \rightarrow 5^{\circ}C$	-130.13	-420.30	215.00	0.00
	$+3^{\circ}C + 10^{\circ}C$	-40.07	-313.04	213.09	0.98
	+3 C - +10 C	-73.39	-303.03	212.80	0.93
1	$+3 C - +10^{\circ}C$	-20.31	-327.96	2/4.94	1.00

ANNEX 14: STATISTICAL RESULTS: BIOMASS N STOCKS

Table A.14(1): Two-way ANOVA of main treatment effects and treatment interactions for total vascular N stock, root N stock, aboveground vascular N stock, non-vascular N stock and total vegetation N stock. Treatments: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C); Grassland (2 levels: GN and GO).

Treatment	p-value	F-value	df	$R^{2}(\%)$
Total vascular N stock				
Ts	< 0.05	2.74	F(4,37)	11.23185
Grassland	< 0.001	49.61	F(1,37)	50.84461
T _s x grassland	0.6920	0.562	F(4,33)	2.420231
Roots vascular N stock				
Ts	< 0.05	3.87	F(4,39)	14.61124
Grassland	< 0.001	51.50	F(1,39)	48.59146
T _s x grassland	0.8812	0.292	F(4,35)	1.189433
Above-ground vascular				
N stock				
Ts	0.81874	0.384	F(4,41)	3.03732
Grassland	< 0.01	8.14	F(1,45)	15.31599
T _s x grassland	0.42739	0.986	F(4,37)	7.804413
Non-vascular N stock				
Ts	0.623	0.660	F(4,43)	5.475162
Grassland	0.115	2.581	F(1,43)	5.350972
T _s x grassland	0.501	0.853	F(4,39)	7.170626
Total vegetation N stock				
T _s	< 0.05	2.664	F(4,46)	13.4023
Grassland	< 0.001	32.854	F(1,36)	41.32279
T _s x grassland	0.7944	0.418	F(4,32)	2.243403

Table A.14(2): One-way ANOVA and Tukey multi comparison of means test of T_s effect for total vascular N stock, root N stock, aboveground vascular N stock, non-vascular N stock and total vegetation N stock in the GN. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GN				
Dependent variable	temperature	Mean Δ	95%	5 CI	n valua	
	cievations	(b – a)	lower bound	Upper bound	p-value	
	Control - +1°C	3.22	-4.19	10.62	0.67	
	Control - $+3^{\circ}C$	-0.15	-7.82	7.51	1.00	
	Control - $+5^{\circ}C$	-0.97	-8.64	6.69	0.99	
	Control - +10°C	-2.03	-9.44	5.37	0.91	
Total vascular	$+1^{\circ}C - +3^{\circ}C$	-3.37	-9.31	2.57	0.43	
IN SLOCK	$+1^{\circ}C - +5^{\circ}C$	-4.19	-10.13	1.75	0.24	
	$+1^{\circ}C - +10^{\circ}C$	-5.25	-10.85	0.35	< 0.10	
	$+3^{\circ}C - +5^{\circ}C$	-0.82	-7.08	5.44	0.99	
	$+3^{\circ}C - +10^{\circ}C$	-1.88	-7.82	4.06	0.86	
	$+5^{\circ}C - +10^{\circ}C$	-1.06	-7.00	4.88	0.98	
	Control - +1°C	2.76	-3.91	9.43	0.71	
	Control - $+3^{\circ}C$	0.05	-6.86	6.95	1.00	
Root N stock	Control - $+5^{\circ}C$	-1.73	-8.64	5.17	0.93	
	Control - +10°C	-2.76	-9.43	3.91	0.71	
	+1°C - +3°C	-2.71	-8.06	2.64	0.54	

	$+1^{\circ}C - +5^{\circ}C$	-4.49	-9.84	0.86	0.12
	+1°C - +10°C	-5.52	-10.56	-0.48	< 0.05
	+3°C - +5°C	-1.78	-7.42	3.86	0.86
	+3°C - +10°C	-2.81	-8.16	2.54	0.51
	$+5^{\circ}C - +10^{\circ}C$	-1.03	-6.38	4.32	0.97
	Control - +1°C	-0.21	-1.38	0.95	0.98
	Control - +3°C	-0.35	-1.52	0.81	0.89
	Control - $+5^{\circ}C$	-0.12	-1.35	1.12	1.00
Above-ground	Control - +10°C	-0.19	-1.35	0.98	0.99
vascular N stock	+1°C - +3°C	-0.14	-1.30	1.03	1.00
(Log transformed)	+1°C - +5°C	0.10	-1.14	1.33	1.00
(Log-transformed)	$+1^{\circ}C - +10^{\circ}C$	0.03	-1.14	1.19	1.00
	+3°C - +5°C	0.23	-1.00	1.47	0.98
	+3°C - +10°C	0.17	-1.00	1.33	0.99
	$+5^{\circ}C - +10^{\circ}C$	-0.07	-1.31	1.17	1.00
	Control - +1°C	-0.04	-1.38	1.30	1.00
	Control - +3°C	0.53	-0.81	1.86	0.76
	Control - +5°C	0.33	-1.01	1.66	0.95
Non-vascular	Control - +10°C	0.10	-1.23	1.44	1.00
N stock	+1°C - +3°C	0.57	-0.77	1.90	0.71
(Log transformed)	+1°C - +5°C	0.37	-0.97	1.70	0.92
(Log-transformed)	$+1^{\circ}C - +10^{\circ}C$	0.14	-1.19	1.48	1.00
	$+3^{\circ}C - +5^{\circ}C$	-0.20	-1.53	1.14	0.99
	+3°C - +10°C	-0.42	-1.76	0.91	0.87
	$+5^{\circ}C - +10^{\circ}C$	-0.22	-1.56	1.11	0.99
	Control - +1°C	1.92	-6.55	10.40	0.95
	Control - +3°C	1.29	-7.48	10.06	0.99
	Control - +5°C	-0.96	-9.73	7.81	1.00
	Control - +10°C	-2.31	-10.79	6.16	0.91
Total vegetation	+1°C - +3°C	-0.63	-7.43	6.16	1.00
N stock	$+1^{\circ}C - +5^{\circ}C$	-2.88	-9.68	3.91	0.69
	$+1^{\circ}C - +10^{\circ}C$	-4.23	-10.64	2.17	0.29
	$+3^{\circ}C - +5^{\circ}C$	-2.25	-9.41	4.91	0.86
	$+3^{\circ}C - +10^{\circ}C$	-3.60	-10.40	3.19	0.50
	$+5^{\circ}C - +10^{\circ}C$	-1.35	-8.15	5.44	0.97

Table A.14(3): One-way ANOVA and Tukey multi comparison of means test of T_s effect for total vascular N stock, root N stock, aboveground vascular N stock, non-vascular N stock and total vegetation N stock in the GO. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GO					
Dependent variable	temperature	Mean Δ	95%	n value			
	cievations	(b – a)	lower bound	Upper bound	p-value		
	Control - +1°C	0.59	-6.34	7.51	1.00		
	Control - $+3^{\circ}C$	-0.01	-6.93	6.92	1.00		
Total vascular	Control - +5°C	-2.78	-10.13	4.56	0.78		
N stock	Control - +10°C	-0.56	-7.91	6.78	1.00		
	+1°C - +3°C	-0.59	-7.52	6.33	1.00		
	$+1^{\circ}C - +5^{\circ}C$	-3.37	-10.72	3.98	0.64		
	$+1^{\circ}C - +10^{\circ}C$	-1.15	-8.50	6.20	0.99		

