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Effects of soil warming on growth processes of unmanaged subarctic grasslands

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Clarification of contribution

I hereby declare that this project is based on my own observations, is written by me, and has not been previously submitted for a higher degree, neither in part nor in whole, and the four accompanying papers are my own work, done under the supervision and with the assistance of my supervisor, Pr. Bjarni Diðrik Sigurðsson, and co-supervisors Dr. Peter Lootens and Dr. Iolanda Filella.

My contributions to the four papers presented in this thesis were as follows:

Paper I: R.P.T. Wandji did the conceptualisation, drafting the paper, methodology and data analysis. Wandji wrote the manuscript, which was later edited by all the co-authors. R.P.T. Credits were attributed in the corresponding chapter under CRediT statement. Wandji was responsible for the correspondence with the scientific journal.

Paper II: R.P.T. Wandji collected the data provided in the study and conducted the statistical analysis. Wandji authored the manuscript, which was subsequently revised by all co-authors. R.P.T. Credits were attributed in the corresponding chapter under CRediT statement. Wandji was responsible for the correspondence with the scientific journal.

Paper III: R.P.T. Wandji contributed to the writing of the original draft, investigation, conceptualisation, methodology, collection of data and reviewing of the manuscript. As stated under CRediT of the paper the original draft, conceptualisation, revision, supervision, and other contributions was equally done collectively by Steven Mortier, Amir Hamedpour, Bart Bussmann, Ruth P. T. Wandji, and co-authors. S. Mortier was responsible for the correspondence with the scientific journal.

Paper IV: R.P.T. Wandji wrote the original document and did the revision of the draft. She also, did the conceptualisation, methodology, investigation, validation, collected data for the years 2020, 2021 and 2022 and last but not least did the data curation and formal analysis. Co-authors participated in data sampling of the years 2013, 2016, 2018, part of the 2020 data analysis, revision and more. Credits were attributed in the corresponding chapter under CRediT statement. R.P.T. Wandji was responsible for the correspondence with the scientific journal.

Ruth Phoebe Tchana Wandji

Abstract

High-latitude plant growth processes involve a range of physiological and biochemical mechanisms that allow plants to progress during relatively short growing seasons from unmature to fully developed organisms capable of reproducing.

During the past decades, terrestrial ecosystems have experienced a lot of alterations from climate change, and high latitude ecosystems are affected at a faster pace compared to other terrestrial ecosystems. Therefore, it is important to study how further warming is likely to affect high-latitude plant communities, including Iceland.

There are now 18 whole-soil warming experiments ongoing worldwide to increase our understanding of how plant and soil communities are likely to respond to further climate warming, and the ForHot research site is one of them. It utilises warm bedrocks below the soil profiles of known age to study the impacts of soil warming.

The ForHot contains six ecosystem-level field experiments that involve different amounts of soil warming, duration of warming and N-availability in different vegetation communities. Whereof I used two grassland experiments. That is the medium-term warming (MTW) site that has been warm since 2008 and the long-term warming (LTW) site with the same type of grassland, but where the warming has been ongoing for >60 years.

To understand how subarctic grassland growth processes respond to soil warming, I looked at the first step in the plant growth processes, that is, the responses in the photosynthetic system. Secondly, I studied the duration of vegetation activity (phenology) throughout the growing season with both traditional and remote-sensing methods. Lastly, I investigated how soil warming and interannual variation affected the aboveground net primary productivity (ANPP).

The main outcomes were that even if the photosynthetic capacity remained unaltered per unit leaf area under warmer conditions, the amount of community leaf area over each m^2 of surface (NDVI) increased and the duration of growth lengthened with warming. Which likely resulted together in more seasonal carbon uptake and the observed increases in ANPP under warming in both grasslands. Nevertheless, the increasing ANPP was associated with a "down-regulation" at the higher warming levels, which was possibly linked to N losses from the warmed soils. Duration of warming was generally not found to be important in plant aboveground responses.

Keywords: Iceland, photosynthesis, reproductive and growth phenology, NDVI, ANPP, remote sensing.

Ágrip

[Áhrif jarðvegshlýnunar á vaxtarferla í náttúrulegum graslendum].

Vaxtarferlar norðlægra plantna eru samspil lífeðlisfræðilegra og lífefnafræðilegra ferla sem tryggja að hæfilegur þroski og vöxtur náist innan fremur stuttra vaxtartímabila þannig að þær verði fullþroska með getu til að fjölga sér.

Á síðustu áratugum hefur veðurfar breyst, einkum á norðurslóðum, sem er farið að valda örari breytingum á gróðurfari þar en víðast annars staðar. Vegna þessa er mikilvægt að rannsaka hvernig frekari hlýnun hefur áhrif á gróður norðurslóða, þ.m.t. á Íslandi.

Það eru 18 rannsóknir í gangi í heiminum þar sem allur jarðvegsprófíllinn er hitaður til að líkja eftir framtíðar hlýnun og áhrifum hennar á gróður og jarðveg. ForHot verkefnið á Íslandi er eitt þeirra. Það nýtir einstakar aðstæður á Íslandi þar sem jarðskjálftavirkni hefur valdið því að berggrunnur hitnar á ákveðnum svæðum og hitar jarðveginn sem á honum hvílir.

Í ForHot eru alls sex vistkerfistilraunir sem skoða áhrif mismikillar hlýnunar, lengd hlýnunar eða N-ákomu í mismunandi gróðurlendum. Í mínu verkefni nýtti ég tvær af þessum tilraunum sem eru í náttúrulegum graslendum, MTW tilraunina sem inniheldur reiti með þar sem hlýnunin hófst árið 2008 og LTW tilraunina með sambærilegum reitum en þar sem hlýnunin hefur staðið yfir í >60 ár.

Til að skilja betur hvernig frekari jarðvegshlýnun hefur áhrif gróður og hvort lengd hlýnunarinnar skiptir máli, þá rannsökuðum við fyrst hvernig áhrifin hafa verið á fyrsta skrefið í vaxtarferli plantna, ljóstillífunargetu. Því næst rannsökuðum við hvort bæði vaxtarferill og æxlunarferill platna breyttist við hlýnunina, bæði með hefðbundnum aðferðum og fjarkönnun Að lokum rannsökuðum við hvernig jarðvegshlýnun hefur breytt ofanjarðar framleiðni (ANPP) í graslendunum og hversu mikill árabreytileiki er í ANPP þeirra.

Helstu niðurstöður voru þær að þrátt fyrir að ljóstillífunargeta gróðurs ykist ekki per laufflatarmál, þá gerði lengri vaxtartími með ljóstillífun og meira laufflatarmál á hvern m^2 (aukið NDVI) það að verkum að ANPP jókst marktækt við hlýnun í báðum graslendunum. Hins vegar minnkað hlutfallsleg aukning í ANPP með aukinni langtíma hlýnun sem sennilega tengdist tapi á köfnunarefni út úr vistkerfinu í hlýnandi jarðvegi. Hversu langt var liðið frá því að hlýnun hófst, áratugur eða >6 áratugir, hafði ekki mikil áhrif á grósku ofanjarðar í graslendunum.

Lykilorð: Ísland, ljóstillífun, æxlunar- og vaxtarferlar plantna, NDVI, ANPP, fjarkönnun.

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Finally, in keeping with personal cultural tradition, I would like to express my appreciation in my mother tongue; *Mə Làbtə Tshúaben Nsi, wù b'a yàm njoṅ nù fa, ben be yàm njoṅ nù fa*

List of original papers

This thesis comprises following publications, which will be referred to in the general discussion by their Roman numerals.

I. **Wandji, R. P. T**., T. Callebaut, I. Filella, P. Lootens, and B. D. Sigurdsson. 2025. Gas exchange response of *Ranunculus acris* to long-term soil warming in subarctic grasslands. [*Manuscript*]

II. **Wandji, R. P. T.**, A. Hamedpour, I. Filella, P. Lootens, and B. D. Sigurdsson. 2025. Subarctic grassland phenological response to soil warming. [*Submitted for publication in Arctic Science Journal– Status: submitted*]

III. Mortier, S., A. Hamedpour, B. Bussmann, **R. P. T. Wandji**, S. Latré, B. D. Sigurdsson, T. De Schepper, T. Verdonck. 2024. Inferring the relationship between soil temperature and the normalized difference vegetation index with machine learning. Ecological Informatics. Volume 82, 102730, ISSN 1574-9541, https://doi.org/10.1016/j.ecoinf.2024.102730. [*Published*] *Included in this thesis with a permission from the journal.*

IV. **Wandji, R. P. T.**, A. Hamedpour, A. Merand, N. Leblans, N. Verbrigghe, I. Filella, P. Lootens, I. Janssens, and B. D. Sigurdsson. 2025. Effects of soil warming and interannual variability on aboveground biomass production of subarctic grasslands. [*Submitted for publication in Arctic, Antarctic, and Alpine Research journal, 2024 – Status: accepted for review*]

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List of Abbreviations

Abbreviation	**Full description**
A	Net assimilation rate
A/C_i	Net photosynthetic assimilation rate at varying intercellular carbon dioxide concentrations
A_{max}	Maximum rate of photosynthesis under conditions of carbon dioxide saturation and high light conditions
AGB_{vasc}	Aboveground vascular plant biomass
Agr	*Agrostis capillaris*
A_{sat}	Maximal light saturated rate of net photosynthesis
A/I	Net photosynthetic assimilation rate at varying irradiance
Al	Aluminium
ANPP	Aboveground Net Primary Productivity
ANOVA	Analysis of Variance
A/T_L	Net photosynthetic assimilation rate at varying leaf temperature
B	Baron
C	Carbon
Ca	Calcium
CO_2	Carbon dioxide
C_i	Intercellular CO_2 concentration
Cu	Copper
DEM	Digital Elevation Model
DOC	Dissolved Organic Carbon
e.g.	*exempli gratia*: “for example”
EOS	End of the growing season
EOT	Ecosystem-Of-Thing
ESR	Early-Stage Researcher
Equi	*Equisetum pratense*
Fe	Iron
G_{max}	Date of maximum growth rate
H	Height
H_2O	Water vapor
I	Light intensity / Irradiance
i.e.	*id est:* “that is”
J_{max}	Maximum rate of photosynthetic electron transport
K	Potassium
L	Length
LAI	Leaf Area Index
LOS	Length of the growing season
LTW	Long-Term Warming
MAT	Mean annual air temperatures
MAT_s	Mean annual soil temperatures

Mg	Magnesium
Mn	Manganese
ML	Machine Learning
MTW	Medium-Term Warming
N	Nitrogen
NDVI	Normalised Difference Vegetation Index
NH_4^+	Ammonium
NPP	Net Primary Production
NO_3^-	Nitrate
n.s.	Non-significant ($p > 0.05$)
O_2	Oxygen
P	Phosphorus
PAR	Photosynthetically Active Radiation
PEAK	Maximum annual NDVI values
PPS	Portable Photosynthesis Systems
Poa	*Poa pratensis*
POS	Peak of the growing season
PRS probes	Plant Root Simulator probes
R_{dark}	Dark respiration
R. acris / Ran	*Ranunculus acris*
S	Sulphur
SHAP	SHapley Additive exPlanations
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
SOS	Start of the growing season
SWC	Soil Water Content
Vc_{max}	Maximum rate of Rubisco carboxylase activity
VPD	Vapour Pressure Deficit
T_a	Air temperatures
T_L	Leaf temperature
T_s	Soil temperature
T_{s10}	Soil temperature values at 10cm depth
$T_{s\text{-}sum}$	Soil temperature sums
TNT	An experiment within the ForHot research infrastructure with varying soil temperature and nitrogen addition rates
Zn	Zinc

1. Introduction

Plant growth processes involve a range of physiological and biochemical mechanisms that allow plants to progress from seeds to fully developed organisms capable of reproducing (Taiz et al., 2015). These involve several key processes, including *e.g.* photosynthesis (Kruger & Volin, 2006), phenology (Körner & Basler, 2010; Schwartz, 2013), nutrient uptake and assimilation (Chapin & Shaver, 1989; Semenchuk et al., 2015), biomass production (Shaver et al., 1996;Yu et al., 2017), and last but not least, the interactions between aboveground and belowground components (Liu et al., 2021; Wardle et al., 2013). Plant growth processes can be influenced both by intrinsic factors such as genetic selection or expression (Wareing & Phillips, 1970), and extrinsic factors, including environmental conditions and interactions with other organisms. These processes can include nutrient dynamics (Christiansen et al., 2012; Jonasson et al., 1999; Semenchuk et al., 2015) and other environmental influences; light (Mølmann et al., 2021), temperatures (Hollister, 2003), water availability (Thorsteinsson et al., 2019), and other soil conditions (Arnalds, 2015; Schmidt et al., 1999).

High-latitude terrestrial ecosystems (>60°N) are characterised by extreme environmental conditions, such as, long, cold winters, and short growing seasons (Bai et al., 2010; Elmendorf et al., 2012). These are abiotic factors that have strongly shaped the characteristics of the plant and animal lifeforms which are able to exist in these environments (Callaghan et al., 2004).

Biota in these ecosystems experience now a lot of alterations from climate change. This phenomenon involves a range of factors including the rising of temperatures and precipitation changes associated with global warming and other anthropic activities (Dentener et al., 2013; IPCC, 2023). One notable aspect of climate change in high-latitude ecosystems, is the rate of warming. The Arctic and subarctic, for instance, is warming at more than twice the global average (IPCC, 2023). This accelerated warming is largely due to Arctic feedback mechanisms, such as changes in albedo and increased greenhouse gas emissions from thawing permafrost, which further enhances climate change impacts (Koven et al., 2011; Schuur et al., 2015). The implications of these changes can be profound, affecting high-latitude biodiversity (Post et al., 2009, Schuur et al, 2015), carbon (C) cycling (Bai et al., 2010; Oberbauer et al., 2007), inducing changes in plant physiological or phenological responses (Starr et al., 2008) and altering various other growth processes (Myers-Smith et al., 2011).

Subarctic grasslands

Unmanaged high-latitude grasslands, including tundra with continuous permafrost, cover approximately 10% of the global terrestrial surface (Chapin et al., 2011) and its soils act as significant C reservoirs, storing approximately 50% of global soil organic carbon (SOC) (Xue et al., 2016).

Icelandic lowland grasslands cover 4400 km² (Ottósson, et al., 2017) , hosting a variety array of perennial plant species which include diverse grass species and herbs (Kristinsson; & Sigurðsson, 2010). They have typically frozen soils during winter, but do not contain permanent permafrost. The local plant species have adapted to environment-specific weather fluctuations and temperatures (Thorvaldsson & Björnsson, 1990; Thorvaldsson & Martin, 2004). Nitrogen (N) supply is often a significant constraint on plant production in most Icelandic ecosystems (Gudmundsson et al., 2004). In these cold ecosystems, characterised by brief periods of plant growth, the breakdown of soil organic matter (SOM) occurs at a slow pace, resulting in a slow cycling of N and other essential elements (Aerts et al., 2012).

Vegetation response to warming

Rising temperatures are expected to directly impact plant activity at higher latitudes through the lengthening of the growing season, and more importantly, enhance the availability of N and other nutrients by promoting decomposition (Pregitzer & King, 2005). Increased availabilities in N can lead to increased aboveground biomass (Paper IV), thus, contributing to changes in the ecosystem C-dynamics.

Soil warming experiments have been established across high latitudes since the 1980s with the aim to get more knowledge into how these ecosystems will respond to increasing temperatures (Gestel et al., 2019). Those studies have shown that experimental (soil) warming can stimulate C-fluxes in grassland ecosystems, leading to increased plant production and soil respiration (Wang et al., 2019). For instance, with soil warming, stronger responses in C-losses from soils have been observed due to enhanced heterotrophic respiration with warming (Marañón-Jiménez et al., 2018; Propster et al., 2023; Shaver et al., 1992). However, how long such C-losses from the soil can continue, also depends on the longer-term impact of warming and on the degree to which canopy photosynthesis and biomass production are enhanced.

Phenology, or the timing of plant life cycle events, has been studied since the mid-19th century (Templ et al., 2018). It has gained more interest in recent decades, as it is generally an important component of how terrestrial ecosystems respond to ongoing and future climate change. Traditional phenological monitoring often relies on direct field observations, where status of specific phenophases are scored for individual plants (Paper II). On the other hand, modern methods, such as remote sensing (Yingying et al., 2018; Zhang et al., 2003) or instrumental monitoring (Paper III, Leblans et al., 2017) which rely on satellite imagery, drones, phenocams, hand-held instruments, are becoming more common. These have enabled the monitoring of plant phenology over large spatial scales (Forkel et al., 2013; Pei et al., 2019), but those methods have rarely been compared to the traditional manual scoring (Paper II).

The start of the growing season in high-latitude ecosystems can be influenced by a variety of factors. Perennial plant species are common in Icelandic grasslands, and they grow and flower in the summer, senescence in the autumn and regenerate from their rootstock in the subsequent spring, provided that environmental circumstances are favourable (Bjarnason, 1993; Hopkins et al., 2014).

As the plants mature, they enter the reproductive phase marked by flowering and production of seeds. The reproductive phase is a complex process regulated by environmental cues, such as the relative length of the days and nights (photoperiod) (Adams & Langton, 2005; Jackson, 2009) and temperature (Bahuguna & Jagadish, 2015; Kerbler & Wigge, 2023; Legris et al., 2017). For those plant species which are not wind pollinated, a successful flowering may also have to be synchronised with the occurrence of key pollinators (Bokhorst et al., 2009). In fact, warming experiments have shown that earlier warming-induced flowering may potentially disrupt plant-pollinator interactions, and lead to mismatches that reduce reproductive success (Post et al., 2018).

At high latitudes, increased temperatures extend the growing season by causing earlier snowmelt and delaying the onset of winter (Yu et al., 2010; Zhu et al., 2012), which can enhance plant growth and biomass production through enhanced seasonal C-uptake by photosynthesis. However, this might possibly disadvantage Arctic species adapted to shorter growing seasons compared to more southern species which can use the longer growing seasons or the new growing space more effectively (Callaghan et al., 2004). This can for example be seen, in the increased occurrence of perennial woody (shrub/tree) species, in areas where previously only Arctic herbaceous plants could survive (Chapin et al., 2011; van Huissteden, 2020).

In the Arctic (Alaskan tundra), a 20% increase in aboveground biomass was recorded as a result of experimental soil warming throughout the year, while increases in summer air temperature alone did not yield similar effects (Natali et al., 2012). The increase in winter and spring temperature also causes shifts in phenology (Bokhorst et al., 2009) and may also strongly influence soil C and nutrient cycling through enhanced soil decomposition rates (Cornelissen & Makoto, 2014; Dubeux et al., 2007), which again can stimulate plant growth and C-uptake (Paper IV).

There have been many studies which have predicted that the effect of future warming on heterotrophic microbial respiration will potentially turn Arctic grasslands into net C-sources (Jahn et al., 2010; Crowther et al., 2019). However, enhanced plant growth could also partially offset this loss or at least create a new steady state after some initial losses, by increasing C-inputs through enhanced vegetation photosynthesis (Hartley et al., 2012).

The relationship between N-availability and plant growth dynamics in high-latitude ecosystems is another critical aspect. Increased decomposition accelerates nutrient cycling, often favouring species that can capitalise on elevated nutrient availability, thereby altering competitive dynamics of the existing ecosystems (Sistla & Schimel, 2013). If, however, the N is not contained in the active N-cycle these ecosystems can remain N-limited for plant production (Chapin & Shaver, 1989).

Even if warming would alleviate the N limitation, there are still other limits to plant productivity in the variable climate of the subarctic region. Research on the seasonal variability of plant growth in fertilised cultivated grasslands in Iceland has indicated that in the absence of N-availability limitations, temperature, irradiance and precipitation fluctuations still were all important drivers for growth rate (Thorvaldsson & Martin, 2004).

Gas Exchange processes

Gas exchange process in plants is often referred to as the movement of gases, primarily carbon dioxide (CO_2), oxygen (O_2) and water vapor (H_2O) between plants and the environment (Pereira, 1995). These processes occur mainly through microscopic openings on the leaf surfaces called the stomata. They are flanked by guard cells that regulate their opening and closing in response to environmental conditions, thereby controlling gas exchange and transpiration rates (Arve et al., 2013; Ueda et al., 2022). During the process of photosynthesis, which is the foundational process by which plants harness energy from sunlight to synthesise glucose from CO_2 and H_2O, plants take in CO_2 from the air via the stomata. The glucose serves as a vital energy source, fueling various metabolic activities and providing the raw materials for growth (Calvin, 1976), and is often considered to be the first step in the growth process of plants. Photosynthesis not only supports the metabolic activity and growth of individual plants but also plays a critical role in the global C-cycle (Amthor, 1995; Chapin et al., 2011; IPCC, 2023).

Models and theories have been developed to understand this process throughout time. The most widely adopted biochemical model that describes the process of carbon fixation and photorespiration in C3 plants, integrating the kinetics of the enzyme Rubisco (Farazdaghi, 2011), and the electron transport rate is the Farquhar model (Farquhar et al., 1980). This model has been instrumental in predicting photosynthetic responses to environmental factors like light intensity (irradiance) (Bellasio, 2019), CO_2 concentration, and temperature (Berry & Bjorkman, 1980; Harley & Tenhunen, 2015). In order to measure photosynthetic rates an infrared gas analyser (IRGA) is mainly used, connected to a cuvette (chamber) that contains known leaf- or surface area, and employed over known time interval. This quantifies the rate of CO_2 uptake (*e.g.* μmol CO_2 m^{-2} leaf area s^{-1}) or the O_2 release by plants by providing a direct measure of photosynthetic activity (Paper I, Harley & Tenhunen, 2015).

So as to understand how plants respond to varying environmental conditions, photosynthetic response curves are used. For example, with the Farquhar model, to derive key biochemical constants that determine how the species in question responds to *e.g.* variations in light intensity and (intercellular) CO_2 concentrations. The most common curves used illustrate the relationship between the net photosynthetic assimilation rate and the intercellular CO_2 concentration (A/C_i curves). Another widely used type shows the plant's (leaf's) response to varying light concentration at the leaf's surface, which is also termed irradiance (A/I curves; Chapin et al., 2011). More rarely such response curves are made for other environmental factors, such as leaf temperature (A/T_L curves; Gudmundsdóttir & Sigurdsson, 2005) or vapour pressure deficit (VPD). Similar response curves can also focus on how the opening or closure of stomates reacts to changing T_L and VPD (Ethier & Livingston, 2004), thereby revealing key parameters for understanding (and modeling) the process of net photosynthesis at both plant- or whole community levels. The key parameters used in such models are usually the saturation points, compensation point (the value of the driver where net-photosynthesis is zero), and others (Crous et al., 2010). The shape of these curves can vary among different plant species and ecosystems, influenced by factors such as nutrient availability, general light and water availability, and temperature fluctuations (Wilcox et al., 2017).

Rising atmospheric CO_2 concentrations enhance photosynthesis in plants as more CO_2 gets into the leaves at the same stomatal opening, which increases the intercellular CO_2 concentration (C_i), which again increases the rate of C-fixation by the Rubisco enzyme (Drake et al., 1997; Lawson et al., 2022).

Increase in air temperatures (T_a) during the day can both increase or decrease photosynthetic rates, depending on what is the optimum temperature for photosynthesis of the plant species in question (Gudmundsdóttir & Sigurdsson, 2005). However, increasing T_a always leads to exponentially greater mitochondrial respiration rates, which may offset gains in photosynthesis, particularly if moisture becomes limiting so the stomates start to close (Zhang et al., 2018).

Moderate warming over longer time periods can enhance photosynthesis at higher latitudes by increasing enzyme activities (Moore et al., 2021), but mainly by extending the growing season, allowing plants to capitalise on longer periods of favourable conditions of growth (Papers II & III). However, as temperatures continue to rise, they may exceed the optimal range of photosynthetic activity Berry & Bjorkman,1980; Hussain et al., 2021), which might lead to a decline in photosynthetic efficiency. Respiration rates are highly sensitive to temperature increase, as previously stated, often rising more sharply than photosynthesis with warming (Dusenge et al., 2019). Enhanced respiration can lead to C-loss from plants potentially offsetting the gains in C-uptake from increased photosynthesis (Pendall et al., 2013). This imbalance between photosynthesis and respiration under warming conditions could indicate a decrease in net C-gain of high-latitude ecosystem, affecting their role as C-sinks.

In high-latitude ecosystems, nutrient availability, particularly N, often limits plant growth more than CO_2 (Shirley et al., 2022). While increased CO_2 and warming may initially boost photosynthesis, the response is constrained if nutrient supplies do not increase accordingly (Keane et al., 2023). Soil warming can release nutrients, but the rapid microbial uptake and altered soil processes, including increased dissolved organic carbon (DOC) and nutrient leaching (Verbrigghe et al., 2022b), can make these nutrients less available to plants, thereby limiting the potential positive effects on gas exchange processes (Pereira, 1995; Paper I).

The ForHot as a case study

The ForHot research infrastructure in Iceland (forhot.is; Figure 1) presents a unique natural laboratory for studying the effects of soil warming on plant communities and other processes. The soil characteristics and unique temperature variations in geothermal regions facilitate the study of plant responses to warming (Sigurdsson et al., 2016), offering some insights into the mechanistic of how present and future climate change may affect plant metabolism and community composition.

The ForHot project was initially started in 2011, following an earthquake in S-Iceland in May 2008 which shifted soil warming gradients from warm bedrocks to new areas (Sigurdsson et al., 2016). The grassland experiment that was established on the warming gradients from 2008 is called Medium-Term Warming (MTW). In 2013, another experiment was established on the same type of grassland that had been warm since at least 1963, and it is called Long-Term Warming (LTW).

Over a decade of data from the ForHot reveal how soil temperature (T_s) affects soil microbial activity and microbial communities (Dahl et al., 2023; Radujković et al., 2018; Soong et al., 2020; Verbrigghe et al., 2022a; Walker et al., 2018), nitrogen dynamics (Marañón Jiménez et al., 2023; Meeran et al., 2023), and many other processes. Moreover, after only six to ten years of warming, it was observed that the soil organic carbon (SOC) had decreased (Poeplau et al., 2017; Walker et al., 2018), and also, the C-loss in the topsoil (SOC) did not continue after the initial five to ten years (Poeplau et al., 2020; Verbrigghe et al., 2022b). (Verbrigghe et al., 2022b), thus, highlighting the ecosystem's "new" steady state.

Further description of the ForHot research infrastructure can be found in the Methods part of this thesis.

The FutureArctic PhD consortium

This PhD dissertation work was conducted within the FutureArctic PhD consortium, which was established in 2019 with a four-year grant from Marie Curie Innovative Training Network, Horizon 2020. Behind the PhD consortium were eight universities and four private companies and within it 15 PhD students were given opportunities to do their dissertation research, which all included some work at the ForHot infrastructure in Iceland.

Half of the PhD projects were within different subjects of environmental and Earth sciences, including my PhD project, while the other half was within engineering, technical- and social sciences.

The aim of the PhD consortium was to do state-of-the-art research on the climate challenge and the particularly important role therein of (sub)Arctic ecosystems. Its main aim in the environmental science part was to:

I) Study how much C will escape from the (sub)Arctic in future climate?

II) How do the multitude of ecosystem processes, driven by plant growth, microbial activities and soil characteristics, interact to determine (sub)Arctic soil carbon storage capacity? Papers I, II, III and IV

Its main aim in the technical part was to:

III) Initiate a new machine-learning approach to analyse large high-throughput environmental data-streams (Paper III)

IV) Install a pioneer "ecosystem-of-thing" (EOT) at the ForHot infrastructure.

V) Develop a rapid assessment of the ecosystem and ambient processes that will provide potentially crucial insight in future carbon cycling.

VI) Pave the way for generalised permanently connected data acquisition systems for key environmental variables and processes.

Figure 1 gives an overview over different work packages of the FutureArctic PhD consortium.

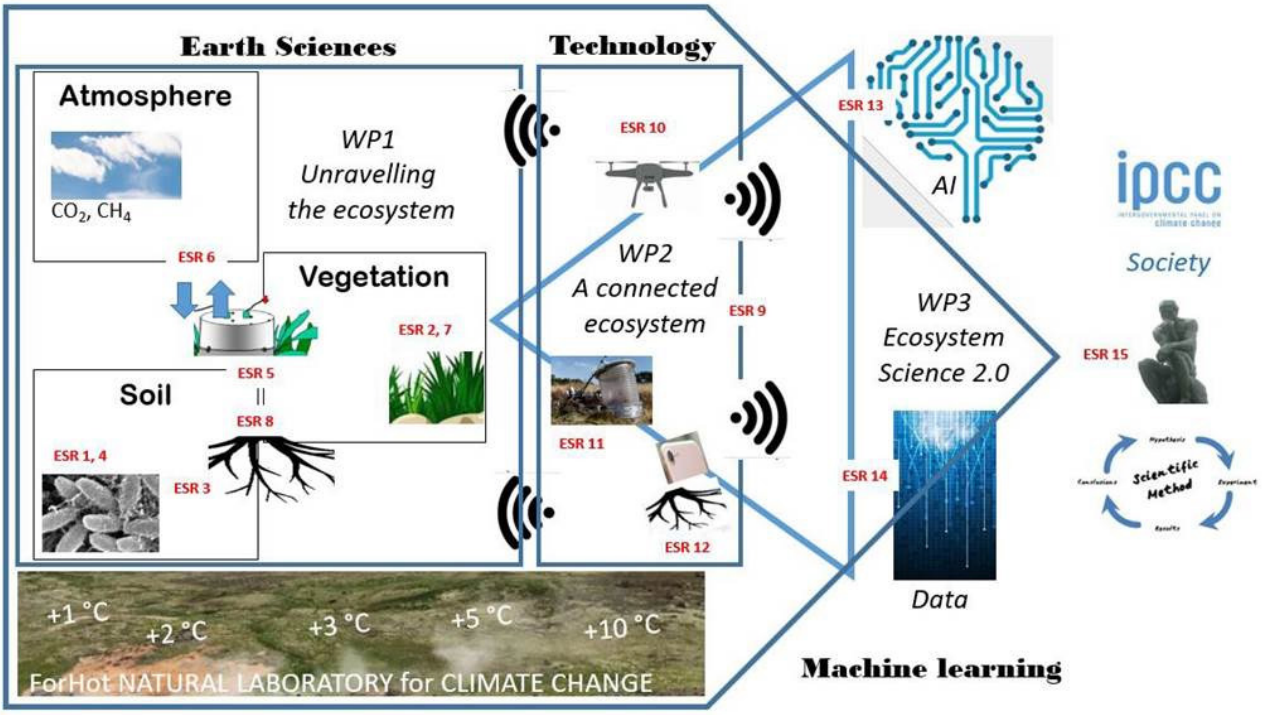

Figure 1: The structure of the FutureArctic PhD consortium (June 2019 – June 2024). Further information can be found at www.futurearctic.be

The background of my studies within the FutureArctic

My research had to large extent been related to the unexpected stabilisation of SOC after the first five to ten years of soil warming. For a new "steady state" in C-losses to occur in a warmer world, there has to be either some decreases in C-losses at a given T_s or the C-inputs from plants has to increase from the previous levels, or both.

I have focused on different aboveground processes in the grassland communities which could help to understand how soil warming is affecting their C-dynamics. Within the FutureArctic my PhD project was ESR7 (Figure 1).

Figure 2: ForHot research site in south of Iceland. Medium-term soil warming site showcasing different experimental setups at the site (forhot.is, futurearctic.be).

Another FutureArctic PhD student at Copenhagen University, Linsey Avila (ESR2; Figure 1), has at the same time been doing measurements with large automatic chambers on how soil warming has affected the grassland community gas exchange (Figure 2). Her data is not yet available.

The third FutureArctic PhD student at Tartu University, Biplabi Bhattarai (ESR3; Figure 1), and other researchers at ForHot have focused on how the warming is affecting the plant belowground net primary production and other C flows from the plants into the soil food web (Bhattarai et al., 2023, 2024; Fang et al., 2023; Meeran et al., 2023).

Ultimately, my work, together with other FutureArctic PhD students and other ForHot colleagues, will contribute to assembling the different pieces of the puzzle to fully understand how the subarctic ForHot grasslands reach a new steady state in ecosystem C balance and cease to lose SOC in a warming world.

1.1 Objective and research questions of the thesis

The main goal of this research was to understand how unmanaged subarctic grasslands respond to prolonged natural soil warming. To tackle the long-term aboveground vegetation responses in a naturally soil-warmed ecosystems, the following research questions were posed:

1. Will the vegetation C-uptake rate be enhanced with soil warming at both MTW and LTW grasslands?

   This research question examined whether the photosynthetic capacity and seasonal C-uptake was enhanced with soil warming at both studied subarctic grasslands. To address this, firstly, the physiological gas exchange responses of a selected key subarctic species (*Ranunuclus acris*) was studied (Paper I). Then secondly, the use of Normalised Difference Vegetation Index (NDVI) was employed to monitor the amount of vegetation (leaf area) across the warming gradients (Paper III).

2. Will the photosynthetic C-uptake span over a longer period with warming? (Paper II and III)

   This study dived into the changes in vegetation seasonal life cycle across the soil temperature gradients at both grasslands (MTW, LTW). The reproductive and vegetative growth phenology of four key Icelandic grassland species was assessed at the species level (Paper II) and at a community level by remote sensing, that is, repeated Digital Elevation Models (Paper II), and NDVI multispectral signatures (Paper III).

3. Will there be more vegetation aboveground biomass production associated to soil warming in Icelandic grasslands? (Paper IV)

   To explore this question, the aboveground vascular plant biomass (AGB_{vasc}) was repeatedly measured so as to look at the annual variation in aboveground net primary production (ANPP) across the soil temperature gradient (from 0°C to +13°C) at both subarctic grasslands during the past decade (Paper IV).

1.2 Summary of investigations

Throughout this dissertation research, one paper has already been published, two have been submitted for scientific review, and lastly one manuscript is close to submission. Other papers have also been co-authored during my PhD research, which are not part of my dissertation.

The assessment of photosynthetic capacity was conducted on one key Icelandic grasslands species (*Ranunculus acris*) across two subarctic grasslands (MTW, LTW). This study is the first paper under this thesis.

Followed by the plant phenological assessment of four key subarctic plant species at the same natural experimental grassland sites (MTW, LTW), where I studied the soil warming effects on the duration of active growth and reproduction. This phenology study encompassed manual measurements (reproductive and vegetative growth phenology) at the species level alongside drone-based remote sensing using the Digital Elevation Model method at the community level (vegetative growth phenology).

The third paper continues in assessing plant vegetative phenology but focused specifically on the interannual variation in vegetation phenology and the amount of active vegetation per unit area. It was also coupled with machine learning to understand different entangled relationships. This research was equally conducted at both the MTW and LTW sites.

Lastly, the fourth paper studies the aboveground net primary productivity (ANPP) response to soil warming at the MTW and LTW sites. The aboveground biomass was studied over the years 2013, 2016, 2018, 2020, 2021, and 2022. Alongside soil warming effect, I equally looked at the interannual variability effect.

2. Material and Methods

2.1 Studying Site

The research was all carried out at the ForHot natural experimental studying site located in the south of Iceland (64.008°N, 21.178°W). The soil characteristics of the site is silandic andosol with a silt loam texture soil type (Arnalds, 2015). Located at an elevation of 100 to 225 m a.s.l and characterised by an oceanic climate. The snow cover throughout the winter is not permanent, and unwarmed soils normally remain frozen for some months in winter (Sigurdsson et al., 2016). One of the first studies at the site was conducted by Sigurdsson et al. (2016), where they thoroughly described the experimental approach used in this research.

Throughout this research, I have focused on two grassland sites, that is, the medium-term warming (MTW) and long-term warming (LTW) – Figure 3. The LTW grassland has had its soils warmed for over 60 years, but the MTW the warming started in 2008 when the geothermal channels in the underlying bedrock were disrupted by an earthquake.

Within these two grassland sites, 60 permanent survey plots (2m x 2m) were established in 2013, encompassing soil warming between 0 to ca. +15°C, but for the different research papers included in this thesis, the soil warming levels were redefined. In Paper III (NDVI), 50 permanent plots were used, that is, 25 per grassland site; five soil warming levels per transect with a total of five transects. For Paper II (phenology), five transects were used for each grassland (MTW, LTW), and within each transect three soil warming levels. In Paper I (gas exchange), three transects were used within each grassland with two soil warming levels. Lastly, for Paper IV (AGB_{vasc}), I used five transects and five soil warming in each grassland.

Figure 3: Sampling sites (MTW – a, LTW – b & c) showing the experimental setup.

The vegetation type at both research sites is quite similar. That is, perennial herbaceous flowering plants, mosses, and lichens (Figure 3). Both sites have limited anthropogenic activities, in the exception of sheep grazing at the LTW, where fences were installed during the setting of the permanent plots in 2013 to exclude it.

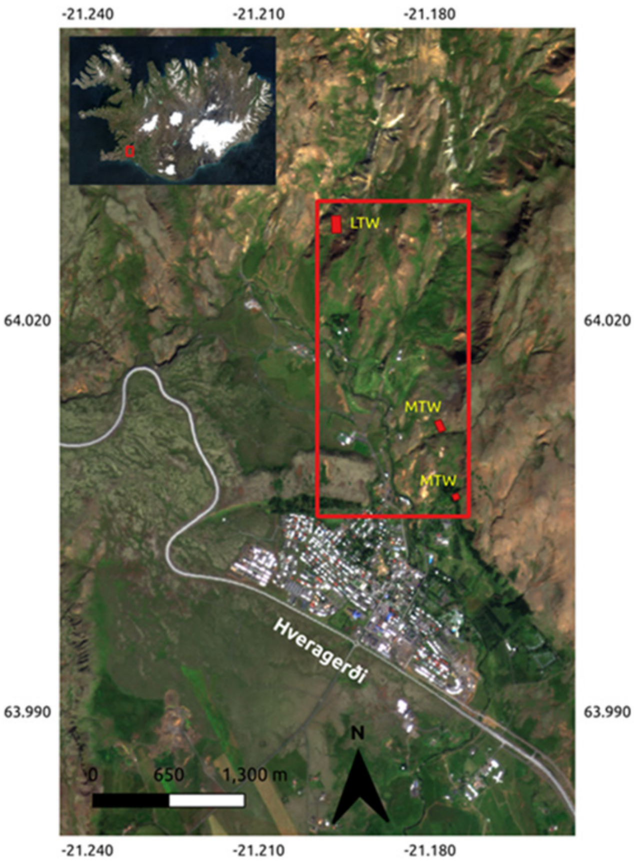

Figure 4: Location of Icelandic grassland (MTW, and LTW) studying sites in the south of Iceland next to Hveragerði village. (map courtesy from FutureArctic fellow Amir Hamedpour – ESR10).

During my own sampling period, that is, from the summer 2020 to the fall 2023, the mean annual atmospheric temperature (MAT, °C) at both Icelandic grasslands in the coldest month 0.7°C and warmest month 11.1°C. With an annual accumulated hourly-daily precipitation (MAP, mm) of 769 mm (Reykjavik station 1, 64°13'N 21°90'E; Icelandic Meteorological Office, 2023).

At the ForHot experimental sites (Figure 4), hourly-daily values of soil temperature (MAT$_s$) were measured in each permanent plot with soil temperature loggers, TidbiT v2 HOBO® data loggers; Onset Computer Corp., Bourne, MA, USA) that were placed at a depth of 10 cm in the soil. Then daily and annual average temperatures, varying from unwarmed (ambient) plots to the highest warmed plots, were calculated for each grassland experiment (Figure 5).

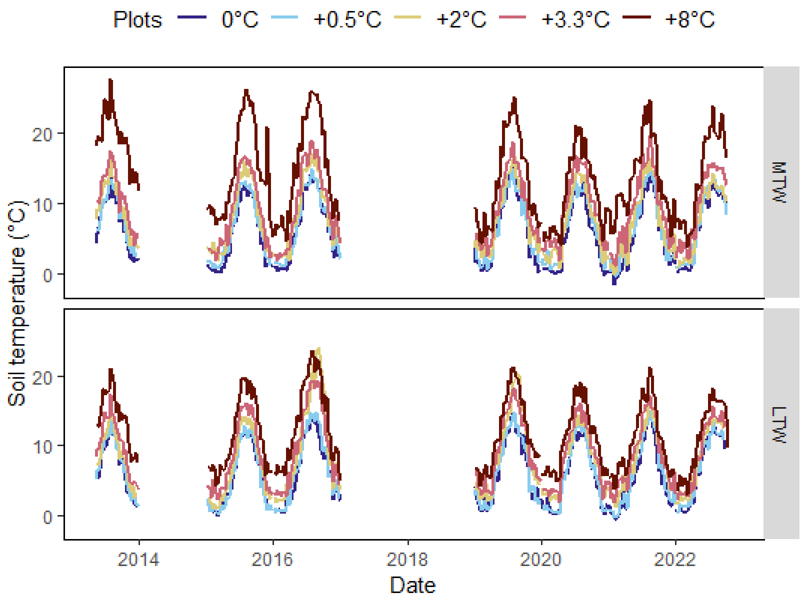

Figure 5: Example of daily soil temperature values at 10cm depth (T_{s10}) recorded during the sampling period. The years 2013, 2016, and 2018 were added as these were used in Paper IV.

The soil water content (SWC, %) was sampled using the ThetaProbe moisture meter (Figure 6, HH2 Moisture Meter, AT Delta-T devices, Burwell, Camb., UK). The SWC probe assess the volumetric soil moisture content at a depth of 5cm by detecting variations in the dielectric constant of moist soil. I specifically chose to show here the SWC values during peak growing season, because soils are normally water saturated before June, because of soil thawing, and after August due to the onset of autumn rains and winter temperatures.

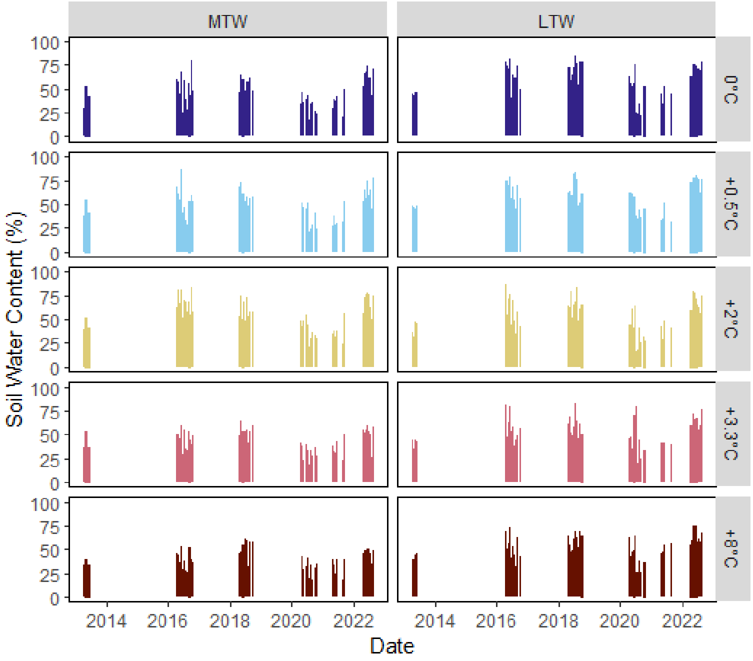

Figure 6: Soil water content recorded during the peak of the growing season throughout the research period with the inclusion of the years 2013, 2016 and 2018 to have an overview of the soil water content values during those years (Paper IV).

Nutrients were measured at the soil and plant level (leaf-N – Paper III). Plant Root Simulator probes (µg nutrient/10 cm^2/time of probe burial; PRS probes, Western Ag Technology, Sk, Canada) were used to measure available nutrients in the soil solution throughout one growing season. Probes were buried and retrieved throughout the growing season *i.e.* from April to September 2021. Campaigns were: two for the spring, one for the summer and one for the winter. The probes were buried for a week, following the protocols provided by Western Ag Innovations.

The plant nutrient concentrations were analysed on 1) vascular plants after aboveground biomass laboratory sampling was done (Paper IV) and 2) leaves harvested from gas exchange measurements (Paper I). All samples underwent ball milling (Restsch, MM200, Haan, Germany) before being transported to respective laboratories for chemical analysis, that is, Akranes – Iceland and University of Tartu – Estonia, respectively.

2.2 Plant and vegetation measurements

Plant and vegetation were assessed using different methodologies, gas exchange measurements, NDVI, plant phenology (manual scoring measurements and remote sensing), and lastly, harvesting the aboveground net primary productivity after the peak growing season.

I. Photosynthetic activity assessment

The photosynthetic assimilation rate (Paper I) was assessed using the LI-6800 Portable Photosynthesis Systems (PPS) to measure gas exchange primarily for two key subarctic plant species (*Agrostis capillaris* and *Ranunculus acris*). Due to difficulties during measurements of *Agrostis capillaris*, resulting in high variability of estimated photosynthetic parameters, I focused only on *Ranunculus acris* (Paper I). During measurements, the instrument was equipped with a large leaf chamber and a compatible light source, allowing for accurate measurements of different attached leaves in the field, but where light, temperature, humidity and CO_2 concentration could be controlled. The leaf area within the chamber was measured using a leaf scanner WinSEEDLE 5.1a leaf scanner (Regent Instruments Inc., Quebec, Canada). The response curves focused on the relationship between net photosynthetic assimilated CO_2 (A) and intercellular CO_2 concentrations (C_i), and the relationship between A and light intensity (irradiance) (I).

II. Seasonal vegetation assessment

Firstly, the individual plant phenology (Paper II) was assessed. The study focused on four key Icelandic grassland species response to soil warming (Table 1). That is: two monocots; Poales (*Agrostis capillaris*, *Poa pratensis*), one pteridophyte; Equisetale (*Equisetum pratense*), and one eudicot; Ranunculale (*Ranunculus acris*). (Figure 7).

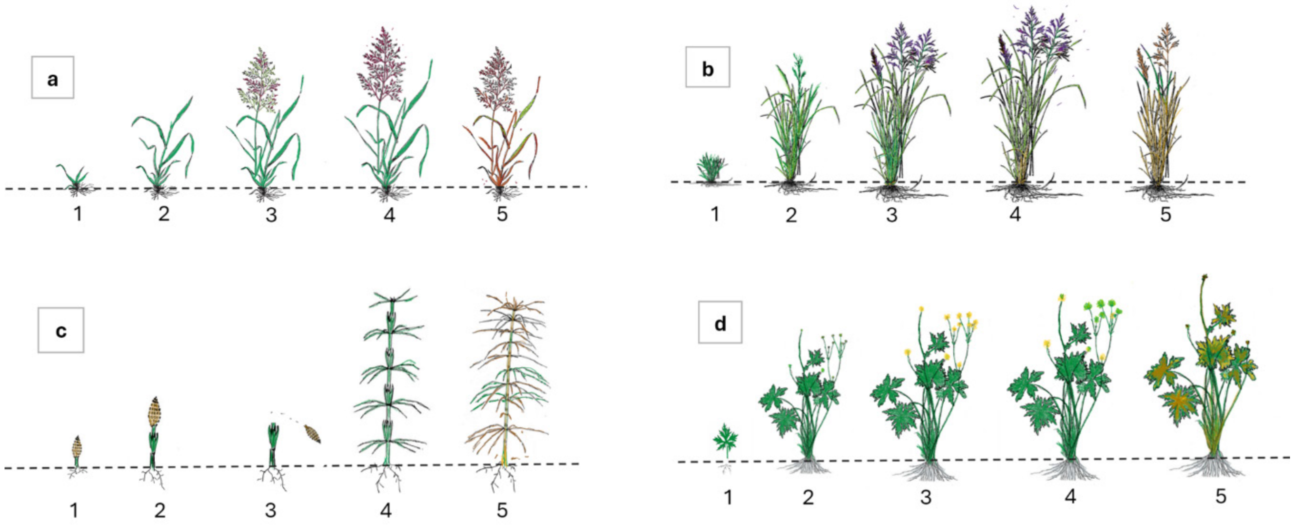

Figure 7: Plant phenological assessment of four Icelandic grassland species; a) *Agrostis capillaris*, b) *Poa pratensis* c) *Equisetum pratense*, d) *Ranunculus acris*. The numbers represent the phenological stages of each species which are explained in Table 1. (Paper II)

Table 1: Phenological stages recorded; vegetative phenology, that is, all growth stages from the first leaves to the senescence of the plant (scoring and measurement - L, cm). The reproductive phenology includes the different scoring of appearance of first reproductive traits. (Paper II)

Growth phenology		**Vegetative and reproductive phenology**
Length growth of first emerging leaf (0.0 cm)	*Agr, Poa*	first leaf (1), first flowering stem (2), first flowers (3), first seeds (4), first signs of senescence (5)
	Equi	first sight of reproductive cone (1), first sight of green stem + cone (2), cone fall (3), first horizontal leaves (4), first signs of senescence (5)
	Ran	first leaf (1), first flowering buds (2), first flowers (3), first seeds (4), first signs of senescence (5)

The plant species were randomly selected and marked in the autumn of 2020. Their emergence was then surveyed in winter/spring 2021 and after first emergence they were scored every 14 Julian days from March to November as they went through their vegetative growth and reproduction phases (Figure 7; Table 1). The phenological phases were divided into reproductive, vegetative phases (where visual scoring was used) and growth phenology (where leaf length was repeatedly measured) (Table 1). Simultaneously, a drone was flown over the grassland sites and repeated digital elevation models (DEM) were constructed to follow vegetation height growth (remote sensing).

Paper III manually measured NDVI with a handheld instrument (SKYE Instruments, SpectraoSense2) was used at both studied sites (LTW, MTW) as a proxy to vegetation phenology to infer the relationship between soil temperature and the sampled parameter with machine learning. Sampling was done from the years 2013 to 2023 with an interval of two weeks from April to November with the exclusion of permanent snow covers periods, usually in early spring and late fall. To avoid over-estimation or under-estimation, sampling was done under optimal weather conditions. However, in Paper III the years used were 2014 to 2019.

III. Plant productivity assessment

Aboveground biomass was harvested in a 0.2 × 0.5 m frame in each plot. Sampling was done for the years 2013, 2016, 2018, 2020, 2021, and 2022 at peak growing season (late July). The AGB_{vasc} samples were then sorted in the laboratory and dried at 105°C for 48 hours to obtain their dry weight. Though vascular plant, moss and lichen biomass was sampled, this study (Paper IV) focused only on the vascular plant biomass (AGB_{vasc}), because of its perennial herbaceous aboveground tissues.

2.3 Statistical Analysis

Analyses were conducted using R (R.4.2.2, R Core Team, 2022) and Python (Raybaut, 2009) software. Regression analysis and machine learning techniques were used to answer our research questions in Paper III.

To assess the plant phenological growth processes in Paper II, I conducted a non-parametric test (Kruskal-Wallis, *kruskal.test* in the *stats* package of R) to look at phenophases and soil warming levels based on reproductive phenology features. I later looked at the vegetation phenology using a double logistic function fitting with Python software (manual recording and DEM). From the fitted curve, the growth phenological parameters were extracted. That is, the start of the season, the maximum growth, the peak of the season, the end of the season and the length of the season. A linear regression was then conducted between various factors to assess the relationship between them.

To look at the effects of warming on calculated photosynthetic parameters (Paper I), an analysis of variance (ANOVA, *aov*, *stats* package - R) was performed to observe the species-specific response to soil warming, and a regression model to look at the direction of the responses.

In Paper IV, a multiple linear regression with interactions was equally performed using the *lm* function in the *stats package* in R with dependent variable AGB_{vasc} and independent variables soil temperature and site. To assess the interannual variability, I looked at the cold plots and performed a linear model with temperature sums and years as independent variables over two three time periods (July to July and November to July and June to July) prior to the harvest measurements. Then, a linear mixed-effect models analysis of variance – ANOVA (*lme* function in the *nlme* package – R) was fitted to the AGB_{vasc} with years as random effects, and environmental variables as fixed effects. Furthermore, the temperature sums were calculated with respect to a baseline temperature of 0°C using these daily mean temperatures, assuming that plant "activate" their growth at this particular baseline temperature.

3. Main Findings

Growth processes at both the community (Papers II, III, IV) and species (Paper I) levels were observed to be affected by warming and other environmental factors when looking at a decade of soil warming manipulation (Paper IV), five years period (Paper III) and one year period (Paper II, I).

3.1 Photosynthetic activity with soil warming

Firstly, the assessment of the photosynthetic activity per unit leaf area, the Farquhar model (Farquhar et al., 1980) and convexity equations were used to estimate key photosynthetic parameters for both the net photosynthetic assimilated CO_2 (A) and the intercellular CO_2 concentrations (C_i) – A/C_i curves – and between A and the irradiance (I) – A/I curves (Paper I). The outcomes showed that there was no significant effect of soil warming, nor duration of warming on the two key Farquhar model parameters (maximum rate of Rubisco carboxylase activity (Vc_{max}) and the maximum rate of photosynthetic electron transport (J_{max}) (Figure 8). This was contrary to the stipulated hypothesis.

Moreover, most other photosynthetic parameters derived from the A/C_i curve convexity formula, such as the maximum rate of photosynthesis under conditions of CO_2 and high irradiance (A_{max}), carboxylation efficiency, and others useful to process-based modeling parameters of carbon dynamics were not significantly altered by prolonged soil warming nor site ($p > 0.05$). In addition, the A/I curve convexity formula parameters: maximal rate of photosynthesis at saturating conditions of light (A_{sat}), and more importantly, the quantum yield (Φ) and dark respiration (R_{dark}), were neither significantly affected by prolonged warming nor site warming ($p > 0.05$)

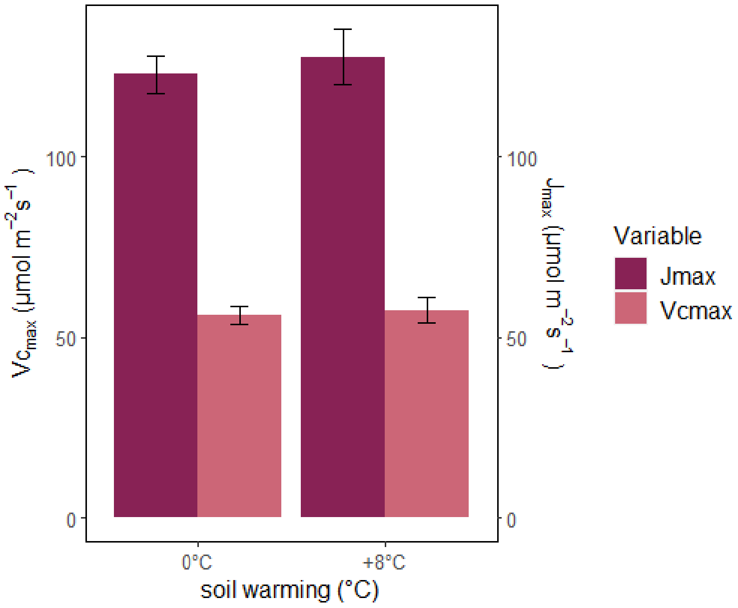

Figure 8. Farquhar model estimated parameters (Vc_{max}, J_{max}) relationship of *Ranunculus acris* to soil warming at the MTW, LTW ForHot sites in Iceland. Neither warming nor site were significantly different. Taken from Paper I. The variable represent the two Farquhar modelled parameters on the y-axes (Vc_{max} and J_{max}) and soil warming levels are the ambient temperatures (0°C) and warmed plots (+8°C) on the x-axis.

Based on the above findings for one of the key species of the subarctic grassland, photosynthetic rates were relatively high but were not affected by prolonged warming or the duration of warming (site differences), when expressed per unit leaf area (m^2 leaf area).

In Paper III, the maximum annual NDVI values (PEAK) were analysed in relation to soil warming in the two grasslands across five warming levels and multiple years. The two sites did not differ significantly and were therefore merged, but there was a significant linear increase in grassland community peak NDVI values with increasing soil warming levels (Figure 8).

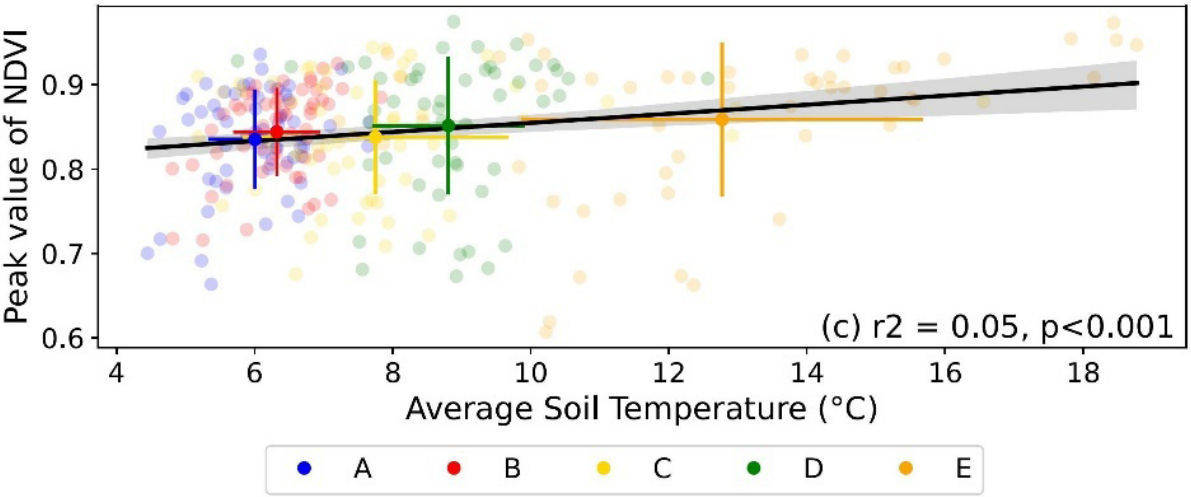

Figure 9: Linear regression between the peak value of NDVI (from Paper III) and mean annual soil temperature at both Icelandic grasslands. The letters (A to E) represent the soil warmed plots ranging from 0°C (A) to +13°C (E).

The linear change in NDVI between 5°C MAT_s and 12°C MAT_s (+7°C) was from 0.83 to 0.86, or ~4% increase (Figure 9). However, since the NDVI is not so sensitive to increases in biomass or leaf area at such high values (see in discussion), the actual increase in leaf area was likely twice as large or even greater. Therefore, even if photosynthetic capacity was not altered per unit leaf area, the daily C-uptake during peak growing season most likely increased as the amount of leaf area increased (maximum NDVI).

3.2 Phenology and warming effect

Next, I focused on phenological plant observations at the species and at the grassland community scale (Papers III and II). Soil warming significantly affected the plants' growing patterns at the grassland sites.

For seasonal C-uptake, the most important factors are whether vegetation becomes active earlier, when the daylength is quite long at the high latitudes, and whether the length of the growing season increases. Indeed, as shown in Figure 10, which examined the start of the growing season (SOS) based on an increase in the plot NDVI signature, the SOS was advanced by 1.5 days for each °C of MAT_s. This means that 4.6°C warming would advance the growing season by one week. It may not seem to be much, but it is significant given the short length of the growing season, and also since this shift occurs during April/May, when the day is much longer than night at high latitudes.

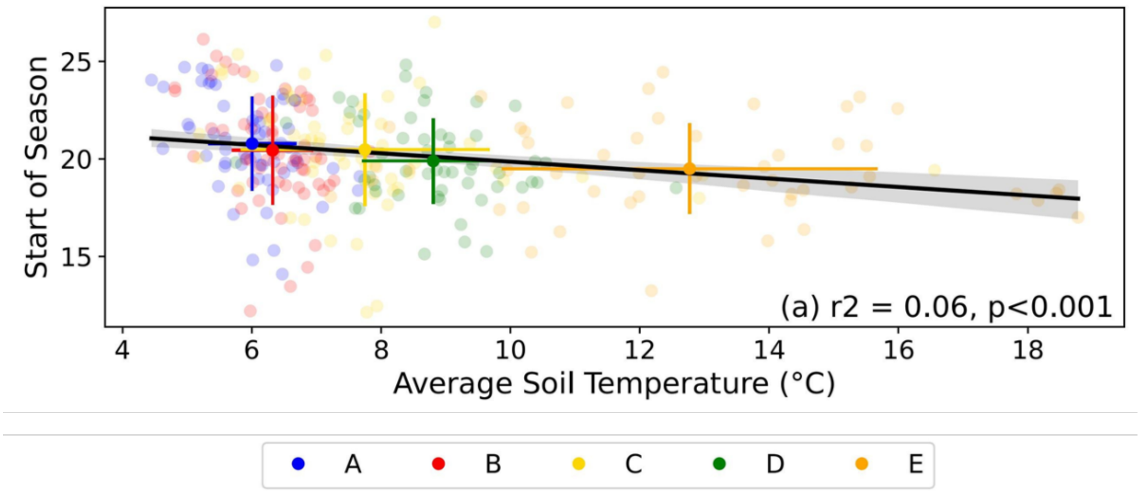

Figure 10: Linear regression between the SOS (from Paper III) and mean annual soil temperature at both Icelandic grasslands. The letters (A to E) represent the soil warmed plots ranging from 0°C (A) to +13°C (E).

Similarly, the vegetative growth phenology analysis in Paper III found comparable, but even stronger, response in SOS to soil warming, both when manual scoring was used to asses leaf growth in four plant species, and when drone imagery was used to track the seasonal curve of height growth (Table 2). The manual observations found the soil warming resulted in 2.3 days earlier SOS per °C of MAT_s and the drone DEMs similarly found 2.2 days earlier onset of height growth per °C of MAT_s (Table 2). A 2.3 earlier SOS means that with +7°C warming the start of the growing season is advanced by 16 days.

It is interesting to note whether an earlier start also leads to an earlier end in the vegetation activity, something that could happen if the subarctic plants are programmed to finish their growth cycle as quickly as possible to avoid the risk of autumn frost damages. Indeed, Paper I showed that the time of the peak NDVI (POS) was advanced as much as SOS, however, the study did not determine the end of the growing season (EOS) or the length of the growing season (LOS). This was addressed in Paper III. Manual measurements showed that the soil warming led to earlier POS by 1.9 days per °C of MAT_s, *i.e.* almost as much as SOS had advanced, while EOS remained nearly unchanged compared the unwarmed treatments (Table 2). The length of season (LOS) was therefore extended by 2.1 days per °C of MAT_s, or 15 days for +7°C soil warming.

The significant lengthening in the growing season will have increased the carbon uptake by the grassland community, even if the photosynthetic capacity per unit time and unit leaf area did not increase.

Table 2: Statistical linear regression output from Paper II showing the relationship between vegetative growth phenological parameters and soil temperature (MAT_s) for both studied sites (MTW, LTW) at both species (manual scoring) and community (DEM) level. Significance level "ns" indicates p-value (p-val.) > 0.05.

Parameters	**Manual scoring**				**Remote sensing (DEM)**				
	slope	intercept	R^2	p-val.		slope	intercept	R^2	p-val.
SOS	-2.3x	150	0.22	<.0001	Both	-2.2x	190	0.30	<0.002
G_{max}	-1.2x	170	0.08	0.022	Both	-6.0x	240	0.21	0.03
POS	-1.9x	250	0.11	0.002	MTW	+0.9x	250	0.02	ns
					LTW	+0.4x	220	0.03	ns
EOS	+0.1x	300	0.002	ns	MTW	+1.4x	270	0.06	ns
					LTW	+0.6x	230	0.05	ns
LOS	+2.0x	160	0.09	0.01	MTW	+5.1x	77	0.42	0.02
					LTW	+3.9x	38	0.63	0.01

G_{max}: date of maximum growth rate

3.3 Species specific phenological responses

At the species level, results of Paper I indicated that *Ranunculus acris*, a eudicot plant species, exhibited a greater phenological response to changes in soil warming compared to the other plant species that were studied: *Agrostis capillaris, Poa pratensis*, and *Equisetum pratense*.

Warming induced in *Ranunculus acris* an earlier emergence of the initial leaves (p = 0.017), buds (p = 0.04), first sign of seed fall (p = 0.03) and first sign of senescence (p = 0.045). Interestingly, the timing of flowering was not significantly altered by the soil warming for *Ranunculus* (p > 0.05).

When observing the two monocots, contrasting phenological responses were found to soil warming. *Agrostis capillaris* displayed an earlier emergence of its first leaves (p = 0.036) and flowering (p = 0.035). *Poa pratensis,* on the other hand, did not exhibit any statistically significant changes in its phenophases in response to soil warming during the study (p > 0.05).

Lastly, *Equisetum pratense*, showed a significant early onset in the reproductive phase, which was the falling of cones, between +2°C and +8°C warming (p = 0.026).

3.4 Net aboveground productivity and response to soil warming

The study in Paper IV, on the vascular plant aboveground biomass (AGB_{vasc}) and response to chronic warming and interannual variation showed no overall difference between the two sites (MTW, LTW; p > 0.05) and were therefore merged for further analysis. Results from the linear regression analysis between annual soil temperature sum ($T_{s\text{-}sum}$) and AGB_{vasc} across all years and both sites, only showed a significant positive relationship with warming levels in some years (Paper IV). That is, the peak season aboveground biomass (~ANPP) was indeed increased by soil warming, but it was only significant in some years (not shown).

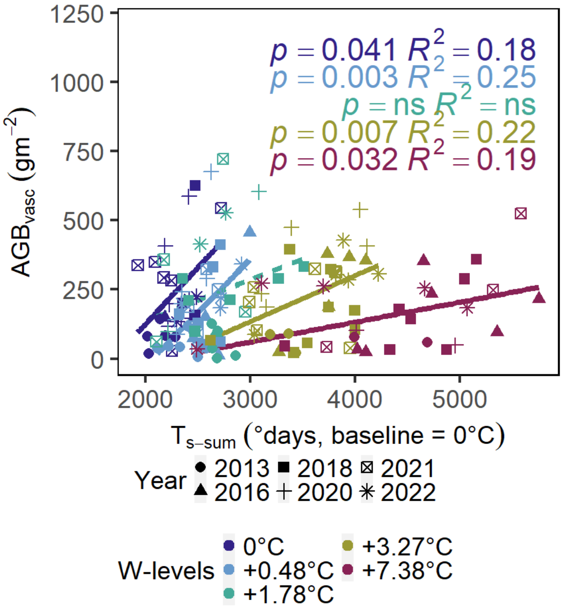

Figure 11: Linear regression from Paper IV, showing a positive relationship between AGB_{vasc} and annual soil temperature sums (July to July) at baseline temperate of 0°C at different soil warming levels (W-levels) over the studied years and grassland sites in Iceland.

Figure 11 shows the data after the realised AGB_{vasc} values were first "normalised" based on the interannual variation of the corresponding "unwarmed plots" within each transect. The proposed explanation for this soil warming-induced response of AGB_{vasc} was the interannual variation in the site's background AGB_{vasc}, which likely caused the strongest "background" (interannual) atmospheric warming response in the unwarmed, control plots, and then progressively smaller "background" effects, depending on the warming level of each plot (Figure 11). Per unit $T_{s\text{-}sum}$ at the unwarmed plots (0°C), there was an increase of 0.41 gm^{-2} degree day^{-1} in average AGB_{vasc} productivity. However, a clear "down-regulation" in this $T_{s\text{-}sum}$ response with increasing soil warming levels was spotted. As a result, the slopes decreased to 0.38, 0.14 (n.s.), 0.17, 0.07 gm^{-2} degree day^{-1} for the +0.48, +1.78, +3.27 and +7.38°C warming levels, respectively (Figure 11). Due to this overshadowing response, the realised chronic soil warming response in AGB_{vasc} which is always part of those curves could become less in the warmed plots in the warmest years, when this "background" response was strongest. Conversely, during "cold" years the chronic warming response was significant.

Another interesting finding in Paper IV was that the best T_{sum} to explain the realised interannual variation included not only the T_s during the growing season, but also the soil temperature from the preceding winter and autumn (data not shown).

3.5 Duration of warming and plant activity

The duration of warming (MTW vs. LTW sites) was not found to be a significant factor affecting plant activity in the subarctic grasslands. It had no significant effect on photosynthetic rates or manual phenological observations on individual plants at the two sites (Paper I and II). Paper III did not report differences between the two sites but merged the NDVI data before doing the analysis. Overall, the minor site differences reported in Paper IV were not indicative of a convincing acclimation or adaptation response in ANPP.

Paper II reported some differences in the seasonality of the grassland vegetation height growth between the two sites (MTW, LTW) (Table 2), where the POS, EOS and LOS were all significantly earlier for LTW that MTW. In Paper II it was, however, more related to vegetation bending (lodging) at the more exposed LTW site, rather than to a change in the plant growth pattern.

3.6 Nutrient dynamics with soil warming in Icelandic grasslands

One of the most unexpected findings in this study was the lack of significant leaf-N differences in *R. acris* across the warming treatments or the two sites (Paper I). My initial hypotheses were assuming that chronic soil warming was going to induce measurable changes in available N in soils and plants, and that would drive most of the functional differences.

Also intriguing, was the finding that the leaf-level N-availabilty played a major role in *R. acris'* photosynthetic assimilation rate across all treatments. Figure 12 shows the significant leaf-N responses in both Vc_{max} and J_{max} (Equations 1 & 2):

$Vc_{max} = 7.1 + 25 \times N$, *Equation 1*

with $R^2 = 0.17$ and $p = 0.003$, and:

$J_{max} = 25 + 50 \times N$, *Equation 2*

with $R^2 = 0.14$ and $p = 0.006$).

To go further with this, I have included here an analysis of auxillary data that is not part of any of the papers included in the thesis, *i.e.* PRS probe data from 2021, to asses whether the unexpected lack of warming-level response in leaf-N concentrations is similar in the soil solution of the permanent plots in MTW and LTW. Indeed, there was no significant warming effect on the availability of any macro or micro soil nutrients (Table 3). This supported the findings in Paper I.

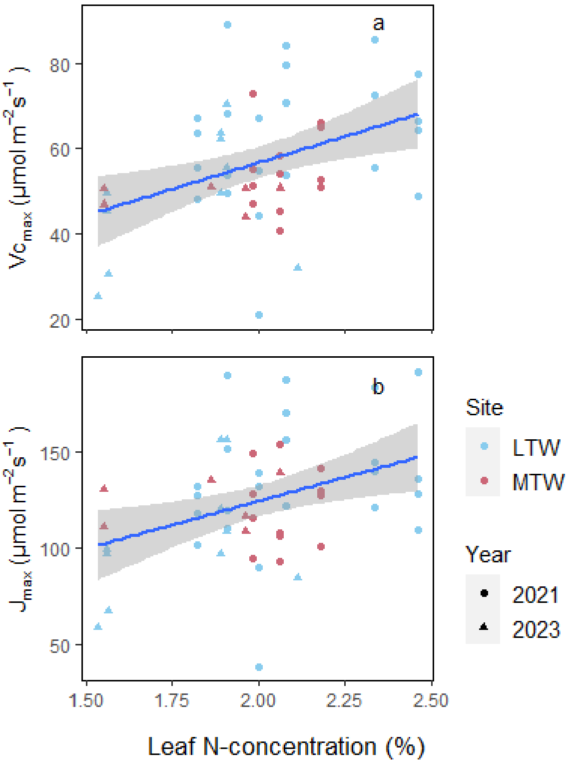

Figure 12: Linear relationship between Farquhar modeled parameters and leaf-N concentrations across all plots in both the MTW and LTW.

Table 3 : PRS probes (μg nutrient/10 cm²/time of probe burial) chemical composition at both sites (MTW, LTW) and sampling campaign dates (spring, summer, autumn) across the soil temperature (T_s) experimental setup in Iceland in 2021. Bold values in the table represent significant differences at $p < 0.05$ and the other values are categorised as non-significant ($p > 0.05$).

Components	T_s	site	date	T_s × site	T_s × date	site × date	T_s × site × date
NO_3-N	0.25	0.59	*0.09*	0.99	0.74	0.91	0.96
NH_4-N	0.19	**<0.01**	0.71	**0.03**	0.57	0.61	0.58
P	0.60	**<0.01**	*0.06*	0.14	0.23	**0.01**	**0.01**
K	0.6	*0.09*	0.55	0.25	0.70	0.60	0.70
Cu	0.70	0.47	0.26	0.96	0.23	0.59	0.55
S	*0.08*	**<0.01**	0.16	*0.06*	0.66	**<0.01**	0.40
Ca	0.38	**0.02**	0.50	0.47	0.84	0.80	*0.09*
Mg	0.80	0.19	0.77	0.53	0.34	0.33	0.20
Fe	0.16	*0.06*	**0.04**	0.83	*0.09*	0.35	*0.07*
Mn	0.20	**<0.01**	**0.02**	0.24	0.19	**0.03**	**<0.01**
Zn	0.13	**0.02**	0.94	0.95	0.43	0.47	0.17
B	0.57	0.11	0.73	0.55	0.49	0.13	0.71
Al	0.81	0.77	*0.06*	0.88	0.11	0.30	**0.03**

The two forms of mineral N, NO_3^- and NH_4^+, were not significantly affected by warming level, nor warming level × date (seasonality) (Table 3). Furthermore, the more mobile form in the soil, NO_3^-, was not significantly different for any of the experimental factors tested. The ammonium (NH_4^+) was significantly different between the two sites and the interaction between soil temperature (T_s) and site (T_s ×site, Table 3), and when looking at the actual data the pattern was that the concentrations were higer in the LTW site (data not shown).

Other nutrients which were significantly different between the two sites were P, S, Ca, Mn and Zn (Table 3). Visual inspection of the data showed that all those nutrients also had higher average concentrations in LTW. The Fe and Mn stood out as the only ones to be affected by the sampling date (Table 3).

3.7 Interannual variability effect on subarctic grasslands

A strong interannual variability in conditions and processes was observed at our Icelandic subarctic study sites, and this variability was found to greatly impact the outcomes of the different research objectives (Papers I, III, IV).

I mentioned parts of it in some detail earlier when I addressed main findings of Paper IV where interannual variability was shown to govern the observed response in AGB_{vasc} to soil warming. It was also shown in Paper IV that the annual $T_{s\text{-}sum}$ prior to the harvest measurements (July-July; including autumn and winter before the growing season in question) gave the most robust relationship with observed AGB_{vasc}, while the “traditional” $T_{s\text{-}sum}$ of the growing season of sampling year (June and July) did not significantly ($p > 0.05$) explain the interannual variation in AGB_{vasc}.

In Paper III, machine learning methods revealed that meteorological variables (air temperature, precipitation and irradiance) were the primary drivers of the interannual variability of NDVI phenology and peak NDVI values, with variable contributing more-or-less equally to the observed interannual response. It was also found in Paper III that the chronic soil warming significantly contributed to the NDVI variation, but explained only the "within site variability", which was relatively small compared to the interannual variability. These findings were similar to what was found for AGB_{vasc} in Paper IV.

In Paper I measurements of photosynthetic capacity per unit leaf area was conducted in two separate years, 2021 and 2023. Even for the gas exchange a significant effect of Year in many of different measured parameters was found ($p < 0.0001$, Paper I).

4. Discussion

4.1 Nutrient availability in Icelandic grasslands and warming effect

High-latitude ecosystem productivity has often been found to be more limited by nutrient availability during the active growing season, specifically N, than, for example, water or carbon (light) (Chapin, 1983; Hobbie et al., 2002; Shirley et al., 2022). A common assumption regarding ongoing global warming at higher latitudes, where soil organic matter (SOM) stocks are generally large due to the previously cold climate, is that warming will likely enhance soil nutrient availability due to stimulated microbial activity, thereby accelerating SOM decomposition and increasing nutrient mineralisation (Chapin et al., 2011; Hobbie et al., 2002). This has indeed been verified in some studies on warming, where SOM decomposition rates have been found to increase (Bölter, 1996; Rustad et al., 2001; Metze et al., 2024), which in turn might have a positive feedback to increasing nutrient availability (Chapin et al., 2012) and an increased nutrient plant uptake and growth (Rinnan et al., 2009). However, there are also studies from high-latitude ecosystems and warming manipulation, where this effect has not been observed. For example, Peplau et al. (2021) and Sun et al. (2024) found that N was not lost from the soil as a result of warming in geothermal subarctic forest soil or alpine grasslands, respectively.

The above expected strong interaction between warming and N-availability at high latitudes was central to many of our working hypotheses at the initial stages of this research. It came therefore as a surprise when we found in Paper I that *R. acris* leaf-N was not significantly altered by medium- and long-term +8°C soil warming.

By adding an analysis of soil PRS probes in the thesis part of this dissertation (Table 3), I was able to confirm the lack of a significant warming response for mineral N (NO_3^- and NH_4^+) availability in the top 5 cm of soil. As PRS probes are measuring "available" nutrients in the topsoil by charged resin films that can (easily) exchange anions or protons with charged ions in the soil solution (Hangs et al., 2002; Western Ag Group of Companies), they can be used as proxies for nutrient availability during the periods they are employed. However, mineralised ions are rarely accumulating in the soil solution under natural conditions in the "cold" subarctic ecosystems, but are assimilated by soil microbes, fungi (mycorrhiza) or plants, or immobilised by clays and other mineral surfaces (Borggaard, 2020; Martini & Chesworth, 1992; Sharma et al., 2025). If any of those competing processes have higher affinity for the ions, then PRS probes are likely to underestimate the nutrient availability (Harrison & Maynard, 2014; Sulewski et al., 2002).

Therefore, a lack of significant PRS probe results may not necessarily indicate that nutrient decomposition and mineralisation did not increase, but rather that the concentrations of ions remained low in the soil solution. The lack of significant effect of soil warming on soil nutrients in the PRS probes could be due to enhanced plant growth (or microbial populations) under warming conditions, which may have absorbed the "extra" available nutrients rather than the resin films of the PRS probes.

Nitrate (NO_3^-) is known to be the most abundant form of N available to the plant as compared to NH_4^+ (Oaks, 2011), which is more prone to be lost (Garwood & Ryden, 1986). One possible explanation for the non-significance in NO_3^- availability, as measured by the PRS probes, is that it had already been lost since the onset of the soil warming via leaching during winter months, a phenomenon that has been observed in Icelandic managed grasslands in high precipitation-parts of Iceland (Thorsteinsson et al., 2019). This would be consistent with the warming-level dependent temperature sum response observed in aboveground net primary productivity, which was found to decrease with increasing warming levels (Paper IV).

Moreover, as was addressed in the Introduction, there is evidence that topsoil (0-10 cm) N-stocks have declined under long-term soil warming at the ForHot grasslands, likely due to increased mineralisation rates combined with (winter) leaching losses (Poeplau et al., 2020; Verbrigghe et al., 2022b).

To complicate matters further, it has been shown that Arctic and subarctic plants are, to large extent, not as dependent on mineralised N (NO_3^- and NO_3^+) as their more southern counterparts (Atkin, 1996). This is because they rely more on symbiosis with mycorrhizal fungi which assimilate the soil N in organic forms (amino acids and other dissolved organic nitrogen forms) thereby bypassing the NH_4^+ and NO_3^- mineralisation step (Atkin, 1996; Celi et al., 2021; Kielland, 1994). There are studies at the ForHot site which have measured and found that amino acid uptake is indeed increased by the soil warming (Zevenhuizen et al., 2025). Therefore, it is not that simple that the N-availability was not at all increased by the warming at the MTW and LTW sites, but it is certainly less pronounced that was expected.

Another reason why leaf nutrient concentrations, especially in perennial plants, may not show substantial changes, is that plants can change the allocation of their resources between different tissues and among various internal processes. This allows them to increase the investment in those tissues/processes that most limit their fitness (Koller et al., 2016; Sigurdsson, 2001; Wolfe et al., 1998). Such conservative "growth processes" have indeed been seen in Arctic plants (Callaghan et al., 2004). Paper I, showed that *R. acri*s already had high photosynthetic capacity in cold soils, and it may therefore have allocated additional nutrients acquired in warmer soils to other growth-related processes than enhancing the photosynthetic system.

4.2 More photosynthetic activity per unit leaf area with warming?

To increase productivity, plants need to accumulate more C per unit of surface area and over time. They have three main possible strategies to do that: i) increasing the rate of C-uptake (photosynthetic rate per unit leaf area), ii) increasing their leaf area per unit ground surface at the community level (also known as leaf area index (LAI)) or iii) extending the duration of the year during which photosynthesis occurs per unit leaf area (Chapin et al., 2012). I will now examine how did soil warming affect these three processes, starting with the photosynthetic capacity per unit leaf area. Then in section 4.3, I will address the leaf area, and in section 4.4 the duration of C-uptake.

One possible interpretation is that any excess N and P in the system may have been fully assimilated to support the observed increase in plant growth (Papers III & IV). There might, therefore, have been insufficient residual nutrients to allow plants to invest in high photosynthetic capacity (Rubisco or chlorophyll content) per unit leaf area (Paper I). This could be linked to studies indicating that low-N-availability leads plants to reduce their investment in Rubisco (Vc_{max}) (Chen et al., 2020; Niinemets et al., 1999). However, the relatively modest increase of AGB_{vasc} that was found with soil warming does not give a strong support for this hypothesis.

Nitrogen is essential for photosynthetic enzymes (Atkin, 1996) and therefore it would be expected that the warming-induced increase in N-availability would enhance the vegetation photosynthetic capacity (Chapin et al., 2012). What was observed in Paper I, albeit for only one of the key grassland species, was that neither net photosynthesis nor photosynthetic capacity was significantly enhanced by the soil warming, when measured as a rate per unit leaf area.

It came as a surprise that, at the leaf level, N-concentration was still found to be the main driver in photosynthetic processes across all the plots, as both Vc_{max} and J_{max} showed a significant positively relationship with leaf-N concentration (Paper I; Figure 12). The general meta-analysis study from Walker et al. (2014) showed Vc_{max} to be strongly related to leaf and high levels of leaf-P that strengthened the sensitivity of Vc_{max} to leaf-N. In this study the findings seemed to be driven by variability in leaf-N at a plot level across all the experimental sites. Nitrogen limitation at the ForHot site was also reported by Meeran et al. (2023) in their study on the interactive effect of soil warming and mineral N fertilisation (the TNT experiment). They showed that N was still a limiting factor for plant growth in warmed plots in those grasslands next to the MTW experiment.

Different conditions between the two sample years could have played a role in the strong apparent N-response of the photosynthetic capacity (Figure 12). For example, sampling at the same phenological stages could be slightly offset between plots or warming-levels, and it is known that N-concentrations can vary throughout the growing season (Rasmussen et al., 2022; Weintraub & Schimel, 2005, Chapin & Shaver, 1989).

4.3 Any changes of the vegetation (leaf area) with warming?

It was observed that there were significant increase in maximum NDVI values with warming (Paper III), which is often interpreted as an increase in chlorophyll content per unit ground surface area (Caruso et al., 2017; Datt, 1998). In the literature, a direct proportional relationship has been observed between

NDVI and plant community leaf area index (LAI) (Rees et al., 2020). While increasing NDVI can occur without substantial changes in vegetation biomass or leaf area (Myers-Smith et al., 2020), particularly if plants exhibit increased chlorophyll concentration per unit leaf area at higher nutrient availability, the outcomes suggested this is unlikely – at least for *R. acris*. Chlorophyll concentrations did not appear to increase in this species, as this would have been reflected in values of Vc_{max} and J_{max} being altered, which was not the case. However, since *R. acris* covered only relatively small portion of each plot (Meynzer et al., 2016), caution is warranted in extending this interpretation to the entire community. It would be good to measure the photosynthetic characteristics of more species to determine whether comparable physiological patterns hold more broadly.

NDVI and LAI have often been found to respond to increases in N and P availability (Chapin et al., 2011; Kizilgeci et al., 2021) and increases in the NDVI have been observed across the Arctic and subarctic in past decades as the climate has warmed (Arctic greening; Frost et al., 2021; Magnússon, 2022).

The above finding also fitted well with past changes in the NDVI values measured across whole Iceland from the 1980s to 2010 (Raynolds et al., 2015). The authors observed that NDVI increased on average by more than 80% on each km^2 during that period, and the atmospheric warming in Iceland was >1.5°C in MAT during the same period. They pointed out that since livestock grazing also was decreasing during the same period it is not clear how much of the NDVI increased was caused by the warming alone. However, as was pointed out by Björnsson et al. (2018) the regional NDVI increase within Iceland closely mirrored the measured regional warming during the same period.

It should be noted that NDVI tends to saturate at values approaching 1 and its relationship with LAI or canopy mass becomes non-linear at NDVI > 0.3 (Rees et al., 2020). Hence, in cases such as this study (Paper III) – where NDVI had high values – NDVI could be considered as not a very good indicator for the actual increase in LAI.

The finding that LAI likely increased with warming when analysed as NDVI across multiple years in Paper III was supported by the multi-year observed response in AGB_{vasc} in Paper IV (see later). This finding supports the research question on how warming could affect vegetation growth (*i.e.* increased LAI). It can, therefore, be suggested that in reality more N-uptake did occur in the warm plots, even if the N-concentration per gram dry mass was not altered. The N might rather therefore be used to create higher LAI (Paper III), rather than increase the "photosynthetic machinery" per unit leaf area. This indirect pathway has been suggested as a likely way for vegetation to increase its NPP under higher N-availability (e.g. Poorter & Evans, 1998).

How could the proposed relative effect of the realised increased NDVI (LAI) on vegetation ANPP be validated? The realised canopy photosynthesis per day would be expected to increase with greater LAI in the grassland community in warmer plots. To calculate how much the daily canopy photosynthesis would increase just by the increased grassland LAI, one would need to parameterise a light-interception model (Beyschlag & Ryel, 2007; Hesketh & Jones, 2018; Sheehy & Cooper, 1973) with information about amount of vegetation per m^2 (Paper IV) and how much of it was leaf biomass. Then, apply representative specific leaf area (SLA) for estimating its leaf area, and the measured height of the canopy (Paper II), but that was outside the scope of this thesis and awaits later processing.