	+3°C - +5°C	-2.78	-10.12	4.57	0.78
	+3°C - +10°C	-0.56	-7.90	6.79	1.00
	+5°C - +10°C	2.22	-5.52	9.96	0.91
	Control - +1°C	1.11	-4.64	6.86	0.98
	Control - +3°C	-0.32	-6.06	5.43	1.00
	Control - +5°C	-1.75	-7.50	3.99	0.89
	Control - +10°C	-1.69	-7.43	4.06	0.90
	+1°C - +3°C	-1.43	-7.18	4.32	0.94
Root N stock	+1°C - +5°C	-2.86	-8.61	2.88	0.58
	$+1^{\circ}C - +10^{\circ}C$	-2.80	-8.54	2.95	0.60
	+3°C - +5°C	-1.44	-7.18	4.31	0.94
	+3°C - +10°C	-1.37	-7.11	4.38	0.95
	+5°C - +10°C	0.07	-5.68	5.81	1.00
	Control - +1°C	-0.02	-0.88	0.83	1.00
	Control - $+3^{\circ}C$	0.27	-0.59	1.12	0.88
	Control - +5°C	0.00	-0.91	0.91	1.00
	Control - +10°C	0.63	-0.28	1.54	0.26
Above-ground	+1°C - +3°C	0.29	-0.57	1.15	0.84
vascular N stock	$+1^{\circ}C - +5^{\circ}C$	0.02	-0.89	0.93	1.00
	$+1^{\circ}C - +10^{\circ}C$	0.65	-0.25	1.56	0.23
	$+3^{\circ}C - +5^{\circ}C$	-0.27	-1.18	0.64	0.90
	+3°C - +10°C	0.36	-0.55	1.27	0.75
	$+5^{\circ}C - +10^{\circ}C$	0.63	-0.33	1.59	0.31
	Control - +1°C	1.13	-0.72	2.99	0.38
	Control - $+3^{\circ}C$	0.14	-1.72	2.00	1.00
	Control - $+5^{\circ}C$	0.32	-1.54	2.17	0.99
	Control - +10°C	0.08	-1.89	2.04	1.00
Non-vascular	+1°C - +3°C	-0.99	-2.85	0.86	0.51
N stock	$+1^{\circ}C - +5^{\circ}C$	-0.82	-2.67	1.04	0.68
	$+1^{\circ}C - +10^{\circ}C$	-1.05	-3.02	0.91	0.51
	+3°C - +5°C	0.18	-1.68	2.03	1.00
	$+3^{\circ}C - +10^{\circ}C$	-0.06	-2.03	1.90	1.00
	+5°C - +10°C	-0.24	-2.21	1.73	1.00
	Control - $+1^{\circ}C$	1.72	-5.02	8.46	0.93
	Control - +3°C	0.13	-6.61	6.88	1.00
	Control - $+5^{\circ}C$	-2.56	-9.72	4.59	0.81
	Control - +10°C	0.15	-7.63	7.94	1.00
Total vegetation	+1°C - +3°C	-1.59	-8.33	5.16	0.95
N stock	$+1^{\circ}C - +5^{\circ}C$	-4.28	-11.44	2.87	0.39
	$+1^{\circ}C - +10^{\circ}C$	-1.57	-9.35	6.22	0.97
	$+3^{\circ}C - +5^{\circ}C$	-2.70	-9.85	4.46	0.78
	$+3^{\circ}C - +10^{\circ}C$	0.02	-7.77	7.81	1.00
	+5°C - +10°C	2.72	-5.43	10.86	0.85

ANNEX 15: STATISTICAL RESULTS: NECROMASS

Table A.15(1): Two-way ANOVA of main treatment effects and treatment interactions for necromass. Treatments: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C); Grassland (2 levels: GN and GO).

Treatment	p-value	F-value	df	$R^{2}(\%)$
Necromass				
Ts	< 0.05	3.26	F(4,44)	17.00
Grassland	< 0.001	19.72	F(1,44)	25.68
T _s x grassland	0.15	1.78	F(4,40)	8.64

Table A.15(2): One-way ANOVA and Tukey multi comparison of means test of T_s effect for necromass in the GN. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GN				
Dependent variable	temperature	Mean Δ	95%	5 CI	n value	
	cievations	(b – a)	lower bound	Upper bound	p-value	
	Control - +1°C	0.21	-0.58	1.00	0.93	
	Control - +3°C	-0.27	-1.07	0.52	0.84	
	Control - $+5^{\circ}C$	-0.58	-1.38	0.21	0.22	
Necromass	Control - +10°C	-0.40	-1.20	0.39	0.57	
	+1°C - +3°C	-0.48	-1.28	0.31	0.39	
(Log-transformed)	$+1^{\circ}C - +5^{\circ}C$	-0.79	-1.59	0.00	< 0.10	
	$+1^{\circ}C - +10^{\circ}C$	-0.61	-1.41	0.18	0.18	
	$+3^{\circ}C - +5^{\circ}C$	-0.31	-1.11	0.48	0.77	
	+3°C - +10°C	-0.13	-0.93	0.66	0.99	
	$+5^{\circ}C - +10^{\circ}C$	0.18	-0.61	0.98	0.96	

Table A.15(3): One-way ANOVA and Tukey multi comparison of means test of T_s effect for necromass in the GO. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GO				
Dependent variable	temperature	Mean Δ	95%	6 CI	n value	
	(b - a	(b – a)	lower bound	Upper bound	p-value	
	Control - +1°C	0.13	-0.35	0.61	0.93	
	Control - +3°C	0.00	-0.48	0.48	1.00	
	Control - +5°C	0.07	-0.41	0.55	0.99	
Necromass	Control - +10°C	-0.29	-0.77	0.19	0.38	
	$+1^{\circ}C - +3^{\circ}C$	-0.13	-0.61	0.35	0.93	
(Log-transformed)	$+1^{\circ}C - +5^{\circ}C$	-0.05	-0.53	0.43	1.00	
	$+1^{\circ}C$ - $+10^{\circ}C$	-0.42	-0.90	0.06	0.10	
	$+3^{\circ}C - +5^{\circ}C$	0.07	-0.41	0.56	0.99	
	$+3^{\circ}C - +10^{\circ}C$	-0.29	-0.77	0.19	0.39	
	$+5^{\circ}C - +10^{\circ}C$	-0.37	-0.85	0.11	0.19	

ANNEX 16: STATISTICAL RESULTS: NECROMASS C STOCKS

Table A.16(1): Two-way ANOVA of main treatment effects and treatment interactions for necromass C stock. Treatments: T_s (5 levels: control, $+1^{\circ}$ C, $+3^{\circ}$ C, $+5^{\circ}$ C and $+10^{\circ}$ C); Grassland (2 levels: GN and GO).