4.4 Longer growing seasons with warming

So, what about the duration of active photosynthesis, did it change? From the results, it was shown that with soil warming there was an earlier emergence of green leaves (SOS) and first signs of senescence (EOS) for individual plants (Paper II). Both manual and remote sensing measurements (DEM, Paper II) found a ~2.25 days earlier SOS per each °C warming of soil temperature (Table 2). These results aligned with previous research at the ForHot sites such as Leblans et al. (2017) who equally observed a lengthening of the growing season with soil warming and found that the SOS was 1.6 day earlier per °C warming of soil temperature during the years 2013-2015. Moreover, in Paper III, it was observed that during the years 2013-2021 the NDVI SOS was advanced by 1.5 days earlier per 4.6 °C warming of MAT_s. These findings also correspond well to Ren et al. (2020), who observed 0.85 days advancement in SOS with 1°C increase in mean air temperature (MAT) in a study on mid-latitude grasslands in the northern hemisphere (between 30°N and 55°N). Also, Piao et al. (2007) showed an earlier vegetation green-up of 3 days $°C^{-1}$ mean seasonal air temperature increase across northern ecosystems.

As for the EOS studied in paper II, it was not significantly affected by warming (Table 2). This could be due to an early shift in EOS, which was, however, not as strong as that of the SOS, thereby, leading to a somewhat increased LOS.

In summary, there was a significant positive effect of soil warming on both the start and length of the growing season at the ForHot grasslands (Paper II, III). This was found both at individual species- (Paper II) and community levels (Paper II, III). Warming resulted in significantly longer growing season, which should have increased the seasonal C-uptake. Adams and Langton (2005) showed how an earlier start of growth, during the long daylight hours at higher latitudes could significantly induce more C-uptake. In these experiments it seems that this effect should have increased the seasonal C-uptake much more than the relatively small increases in maximum NDVI values, albeit the potential underestimate of LAI changes from high NDVI value changes.

4.5 The observed warming response on ANPP

What was then the realised effect of stable photosynthetic rates, slightly increased LAI and much longer growing season on ANPP?

From literature, increased soil nutrients, such as inorganic N and P, lead to improved conditions for plant growth, resulting in higher vegetation biomass (Wild et al., 2016). This highlights the interconnected nature of nutrient availability (Paper I, Table 3) and plant productivity in subarctic grasslands undergoing warming.

Diving into the changes in AGB_{vasc} at the sites between 2013 and 2022 (AGB_{vasc}; Paper IV), which here is used as a proxy for ANPP, there was some increase in biomass across the soil warming levels (on average; see later). Therefore, the proposed warming-induced pathway for increased ANPP was at least partly active.

Earlier research at ForHot has, however, claimed that ANPP was negatively affected by the soil warming at MTW (Fang et al., 2023) and MTW and LTW (e.g. Verbrigghe, 2021). Fang et al. (2023) related this apparent reduction in ANPP to the observed reduction in topsoil N stocks that have decreased since 2008 in relation to soil warming level. However, in both those studies, they only used one year's harvest data (2018), which was an extreme year with unusually wet and warm climate during winter, spring, and summer (Icelandic Meteorological Office, 2023). This resulted in unusually high biomass in the unwarmed grassland plots that year, though, the warmed plots did not respond as strongly to this interannual variability (Paper IV). The fact that multiple years were used to derive the warming impact on vascular plant biomass, it explained these contrasting findings.

Another complicating factor in earlier studies is also that they have included both vascular- and non-vascular plant biomass in their ANPP approximations (Fang et al., 2023; Verbrigghe 2021). The non-vascular plants are, however, long-lived and evergreen and cannot simply be added to the otherwise deciduous grassland vascular plant biomass as ANPP. They are also likely not as affected by the soil warming as they do not have roots and mostly rely on nutrients brought by rain and dry deposition (Cornelissen et al., 2007; Guðmundsdóttir, 2012). Moreover, they also form the field layer in the subarctic grasslands at the ForHot sites and when the vascular plants increase their biomass, the non-vascular plants give away in the shadow below the denser canopy (Guðmundsdóttir et al., 2014). This can mask the dynamic growth responses of the vascular plant vegetation.

It should also be stated here that in the multi-year dataset in Paper IV, although an increase was observed in AGB_{vasc} with warming, there was a clear "down-regulation" of this positive response as the level of the chronic warming intensified (Figure 11). This could, possibly be related with reduced amounts of topsoil soil organic matter (SOM) stocks with increasing warming levels. SOM has been shown to be important in maintaining soil nutrient and water cycles in a complex way (Chapin et al., 2011). Both Poeplau et al. (2020) and Verbrigghe et al. (2022b) have shown that there have been large losses of SOM following the onset of the soil warming and the loss has been in direct relation to the warming level, which has also decreased the amount of microbial biomass (Verbrigghe et al., 2022a).

In summary, I did find the expected increase in ANPP, albeit it should be stated that the increase was less than expected. However, it can partly explain why SOC losses have been found to stabilise at the ForHot grasslands. From pulse-labelling studies at MTW grasslands it is also clear that only smaller part of the assimilated carbon is used for ANPP, but large parts of it are used for belowground processes (Meeran et al., 2023). Therefore, ANPP and aboveground litterfall may not be the domination pathway for C into the soil ecosystem in these subarctic grasslands. Other studies at the ForHot sites have found enhanced mycorrhizal turnover (Zhang et al., 2020) and carbohydrate exudation (Ivika Ostonen, unpublished) that may all be more important pathways than ANPP at our sites.

4.6 Duration of warming (MTW vs. LTW differences)

Duration of warming turned out to have relatively small impact on the growth processes of the studied grassland species.

When observing different processes, it can be hypothesised warming duration might have a cumulative effect on aboveground processes (Papers I, II, IV), potentially leading to different growth responses over time between MTW and LTW. Walker et al. (2020), based on his analysis of >150 ecosystem variables at the MTW and LTW sites, suggested that the grassland ecosystems initially exhibited a significant overreaction to short- and medium-term soil warming, which led to pronounced changes in (soil) biotic composition and ecosystem processes but eventually stabilised into a new steady state at LTW. Such a long-term acclimation was not found in the processes I focused on in my research.

Figure 13: Visual observation of low plant turn-over (mortality) at the ForHot site in Iceland. a – flags set at the plots and species individuals marked in 2020 for phenology monitoring in 2021's growing season. b and c – red circle show the re-appearance of the marked individuals.

One possible explanation is the vegetation composition at the sites consists mostly of perennial plants, which are likely present for a long period of time at both grassland sites (Meynzer et al., 2016). This could limit genetic adaptation to the changed conditions, if the plant turnover was not high. Individual plant mortality has not yet been studied at our research site. However, when doing the preparation for monitor phenology in the year 2021, I marked all the individuals already in summer of 2020. They all re-appeared during spring of 2022 (Figure 13). This is an indirect observation of low plant turnover.

However, even if plants do not differ genetically, they might instead show a plastic response to the warming. For example, Wickander et al. (2021) observed that the alpine bistort (*Persicaria vivipara*), a dicot species found in Iceland, showed significant plasticity to elevated temperature, but again, this was not found in our study.

Apparent differences were observed in the remote-sensed vegetation height-growth measurements at the community level between MTW vs. LTW (Paper II). Those differences were, however, not validated by the manual measurements and were likely not caused by differences in plant growth, but rather in exposure differences between the sites, which led to difference in lodging of the grassland plants.

4.7 Effect of Interannual variability in subarctic grasslands growth processes

From this study (Papers I, III, IV), it was observed that interannual variability played an important role in shaping responses to soil warming in subarctic grasslands. Across the two sampling years of the gas exchange (Paper I) there was a significant effect of years treatment in most estimated photosynthetic variables. Although interannual variation could be an indirect factor in the observed responses (Werner et al., 2020), caution should still be employed when interpreting short-term interannual trends, since plant communities could hypothetically be stable on year-to-year basis which incorporates short-term fluctuations that might shadow long-term trends (Morecroft et al., 2016).

The study also assessed six years of environmental variables' (air temperature, precipitation, irradiance) interannual variability on NDVI with the use of machine learning (ML) techniques and SHapley Additive exPlanations (SHAP) values (Paper III). The findings of the ML analysis demonstrated that the studied variables all had a considerable impact on the predictions of the SOS, POS, and maximum annual NDVI values. It was also observed that the three meteorological factors had a far higher impact on the forecasts of NDVI each year than the soil warming data. It is noteworthy that, within this investigation, the SHAP values did not find significant differences in the explainability of the three different climatic factors.

Furthermore, when looking at the variation in soil temperatures on AGB_{vasc} interannual response (Paper IV), only when the annual $T_{s\text{-}sum}$ (July-July) with a baseline temperature of 0°C was used, a significant linear relationship appeared with observed AGB_{vasc} across both the MTW and LTW. This finding was interesting and unexpected, as the traditional growing season $T_{s\text{-}sum}$ did not significantly explain the interannual variation. The response was weaker and insignificant when only adding the previous winter (November-July). This implies that conditions in the previous late summer and winter influenced the growth dynamics of the plants in the next growing season. At our field site, the grassland species are perennial (Sigurdsson et al., 2016), so they store their energy and available nutrients in their root systems for the next growing season. One reason for the conditions from the previous growing season to play a role in the next growing season's productivity can be that the uptake and storage of resources at the end of the season and in the winter will increases growth in the following growing season. Such dynamics are well known in Arctic environments (Peterson, 2014). Such winter and spring temperature impacts have also been reported as important control variables of yield in managed hayfields in Iceland (Thorvaldsson & Martin, 2004).

4.8 Limitation of the research

Soil warming vs atmospheric warming

Natural geothermal gradients, such as the ForHot study site in Iceland, provide an excellent context for examining long-term ecological responses to soil warming under realistic natural field circumstances (Sigurdsson et al., 2016). The presence of both short- and long-term warming gradients permits the investigation of temporal dynamics in assessing distinct ecosystem process that is novel with this setup (forhot.is).

Even though in different papers (I, II, III, IV) the apparent responses gave us a proxy to interpret these results in relation to an ongoing changing climate, precaution should be exercised when interpreting outcomes in relation to global warming, as the heat comes only from below, but in more natural conditions the soils are warmed through atmospheric warming from above. This may mean that soil warming systems might potentially underrepresent the complete spectrum of aboveground biological reactions, which are more directly governed by air temperature (Leblans et al., 2017; Sigurdsson et al., 2016).

Moreover, the step commencement of geothermal warming after an earthquake might not adequately mimic the steadily increasing trajectory of climate change, as described by Sigurdsson et al. (2016). This last limitation is, however, shared with almost all climate manipulation experiments in the world.

Sampling

Although we did not observe a response to warming in photosynthetic activity per unit leaf area for the single key species monitored, it should be kept in mind that the overall community response may be more variable.

5. Conclusion

This study aimed to assess different growth processes of subarctic grasslands species and their response to soil warming at the ForHot experiment in Iceland. From this research, I could conclude and suggest the different following outputs which are also shown graphically in Figure 14:

- There was little or no effect of soil warming on the photosynthetic capacity per unit leaf area at the species level (*Ranunculus acris*, Paper I).

- Still the main explanatory variable of differences in photosynthetic capacity among individual plants was leaf-N concentration (Paper I).

- On the other hand, an indirect measure of photosynthetic activity per unit surface area via peak NDVI showed a slight increase with soil warming (Paper III). Thereby suggesting that, although the vegetation is not more active per unit leaf area it might still be able to assimilate more C per unit surface area.

- Soil warming induced earlier onset of growth of plants, and it was found that the duration of the season was longer (Papers II, III). This has also increased the annual C assimilation and was probably the most important process for increased C-inputs and the observed stabilisation of SOC stocks in the warmer plots.

- The increased seasonal carbon assimilation per unit surface area led to increases in ANPP with soil warming, on average (Papers III & IV). However, this increase was less than expected and there was a "down-regulation" of the $T_{s\text{-}sum}$:AGB_{vasc} relationship with warming level (Paper IV).

- Interannual variability can mask responses to soil warming in subarctic grasslands (Paper, I, III, IV). It is an important variable to consider when studying subarctic grasslands response to environmental factors.

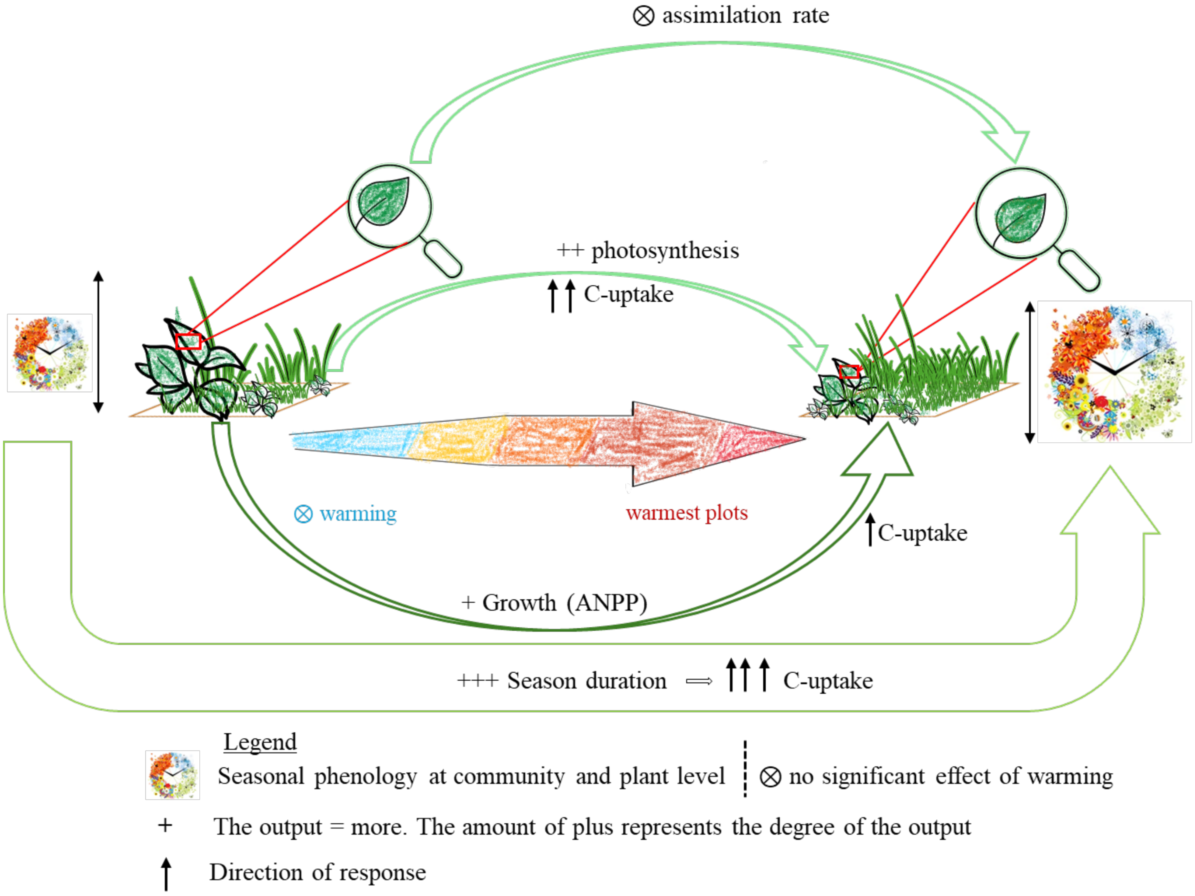

Figure 14: Graphical conclusion of plant growth processes in subarctic grasslands in Iceland under natural geothermal warming.

6. Future Work

This thesis has found new knowledge and reinforced previous knowledge in subarctic grassland species response to soil warming. By doing so, numerous gaps of knowledge have also been identified that should be addressed:

- The most obvious and planned additions are merging different aboveground and belowground phenology datasets with ESR3 (Biplabi Bhattarai) to be able to get a quite unique picture of seasonal growth patterns of aboveground parts and roots in these subarctic grasslands.

- Also planned in the future is collaboration with ESR 6 (Linsey Avila; whole community GPP with four automated chambers on different MTW plots over >2 years) and ESR 10 (Amir Hamedpour; repeated drone flights with multispectral sensors) to put together the whole GPP story. That would greatly help our understanding of how much C-inputs have in fact increased at different warming levels and how important those processes are in explaining the stabilisation of SOC stocks in the warmer world.

- Some work remains to strengthen the estimates of ANPP. Especially to look at the insect herbivory effect which can lead to an underestimate of ANPP derived from peak season harvest data. During sampling, the effects of soil fauna herbivory on plant growth was recorded. In the same manner little pitfall traps were installed to capture insects or soil fauna at different soil warmed plots. This data awaits further processing. Moreover, adding a paper on the livestock grazing effect and its interaction with soil warming on various gas exchange, phenology and plant growth variables, inside and outside the enclosures at the LTW site, can be an option as the data is available for processing.

- Increasing the number of plant species in assessing the photosynthetic capacity would strengthen the storyline of the carbon assimilation. Data exists for *Agrostis capillaris* but needs further work due to various problems encountered during the measurements of the small and thin grass leaves.

- Another important consideration is the use of the LAI, measured with the LI-2200C Plant Canopy Analyser (LI-COR Inc., Lincoln, NE, USA), to support the findings presented in Paper III. This measurement provides additional validation for the observed increase in vegetation growth under warming conditions, as indicated by NDVI analyses conducted over multiple years.

- Furthermore, in Paper I, additional analyses will be undertaken to explore potential variability in the photosynthetic response across different N-levels at both the MTW and LTW sites. This will involve selecting a specific range of N-concentrations to examine whether soil temperature differentially affects photosynthetic parameters. Such analysis could elucidate specific mechanism underlying the interaction between warming and nutrient availability. Moreover, it will also be beneficial to investigate the relationship between the SLA and plant photosynthetic assimilation rates, expressed per unit mass (μmol CO_2 g^{-1} s^{-1}), rather than per square meter (μmol CO_2 m^{-2} s^{-1}). The mass-based normalises the assimilation rate to leaf mass which might offer a deeper insight into the efficiency of individual leaves in converting atmospheric CO_2 into biomass. Lastly, a careful review of phenological sampling across both sampling years will be conducted to account from minor differences in plant developmental stages at the time of sampling. Such phenological variability could influence photosynthetic measurements, especially under the hypothesis that N-concentration and gas exchange responses vary throughout the growing season and are sensitive to subtle shifts in phenological timing.

Collaborations on joint synthesis papers are ongoing within the ForHot consortium and are drafted at annual workshops that bring together all those who are interested.

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9. Appendices

I

Gas exchange response of *Ranunculus acris* to long-term soil warming in subarctic grasslands

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Abstract

Photosynthetic assimilation rate (*A*) is highly sensitive to environmental factors. With ongoing climate change, at high-latitudes (>60°N), where temperatures increases are projected to exceed the global average, ecosystems experience this phenomenon at an accelerated pace. These environmental factors are both direct, such as changes in light or air temperature, and indirect, such as changes in soil water availability or nutrient availability in a warmer world. The aim of this research was to assess the effect of prolonged soil warming (+8°C) on the photosynthetic characteristics of *Ranunculus acris* growing in natural subarctic grasslands that had been exposed to warming since 2008 (medium-term warming; MTW) or since at least 1960s (long-term warming; LTW). To determine the photosynthetic characteristics, both CO_2-reponse (A/C_i) and light-response (A/I) curves were measured, as well as indications of indirect changes through water limitations like the stomatal conductance, stable isotope ratio or through changes in nitrogen (N) availability. Our results showed little to no significant effect of prolonged soil warming on the characteristics of the A/C_i or A/I curve parameters, indicating a conservative response in C uptake per unit leaf area. Same was true for stomatal conductance (g_s), stable isotope ratio ($\delta^{13}C$) and leaf-N between the soil warming treatments, indicating that the expected indirect effects of the prolonged soil warming were not apparent. However, across the whole experiment, *R. acris* showed a strong positive response to leaf-N concentrations, indicating that variability in plant N status was still the primary indirect driver of photosynthetic capacity in these subarctic grasslands. However, this relationship was not significantly affected by the warming treatments or the duration of the soil warming.

Keywords

Photosynthesis, A/C_i curves, A/I curves, stomatal conductance, leaf nitrogen, stable isotopes

Introduction

Climate change is driving significant environmental shifts in ecosystems, particularly in high-latitude (>60°N) ecosystems, where temperatures increases are projected to exceed the global average (IPCC, 2023; Vaughan et al., 2007). These ecosystems are characterised by short but intense growing seasons (Callaghan et al., 2004), with low temperatures and limited sunlight prevailing for most of the year (Chapin et al., 2000). In these regions, a slight increase in temperature can have effects on ecosystem dynamics (Post et al., 2009). A critical aspect of understanding these changes lies in assessing how warming affects photosynthetic processes which are pivotal for plant productivity and ecosystems functioning (Cavender-Bares & Bazzaz, 2004; Jonasson et al., 2019).

Photosynthesis, the primary mechanism through which plants fix atmospheric carbon dioxide (CO_2) into organic compounds (Gest, 2002), is highly sensitive to environmental factors such as temperature, light and nutrient availability (Chapin, 1983; Jonasson et al., 1999; Shaver & Kummerow, 1992). The photosynthetic response to warming often involves direct and indirect effects (Chapin, 1983; Liang et al., 2013). At the molecular level, temperature influences enzyme kinetics, electron transport rates, and the efficiency of the carbon (*C*) assimilation pathway (Baker, 1996). Typically, moderate warming may enhance photosynthetic rates (Starr et al., 2008) by accelerating enzyme activities, particularly those related to Rubisco and the Calvin's cycle (Farquhar et al., 1980).

In the subarctic and Arctic ecosystems, plants often display conservative physiological strategies to cope with low temperatures (Billings & Mooney, 1968). As a result, photosynthetic parameters such as maximum carboxylation rate (Vc_{max}) and maximum electron transport rate (J_{max}) may exhibit limited plasticity in response to warming (Schedlbauer et al., 2018). Additionally, increased temperatures can indirectly alter photosynthesis by affecting water availability and nutrient cycling (Callaghan et al, 2004), especially nitrogen (*N*) availability; a key limiting nutrient in high-latitude ecosystems (Atkin, 1996).

Icelandic natural grasslands cover approximately 4400 km² of the subarctic lowlands (Ottósson et al., 2017). These grasslands are characterised by a unique assemblage of vegetation, adapted to Iceland's subpolar oceanic climate (Sigurdsson et al., 2016), and volcanic soils (Arnalds, 2015). The vegetation consists primarily of perennial species, including diverse grass species and herbs (Kristinsson; & Sigurðsson, 2010) such as *Ranunculus acris* L. The Icelandic subarctic vegetation might have developed physiological and morphological adaptations to cope with temperature fluctuations and extreme weather conditions typical of high-latitude environments (Thorvaldsson & Björnsson, 1990; Thorvaldsson & Martin, 2004).

Soil warming experiments have shown to contribute considerably into the impacts of climate change by providing knowledge on how ecosystems will respond to increasing temperatures (Gestel et al., 2019). It has been shown that prolonged exposure to soil warming could increase plant respiration rates and/or accelerate *C* loss (Schindlbacher et al., 2009; Walker et al., 2018). Moreover, in soil warming manipulation experiments, tundra plant species, showed high photosynthetic rates in forbs, while dwarf evergreen shrubs exhibited the lowest rates (Starr et al., 2008). In addition, at a more whole-ecosystem warming, van de Weg et al. (2013), studied the contrasting effect of *N*-addition on photosynthesis in Arctic ecosystems, and suggested that under "low" *N* availability, Arctic species either already optimised their photosynthetic capacity per leaf area, or were limited by other nutrients.

One major soil warming manipulation experiment in Iceland is the ForHot which has natural geothermal grasslands. Previous studies at the ForHot grasslands have not looked much into photosynthetic characteristics at an individual plant level. It has, however, been shown that soil warming has enhanced grassland Normalised Difference Vegetation Index (NDVI) which is indicative of vegetation density and greenness (Leblans et al., 2017; Mortier et al., 2024). Wandji et al., (202xa - *in review AAAR*) have also shown that soil warming has indeed enhanced aboveground Net Primary Productivity (ANPP) at the sites, which could indicate an increase in seasonal photosynthetic assimilation rate (*A*). However, in a recent pulse-labelling study at ambient light at one of the grassland sites, ecosystem gross primary production (ecosystem photosynthesis rate per unit surface area; gross primary productivity – GPP) was not found to increase (Meeran et al., 2023), which made it interesting to look further into the photosynthetic response.

In this research we focused on the photosynthetic characteristics of only one perennial vascular plant species that was found in all experimental plots, *R. acris*. It is commonly known as meadow buttercup. It is among the seven most frequent vascular plant species in the ForHot natural grasslands, and which contain 32-38 species in the plant community within the studied warming levels (Meynzer, 2016). It is a widely distributed forb in subarctic grasslands and has a C_3 photosynthetic pathway (Hoffmann et al., 2010; Sarukhan & Harper, 1973). It was therefore chosen as a good representative for the whole community.

Here we report *R. acris* ecophysiological responses to ca. +8°C prolonged soil warming in two subarctic grasslands at the ForHot site, that experienced the soil warming for 13-15 years (medium-term soil warming; MTW or for at least 60 years (long-term soil warming; LTW). The following hypotheses were tested:

a) Parameters explaining photosynthetic capacity and quantum yield (variables from both A/C_i and light-response (A/I) curves) will exhibit limited plasticity in response to soil warming.

b) Feedback response 1. Net photosynthetic rate in ambient air is, however, expected to be more limited by stomatal closure in warmed plots, due to less water availability, also seen in a lower carbon stable isotope ratio ($\delta^{13}C$).

c) Feeback response 2 (duration of warming): The characteristics of the A/C_i and A/I response curves will, however, not be the same at MTW and LTW due to the longer time at LTW for acclimation. This was expected to be because of longer time in LTW for accelerated *N* mineralisation of soil organic matter which was expected to shift the plant *N*-status there more than in MTW.

MATERIALS AND METHODS

Studying Site

This study was conducted in the south of Iceland (64.008°N, 21.178°W), at the ForHot (forhot.is) experimental site (Figure 1). Soils are silandic andosol (Arnalds, 2015) and have been naturally warmed by geothermal activity within the bedrock below the soil profile (Sigurdsson et al., 2016; Arnalds, 2015). Here, two natural grasslands sites were selected to represent duration of soil warming, that is, medium-term (MTW) and long-term soil warming (LTW). The MTW site has been warmed since 2008 when an earthquake disrupted the bedrock channels, and at the LTW the warming has been ongoing for >60 years.

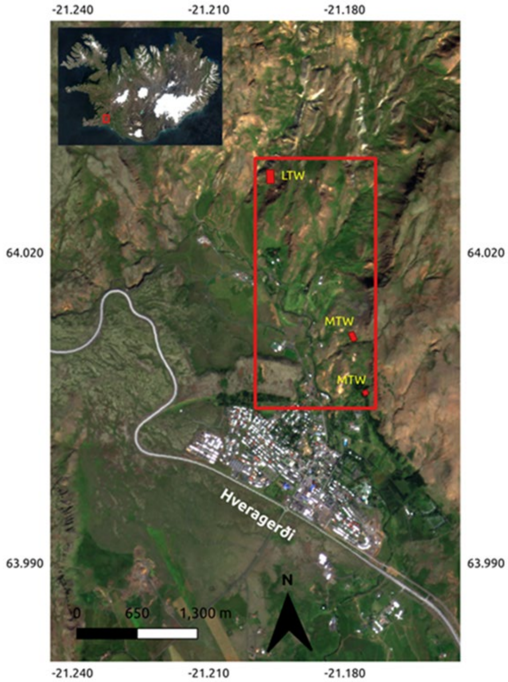

Figure 1: Location of both natural experimental grassland sites, that is, MTW (15-17 years of warming) and LTW (>60 years of warming) in the south of Iceland next to Hveragerði village. (Map courtesy: Amir Hamedpour)

The research area has a subarctic oceanic climate, with the mean annual air temperature (MAT), mean annual precipitation (MAP) and mean wind speeds of +5.2 °C, 1457 mm and 6.6 m s^{-1} at the closest synoptic station (Icelandic Meteorological Office, 2023). The mean air temperature of the warmest and coldest months, July and December, were 12.2 °C and -0.1 °C. The length of the growing season is only ca. 3.5 months at ambient conditions (Leblans et al., 2017). Despite snow cover throughout the winter, it is not permanent, and the soil remains frozen for some months during mid-winter (Sigurdsson et al., 2016).

Measurements were done during summers of 2021 and 2023, and within these two years the region experienced MAT of 5.8°C and 5.4°C, respectively. The maximum monthly (Aug.) MAT was 15.7°C in both years, and the minimum monthly (Jan.) MAT were -6.9°C and -11.3°C in 2021 and 2023, respectively. The annual precipitation (MAP, mm) for both years was recorded to be 1188mm and 1326mm in 2021 and 2023, respectively (Icelandic Meteorological Office, 2023).

Experimental Design

The ForHot manipulation experiment is a natural experimental site harbouring diverse research interest in the atmosphere-plant-soil continuum (forhot.is, futurearctic.be). Both the MTW and the LTW original experimental setup consist of 30 permanent plots (2m x 2m plots) per site ranging from 0°C to ~+15°C along five transects (six plots per transect). In this study we selected two soil temperature (T_s) ranges within three transects, ambient soil temperatures (+0°C) and a warm plot (+8°C) for measuring photosynthetic response curves.

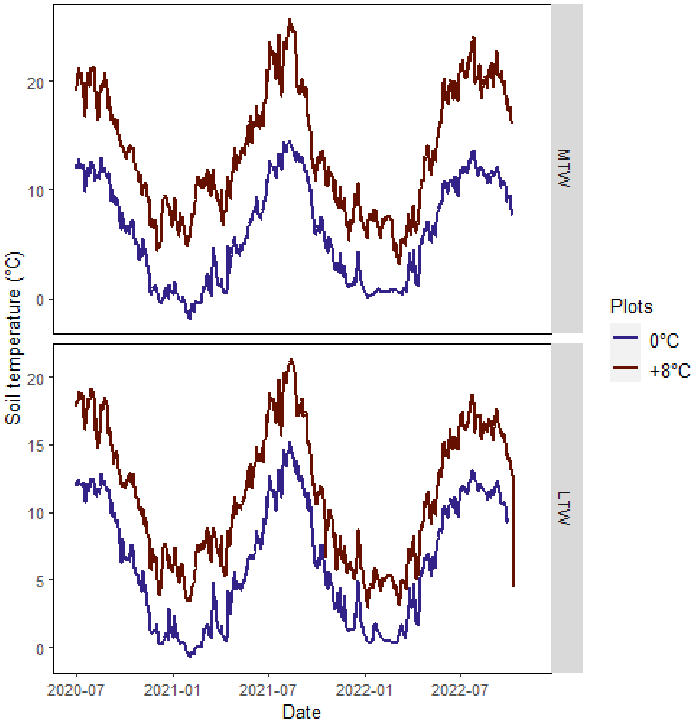

Figure 2: Daily mean soil temperature (MAT$_s$) at 10 cm depth for unwarmed (+0°C) and +8°C permanent plots for both Icelandic grasslands (MTW, LTW).

Soil temperature (T_s) in each permanent study plot was measured with TidbiT v2 HOBO® data loggers (Onset Computer Corp., Bourne, MA, USA) installed at 10 cm depth. For every treatment, hourly-daily mean temperatures from cold to the warmed plots were calculated (Figure 2). The seasonal T_s mirrored quite well the seasonal changes in air temperatures (Supp. Figure 1).

In the studied natural grasslands, subarctic perennial plant species characterise the flora. Plant species present in the two grasslands consisted of 32-38 species, where *Agrostis capillaris, Anthoxanthum odoratum, Festuca vivipara, Ranunculus acris, Equisetum sp., Poa pratensis* are the most frequent (Meynzer et al., 2016). However, for this research only one species, *Ranunculus acris* (Ranunculaceae – eudicot), was selected due to time restrains during sampling. The measurements were carried out during summer (Jul-Aug) of both the year 2021 and 2023, considering the phenological stages of the *R. acris* plants to be similar.

Gas-exchange measurements

The gas exchange measurements were conducted using the LI-6800 Portable Photosynthesis Systems (LI-COR Biosciences Inc., Lincoln, NE). Attached to the instrument was a leaf and needle cuvette (6800-13), with a compatible light source (6800-03). Leaf gas-exchange was measured on leaves on three to five healthy mature *R. acris* individuals in each individual plot to maximise the sampling size. These measurements were carried out from mid-July to the end of August and considering long days in the subarctic measurements were equally done during periods of peak solar radiation (11 AM to 7 PM) to maximise the amount of photosynthesis. Due to time sensitivity and consideration of stabilisation of leaves in the chamber during each sampling, one leaf was sample per individual plant. The response curves studied were the relationship between light-saturated net photosynthetic rate (A) and the intercellular CO_2 concentrations (C_i; so-called A/C_i curves) and the relationship between net photosynthetic rate and irradiance (I; so-called A/I curves or light-response curves).

i. *Light-saturated net photosynthetic rate and the intercellular CO_2 concentrations curves (A/C_i)*

We set the leaves in the cuvette at saturating light (2000 μmol photons m^{-2} s^{-1} PAR) and low CO_2 concentration (30 μmol mol^{-1}) with air temperature of 20°C, vapor pressure deficit (VPD) of 1.1 kPa at stable conditions in the cuvette. Stomatal conductance (g_s) was recorded at the beginning before it had reacted to the changing conditions, but after steady state had been reached in A after ca. 3-7 minutes, the atmospheric CO_2 concentrations (C_a) were changed starting with low and ending with high concentrations, that is; 30, 100, 200, 300, 400, 700, 1000 and 1500 ppm. Leaf temperature (T_{leaf}) was on average 22°C during the measurements.

ii. Light response curves (A/I)

The *A/I* response curves were obtained directly after finishing the A/C_i measurements, and the C_a was kept at 1500 ppm. The light source was used to change the light levels at the leaf surface from high to low, that is; 2000, 1250, 1000, 750, 500, 250, 100, 50 and 0 µmol photons m^{-2} s^{-1} PAR.

At the end of the sampling, the leaves were harvested and stored in a cooler while transported to the laboratory. There the leaf area within the chamber was measured accurately with the WinSEEDLE 5.1a leaf scanner (Regent Instruments Inc., Quebec, Canada). After that the leaves were dried at 40°C for 48 hours, weighed for dry mass (*g*) and then ball-milled (Restsch, MM200, Haan, Germany) and stored for later nutrient analysis.

Leaf N and stable isotopes

The stable isotope and nutrient analysis were done at an external laboratory (Department of Geology, University of Tartu, Estonia) where leaf-*C* concentration (%), leaf-*N* concentration (%), the stable isotopic *C* ($\delta^{13}C$, $^{0}/_{00}$ V- PDB), stable isotopic *N* ($\delta^{15}N$, $^{0}/_{00}$ air N_2), and final dry weight (*g* dry mass, at 105°C) were measured.

Data analysis

Specific Leaf Area

The computation of specific leaf area (*SLA*, cm^2 g^{-1} dry mass) was calculated for the leaf samples (Equation 1)

$$SLA = \frac{A\_s}{W} \qquad (1)$$

where *A_s* is the leaf area (cm^2), and *W* is the dry mass (*g*).

Estimated Parameters from the A/C_i curves

To estimate the basic photosynthetic parameters from A/C_i curves done on individual leaves, the so-called convexity equation (Equation 2) was used (Heberling & Fridley, 2013; Roberntz & Stockfors, 1998). Sigurdsson et al., 2002)

$$A/C_i = \frac{\alpha.C_i + A_{max_{ci}} - \sqrt{(\alpha.C_i + A_{max_{ci}})^2 - 4.\alpha.C_i.A_{max_{ci}}.\theta}}{2\theta} - R_{day} \ (2)$$

Where α = the initial slope of the A/C_i curve represents the rate at which the concentration of CO_2 limits the activity of Rubisco. C_i = intercellular concentration of CO_2. A_{max} = The maximum rate of photosynthesis under conditions of carbon and light saturation., θ = convexity, *i.e.* the curvature factor of the A/C_i curve.

From the fitted A/C_i curves other parameters such as; the carbon compensation point (*CCP*), the theoretical carbon saturation point (*CSP*) at 50% of attained maximal assimilation (A_{max}), the maximal rate of photosynthesis at ambient carbon and light saturating conditions (A at C_a = 400ppm), assimilation rate at an intercellular CO_2 of 400 ppm (A at C_i = 400ppm), and the intercellular CO_2 concentration (C_i) at the ambient atmospheric CO_2 concentration (C_i at C_a = 400 ppm). Furthermore, the relative stomatal limitation was estimated from each A/C_i curve (L_s, Equation 3, Sigurdsson et al., 2002).

$$L_s = \left(1 - \frac{A_{net2000}}{A_{sat_I}}\right) \times 100 \; (3)$$

Where $A_{net2000}$ was the rate of light-saturated photosynthesis at the ambient atmospheric CO_2 concentration (C_a = 400 ppm), and $A_{sat\,I}$ was the light-saturated photosynthetic rate when intercellular CO_2 concentration was equal to C_a of 400 ppm.

Finally, the individual A/C_i curves were fitted by the Farquhar, von Caemmerer and Berry model (Farquhar et al., 1980). The model incorporates estimates when the net assimilation rate (A) is limited by the Rubisco enzyme activity and when it is limited by the RuBP-regeneration (light-harvesting processes). (Wullschleger, 1993)The model fitting followed the Duursma, (2015) hyperbolic minimum equation, which provided the transition point for every curve (Roberntz & Stockfors, 1998) (Supp. Figure 2) derived using the *plantecophys* package in R were the maximum rate of Rubisco carboxylase activity (Vc_{max}), the maximum rate of photosynthetic electron transport (J_{max}), the leaf mitochondrial respiration in the light (R_{day}) excluding of photorespiration (Wullschleger, 1993).

Estimated Parameters from the A/I curves

The methodology used in analysing the *A/I* response curves was similar to that of the A/C_i curves, i.e. we used the convexity formula to model the curves (Equation 4, Supp. Figure 3).

$$A_I = \frac{\beta.I + A_{sat} - \sqrt{(\beta.C_i + A_{sat})^2 - 4.\beta.I.A_{sat}.\theta}}{2\theta} - R_{dark} \; (4)$$

Where β is the apparent quantum yield, I is the irradiance (intensity of incident PAR), A_{sat} is the maximal light saturated rate of net photosynthesis at CO_2 saturation (C_a = 1500 ppm). θ is the curvature factor of the curve (convexity) and R_{dark} is the mitochondrial respiration in dark, usually termed dark respiration.

Statistical analysis

All data and statistical analyses were conducted with R software (4.2.2, R Core Team, 2022), where computation and modelling of different parameter was conducted before diving into statistical analysis. The normality of the data was tested using the Shapiro-Wilk test ($\alpha = 0.05$).

For each parameter from the A/C_i curves, A/I curves, leaf isotopic and structural traits, the mean ± standard error was calculated for across both ambient (0°C) and warmed (+8°C) plots for both grasslands sites (MTW and LTW) measured in different years (2021, 2023). An analysis of variance (ANOVA, aov, *stats* package) was then performed to observe how the observed variation in each parameter could be related to the three experimental variables (warming, sites and year) and interactions between those. Furthermore, a multiple linear regression was later conducted on the most relevant relationships with plot mean annual soil temperature (MAT_s) and leaf-N using the *lm* function from the *stats* package. Since linear regression models require normally distributed and homoscedastic residuals, we verified these assumptions specifically on the model residuals. For each linear model (LM) we examined normality of residuals with Q-Q plots and assessed homoscedasticity.

RESULTS

Basic measurements

The net light saturated photosynthetic rate measured at ambient atmospheric CO_2 concentration of 400 ppm showed no significant effect with soil warming level ($p = 0.8$) and no significant difference was found between the sites (Figure 3; Table 1). On average the rate of $Anet_{2000}$ was 13.2 µmol CO_2 m^{-2} s^{-1}.

Surprisingly, the leaf-*N* concentration (%) was not either significantly affected by the prolonged soil warming ($p = 0.63$), nor for the duration of warming (Figure 3; Table 1). The average leaf-*N* was 1.9% of dry leaf dry mass. Similarly, SLA (cm^2 g^{-1}) did not significantly vary between soil warming levels ($p = 0.62$) and no significant difference was found between the sites or interactions. It was on average 179.8 $cm^2 g^{-1}$ across all the treatments (Figure 3; Table 1).

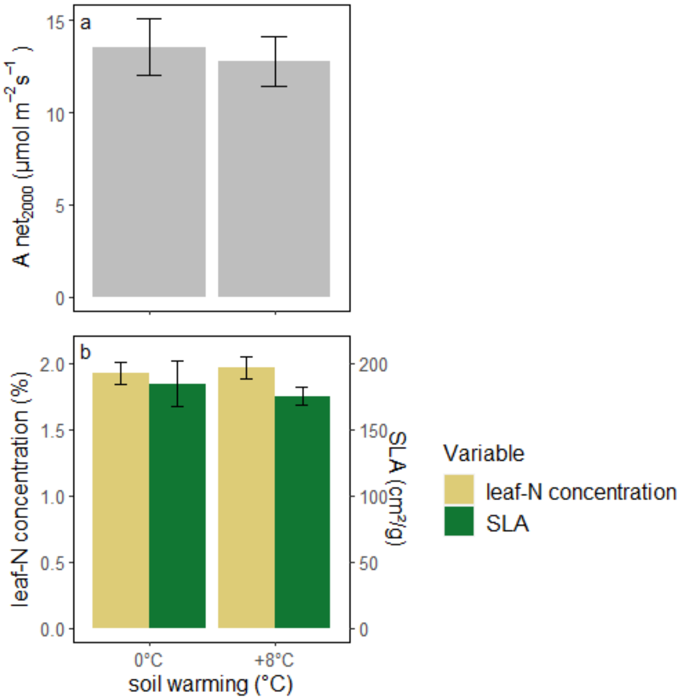

Figure 3: Mean and standard error visualisation of: a – the net assimilation rate at atmospheric ambient CO_2 (on average 400 ppm), b – leaf nitrogen concentration and specific leaf area, against the two studied soil warming levels unwarmed (0°C) and warmed (+8°C) for both subarctic grasslands (MTW, LTW). The legend shows variables from both y-axes across the studied warming levels.

Table 1: Analyse of variance of the effects of site, soil warming (T_s), leaf-*N* concentrations (*N*), and interaction terms of photosynthetic parameters alongside looking at the site, and T_s effects of *N* concentration. Displayed results are the p-values with significance level $p < 0.05$ and non-significance $p > 0.05$. Values annotated “n.d.” non-determined values.