Treatment	p-value	F-value	df	$R^{2}(\%)$
Necromass C stock				
Ts	< 0.05	2.89	F(4,40)	13.44
Grassland	< 0.001	25.94	F(1,40)	30.11
T _s x grassland	< 0.10	2.16	F(4,40)	10.03

Table A.16(2): One-way ANOVA and Tukey multi comparison of means test of T_s effect for necromass C stock in the GN. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GN				
Dependent variable	temperature	Mean Δ	95%	6 CI	n velue	
	cievations	(b – a)	lower bound	Upper bound	p-value	
	Control - +1°C	0.23	-0.57	1.03	0.91	
	Control - $+3^{\circ}C$	-0.24	-1.04	0.56	0.89	
	Control - $+5^{\circ}C$	-0.57	-1.38	0.23	0.24	
N	Control - +10°C	-0.37	-1.18	0.43	0.64	
Necromass C at a alv	+1°C - +3°C	-0.47	-1.27	0.33	0.43	
C SLOCK	$+1^{\circ}C - +5^{\circ}C$	-0.80	-1.61	-2.26e-05	< 0.05	
	$+1^{\circ}C - +10^{\circ}C$	-0.60	-1.41	0.20	0.20	
	$+3^{\circ}C - +5^{\circ}C$	-0.33	-1.14	0.47	0.73	
	+3°C - +10°C	-0.13	-0.94	0.67	0.99	
	$+5^{\circ}C - +10^{\circ}C$	0.20	-0.60	1.00	0.94	

Table A.16(3): One-way ANOVA and Tukey multi comparison of means test of T_s effect for necromass C stock in the GO. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GO				
Dependent variable	temperature	Mean Δ	95%	5 CI	n velue	
	elevations	(b – a)	lower bound	Upper bound	p-value	
	Control - +1°C	21.09	-44.56	86.75	0.87	
	Control - +3°C	11.25	-54.41	76.91	0.99	
	Control - +5°C	21.88	-43.77	87.54	0.85	
N	Control - +10°C	-22.62	-88.28	43.03	0.84	
Necromass C stock	+1°C - +3°C	-9.84	-75.50	55.82	0.99	
C SLOCK	$+1^{\circ}C - +5^{\circ}C$	0.79	-64.87	66.45	1.00	
	$+1^{\circ}C - +10^{\circ}C$	-43.72	-109.38	21.94	0.31	
	$+3^{\circ}C - +5^{\circ}C$	10.63	-55.03	76.29	0.99	
	$+3^{\circ}C - +10^{\circ}C$	-33.88	-99.54	31.78	0.55	
	+5°C - +10°C	-44.51	-110.17	21.15	0.29	

ANNEX 17: STATISTICAL RESULTS: NECROMASS N STOCKS

Table A.17(1): Two-way ANOVA of main treatment effects and treatment interactions for necromass N stock. Treatments: T_S (5 levels: control, +1°C, +3°C, +5°C and +10°C); Grassland (2 levels: GN and GO).

Treatment	p-value	F-value	df	$R^{2}(\%)$
Necromass				
Ts	0.57	0.74	F(4,44)	3.90
Grassland	< 0.001	29.31	F(1,48)	37.91
T _s x grassland	0.40	1.04	F(4,40)	5.46

Table A.17(2): One-way ANOVA and Tukey multi comparison of means test of T_s effect for necromass N stock in the GN. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GN				
Dependent variable	temperature	Mean Δ	95%	5 CI	n valuo	
	elevations	(b – a)	lower bound	Upper bound	p-value	
	Control - +1°C	-0.19	-1.01	0.64	0.96	
	Control - +3°C	-0.33	-1.15	0.49	0.74	
0	Control - +5°C	-0.55	-1.37	0.27	0.30	
Necromass	Control - +10°C	-0.24	-1.06	0.59	0.91	
N stock	$+1^{\circ}C - +3^{\circ}C$	-0.15	-0.97	0.67	0.98	
(Log_transformed)	$+1^{\circ}C - +5^{\circ}C$	-0.36	-1.18	0.46	0.68	
(Log-transformed)	$+1^{\circ}C$ - $+10^{\circ}C$	-0.05	-0.87	0.77	1.00	
	$+3^{\circ}C - +5^{\circ}C$	-0.21	-1.04	0.61	0.93	
	$+3^{\circ}C - +10^{\circ}C$	0.10	-0.72	0.92	1.00	
	$+5^{\circ}C - +10^{\circ}C$	0.31	-0.51	1.13	0.78	

Table A.17(3): One-way ANOVA and Tukey multi comparison of means test of T_s effect for necromass N stock in the GO. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison		GO				
Dependent variable	temperature	Mean Δ	95%	6 CI	n voluo		
	(b – a)	(b – a)	lower bound	Upper bound	p-value		
	Control - +1°C	0.59	-1.02	2.20	0.81		
	Control - +3°C	0.20	-1.41	1.81	1.00		
	Control - +5°C	0.30	-1.31	1.91	0.98		
NY.	Control - +10°C	0.33	-1.28	1.94	0.97		
Necromass	+1°C - +3°C	-0.39	-2.00	1.22	0.95		
IN SLOCK	$+1^{\circ}C - +5^{\circ}C$	-0.29	-1.90	1.32	0.98		
	+1°C - +10°C	-0.26	-1.87	1.35	0.99		
	$+3^{\circ}C - +5^{\circ}C$	0.10	-1.51	1.71	1.00		
	+3°C - +10°C	0.13	-1.48	1.74	1.00		
	$+5^{\circ}C - +10^{\circ}C$	0.03	-1.58	1.64	1.00		

ANNEX 18: STATISTICAL RESULTS: SOIL C STOCKS

Table A.18(1): Two-way ANOVA of main treatment effects and treatment interactions for soil C stock. Treatments: T_S (5 levels: control, +1°C, +3°C, +5°C and +10°C); Grassland (2 levels: GN and GO).

Treatment	p-value	F-value	df	$R^{2}(\%)$
Soil C stock				
Ts	< 0.001	6.20	F(4,44)	23.43
Grassland	< 0.001	37.02	F(1,44)	34.99
T _s x grassland	0.59	0.70	F(4,40)	2.73

Table A.18(2): One-way ANOVA and Tukey multi comparison of means test of T_s effect for soil C stock in the GN. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GN				
Dependent variable	temperature	Mean Δ	95%	6 CI	n value	
	cievations	(b – a)	lower bound	Upper bound	p-value	
	Control - $+1^{\circ}C$	-0.24	-1.15	0.66	0.93	
	Control - +3°C	-0.32	-1.22	0.59	0.83	
	Control - $+5^{\circ}C$	-0.97	-1.88	-0.06	< 0.05	
Soil C stock	Control - +10°C	-0.83	-1.74	0.08	< 0.10	
	+1°C - +3°C	-0.07	-0.98	0.83	1.00	
(Log-transformed)	$+1^{\circ}C - +5^{\circ}C$	-0.73	-1.63	0.18	0.16	
	$+1^{\circ}C - +10^{\circ}C$	-0.59	-1.49	0.32	0.33	
	$+3^{\circ}C - +5^{\circ}C$	-0.65	-1.56	0.25	0.24	
	+3°C - +10°C	-0.51	-1.42	0.39	0.46	
	$+5^{\circ}C - +10^{\circ}C$	0.14	-0.77	1.05	0.99	

Table A.18(3): One-way ANOVA and Tukey multi comparison of means test of T_s effect for soil C stock in the GO. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GO				
Dependent variable	temperature	Mean Δ	95%	6 CI	n volvo	
	elevations	(b – a)	lower bound	Upper bound	p-value	
	Control - +1°C	-245.79	-3954.91	3463.32	1.00	
	Control - +3°C	776.53	-2932.58	4485.65	0.97	
	Control - +5°C	-1949.81	-5658.93	1759.30	0.53	
	Control - +10°C	-3230.38	-6939.49	478.74	0.11	
Soil C stock	+1°C - +3°C	1022.33	-2686.79	4731.44	0.92	
	$+1^{\circ}C - +5^{\circ}C$	-1704.02	-5413.13	2005.09	0.65	
	$+1^{\circ}C - +10^{\circ}C$	-2984.58	-6693.70	724.53	0.15	
	$+3^{\circ}C - +5^{\circ}C$	-2726.35	-6435.46	982.77	0.22	
	$+3^{\circ}C - +10^{\circ}C$	-4006.91	-7716.03	-297.80	< 0.05	
	$+5^{\circ}C - +10^{\circ}C$	-1280.56	-4989.68	2428.55	0.84	

ANNEX 19: STATISTICAL RESULTS: SOIL C STOCKS – NORMALIZED FOR SOIL DEPTH

Table A.19(1): Two-way ANOVA of main treatment effects and treatment interactions for soil C stock normalized for soil depth. Treatments: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C); Grassland (2 levels: GN and GO).