	Site	**T_s**	**N**	**Site × T_s**	**Site × N**	**T_s × N**	**Site × T_s × N**
$A_{net2000}$	0.34	0.81	0.36	0.52	*0.08*	0.89	0.84
N	0.87	0.63	n.d.	0.59	n.d.	n.d.	n.d.
SLA	0.20	0.62	0.19	0.46	0.58	0.14	0.19

Change in light-saturated photosynthesis system (A/C_i curves)

There was no effect of warming ($p > 0.7$), nor duration of warming ($p > 0.10$) on both main Farquhar-model photosynthetic parameters (Figure 4, Table 2). On average, the observed rates were 56.7 and 124.6 µmol m^{-2} s^{-1} for Vc_{max} and J_{max}, respectively.

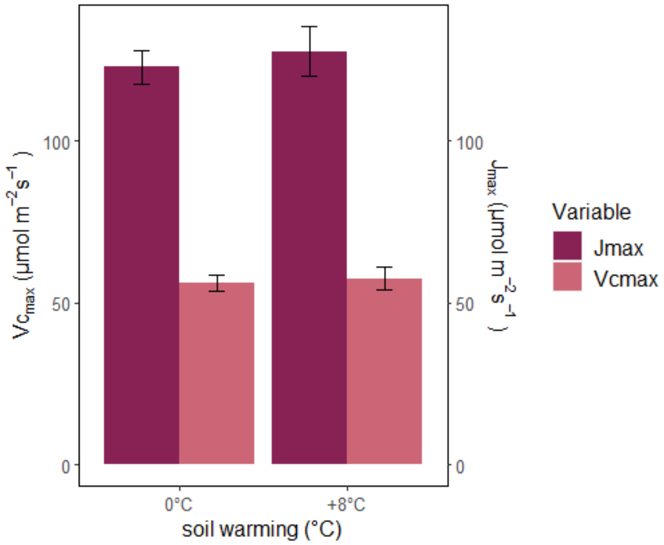

Figure 4: Farquhar model estimated parameters (Vc_{max}, J_{max}) relationship to the two studied soil warming levels unwarmed (0°C) and warmed (+8°C) for both subarctic grasslands (MTW, LTW). The legend shows variables from both y-axes across the studied warming levels.

Table 2: ANOVA statistics of the effects of year (*Y*), site, warming (T_s), leaf-*N* concentration (*N*), and interaction terms of estimated A/C_i, and *A/I* photosynthetic parameters in Table 3. Other interactions were not included in the table due to their non-significance; only relevant interactions were displayed. Results show the p-values with significance level $p < 0.05$ (bold values) and non-significance $p > 0.05$. The list of abbreviations is displayed in Supp. Table 1.

	T_s	**Site**	**N**	**Y**	**T_s × Y**	**N × Y**	**Site × T_s**	**T_s × N**	**Site × T_s × N**
Vc_{max} (μmol s^{-1} m^{-2})	0.92	0.13	**<0.01**	0.18	0.19	0.89	0.43	0.96	0.43
J_{max} (μmol s^{-1} m^{-2})	0.68	0.67	**<0.01**	0.55	0.30	0.88	0.22	0.78	0.23
R_{day}	0.84	0.98	**<0.01**	**<0.01**	*0.07*	**0.03**	**0.03**	0.50	0.86
$A_{max\ A/Ci}$ (μmol s^{-1} m^{-2})	0.64	0.30	**<0.01**	0.57	0.61	0.87	0.33	0.88	0.89
α	0.76	0.19	**<0.01**	0.31	0.72	0.88	0.53	0.93	0.65
R_{day}	0.93	*0.07*	**0.03**	**<0.01**	0.30	0.10	**0.04**	0.85	0.21
Θ	0.98	0.30	0.55	0.41	0.40	0.64	0.28	0.51	0.53
CCP (ppm)	0.93	0.24	**<0.01**	**<0.01**	0.42	0.9	*0.06*	0.82	0.16
CSP (ppm)	**0.03**	0.41	**<0.01**	**<0.01**	0.23	0.26	**<0.01**	0.78	0.68
C_i at C_a = 400_{ppm}	0.74	0.79	**0.02**	0.57	0.60	0.26	0.31	0.62	0.13
A at C_i = 400_{ppm} (μmol CO_2 m^{-2} s^{-1})	0.81	0.35	**<0.01**	**<0.01**	0.58	0.84	0.32	0.94	0.99
A at C_a = 400_{ppm} (μmol CO_2 m^{-2} s^{-1})	0.44	0.58	**<0.01**	**<0.01**	0.83	0.90	0.75	0.97	0.56
L_s (%)	0.88	0.55	0.84	0.51	0.48	0.49	*0.05*	0.57	0.48

Photosynthetic parameters derived from the A/C_i curves (Table 3) did not show significant responses to warming levels or site differences, except for the theoretical carbon saturation point (*CSP*) differed significantly between warming levels (p = 0.03) and showed a significant interaction between warming level and site (p = 0.001; Table 2). *CSP* represents the C_i concentration at which 50% of A_{max} is reached, it was close to the realised C_i at C_a in the MTW treatments, while it was higher in the LTW treatments, especially for the warmed plots. Since the realised *A* at C_a = 400 ppm was not significantly affected, then this finding is of limited relevance for actual carbon uptake under ambient conditions. The average value across all the measurements for A_{max} was 28.2 µmol m^{-2} s^{-1}, and the carboxylation efficiency (α) was 0.063 mol m^{-2} s^{-1} (Table 3).

Table 3: A/C_i – Mean and standard error values from the Farquhar model (R_{day}), convexity parameters (A_{max}, *Alpha,* R_{day}, Θ), other derived parameters: the *CCP, CSP,* C_i at C_a = 400 ppm, A at C_i = 400 ppm, and A at C_a = 400 ppm for both Icelandic grassland sites (MTW, LTW) at ambient (0°C) and warmed (+8°C) soil temperature conditions.

	MTW$_{ambient}$		**MTW$_{warmed}$**		**LTW$_{ambient}$**		**LTW$_{warmed}$**	
	mean ± SE		mean ± SE		mean ± SE		mean ± SE	
R_{day} (Farquhar model)	1.3	0.3	0.8	0.2	1.2	0.3	1.0	0.3
$A_{max\ A/Ci}$ (μmol s^{-1} m^{-2})	26.3	1.9	28.0	1.5	29.2	1.9	29.2	1.9
α	0.1	0.004	0.1	0.004	0.1	0.01	0.1	0.01
R_{day}	3.1	0.5	2.7	0.17	3.9	0.5	3.6	0.4
Θ	1.0	0.2	0.97	0.01	1.0	0.01	1.0	0.01
CCP (ppm)	55.7	0.02	44.9	2.4	68.4	11.3	58.6	9.1
CSP (ppm)	344.1	6.4	317.6	13.7	369.2	30.8	401.3	51.4
C_i at C_a = 400$_{ppm}$	329.5	4.9	322.8	5.9	329.0	3.2	326.7	3.3
A at C_i = 400$_{ppm}$ (μmol CO$_2$ m^{-2} s^{-1})	16.1	1.3	17.3	1.0	17.0	1.6	18.1	1.7
A at C_a = 400$_{ppm}$ (μmol CO$_2$ m^{-2} s^{-1})	14.9	1.2	15.9	0.9	15.0	1.5	15.5	1.2

Light response of the photosynthesis system (*A/I* curves)

Similarly, the main light response curve parameters, apparent quantum yield (Φ) and dark respiration were not significantly affected by warming level or site (Figure 5, Table 4). Their average values were 0.058 μmol CO$_2$ μmol photons^{-1} and 2.80 μmol m^{-2} s^{-1} for the Φ and R_{dark}, respectively.

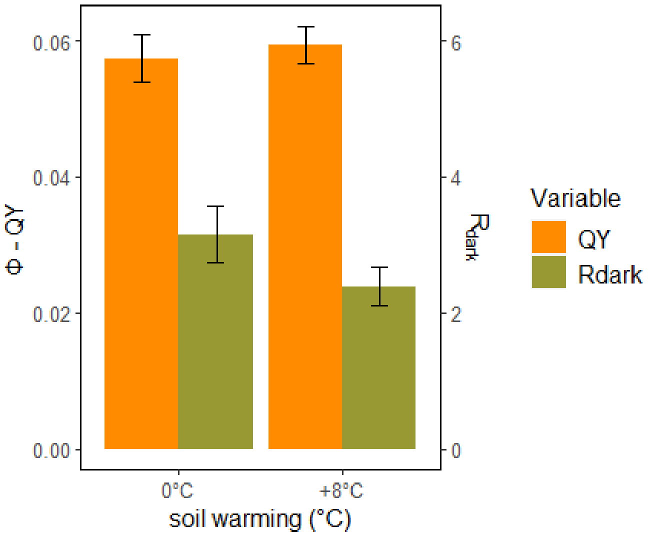

Figure 5: Visualisation of the average and standard error of light response curve convexity parameters (Φ - *QY*, R_{dark}) against the two studied soil warming levels unwarmed (0°C) and warmed (+8°C) for both subarctic grasslands (MTW, LTW). The legend shows variables from both y-axes across the studied warming levels.

Table 4: ANOVA statistics of the effects of year (*Y*), site, warming (T_s), leaf-*N* concentration (*N*), and interaction terms of estimated *A/I* photosynthetic parameters in Table 3. Other interactions were not included in the table due to their non-significance; only relevant interactions were displayed. Results show the p-values with significance level $p < 0.05$ (bold values) and non-significance $p > 0.05$. The list of abbreviations is displayed in Supp. Table 1.

	T_s	**Site**	**N**	**Y**	**T_s × Y**	**N × Y**	**Site × T_s**	**T_s × N**	**Site × T_s × N**
A_{sat} (µmol m^{-2} s^{-1})	0.31	0.45	**<0.01**	*0.05*	0.66	0.22	*0.09*	0.56	0.78
Φ	0.46	0.53	*0.08*	**0.03**	0.37	0.96	0.36	0.69	0.61
R_{dark} (µmol m^{-2} s^{-1})	0.50	0.11	**<0.01**	**<0.01**	0.26	0.12	0.44	0.26	0.86
Θ	**0.03**	0.73	0.88	**<0.01**	0.41	**0.01**	0.56	0.58	0.23
LCP µmol m^{-2} s^{-1} PAR	0.47	0.39	**<0.01**	0.11	0.67	0.82	*0.07*	0.89	0.96
LSP µmol m^{-2} s^{-1} PAR	0.74	0.37	0.35	*0.07*	0.19	**<0.01**	0.89	0.62	0.60

Photosynthetic parameters derived from the *A/I* curves are shown in Table 5. The average theoretical fitted maximum light-saturated net photosynthetic rate (A_{sat}) was quite high, or 32.4 µmol m^{-2} s^{-1}, which was also much higher than the measured A_{max} from the A/C_i curves, indicating that the *A* had not reached stable values at the highest measured irradiance of 2000 µmol PAR (see Supp. Figure 2). None of the parameters in Table 5 differed significantly between warming treatments or sites, except the curvature factor (Θ), which significantly decreased with warming ($p = 0.02$; Table 4). This indicates more gradual light saturation in the warmed treatments due to some morphological changes of the leaves. The light compensation point (*LCP*) was on average low, or only 23.7 µmol PAR, and the light saturation point showing 75% of the theoretical A_{sat} was very high, or 1751.1 µmol PAR, on average (Table 5).

Table 5: A/I – Mean ± standard error values of convexity light response curve parameters (A_{sat}, Θ), along with other derived parameters—namely the light compensation point (*LCP*) and light saturation point (*LSP*)—for both Icelandic grassland sites (MTW and LTW) under ambient (0°C) and warmed (+8°C) soil temperature conditions.

	$MTW_{ambient}$		**MTW_{warmed}**		**$LTW_{ambient}$**		**LTW_{warmed}**	
	mean ± SE		mean ± SE		mean ± SE		mean ± SE	
A_{sat} (μmol m^{-2} s^{-1})	27.8	1.6	34.1	4.4	33.8	3.2	33.9	3.5
Θ	0.7	0.1	0.3	0.3	0.4	0.2	0.1	0.5
LCP (μmol m^{-2} s^{-1} PAR)	21.9	1.6	24.2	1.4	24.2	1.8	24.6	1.9
LSP (μmol m^{-2} s^{-1} PAR)	1197.2	113.5	2019.0	599.9	1920.6	392.5	1903.9	466.6

Indicators of plant water status

Stomatal conductance was relatively high during the measurement days, or 0.35 mol H_2O m^{-2} s^{-1} on average (Figure 6a). The soil warming did not significantly alter g_s ($p = 0.4$), so water stress was not detected before response-curve measurements were done. This finding was supported with the calculated stomatal limitation (L_s) derived from the A/C_i curves did not show significant effects of soil warming (Figure 6b, Table 2). It was further supported that drought had not been affecting *R. acris* more in the warmed treatments, as the leaf stable isotope ratio in leaf tissues was not significantly different ($\delta^{13}C$, $p = 0.09$), nor were there significant interactions between warming and sites ($p > 0.05$). On average, the $\delta^{13}C$ was -29.3 $^{0}/_{00}$ across all treatments (Figure 6a).

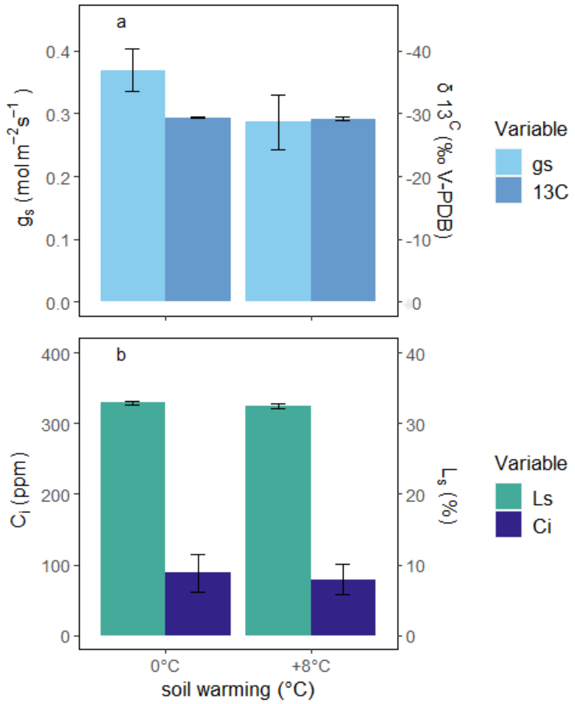

Figure 6: Average and standard error values of physiological parameters (g_s, $\delta^{13}C$, L_s, C_i) depicting the soil water status at the leaf level in warming levels (0° - ambient, +8°C – warmed), for both subarctic grasslands (MTW, LTW). The legends show variables from both y-axes across the studied warming levels.

Duration of warming feedback (Site differences)

Duration of warming (MTW vs. LTW) did not significantly influence any of the photosynthetic parameters for *R. acris* as a main factor (Table 2, Table 4), and neither did the chemical or morphological traits significantly differ between the sites (Table 1). This was contrary to our hypothesis. However, the $\delta^{13}C$, showed a significant site difference ($p = 0.02$, Supp. Table 2b), where LTW plots had on average -29.6 $^{0}/_{00}$ while MTW had -29.1 $^{0}/_{00}$. The less negative ratio (MTW) across both warming levels, indicated that stomates had been more open on average during the formation of the leaf tissue there. This effect is unlikely to be related temperature nor any acclimation or adaptation by the *R. acris* plants, but rather to site-specific differences in hydrology.

There were few instances of a significant *warming* × *site* interaction, *e.g.* for R_{day} in both the Farquhar model and the A/C_i convexity curve analysis (Table 2), while R_{dark} did not show such a response (Table 4). Same was true of *CSP* (Table 2). It remains, however, unclear if these parameters had any practical implications for the realised gas exchange at the two sites, as they are not directly controlling photosynthetic rates at ambient conditions.

N availability feedback

As was stated earlier, the leaf-*N* concentration (%) was not significantly affected by soil warming ($p = 0.63$) neither did the significant interactions between warming and site (Figure 3; Table 1). This was contrary to our hypothesis.

However, when *N* concentration was used as an independent factor in the ANOVA models, it was highly significant for most measured parameters (Table 2; Table 4). To illustrate this strong *N* relationship that existed across all treatments within the ForHot grassland experiment, we show it here for the estimated Farquhar parameters (Figure 7).

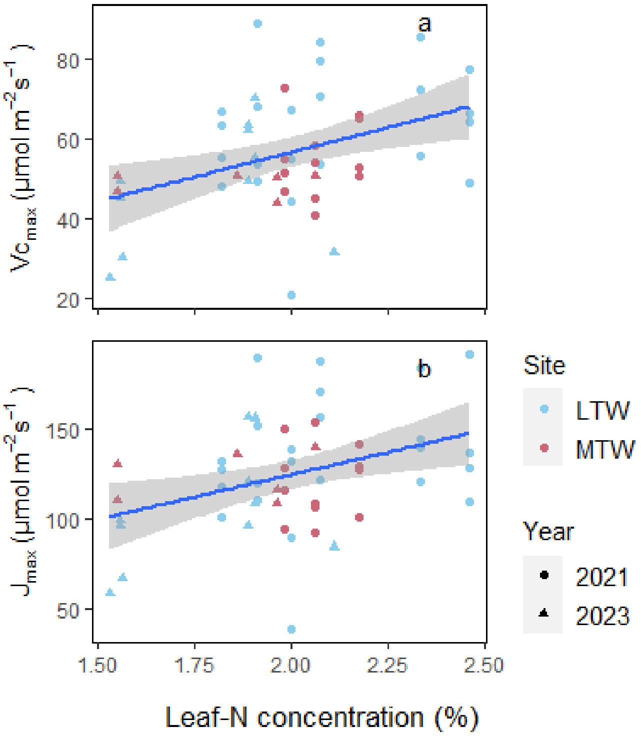

Figure 7: Linear relationship between Farquhar modelled parameters and leaf-*N* concentrations across both the MTW and LTW.

There was an overall significant response of the estimated Farquhar parameters with N concentration ($p < 0.002$, Table 2) across all treatments. The response was linear and is shown in Figure 7. They were positive and the regression equations were:

$Vc_{max} = 7.1 + 25 \times N$ (5)

$J_{max} = 25 + 50 \times N$ (6)

where $R^2 = 0.17$ and 0.14, and $p = 0.003$ and 0.006 for the Vc_{max} and J_{max}, respectively (Figure 7). The other photosynthetic parameters derived from the A/C_i curve convexity formula (Equation 2), including the A_{max}, α and R_{day} responded significantly positively to leaf-N concentration across all treatments (Table 2), and here we show:

$A_{max} = 2.6 + 13 \times N$ (7)

$\alpha = 0.001 + 0.03 \times N$ (8)

which both had R^2 of 0.2 and $p = 0.0002$ (data not shown). Other parameters showing photosynthetic capacity at different conditions had similar positive N concentration responses across all treatments (Table 2). The only parameter in the A/C_i curve convexity formula that did not have a significant response to the leaf-N concentration was Θ (Table 2).

Moreover, the calculated value of the intercellular CO_2 concentration (C_i) at atmospheric CO_2 (C_a) was a parameter where the leaf-N concentration led to a significant negative reaction:

C_i at $C_a = 370 – 22 \times N$ (9)

where $R^2 = 0.10$, $p = 0.012$ (data not shown). This negative reaction indicates that g_s did not open sufficiently to compensate for the increased photosynthetic capacity of the N-richest leaves.

In addition, the light response curve parameters had mostly similar overall positive reaction to leaf-N concentration across all treatments, with the exception of the Θ and the apparent quantum yield (Φ), which was only marginally positively affected ($p = 0.08$; Table 4). An unexpected negative N response was detected for R_{dark} when expressed per m^2 leaf area:

$R_{dark} = 7.2 – 2.2 \times N$ (10)

where R^2 was 0.07 and $p = 0.03$ (data not shown).

It was noteworthy, but not unexpected, that many of the response curve parameters also showed significant differences between year of measurement (Table 2 and Table 4), which was also significant for the leaf-N concentration ($p = 0.02$; data not shown).

DISCUSSION

In this study, we investigated how soil warming influenced plant ecophysiological responses of one common species in the Icelandic subarctic grasslands, *Ranunculus acris*, which we used as a representative species. We were especially interested in how 1) prolonged soil warming would affect the photosynthetic characteristics of this species, but also how other possible long-term feedback would be apparent, including 2) possible more water limitation to gas exchange at warmed plots, 3) whether the duration of soil warming influences leaf-*N* concentration.

Effect of soil warming on photosynthetic parameters

Our findings support the first hypothesis, which proposed that the estimated photosynthetic parameters, when expressed per unit leaf area, would not be strongly affected by soil warming. Both Vc_{max} and J_{max} remained unaltered across the two warming levels. *R. acris* showed limited plasticity or adaptation to the prolonged soil warming, at a leaf level. Although photosynthetic rates are directly influenced by light intensity, temperature and intercellular CO_2 concentrations at the molecular level (Geider & Osborne, 1992), plant responses at high-latitude ecosystems tend to be less sensitive to atmospheric temperature changes than more southern plants (Chapin, 1983). This suggests that other environmental factors, such as nutrient limitation (Chapin, 1983), carbon allocation strategies (Chapin et al., 2012) or species-specific adaptations to high-latitude habitats (Bliss, 1962) may play a stronger role in regulating photosynthetic activity.

This finding is consistent with other studies in the tundra or low-arctic ecosystems, which have showed a limited physiological plasticity in response to warming per unit leaf area (Starr et al., 2008; Chapin, 1983). Starr et al., (2008) proposed that the absence of change in physiological capacity (A_{max}) in respect to climate warming may reflect internal constraints that limit their ability to alter the maximum physiological response. (Chapin, 1983)

It should still be noted that *R. acris* has a quite effective photosynthesis system and is capable of having high *C* uptake rates during high light, air temperatures and CO_2 conditions (open stomates), even if core photosynthetic machinery remains unchanged with warming. Its Vc_{max} and J_{max} were on average 56.7 and 124.6 µmol m^{-2} s^{-1}, which can be compared to "global average" of 64 and 134 µmol m^{-2} s^{-1} for *C3* species (Wullschleger, 1993). This helps this perennial species complete its annual growth cycle within a relatively short growing season.

Notably, among other photosynthetic parameters assessed, the *CSP* from the A/C_i curve showed a significant positive response to warming. Thereby, suggesting that under elevated temperatures, *Ranunculus acris* may have a slight increase in *C* uptake capacity.

Effect of plant water status in warmed soils

We had hypothesised that realised gas exchange could be limited by more soil water limitation at the warmed plots; but soil drought has often been found to be an important driver in (soil) warming manipulation experiments (Schaeffer et al., 2013; Welker et al., 2004). The key parameter to detect this is the stomatal conductance (g_s), which regulates both *C* uptake and transpiration of water from the *R. acris* leaves. However, g_s did not show any significant reductions with warming in this study at the times of measurements. Similarly, there was no significant effect of warming on the $\delta^{13}C$ which provides insights on the water-using processes of plants change in terrestrial ecosystems (Yan et al., 2020), and indicates that partial stomatal closure did not much occur in the studied species under warming manipulations during the time of active leaf growth.

The "lack" of warming-related "drought" response corresponded, however, well with relatively small changes in the volumetric water contents of the top 5 cm of soil at the warmed plots compared to ambient plots, while water content would vary across the whole experiment in relation to rainfall patterns (Helgadóttir et al., 2013; Sigurdsson et al., 2016). This was explained by the site's high and frequent precipitation throughout the year and the relatively low air temperatures that limit excessive evapotranspiration and soil drying from the warmed plots (Sigurdsson et al., 2016).

Other studies on Arctic plants have found a different response to warming manipulations, especially for monocot species that displayed a higher drought sensitivity with lower A_{max} and g_s values, alongside negative corelation of $\delta^{13}C$ with warming, suggesting strong stomatal conductance limitation (Cahoon et al., 2016). Lower $\delta^{13}C$ is typically associated with higher stomatal conductance and consequently less water-limitations (McDowell et al., 2004).

It was noteworthy that the MTW site generally had a lower $\delta^{13}C$ value in the *R. acris* leaf tissues than when it grew at the LTW, but even if the LTW has somewhat deeper soils than MTW it is located in a more flat grasslands (Sigurdsson et al., 2016), and its soil moisture is therefore maybe less affected by sub-surface soil water movements from up-slope during drier periods during summer, possibly making the site more prone to short-term droughts.

Duration of warming feedback response

It was expected for the MTW and LTW to show different response to warming from our hypothesis, which would indicate some types of acclimatation, or adaptation would have taken place where the warming had been ongoing for >60 years. Such "duration of warming" responses has been shown for many different ecosystem processes and community structure variables within the ForHot grassland experiment (Walker et al., 2020). Our results showed, however, that the duration of warming did not significantly influence the photosynthetic parameters for *R. acris*. An earlier study on the plant metabolomes at the ForHot grassland experiments has, indeed, shown that *R. acris* did not have any significant reactions to the duration of warming, contrary to the monocot *Agrostis capillaris* which reacted strongly to the duration of warming (Gargallo-Garriga et al., 2017). It is therefore possible that our choice of species may have affected this unexpected outcome.

The lack of significant difference in leaf-*N* concentrations, between the MTW and LTW sites, was unexpected finding. But many of the expected feedback responses had been based on the assumption that LTW would have had more alterations in its *N*-cycle.

Feedback response to change in the *N* availability

Atmospheric warming has been showed to enhance soil *N* mineralisation in high-latitude ecosystems (Rustad et al., 2001). If the *N* is taken up by the plants and kept in an active *N*-cycle within the plant-soil system, this can theoretically create a positive feedback loop where increased *N* availability supports higher photosynthetic capacity (Hartley et al., 2010). However, in our study, as previously stated, warming did not directly affect most photosynthetic parameters nor the leaf-*N* concentrations. So, this hypothesis could not be validated within this study.

Interestingly, *N* seemed to remain a limiting factor for *R. acris* ecophysiology across all the treatments at the ForHot grassland experiment, with strong *N* relationships found for most photosynthetic parameters. That warmed plots are *N*-limited was also found in the pulse-labelling study of Meeran et al. (2023) at the MTW sites. This unexpected finding could be partly explained by the results of Marañón-Jiménez et al., (2019), who found that the mineralised *N* was not kept in the active soil-plant *N*-cycle in the warmed treatments at ForHot but had been lost. This conundrum in the ForHot site *N*-cycle is now being actively studied.

Temporal and spatial variations in *N* availability is therefore one of the strongest controlling factors for *R. acris* photosynthetic capacity (Vc_{max}, J_{max}, A_{max}, etc.), while it is not particularly affected by the warming treatments. Leaf-*N* is known to both strongly affect the Rubisco-limited (Vc_{max}) and electron transport-limited (J_{max}) stages of photosynthesis (Evans, 1989; Field & Mooney, 1986; Walker et al., 2014).

Furthermore, there were indications that one of the strongest contributing factors in the leaf-*N* concentrations in this study was the year of measurements, which may indicate some differences in the annual *N*-mineralisation or plant phenology at the time of measurements.

Limitation of the study

There was an effect of *year* in most estimated photosynthetic variables (Table 2, 4). Though interannual variation could be an indirect factor in responses observed (Werner et al., 2020), when interpreting the generality of limited warming response some points should be taken into account. For instance, perennial plant communities could hypothetically be stable on year-to-year basis which incorporates short-term fluctuations that might shadow long-term trends (Morecroft et al., 2016). Also, other studies at ForHot have indeed found that annual variation may be much larger than the underlying soil warming impacts for NDVI (Mortier et al., 2024) or aboveground production in the ForHot grasslands (Wandji et al., 202xa - *in review AAAR*).

This study showcases only one species amongst ca. 35 vascular plant species present at the studying site, albeit one of the more common ones. Therefore, a certain caution must be made before drawing too strong conclusions about the whole grassland photosynthesis per unit leaf area, as photosynthetic response to warming may vary among different plant species (Starr et al., 2008).

It is also important to remember that seasonal *C* uptake can still increase with unaltered photosynthetic capacity per unit leaf area, if a) the duration of active leaves is extended in the community or if b) amount of leaf area per surface area is increased in the plant community. According to Mortier et al. (2024) and (Wandji et al., 202xb - *submitted at the Arctic Science Journal*) both seemed to take place in the ForHot grasslands as a response to soil warming.

Finally, the natural experimental setup of our research at the ForHot in Iceland mainly focuses on belowground warming and caution should be employed when drawing conclusions to what would happen after climate warming, as belowground warming might not fully capture the range of aboveground responses to atmospheric warming (Sigurdsson et al., 2016).

CONCLUSION

Based on the findings, *R. acris* was not more photosynthetically active under prolonged soil warming. The lack of significant change in key photosynthetic parameters with warming indicated that the species had a conservative response to changing environmental conditions, as has been found for many subarctic plants.

R. acris leaf-*N* concentration was not significantly affected by the prolonged warming, but still the *N* concentration showed strong relationship with photosynthetic performance across all the treatments. This suggests that *N* availability, rather than temperature change, was a primary factor influencing photosynthetic activity of *R. acris* at these subarctic sites. These findings underscore the importance of accounting for nutrient dynamics when assessing plant responses to soil warming.

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Competing Interest

The authors declare no conflict of interest.

Author Contribution

Ruth Phoebe Tchana Wandji: Conceptualisation, Writing – Original Draft, Writing – Review & Editing, Validation, Visualisation, Methodology, Investigation, Formal analysis, Data Curation and Analysis. **Timon Callebaut**: Writing – Review & Editing, Methodology, Data Analysis, Data Sampling. **Iolanda Filella**: Writing – Review & Editing, Funding Acquisition, Supervision. **Peter Lootens:** Writing – Review & Editing, Funding Acquisition, Supervision. **Bjarni Diðrik Sigurdsson**: Investigation, Conceptualisation, Writing - Review & Editing, Funding Acquisition, Supervision, Project Administration.

Data Availability

The data used in this manuscript can be found at the ZENODO Database and it is Open Access.

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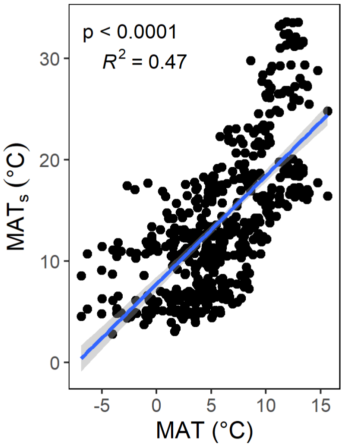
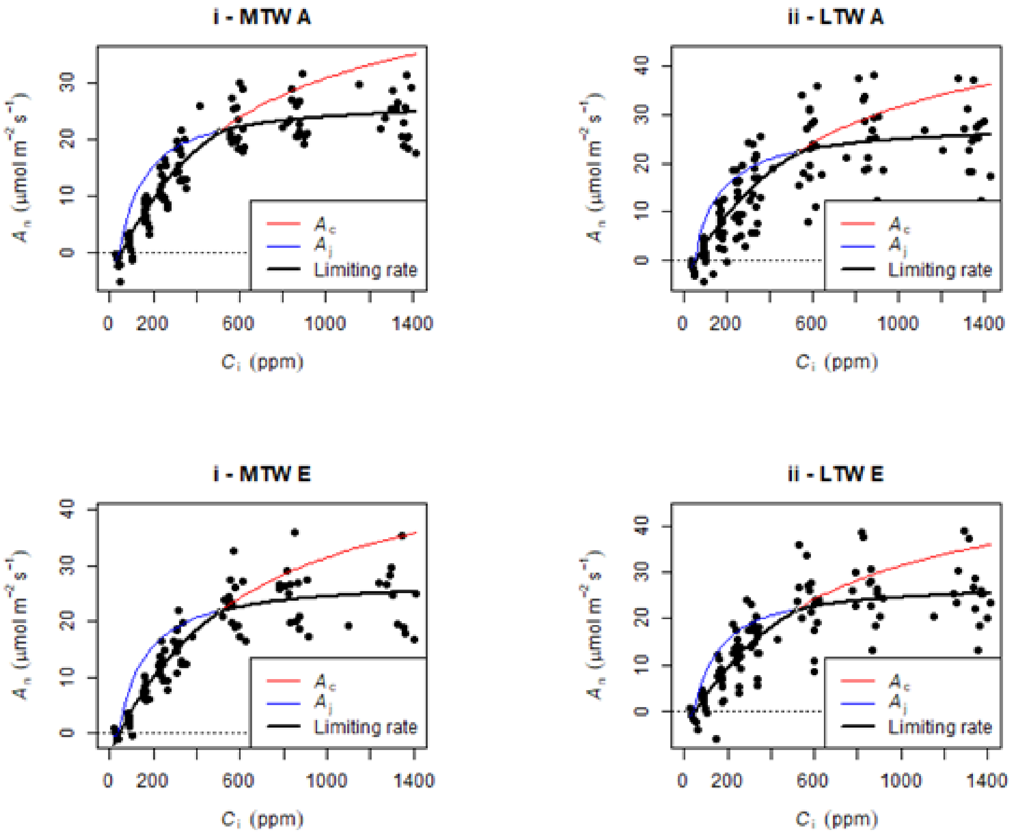
Supporting Materials

Supp. Table 1: List of abbreviations utilised in different result tables or with the document's text.

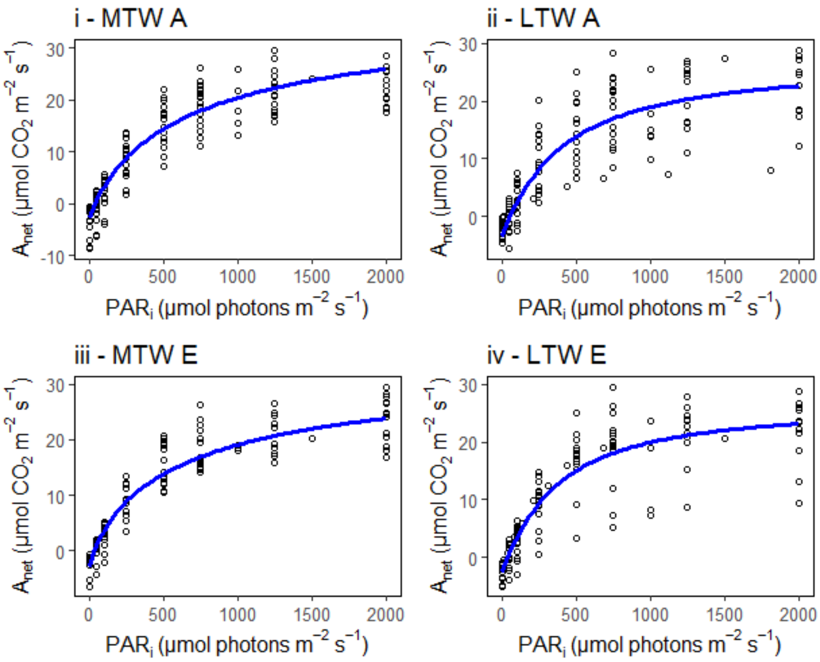
Abbreviations	Full description
C_a (ppm)	Atmospheric CO_2
C_i (ppm)	Intercellular CO_2
$A_{net2000}$ (µmol m^{-2} s^{-1})	Rate of light-saturated photosynthesis at the ambient atmospheric CO_2 concentration
Vc_{max} (µmol m^{-2} s^{-1})	Maximum rate of Rubisco carboxylase activity
J_{max} (µmol m^{-2} s^{-1})	Maximum rate of photosynthetic electron transport
$A_{max\ A/Ci}$ (µmol m^{-2} s^{-1})	The maximum rate of photosynthesis under conditions of carbon and high light conditions.
α	Initial slope of the A/C_i curve
R_{day}	Leaf mitochondrial respiration in the light excluding of photorespiration
ϴ	Convexity parameter, *i.e.* the curvature factor of the A/C_i and A/I curves
CCP (ppm)	Carbon compensation point
CSP (ppm)	Theoretical carbon saturation point at 50% attained maximal assimilation
C_i at C_a = 400ppm	Intercellular CO_2 concentration at atmospheric CO_2 concentrations
A at C_i = 400ppm (µmol CO_2 m^{-2} s^{-1})	Assimilation rate at an intercellular CO_2 of 400 ppm
A at C_a = 400ppm (µmol CO_2 m^{-2} s^{-1})	Maximal rate of photosynthesis at ambient carbon and light saturating conditions.
L_s (%)	Relative stomatal conductance
A_{sat} (µmol m^{-2} s^{-1})	Maximal light saturated rate of net photosynthesis at CO_2 saturation
Φ	Apparent quantum yield; initial slope of the light response curve.
R_{dark} (µmol m^{-2} s^{-1})	Dark respiration
LCP µmol m^{-2} s^{-1} PAR	Light compensation point
LSP µmol m^{-2} s^{-1} PAR	Theoretical light saturation point at 75% attained maximal assimilation



Supp. Figure 1: Positive linear regression between annual atmospheric temperature (MAT, °C), and the annual mean soil temperature (MAT_s, °C) for the year 2021 at both studied Icelandic grassland sites (MTW, LTW).



Supp. Figure 2: Mean estimation of *A/C_i* curves for both MTW and LTW warming at the ForHot site. Where A = unwarmed plots (0°C) and E = warmed plots (+8°C).



Supp. Figure 3: Mean estimation of *A/I* curves for both MTW and LTW warming at the ForHot site. Where A = unwarmed plots (0°C) and E = warmed plots (+8°C).

Supp. Table 2: a – Mean and standard error calculations of leaf *C* concentrations (%), and stable isotope ($\delta^{15}N$ – ‰ air N_2) for both Icelandic grassland sites (MTW, LTW) at ambient (0°C) and warmed (+8°C) soil warming levels. b - statistical analysis of effects of the site, warming (T_s), leaf-*N* concentrations (*N*), and interaction terms on leaf isotopic. Results show p-values at significance level $p < 0.05$ and non-significance $p > 0.05$.

a	**MTW$_{\text{ambient}}$**		**MTW$_{\text{warmed}}$**		**LTW$_{\text{ambient}}$**		**LTW$_{\text{warmed}}$**	
	mean ± SE		mean ± SE		mean ± SE		mean ± SE	
C	42.6	0.6	42.2	0.6	42.2	0.6	42.4	0.7
δ^{15}N	-2.0	0.9	-1.9	0.4	-3.3	0.7	-1.9	0.5

b	Y	Site	T$_s$	T$_s$ × Y	Site × T$_s$
C	**<0.01**	0.35	0.70	0.99	0.99
δ^{13}C	0.18	**0.02**	*0.09*	0.40	*0.06*
N	**0.02**	0.84	0.57	0.22	0.26
δ^{15}N	**0.03**	0.26	0.14	*0.08*	0.37
SLA	0.40	0.24	0.62	0.85	*0.09*

Supp. Table 3: A/C_i – Mean and standard error values from the Farquhar model (Vc_{max}, J_{max}, R_{day}), convexity parameters (A_{max}, $Alpha$, R_{day}, Θ), other derived parameters such as the CCP, CSP, C_i at C_a = 400 ppm, A at C_i = 400ppm, A at C_a = 400ppm, and the L_s for both Icelandic grassland sites (MTW, LTW) at ambient (0°C) and warmed (+8°C) soil warming levels. A/I – Average and standard error values from convexity light response curve parameters (A_{sat}, $\Phi – QY$, R_{dark}, Θ), and other derived parameters, *i.e.*, *LCP*, and *LSP*.

		MTW$_{ambient}$				**MTW$_{warmed}$**				**LTW$_{ambient}$**				**LTW$_{warmed}$**			
A/C$_i$		mean ± SE		mean ± SE		mean ± SE		mean ± SE		mean ± SE		mean ± SE		mean ± SE		mean ± SE	
		Y$_1$		Y$_2$		Y$_1$		Y$_2$		Y$_1$		Y$_2$		Y$_1$		Y$_2$	
	Vc_{max} (μmol s^{-1} m^{-2})	54.9	2.7	47.1	1.9	56.7	3.6	50.8	0.1	63.4	5.4	47.4	5.1	62.7	3.6	47.7	22.7
	J_{max} (μmol s^{-1} m^{-2})	120.7	6.0	116.6	6.9	118.1	7.9	137.4	1.9	135.5	11.9	96.0	8.5	134.7	8.5	107.4	48.7
	R_{day}	0.8	0.2	3.4	0.2	0.8	0.1	1.4	0.9	0.6	0.1	2.6	0.3	0.7	0.2	3.1	0.2
	$A_{max\ A/Ci}$ (μmol s^{-1} m^{-2})	28.1	1.3	22.1	5.7	27.5	1.7	40.0	0.4	31.3	2.7	25.6	2.2	30.8	1.9	24.5	4.8
	Alpha	0.1	0.003	0.1	0.1	0.1	0.004	0.1	0.004	0.1	0.01	0.1	0.01	0.1	0.01	0.1	0.01
	R_{day}	2.6	0.2	4.2	1.4	2.9	0.2	2.4	0.6	2.9	0.2	5.6	0.9	2.8	0.3	5.9	0.9
	Θ	1.0	0.01	1.0	0.1	1.0	0.01	1.0	0.01	1.0	0.01	1.0	0.02	1.0	0.01	0.9	0.02
	CCP (ppm)	42.0	1.8	93.5	10.43	43.7	1.4	51.1	16.6	41.6	2.0	114.3	21.8	41.4	2.8	110.2	19.3
	CSP (ppm)	304.2	11.6	453.8	22.8	300.5	8.1	403.1	45.8	297.4	9.3	492.2	58.8	305.1	7.7	689.7	123.8
	C_i at C_a = 400$_{ppm}$	323.2	3.3	344.5	13.4	325.7	6.7	306.6	1.84	331.8	3.8	324.7	5.8	323.8	4.3	33.8	1.7
	A at C_i = 400$_{ppm}$ (μmol CO$_2$ m^{-2} s^{-1})	12.7	1.13	10.4	2.3	17.5	1.2	15.9	1.5	20.5	1.8	11.0	1.2	20.5	1.4	10.9	3.8
	A at C_a = 400$_{ppm}$ (μmol CO$_2$ m^{-2} s^{-1})	16.8	0.8	10.4	2.5	16.4	0.9	12.9	2.1	18.4	1.6	9.7	1.3	17.1	0.9	11.4	3.4
	L_s (%)	9.7	2.0	7.7	9.3	8.6	1.8	19.6	5.9	7.7	3.1	10.1	11.1	11.2	2.3	-7.5	9.4
A/I	A_{sat} (μmol s^{-1} m^{-2})	28.0	1.9	27.1	5.0	29.0	2.8	64.6	14.1	32.7	3.1	35.9	7.1	31.9	2.4	40.0	12.7
	Φ - QY	0.1	0.004	0.1	0.01	0.1	0.01	0.1	0.01	0.1	0.01	0.1	0.01	0.1	0.03	0.04	0.01
	R_{dark}	1.9	0.5	6.3	1.1	0.6	0.1	6.0	0.5	1.9	0.3	5.1	1.0	1.9	0.26	4.4	0.4
	Θ	0.7	0.03	0.5	0.1	0.6	0.1	-1.2	1.3	0.7	0.04	-0.1	0.4	0.7	0.03	-1.6	1.8
	LCP (μmol s^{-1} m^{-2})	23.9	1.2	17.1	4.0	23.5	1.5	28.4	0.8	27.1	2.4	19.3	1.4	26.9	1.6	17.5	4.2
	LSP (μmol s^{-1} m^{-2})	1134.2	84.6	1348.5	346.4	1298.7	267.0	6341.1	2499.4	1648.4	254.9	2387.1	996.5	1528.5	262.2	3030.2	1742.0

II

Subarctic grassland phenological response to soil warming

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ABSTRACT

High-latitude ecosystems are experiencing rapid changes due to warming, thereby influencing key ecological processes including plant phenology. We studied the effects of soil warming on reproductive and growth phenology, incorporating both repeated manual measurements and drone-based Digital Elevation Models (DEM), at the geothermally warmed grassland sites in Iceland. Warming led to a significantly earlier appearance in reproductive traits for most species, with *R. acris* showing the strongest response. On average, start of the season (SOS) moved 2.2 to 2.3 days earlier for each °C increase in warming depending on the method used. The date of maximum growth rate was also reached significantly earlier with warming (1.2 days°C^{-1} and 6.0 days°C^{-1} for manual and DEMs, respectively). The peak of the season was advanced by 1.9 days°C^{-1} on average for all species, while it did not show a significant warming response for the DEMs. The end of season was not significantly affected by warming, irrespective of the method. However, due to the earlier SOS, the length of the season was also significantly increased by warming by 2.2 or 4.5 days for the manual or DEM-based measurements. The strong warming-induced responses in the phenology of the native subarctic vegetation may indicate that it will utilise longer growing seasons in the future, making it less likely that it will be easily replaced by new herbaceous species.