Treatment	p-value	F-value	df	$R^{2}(\%)$
Soil C stock normalized				
Ts	< 0.001	7.39	F(4,44)	32.09
Grassland	< 0.001	18.53	F(1,44)	20.12
T _s x grassland	0.54	0.79	F(4,40)	3.50

Table A.19(2): One-way ANOVA and Tukey multi comparison of means test of T_s effect for soil C stock normalized for soil depth in the GN. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GN				
Dependent variable	temperature	Mean Δ	95%	95% CI		
	elevations	(b – a)	lower bound	Upper bound	p-value	
	Control - +1°C	-0.18	-0.76	0.39	0.87	
	Control - +3°C	-0.23	-0.80	0.34	0.74	
Soil C stock	Control - $+5^{\circ}C$	-0.60	-1.17	-0.03	< 0.05	
normalized for	Control - +10°C	-0.56	-1.13	0.01	< 0.10	
soil depth	+1°C - +3°C	-0.05	-0.62	0.52	1.00	
	$+1^{\circ}C - +5^{\circ}C$	-0.42	-0.99	0.15	0.22	
(Log-transformed)	$+1^{\circ}C - +10^{\circ}C$	-0.38	-0.95	0.19	0.31	
	$+3^{\circ}C - +5^{\circ}C$	-0.37	-0.94	0.20	0.33	
	+3°C - +10°C	-0.33	-0.90	0.24	0.44	
	+5°C - +10°C	0.04	-0.53	0.61	1.00	

Table A.19(3): One-way ANOVA and Tukey multi comparison of means test of T_s effect for soil C stock normalized for soil depth in the GO. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

Dopondont	Comparison	GO				
variable	temperature elevations	Mean Δ	95%	6 CI	n-value	
		(b – a)	lower bound	Upper bound	r · · · · ·	
	Control - +1°C	-157.85	-3402.81	3087.12	1.00	
	Control - +3°C	991.51	-2253.45	4236.48	0.89	
	Control - +5°C	-2086.62	-5331.58	1158.35	0.34	
Soil C stock	Control - +10°C	-2878.59	-6123.56	366.37	< 0.10	
normalized for	+1°C - +3°C	1149.36	-2095.60	4394.33	0.82	
soil depth	$+1^{\circ}C - +5^{\circ}C$	-1928.77	-5173.74	1316.19	0.41	
	$+1^{\circ}C - +10^{\circ}C$	-2720.74	-5965.71	524.22	0.13	
	$+3^{\circ}C - +5^{\circ}C$	-3078.13	-6323.10	166.83	< 0.10	
	$+3^{\circ}C - +10^{\circ}C$	-3870.11	-7115.07	-625.14	< 0.05	
	+5°C - +10°C	-791.97	-4036.94	2452.99	0.95	

ANNEX 20: STATISTICAL RESULTS: SOIL N STOCKS

Table A.20(1): Two-way ANOVA of main treatment effects and treatment interactions for soil N stock. Treatments: T_S (5 levels: control, +1°C, +3°C, +5°C and +10°C); Grassland (2 levels: GN and GO).

Treatment	p-value	F-value	df	$R^{2}(\%)$
Soil N stock				
Ts	< 0.01	4.11	F(4,44)	20.58
Grassland	< 0.001	19.51	F(1,44)	24.39
T _s x grassland	0.58	0.72	F(4,40)	3.68

Table A.20(2): One-way ANOVA and Tukey multi comparison of means test of T_s effect for soil N stock in the GN. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GN				
Dependent variable	temperature	Mean Δ	95%	6 CI	n value	
	cievations	(b – a)	lower bound	Upper bound	p-value	
	Control - +1°C	-0.27	-1.25	0.71	0.92	
	Control - +3°C	-0.35	-1.33	0.63	0.82	
	Control - $+5^{\circ}C$	-1.01	-1.99	-0.03	< 0.05	
	Control - +10°C	-0.93	-1.91	0.05	< 0.10	
Soil N stock	+1°C - +3°C	-0.08	-1.06	0.90	1.00	
(Log-transformed)	$+1^{\circ}C - +5^{\circ}C$	-0.74	-1.72	0.24	0.20	
	$+1^{\circ}C - +10^{\circ}C$	-0.66	-1.64	0.32	0.29	
	$+3^{\circ}C - +5^{\circ}C$	-0.66	-1.64	0.32	0.29	
	+3°C - +10°C	-0.58	-1.56	0.40	0.42	
	$+5^{\circ}C - +10^{\circ}C$	0.08	-0.90	1.06	1.00	

Table A.20(3): One-way ANOVA and Tukey multi comparison of means test of T_s effect for soil N stock in the GO. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GO				
Dependent variable	temperature	Mean Δ	95%	5 CI		
	elevations	(b – a)	lower bound	Upper bound	p-value	
	Control - +1°C	49.29	-235.26	333.85	0.98	
	Control - +3°C	8.87	-275.69	293.42	1.00	
	Control - +5°C	-80.10	-364.66	204.45	0.91	
	Control - $+10^{\circ}C$	-157.25	-441.81	127.30	0.48	
Soil N stock	+1°C - +3°C	-40.43	-324.98	244.13	0.99	
	$+1^{\circ}C - +5^{\circ}C$	-129.40	-413.95	155.16	0.66	
	$+1^{\circ}C - +10^{\circ}C$	-206.54	-491.10	78.01	0.23	
	$+3^{\circ}C - +5^{\circ}C$	-88.97	-373.52	195.59	0.88	
	$+3^{\circ}C - +10^{\circ}C$	-166.12	-450.67	118.44	0.43	
	+5°C - +10°C	-77.15	-361.70	207.41	0.92	

ANNEX 21: STATISTICAL RESULTS: SOIL N STOCKS – NORMALIZED FOR SOIL DEPTH

Table A.x(1): Two-way ANOVA of main treatment effects and treatment interactions for soil N stock normalized for soil depth. Treatments: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C); Grassland (2 levels: GN and GO).

Treatment	p-value	F-value	df	R^{2} (%)
Soil N stock normalized				
Ts	< 0.001	7.22	F(4,45)	39.10
Grassland	0.34	0.93	F(1,44)	1.26
T _s x grassland	0.47	0.90	F(4,40)	4.95

Table A.21(2): One-way ANOVA and Tukey multi comparison of means test of T_s effect for soil N stock normalized for soil depth in the GN. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GN				
Dependent variable	temperature	Mean Δ	95%	n voluo		
	elevations	(b – a)	lower bound	Upper bound	p-value	
	Control - +1°C	-50.69	-267.37	165.99	0.95	
	Control - +3°C	-121.01	-337.69	95.67	0.47	
	Control - $+5^{\circ}C$	-230.74	-447.41	-14.06	< 0.05	
Soil N stock	Control - +10°C	-319.12	-535.80	-102.44	< 0.01	
normalized for	$+1^{\circ}C - +3^{\circ}C$	-70.32	-287.00	146.36	0.86	
soil depth	$+1^{\circ}C - +5^{\circ}C$	-180.04	-396.72	36.64	0.13	
	$+1^{\circ}C$ - $+10^{\circ}C$	-268.43	-485.11	-51.75	< 0.05	
	$+3^{\circ}C - +5^{\circ}C$	-109.72	-326.40	106.96	0.56	
	$+3^{\circ}C - +10^{\circ}C$	-198.11	-414.79	18.57	< 0.10	
	$+5^{\circ}C - +10^{\circ}C$	-88.39	-305.06	128.29	0.74	

Table A.21(3): One-way ANOVA and Tukey multi comparison of means test of T_s effect for soil N stock normalized for soil depth in the GO. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

Dependent	Comparison	GO				
variable	temperature elevations	Mean Δ	95% CI		p-value	
		(b – a)	lower bound	Upper bound	ľ	
	Control - +1°C	56.91	-180.90	294.72	0.95	
	Control - $+3^{\circ}C$	27.48	-210.33	265.29	1.00	
	Control - $+5^{\circ}C$	-91.95	-329.76	145.86	0.77	
Soil N stock	Control - +10°C	-126.79	-364.60	111.01	0.52	
normalized for	+1°C - +3°C	-29.43	-267.24	208.38	1.00	
soil depth	$+1^{\circ}C - +5^{\circ}C$	-148.86	-386.66	88.95	0.36	
	$+1^{\circ}C - +10^{\circ}C$	-183.70	-421.51	54.11	0.18	
	+3°C - +5°C	-119.43	-357.23	118.38	0.57	
	+3°C - +10°C	-154.27	-392.08	83.54	0.33	
	+5°C - +10°C	-34.85	-272.65	202.96	0.99	

ANNEX 22: STATISTICAL RESULTS: ECOSYSTEM C STOCKS

Table A.22(1): Two-way ANOVA of main treatment effects and treatment interactions for ecosystem C stock. Treatments: T_s (5 levels: control, $+1^{\circ}$ C, $+3^{\circ}$ C, $+5^{\circ}$ C and $+10^{\circ}$ C); Grassland (2 levels: GN and GO).

Treatment	p-value	F-value	df	R^{2} (%)
Ecosystem C stock				
Ts	< 0.001	7.56	F(4,36)	29.26638
Grassland	< 0.001	37.07	F(1,36)	35.88289
T _s x grassland	0.47	0.90	F(4,32)	3.54158

Table A.22(2): One-way ANOVA and Tukey multi comparison of means test of T_s effect for ecosystem C stock in the GN. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GN				
Dependent variable	temperature	Mean Δ	95%	5 CI	n voluo	
	cievations	(b – a)	lower bound	Upper bound	p-value	
	Control - +1°C	-1045.91	-5539.02	3447.21	0.95	
	Control - $+3^{\circ}C$	-1663.65	-6314.46	2987.17	0.80	
	Control - $+5^{\circ}C$	-3240.35	-7891.17	1410.46	0.25	
	Control - +10°C	-3480.99	-7974.11	1012.12	0.17	
Ecosystem	+1°C - +3°C	-617.74	-4220.24	2984.77	0.98	
C SLOCK	$+1^{\circ}C - +5^{\circ}C$	-2194.44	-5796.95	1408.06	0.37	
	+1°C - +10°C	-2435.08	-5831.56	961.39	0.23	
	$+3^{\circ}C - +5^{\circ}C$	-1576.71	-5374.08	2220.67	0.71	
	+3°C - +10°C	-1817.35	-5419.85	1785.16	0.54	
	$+5^{\circ}C - +10^{\circ}C$	-240.64	-3843.15	3361.86	1.00	

Table A.22(3): One-way ANOVA and Tukey multi comparison of means test of T_s effect for ecosystem C stock in the GO. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GO				
Dependent variable	temperature	Mean Δ	95%	6 CI	n voluo	
	(b	(b – a)	lower bound	Upper bound	p-value	
	Control - +1°C	-165.62	-3899.00	3567.75	1.00	
	Control - +3°C	784.12	-2949.26	4517.49	0.97	
	Control - +5°C	-1141.55	-5101.39	2818.29	0.90	
	Control - $+10^{\circ}C$	-3928.41	-8239.34	382.52	< 0.10	
Ecosystem	$+1^{\circ}C - +3^{\circ}C$	949.74	-2783.64	4683.12	0.93	
C stock	$+1^{\circ}C - +5^{\circ}C$	-975.93	-4935.77	2983.92	0.94	
	$+1^{\circ}C - +10^{\circ}C$	-3762.79	-8073.72	548.14	0.10	
	$+3^{\circ}C - +5^{\circ}C$	-1925.67	-5885.51	2034.18	0.59	
	$+3^{\circ}C - +10^{\circ}C$	-4712.53	-9023.46	-401.60	< 0.05	
	$+5^{\circ}C - +10^{\circ}C$	-2786.86	-7295.34	1721.62	0.36	

ANNEX 23: STATISTICAL RESULTS: ECOSYSTEM C STOCKS – NORMALIZED FOR SOIL DEPTH

Table A.23(1): Two-way ANOVA of main treatment effects and treatment interactions for ecosystem C stock normalized for soil depth. Treatments: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C); Grassland (2 levels: GN and GO).

Treatment	p-value	F-value	df	$R^{2}(\%)$
Ecosystem C stock				
normalized				
Ts	< 0.001	7.50	F(4,36)	36.45
Grassland	< 0.001	16.33	F(1,36)	19.83
T _s x grassland	0.47	0.909	F(4,32)	4.46

Table A.23(2): One-way ANOVA and Tukey multi comparison of means test of T_s effect for ecosystem C stock normalized for soil depth in the GN. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

Denendent	Comparison	GN				
variable	temperature elevations	Mean Δ	95% CI		n-value	
		(b – a)	lower bound	Upper bound	p (all de	
	Control - +1°C	-1045.91	-5539.02	3447.21	0.95	
	Control - +3°C	-1663.65	-6314.46	2987.17	0.80	
	Control - $+5^{\circ}C$	-3240.35	-7891.17	1410.46	0.25	
	Control - +10°C	-3480.99	-7974.11	1012.12	0.17	
Ecosystem C	+1°C - +3°C	-617.74	-4220.24	2984.77	0.98	
for soil depth	+1°C - +5°C	-2194.44	-5796.95	1408.06	0.37	
for son deput	$+1^{\circ}C - +10^{\circ}C$	-2435.08	-5831.56	961.39	0.23	
	$+3^{\circ}C - +5^{\circ}C$	-1576.71	-5374.08	2220.67	0.71	
	+3°C - +10°C	-1817.35	-5419.85	1785.16	0.54	
	$+5^{\circ}C - +10^{\circ}C$	-240.64	-3843.15	3361.86	1.00	