Keywords

Growth phenology, reproductive phenology, close remote sensing, DEM, drone

1. INTRODUCTION

Subarctic and Arctic ecosystems are experiencing warming at a higher rate compared to other terrestrial ecosystems, with surface temperatures increasing at twice to four time the global average (IPCC, 2021). This warming has far-reaching impacts on environmental processes in the region, including permafrost thaw and shifts in species distribution (Post et al., 2009; Schuur et al., 2015), changes in plant phenological cycles (Lu et al., 2025; Oberbauer et al., 2013) and various other growth processes (Myers-Smith et al., 2011). The timing of recurring life cycle events in plants (phenology), is a vital aspect of plant ecology and serves as a sensitive indicator of environmental changes, particularly climate change (Cleland et al., 2007; Høye et al., 2007; Zeng et al., 2011). Phenophases, that is, specific stages in the phenological cycle such as budburst, flowering, pollination, seed set, and senescence, are significantly influenced by local environmental factors such as temperature and photoperiod (Ettinger et al., 2021; Oberbauer et al., 2013).

Most field studies on vegetative phenology use manual methods with explicit repeated visual scorings of individual plants or plant parts. For example, first date where different vegetative phases are seen, such as budburst, start of leaf expansion, and onset of bud-set and leaf senescence, where plants prepare for the harsh winter by shedding leaves and enter dormancy (Cleland et al., 2007; Menzel, 2003).

Furthermore, a subset of vegetative phenology is often termed as growth phenology (Hilty et al., 2021). It is usually derived from repeated manual measurements of elongation of specific tissues on individual plants (Fitchett et al., 2015; Hilty et al., 2021). However, in recent years, technological advancements in remote sensing — such as repeated recordings using mounted cameras, drones, or satellite observations — have allowed researchers to derive changes in the growth phenology of whole plant communities through image analysis (Crimmins & Crimmins, 2008; Pierrat et al., 2025). Most of these studies often focus on the entire seasonal growth cycle of the plant communities including the start of the season (SOS), peak of the season (POS), and end of season (EOS) (Mortier et al., 2024; W. Yang & Zhang, 2012).

Similarly, reproductive phenology normally focuses on the timing of when the reproductive tissues (e.g. flower stems) start to grow, passing through the fertilisation stage, or when seeds have developed or fell to when they have fully developed (Collins et al., 2021; Segrestin et al., 2018).

“Traditional” ground-based observation methods, such as manual records, phenological gardens, and weather station data (Schwartz, 2013), have long provided essential and detailed insights into phenological patterns. These methods are still considered a fundamental approach for recording plant phenology, particularly for fine-scale studies and historical datasets (Menzel et al., 2020). Manual observations involving direct visual scoring of plant developmental stages, provide highly accurate, species-specific data. The BBCH-scale (Lancashire et al., 1991) is widely used to standardise plant growth stages across various species, allowing for consistent comparisons in phenology studies. Additionally, flowering counts and leaf expansion tracking are commonly employed to assess vegetative and reproductive phases.

Despite the challenges associated with manual data collection in extreme environments, this methodology remains indispensable for validating remote sensing models and providing high-resolution, species-specific data for phenological research (Yang et al., 2017). High-resolution mapping techniques employed, enable the creation of accurate Digital Elevation Models (DEMs) (Bareth & Schellberg, 2018; Brown et al., 2024; Fraser et al., 2022). The DEMs allow for a precise tracking of vegetation height changes over time, providing valuable insights into plant growth dynamics (Chang et al., 2017; Wang et al., 2023). While remote sensing may not always provide exact height measurements, it excels in detecting temporal changes in vegetation structure, offering a robust method for monitoring phenological shifts in large-scale studies (Bendig et al., 2013).

In high-latitude regions, the flora is dominated by perennial low-stature plant species that are generally well adapted to high interannual variations in climate (Jónsdóttir et al., 2023), but where a relatively short growing period has to be utilised without risking damage by early or late frosts (Caradonna & Bain, 2016). Many Arctic plant species may rely on other organisms for ensuring pollination, so phenological mismatches can occur where rapid changes in species' reproductive phenology have been induced (Forrest, 2015; Johner & Ehrlén, 2019). At present, when climate warming has been ongoing for some time in the region, it is of interest to study how further warming is going to affect both the vegetative and reproductive phenology of subarctic and Arctic plant species and whole plant communities. Different experiments use various approaches, including buried heated cables (Chen et al., 2022), open-top chambers (OTCs) (Duchicela et al., 2021; Johnson et al., 2013), and other experimental soil or atmospheric-warming techniques (Diepstraten et al., 2018; Ehrlén et al., 2023) to tackle the warming effects.

One of the most influential experimental warming networks, the International Tundra Experiment (ITEX) established in 1990, has provided valuable long-term insights to tundra plant phenology to experimental and ambient warming (Henry et al., 2022). Findings from Collins et al., (2021) indicated that rapid climate warming leads to inconsistent shifts in tundra plant phenophases, with reproductive stages advancing more than vegetative phases, while leaf senescence exhibits a delayed response. When considering vegetative growth phenology, higher temperatures have generally been linked to an earlier onset of spring green-up in ITEX (Zheng et al., 2022). Moreover, in Iceland, where geothermally

warmed soils provide a unique opportunity to study soil temperature-driven phenological shifts, Ehrlén et al., (2023) demonstrated that plants growing in warmer soils can flower earlier. Also, Leblans et al. (2017) showed at the community level that, the length of the growing season (LOS) extends by approximately 2.1 days for each degree of soil warming. Mortier et al. (2024) expanded on these findings, using machine learning to analyse key phenological markers specifically, the start (SOS) and peak (POS) of the growing season. Their results underscored soil temperature as a major driver of intra-annual phenological variation at the ForHot sites. Additionally, nutrient availability, often expected to increase with warming (Pold et al., 2021; Prendin et al., 2022). However, at the ForHot it has shown that there is a depletion of nutrient availability with increase in soil warming (Jiménez Sara et al., 2019), which makes it crucial to observe the evolution of phenological stages with warming. Sigurdsson et al. (2001) focused on nutrient availability's role in growth and leaf senescence under elevated CO_2, showing that nutrient levels could significantly extend growth periods. While this study centred on *Populus trichocarpa* in southern Iceland, the findings suggest that similar nutrient-dependent adaptations may apply to grassland species at ForHot. When viewed alongside Leblans et al.'s (2017) results, Sigurdsson, (2001) study indicates that both soil temperature and nutrient levels critically influence plant growth responses, with nutrient dynamics potentially affecting adaptation pathways under warming conditions.
These phenological events are pivotal not only for the survival and fitness of individual species but also influence broader ecological dynamics, including nutrient cycling (Steltzer & Post, 2009).

The interdisciplinary approach in the methodology used in this study pinpoints its novelty as it combines both growth-based phenological observations at the plant level with high-resolution at the community level. In this study, firstly, we looked at four key Icelandic species (*Agrostis capillaris*, *Poa pratensis*, *Ranunculus acris*, *Equisetum pratense)* aboveground phenological responses to soil warming in two grasslands (medium-term soil warming – MTW, long-term soil warming – LTW). Secondly, we extend our analysis to the community level by using DEMs to investigate vegetation height changes in 2x2 m permanent plots in these two grasslands. By capturing temporal variations in plant height and phenology, we assess how soil warming influences overall vegetation dynamics in community level.

We hereby tested the following hypotheses:

a) Soil warming will promote an earlier onset of all *reproductive phenology* phenophases (flowering, seed set and seed fall).

b) Soil warming will promote an earlier onset of the *visual scoring of growth phenology*. That is, both the emergence of the first leaves and the first signs of senescence of individual plants of four key plant species in the subarctic grasslands will be advanced. Further we expected different species in the subarctic grassland plant community to have shifted phenology, which would increase their fitness by better utilisation of the growing space.

c) Soil warming will speed up and increase all *growth phenology*, calculated from *repeated manual* individual plant leaf growth- or remote sensed measurements of whole monitoring plots. Even if all variables would shift with warming, we expected the length of the growing season to increase with warming, because of a stronger response in the start of the season in spring than its end in autumn.

d) We expect the manual and remote sensing measurements to give comparable results for all measured phenology parameters.

2. MATERIALS AND METHODS

2.1 Studying Site

The sampling was carried out at the ForHot research site in the south of Iceland (64.008°N, 21.178°W; 100 to 225 m a.s.l) during 2021. The research site has an oceanic subarctic climate with mean annual temperature (MAT) of 5.8°C and annual precipitation (MAP) of 1188mm during the sampling year (Icelandic Meteorological Office, 2023). That is relatively close to the 30-year average of 5.0°C and 910.4mm (Icelandic Meteorological Office, 2023). During winters, the site does not have a permanent snow cover, but top ca. 30 cm of soil frozen during December to March of the winter season, resulting in a relatively short growing season growing season from May until late August, at ambient, that is, unwarmed conditions. (Sigurdsson et al., 2016).

We selected two similar natural grasslands that have had different duration of soil warming, that is, grassland soils which have been naturally warmed over 60 years (long-term warming – LTW) and another similar grassland where the soils have been warmed since May 2008, due to the disruption of the bedrock due an earthquake (medium-term warming – MTW) (Fig. 1). Both natural grasslands are characterised by subarctic perennial plant species such as *Anthoxanthum odoratum*, *Festuca vivipara*, *Ranunculus acris*, *Agrostis capillaris*, *Equisetum pratense*, *Poa pratensis*, just to name a few. At the research site, the soil is a silandic andosol with a silt loam texture (Sigurdsson et al., 2016).

Both MTW and LTW consist of five ca. 50 m long transects and within each transect three soil warming levels were studied (2 m x 2 m plots). Soil temperature (T_s) was measured hourly at 10 cm depth in each plot (TidbiT v2 HOBO® data loggers; Onset Computer Corp., Bourne, MA, USA). In this study we used plots that had no soil warming (0°C) and approximately +2°C, and +8°C mean annual soil warming (Fig. 2).

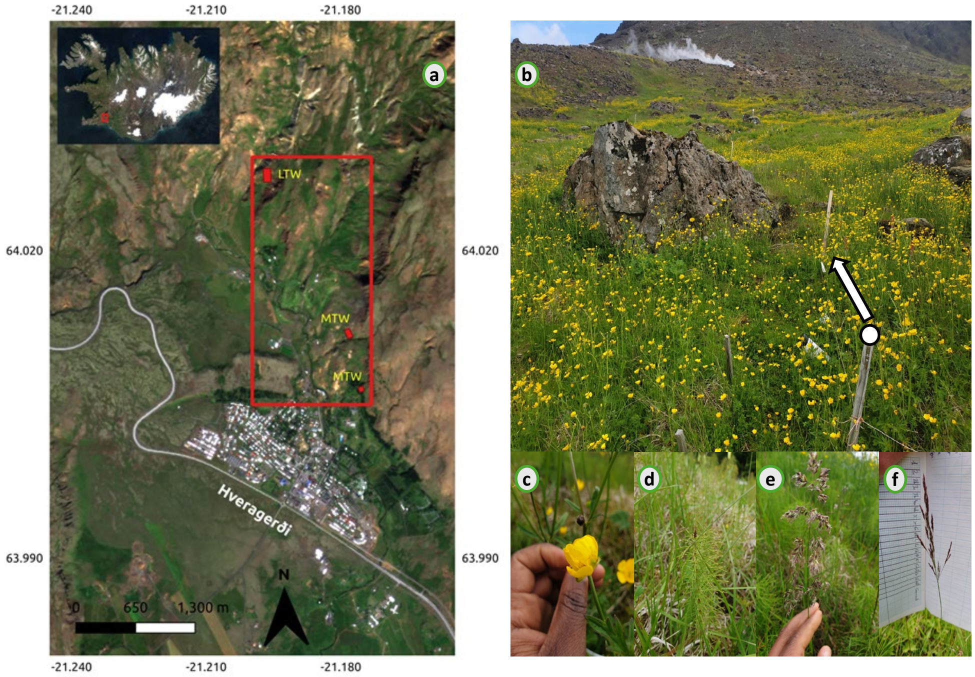

Fig. 1: ForHot sites in the South of Iceland next to Hveragerði village. (a) Location of both grasslands, that is, MTW (17 years of warming) and LTW (>60 years of warming). (b)Visual outlook of grassland site during the peak growing season of the year 2021– example of MTW 4 & 5. The white circle indicates the start of a transect (unwarmed plot, 0°C) till the end of the transect (warmest plot, +8°C) at the steam’s proximity. The (c), (d), (e), and (f) are *Ranunculus acris, Equisetum pratense*, *Poa pratensis*, and *Agrostis capillaris* respectively. These species are present in all sampled plots for both studied grasslands.

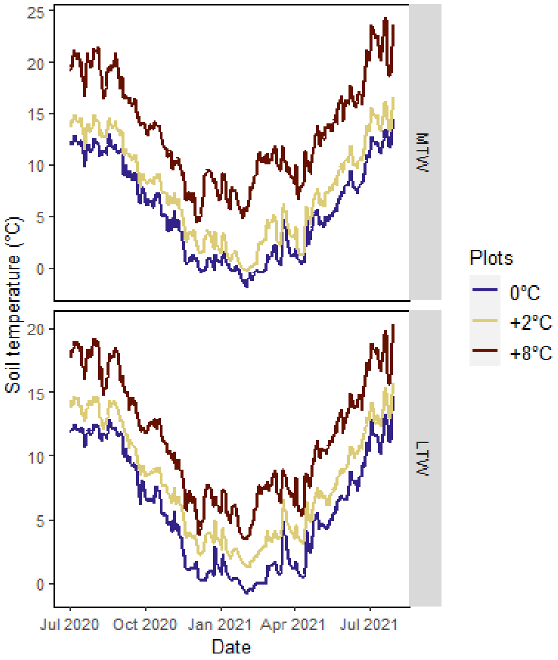

Fig. 2: Daily mean soil temperature (MAT_s) at 10 cm depth for unwarmed (0°C), +2°C, and +8°C for both Icelandic grasslands – MTW and LTW for the year 2021 (from peak growing season 2020 – end of growing season 2021).

2.3 Phenological sampling

In this research, we focused on four key Icelandic grassland species (Fig. 1c, 1d, 1e, 1f). Two monocots; Poales (*Agrostis capillaris* – "*Agr*", *Poa pratensis* – "*Poa*"), one pteridophyte (ferns); Equisetale (*Equisetum pratense* - "*Equi*"), and one eudicot; Ranunculale (*Ranunculus acris* – "*Ran*"). The phenological phases (phenophases) were divided into two main groups, *i.e.,* the reproductive and vegetative growth phase. The reproductive phase told us more about the timing of periodic life-history events, while the vegetative growth phase aided in the monitoring of the seasonal greenness and brownness occurring in high-latitude (>60°N) ecosystems.

Individual plants of the four chosen species were randomly selected in the autumn of 2020 and marked within the 2 m x 2 m plots. Their emergence, different developmental stages and senescence were then followed every 14 days from March to November in 2021. We adapted the BBCH-scale (Lanchashire et al., 1991) to mark the phenophases. The growing season at the field site normally begins in late May and ends in late August (Sigurdsson et al., 2016). However, in the year 2021, there was a "warm spell" in the late winter, which made some plants start to emerge (in warmed plots), which was the reason for the recording of this long time period (March to November). Phenophases were visually scored based on the first appearance of the traits. The traits measured are outlined in Table 1 and Fig. 3. Note that the pteridophyte studied has a reverse growing cycle in comparison to the monocots and eudicots (Table 1,

Fig. 3), where the reproductive stage starts before the vegetative stage. Moreover, the growth phenology was manually measured by taking the length (L, cm) of the individual plant with a graded field ruler from the first emerging leaf to the length at the end of the growing season.

Table 1: Phenological stages recorded; vegetative phenology, that is, all growth stages from the first leaves to the senescence of the plant (scoring and measurement (L, cm)). The reproductive phenology includes the different scoring of appearance of first reproductive traits.

Growth phenology	**Vegetative and reproductive phenology**	
Length growth of first emerging leaf (0.0 cm)	*Agr, Poa*	first leaf (1), first flowering stem (2), first flowers (3), first seeds (4), first signs of senescence (5)
	Equi	first sight of reproductive cone (1), first sight of green stem + cone (2), cone fall (3), first horizontal leaves (4), first signs of senescence (5)
	Ran	first leaf (1), first flowering buds (2), first flowers (3), first seeds (4), first signs of senescence (5)

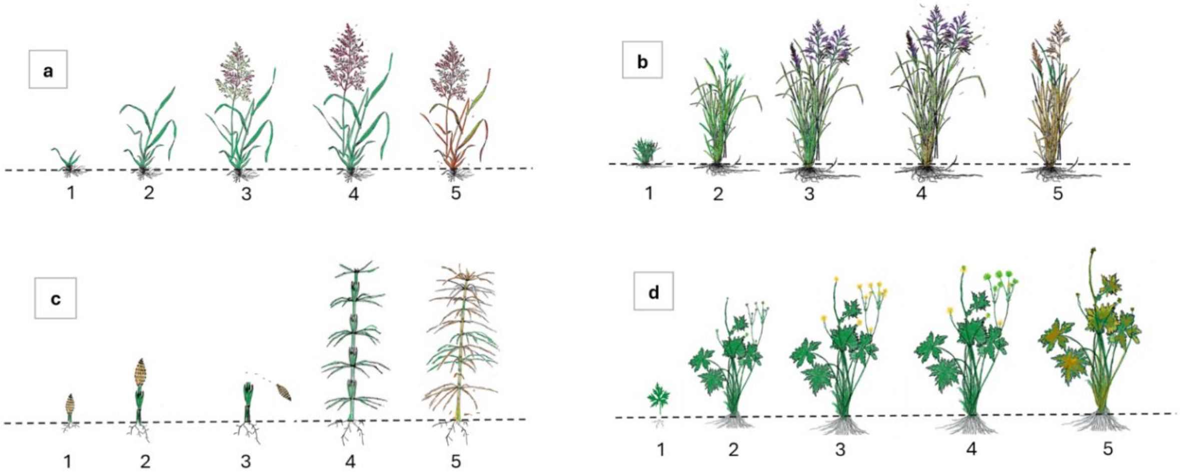

Fig. 3: Visual illustration of different phenological phases outlined in Table 1 for the four studied species at both grassland sites (MTW, LTW). Where (a) *Agrostis capillaris*, (b) *Poa pratensis*, (c) *Equisetum pratense*, (d) *Ranunculus acris*. With the number outlined following the same outline as in Table 1 for the respective species (a – d).

2.3 Digital Elevation Modelling (DEM)

To assess plant growth dynamics and phenological changes over time, Digital Elevation Models (DEMs) derived from high-resolution drone imagery were used. This approach allowed for monitoring vegetation height variations and detecting seasonal changes across different soil warming treatments. A DJI M600 drone (DJI Technology Co. Ltd.) was used for aerial imaging. The drone was equipped with a Micasense dual-camera system, and images were acquired frequently from the end of May until the end of October 2021. The collected imagery was processed using photogrammetry to create three-dimensional DEMs. The generated DEMs were geo-referenced accurately using ground control points (GCPs) installed in the field, to achieve high spatial accuracy. Vegetation height dynamics within the study plots were quantified over time using height values derived from Digital Elevation Models (DEMs), which were subsequently compared to the mean height recorded in each plot at previous time points. The DEMs were constructed from high-resolution drone imagery with a spatial resolution of 3 cm. To assess accuracy, DEM-derived height estimates were validated against independent ground control points measured across various field locations and the computed root mean square error (RMSE) was approximately 3 cm. Given that the DEM spatial resolution is also 3 cm, this RMSE value is within an acceptable range, which is showing the reliability of the dataset for detecting fine-scale vegetation height variations. To facilitate comparability across temporal observations, all height data were standardised using min-max normalisation.

2.4 Statistical Analysis

The analysis was conducted using Python (Raybaut, 2009) and R (R.4.2.2,R Core Team, 2022). While most analyses were performed in R, the double logistic function fitting was carried out in Python.

We conducted an analysis of variance (ANOVA, *aov*, *stats* package) to look at the effects of duration of warming on sub-Arctic grasslands phenology.

A non-parametric test (Kruskal-Wallis, *kruskal.test* in the *stats* package) was employed to assess the difference between species and the association between soil warming levels and phenophases.

For the growth phenology at manual scoring measurements, we looked at the estimated seasonal parameters from the sampled L (cm) by using the double logistic function method described by (Mortier et al., 2024). We fitted two logistic functions (Equation 2) to our data set, one for the greening phase (an ascending curve; SOS) and one for senescence (EOS), to evaluate the growth phenology (Figure 2). Following curve fitting of all data points, the second derivative of the first part of the logistic greening function was used to determine the SOS at 10% of green-up at both manual scoring measurement and DEM. The date of maximum growth rate was the maximum point of first derivative also known as the inflexion point (G_{max}). The POS represented the curve's maximum length. Due to snow cover, phenology sampling ended in November, resulting in an uncompleted recording of the EOS throughout the winter. To estimate the EOS, we took 10% at manual scoring and 20% for DEM of senescence attained by all the data set (Equation 3). We then calculated the LOS by taking the difference between the EOS and the SOS.

$$L(t) = \begin{cases} \frac{c}{1+e^{b_1(t-a_1)}} + d \; if \; t \leq p \\ -\frac{c}{1+e^{b_2(t-a_2)}} + d + c \; if \; t > p \end{cases} (2)$$

$$EOS \; = \; a_2 - \frac{\ln\left(\frac{0.90 \cdot c}{c - 0.90 \cdot c}\right)}{b_2} (3)$$

Where *t* is time in days, L*(t)* is the plant length (cm = at a given time, t), a_1, a_2, b_1, b_2, c, d and p are fitted parameters to the seasonal plant length (cm).

Furthermore, the Interquartile Range (IQR) approach to filter out the outliers and eliminate them without affecting the remaining data points.

To investigate the effect of MAT_s on the double logistic function derived growth phenological parameters, we first compared different regression lines to see if their intercept and slope coefficients were different for parameters derived from the individual growth phenological fitted curves. Considering that, if the outcome of the comparison of the two regression lines to each other showed no statistically significant difference, we could proceed and do a final linear regression of the derived growth phenology parameters against MAT_s on an overall dataset including both species and sites.

To gain an initial understanding of the dataset, we tested data for normality using the Shapiro-Wilk test (α=0.05). However, as linear regression models require normally distributed and homoscedastic residuals, so we verified these assumptions specifically on the model residuals. For each linear model (LM) we examined normality of residuals with Q-Q plots and assessed homoscedasticity.

3. RESULTS

There were no significant differences between the two grassland sites (MTW, LTW; $p = 0.6$, supplementary material Fig. S1) in the scoring data on plant vegetative and reproduction phenology. Hence, the duration of warming was not a significant factor for those data, and we therefore combined the sites when looking further into their warming responses.

3.1 Species differences in reproductive phenology

As expected, there were a significant species differences in the timing of different reproductive traits across the warming gradients. Those species difference included first day of the flowering buds/stem ($p < 0.0001$, df = 3, $\chi^2 = 41.26$), with *Equi* appearing the first in the plots then *Poa* followed by *Ran* and last *Agr (Equi* < *Poa* < *Ran* < *Agr*). There was equally a significant difference for the first flowering ($p < 0.0001$, df = 3, $\chi^2 = 47.19$; *Equi* < *Poa* < *Ran* < *Agr*) and first seed development ($p < 0.0001$, df = 3, $\chi^2 = 32.21$; *Equi* < *Ran* < *Agr* < *Poa*) (data not shown).

3.2 Impact of warming on reproductive phenology

Ran (eudicot) responded more strongly to warming level (WL) compared to the other species (*Agr, Poa, Equi)*. Its flowering buds appeared significantly earlier with increasing WL ($p = 0.041$; 4.1 days $°C^{-1}$ MAT_s) while the other species had no significant change in the onset of the reproductive cycle with WL ($p > 0.05$, Fig 4). This initial response of *Ran* had, however, disappeared when the first flowers opened, and that trait had no significant WL impact in any of the studied species ($p > 0.05$, Fig. 4). The pair-wise comparisons between individual WLs (Fig. 4) showed flowers onset to differ only between the no and maximum levels of soil warming for *Agr,* that is, between +2°C and +8°C ($p = 0.035$).

At the end of the reproductive period, there as a significant positive WL effect in the timing of the first seeds fall in the dicot (*Ran*) between no and maximum warming ($p= 0.032$; 3.8 days $°C^{-1}$ MAT_s). The monocots did not have any significant effect of WL on seed fall ($p > 0.05$). *Equi* on the other hand, showed a significant positive WL effect in the time of cone fall between +2°C and +8°C ($p = 0.026$).

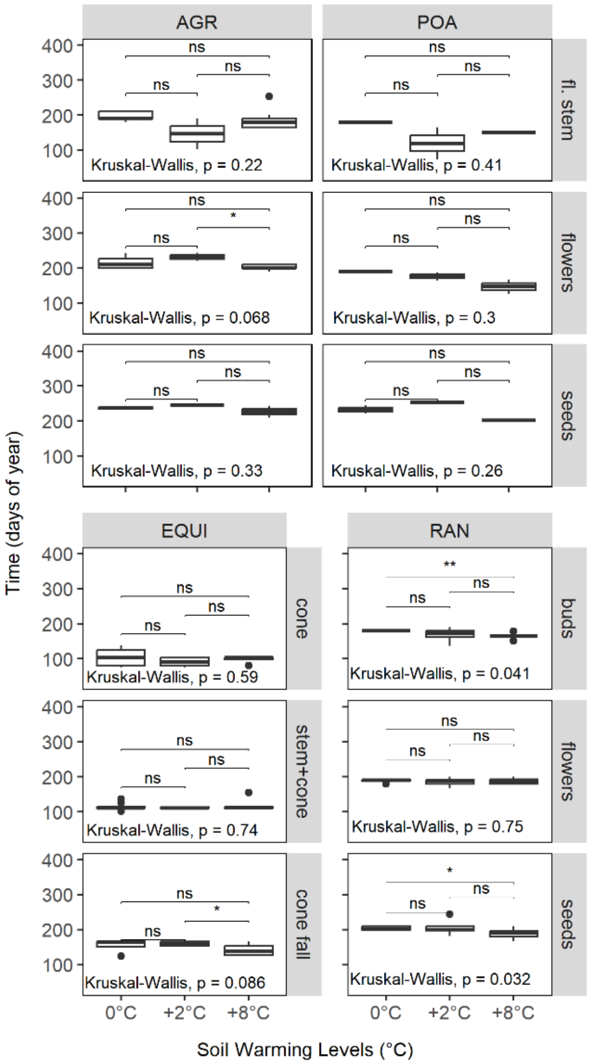

Fig. 4: Non-parametric analysis (Kruskal-Wallis) performed on the reproductive phenology, that is, the flowering (fl.) stem, buds, cone, flowers, stem and cone, seeds or cone fall. The figure shows the relationship between soil warming levels (0°C, +2°C, +8°C) and the appearance of the first reproductive traits for the studied species (*Agrostis capillaris*, *Poa pratensis*, *Ranunculus acris*, *Equisetum pratense*). Non-significant (ns, $p > 0.05$) pair-wise comparisons were not reported and significant relationship were shown according to a significant level. Significant level: *: $p <= 0.05$; **: $p <= 0.01$; ***: $p <= 0.001$; ****: $p <= 0.0001$.

3.3 Growth Phenology

The vegetative growth phenology was assessed via three different methods: i) visual phenology scoring, ii) double logistic fitting of repeated measurements of leaf length (cm), and iii) double logistic fitting of vegetation community height from repeated measurements of digital elevation models (DEMs) by a drone over each permanent plot.

Visual scoring

The growth phenology scoring showed an early onset of the first leaves at the SOS with WL for *Ran*. (p= 0.017, 7.0 days °C^{-1} MAT$_s$ between 0°C and +8°C), *Agr* (p = 0.036). As for *Poa* and *Equi* the first leaves appearance was not affected by WL (p = 0.7 & p = 0.23 respectively). The EOS was not affected for the monocots and pteridophyte by WL (p > 0.05). However, *Ran* EOS was affected by WL (p = 0.045, 7.1 days °C^{-1} MAT$_s$ between 0°C and +8°C).

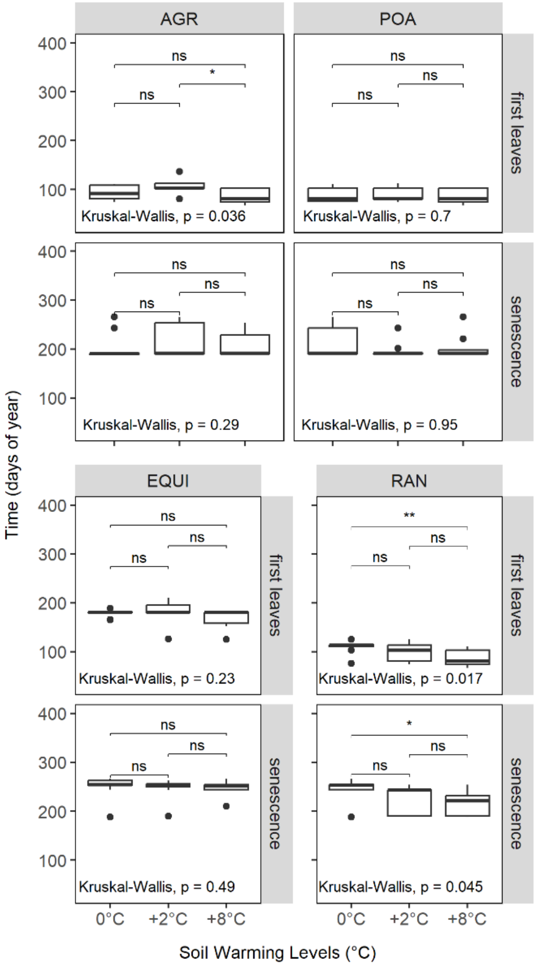

Fig. 5: Non-parametric analysis (Kruskal-Wallis) performed on the growth scoring phenology. The figure shows the relationship between soil warming levels (0°C, +2°C, +8°C) and the appearance of the first growth phenology traits for the studied species (*Agrostis capillaris*, *Poa pratensis*, *Ranunculus acris*, *Equisetum pratense*). Non-significant (ns, p > 0.05) pair-wise comparisons were not reported and significant relationship were shown according to a significant level. Significant level: *: p <= 0.05; **: p <= 0.01; ***: p <= 0.001; ****: p <= 0.0001.

Repeated growth phenology measurements

Prior to looking in depth at how different species phenological phases react to soil warming, we first visualised the average fitted curves at the individual leaf/species- (Fig. 6a) and community levels (Fig.

6b). At the species level, the visualisation was done on average across the four studied species for both sites and all WLs (0°C to +8°C). At the community level (DEM) the average was taken for both sites and all WLs. The visualisation of the normalised length and height of individuals across the time of the year, show how they "fill up" the growing space over the season – but this step does not involve statistical analysis.

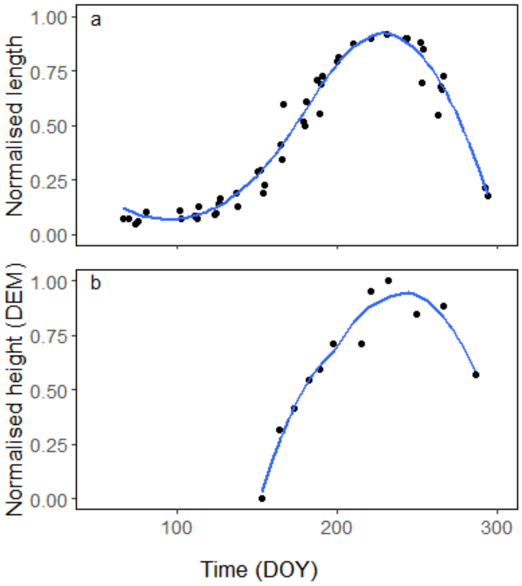

Fig. 6: Representation of normalised growth (length and height) phenology fitted with a double sigmoid function at the species (a) and community level (b) at the MTW and LTW across all soil warming levels.

The repeated manual scoring of individual leaves and the DEMs produced good fits (R^2 0.64-0.94 for individual species; >0.97 for plot DEMs) for the double logistic curves for most individuals/plots (data not shown). Individual curves that did not have good fits (low R^2 values) were omitted from the analyses. No significant differences were found between the four species in the individual leaf measurements (Fig 6a; Table 2) and their responses were therefore merged when their response to WL was investigated (Table 2; Fig. 7).

The SOS was advanced by 2.3 days °C^{-1} of MAT_s at the individual leaf level across the four species and 2.2 days °C^{-1} of MAT_s at the community level (DEM) (Table 2, Fig. 7a, b). Similarly, the date of maximum growth rate – G_{max} was advanced by 1.2 days °C^{-1} of MAT_s at the individual leaf level, but as much as 6.0 days °C^{-1} of MAT_s for the community level (Table 2, Fig. 7c, d).

The POS, which is the time of maximum leaf length (manual scoring) / vegetation height (DEM) happened 1.9 days earlier °C^{-1} of MAT_s at the individual leaf/species level, but did not change significantly with WL when the DEMs for whole plant communities were analysed (Table 2, Fig. 7e, f).

The EOS was not significantly affected by MAT_s, neither at individual leaf level nor at community level (Table 2, Fig. 7g, h). However, when looking at the LOS, there was an increase in the LOS with MAT_s at both levels (Table 2, Fig. 7i, j). That is, 2.0 days for the manual scoring, and 5.1 days and 3.9 days in the MTW and LTW for the DEMs, respectively °C^{-1} of MAT_s.

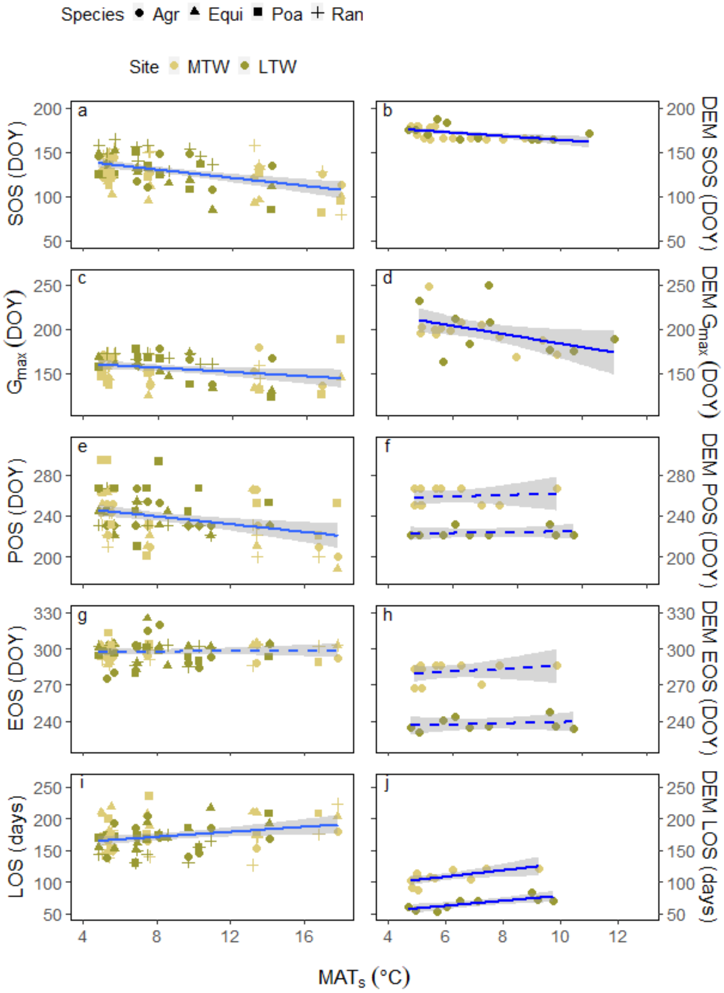

Fig. 7: Linear regression showing the relationship between growth phenological parameters (SOS, POS, G_{max}, EOS, LOS) estimated at both species (*Agr, Poa, Equi, Ran*; a, c, e, g, i) or community level (b, d, f, h, j) and soil (MAT_s, °C) for both studied Icelandic grasslands (MTW, LTW). a & b - start of the season (SOS, doy), c & d - date of maximum growth rate (G_{max}, doy), e & f - peak of the season (POS, doy), g & h - end of the season (EOS, doy), i & j - length of the season (LOS, days). Solid lines indicate significant trends ($p < 0.05$), whereas dashed lines indicate non-significance ($p > 0.05$).

Table 2: Statistical linear regression output (Fig. 5) of the relationship between growth phenological parameters and soil temperature (MAT_s) for both studied sites (MTW, LTW) at both species (manual scoring) and community (DEM) level. Significance level "ns" indicates p-value (p-val.) > 0.05.

Parameters	**Manual scoring**					**Remote sensing (DEM)**			
	slope	intercept	R^2	p-val.		slope	intercept	R^2	p-val.
SOS	-2.3x	150	0.22	<.0001	Both	-2.2x	190	0.30	<0.002
G_{max}	-1.2x	170	0.08	0.022	Both	-6.0x	240	0.21	0.03
POS	-1.9x	250	0.11	0.002	MTW	+0.9x	250	0.02	ns
					LTW	+0.4x	220	0.03	ns
EOS	+0.1x	300	0.002	ns	MTW	+1.4x	270	0.06	ns
					LTW	+0.6x	230	0.05	ns
LOS	+2.0x	160	0.09	0.01	MTW	+5.1x	77	0.42	0.02
					LTW	+3.9x	38	0.63	0.01

3.4 Comparing manual scoring measurements to remote sensing

DEM-based remote sensing successfully captured overall trends in plant growth phenology in comparison with the manual scoring (Fig. 6a, b). Both methods showed essentially the same significant earlier start of growth (SOS) by ~2.25 days °C^{-1} of MAT_s, even if the actual DOY first detected growth was ca. 12 weeks later in the year for the DEMs than for the individual leaf/plant scoring (Fig. 7a, b). Both SOS and G_{max} had no significant differentiation between MTW and LTW in their WL-response in neither method (Fig. 7c, d) and both methods showed a significantly earlier G_{max} with WL. However, for this parameter the G_{max} WL-response was steeper for the DEMs (-6.0 days °C^{-1} MAT_s) compared to manual scoring (-1.2 days °C^{-1} MAT_s).

The DEM-estimates of POS, EOS, and LOS were all significantly different between MTW and LTW (Fig. 5g, h, j; intercepts $p < 0.05$) and therefore the site-specific responses are shown in Table 2.

POS did not change significantly with MAT_s in the DEM-estimates (Fig. 5g; $p > 0.05$, Table 2), unlike the significantly earlier POS response with WL for the individual leaf length measurements. The difference in POS between the sites was noteworthy in the DEM data, where MTW had ~ 4.5 weeks later POS (ca. 7th Sept.) than LTW (ca. 9th Aug.), while the individual leaf measurements showed an average POS at the mean WL to be around mid-August (Fig. 7e).

Neither method found any significant change in EOS with MAT_s (Fig. 5h, i; $p > 0.05$), but it was noteworthy that the average DOY for EOS was much earlier at the LTW site than at MTW. It occurred

~5.5 weeks earlier at LTW than MTW (Fig. 7h), and this then caused similar shift in the LOS for the two sites. Additionally, the LOS was found to be significantly longer with increased MAT_s in both methods ($p < 0.05$; Table 2).

However, some trends showed a clear offset of timing between the two methods (Fig. 7a, b), the DEM detected the SOS later in the season, that is around DOY of 150 – 200 (30th May – 19th Jul.) in comparison to the manual scoring which dealt with individual leaves emerging and were typically first detected (within the litter layer) around DOY of 100 – 140 (10th of Apr. – 20th May). Same observation was made for the G_{max}, the DEM detected it around DOY of 160 – 240 (9th Jun. – 28th Aug.), while the leaf length growth measurements found it to take place around DOY of 124 – 190 (4th May – 9th Jul.) (Fig. 7c, d). There were some differences found between the two methods for POS, but it was noteworthy that the DEM-derived estimates of POS for MTW and LTW were just after and before the average POS for the manual measurements (Fig. 5f, g). The EOS had non-significant trends for both methods. Despite the non-significance observed, the EOS occurred at the DEM earlier, that is, around DOY of 230-250 (18th Aug.-7th Sept.) at the LTW and DOY of 260-280 (17th Sept. – 7th Oct.) for the MTW (Fig. 7g, h). The EOS at manual scoring was observed later around DOY of 275-325 (2nd Oct. – 21st Nov.). The LOS in consequent happened earlier on average for both sites for the DEM compared to the manual scoring with ~84 days difference. In spite of the difference in the LOS, both methods showed similar trends in response to MAT_s (Fig. 7i, j).

4. DISCUSSION

4.1 Effects of soil warming on reproductive phenology

Reproductive phenology, the timing of flowering, pollination, seed production, and dispersal, has been a central topic in plant ecology for a long time. Early studies established the foundational understanding of seasonal reproductive cycles, linking temperature and day length to flowering patterns (An et al., 2004; Haggerty & Galloway, 2011; Lutz et al., 2015). Over time, research expanded to assess and examine species-specific phenological adaptation to environmental conditions, documenting long-term shifts in flowering time due to climate change (Fitter & Fitter, 2002). Within this study, we borrowed a similar species-specific response pathway looking at the impact of soil warming, by stipulating that an increase in soil warming would accelerate the phenological development of the deciduous herbal subarctic plant species. Therefore, leading to an earlier onset of phenophases.