Table A.23(3): One-way ANOVA and Tukey multi comparison of means test of T_s effect for ecosystem C stock normalized for soil depth in the GO. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GO				
Dependent variable	temperature	Mean Δ	95% CI		n-value	
	elevations	(b – a)	lower bound	Upper bound	F	
	Control - +1°C	-77.68	-3547.20	3391.84	1.00	
	Control - +3°C	999.09	-2470.43	4468.62	0.90	
	Control - $+5^{\circ}C$	-1669.23	-5349.21	2010.76	0.65	
Ecosystem C	Control - +10°C	-3804.64	-7810.90	201.62	< 0.10	
stock normalized	$+1^{\circ}C - +3^{\circ}C$	1076.77	-2392.75	4546.30	0.88	
for soil depth	$+1^{\circ}C - +5^{\circ}C$	-1591.55	-5271.53	2088.44	0.69	
	$+1^{\circ}C - +10^{\circ}C$	-3726.96	-7733.22	279.30	< 0.10	
	$+3^{\circ}C - +5^{\circ}C$	-2668.32	-6348.31	1011.66	0.22	
	+3°C - +10°C	-4803.73	-8809.99	-797.47	< 0.05	
	+5°C - +10°C	-2135.41	-6325.26	2054.44	0.55	

ANNEX 24: STATISTICAL RESULTS: ECOSYSTEM N STOCKS

Table A.24(1): Two-way ANOVA of main treatment effects and treatment interactions for ecosystem N stock. Treatments: T_S (5 levels: control, +1°C, +3°C, +5°C and +10°C); Grassland (2 levels: GN and GO).

Treatment	p-value	F-value	df	$R^{2}(\%)$
Ecosystem N stock				
Ts	< 0.01	4.30	F(4,36)	21.90
Grassland	< 0.001	25.26	F(1,36)	32.20
T _s x grassland	0.67	0.59	F(4,32)	3.15

Table A.24(2): One-way ANOVA and Tukey multi comparison of means test of T_s effect for ecosystem N stock in the GN. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GN				
Dependent variable	temperature	Mean Δ	95%	6 CI	1	
	elevations	(b – a)	lower bound	Upper bound	p-value	
	Control - +1°C	-83.56	-489.83	322.71	0.97	
	Control - $+3^{\circ}C$	-119.70	-540.23	300.83	0.90	
	Control - $+5^{\circ}C$	-235.30	-655.83	185.23	0.45	
	Control - +10°C	-260.04	-666.31	146.23	0.32	
Ecosystem Natock	+1°C - +3°C	-36.14	-361.88	289.60	1.00	
IN SLOCK	$+1^{\circ}C - +5^{\circ}C$	-151.74	-477.48	174.01	0.61	
	$+1^{\circ}C - +10^{\circ}C$	-176.48	-483.59	130.63	0.42	
	$+3^{\circ}C - +5^{\circ}C$	-115.60	-458.96	227.77	0.83	
	$+3^{\circ}C - +10^{\circ}C$	-140.34	-466.08	185.40	0.68	
	$+5^{\circ}C - +10^{\circ}C$	-24.74	-350.48	301.00	1.00	

Table A.24(3): One-way ANOVA and Tukey multi comparison of means test of T_s effect for ecosystem N stock in the GO. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GO				
Dependent variable	temperature	Mean Δ	95% CI		1	
	(b – a)	lower bound	Upper bound	p-value		
	Control - +1°C	51.60	-209.96	313.16	0.97	
	Control - +3°C	9.20	-252.36	270.75	1.00	
	Control - +5°C	-0.79	-278.21	276.64	1.00	
	Control - +10°C	-180.73	-482.75	121.29	0.39	
Ecosystem	+1°C - +3°C	-42.40	-303.96	219.15	0.99	
IN SLOCK	$+1^{\circ}C - +5^{\circ}C$	-52.39	-329.81	225.03	0.98	
	+1°C - +10°C	-232.33	-534.35	69.69	0.18	
	$+3^{\circ}C - +5^{\circ}C$	-9.98	-287.41	267.44	1.00	
	+3°C - +10°C	-189.93	-491.95	112.09	0.35	
	+5°C - +10°C	-179.95	-495.81	135.91	0.44	

ANNEX 25: STATISTICAL RESULTS: ECOSYSTEM N STOCKS – NORMALIZED FOR SOIL DEPTH

Table A.25(1): Two-way ANOVA of main treatment effects and treatment interactions for ecosystem N stock normalized for soil depth. Treatments: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C); Grassland (2 levels: GN and GO).

Treatment	p-value	F-value	df	R ² (%)
Ecosystem N stock				
normalized				
Ts	< 0.001	6.40	F(4,37)	40.88
Grassland	0.15	2.20	F(1,36)	3.41
T _s x grassland	0.82	0.39	F(4,32)	2.57

Table A.25(2): One-way ANOVA and Tukey multi comparison of means test of T_S effect for ecosystem N stock normalized for soil depth in the GN. Treatment: T_S (5 levels: control, +1°C, +3°C, +5°C and +10°C).

Dopondont	Comparison	GN				
variable	temperature elevations	Mean Δ	95%	6 CI	p-value	
		(b – a)	lower bound	Upper bound	1	
	Control - +1°C	-5.82	-304.12	292.48	1.00	
	Control - $+3^{\circ}C$	-113.00	-421.77	195.77	0.79	
	Control - $+5^{\circ}C$	-174.98	-483.75	133.79	0.44	
Ecosystem N	Control - +10°C	-278.81	-577.11	19.50	< 0.10	
stock normalized	+1°C - +3°C	-107.18	-346.36	131.99	0.65	
for soil depth	+1°C - +5°C	-169.16	-408.34	70.01	0.24	
	+1°C - +10°C	-272.99	-498.48	-47.49	< 0.05	
	$+3^{\circ}C - +5^{\circ}C$	-61.98	-314.09	190.13	0.94	
	+3°C - +10°C	-165.81	-404.98	73.37	0.25	
	$+5^{\circ}C - +10^{\circ}C$	-103.83	-343.00	135.35	0.67	

Table A.25(3): One-way ANOVA and Tukey multi comparison of means test of T_s effect for ecosystem N stock normalized for soil depth in the GO. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GO				
variable	temperature	Mean Δ	95%	ó CI	n-value	
	elevations	(b – a)	lower bound	Upper bound	p (mae	
	Control - +1°C	59.22	-180.07	298.50	0.94	
	Control - +3°C	27.81	-211.47	267.09	1.00	
	Control - +5°C	-46.47	-300.27	207.33	0.98	
Ecosystem N	Control - +10°C	-170.02	-446.32	106.28	0.37	
stock normalized	$+1^{\circ}C - +3^{\circ}C$	-31.41	-270.69	207.88	0.99	
for soil depth	$+1^{\circ}C - +5^{\circ}C$	-105.69	-359.48	148.11	0.71	
	$+1^{\circ}C - +10^{\circ}C$	-229.23	-505.53	47.07	0.13	
	$+3^{\circ}C - +5^{\circ}C$	-74.28	-328.08	179.52	0.90	
	+3°C - +10°C	-197.83	-474.12	78.47	0.23	
	$+5^{\circ}C - +10^{\circ}C$	-123.55	-412.51	165.41	0.69	

ANNEX 26: STATISTICAL RESULTS: BIOMASS OF VEGETATION TYPES

Table A.26(1): Two-way ANOVA of main treatment effects and treatment interactions for monocotyledon biomass, dicotyledon biomass, equiseta biomass, moss biomass and lichen biomass. Treatments: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C); Grassland (2 levels: GN and GO). NA = not assessable (to our knowledge there is no two-way alternative in RStudio; see § 2.5)

Treatment	p-value	F-value	df	R^{2} (%)
Monocotyledon biomass				
Ts	< 0.10	2.18	F(4,39)	12.39
Grassland	< 0.001	14.31	F(1,39)	20.32
T _s x grassland	< 0.10	2.10	F(4,39)	11.91
Dicotyledon biomass				
Ts	0.73	0.51	F(4,40)	4.67
Grassland	0.95	0.004	F(1,40)	0.74
T _s x grassland	0.83	0.37	F(4,40)	3.38
Equiseta biomass				
T _s	0.40	1.03	F(4,40)	9.05
Grassland	0.68	0.17	F(1,40)	0.38
T _s x grassland	0.89	0.28	F(4,40)	2.50
Moss biomass				
Ts	0.53	0.81	F(4,40)	6.85
Grassland	0.23	1.48	F(1,40)	3.14
T _s x grassland	0.66	0.60	F(4,40)	5.11
Lichen biomass				
Ts	N.A.	N.A.	N.A.	N.A.
Grassland	N.A.	N.A.	N.A.	N.A.
T _s x grassland	N.A.	N.A.	N.A.	N.A.

Table A.26(2): One-way ANOVA and Tukey multi comparison of means test of T_s effect for monocotyledon biomass, dicotyledon biomass, equiseta biomass, moss biomass in the GN. Non-parametric Kruskal-Wallis chi-squared test was used to analyse the T_s effect on lichens. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GN				
Dependent variable	temperature	Mean Δ	95%	o CI	n volvo	
	cievations	(b – a)	lower bound	Upper bound	p-value	
	Control - +1°C	0.16	-0.80	1.12	0.99	
	Control - +3°C	-0.35	-1.31	0.61	0.81	
	Control - $+5^{\circ}C$	-0.44	-1.46	0.58	0.70	
Monocotyledon	Control - +10°C	0.25	-0.71	1.21	0.93	
biomass	+1°C - +3°C	-0.51	-1.47	0.45	0.51	
(Log_transformed)	+1°C - +5°C	-0.60	-1.62	0.42	0.42	
(Log-transformed)	$+1^{\circ}C - +10^{\circ}C$	0.09	-0.87	1.05	1.00	
	$+3^{\circ}C - +5^{\circ}C$	-0.09	-1.10	0.93	1.00	
	+3°C - +10°C	0.60	-0.36	1.56	0.36	
	$+5^{\circ}C - +10^{\circ}C$	0.69	-0.33	1.71	0.29	
	Control - +1°C	-10.88	-63.65	41.88	0.97	
Dicotyledon	Control - +3°C	-5.11	-57.88	47.66	1.00	
	Control - +5°C	26.83	-25.94	79.59	0.56	
UIUIIIASS	Control - +10°C	-12.91	-65.67	39.86	0.95	
	+1°C - +3°C	5.77	-46.99	58.54	1.00	

	+1°C - +5°C	37.71	-15.06	90.47	0.24
	$+1^{\circ}C - +10^{\circ}C$	-2.03	-54.79	50.74	1.00
	+3°C - +5°C	31.94	-20.83	84.70	0.39
	+3°C - +10°C	-7.80	-60.56	44.97	0.99
	$+5^{\circ}C - +10^{\circ}C$	-39.73	-92.50	13.03	0.20
	Control - +1°C	0.22	-1.38	1.82	0.99
	Control - +3°C	0.51	-1.09	2.11	0.87
	Control - +5°C	0.81	-0.79	2.41	0.56
Equiseta biomass	Control - +10°C	0.06	-1.54	1.65	1.00
(I a s transformed)	+1°C - +3°C	0.29	-1.31	1.89	0.98
(Log-transformed)	+1°C - +5°C	0.59	-1.01	2.19	0.80
	+1°C - +10°C	-0.17	-1.77	1.43	1.00
	+3°C - +5°C	0.30	-1.30	1.90	0.98
	+3°C - +10°C	-0.46	-2.06	1.14	0.91
	+5°C - +10°C	-0.76	-2.36	0.84	0.62
	Control - +1°C	-3.03	-278.35	272.30	1.00
	Control - +3°C	0.70	-274.63	276.03	1.00
	Control - +5°C	-48.22	-323.55	227.10	0.98
	Control - +10°C	-22.12	-297.45	253.20	1.00
Masshiemass	$+1^{\circ}C - +3^{\circ}C$	3.73	-271.60	279.05	1.00
Moss biomass	$+1^{\circ}C - +5^{\circ}C$	-45.20	-320.52	230.13	0.99
	$+1^{\circ}C - +10^{\circ}C$	-19.10	-294.42	256.23	1.00
	+3°C - +5°C	-48.92	-324.25	226.40	0.98
	+3°C - +10°C	-22.82	-298.15	252.50	1.00
	$+5^{\circ}C - +10^{\circ}C$	26.10	-249.23	301.43	1.00
	Control - +1°C	0.00	N.A.	N.A.	> 0.10
	Control - +3°C	6.00	N.A.	N.A.	> 0.10
	Control - +5°C	9.60	N.A.	N.A.	> 0.10
T · 1 · 1 ·	Control - +10°C	4.40	N.A.	N.A.	> 0.10
Lichen biomass	$+1^{\circ}C - +3^{\circ}C$	6.00	N.A.	N.A.	> 0.10
(Non-parametric)	$+1^{\circ}C - +5^{\circ}C$	9.60	N.A.	N.A.	> 0.10
(iton parametric)	$+1^{\circ}C - +10^{\circ}C$	4.40	N.A.	N.A.	> 0.10
	$+3^{\circ}C - +5^{\circ}C$	3.60	N.A.	N.A.	> 0.10
	$+3^{\circ}C - +10^{\circ}C$	1.60	N.A.	N.A.	> 0.10
	$+5^{\circ}C - +10^{\circ}C$	5.20	N.A.	N.A.	> 0.10

Table A.26(3): One-way ANOVA and Tukey multi comparison of means test of T_s effect for monocotyledon biomass, dicotyledon biomass, equiseta biomass, moss biomass in the GO. Non-parametric Kruskal-Wallis chi-squared test was used to analyse the T_s effect on lichens. Treatment: T_s (5 levels: control, +1°C, +3°C, +5°C and +10°C).

	Comparison	GO				
Dependent variable	temperature	Mean Δ	95% CI		1	
	elevations	(b – a)	lower bound	Upper bound	p-value	
	Control - +1°C	2.09	-113.12	117.30	1.00	
	Control - +3°C	59.46	-55.75	174.67	0.55	
Monocotyledon	Control - +5°C	25.66	-89.55	140.87	0.96	
biomass	Control - +10°C	119.09	3.88	234.30	< 0.05	
	+1°C - +3°C	57.36	-57.85	172.57	0.58	
	$+1^{\circ}C - +5^{\circ}C$	23.57	-91.64	138.78	0.97	
	+1°C - +10°C	117.00	1.79	232.21	0.05	