The outcome confirmed the stipulated hypothesis. Results showed that, the eudicot (*Ran. acris*) responded more to changes in warming levels compared to the monocots and the pteridophyte (Figs. 4 and 5). It had a significantly earlier onset of first leaves, flower buds, seed set and early senescence stage in warmer soil. Interestingly the time of flowering was, however, not significantly affected (Fig. 4).

Could this intriguing stronger response observed in *Ranunculus acris* as compared to other studied species be an adaptative strategy of eudicots to warming? That is, the presence of a higher capacity for phenotypic plasticity, which allows them to adjust their growth and development rapidly in response to environmental changes. Looking at other studies on soil warming and species-specific responses Wickander et al., (2021) observed that the alpine bistort (*Persicaria vivipara*), a dicot species found in Iceland, showed significant plasticity to elevated temperature. The earlier sprouting of the species and production of more leaves at higher soil temperatures was observed, but, without any evidence of genetic differentiation. Similarly, Valdés et al., (2022) in their study found an advancement in flowering but a decrease in fitness with increasing soil temperatures in subarctic grassland for the species *Pinguicula vulgaris*. Perhaps, we could suggest that these eudicots could exhibit some adaptive responses in soil warmed grasslands. Taking into consideration this might be context-dependent and varying across species.

In addition, if the time of flowering shifts much, then insect pollinated plant species may have reduced fitness due to a mismatch in pollinator appearance if the pollinators do not respond equally strong to the environmental changes (Høye et al., 2013), particularly those dependent on specialist pollinators (Forrest, 2016). *Ran* is insect pollinated according to Harper, (1957). It was therefore noteworthy that even if most phenophases of *Ran* were significantly advanced by the warming, then the time of flowering was not significantly moved. A marginal significant earlier flowering was seen for *Agrostis capillaris* at higher warming, but it has been shown to be wind pollinated and could also be dispersed by vertebrates or water (IUCN GISD, 2025).

4.3 Soil warming impacts on vegetative growth phenology and the duration of growing season

We expected earlier emergence of green leaves (SOS) and first signs of senescence (EOS) for individual plants in a warmer world. Further we expected that this would be mirrored in the remote sensed plant community growth parameters (SOS, EOS), but in both cases we expected that the earlier shift of EOS would not be as strong as of SOS leading to somewhat increased LOS. Overall, our hypotheses were validated.

Both methods found a ~2.25 days earlier SOS per each °C warming of MAT_s (Table 2, Fig. 7a, b). This corresponds well to an earlier study (2013-2015) done at the ForHot subarctic grasslands, which found that the SOS was 1.6 day earlier per °C warming of MAT_s (Leblans et al., 2017) and (Mortier et al., 2024) recently reported that during 2013-2021 the NDVI SOS was advanced by 1.5 days earlier SOS

per 4.63 °C warming of MAT_s. This also corresponds well to Ren et al., (2020) observing 0.85 days advance in the SOS with 1°C increase in mean air temperature in study on mid-latitude grasslands in the Northern hemisphere (between 30°N and 55°N). Moreover, Piao et al., (2007) showed an earlier vegetation green-up of 3 days $°C^{-1}$ with air temperature.
On the other hand, the EOS showed no significant effect of soil warming with both methods. Though no significant effect was observed, a visible disparity (Fig. 7h) was seen between the MTW and LTW is detecting the EOS with DEM, which might be due to the influence of other environmental factors. Leblans et al (2017), equally saw no effect of warming on two studied years (2013, and 2014) but found a slight delay in the EOS in a similar way at both sites (MTW, LTW) of 0.93 days $°C^{-1}$ MAT_s over the three-year period. Gill et al., (2015) found at 50% leaf fall a delay of 2.14 d $°C^{-1}$ in mean October air temperatures, and overall, by 1.20 d $°C^{-1}$ at both low and high-latitudes.

As for the LOS, the manual scoring of individual leaves of four species resulted on average in 2 days extension of LOS per °C increase of MAT_s, while the remote sensing of the whole plant community found the extension of LOS to be ~4 – 5 days $°C^{-1}$ of MAT_s (Table 2, Fig. 7i, j). Earlier remote sensing research at ForHot on the NDVI vegetation index LOS found it to increase by 2.1 days $°C^{-1}$ soil warming on average (Leblans et al., 2017). Other studies at high-latitude ecosystems look at air temperature effects showed an increase of the LOS by 0.38 days yr^{-1} from 1988 till the 2000s (Euskirchen et al., 2006), and 0.30 days yr^{-1} since 1980s (Piao et al., 2007). Moreover, the positive significant relationship between the LOS and the MAT_s suggests that the lengthening of the growing season might stimulate plant productivity (Bjorkman et al., 2019) without necessarily major changes in vegetation community. This could be found true as recent studies at the ForHot (subarctic) showed an increase in aboveground vascular plant biomass with annual T_{sum} (growing °days) (Wandji et al., in review). Implications of the extension of the growing season has visible changes such as one large-scale ecological response to global warming known as the "greening of the Arctic" (Liu et al., 2021; Zeng et al., 2011; Zheng et al., 2022). Though our study does not explore atmospheric warming, the existing strong correlation (supplementary material Fig. S2) between both soil and atmospheric warming gives us a proxy to interpret our results. Suggesting that the "greening" of high-latitude ecosystem could also be driven by soil warming.

4.4 Soil warming impacts on the timing of maximum vegetative growth

The timing of maximum vegetative growth rate (G_{max}) and maximum standing vegetative biomass (POS) has received much less attention in the phenology literature so far than the onset (SOS) and the end (EOS) of the growing season in a warmer climate (Park et al., 2016; Shi et al., 2025; Wen et al., 2021). This information is, however, very important because it can help in predicting if e.g. plant-animal interactions are likely to be affected in future climate due to mismatch in their phenological cycles (Gerlich et al., 2025; Schmidt et al., 2017).

Both methods showed a significant advancement in the time of G_{max} with warming in the subarctic grasslands of 1.2 – 6 days °C^{-1} of MAT_s and the time of maximum individual green leaf length (POS) was similarly advanced by 1.9 days °C^{-1} of MAT_s, while the whole plant community height (remote sensed POS) did not significantly change with warming (Fig. 7e, f; Table 2). One earlier remote sensing study from the ForHot sites looked at changes in POS of the NDVI vegetation index and found that on average during 2013-2021 the POS was advanced by 1.65 days per 4.25 °C warming of MAT_s (Mortier et al., 2024). More studies are needed on this phenological aspect of individual plant species and vegetation communities in future warming experiments.

4.5 Individual species' response to warming

It was unexpected that there were no significant shifts in the growth phenology of the four plant species that we had selected from the subarctic grasslands to represent different functional groups. Based on the competitive exclusion principle in plant ecology (Bengtsson et al., 1994; den Boer, 1986), we would have expected the species not to have such a synchronised growth pattern, in order to utilise the growing space more effectively over the whole season. Some other studies have found that individual plants in a community have responded to warming in a non-synchronised way (Sherry et al., 2007). There are different possible reasons for why this was not observed in the present study: i) the methodology: we preselected and marked individual plants in the autumn before the experiment started without any standardisation of size, which led to quite high plant-to-plant variation in the following year when we monitored their phenology and growth. This high intra-species variation may have resulted in lack of statistical power to differentiate species-dependent growth patterns. ii) The plants in the subarctic ecosystem may be more limited by other environmental factors than GPP and aboveground growing space, which would then make the timing of aboveground growth not a key variable in the competitive exclusion principle. One such important variable would be belowground competition for available nitrogen (N) as has been shown in other studies from the ForHot grasslands (Bhattarai et al., 2023; Fang et al., 2023). iii) possibly the current species pool in and around the subarctic grassland has gone through such as strong selection towards the short growing seasons of the past (sub)Arctic environment that the present plant species are all more or less tuned to finish their growth cycle in as short time as possible (Wookey, 1993).

4.6 Comparing manual scoring of phenology to remote sensing

Upon reviewing the literature, we found no studies which specifically explored the relationship between DEM-derived grassland community height and soil warming. While there are independent studies on DEM applications in (mostly woody) vegetation monitoring, and also remote sensing (e.g. multispectral vegetation indices) on soil warming effects on vegetation, the intersection between DEMs and vegetation structure in warming experiments remains unexplored. This shows a new aspect of this study, emphasising the importance of integrating high-resolution topographic data with warming effects on phenology. By using this approach, this research bridges a crucial knowledge gap, providing new insights into how plant growth responses can also be quantified using DEMs under varying soil temperature conditions.

We hypothesised that both manual (individual plant level) and remote sensing (plant community level) to show similar trends between the relationship of estimated phenological parameters and soil warming. This was only partly supported by this study. Even though both methodologies showed strong similarities in the general trends of most estimated phenological parameters, there were some discrepancies observed between remote sensing and manual measurements. For example, the DEM did have a large delay (12 weeks) until it was able to capture the early plant growth (SOS) as compared to manual scoring (Fig. 7a, b; Table 2), which partly also explained its much shorter estimated LOS than was observed in the manual measurements (Fig. 7i, j; Table 2). This aspect can partly be explained by the fact that the subarctic grassland has a partly upright dead standing litter from the previous year, which interferes with the ability of the DEM to detect early structural height changes of the emerging plants early in the season. Meanwhile manual measurements see the new green leaves already when they emerge within the litter layer. The same trend was true of G_{max}, but it that case the manual measurements were focusing on the elongation growth of leaves which do not grow vertically in the grassland canopy, while the DEMs were likely more prone to detect the elongation growth of the flowering stems of both the dicot and the monocots; *i.e.* they were not really capturing the growth of the same plant tissues. The flowering stems may not be as good indicators of the community's GPP as the length of the green leaves. So, this finding was an important reminder for those who only relay on remote sensing. Though remote sensing has the ability to conduct large-scale assessments in a more efficient way than traditional manual scoring (Ma et al., 2022), there are however some important limitations. DEMs primarily measure vegetation height but sometimes struggle to capture early plant growth stages where manual scoring gives a more accurate assessment with the measurement of the plant's length. However, to have better accuracy, multispectral remote sensing assessment of plant biomass is recommended, as seen in (Mortier et al., 2024). To follow the gross- or net primary productivity (GPP, NPP) while doing manual scoring, we recommend looking at the “green part of the plant" rather to measure maximum canopy height of a plant or a plant community.

Another aspect of the DEM remote sensing came as a surprise. It was the significant difference in the growth patterns of MTW and LTW, that were not detected by the manual measurements (Fig. 7f, h, j; Table 2). In this case it may also be explained by what the DEM really measures. It only senses the vertical height change of the plant community and does not “care” if that structure is living or dead. It was noteworthy that POS was according to the DEM occurring a little earlier than the manual leaf length measurements found at LTW site, but much later at the MTW site. We think that this was due to the difference in the wind exposure at the two sites; the LTW is situated on a hill shoulder in a valley, while MTW is situated on a more sheltered mountain slope (Fig. 1; Sigurdsson et al., 2016). We noticed that the grassland canopy started to lodge at the LTW site during the late summer due to the generally higher wind speeds there (Supplementary F3S1). Unfortunately, we only have wind speed measurements from the LTW site, so we cannot proof this hypothesis.

During the EOS phase, plant behaviour can vary depending on species and environmental factors (Thomas, 2013). The observed earlier EOS in DEM-based estimates is likely due to structural vegetation collapse (*e.g.*, wind-driven bending and/or precipitation-induced lodging), which reduces measurable plant height before full physiological senescence. Some plants bend as they approach growth inactivity, while others remain upright (Nelson & Moore, 2020), but our herbaceous subarctic grassland species are all prone to bending. Such wind effects of plant and vegetation stature are likely to contribute to spatial variability in high-latitude ecosystems (Thórhallsdóttir, 2021; Wielgolaski & Inouye, 2003), as plants may be bent earlier or later. This should be kept in mind in future remote sensing applications of vegetation growth and phenology under such conditions.

4.6 Methodological warming issues

Natural geothermal gradients, such as the ForHot research site in Iceland, provide an invaluable framework for examining long-term ecosystem responses to warming under realistic natural field conditions (Sigurdsson et al., 2016). The presence of both short- and long-term warming gradients enables the study of temporal dynamics in assessing different ecosystem process (forhot.is). However, precaution should be employed when interpreting outcomes related to soil warming in these systems in relation to global warming as the warming is belowground. Thereby, potentially underrepresenting the full spectrum of aboveground biological responses, which are more directly regulated by air temperature

(Leblans et al 2017; Sigurdsson et al., 2016). Moreover, the abrupt onset of geothermal warming might not fully showcase the gradual trajectory of climate change as discussed by Sigurdsson et al., (2016).

5. CONCLUSION

Soil warming promoted a stronger response in most reproductive traits, especially for the eudicot compared to monocots or a pteridophyte, suggesting taxon-specific adaptative responses in warmed subarctic grasslands.

The vegetative phenology equally showed an advancement in most phenophases with soil warming, including the onset of growth and the time of maximum greening point and maximum leaf length or grassland canopy height. The end of season was, however, not affected by warming, indicating that the native subarctic vegetation was able to utilise the extending length of the growing season to the fullest, making it less likely that it will be replaced by new species which would be better adapted to the prolonged autumn season.

Overall, manual methods and remote sensing found similar phenological responses to warming in the subarctic grasslands. However, the exact timing and size (duration) of the phenological responses often differed. This was mostly explained by different structures being measured with the two methods and was an important reminder of methodological issues that need to be kept in mind when remote sensing is used for monitoring impacts of climate change on vegetation structure and phenology.

CRediT Author Statement

Ruth Phoebe Tchana Wandji: Investigation, Conceptualisation, Writing – Original Draft, Writing – Review & Editing, Validation, Visualisation, Methodology, Formal analysis, Data Curation and Analysis. **Amir Hamedpour**: Writing – Contribution to Original Draft, Writing – Review & Editing, Contribution to Data Analysis. **Iolanda Filella**: Funding Acquisition, Supervision, Writing – Review & Editing. **Peter Lootens:** Funding Acquisition, Supervision, Writing – Review & Editing. **Bjarni Diðrik Sigurdsson**: Investigation, Writing - Review & Editing, Funding Acquisition, Supervision, Project Administration.

DECLARATION OF COMPETING INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY

The data used in this manuscript can be found at the ZENODO Database (10.5281/zenodo.15576205) and it is Open Access.

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Subarctic grassland phenological response to soil warming

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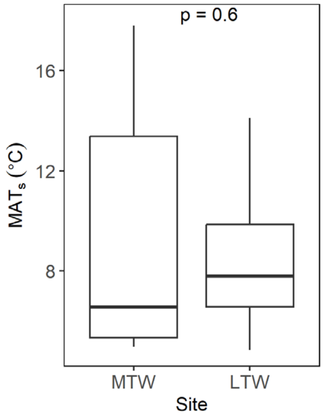
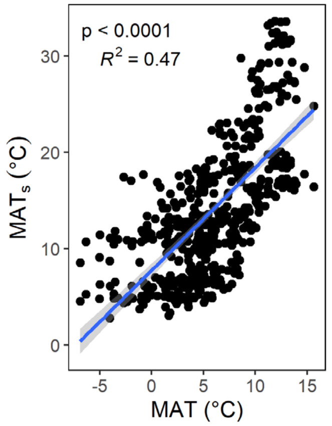
Supporting Information

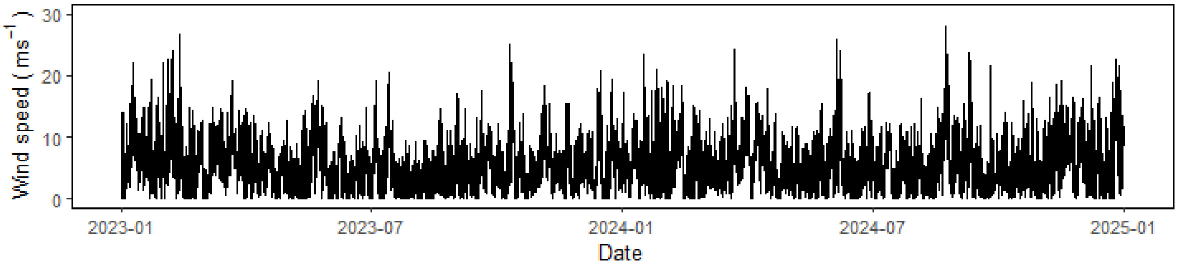
Figures



Supplementary material Fig. S1: ANOVA boxplot of mean annual soil temperature (MAT$_s$, °C) for the sampling year 2021 for both studied Icelandic grassland sites (MTW and LTW).



Supplementary material Fig. S2: Positive linear regression between annual atmospheric temperature (MAT, °C), and the annual mean soil temperature (MAT$_s$, °C) for the year 2021 at both studied Icelandic grassland sites (MTW, LTW).



Supplementary material Fig. S3: Figure showing the high frequent winds (wind speed, ms^{-1}) at the LTW site, example for the years 2023 and 2024.

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Inferring the relationship between soil temperature and the normalized difference vegetation index with machine learning

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ABSTRACT

Changes in climate can greatly affect the phenology of plants, which can have important feedback effects, such as altering the carbon cycle. These phenological feedback effects are often induced by a shift in the start or end dates of the growing season of plants. The normalized difference vegetation index (NDVI) serves as a straightforward indicator for assessing the presence of green vegetation and can also provide an estimation of the plants' growing season. In this study, we investigated the effect of soil temperature on the timing of the start of the season (SOS), timing of the peak of the season (POS), and the maximum annual NDVI value (PEAK) in subarctic grassland ecosystems between 2014 and 2019. We also explored the impact of other meteorological variables, including air temperature, precipitation, and irradiance, on the inter-annual variation in vegetation phenology. Using machine learning (ML) techniques and SHapley Additive exPlanations (SHAP) values, we analyzed the relative importance and contribution of each variable to the phenological predictions. Our results reveal a significant relationship between soil temperature and SOS and POS, indicating that higher soil temperatures lead to an earlier start and peak of the growing season. However, the Peak NDVI values showed just a slight increase with higher soil temperatures. The analysis of other meteorological variables demonstrated their impacts on the inter-annual variation of the vegetation phenology. Ultimately, this study contributes to our knowledge of the relationships between soil temperature, meteorological variables, and vegetation phenology, providing valuable insights for predicting vegetation phenology characteristics and managing subarctic grasslands in the face of climate change. Additionally, this work provides a solid foundation for future ML-based vegetation phenology studies.

1. Introduction

In-situ monitoring of changes in vegetation in inaccessible Arctic regions is challenging, prompting many such studies to rely on remote sensing techniques (Zmarz et al., 2018). In the field of remote sensing, vegetation indices such as the Normalized Difference Vegetation Index (NDVI) are used to quantify and qualify vegetation cover (Huang et al., 2021). This is achieved through airborne or satellite spectral methods (Ryu et al., 2021; Zhao et al., 2021) or ground-level measurements, using handheld instruments (Balzarolo et al., 2011; Ferrara et al., 2010). Vegetation activity monitoring using NDVI has shown both intra-annual and inter-annual variations that can give valuable insights into ecosystem changes (Beck et al., 2006; Rhif et al., 2022). Some parameters that can be derived from such intra-annual seasonal NDVI curves are the start of the season (SOS), peak of the season (POS), and maximum annual NDVI value (PEAK) (Li et al., 2017; Ma et al., 2022).

In high latitudes, the intra-annual temperature and irradiance variation are important factors that control the cycles in the growth and

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Abbreviations: NDVI, Normalized Difference Vegetation Index; SOS, start of the season; PEAK, maximum annual NDVI value; POS, peak of the season; ML, machine learning; ANN, artificial neural network; MLP, multilayer perceptron; MSE, mean squared error; MAE, mean average error; CV, cross validation; SHAP, SHapley Additive exPlanations; LIME, Local Interpretable Model-Agnostic Explanations; xAI, explainable artificial intelligence.

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reproduction of the flora (Mølmann et al., 2021; Odland et al., 2003). Over the last decades, different life-cycle events of vegetation (phenology) have been observed to change in this region (Epstein et al., 2013). This has been related to ongoing climate change (IPCC, 2021), which has started to affect vegetation phenological cycles, productivity, and community structure (Semenchuk et al., 2016). Inter-annual analyses found relationships between climate change and these changes in vegetation dynamics, particularly with regard to the increase in surface temperature, resulting in an increased PEAK NDVI and with a notable impact on the length of the growing seasons (Arndt et al., 2019; Potter and Alexander, 2020). Starting from the year 2000, scientists started to name this phenomenon of an increasing PEAK "Arctic greening" (Merrington, 2019). This phenomenon was hypothesized to persist with continued climate warming, based on the compelling evidence of increased PEAK NDVI (Beck and Goetz, 2011), plant productivity (Loranty and Goetz, 2012), phenology (Semenchuk et al., 2016), and vegetation composition (Walker et al., 2012b) between 1980s and early 2000s (Epstein et al., 2012, 2013).

Interestingly, the "Arctic greening" effect has not occurred everywhere at high latitudes and since the early 2000s, the relationship between PEAK NDVI with an increase in surface temperature has weakened in many places (Bhatt et al., 2013; Myers-Smith et al., 2020). In fact, in some regions, this relationship has even become negative, introducing the term "Arctic browning" (Beck and Goetz, 2011). It is generally believed that the shift towards browning must indicate that other meteorological drivers (e.g., temperature, precipitation, wind, photoperiod) or biological drivers (e.g., insect grazing, drought, etc.) are in play. However, the issue still requires further study.

In Iceland, the same strong "Arctic greening" trend was shown to occur during the 1980s–2000s as in many other high-latitude regions, but with a notable stagnation of the national PEAK NDVI during 2000–2010, even if the surface temperatures continued to increase in Iceland during that period (Björnsson et al., 2007; Raynolds et al., 2015). What happened in Iceland after 2010 is unclear, but a recent study showed that the inter-annual variation in the national average PEAK NDVI has been large during 2001–2019 period (Olafsson and Rousta, 2021). Therefore, it is of interest to further study how the NDVI of Icelandic ecosystems responds to further warming.

The impact of climate change on high latitude vegetation is not only limited to the air temperature increases. Soil warming studies have revealed significant insights into how soil warming affects soil processes and, consequently, vegetation. Soil warming experiments in high latitudes have demonstrated that increased soil temperatures can lead to changes in nutrient availability, microbial activity, plant composition and biomass, all of which influence plant growth and ecosystem dynamics (Fang et al., 2023; Metze et al., 2024; Verbrigghe et al., 2022b). For example, Bhattarai et al. (2023) found that soil warming resulted in changes to below-ground plant biomass and fine root biomass, under different warming conditions. These changes were associated with shifts in plant community composition and soil chemistry, highlighting the complex adaptation mechanisms of subarctic grasslands to prolonged soil warming.

Continued climate change is expected to cause relatively higher increases in surface temperatures at higher latitudes in the coming decades (IPCC, 2021), which will likely lead to relatively more ecosystem changes in plant productivity than at lower latitudes (Chen et al., 2021). Potential changes include further temporal shifts in parameters that characterize growing seasons (Semenchuk et al., 2016) and increases in plant productivity (Street and Caldararu, 2022; Van Der Wal and Stien, 2014). However, it is important to further investigate the warming impacts on NDVI to better underpin such predictions for future changes. Combining data from manipulation (warming) experiments offer possibilities to study future high-latitude ecosystem NDVI responses (Bjorkman et al., 2020; Leblans et al., 2017).

To relate changes in vegetation composition, biomass or NDVI to environmental parameters, traditional statistical methods like (non-) linear regression or linear mixed models have been most commonly used (Estrella et al., 2021; Hope et al., 1993; Leblans et al., 2017; Mehmood et al., 2024; Walker et al., 2012a; Wang et al., 2021). Additionally, multivariate methods have also been used, for example multivariate analysis of variance tests (Michielsen, 2014).

Despite massive advancements in the field of machine learning (ML) during the last decade, ML is not yet often used for vegetation studies. ML models can be used for various tasks, among which are classification, regression, and image segmentation. In ML, models extract knowledge from data and use this knowledge to produce an output relevant to the task at hand. These models use three main learning paradigms: supervised learning, unsupervised learning or reinforcement learning. This study only considers the first paradigm, as we build a regression model. Within supervised learning, there are a multitude of model types, for example, support vector machines (Hearst et al., 1998), boosted tree ensembles (e.g., XGBoost (Chen and Guestrin, 2016) or LightGBM (Ke et al., 2017)) and artificial neural networks (ANNs) (McCulloch and Pitts, 1943). This analysis will use ANNs, particularly multilayer perceptrons (MLPs), which are fully connected feedforward neural networks that consist of multiple layers of nodes that are connected with each other by weighted edges.

Recently, ML has also shown promising results in the field of ecology (Christin et al., 2019; Thessen, 2016), for use cases such as species identification (Barhate et al., 2023; Barré et al., 2017; Chen et al., 2020; Wäldchen and Mäder, 2018), behavioral studies (Clapham et al., 2020; Schofield et al., 2019), ecological modeling and forecasting (Cho et al., 2009; Strydom et al., 2021; Ye and Cai, 2011), remote sensing (Guo et al., 2020; Li et al., 2020) and climate change studies (Kumar, 2023; O'Gorman and Dwyer, 2018; Rolnick et al., 2022), among others. The utilization of ML techniques has opened new avenues for understanding complex ecological phenomena and predicting ecological responses. Considering the proven potential of ML in addressing research questions in the broad field of ecology (Gao et al., 2024; Jemeļjanova et al., 2024), we propose to apply ML methods to investigate the relationship between vegetation phenology and environmental drivers in subarctic grasslands.

Unfortunately, MLPs are black-box models. This means that, while they can approximate any function, it is nearly impossible to determine the structure of the approximated function. This led to a whole new field within ML, explainable artificial intelligence (xAI), which tries to create methods that allow human users to understand the predictions made by an ML model (Vilone and Longo, 2021). Some popular examples include sensitivity analysis (Zeiler and Fergus, 2014), Local Interpretable Model-Agnostic Explanations (LIME) (Ribeiro et al., 2016), and SHapley Additive exPlanations (SHAP) values (Lundberg et al., 2017). This study uses the last method, as it is gaining in popularity and is now often used in ecology. For example, Masago and Lian (2022) use SHAP values to investigate how inter-annual variation in the daily average temperature affected the first flowering date or the full blossom date of the Yoshino cherry trees in Japan. He et al. (2022) construct a seagrass distribution model and explain the importance of environmental variables in the model and subsequent predictions. In Park et al. (2022), an XGBoost model is trained to predict chlorophyll concentration, and they use SHAP values to perform feature selection, as well as investigate feature importance. SHAP values have a number of advantages over other methods for understanding the output of a model. First, SHAP values are model-agnostic, which means that they can be used with any ML model (Lundberg et al., 2017). Second, SHAP values are able to account for interactions between features, which is something other methods are not able to do. Third, SHAP values have an intuitive interpretation, which means that they are easy to understand and explain to others. Finally, SHAP values have some desirable mathematical properties, such as local accuracy, missingness, and consistency (Aas et al., 2021).

An earlier study was conducted by Leblans et al. (2017) at the same research sites in Iceland (Sigurdsson et al., 2016), focusing on the phenology of subarctic grasslands. They used a short-term temporal dataset from 2013 to 2015 with curve function fitting analyses based on the methodology proposed by Zhang et al. (2003) to determine seasonal (intra-annual) parameters (e.g. SOS). They found that the response towards earlier SOS in the warmed subarctic grasslands did not saturate at higher soil warming levels (i.e., +10 °C). Therefore they concluded that growing seasons at high-latitudes grasslands are likely to continue lengthening with future warming. However, there was still quite a large unexplained inter-annual variability in their 3-year dataset, that warranted a further study (Leblans et al., 2017). In this study, we extended the analysis period to six years, compared to the three years used by Leblans et al. (2017). This enabled a more comprehensive examination of the inter-annual variability in NDVI phenology and annual maximum values. Specifically, the variables used to study NDVI phenology were the annual day numbers of the SOS and POS, as well as the PEAK, in each plot. Our primary objective was to reanalyze the soil warming effects using conventional linear statistics, as performed by Leblans et al. (2017), and to assess the robustness of these relationships over a longer timeframe. Additionally, our study extends previous research by employing ML algorithms to identify further drivers of the unexplained inter-annual variation in the studied variables. Specifically, we added a set of three meteorological variables, namely air temperature, precipitation, and irradiance. However, as predictions made by ML are often not intuitive, we used xAI methods, providing deeper insights into the model outputs.

Our objective was to study the relationship between soil temperature and vegetation phenology. More specifically, we studied this relationship using three vegetation phenology characteristics: SOS, POS and PEAK. Additionally, we investigated the effect of other meteorological variables on these characteristics. To this end, we postulated following hypotheses:

A **Soil warming**
  i. A higher soil temperature will introduce significantly earlier SOS, as was found by Leblans et al. (2017) for individual years.
  ii. The POS will take place at a similar time each year, regardless of the soil temperature. Plants must use some external trigger to “know” when to start to slow down growth and prepare for autumn. The prevailing theory suggests that for most plants, this is triggered by the length of the day (Adams and Langton, 2005; Roeber et al., 2022), which remains consistent across different years, and is mediated through the phytochrome system (Sigurdsson, 2001).
  iii. The PEAK value will not be significantly related to soil temperature, as Verbrigghe et al. (2022a) showed that there was no difference in above-ground biomass between the warming treatments.

B **Other meteorological variables**

We expect that ML can identify other important controls for the previously observed inter-annual variability of NDVI phenology and PEAK values. Additionally, we expect that ML can identify the importance of meteorological variables compared to the soil temperature. Out of the three additional meteorological variables, we hypothesized for both phenology and PEAK values:
  i. Larger impact of meteorological variables compared to the soil temperature, as they can also impact the soil temperature (Beer et al., 2018; Tan et al., 2022; Xie et al., 2021).
  ii. Within the meteorological variables, air temperature's influence is expected to be the smallest due to its regulation of soil temperature, while precipitation may have an intermediate effect given consistently high soil water content in these areas (Sigurdsson et al., 2016). Additionally, a substantial impact of irradiance is hypothesized, particularly in consistently cloudy sub-Arctic climates (Hou et al., 2015).

Ultimately, the contributions of this research advance our understanding of the relationships between soil temperature, other meteorological variables, and vegetation phenology. We achieve this goal by employing a methodology that exceeds standard practice, using ML and SHAP values.

2. Materials and methods

2.1. Data

The study was carried out in the south of Iceland near the village of Hveragerdi on the ForHot site (Sigurdsson et al., 2016), as shown in Fig. 1. Following an earthquake in May 2008, the bedrock of one unmanaged (cold) grassland field site underwent a disruption, resulting in the creation of areas with differently warmed soils. Another nearby grassland field site had had such warmed soil gradients for at least six decades, and those were not disturbed by the earthquake in 2008 (Sigurdsson et al., 2016). In spring 2013, five transects were selected in each field site, each with five permanent plots across the natural soil temperature gradients, resulting in a total of 50 studied plots. We categorized the plots according to their annual soil temperature range, as indicated in Table 1.

2.1.1. NDVI data

To be able to estimate vegetation phenology characteristics, we measured the NDVI of all studies plots using a handheld instrument from SKYE Instruments (SpectraoSense2). From 2014 to 2019, NDVI measurements were done approximately bi-weekly from April to November, except during periods with continuous snow cover in early spring, late autumn, or winter. The measurements were always conducted on a clear day. We refer to Leblans et al. (2017) for further information about the NDVI measurements. As can be seen in Fig. 2, the NDVI data clearly

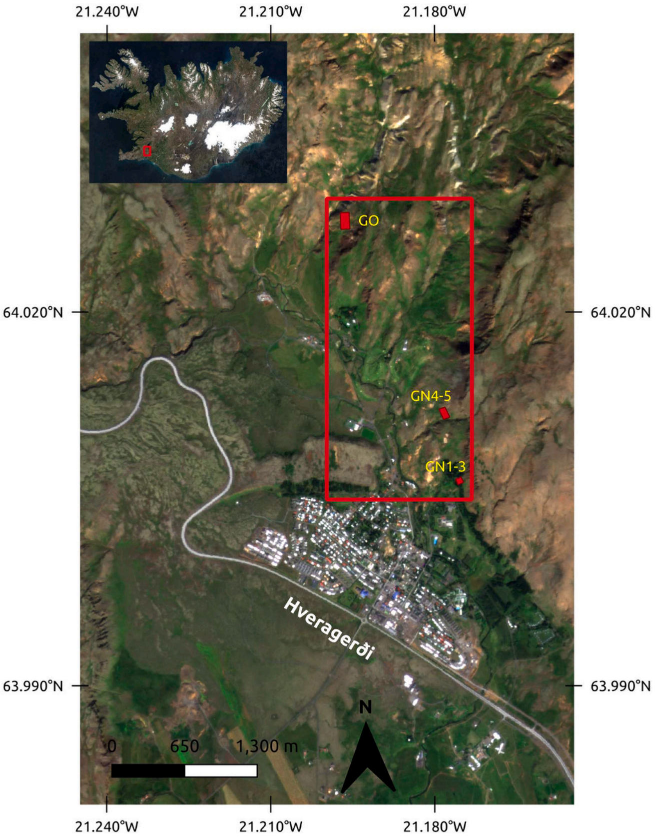

Fig. 1. Map depicting the research site locations near the village of Hveragerdi, Iceland. “GO” (grassland old) marks the sites where the soil has been warming for over six decades, and “GN” (grassland new) denotes the sites where soil warming began following the May 2008 earthquake.

Table 1
Category of the temperature range of the plots.

Category	Temperature Range
A	Ambient
B	+0.5 to 1 °C
C	+2 to 3 °C
D	+3 to 5 °C
E	+5 to 10 °C

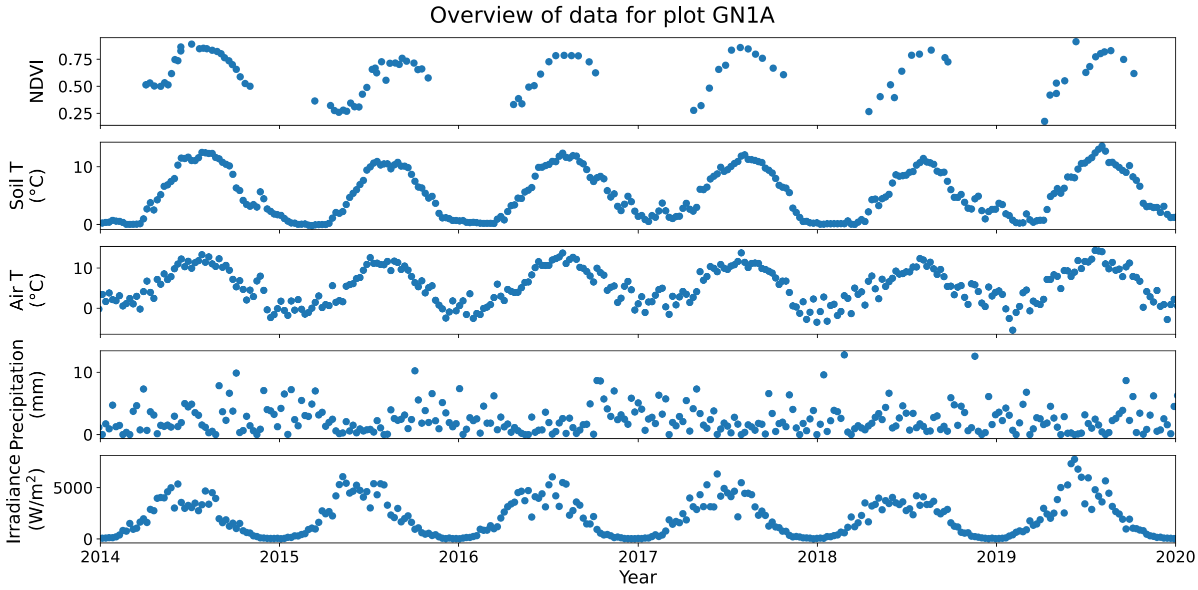

Fig. 2. Overview of all available variables for plot GN1A (unwarmed control plot). Whereas the NDVI and soil temperature (upper two figures) are unique for all 50 plots, the meteorological variables (bottom three figures) are the same for every plot.

showed a seasonal pattern, with a higher NDVI in the summer months.

2.1.2. *Soil temperature data*

The soil temperature at a depth of 10 cm was monitored in all the permanent plots using HOBO TidbiT v2 Water Temperature Data Loggers (Onset Computer Corporation, USA) since the spring of 2013 (Sigurdsson et al., 2016). In Table 1, the different soil warming categories with their accompanying temperature range are given, while Fig. 2 shows the data for one of the 50 plots used in this study. The main soil warming effect was an approximately constant shift in temperature across the seasons, as shown by Sigurdsson et al. (2016).

2.1.3. *Meteorological data*

In addition to NDVI and soil temperature data, we also used meteorological data. As the measurement of meteorological variables such as irradiance (global radiation), precipitation, and air temperature at the Forhot site only began in 2019, we relied on data from another source. Specifically, we obtained the aforementioned meteorological variables from a weather station in Reykjavík,[1] located approximately 40 km from the research site, as this is the closest station where irradiance is measured. We aggregated the data by taking the average on a weekly resolution scale, and assumed that the weather conditions are the same for all plots during each year. Given the distance between the weather station and the research plots, the data serve as a proxy for the actual weather conditions at the ForHot site. In Fig. 2, the three bottom panes show all meteorological variables measured in the relevant period.

2.2. *Data analysis*

2.2.1. *Estimating the NDVI seasonal characteristics*

To extract the intra-annual vegetation phenology characteristics (SOS, POS and PEAK) in each plot during each growing each growing season, we first fitted a curve to the measured NDVI data. Based on the approach of Zhang et al. (2003), we used a double logistic curve. We require that the two logistic curves transition into each other continuously, such that the resulting function is differentiable at every point. These requirements result in the following formula for the estimated NDVI:

$$\widehat{NDVI}(x) = \begin{cases} \dfrac{c}{1+e^{b_1\cdot(x-a_1)}}+d & x \leq p \\ -\dfrac{c}{1+e^{b_2\cdot(x-a_2)}}+d+c & x > p \end{cases} \tag{1}$$

where the parameters a_1, a_2, b_1, b_2, c, d and p are fitted to a season's NDVI data and x represents the week number $(x \in 0, 1, \dots, 52)$ of the year. The parameter p has an important interpretation, as it is defined as the date of the POS, i.e., where the maximal NDVI value is reached.

The best fit for the curve parameters is found using the Trust Region Reflective algorithm (Conn et al., 2000). This generally robust optimization method finds the optimal set of parameters by minimizing the mean squared error (MSE) between the predicted NDVI curve and the NDVI data points. After the curve parameters have been fitted, we extracted the start SOS, POS and PEAK for each plot in each year.

The SOS is considered to be the time of year when the NDVI increases the fastest, i.e., the curvature of the NDVI curve increases the most. This can be calculated using the second derivative of the fitted curves. As shown in Fig. 3, the estimated start of season is the moment in time when the second derivative of the first logistic function is maximal. Combined with the aforementioned definition of the POS, we establish the following equation for calculating the relevant vegetation phenology characteristics:

$$\widehat{SOS} = \underset{x}{\operatorname{argmax}} - \frac{cb_1^2 e^{b_1(x-a_1)}\left(-e^{b_1(x-a_1)}+1\right)}{\left(1+e^{b_1(x-a_1)}\right)^3} \tag{2}$$

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[1] Data courtesy of the Icelandic Meteorological Institute.

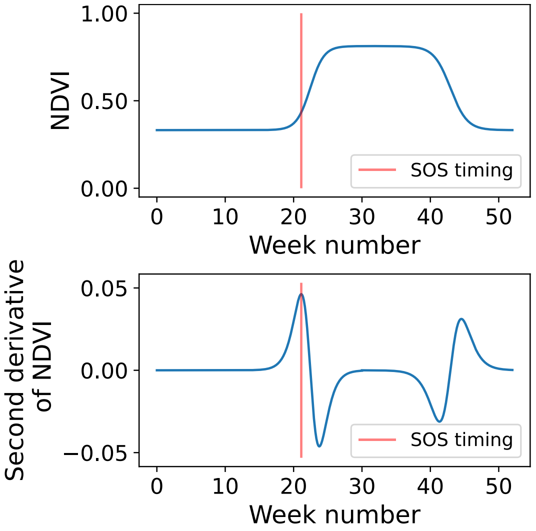


Fig. 3. The SOS is estimated based on the second derivative of the fitted NDVI curve. The SOS is defined as the week when the NDVI curvature increases the most, and is indicated with a red line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

$$\widehat{POS} = p \tag{3}$$

$$\widehat{PEAK} = \widehat{NDVI}(p) \tag{4}$$

where $\widehat{SOS}$ indicates the estimated start of the season, $\widehat{POS}$ the date of the peak of the season, and $\widehat{PEAK}$ the maximum value of the NDVI.

2.3. Statistical modeling and machine learning

2.3.1. Linear regression

After identifying the start and peak of the season for each plot and year, we performed a linear regression analysis. In this analysis, we used the SOS, POS, and PEAK as depenent variables, with the average soil temperature in each plot an year as the independent variable. We conducted this analysis using the ordinary least squares method available in the statsmodels library (version 0.13.2) for Python 3.9.13 (Seabold and Perktold, 2010). This approach also enabled us to compute the *p*-values for the slope and intercept of the linear model through a *t*-test. These p-values help use determine the statistical significance of the relationship between soil temperature and vegetation phenology characteristics by indicating whether the observed relationships are likely due to chance or reflect a genuine underlying pattern.

2.3.2. Machine learning

To better understand the inter-annual variability in our results, we used ML techniques to create models predicting different vegetation phenology characteristics. Specifically, we trained three separate MLPs: one to predict the start of the season, another to predict the peak of the season, and a third to predict the height of the peak season. An MLP is a type of ANN designed to mimic the way the human brain processes information. It consists of multiple layers of nodes (neurons): an input layer, one or more hidden layers, and an output layer which is used to provide the final predictions. Each node in a layer connects to every node in the next layer, with each connection having a specific weight. During training, the MLP adjusts these weights to minimize the difference between its predictions and the actual outcomes using an algorithm called backpropagation, allowing it to learn complex patterns in the data (Werbos, 1990).

Contrary to the linear models introduced in Section 2.3.1, the MLPs also take meteorological variables into account. This meant that in total, each MLP used 79 input variables, which included the average weekly air temperature, precipitation and solar irradiance for the first 26 weeks of the year, as well as the average soil temperature over the entire year. We implemented the MLPs using the MLPRegressor class from the scikit-learn package (version 1.1.3) (Pedregosa et al., 2011). To ensure the models were as accurate as possible, we optimized their hyperparamaters – the parameters that control the learning process – through a process called grid search, which we performed using Optuna (version 3.1.0) (Akiba et al., 2019). This process involved testing different combinations of hyperparameters to find the best settings for each of the three target variables. A description of these hyperparameters, the ranges we explored, and the optimal values we found are provided in Table 2.

To evaluate how well the models performed, we used three standard metrics: MSE, mean average error (MAE), and the coefficient of determination (r^2). For the grid search, we focused on minimizing the MSE to identify the optimal set of hyperparameters. Prior to conducting the grid search, we divided our data into a training set (80% of the data) and a test set (20% of the data). This split ensures that the models are trained on one portion of the data and tested on a separate, previously unseen portion, allowing us to assess their ability to generalize to new, unseen data accurately.

2.3.3. SHAP values

The 79 input features we used are not equally important, and each one influences the model's predictions differently. To understand which features are most significant, and what the direction of their impact is, we use SHAP values. They are calculated by by examining how the model's predictions change when a specific feature is included or excluded, considering all possible combinations of features (Lundberg et al., 2017). By averaging these effects, SHAP values provide a clear and fair measure of each feature's contribution to the final prediction. This method ensures that the importance of each feature is assessed in the context of all other features in the model. In the end, SHAP values can break down each prediction made by the model, showing the contribution of each feature. The sum of the SHAP values for all features then equals the model's output.

After training the MLP models, we computed SHAP values using the model-agnostic Kernel SHAP method to understand which features are most important in predicting the start and (height of the) peak of the greening season. We used the implementation in the Python SHAP package for this analysis Lundberg et al. (2017).

Table 2
Overview of the explored ranges of hyperparameters used in the Optuna grid search. The optimal values for the three different regression tasks are displayed in the right-most three columns.

Description	Range	SOS	POS	PEAK
Number of neurons in first layer	int: 10, 20, …, 100	100	70	30
Number of neurons in second layer	int: 0, 10, …, 100	0	0	100
Strength of the L2 regularization term	float: 1e-4 — 1e-1 logscale	0.0290	0.0010	0.0606
the solver for weight optimization	adam, lbfgs	adam	adam	adam
initial learning rate	float: 1e-4 — 1e-1 logscale	0.0031	0.0003	0.0028
learning rate schedule for weight updates	constant, adaptive	constant	adaptive	adaptive
maximum number of iterations	int: 1000, 2000, …, 10,000	8000	8000	8000
maximum number of iterations with no improvement	int, 10, 20, …, 100	20	50	100

3. Results

3.1. The logistic fitting

For most plots and years, good fits were found for the double logistic curves that were fitted to the intra-annual individual plot NDVI data, with an average r^2 of 0.942 ($\pm$ 0.095). However, for 5.8% of all plots and years, the data did not follow a double sigmoid curve, and the r^2 value was lower than 0.80. These curves were not included in the analysis. The mean estimated SOS was week 20.41 ($\pm$ 2.40), the mean estimated POS was week 29.97 ($\pm$ 3.27), and the mean estimated PEAK was 0.842 ($\pm$ 0.071) across all the soil warming treatments.

3.2. The average response to soil temperature

Fig. 4 shows the linear relationship found between the average annual soil temperature and the three NDVI characteristics found by the double-logistic curves. The parameters of the linear model are given in Table 3. A significant linear relationship was found between average soil temperature and SOS ($p < 0.001$), POS ($p = 0.001$) and PEAK NDVI ($p < 0.001$) (Fig. 4 and Table 3). The relationship between soil temperature and SOS was negative, with an estimated coefficient of -0.2160 ($\pm$ 0.053). This means that for every 4.63 degrees of soil warming, the greening season starts a week earlier. Otherwise stated, the SOS happens 1.52 days earlier per degree of soil warming when derived across multiple years. Similarly, we see that the date of the NDVI peak shifted forward. The estimated coefficient of -0.2353 ($\pm$ 0.07) indicates that for every 4.25 degrees of soil warming, the NDVI peaks a week earlier, or the POS occurs 1.65 days earlier per degree of soil warming. Finally, the PEAK value of the NDVI curve increased slightly with increasing soil temperature.

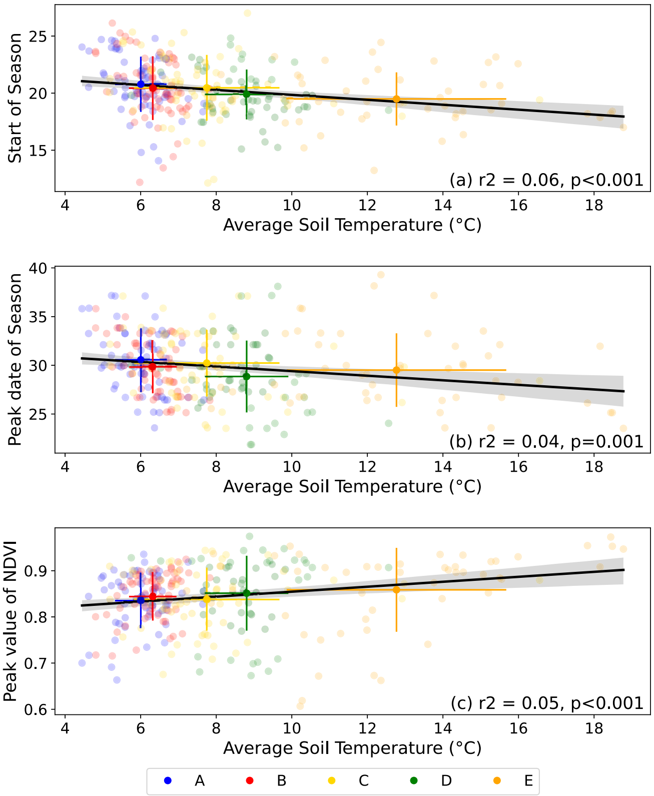


Fig. 4. Linear model that predicts the start of the season (a), the peak date of the season (b) and the peak value of NDVI (c), based on the average annual soil temperature. The filled circles represent the mean values for each category (A to E) of average soil temperature, with error bars indicating the standard deviation. The semi-transparent circles represent individual observations. The colour indicates the soil warming category where the blue points are A plots, the red points are B plots, the yellow points are C plots, the green points are D plots, and the orange points are E plots. All models had a significant relationship between the average soil temperature and the studied NDVI curve parameter (See Table 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
The parameters describing the results of the linear models, where different variables are fitted against the average soil temperature over a whole year. The SOS and POS are measured in weeks, while the intercept is measured in degrees Celsius.

Target variable	Slope	Intercept	r^2	p-value
SOS	$-$ 0.216 $\pm$ 0.052	22.011 $\pm$ 0.454	0.06	<0.001
POS	$-$ 0.235 $\pm$ 0.070	31.755 $\pm$ 0.607	0.04	0.001
PEAK	0.005 $\pm$ 0.001	0.801 $\pm$ 0.013	0.05	<0.001

Although the linear relationships that were observed between average soil temperature and SOS, POS, and PEAK were significant (Fig. 4), we also observed a lot of unexplained variance, which is indicated by the relatively low r^2 values in Table 3.

3.3. The machine learning approach

To explain a larger part of the variance, the possibility of predicting characteristics of the NDVI curve using MLPs, based on both the soil temperature and meteorological variables, was investigated. The performance of the MLPs can be found in Table 4. From Tables 3 and 4, it becomes evident that the inclusion of the meteorological variables and the utilization of MLPs enabled us to explain a significantly larger part of the variance compared to the linear models.

To investigate the impact of a given feature on the predictions made by the model, we calculated SHAP values for all three MLPs. These can be found in Fig. 5, Fig. 6 and Fig. 7 for the SOS, POS and PEAK, respectively. In these figures, we separate the six years to investigate the annual variation in the SHAP values. To obtain the SHAP value for one meteorological variable, we summed the SHAP values of the 26 weekly averages, as shown in Eq. (5). Next, we calculated the sum of absolute values of the SHAP values A_SHAP for the four remaining features for all n samples, as shown in Eq. (6). By taking the absolute value and adding it over all years, we can investigate the total impact of a feature on the prediction, regardless of the direction of the impact. The results for the (A_SHAP) values are shown in Fig. 8.

$$\mathrm{SHAP}_{feature} = \sum_{week=1}^{26} \mathrm{SHAP}_{feature,week} \tag{5}$$

$$\mathrm{A\_SHAP}_{feature} = \sum_{i}^{n} |\mathrm{SHAP}_{feature,i}| \tag{6}$$

When interpreting Figs. 5 and 8a, we see that the meteorological variables had the largest impact on the prediction of the SOS. However, within each year, this impact was approximately constant. The intra-annual variation in the SOS was clearly the result of soil warming. In fact, the Pearson correlation between soil temperature and its accompanying SHAP values was -0.93, meaning that the higher the soil

Table 4
Model performance of MLP after a 5-fold cross validation (CV) grid search. The test set consists of 20% of the total data, and is split evenly across the years of data taking. The naive MSE (MAE) is the MSE (MAE) when the mean of all training samples is used as the prediction.

Target	5-fold CV MSE	Test MSE (naive)	Test MAE (naive)	Test r^2
SOS	3.408	4.760 (7.102)	1.521 (2.095)	0.322
POS	7.933	8.943 (11.103)	2.473 (2.696)	0.192
PEAK	0.004	0.004 (0.006)	0.053 (0.063)	0.248

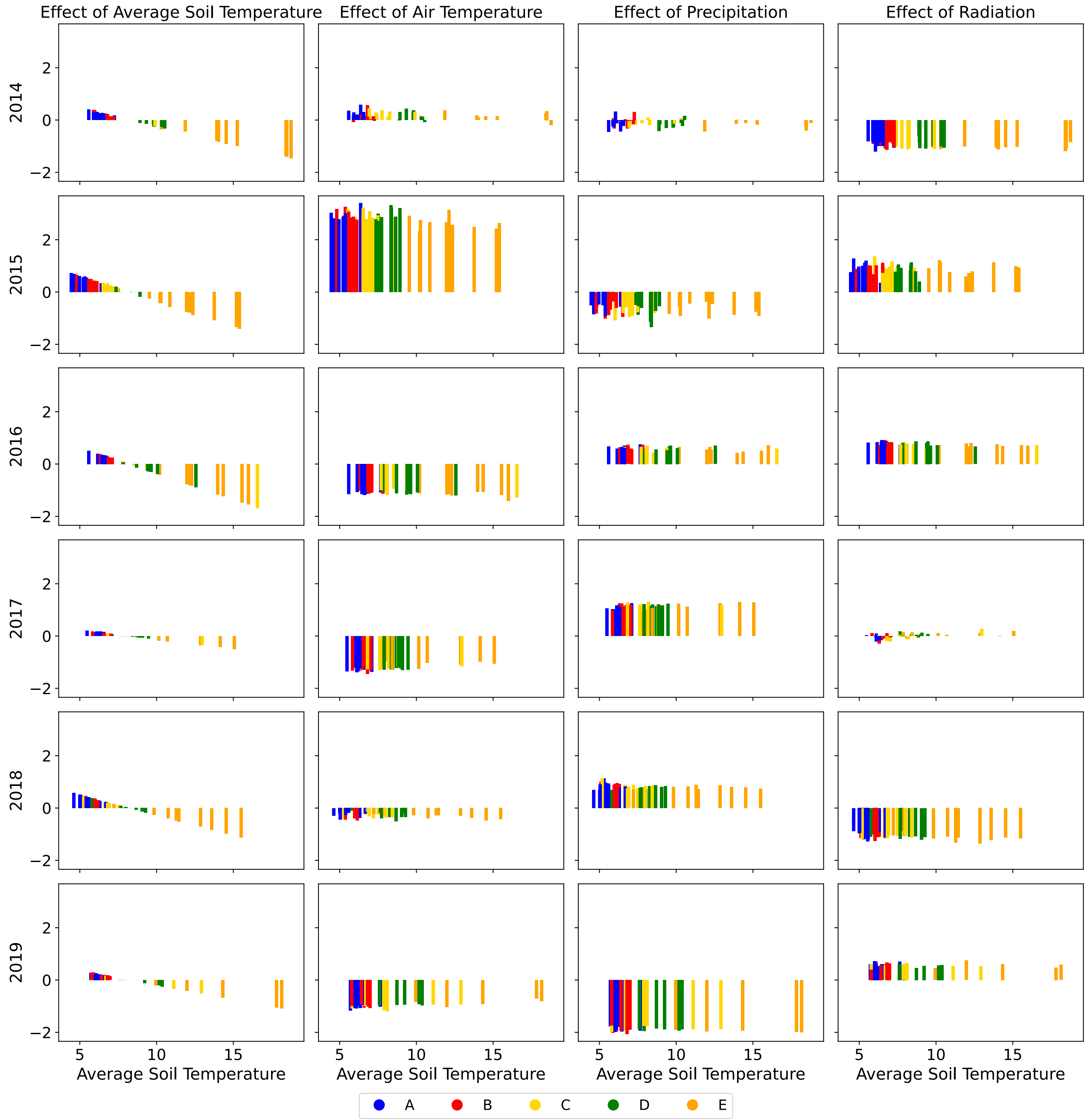


Fig. 5. SHAP values of multi-layer perceptron that predicts the start of the greening season based on the average soil temperature, air temperature, precipitation, and radiation. The colour indicates the soil warming category where the blue bars are A plots, the red bars are B plots, the yellow bars are C plots, the green bars are D plots, and the orange bars are E plots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

warming, the earlier the season started each year. All Pearson correlation values can be found in Table 5.

From Fig. 8b and c, we can also conclude that the three meteorological variables also had the largest impact on the predictions of the POS and PEAK. From Table 5, we can see that the POS was earlier and the PEAK value of the NDVI was higher with increasing soil temperature, as they had a Pearson correlation coefficient of −0.85 and 0.91, respectively. For the POS, Fig. 6 indicates that the size and direction of the SHAP effect for the three meteorological variables shifts significantly over the years, while the smaller effect of the soil temperature is relatively stable across the six years and drives the intra-annual variation within the dataset.

4. Discussion

The purpose of this study was to explore the relationship between soil temperature and NDVI, along with the impact of meteorological variables, utilizing ML techniques. The discussion will focus on emphasizing the novelties of this work, addressing the hypotheses presented in the paper, discussing the findings in relation to previous research, and highlighting the implications of the results.

4.1. *Using machine learning to study vegetation phenology*

Currently, the standard practice in vegetation phenology studies using NDVI consists of using traditional statistical methods such as (non-)linear regression or linear mixed models (Estrella et al., 2021; Hope

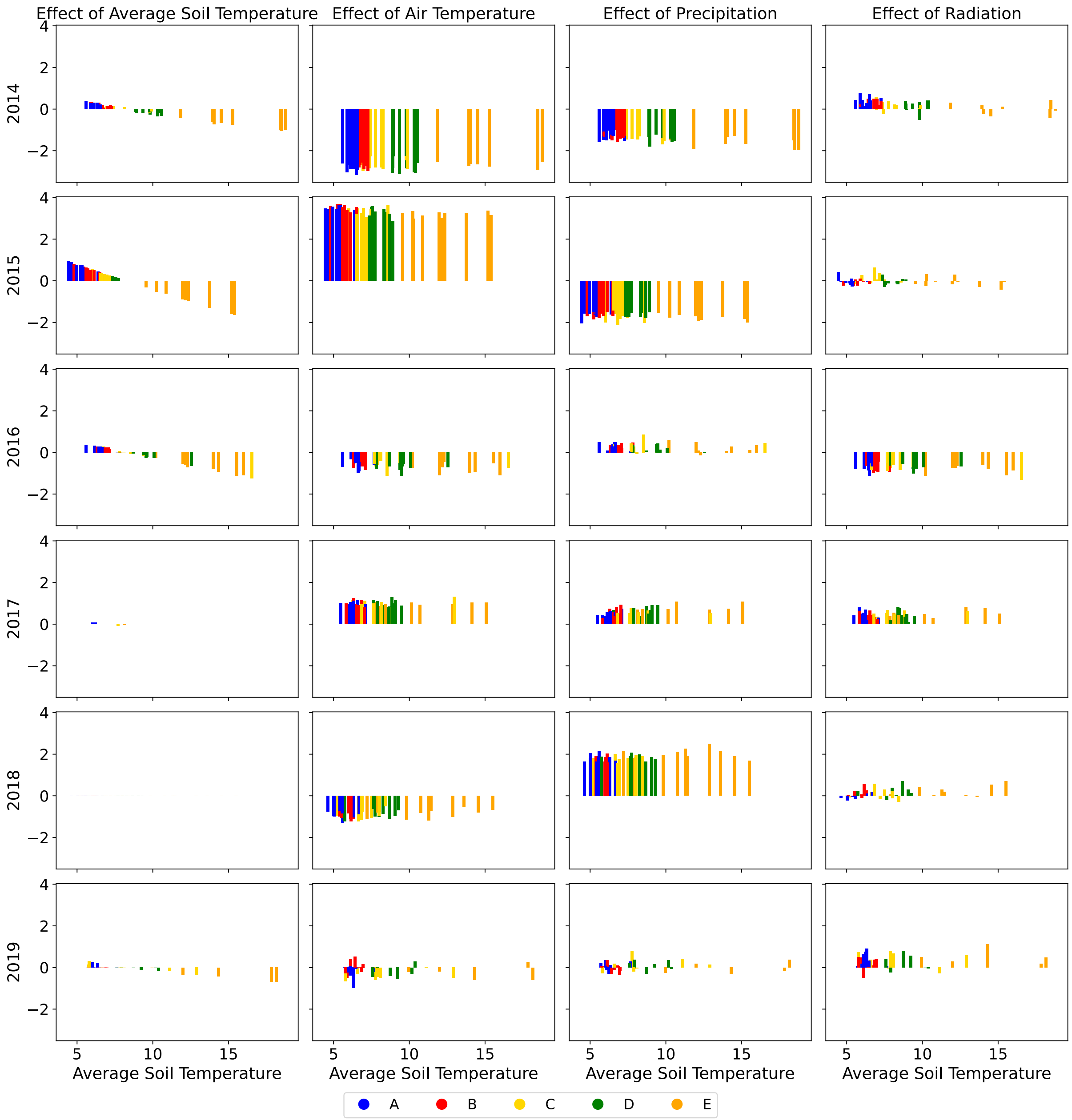


Fig. 6. SHAP values of multi-layer perceptron that predicts the peak of the greening season (POS) based on the average soil temperature, air temperature, precipitation, and radiation. The colour indicates the soil warming category where the blue bars are A plots, the red bars are B plots, the yellow bars are C plots, the green bars are D plots, and the orange bars are E plots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

et al., 1993; Leblans et al., 2017; Mehmood et al., 2024; Walker et al., 2012a; Wang et al., 2021). However, our results clearly indicate that, after applying linear regression, a large amount of unexplained variance remains. Our study advances the traditional approach by using ML models, specifically MLPs, which integrate meteorological variables to capture nonlinear relationships. This method allowed us to explain a larger portion of inter-annual variance compared to traditional methods. The use of SHAP values further provided insights into a deeper understanding of the complex interactions between soil temperature, meteorological variables, and NDVI dynamics.

4.2. Effect of the soil temperature on SOS, POS, and PEAK in subarctic grasslands

The first hypothesis stated that a higher soil temperature would lead to an earlier SOS based on previous research by Leblans et al. (2017). Such responses have also been found when past changes in NDVI have been related to changes in annual, seasonal or monthly temperatures (Arndt et al., 2019; Karlsen et al., 2014; Potter and Alexander, 2020).

The findings of this study supported this hypothesis, as a significant relationship was observed between average soil temperature and the start of the greening season. The negative coefficient (−0.2160) indicates that SOS occurs 1.5 days earlier per degree of soil warming across the six years. This finding was consistent with a recent analysis from the International Tundra Experiment covering up to 20 years of

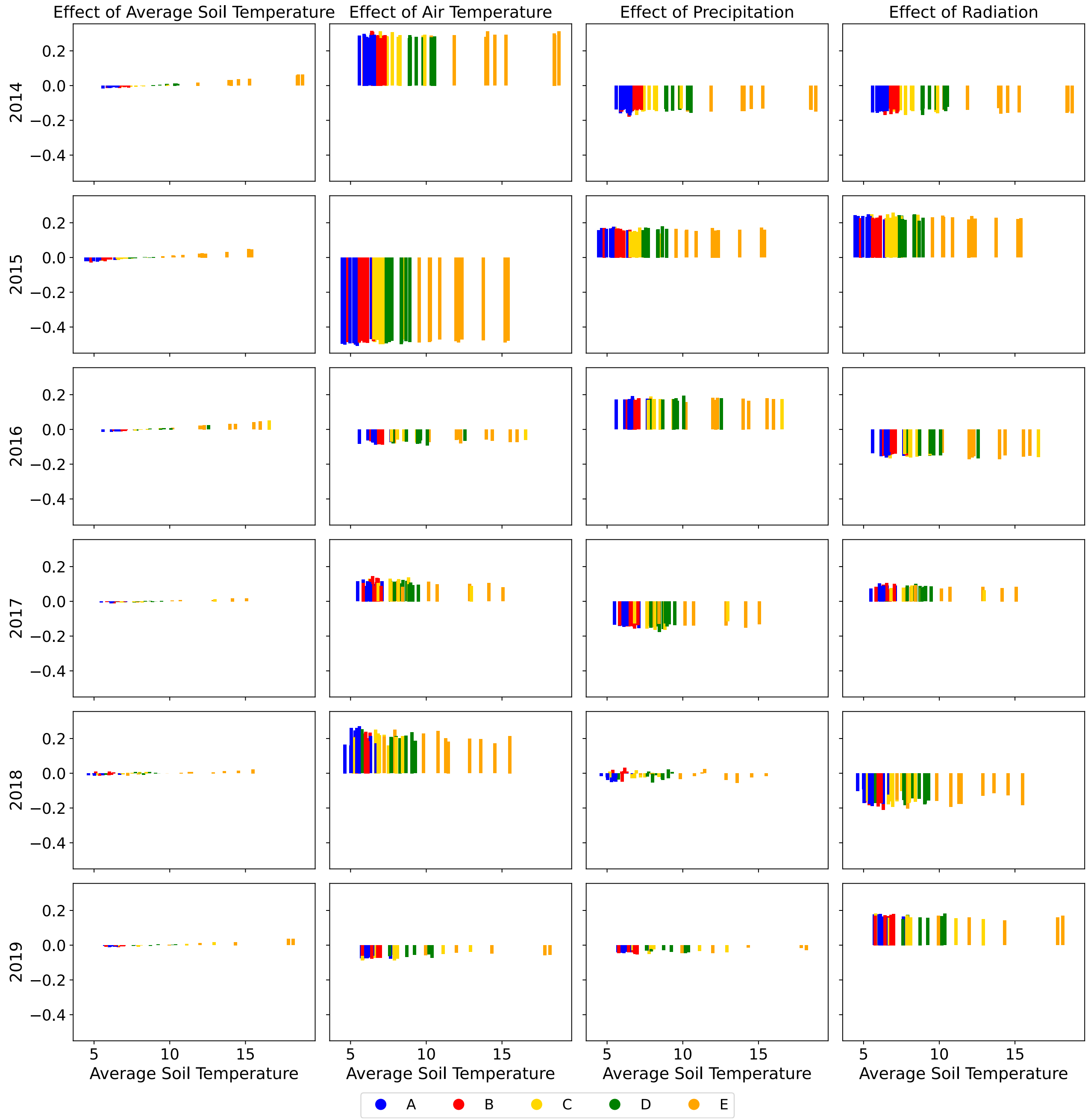
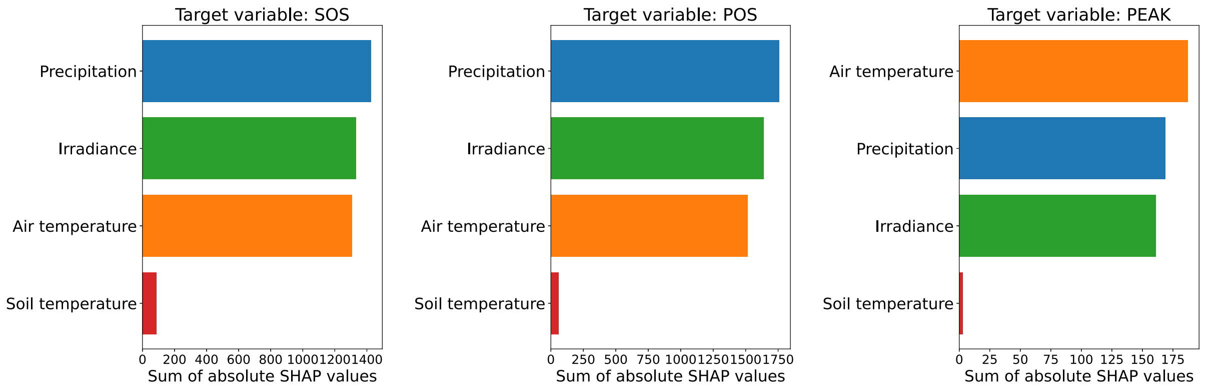


Fig. 7. SHAP values of multi-layer perceptron that predicts the peak NDVI based on the average soil temperature, air temperature, precipitation, and radiation. The colour indicates the soil warming category where the blue bars are A plots, the red bars are B plots, the yellow bars are C plots, the green bars are D plots, and the orange bars are E plots. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

data from 18 sites and 46 open-top chamber warming experiments across the Arctic, sub-Arctic, and alpine ecosystems (Collins et al., 2021). They observed a 0.73-day earlier start of the greening season, in an environment where the average air warming was 1.4 °C and the soil warming approximately half of that (Collins et al., 2021). Our finding was also consistent with previous research at the same ForHot site, as Leblans et al. (2017) found that on average, the SOS occurred 1.6 days earlier for every degree of soil warming.

Day length has traditionally been considered a dominant factor in regulating the phenology of many plant species (Adams and Langton, 2005; Roeber et al., 2022), particularly in high-latitude ecosystems where day length changes significantly throughout the growing season. Therefore in this study, the second hypothesis stated that the date of the POS would occur at a similar time each year, regardless of soil temperature, as the day length remains consistent across all years. However, our results indicate that temperature conditions in the soil can have a considerable influence on the timing of POS. The hypothesis was therefore rejected. This finding suggests that, in our sub-Arctic grasslands, day length might not be the primary factor influencing the timing of the POS. While previous studies have highlighted the interplay between day length and air temperature in determining phenological events (Malyshev et al., 2014), our study is unique in demonstrating the notable impact of soil temperature. This underscores the need to consider soil temperature as an influential factor in phenological studies, particularly in the context of climate change where both soil and air temperatures are rising.

The third hypothesis proposed that the PEAK NDVI would not be significantly related to soil temperature, based on previous research by



(a) Start of season

(b) Peak of season

(c) Peak NDVI value

Fig. 8. Sum of the absolute SHAP values as defined in Eq. (6).

Table 5
Pearson correlation coefficient between the average soil temperature and its corresponding SHAP values.

Target variable	Pearson correlation
SOS	−0.93
POS	−0.85
PEAK	0.91

Verbrigghe et al. (2022a), who had not found significant differences in vegetation biomass across the warming gradients. However, the findings of this study indicate a slight increase in the PEAK value with increasing soil temperature. Although the relationship was not as strong as for the SOS and POS, it suggests that higher soil temperatures may contribute to higher NDVI peak values. It is worth noting that while NDVI is often used to estimate vegetation biomass (Bui et al., 2024; Lumbierres et al., 2017; Perry et al., 2022; Zhang et al., 2016), it is not measuring it directly, but rather the amount of chlorophyll per surface area (Huang et al., 2021). Therefore, “Arctic greening” measured using the NDVI, could occur without any changes in vegetation biomass, if the plants are getting “greener” due to a higher nutrient content in warmer soils. Further research is needed to better understand this relationship and its underlying mechanisms.

4.3. Effect of the other meteorological variables

Hypothesis B focused on the impact of other meteorological variables (air temperature, precipitation, and irradiance) on the inter-annual variability of the NDVI phenology and PEAK values, and the potential of ML to identify their importance. The results of the ML analysis using MLPs showed that these variables have a strong impact on the predictions of the SOS, POS, and PEAK, and the r^2 values of the MLPs were much higher than those obtained by the linear regression.

The SHAP values also provided information on the relative importance of these variables. It was noteworthy that the three meteorological variables had a much larger impact on the predictions than the soil warming data. These findings align with other studies that emphasize the significance of climatic variables over soil conditions because of their influence on soil temperature in predicting vegetation responses (Beer et al., 2018; Tan et al., 2022). However, the intra-annual variation in the SOS, POS, and PEAK was found to be influenced by the soil temperature. This influence of soil temperature highlights the significant role of below-ground processes in driving vegetation phenology and productivity (Fang et al., 2023). Studies have shown that soil temperature can affect root growth, nutrient availability, and microbial activity, all of which are crucial for plant development (Bhattarai et al., 2023; Metze et al., 2024; Verbrigghe et al., 2022a). Understanding these interactions is essential for accurately predicting how the ecosystems will respond to ongoing climate change.

The SHAP values did not indicate significant differences among the meteorological parameters, making it challenging to prioritize their impact as hypothesized. This contradicts our hypothesis that air temperature's influence would be minimal due to its regulation of soil temperature, precipitation would have an intermediate effect, and irradiance would have a substantial impact, especially in cloudy sub-Arctic climates (Hou et al., 2015; Sigurdsson et al., 2016). However, collectively, these meteorological factors exhibited a considerably higher influence on the predictions compared to the soil warming data. Therefore, our findings not only contribute to understanding the dominant impact of meteorological parameters on vegetation dynamics, but also emphasize the need for continued research to explain the interdependencies and potential interactions between these factors.

4.4. Methodological considerations

It is important to note some limitations of the study. The analysis focused on a specific location in Iceland, and the results may not be directly applicable to other regions. The study period also covered a limited period of time (2014–2019), and longer-term data would provide a more comprehensive understanding of the inter-annual variation in NDVI. Furthermore, the meteorological data does not have the same spatial resolution as the NDVI or soil temperature data. Indeed, as we relied on the measurements of the nearest weather station, we had to assume that the weather conditions were the same across all plots.

The SHAP values should also be interpreted with caution. Although they are model-agnostic, we can only draw valid conclusions if the model generalizes well. That is, if it has an acceptable test set performance (Molnar et al., 2020). Furthermore, the SHAP values do not have a causal interpretation (Frye et al., 2020). We cannot assume that if the variable X has a large impact on the prediction of Y, then X causes Y. On the contrary, Y might cause X, X and Y could both be caused by a confounding variable, or they could have no causal relationship at all.

Nevertheless, this study produces valuable insights and provides clear directions for future research. Our promising results, achieved by applying ML in a vegetation phenology study, emphasize the potential of this approach in advancing our understanding of seasonal plant characteristics based on NDVI data. They can also be viewed as a starting point for other analyses in a broader ecological context.

In the future, it would be interesting to consider other model architectures or methodologies, for example, XGBoost (Chen and Guestrin, 2016). Additionally, other xAI approaches like LIME (Ribeiro et al., 2016) could be considered, allowing comparison between different xAI approaches.

5. Conclusions

Our results only partly supported our hypotheses regarding the effect of soil temperature on the timing of the SOS, timing of the POS, and peak NDVI values. We observed a significant relationship between soil warming and the timing of SOS and POS, indicating that higher soil temperatures advance the onset of the growing season. Unexpectedly, this also led to a corresponding shift in the timing of POS. Moreover, the peak NDVI values showed a slight increase with higher soil temperatures. Furthermore, we explored the impact of meteorological variables, more specifically air temperature, precipitation, and irradiance, on vegetation phenology and its inter-annual variation. The use of SHAP values allowed us to gain insight into the relative importance and contribution of each meteorological variable to the predictions. It became evident that the three meteorological variables had the largest impact on the prediction of SOS, POS, and PEAK NDVI values across the six years. However, within a given year, the impact of the three meteorological variables remained approximately equal, while the variations in phenological characteristics were primarily driven by soil temperature.

For future work, we suggest further exploration of the underlying mechanisms driving the observed relationships between soil temperature and phenology. Investigating the physiological responses of plant species to soil temperature variations and exploring the interactions between soil temperature and other environmental factors at finer temporal and spatial scales would provide a more comprehensive understanding. Additionally, collecting data considering the soil characteristics, e.g., soil chemistry or nutrient availability, could improve the performance of the ML models, and further increase the explained variance.

In addition, incorporating advanced remote sensing techniques, such as satellite imagery, in conjunction with ground-based measurements can improve the accuracy and comprehensiveness of phenological studies in subarctic grassland ecosystems. Long-term monitoring at multiple sites and the incorporation of various geographical locations would provide valuable information on the generalizability of our findings and the response of subarctic grasslands to ongoing climate change.

This study contributes to our knowledge of the relationships between soil temperature, other meteorological variables, and vegetation phenology in subarctic grassland ecosystems. The findings enhance our understanding of the mechanisms driving ecosystem dynamics in these regions and have implications for predicting and managing subarctic grasslands in the face of environmental change. Finally, this work also functions as a proof-of-concept for ML-based vegetation phenology studies, and thereby provides a solid foundation for future research in this domain.

CRediT authorship contribution statement

Steven Mortier: Conceptualization, Methodology, Software, Validation, Formal analysis, Data Curation, Writing - Original Draft, Visualization, Writing - Review & Editing. **Amir Hamedpour:** Conceptualization, Methodology, Investigation, Writing - Original Draft, Visualization, Writing - Review & Editing. **Bart Bussmann:** Conceptualization, Methodology, Software, Data Curation. **Ruth Phoebe Tchana Wandji:** Conceptualization, Methodology, Investigation, Writing - Original Draft, Writing - Review & Editing. **Steven Latré:** Funding acquisition, Supervision. **Bjarni D. Sigurdsson:** Investigation, Writing - Review & Editing, Funding acquisition, Supervision, Project administration. **Tom De Schepper:** Conceptualization, Methodology, Writing - Review & Editing, Supervision. **Tim Verdonck:** Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision.

Data availability

The data used in this study is made available as open data and can be found here: `http://dx.doi.org/10.17632/C9T7FX9N4H.1` (Mortier et al., 2023).

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IV

Effects of soil warming and interannual variability on aboveground biomass production of sub-Arctic grasslands

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ABSTRACT

Long-term field experiments with multiple warming levels are rare, but they can clarify how future ecosystem processes are likely to change and if an ecosystem will cross some thresholds in its response to warming. We used 10 years of harvest data of aboveground vascular plant biomass (AGB_{vasc}) from unmanaged grasslands exposed to chronic increases in soil temperature (T_s) ranging between +0°C to +13°C since 2008 (medium-term warming; MTW) or for >60 years (long-term warming; LTW). Our results showed that the effects of chronic (decadal) warming was smaller than the interannual variability in response of $AGB_{vasc.}$ After normalising for the interannual variability, chronic warming was still found to enhance AGB_{vasc} on average, but the enhancement decreased strongly as the level of chronic warming increased (apparent down-regulation). Further analysis with linear mixed models confirmed this. Moreover, we could observe that the LTW had a significantly stronger positive chronic warming response than MTW, and the accumulated T_s-sum during the 12 months prior to harvest, in combination with annual soil water content and other warming-level changes, were the most important drivers in the observed interannual responses. Our findings highlight the importance of including more than one warming level in ecosystem manipulation studies.

KEYWORDS

geothermal warming; plant growth; sub-Arctic ecosystems, temperature response, aboveground plant biomass

INTRODUCTION

Climate change is a threatening global phenomenon affecting all ecosystems on Earth (e.g. IPCC, 2023; Post et al., 2019). Arctic and sub-Arctic ecosystems are experiencing some of the most rapid and severe climate changes on Earth, leading to some significant ecological transformations and affecting everything from the micro-level of soil chemistry to the macro-level of global climate patterns (IPCC, 2023).

High-latitude grassland ecosystems (>60° N), encompass a unique range of environments from the Arctic tundra to sub-Arctic meadows. These ecosystems are integral to global climate regulation due to their substantial carbon storage capabilities and their status as critical biodiversity hotspots (Myers-Smith et al., 2011). Unlike temperate or tropical grasslands, which may experience more stable climatic conditions and longer growing seasons, high-latitude grasslands are characterised by a shorter growing season, often limited by low temperatures and the duration of sunlight (Elmendorf et al., 2012; Bai et al., 2010; Mortier et al., 2024). The annual solar radiation received in these regions is typically lower than that in lower latitude grasslands, which can further constrain plant growth (Mølmann et al., 2021). Precipitation patterns also differ, with high-latitude grasslands often experiencing a more pronounced seasonality in moisture availability, impacting the overall productivity of these ecosystems (Bai et al., 2010). Additionally, soil characteristics in high-latitude regions, such as water-holding capacity, nutrient availability, and pH levels, exhibit distinct variations compared to grasslands in other climatic zones (Arnalds, 2015; Epstein et al., 2004; Gough et al., 2000). Furthermore, the standing plant biomass in Arctic and sub-Arctic grassland ecosystems is significantly influenced by biological factors, particularly the grazing pressures exerted by both vertebrate and invertebrate herbivores, which can differ in intensity and impact compared to those in lower latitude grasslands (Pedersen & Post, 2008). These unique environmental and biological factors underscore the distinct ecological dynamics of high-latitude grassland ecosystems, highlighting their importance in the broader context of global biodiversity and climate regulation.

Since the 1980s, soil warming experiments have been established across high-latitudes with the goal to have more insights into how these ecosystems will respond to increasing temperatures (Gestel et al., 2019). Long-term climate warming experiments across the Arctic have demonstrated that many ecosystem types respond to warming (Yu et al., 2017). Studies such as Bjorkman et al., (2018) who looked at the tundra biome found an overall increase in vegetation productivity with warming. The International Tundra Experiment (ITEX) used open-top chambers across multiple sites to study plant growth and phenological changes due to 1-2 °C warming, revealing that warming accelerates the developmental processes of plants within tundra ecosystems (Elmendorf et al., 2012). Equally, open-top chamber warming experiments in Abisko, Sweden, showed that an increase in temperatures led to higher biomass of vascular plants (Dorrepaal et al., 2004; Alatalo et al., 2016).

High-latitude grasslands exhibit larger relative and absolute interannual variability in aboveground net primary productivity (ANPP) compared to other ecosystems (Frolking et al., 2005) which could be driven by the high climate variability (Xie et al., 2022). The variability in ANPP is largely driven by changes in environmental factors such as temperature (Post & Pedersen, 2008; Butterfield et al., 2020) and precipitation patterns (Fay et al., 2003). Grasslands with intermediate levels of precipitation tend to have the greatest fluctuation in productivity from year to year (Cleland et al., 2013). Moreover, fluctuations in annual temperature can significantly affect the length of the growing season (Post & Pedersen, 2008) and the timing of snowmelt in spring (Callaghan et al., 2011), which directly impacts plant growth at higher latitudes (Post & Pedersen, 2008) and productivity.

In Iceland, natural grasslands cover 4400 km^2, mainly within the sub-Arctic lowland zone (Ottósson et al., 2017). These ecosystems host a diverse array of perennial species which include a variety of grass species and herbs (Kristinsson & Sigurðsson, 2010) that have adapted to weather fluctuations and temperatures typical of high-latitude environments (Thorvaldsson & Martin, 2004; Thorvaldsson & Björnsson, 1990). In most Icelandic grasslands and heathlands, nitrogen (N) availability is an important limiting factor for plant production (Gudmundsson et al., 2004). In these control ecosystems with short growing seasons, the decomposition of soil organic matter is slow, implying slow turnover of N and other nutrients (Aerts et al., 2012). Therefore, an increase in temperatures is likely to affect plant productivity directly and indirectly through enhanced decomposition and N mineralisation on nutrient availability.

Meeran et al. (2023) in their study on the same Icelandic grasslands showed that N was a limiting factor for plant growth at the ecosystem level, and Callebaut et al. (2022) showed that such N-limitation can also be at the species-level (plant net-photosynthesis rate). However, research on the seasonal variability of plant growth in fertilised cultivated grasslands in Iceland has indicated that in the absence of N-availability limitations, temperature, irradiance and precipitation fluctuations were all important drivers for growth rate (Thorvaldsson & Martin, 2004). While they showed a strong influence of weather on growth rate within seasons, weather variables only explained a part of the interannual variation in growth rate in their study (Thorvaldsson & Martin, 2004). Other factors such as grazing pressure, soil conditions such as warming, or moisture levels along with other abiotic or biotic components, could also contribute to the interannual variability in response of ANPP. Thereby, underscoring the complex interactions that influence plant productivity.

As many of the responses to warming in the sub-Arctic areas are mediated through changes in soil processes, many experimentalists have chosen to only warm the soil to better understand those responses. In Iceland, with its volcanic activity, natural grassland ecosystems are sometimes exposed to natural soil warming when shifts in geothermal activity warm up the underlying bedrock. This offers a unique natural laboratory for studying the impacts of warming (O'Gorman et al., 2014; Sigurdsson et al., 2016).

Studies have shown that experimental warming can stimulate carbon fluxes in grassland ecosystems, leading to increased plant production and soil respiration (Wang et al., 2019). Relatively strong responses have been seen in carbon (C) loss from the soils because of enhanced heterotrophic respiration with warming (Propster et al., 2023; Marañón-Jiménez et al., 2018, 2019; Shaver et al., 1992). However, the longer-term impact of warming on the ecosystem C balance also depends on the degree to which biomass production is enhanced.

This study examined the annual aboveground vascular plant biomass growth (AGB_{vasc}) across a soil warming gradients from 0°C to +13°C in two unmanaged Icelandic grasslands, with different warming durations categorised as Medium-Term Warming (MTW) and Long-Term Warming (LTW). The following hypotheses were tested:

1. The interannual variability in response of AGB_{vasc} is expected to have smaller impact on ANPP (AGB_{vasc}) than chronic soil warming. In control plots, interannual variability is expected to play a more significant role, whereas in soil-warmed plots, its impact is anticipated to be smaller.
2. The interannual variability in response of AGB_{vasc} in control plots is expected to be primarily influenced by growing-season temperature, alongside other growth-limiting factors such as soil water content, which is also expected to contribute significantly to the year-to-year variability of the AGB_{vasc}.
3. Chronic warming is expected to increase AGB_{vasc} due to enhanced nutrient availability resulting from an increased decomposition rate. However, in warmer plots, we also expect soil moisture to become a stronger limiting factor for ANPP due to increased evapotranspiration.
4. The duration of warming might have a cumulative effect on biomass production, potentially leading to more pronounced growth responses over time. Research by Walker et al. (2019) suggested that ecosystems initially exhibit a significant overreaction to short-term soil warming, which leads to pronounced changes in biotic composition and ecosystem processes, but eventually stabilise into a new steady state after prolonged exposure to warming. Therefore, in the LTW, we expect to see a clearer warming-induced response in AGB_{vasc} in comparison to the MTW.