	$+3^{\circ}C - +5^{\circ}C$	-33.79	-149.00	81.42	0.90
	+3°C - +10°C	59.63	-55.58	174.84	0.54
	$+5^{\circ}C - +10^{\circ}C$	93.42	-21.79	208.63	0.15
	Control - +1°C	0.05	-1.33	1.42	1.00
	Control - +3°C	0.04	-1.34	1.41	1.00
	Control - +5°C	0.22	-1.16	1.59	0.99
Dicotyledon	Control - +10°C	0.17	-1.21	1.55	1.00
biomass	+1°C - +3°C	-0.01	-1.39	1.36	1.00
	+1°C - +5°C	0.17	-1.21	1.55	1.00
(Log-transformed)	+1°C - +10°C	0.12	-1.25	1.50	1.00
	+3°C - +5°C	0.18	-1.20	1.56	0.99
	+3°C - +10°C	0.13	-1.24	1.51	1.00
	$+5^{\circ}C - +10^{\circ}C$	-0.05	-1.42	1.33	1.00
	Control - +1°C	0.01	-0.01	0.02	0.64
	Control - +3°C	0.00	-0.01	0.01	1.00
	Control - +5°C	0.00	-0.01	0.01	1.00
Equiseta biomass	Control - +10°C	0.00	-0.02	0.01	0.93
(her eer	$+1^{\circ}C - +3^{\circ}C$	-0.01	-0.02	0.01	0.62
(DOX COX- tranformed)	$+1^{\circ}C - +5^{\circ}C$	-0.01	-0.02	0.01	0.78
trainormed)	$+1^{\circ}C$ - $+10^{\circ}C$	-0.01	-0.02	0.00	0.23
	$+3^{\circ}C - +5^{\circ}C$	0.00	-0.01	0.01	1.00
	$+3^{\circ}C - +10^{\circ}C$	0.00	-0.02	0.01	0.94
	$+5^{\circ}C - +10^{\circ}C$	0.00	-0.02	0.01	0.84
	Control - +1°C	0.35	-0.88	1.58	0.91
	Control - $+3^{\circ}C$	0.01	-1.22	1.23	1.00
	Control - $+5^{\circ}C$	0.08	-1.15	1.31	1.00
	Control - $+10^{\circ}C$	-0.85	-2.08	0.37	0.27
Moss biomass	$+1^{\circ}C - +3^{\circ}C$	-0.34	-1.57	0.89	0.92
WIOSS DIOIIIASS	$+1^{\circ}C - +5^{\circ}C$	-0.27	-1.50	0.96	0.96
	$+1^{\circ}C - +10^{\circ}C$	-1.20	-2.43	0.03	0.06
	$+3^{\circ}C - +5^{\circ}C$	0.07	-1.15	1.30	1.00
	+3°C - +10°C	-0.86	-2.09	0.37	0.26
	$+5^{\circ}C - +10^{\circ}C$	-0.93	-2.16	0.29	0.19
	Control - +1°C	2.60	N.A.	N.A.	> 0.10
Lichon biomass	Control - +3°C	2.40	N.A.	N.A.	> 0.10
	Control - $+5^{\circ}C$	0.00	N.A.	N.A.	> 0.10
	Control - +10°C	0.00	N.A.	N.A.	> 0.10
Lichen biomass	$+1^{\circ}C - +3^{\circ}C$	0.20	N.A.	N.A.	> 0.10
(Non-parametric)	$+1^{\circ}C - +5^{\circ}C$	2.60	N.A.	N.A.	> 0.10
	$+1^{\circ}C - +10^{\circ}C$	2.60	N.A.	N.A.	> 0.10
	$+3^{\circ}C - +5^{\circ}C$	2.40	N.A.	N.A.	> 0.10
	$+3^{\circ}C - +10^{\circ}C$	2.40	N.A.	N.A.	> 0.10
	$+5^{\circ}C - +10^{\circ}C$	0.00	N.A.	N.A.	> 0.10

ANNEX 27: STATISTICAL RESULTS: N CONCENTRATION

Table A.27(1): Two-way ANOVA of main treatment effects and treatment interactions for N concentration. Treatments: vegetation type (5 levels: mosses, monocotyledons, dicotyledons, equiseta and lichens); Grassland (2 levels: GN and GO).

Treatment	p-value	F-value	df	$R^{2}(\%)$
Vegetation Type	< 0.001	93.74	F(4,161)	64.45
Grassland	< 0.001	22.80	F(1,161)	3.92
T _S x grassland	< 0.001	5.75	F(4,161)	3.95

Table A.27(2): One-way ANOVA and Tukey multi comparison of means test of vegetation type effect for N concentration in the GN. Treatment: vegetation type (5 levels: mosses, monocotyledons, dicotyledons, equiseta and lichens).

Comparison vegetation types	GN				
(how some transformed)	$\begin{array}{c} \text{Mean } \Delta \\ (b-a) \end{array}$	95%	n velue		
(box cox-transformed)		lower bound	Upper bound	p-value	
Mosses – Monocotyledons	0.38	0.16	0.60	< 0.001	
Mosses – Dicotyledons	0.47	0.23	0.71	< 0.001	
Mosses - Equiseta	0.77	0.50	1.03	< 0.001	
Mosses - Lichens	1.26	0.95	1.57	< 0.001	
Monocotyledons - Dicotyledons	0.09	-0.15	0.33	0.97	
Monocotyledons - Equiseta	0.39	0.12	0.65	< 0.001	
Monocotyledons - Lichens	0.88	0.57	1.19	< 0.001	
Dicotyledons - Equiseta	0.29	0.01	0.58	< 0.05	
Dicotyledons – Lichens	0.79	0.46	1.12	< 0.001	
Equiseta - Lichens	0.49	0.15	0.84	< 0.001	

Table A.27(3): One-way ANOVA and Tukey multi comparison of means test of vegetation type effect for N concentration in the GO. Treatment: vegetation type (5 levels: mosses, monocotyledons, dicotyledons, equiseta and lichens).

Comparison vegetation types	GO				
(box cox-transformed)	$\begin{array}{l} \text{Mean } \Delta \\ (b-a) \end{array}$	95%	n velue		
(box cox-transformed)		lower bound	Upper bound	p-varue	
Mosses – Monocotyledons	0.42	0.20	0.64	< 0.001	
Mosses – Dicotyledons	0.91	0.67	1.14	< 0.001	
Mosses - Equiseta	0.87	0.60	1.14	< 0.001	
Mosses - Lichens	1.10	0.53	1.66	< 0.001	
Monocotyledons - Dicotyledons	0.49	0.26	0.72	< 0.001	
Monocotyledons - Equiseta	0.46	0.19	0.73	< 0.001	
Monocotyledons - Lichens	0.68	0.11	1.25	< 0.01	
Dicotyledons - Equiseta	-0.03	-0.31	0.25	1.00	
Dicotyledons – Lichens	0.19	-0.38	0.76	0.99	
Equiseta - Lichens	0.22	-0.36	0.81	0.97	

ANNEX 28: DATA OWNER LIST

Table A.28: Data owner list.

Data	Owner	
Biomass (except spring roots)	Vande Velde Katherine	
Biomass C stocks (except spring roots)	Vande Velde Katherine	
Biomass N stocks (except spring roots)	Vande Velde Katherine	
Spring root biomass	Leblans Niki	
Spring root C stocks	Leblans Niki	
Spring root N stocks	Leblans Niki	
Necromass	Vande Velde Katherine	
Necromass C stocks	Vande Velde Katherine	
Necromass N stocks	Vande Velde Katherine	
Soil C stocks	Vande Velde Katherine	
Soil N srocks	Vande Velde Katherine	

ANNEX 29: ABBREVIATION LIST

Abbreviation	Meaning
С	carbon
GN	new grassland
GO	old grassland
Ν	nitrogen
Ts	soil temperature

Table A.29: Alphabetical list of frequently used abbreviations.