Materials and Methods

STUDY AREA

The samples were collected at the ForHot site (*forhot.is*), located in the South of Iceland near Hvergeraði village (64.008°N, 21.178°W). The studied grasslands are protected from livestock grazing with fences and are dominated by sub-Arctic perennial plant species (example: *Agrostis capillaris*, *Ranunculus acris*, *Poa pratensis*, *etc*), with a growing season usually from May to late August. The soil type at the grasslands is silandic andosol with a silt loam texture, and the unwarmed area has a sub-Arctic climate with oceanic weather, thus non-permanent snow cover but the soils remain frozen for two to three months during the middle of the winter (Sigurdsson et al., 2016).

The ForHot site is an exceptional study site with naturally warmed soils, thus, crucial in the assessment of long-term effects of global warming. At the research site, one grassland ecosystem has soils that have been warmed for over 60 years (LTW) and the other since 2008 (MTW), when an earthquake disrupted geothermal channels in the underlying bedrock (Sigurdsson et al., 2016). Sixty permanent 2×2 m survey plots were established in the autumn of 2012 along the two grasslands' soil temperature gradients. In this study, we utilised 50 of these plots, that is, 25 per grassland site; that is, five plots per transects, replicates located perpendicular on the five-soil warming level (0°C, +0.48°C, +1.78°C, +3.27°C, and +7.38°C) (Sigurdsson et al., 2016).

DATA

Soil Temperature

Soil temperature (T_s) loggers (TidbiT v2 HOBO® data loggers; Onset Computer Corp., Bourne, MA, USA) were installed in every permanent survey plot at 10 cm soil depth. The hourly and daily mean temperatures were calculated for each plot.

Meteorological Data

For this study, we used the weather station located in Reykjavik (64°13’N 21°90’E), approximately 40 km from the sampling areas, with a mean annual (2012-2022) temperature (MAT, °C) of 5.1°C and annual (2012-2022) accumulated hourly and daily precipitation of 886 mm (Icelandic Meteorological Office, 2023).

Soil Water Content

The soil water content (SWC, %) was sampled manually using the ThetaProbe moisture meter (HH2 Moisture Meter, AT Delta-T devices, Burwell, Camb., UK). The probe measures the volumetric soil moisture content in response to changes in the dielectric constant in the topsoil layer (0-7 cm soil depth). We used the SWC values at peak season at the time of harvesting, considering the fact that the soils can be saturated before July due to spring thawing and after August due lower evapotranspiration in the cool and wet climate.

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Aboveground Biomass

The AGB_{vasc} was harvested to the ground level at peak growing season (July) in a 0.2 × 0.5 m area in each plot for the years 2013, 2016, 2018, 2020, 2021, and 2022. The AGB_{vasc} samples were then sorted in the laboratory into vascular plants, moss, lichens and dried at 105°C for 48 hours to obtain the dry weight. In this study we focused on vascular plant biomass only, excluding moss and lichens biomass.

DATA AND STATISTICAL ANALYSIS

All statistical analyses were performed with the R software (R.4.2.2, http://CRAN-R-project.org). We used daily mean plot level soil temperatures T_s from: i) the previous summer to the sampled summer (July to July, annual temperatures), ii) the previous winter to the sampling time of the corresponding AGB_{vasc} harvest (November to July; Thorvaldsson & Bjornsson, 1990), and iii) the summer temperatures of the sampled year (June to July of sampled year; Van Der Wal and Stein, 2014), we calculated the temperature sums (T_{sum}, Equation (1)) using the baseline daily temperature of 0°C.

$$T_{sum} = \sum_{i=1}^{n} (T_{mean} - T_{base})\Delta t \quad (1)$$

Where T_{sum} = temperature sums (atmospheric, soil); T_{mean} = average temperature, T_{base} is the baseline temperature, Δt = time step (1 day). We evaluated other baseline temperatures (+2°C, +5°C) alongside 0°C, assessing each based on the clarity of results as indicated by higher R^2 values. The 0°C baseline was ultimately retained, as it produced the most robust model fit and the clearest results, evidenced by its higher R^2 compared to other baseline temperatures. This approach allowed for a more accurate representation of the data, ensuring that our analysis captured the primary trends in the dataset.

Although the permanent survey plots were established in the autumn of 2012, the data collection of T_s started in spring (May) of the year 2013. Therefore, the annual (July – July) and winter (November – July) T_s were obtained through extrapolation with a plot-level correction factor. The correction factor was calculated from available T_s data from a proximate plot at the research site (not included in this study).

Moreover, in the sampling of AGB_{vasc} across the six studied years, a methodological difference in 2018 led to an inflated AGB_{vasc} estimate, as litter was inadvertently included. To correct for this discrepancy, we calculated a litter-to-AGB_{vasc} ratio using data from previous years where litter was separately categorised (2013, 2016), enabling adjustment of the 2018 values for consistency across all sampling years.

Furthermore, outliers were detected using boxplots, which identifies values that fall beyond 1.5 times the interquartile range (IQR) from the first (Q1-1.5*IQR) or third quartiles (Q3+1.5*IQR and thereby removed throughout the dataset (*ggstatsplot;* Patil, 2021) , and a Cleveland dot plot (Cleveland, 1985) was employed to thoroughly consider data points as not every outlier is a wrong value.

To gain an initial understanding of the dataset, we tested data for normality using the Shapiro-Wilk test ($\alpha = 0.05$). However, as linear regression models require normally distributed and homoscedastic residuals—not necessarily normally distributed response or explanatory variables—we verified these assumptions specifically on the model residuals. For each linear model (LM) and linear mixed model (LMM), we examined normality of residuals with Q-Q plots and assessed homoscedasticity using the Breusch-Pagan test ($\alpha = 0.05$). Additionally, we performed a correlation analysis among variables using Pearson's correlation ($\alpha = 0.05$).

For data that did not meet normality assumptions based on residual analysis, we applied a logarithmic transformation to the response variable to improve model fit. Following model fitting, we interpreted and visualised results by retransforming estimates for clearer interpretation. This approach allowed us to maintain straightforward interpretations of model effects, with each unit increase in predictor variables corresponding to a proportional change in the response variable, despite the transformation. Additionally, the model structure was cross-checked using the approach of Zuur et al. (2009) to ensure effective and accurate model construction.

To assess the T_s and grassland ecosystem (site) effect per year on the AGB_{vasc}, we performed a linear regression with interaction (*lm* function in the *stats* package) with independent variables T_s and site. Furthermore, the interannual variability was assessed by looking at the control plots and performed a linear model with soil temperature sum ($T_{s\text{-}sum}$) and years as independent variables over two annual periods (July to July and November to July respectively) and one seasonal period (June to July of sample year). Then, we fitted a linear mixed-effect models analysis of variance – ANOVA (*lme* function in the *nlme* package) to the AGB_{vasc} with years as random effects, and environmental variables as fixed effects.

$$log\ (AGB_{vasc})_{ij} = \alpha + \beta_1 \times (T_{s\text{-}sum})_{ij} + \beta_2 \times Site_{ij} + \beta_3 \times SWC_{ij} + \beta_4 \times (T_{s\text{-}sum})_{ij} \times Site_{ij} + \beta_5 \times (T_{s\text{-}sum})_{ij} \times SWC_{ij} + \beta_6 \times (T_{s\text{-}sum})_{ij} \times Site_{ij} \times SWC_{ij} + \mathrm{a}_i + \varepsilon_{ij} \quad (2)$$

Where AGB_{vasc} (aboveground vascular biomass) is the response variable; α is the intercept of the model; β_n, β_{n+1} is the slope, of the independent variable, that is, $T_{s\text{-}sum}$ the soil temperature sum, Site = MTW and LTW, SWC = soil water content; a_i = the random effect accounting for the variability that is unique to each site, which is not explained by the fixed effects; ε_{ij} = the error term capturing any residual variability that is not explained by the model.

To effectively remove the multi-year variability in response of AGB_{vasc} using the reference control plots across all soil warming levels, we normalised the data by first calculating annual AGB_{vasc} baseline values for the control plots, then we proceeded by calculating the annual deviation from the baseline values established by the control plots for each site combination. The data was finally normalised specifically for each year, site, transect, and gradient combination, by taking the difference between AGB_{vasc} values and the annual deviation from the calculated baseline. Then, a linear regression was conducted to assess the effect of chronic warming on AGB_{vasc}, with soil warming levels, $T_{s\text{-}sum}$ and years as independent variables.

A linear mixed-effect models analysis of variance – ANOVA with years as random effects, and environmental variables as fixed effects was also performed. For model selection in all linear mixed models, we followed Zuur et al. (2009) steps and for the restricted maximum likelihood (REML), and goodness of fit, the lowest Akaike Information Criterion (AIC) was chosen. The random and fixed effect selection followed as well Zuur et al. (2009) steps.

$$log\ (AGB_{vasc})_{ij} = \alpha + \beta_1 \times (T_{s\text{-}sum})_{ij} + \beta_2 \times Site_{ij} + \beta_3 \times SWC_{ij} + \beta_4 \times (T_{s\text{-}sum})_{ij} \times Site_{ij} + \beta_5 \times (T_{s\text{-}sum})_{ij} \times SWC_{ij} + \beta_6 \times (T_{s\text{-}sum})_{ij} \times W\text{-}level_{ij} + \beta_7 \times (T_{s\text{-}sum})_{ij} \times Site_{ij} \times SWC_{ij} + \mathrm{a}_i + \varepsilon_{ij} \quad (3)$$

AGB_{vasc} (aboveground vascular biomass) is the response variable; α is the intercept of the model; β_n, β_{n+1} is the slope, of the independent variable, that is, $T_{s\text{-}sum}$ the soil temperature sum, Site=MTW and LTW, SWC = soil water content, W-level = soil warming levels; a_i = the random effect accounting for the variability that is unique to each site, which is not explained by the fixed effects; ε_{ij} = the error term capturing any residual variability that is not explained by the model.

Moreover, Equation (3) extends Equation (2) by incorporating W-level as an additional factor, allowing for a more comprehensive analysis of how soil temperature, soil water content, and site interactions affect the AGB_{vasc}. While both models account for interannual variability using random effects for years and follow the model selection steps outlined by Zuur et al. (2009).

Results

OBSERVED RESPONSES IN PRODUCTIVITY

The observed patterns in the whole multi-year dataset on AGB_{vasc} across the soil warming gradients did not follow the expected patterns (Figure 1).

When looking at the overall dataset, none of the years in the MTW had a significant change ($p > 0.05$) in AGB_{vasc} across the ca. +13°C soil warming gradients. In the LTW, the years 2013 (AGB_{vasc} [gm^{-2}] = 62 + 13 × MAT_s [°C], $p = 0.002$, $R^2 = 0.36$; Figure 1), 2016 (AGB_{vasc} [gm^{-2}] = 130 + 17 × MAT_s [°C], $p = 0.023$, $R^2 = 0.22$; Figure 1) , and 2020 (AGB_{vasc} [gm^{-2}] = 0.8 + 67× MAT_s [°C], $p = 0.036$, $R^2 = 0.34$; Figure 1) showed a positive increase in AGB_{vasc} with soil warming. On the other hand, the years 2018, 2021 and 2022 were not significant (Figure 1) with soil warming.

It should be noted that for LTW part of the warmest plots harvested from 2018 and onwards had to be excluded from the analysis because of non-stable annual warming, which reduced the warming range in those later years making it more difficult to observe significant changes across the warming gradient (Figure 1-LTW). However, the most striking observation from Figure 1 is that the interannual variability induced changes in AGB_{vasc} that could amount to >900 gm^{-2}. While when comparing different regression lines to see if their intercept and slope coefficients are different, the chronic soil warming only induced significant changes in AGB_{vasc} that amounted to loss of 92 gm^{-2} by +10 °C (data not shown) warming in MTW.

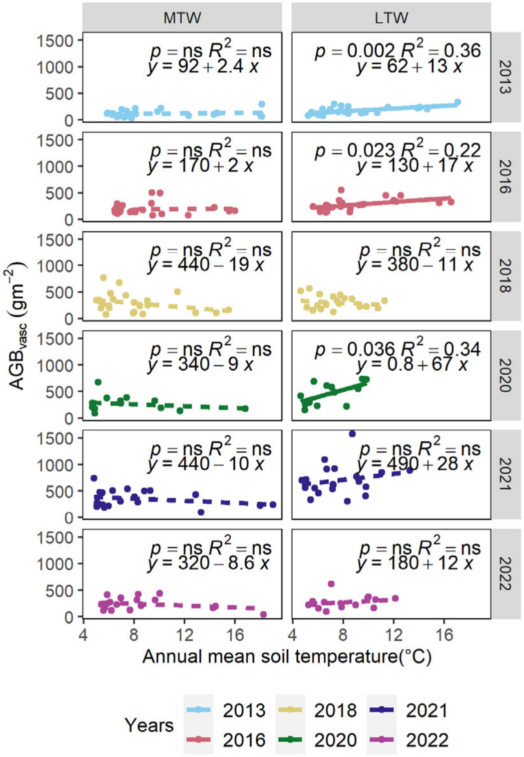

Figure 1: Linear regression between the AGB_{vasc} (gm^{-2}) and the annual mean soil temperature (MAT_s, °C) across different years (2013, 2016, 2018, 2020, 2021, 2022) for both the medium-term (MTW) and long-term (LTW) soil-warming grasslands in S-Iceland. Solid lines indicate significant relationships ($p < 0.05$), whereas dashed lines indicate non-significant trends ($p > 0.05$).

INTERANNUAL VARIABILITY OF COLD GRASSLANDS

To delve further into the causes of the substantial AGB_{vasc} interannual variability response observed in Figure 1, we confined our analysis to the AGB_{vasc} in the control plots across various years. The initial inquiry focused on whether the average soil temperature (T_s) accurately reflected the variability observed in the air temperature (T_a) measured near the sites. If that was not the case, then that could possibly explain the unexpectedly stronger interannual response of AGB_{vasc} compared to a chronic change in T_s. A linear regression analysis between monthly T_a and T_s showed, however, a strong positive relationship across all the years (Figure 2; T_s [°C] = $0.93 + 0.9 \times T_a$ [°C], $R^2 = 0.76$, $p < 0.0001$), indicating that one could be representing the other.

The observed decreasing sensitivity trend of T_s (more spread in T_a around average T_s) towards the lowest T_a values was expected and represented winter conditions when soil is either frozen or covered by snow which gives an insulation from T_a fluctuations (Figure 2). Moreover, there was no significant difference ($p > 0.05$) between the MTW and the LTW sites in this T_a versus T_s response. These findings support that annual differences in measured individual (control) plot T_s can largely be interpreted as differences in T_a in the respective year.

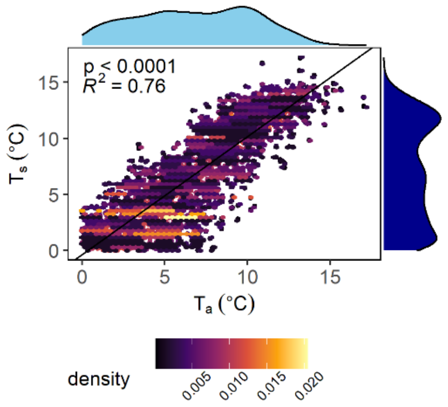

Figure 2: Regression showing a significant positive trend ($p < 0.0001$, $R^2 = 0.76$) between control plots annual soil temperatures (T_s) and annual air temperature (T_a) across the sampled years (2013, 2016, 2018, 2020, 2021, 2022) for both studied sites (MTW, LTW) in S-Iceland, with density indicated by colour intensity and marginal distributions highlighting seasonal clustering.

Looking at MAT_s seasonal averages (June – July) or annual averages (November-July, July-July) of T_s values for different years in the control plots, there was no significant relationship with AGB_{vasc} across the years for the MAT_s (November-July) and seasonal averages (June-July) (data not shown). However, when we used temperature sum ($T_{s\text{-}sum}$) that accumulated daily T_s above the baseline temperature of 0°C from the peak of the growing season of the previous year to that of the sampling year (July-July) we found a positive significant relationship across merged sites – MTW, LTW (AGB_{vasc} [gm^{-2}] = $-322.5 + 0.25 \times T_{s\text{-}sum}$ [°days]; $p = 0.044$, $R^2 = 0.10$, non-significant intercept, Figure 3c). When the $T_{s\text{-}sum}$ was calculated for the current growing season (June-July) no positive trend emerged (Figure 3a, $p = 0.14$) and when the $T_{s\text{-}sum}$ accumulation was started from the beginning of previous winter (Nov.-Jul.) the positive trend was still not significant (Figure 3b, $p = 0.46$). Late growing season conditions in the previous year and winter conditions were apparently more important than temperatures during the current growing season in influencing the AGB_{vasc} measured in late July.

Still, a better relationship that only explained 10% of the observed variation in AGB_{vasc} in the control plots across all years.

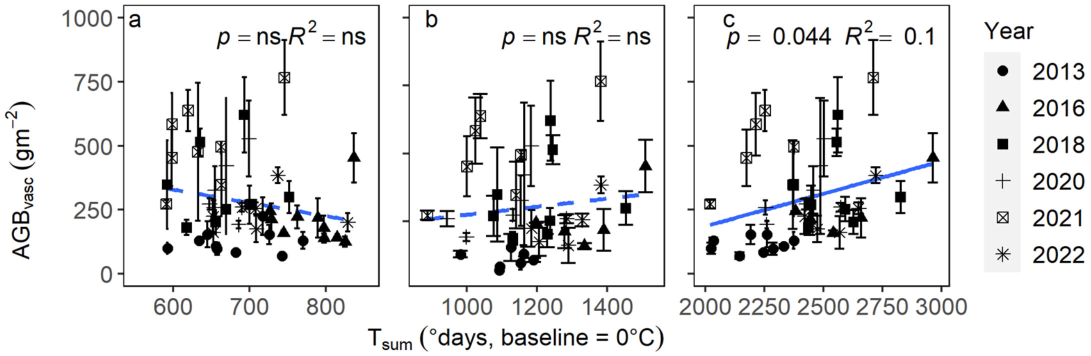

Figure 3: Linear regression showing the relationship between vascular aboveground biomass (AGB_{vasc}, gm^{-2}) and soil temperature sums ($T_{s\text{-}sum}$, growing degree days) in the control plots grassland plots in S-Iceland. a – $T_{s\text{-}sum}$ for the peak growing season of sampling year (June to July); b – $T_{s\text{-}sum}$ from the winter of the previous year to the peak of the growing season of the sampling year (November to July); c – annual $T_{s\text{-}sum}$ from the peak of the growing season of the previous year to that of the sampling year (July to July). Solid lines indicate significant trends ($p < 0.05$), whereas dashed lines indicate non-significance ($p > 0.05$).

A linear mixed effects model (Table 1) was used to examine the relationship between AGB_{vasc} and the available environmental factors from each plot across all years. This approach aimed to further explore potential explanations for the observed annual variability. The best model we found included soil water content (SWC), site difference (MTW, LTW) and interactions with control annual $T_{s\text{-}sum}$ at a baseline temperature of 0°C (Table 1). Out of the three fixed effects variables, only the $T_{s\text{-}sum}$ had a significant effect on AGB_{vasc} in the control plots ($p = 0.003$, $F = 10.59$) and the site difference was not significant ($p > 0.05$), alongside the SWC or interactions among fixed factors. The test of the random effect in the model had a likelihood ratio indicating that the model with the random intercept was considerably better ($L = 6.73$, $df = 14$, $p = 0.035$). We can, therefore, assume that the relationship between AGB_{vasc} and $T_{s\text{-}sum}$ changed randomly between years, with an increase in AGB_{vasc} with $T_{s\text{-}sum}$ for each °C accumulated $T_{s\text{-}sum}$ (slope = 1.003, ICC= 0.68, Table 1).

Table 1: Linear mixed effect model (Equation (2)) ANOVAs testing whether AGB_{vasc} at control temperatures (0°C, no warming), significantly responded to various environmental variables measured in the unwarmed sub-Arctic grassland plots in S-Iceland ($T_{s\text{-}sum}$, Site, SWC as fixed factors) during different years (2013, 2016, 2018, 2020, 2021, 2022; as random factor).

Source	***numDF***	***denDF***	***F-value***	***P-value***
Fixed effects				
$T_{s\text{-}sum}$	1	31	**10.59**	**0.003***
Site	1	29	3.59	0.068
SWC	1	31	0.08	0.783
$T_{s\text{-}sum}$ × Site	1	29	1.91	0.177
$T_{s\text{-}sum}$ × *SWC*	1	29	2.25	0.145
$T_{s\text{-}sum}$ × Site × *SWC*	2	31	3.16	0.057
Random effect: Year				
ε_{ij}	0.20	N_{Year}	6	Observations
σ^2 $_{Year}$	0.09	AIC	127.2	41
ICC	0.68	BIC	142.6	
Marginal R^2 / Conditional R^2	0.15/ 0.73	RMSE	0.261	

σ^2 $_{Year}$ = variance; ε_{ij} = residual term; ***significance $p < 0.05$**; "×" interaction term
SWC = soil water content
$T_{s\text{-}sum}$ = soil temperature sums (growing °days)
Site = grassland ecosystem (MTW vs. LTW unwarmed plots)

EFFECT OF CHRONIC WARMING ON AGB_{vasc}

To address the effects of chronic warming on AGB_{vasc}, we first normalised all the data with the observed interannual differences of AGB_{vasc} on the control plots in each transect. Thereafter we stratified the plots' soil warming levels (W-levels) into five groups, with 0°C as control plots, then +0.48°C, +1.78°C, +3.27°C, and +7.38°C (Figure 4). There was no overall difference between the two sites in the regression lines in the normalised data for different years ($p > 0.05$; data not shown). When performing a regression analysis between annual $T_{s\text{-}sum}$ and AGB_{vasc} across all years and both sites, a significant W-level pattern appeared (Figure 4). Per unit $T_{s\text{-}sum}$ at the control plots, there was an increase of 0.41 gm^{-2} in average AGB_{vasc} productivity. There was, however, a clear "down-regulation" in this $T_{s\text{-}sum}$ response with increasing W-level, leading to the slopes decreasing to 0.38, 0.14 (n.s.), 0.17, 0.07 gm^{-2} for the +0.48, +1.78, +3.27 and +7.38°C W-levels, respectively (S3 Table1). Still, the positive impacts of the chronic soil warming on average AGB_{vasc} were significant for four W-levels (Figure 4).

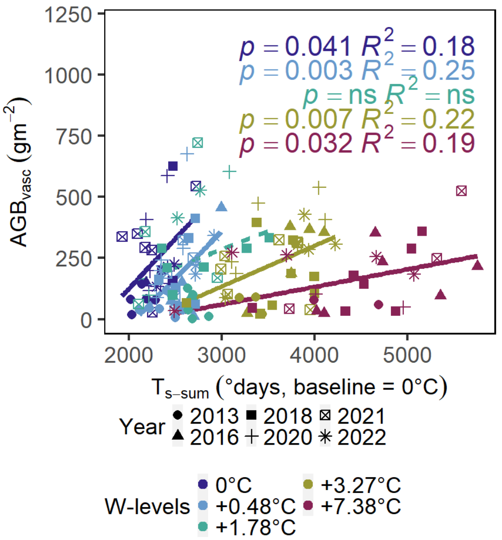

Figure 4: Linear regression showing a positive relationship between $\mathrm{AGB_{vasc}}$ and annual $\mathrm{T_{s\text{-}sum}}$ (July to July) at baseline temperate of 0°C at different soil warming levels (W-levels) over the studied years and studied grassland sites in Iceland.

The analysis of the chronic warming was taken further by applying a similar linear mixed effect model between $\mathrm{AGB_{vasc}}$ and environmental factors that we had available from all control and warmed plots across all the years and both sites, without applying any normalisation of the data but keeping the W-levels as a group (Table 2). The chosen best model (AIC = 1793) demonstrated a robust association between $\mathrm{AGB_{vasc}}$ and various environmental factors changing randomly between years ($L = 133.94$, $df=9$, $p < 0.0001$, Conditional $R^2 = 0.60$; Table 2). However, it should be noted that the marginal R^2 of the fixed effects explained ca. 21% of the variability. The model found that the two sites (MTW, LTW) responded significantly differently to the chronic warming in their $\mathrm{AGB_{vasc}}$, but still within a relatively small marginal R^2 that fits well with the small opposite trends seen in the actual data shown in Figure 1.

The interaction between $\mathrm{T_{s\text{-}sum}}$ × W-level was significant, which confirmed the earlier finding with down-regulation of the $\mathrm{T_{s\text{-}sum}}$: $\mathrm{AGB_{vasc}}$ relationship between W-levels as was found in Figure 4. Soil water contents did still play some part in different $\mathrm{AGB_{vasc}}$ at higher $\mathrm{T_{s\text{-}sum}}$ ($\mathrm{T_{s\text{-}sum}}$ × SWC).

Table 2: Linear mixed effect model (Equation (3)) ANOVAs testing whether environmental variables (fixed effect) affect the AGB_{vasc} at both medium-term warming (MTW) and long-term warming (LTW) in sub-Arctic grasslands in S-Iceland with different years (2013, 2016, 2018, 2020, 2021, 2022) as random effect.

Source	***numDF***	***denDF***	***F-value***	***P-value***
Fixed effects				
$T_{s\text{-}sum}$	1	114	1.78	0.185
Site	**1**	**114**	**16.23**	**0.0001**
SWC	**1**	**114**	**9.34**	**0.003**
$T_{s\text{-}sum}$ × Site	**1**	**122**	**14.98**	**0.0002**
$T_{s\text{-}sum}$ × SWC	**1**	**122**	**7.86**	**0.006**
$T_{s\text{-}sum}$ × W-level	**4**	**118**	**7.30**	**<0.0001**
$T_{s\text{-}sum}$ × SWC × Site	1	114	0.11	0.737
***Random effect**: Year*				
ε_{ij}	0.163	N_{Year}	6	Observations
σ^2 Year	0.159	AIC	1793	130
ICC	0.49	BIC	1830	
Marginal R^2 / Conditional R^2	0.21 / 0.60	RMSE	0.379	

σ^2 Year = variance; ε_{ij} = residual term; ***significance p < 0.05**; "×" interaction term
SWC = soil water content
$T_{s\text{-}sum}$ = soil temperature sums (growing °days)
Site = ecosystem (MTW, LTW; duration of warming)
W-level = soil warming levels

Discussion

RELATIVE IMPACTS OF INTERANNUAL VARIABILITY

The study did not validate our first hypothesis that the interannual variability in response of AGB_{vasc} would have a smaller impact on AGB_{vasc} in comparison to large-scale chronic warming. We observed that interannual variability exerted more influence on grassland productivity than previously anticipated, even surpassing the effects of chronic soil warming (Figure 1). This could indicate either that the temperature is not an important driver for AGB_{vasc} in the sub-Arctic grassland ecosystems we studied, or that the AGB_{vasc} may have changed during the long exposure to soil warming. We will address this in more detail in the following sections.

INTERANNUAL VARIABILITY OF COLD GRASSLANDS

To get a better understanding of the dynamics of the observed interannual variability of AGB_{vasc}, in the control plots we first looked at the relationship between AGB_{vasc} and annual $T_{s\text{-}sums}$. Then tested whether, at control temperatures, environmental variables affected the AGB_{vasc} at both medium-term warming (MTW) and long-term warming (LTW) with different studied years (linear mixed effect model).

We primarily showed that in the absence of active soil warming, soil temperature variations closely mirrored air temperature fluctuations (Figure 2). When only considering the variation in soil

temperatures (Figure 3), our data showed that only when the annual $T_{s\text{-}sum}$ (July-July) with a baseline temperature of 0°C was used, a significant linear relationship appeared with observed AGB_{vasc} across both sites. This finding was interesting and unexpected, as the traditional growing season $T_{s\text{-}sum}$ did not significantly explain the interannual variation. The response was weaker and insignificant when only adding the previous winter (November-July). This implies that conditions in the previous late summer and winter influenced the growth dynamics of the plants in the next growing season.
At our field site, the grassland species are perennial (Sigurdsson et al., 2016), so they store their energy and available nutrients in their root systems for the next growing season. One reason for the conditions from the previous growing season to play a role in the next growing season's productivity can be the uptake and storage of resources at the end of the season and in the winter will increases growth in the following growing season. Such dynamics are well known in Arctic environments (Peterson, 2014).
Thorvaldsson and Björnsson (1990) partly support our results, showing that higher winter temperatures (October to May) stimulated the productivity of cultivated grassland species in Iceland in the following growing season. However, their study did not account for autumn temperatures as ours did, which may be more relevant in the sub-Arctic where autumns and winters are milder than in the Arctic. In contrast, Van Der Wal & Stien (2014) found a strong relationship between plant biomass and summer temperature in the high Arctic, with little evidence of a lagged effect from the previous year's biomass.

The outcome of applying a mixed linear model that included T_{sum}, soil water availability and site differences between the control plots, only found $T_{s\text{-}sum}$ to significantly affect AGB_{vasc} across the years investigated (Table 1). This was partly supporting our Hypothesis 2, where we expected that T_{sum} would be a stronger explaining factor at these sites than soil water, as the area normally receives >800 mm of precipitation annually (Sigurdsson et al., 2016). However, it should be noted that we did not include other potential limiting factors, such as irradiance (Muraoka et al., 2008) or nutrients (Meeran et al., 2023), as we did not have those variables measured at plot level for all the different years. Also, it should be noted that our model could only explain a limited part of the observed interannual variability in the control plots (Table 1; marginal R^2 of 0.15).

Moreover, recent studies suggest that interannual variability could have a significant influence on AGB_{vasc} in control environments, potentially playing a larger role in feedback mechanisms than previously understood. For instance, Post & Pedersen, (2008) reported that the timing of snowmelt, which varies significantly from year to year, profoundly affects the length of the growing season and productivity in high-latitude ecosystems, such as the Greenlandic low shrub tundra. In Icelandic grasslands, Leblans et al. (2017) found that the growing season lengthened linearly by an average of 2.1 days per °C of soil warming, potentially extending it up to one month at the highest warming levels. Additionally, interannual variability can impact biodiversity and ecosystem functions. Myers-Smith et al. (2011) demonstrated that significant year-to-year variations in plant community structure, driven by fluctuations in temperature and other environmental factors, can be more pronounced than changes caused by prolonged warming. These factors highlight the importance of considering interannual variability alongside chronic warming in studies of Arctic and sub-Arctic ecosystems, as they can significantly influence ecosystem dynamics and productivity.

CHRONIC SOIL WARMING EFFECTS ON AGB_{vasc}

We expected that chronic soil warming would increase productivity (hypothesis 3). Our findings showed when normalised for the interannual variability that AGB_{vasc} increased significantly with the level of the chronic warming, however, with a "down-regulation" in the realised AGB_{vasc} across most of the warming-levels (Figure 4).

The increase in productivity throughout soil warming levels may have an indirect biological explanation, such as an increase in microbial decomposition of organic matter (Metze et al., 2024) and an increased nutrient release for plant uptake and growth (Rinnan et al., 2009). Research has demonstrated that increased soil nutrients, such as inorganic nitrogen (N) and phosphorus (P), can lead to improved conditions for plant growth, resulting in higher vegetation biomass (Wild et al., 2016). This highlights the interconnected nature of nutrient availability and plant productivity in sub-Arctic ecosystems undergoing warming. Unfortunately, we were unable to incorporate nutrient availability in our study to support this point since such data were not available for all the years. However, in another recent paper (Mortier et al., 2024) where we looked at Normalised Difference Vegetation Index (NDVI) during similar period in the grassland’s sites, we observed a significant increase in maximum NDVI value with soil warming. NDVI responds to chlorophyll content per unit area (Caruso et al., 2017; Datt, 1998), which again is affected by N and P availability (Chapin et al., 2011; Kizilgeci et al., 2021). This is an indirect proof that the underlying driver (nutrient availability) could be accounted for a positive response in AGB_{vasc} to chronic soil warming.
The observed “down-regulation” of the positive response of AGB_{vasc} with chronic warming could also be explained with results from other studies conducted on the same grasslands, through the effects on topsoil soil organic matter (SOM) stocks. Both Poeplau et al. (2020) and Verbrigghe et al. (2022a) have shown that there have been large losses of SOM following the onset of the soil warming and the loss has been in direct relation to the warming level, which has also decreased the amount of microbial biomass (Verbrigghe et al., 2022b). Walker et al. (2018) have also shown that the heterotrophic respiration rates (breakdown of SOM) have decreased with the warming level at the LTW site, due to less microbial biomass at warmer levels, and in fact found almost total down-regulation in decomposition rates in warmer soils. Additionally, research at the sites showed that the soil organic carbon (SOC) stocks have also decreased to the same extent as the SOM stocks

(unpublished data). That means, in combination with the above findings, that we can explain why the observed low positive AGB_{vasc} response to chronic warming is so low, compared to the interannual variability response of AGB_{vasc} effects on the growing conditions, which also includes the mineralisation rates of the remaining SOM at different warming levels, and other factors. At exceptional good environmental conditions for decomposition, the smaller SOM stocks in warmer soils may act as a bottleneck, limiting the magnitude of AGB_{vasc} interannual growth response. Other warming studies have also found similar indications for reduced mineralisation rates of N or vegetation NPP in warming manipulation experiments on grassland and tundra ecosystems at high latitudes (e.g. Sistla & Schimel, (2013); Vande-Velde, (2014)), but not having as detailed information on both structural and biological changes on the soil ecosystems in their experiments, to fully explain the dynamics of their observed responses.

Another common limiting factor for vegetation responses in manipulation experiments including warming can be reduced soil water availability, through enhanced evapotranspiration at the warmer conditions (Aalto et al., 2013). However, Sigurdsson et al. (2016) have shown that such indirect effects are rare in this particular experiment in the very wet climate of S-Iceland, and therefore unlikely to significantly play into the effect of the chronic warming. However, when dry spells do happen during summer, such effects can contribute to the interannual variation in at higher warming levels. The second run with the linear mixed model, including other environmental parameters than temperature sums (Table 2), showed significant interactions between $T_{s\text{-}sum}$, SWC, site (MTW, LTW) that influenced the observed (interannual) variation in AGB_{vasc}.

DURATION OF WARMING

Overall, there were relatively small differences in how the two sites responded with chronic warming, but in the LTW some years showed a positive response, while in the MTW pooling certain years showed a slight negative change in AGB_{vasc} with warming (Figure 1). Which is in contrast to our final hypothesis, where we expected the duration of warming will have cumulative effects on the response of biomass production by potentially leading to a more pronounced growth response over time in the LTW. The second run with the linear mixed model (Table 2) also confirmed that across all the years the LTW was significantly more responsive than MTW. Still, the relatively small difference in the response between the two sites can partly be explained by both Walker et al. (2018) and (Verbrigghe et al., (2022a). They showed that the chronic warming-induced losses of SOM took place during the first decade of warming and thereafter a new steady-state seemed to be achieved, explaining similar responses across both the sites. However, Walker et al., (2020) compared a wide array of ecosystem parameters between the two sites and found a general negative warming-induced “over-reaction” in the MTW site compared to LTW. Something that our observations of AGB_{vasc} seem to support, although the difference is relatively small between the sites. Suggesting that, a prolonged exposure to increased temperatures can lead to changes in soil carbon feedback mechanisms, potentially altering nutrient availability and microbial activity over decades (Melillo et al., 2017).

Conclusion

In conclusion, our research sheds light on the complex interplay of soil warming, interannual variability in response of AGB_{vasc}, and chronic warming on grassland productivity in sub-Arctic grasslands with a case study in Iceland. Our findings underscore the importance of considering interannual variations, highlighting the role of previous growing seasons in shaping the next season's productivity. Thereby, highlighting the importance of considering the interannual variability in response of AGB_{vasc} alongside chronic warming in studies of Arctic and sub-Arctic ecosystems, as they can significantly influence ecosystem dynamics and productivity. Moreover, the chronic warming surprisingly, led to relatively small changes in aboveground productivity. We showed that this response was mediated through a down-regulation across the soil warming levels, potentially due to decreases in SOM, microbial biomass and N-stock. These insights contribute to our understanding of likely ecosystem responses to long-term global warming in sub-Arctic regions.

CRediT Author Statement

Ruth Phoebe Tchana Wandji: Conceptualisation, Writing – Original Draft, Writing – Review & Editing, Validation, Visualisation, Methodology, Investigation, Formal Analysis, Data Sampling (2020, 2021, 2022), Data Curation. **Amir Hamedpour:** Writing – Review & Editing **Agathe Merand**: Data Sampling and preliminary analysis (2020). **Niki Leblans:** Data Sampling (2013). **Niel Verbrigghe**: Data Sampling (2016, 2018). **Iolanda Filella**: Funding Acquisition, Writing - Reviewing & Editing, Supervision. **Peter Lootens:** Funding Acquisition, Writing - Reviewing & Editing, Supervision. **Ivan Janssens:** Data Sampling (2016, 2018), Funding Acquisition, Project Administration. **Bjarni D. Sigurdsson**: Investigation, Conceptualisation, Writing - Review & Editing, Funding Acquisition, Supervision, Project Administration.

DECLARATION OF COMPETING INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY

The data used in this manuscript can be found at the Open Access ZENODO Database, https://doi.org/10.5281/zenodo.13737201.

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Supporting Information

S1 Figure 1

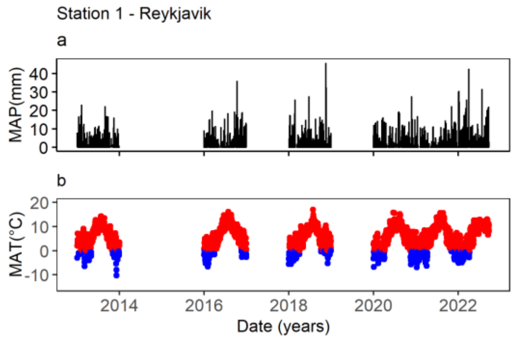

Figure 1: Atmospheric variables (a – daily accumulated precipitation (MAP, mm) b- daily mean air temperature (MAT, °C)), observed at the ForHot research site from Reykjavik station 1 (Icelandic et. Office) for both studied grasslands in Iceland. The atmospheric variables comprise the studied years; 2013, 2016, 2018, 2020, 2021, 2022. At (b) the blue colour represents values below 0°C and red values above 0°C.

S2 Figure 2

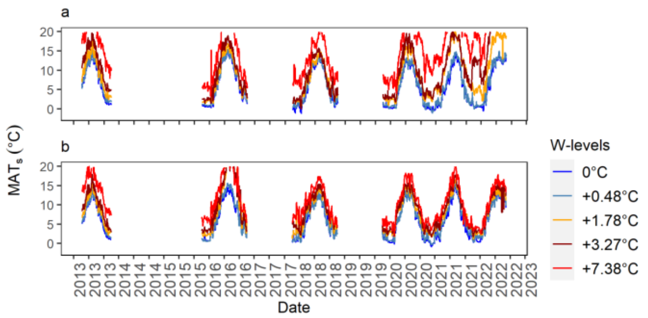

Figure 2: Daily soil temperature (°C) sampled at the ForHot research site for the years 2013, 2016, 2018, 2020, 2021, 2022 at both studied grasslands (a – medium term soil warming (MTW) b- long term soil warming (LTW)), in Iceland. W-levels are the soil warming levels at both studied grasslands.

S3 Table 1

Table 1: Linear statistical relationship between AGB_{vasc} and annual $T_{s\text{-}sum}$ (July to July) with 0°C of baseline temperate at different soil warming levels (W-levels) over the studied years and grassland sites in Iceland.

W-levels	Intercept	Slope	Std Error	p-value	R^2
0°C	-21.62	**0.31**	**0.12**	**0.017***	**0.13**
+0.48°C	-30.93	**0.19**	**0.07**	**0.013***	**0.13**
+1.78°C	86.56	**0.14**	**0.07**	**0.042***	**0.09**
+3.27°C	53.08	**0.11**	**0.04**	**0.008****	**0.14**
+7.38°C	86.94	**0.07**	**0.03**	**0.018***	**0.12**

Significance codes p-value: 0 '*' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1**

Effects of soil warming and interannual variability on aboveground biomass production of sub-Arctic grasslands

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Table 1: Linear mixed effect model (Equation (2)) ANOVAs testing whether AGB_{vasc} at control temperatures (0°C, no warming), significantly responded to various environmental variables measured in the unwarmed sub-Arctic grassland plots in S-Iceland ($T_{s\text{-}sum}$, Site, SWC as fixed factors) during different years (2013, 2016, 2018, 2020, 2021, 2022; as random factor).

Source	***numDF***	***denDF***	***F-value***	***P-value***
Fixed effects				
$T_{s\text{-}sum}$	1	31	**10.59**	**0.003***
Site	1	29	3.59	0.068
SWC	1	31	0.08	0.783
$T_{s\text{-}sum}$ × Site	1	29	1.91	0.177
$T_{s\text{-}sum}$ × *SWC*	1	29	2.25	0.145
$T_{s\text{-}sum}$ × Site × *SWC*	2	31	3.16	0.057
Random effect: Year				
ε_{ij}	0.20	N_{Year}	6	Observations
$\sigma^2{}_{Year}$	0.09	AIC	127.2	41
ICC	0.68	BIC	142.6	
Marginal R^2 / Conditional R^2	0.15/ 0.73	RMSE	0.261	

$\sigma^2{}_{Year}$ = variance; ε_{ij} = residual term; ***significance $p < 0.05$**; "×" interaction term
SWC = soil water content
$T_{s\text{-}sum}$ = soil temperature sums (growing °days)
Site = grassland ecosystem (MTW vs. LTW unwarmed plots)

Table 2: Linear mixed effect model (Equation (3)) ANOVAs testing whether environmental variables (fixed effect) affect the AGB_{vasc} at both medium-term warming (MTW) and long-term warming (LTW) in sub-Arctic grasslands in S-Iceland with different years (2013, 2016, 2018, 2020, 2021, 2022) as random effect.

Source	*numDF*	*denDF*	*F-value*	*P-value*
Fixed effects				
$T_{s\text{-}sum}$	1	114	1.78	0.185
Site	**1**	**114**	**16.23**	**0.0001**
SWC	**1**	**114**	**9.34**	**0.003**
$T_{s\text{-}sum}$ × Site	**1**	**122**	**14.98**	**0.0002**
$T_{s\text{-}sum}$ × SWC	**1**	**122**	**7.86**	**0.006**
$T_{s\text{-}sum}$ × W-level	**4**	**118**	**7.30**	**<0.0001**
$T_{s\text{-}sum}$ × SWC × Site	1	114	0.11	0.737
Random effect: Year				
ε_{ij}	0.163	N_{Year}	6	Observations
σ^2_{Year}	0.159	AIC	1793	130
ICC	0.49	BIC	1830	
Marginal R^2 / Conditional R^2	0.21 / 0.60	RMSE	0.379	

σ^2_{Year} = variance; ε_{ij} = residual term; ***significance** $p < 0.05$*; “×” interaction term

SWC = soil water content

$T_{s\text{-}sum}$ = soil temperature sums (growing °days)

Site = ecosystem (MTW, LTW; duration of warming)

W-level = soil warming levels